Manipulation of canopy architecture and possible vigour control mechanisms in kiwifruit

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Abstract

Dwarfing or vigour-controlling rootstocks have been used in many fruit trees to reduce scions growth, improve precocity and yield efficiency, but they are not currently available for kiwifruit. Therefore, there is a strong need to evaluate the vigour-controlling rootstocks and/or other growth manipulation techniques for controlling excessive growth of kiwifruit. In this study, the initial growth and architecture of 'Hayward' scions may have been modified by the inter-specific hybrid kiwifruit rootstocks, during the first- and second- year of growth following grafting. Rootstocks modified the trunk cross-sectional area and proleptic bud break of the 'Hayward' primary shoots. The lengths of long and short proleptic shoots of the scions from particular rootstocks were also slightly reduced, thus reducing the total length of proleptic shoots on grafted scions. In the field, inter-specific hybrid kiwifruit rootstocks affected the duration and compactness of scions bud break. The most notable effect of hybrid rootstocks was on the growth rate of long proleptic axillary shoots of scions during early spring growth with 'Hayward' scions on particular rootstocks had the slowest growth rate compared to other rootstocks. Rootstocks may affect scions floral precocity, with 'Hayward' scions on particular kiwifruit rootstocks tended to produce higher flower numbers when they were first planted on the field. There was a strong trend that rootstocks affected the proportion of long shoots and this effect had contributed to the differences in the proportion of non-terminated and terminated shoots of the scions. Auxin transport inhibitor, 1-N-naphthylphthalamic acid (NPA) applied to the stem junction at graft-union on some rootstocks had decreased the length, node number and cross-sectional area of scion primary shoots. However, NPA treatment on particular rootstocks did not affect the growth of scion primary shoots on some of the rootstocks, suggesting that restriction of IAA did not influence the level of IAA transported from shoot to root system of those kiwifruit rootstocks. NPA reduced the leaf size of scions, indicating that sufficient IAA is needed for the leaf growth of kiwifruit, but it may be regulated by the rootstocks. The transport and uptake of radioactivity of IAA in the stem segment varied between the rootstocks, suggesting that the level of IAA in the stem tissues of inter-specific hybrid kiwifruit rootstocks may vary depending on the vigour and genetics of the kiwifruit rootstocks. Restriction of IAA by inverting a single piece of bark (180-degree orientation) and grafted back to the main stem did not completely reduce the vigour of young 'Hort16A' vines. However,

the growth and vigour of young ‘Hort16A’ vines in terms of total length, total node number and total leaf area were greatly reduced when grafted three rings of bark from other cultivars in an inverted orientation. In the field, the bark grafting treatments along with girdling were evaluated to regulate the characteristics of ‘Hayward’ fruits. All treatments did not consistently produce similar effects in each season and year. Comparison between treatment, season (i.e. early and late summer) and year indicated that the treatment effects on fruit fresh weight, dry weight and dry matter concentration were only evident in the first harvesting year, and the effects were lessened in the following year. In this study, four distinct phenotypes were found from the kiwifruit seedlings population based on their main primary shoots; i) Long Multiple Stems (LMS), ii) Short Multiple Stems (SMS), iii) Long Single Stem (LS), and iv) Short Single Stem (SS). Gibberellins (GA_3+GA_{4+7}) treatment on these phenotypes at an early stage of bud break has transformed the morphology and characteristics of proleptic axillary shoots. The mean total number of proleptic and sylleptic axillary shoots (i.e. branching) was increased with gibberellins treatment, suggesting that gibberellins can promote meristematic activity by regulating both apical and sub-apical meristem of kiwifruit shoots.

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"IN THE NAME OF ALLAH, THE MOST BENEFICENT, THE MOST MERCIFUL"

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List of abbreviations

A.D.	Anno Domini
ABA	Abscisic acid
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BAP	Benzylaminopurine
CCD_n	Carotenoid Cleavage Dioxygenase $_n$ denotes the number
CG.4213	Cornell-Geneva 4213
CG.7037	Cornell-Geneva 7037
CG.8534	Cornell-Geneva 8534
CK	Cytokinins
cm	Centimetre
CO_2	Carbon dioxide
cv.	Cultivar
CSA	Cross-Sectional Area (mm^2)
DMC	Dry matter concentration
dpm	Disintegrations per minute
EMLA.27	East Malling/Ashton Long 27
G.16	Geneva 16
G.935	Geneva 935
GA	Gibberellins
GA_n	Gibberellin $_n$ denotes the number
GLM	General linear model
*GM*1	Growth manipulation 1
*GM*2	Growth manipulation 2
*GM*3	Growth manipulation 3
GN	Green cuttings (self-rooted control)
GR24	Synthetic strigolactones
h	Hour
Hi-Cane	Hydrogen cynamide
HV	High-Vigour rootstock group
IAA	Indole-3-acetic acid
IBA	Indole-3-butaric acid
IV	Intermediate-Vigour rootstock group
K	Kalium
L	Litre
LAI	Leaf Area Index
LMA	Leaf Mass per Area
LMS	Long Multiple Stems
LS	Long Single Stem
LSD	Least Significant Difference
LV	Low-Vigour rootstock group
lsmeans	Least square means
m	Meter
MAX	More Axillary Growth (*n*) denotes the number
M.104	Merton 104
M.16	Malling 16
M.793	Malling 793
M.9	Malling 9
mm	Millimetre
MM.106	Malling Merton 106
MM.111	Malling Merton 111
MM.25	Malling Merton 25
MM.27	Malling Merton 27
N	Nitrogen
NAA	1-Naphthaleneacetic acid
No.	Rootstock number
NPA	1-N-napthylphthalamic acid

O_2	Oxygen
P	Phosphorus
PCA	Principal Component Analysis
PFR	Plant and Food Research
PGU	Plant Growth Unit
PGR	Plant Growth Regulator
PSA	*Pseudomonas syringae* pv. *Actinidiae*
QTLs	Quantitative Trait Loci
RCBD	Randomised Completed Block Design
SAM	Shoot Apical Meristem
SAS	Statistical Analysis System
SLs	Strigolactones
SMB	Short Multiple Stems
spp.	Species
SS	Short Single Stem
TIBA	2,3,5,-Triiodobenzoic acid
UK	United Kingdom
USA	United State of America
VLV	Very Low-Vigour rootstock group
[^{14}C]-IAA	Carboxyl-labelled indole-3-acetic acid
[^{14}H]-IAA	Tritiated-labelled indole-3-acetic acid

Defination of terms

TERM	DEFINATION
Proleptic shoots	The shoots that developed from buds which has been dormant for some period of time. The leaves scale usually enclose the bud.
Sylleptic shoots	The shoots that developed from lateral buds with any period of dormancy. The leaves scale do not usually enclose the bud.
Girdling	The completely removal of a strip of bark around the entire circumference of a vines (consisting of cambium or phloem).
Phenology	The scientific study of periodic biological phenomena (e.g. shoot, flowering and fruiting stage).
Precocity	The ability of fruit trees or vines to induce fruitfulness without the need for completing the juvenile phase.
Bud break	The opening of a dormant bud when the shoot begins to grow and usually during early spring.
Phenotype	The physical appearance, observable characteristics or particular traits (e.g. plant morphology).
Genotype	The set of genes, which is responsible for a particular trait.
Long shoots	The kiwifruit shoots that have a number of neofomed nodes and the total number of nodes per shoot is up to 90, and non-terminated.
Medium shoots	The kiwifruit shoots that have more than nine nodes and terminated.
Short shoots	The kiwifruit shoots that have nine or less nodes and terminated.
Clonal rootstock	A vegetatively propagated or cloned rootstock as opposed to a germinated seedling rootstock.
Leader pruning	Pruning or removing all vigorous vegetative shoots that closed to the central leader of kiwifruit vines.

List of publications and conferences

Abdullah, F. and Woolley, D. J. (2012). Effects of bark inversion on fruit weight, size and dry matter concentration of green kiwifruit (*Actinidia deliciosa* cv. 'Hayward'). *Acta Horticulturae, 1012*, 213-218.

Abdullah, F., Woolley, D.J., B.M. van Hooijdonk and A.P. Friend (2012). Effect of bark insert-grafting on initial shoot architecture and vegetative growth of newly-propagated gold kiwifruit cv. 'Hort16A'. Poster paper presented in 10th International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems. South Africa, 2012. (Part of Chapter 5)

Abdullah, F., Woolley, D.J., B.M. van Hooijdonk and A.P. Friend (2013). Control of vegetative vigour and crop architecture in kiwifruit. Oral paper presented in New Zealand Institute of Agriculture and Horticulture Plant Science Conference 2013, Massey University, Palmerston North, New Zealand.2-4 July 2013. (Part of Chapter 2)

Abdullah, F., Woolley, D.J., K.A. Funnell, B.M. van Hooijdonk and A.P. Friend (2013). Preliminary observation on the initial architecture of kiwifruit seedlings obtained from specific crosses. Poster paper presented in New Zealand Institute of Agriculture and Horticulture Plant Science Conference 2013, Massey University, Palmerston North, New Zealand. 2-4 July 2013. (Part of Chapter 6)

Abdullah, F., Woolley, D.J., B.M. van Hooijdonk and A.P. Friend (2015). Interspecific hybrid kiwifruit rootstocks have potential to modify scion architecture and vigour of young 'Hayward' vines. *Acta Horticulturae, 1096*, 241-246. (Chapter 2)

Chapter One

1. General Introduction and literature review

1.1 Overview of the thesis

The horticultural industry makes a major contribution to the New Zealand economy. In 2014, New Zealand's exports of horticultural products (mainly fruits, vegetables and flowers) to the five major markets were valued at $2.45 billion. These horticultural products were exported to over 100 countries, especially to Australia, Japan, UK and Ireland, Continental Europe and North America (FreshFacts, 2014). In the fruit sector, kiwifruit and apple are the main fruit types that have been exported. In 2014, the total export of kiwifruit was valued at $931 million, slightly less (0.4%) than 2013 ($934 million), possibly due to the impact of the bacterial canker disease specific to kiwifruit *Pseudomonas syringae* pv. *actinidiae* (termed PSA) that affected the kiwifruit production areas. Nevertheless, the New Zealand horticultural industry was strongly supported by the research and development in various horticultural aspects, especially from Plant and Food Research in collaboration with other research agencies, such as Massey University, New Zealand.

Kiwifruit is one of the main fruit types in New Zealand fresh fruit sector. There are two main commercial kiwifruit cultivars, green kiwifruit (*Actinidia deliciosa* cv. 'Hayward') and gold kiwifruit (*Actinidia chinensis* cv. 'Hort16A'). These cultivars have been successfully developed by the HortResearch breeding programme in New Zealand and commercialised under the name ZESPRI^{TM} GREEN Kiwifruit and GOLD Kiwifruit (Ferguson & Huang, 2007). The introduction of 'Hort16A' cultivar in New Zealand significantly boosted the kiwifruit industry. 'Hort16A' was developed from controlled crosses between accessions of kiwifruit. Currently, three new cultivars have been developed by Plant and Food Research and were released by ZESPRI^{TM} which are, SunGold G3, and G9, and a new SweetGreen G14.

Despite the releasing of new cultivars, kiwifruit still had a problem in terms of vigour management. One of the major problems in kiwifruit is excessive vegetative vigour. This has caused an increase in production cost and time for summer and winter pruning (Miller et al., 2001). Excessive vigour can affect fruit yield (McAneney et al., 1989) and lower the dry matter concentration (DMC) (Lancaster & MacRae, 2000) because of insufficient light exposure in the kiwifruit canopies, which is known to be necessary for the fruit quality (Biasi et al., 1995). Currently, Currie et al. (2008) reported that early trunk girdling during late December and an early January can partly control vigour and improve fruit size, while late trunk girdling during mid-February can sometimes increase the DMC. Kiwifruit is a vine crop, that is still lacking in size-controlling rootstocks with only a few limited kiwifruit rootstocks available (Clearwater et al., 2004; 2007a). In New Zealand, growers still rely on 'Bruno' seedling rootstock (Clearwater et al., 2007b; Warrington, 2000) and 'D1' rootstock in Italy (Viti et al., 1990). However, clonal rootstock may offer many advantages for improving productivity and fruit quality in kiwifruit. According to Lowe (1989), only one clonal kiwifruit rootstock namely 'Kaimai' has been registered in New Zealand. Therefore, the development of the clonal rootstocks that can control scions growth, increase fruit quality, extend storage-life, increase DMC and resistances to soilborne disease is needed for the kiwifruit industry (Clearwater et al., 2007b; Palmer, 2007; Warrington, 2000).

In order to search for promising rootstocks for kiwifruit, a rootstock breeding programme has been developed by the HortResearch and funded by ZESPRI Kiwifruit New Zealand (Clearwater et al., 2007b). Initially, kiwifruit seedlings produced from any breeding programme (seedlings in the germplasm collection) were screened. Limited ranges of potential rootstock clones from *Actinidia* species were tested and evaluated with 'Hayward' as the scions, to identify desirable attributes that could be imparted by these rootstocks. Potential characteristics were monitored such as control of scion vigour, improve flowering and fruit quality (especially DMC and size), as well as early maturity. According to Lowe (1989), 'Kaimai' rootstock was selected from eight different *Actinidia* clones. After that, a few series selections were carried out for further testing as potential commercial rootstock cultivars. Besides germplasm selections, several breeding trials involve crossing of potential kiwifruit (hybridization) such as *A. macrosperma* and *A. polygama* to breed a new range of promising rootstock hybrid

clones. This 'inter-specific hybridization' may help to increase the range of rootstock materials. In the present study, thirteen inter-specific hybrid clones of *Actinidia*, grafted with *A. deliciosa* cv. 'Hayward' scions (green kiwifruit) were used for evaluation of potential vigour controlling rootstocks in kiwifruit (Table 1.1). Details of literature on the kiwifruit rootstocks were discussed in Section 1.2.

In some fruit tree species, the relationship between vigour of trees and anatomical structure has been established, for example in apple (Beakbane & Thompson, 1939; Jaumien & Faust, 1984; Miller, 1977b), avocado (Lopez-Jimenez & Barrientos-Priego, 1987), cherries (Floor, 1957; Misirli et al., 1996), mango (Kurian & Iyer, 1992) and citrus (Saeed et al., 2010). However, in kiwifruit, there is little-published information regarding anatomical studies of rootstocks. For example, a study on the root structure of kiwifruit has been described by Lemon & Considine (1993), but their study only focused on one species of kiwifruit, *A. deliciosa* and no comparison was made with other species. Only Wang et al. (1994a) described the root anatomy of a few selections such as *A. deliciosa, A. chinensis, A. hemsleyana, A. eriantha* and *A. rufa*. However, they only presented the anatomical features that could be associated with flowering, not vigour control. In addition, less works have been done on the screening of potential vigour controlling rootstocks in kiwifruit. In the present study, we did not carry out the experimental work on anatomical aspects of dwarfing, but the changes in vigour and architectural structures of 'Hayward' scions grafted onto the inter-specific hybrid kiwifruit rootstocks (Table 1.1) will be described and discussed. Through this study, some potential kiwifruit rootstocks that can control the vigour of grafted vines could be identified and described.

Grafting of plants as composite trees or vines might be altering the translocation of nutrients, water uptake and endogenous translocation of hormones between rootstock and scion (reviews by Atkinson & Else, 2001; Gregory et al., 2013; Lockhard & Schneider, 1981; Webster, 2004). However, the mechanisms on how the rootstocks may control the scions growth, especially on the relationship between endogenous hormones are still not fully understood. In fruit trees, the auxin, indole-3-acetic acid (IAA), appears to be an important hormone related to vigour control, together with interactions with the other hormones such as cytokinins (CK) and gibberellins (GA) that may affect plant growth. A recent study on the dwarfing apple rootstock has provided clear

evidence that an 'endogenous signalling mechanism' was involved in the shoot to root and root to shoot relationships (van Hooijdonk, 2009). For example, dwarfing apple rootstocks such as 'M.9' may reduce scion vigour by decreasing the basipetal transport of IAA to the root system, thus reducing the CK and GA transported from root to shoot (van Hooinjdonk et al., 2010), which causes a modification in scion architecture by decreasing branching and reducing the duration of shoot growth. However, there is little published information regarding the mechanism of hormonal control in kiwifruit vines. Therefore, the knowledge of plant hormonal interactions is needed for a basic understanding of hormonal control in kiwifruit. In this present study, the effects of inter-specific hybrid of kiwifruit rootstocks on the hormonal mechanisms in relation to vigour control was assessed and evaluated. Overall, findings from these studies perhaps can reveal the actual mechanism (s) of hormonal control in kiwifruit.

Table 1.1 The inter-specific hybrid *Actinidia* species that were used as rootstocks in this study that have been grafted with *A. deliciosa* cv. 'Hayward' kiwifruit.

Rootstock selections	Parentages	ǂVigour
No. 8	*A. chinensis x A. macrosperma*	7
No.18	*A. chinensis x A. macrosperma*	8
No.19	*A. chinensis x A. macrosperma*	2
No.21	*A. chinensis x A. macrosperma*	1
No.45	*A. polygama x A. chinensis*	11
No.55	*A. polygama x A. chinensis*	12
No.71	*A. polygama x A. chinensis*	6
No.84[x]	*A. polygama*	?[x]
No.85	*A. macrosperma*	5
No.86	*A. macrosperma*	9
No.87	*A. polygama*	3
No.100	*A. macrosperma x A. melanandra*	4
No.101	*A. macrosperma x A. melanandra*	10

ǂVigour rating was based according to the previous trials with 'Hort16A' scion grafted on each rootstock (Friend et al., 2014). Rating scale: 1- low vigour, 12- high vigour

[x]Unknown vigour.

1.1.1 The importance of rootstocks in fruit tree management

Rootstocks have been used for propagation of fruit trees for more than 2000 years ago (Rom & Carlson, 1987; Webster, 1995a). Grafting a potential scion onto rootstock or interstock produces a composite tree comprised of the shoot system (scion and graft union) and root system (rootstock stem) (Figure 1.1). Sometimes, interstock is used between the shoot (scion) and root system (rootstock) (Figure 1.1). Rootstocks in fruit trees play an important role in controlling vigour of the tree growth, improve in precocity and efficiency of cropping, as well as fruit quality (Castle, 1995; Rom & Carlson, 1987; Webster, 1995a). Furthermore, rootstocks also can be used to improve adaptation of scions to unfavourable climatic conditions and increase resistance to pests and diseases (Mudge et al., 2009; Rom & Carlson, 1987). Rootstocks must be chosen correctly by the fruit growers because many factors can influence the rooting system in a tree, such as soil moisture, temperature, chemical residues, biotoxins, soil CO_2 and O_2 content, compaction, pH, mychorrhiza and genetic of the root itself (Hartmann et al., 2011; Rom & Carlson, 1987). Most rootstocks are propagated using vegetative techniques to produce clonal rootstock (Hartmann et al., 2011; Webster, 1995b). Propagation techniques such as stooling, layering and cuttings are widely used for propagation of clonal rootstocks.

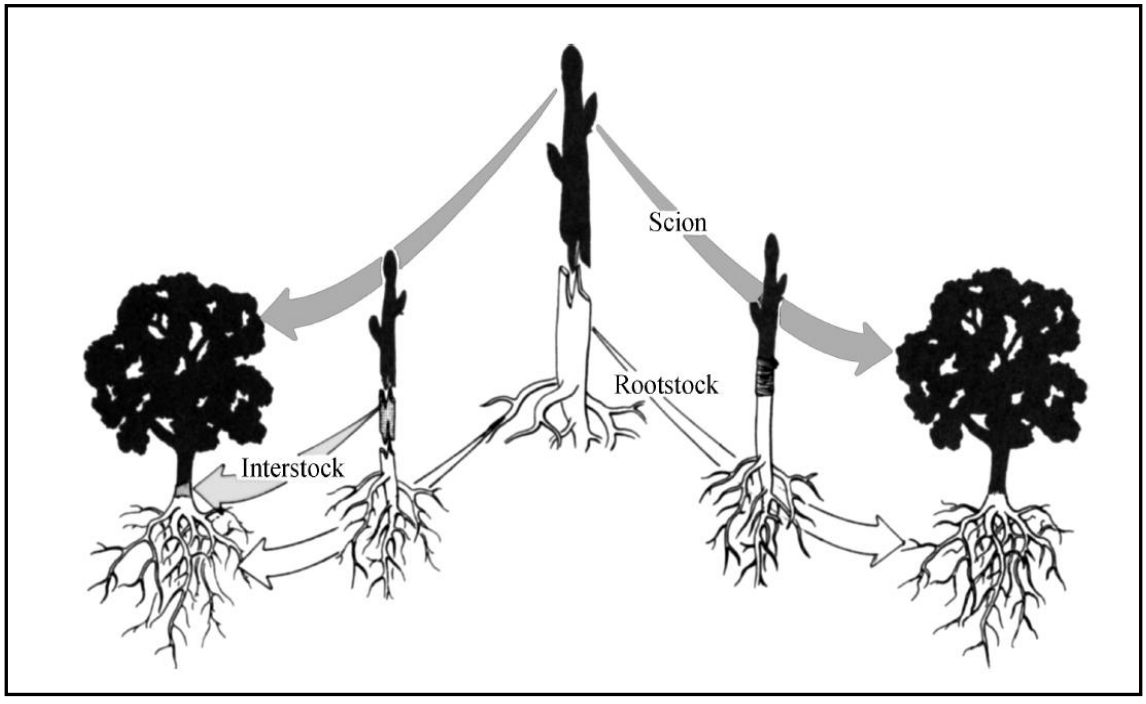

Figure 1.1 A grafted composite tree and its component parts (Mudge et al., 2009)

In temperate fruit trees, rootstocks offer the principal technique of controlling the excessive inherent vigour of the scion cultivars. The vigour of apple, plum, pear, sweet cherry can be controlled very effectively by suitable dwarfing rootstocks. Controlling excessive scion vigour in fruit trees through dwarfing rootstocks has become a priority as the economic viability of fruit tree productions (Webster, 2002). This option has been commercially available for apple, pear and sweet cherries (Webster, 2006), but unfortunately not available for kiwifruit (Clearwater et al., 2004; 2007b). Dwarfing rootstocks generally promote early cropping (precocity) and better yield than vigorous rootstocks. For example, in apple, Ferree et al. (1995) reported dwarfing rootstocks or interstocks can increase the number of floral clusters per branches as well as flower quality. Similarly in kiwifruit, rootstocks from *A. hemsleyana* namely 'Kaimai' also can increase flowering in kiwifruit (Clearwater et al., 2002; Wang et al., 1994b). In trials with 'Hayward' and 'Hort16A' scions, 'Kaimai' rootstocks had improved budburst and produced more flower numbers compared to standard clonal *A. deliciosa* rootstocks. However, no reports were made regarding on the vigour control on this type of rootstocks. Therefore, there is a strong need to develop vigour controlling rootstocks for kiwifruit through breeding programme or selection from potential cultivars.

1.1.2 Hormonal physiology in fruit tree architecture

Manipulation of both vegetative and reproductive growth in fruit tree architecture is important to optimise production and yield (Costes et al., 2006b; Fideghelli et al., 2002). The most important part in plant architecture of fruit trees is largely determined by their branching patterns. It was notable that branching processes of fruit trees have been shown to be correlated with apical dominance and bud dormancy (Cline, 1991; Costes et al., 2006b; Reinhardt & Kuhlemeier, 2002; Ward & Leyser, 2004). Branching in trees is developed from the formation of the axillary meristem initiated in leaves axils (axillary or lateral shoots). In most plants, the development of axillary meristems is initially suppressed by the main shoot apex, a physiological phenomenon known as 'apical dominance' (Cline, 1991; Forshey & Elfving, 1989; Phillips, 1975). This phenomenon is an important mechanism in controlling plant shoot architecture (Tamas, 1995).

Many studies have postulated that apical dominance is mainly under the control of endogenous hormones such as IAA and CK (Bangerth, 1994; Cline, 1994; Shimizu-Sato et al., 2009) and the nutritional condition of the axillary meristems (review by Cline, 1991; Martin, 1987; Phillips, 1975). Even though some of the branching mechanisms have been revealed through genetic studies, for example in *Arabidopsis* (reviews by Evers et al., 2011; Ferguson & Beveridge, 2009; Leyser, 2009), the physiological mechanisms underlying apical dominance and branching patterns are still not fully understood, especially in kiwifruit. Recently, a new class of plant hormones called 'strigolactones' that was found to be involved in controlling branch architecture of plants. Strigolactones, compounds derived from terpenoid lactones, has been implicated in inhibition of shoot branching (Gomez-Roldan et al., 2008; Umehara et al., 2008). Strigolactones were found in root exudates that triggered germination of the parasitic weeds, *Striga* spp. and *Orobanche* (Figure 1.6) (Bouwmeester et al., 2003; Goldwasser et al., 2008; Humphrey & Beale, 2006; Matusova et al., 2005). This new hormone also might be involved in controlling axillary shoot branching in kiwifruit (Honda et al., 2011; Ledger et al., 2010). In addition, preliminary observation by Manandar (2011) found high germination of *Orobanche* seeds when treated with xylem exudates of kiwifruit, suggesting the present of strigolactones in kiwifruit. Therefore, it can be suggested that strigolactones may be involved in branching of kiwifruit. However, more detailed study is needed to reveal the actual mechanism (s) in controlling branching of kiwifruit vines.

1.1.3 Rationale of the thesis, hypotheses and major objectives

Kiwifruit rootstock breeding programmes in New Zealand have the potential to greatly increase production and marketing of kiwifruit to the world. Besides the two main commercial kiwifruit cultivars, 'Hayward' (*Actinidia deliciosa*, green kiwifruit) and 'Hort16A' (*Actinidia chinensis*, gold kiwifruit), the introduction of new cultivars (G3, G9 and G14) may also significantly boost the horticultural industry in New Zealand. However, kiwifruit growers are still facing a major problem in terms of excessive vegetative growth. Although girdling is widely used in vigour control management of kiwifruit, this technique only offers a short-term solution in controlling the vegetative growth of kiwifruit. Thus, there is a strong need to develop vigour controlling

techniques in kiwifruit, for example through vigour controlling rootstocks because it can offer great advantages such as low-cost technique with the long-term effects, at the same times can greatly improve production efficiencies (Palmer, 2007; Warrington, 2000; Webster, 1995a). In this study, research was focused on the evaluation of the inter-specific hybrid rootstocks for vigour control and also growth manipulation (i.e. bark inserts/grafting) that could be an alternative method for vigour control in kiwifruit. In addition, the architecture of seedlings from specific crosses and their responses to plant growth regulator also have been evaluated. Details of materials and methods will be elaborated in each chapter. Proposed experiments and chapter's arrangement are presented in Figure 1.2. Thesis hypotheses and general approaches to achieve the objectives of the present study are stated and outlined below:

Hypothesis I: The effects of hybrid rootstocks on composite kiwifruit vines can be expressed during the early stage of vines growth following grafting, thus will affect the initial shoot architecture of grafted scion.

Hypothesis II: The vigour and architecture structure of scion can be influenced by the hybrid kiwifruit rootstocks when planted in field and the vigour of grafted scion can be related to the parentage of rootstock.

Hypothesis III: Inhibition of auxin (IAA) signalling on the stem junction (i.e. graft-union) by the auxin transport inhibitor (NPA) may influence the transport of IAA from the shoot (i.e. scion) to root (i.e rootstock), thus may affect the growth and architecture structure of grafted kiwifruit vines.

Hypothesis IV: The auxin (IAA) transport from shoot to root may be reduced by the inverted bark grafting, thus may affect the growth, shoot architecture structure and possibly affecting fruit characteristics of kiwifruit vines.

Hypothesis V: Differences in the initial architecture structure of kiwifruit seedlings may be attributed to the different level of GA and exogenous supply of GA may alter the growth and vigour of kiwifruit seedlings.

1.1.3.1 Assessment of 'Hayward' scions grafted onto inter-specific hybrid rootstocks in the nursery stage and field planting

In fruit trees, the architectural structure and branching habit of the scion are modified by the genetics of the rootstock, as early as in the nursery stage. Evidence has been demonstrated in studies with apple (Costes et al., 2001; Fazio & Robinson, 2008; Seleznyova et al., 2007; van Hooijdonk et al., 2010), pear (Watson et al., 2012) and grape (Cookson et al., 2012; Tandonnet et al., 2010). However, the precise time of modification of kiwifruit rootstocks on the scion growth is largely unknown (**Hypothesis I**). Therefore, the architectural changes of scions imposed by the inter-specific hybrid kiwifruit rootstocks (Table 1.1) were evaluated in the first and second growing season following grafting. Growth characteristics such as the trunk cross-sectional area (TCA) of rootstocks, scions, and scion primary shoots, including the node number and internode length of the scion primary shoots were recorded in the first growing season following grafting. In the second growing season, the shoot architectural structures such as spring bud break, branching and proleptic shoot characteristics were measured and recorded. In the next following season, these composite kiwifruit vines were transplanted in the field in order to evaluate their performances in the orchard level (**Hypothesis II**). Explanation from these studies is necessary to identify how the scions vigour and growth in kiwifruit were initially modified by the inter-specific hybrid rootstocks.

1.1.3.2 Hormonal assessment on grafted inter-specific hybrid rootstocks of kiwifruit in relation to their vigour

Recent research has provided clear evidence of the role of endogenous hormones in grafted trees and how their interactions can affect tree architecture (van Hooijdonk, 2009). However, little work has been done in kiwifruit, particularly the underlying physiological mechanism (s) that is related to hormonal interactions. Thus, elucidating the hormonal signalling from the shoot to root and/or from the root to shoot system could improve our understanding of rootstock effects on scion vigour, particularly in kiwifruit (**Hypothesis III**). Growth manipulation studies were carried out in order to improve our understanding of the signalling of endogenous hormones that may have affected shoot growth in kiwifruit. Auxin transport inhibitor, '1-N-napthylphthalamic

acid' (NPA) was applied to the rootstocks stem according to the methods described by van Hooijdonk et al., (2011). NPA was chosen as a compound of choice as it seemed not to give any toxic effects to the plants as compared to other products (2,3,5,-Triiodobenzoic acid (TIBA) (van Hooijdonk et al., 2011). The changes in plant architecture (i.e. vegetative growth) have been monitored throughout the experimental season. Elucidation of these studies would provide important information of hormonal mechanism (s) related to the vigour of composite vines in kiwifruit. In addition, a study on the transport and uptake of IAA was also conducted on selected inter-specific hybrid kiwifruit rootstock stems in order to reveal more information on the endogenous hormones in relation to their vigour control in kiwifruit.

1.1.3.3 Vigour manipulation of kiwifruit by bark inserts/grafting

The use of growth manipulation such as bark inserts/grafting has shown potential results (Arakawa et al., 1996; Lockhard & Schneider, 1981). Therefore, this technique is worthwhile testing. Since the polarity of cells of stem can be retained regardless of orientation (Antoszewski et al., 1978; Sheldrake, 1973), therefore, by theory, the polarity of the basipetal transport of IAA in the stem can be manipulated by grafting a piece of bark to the stem of young kiwifruit vines either in normal or in an inverted orientation (**Hypothesis IV**). Besides that, our preliminary study has shown that bark insert/grafting in an inverted orientation on the kiwifruit stem increased size, dry matter concentration and weight of fruit at harvest compared with non-treated vines (Abdullah, 2011; Abdullah & Woolley, 2012).Therefore, the objective of this study was to evaluate the bark inserts/grafting as a method for vigour manipulation and also regulating fruit quality in kiwifruit. Newly propagated gold kiwifruit vines were subjected to bark inserts (normal and inverted position). Data on vegetative growth and vigour of vines (i.e. shoot growth and development, leaf area, etc.) were collected and analysed. For field-grown of kiwifruit vines, bark inserts were evaluated as a method for manipulating fruit characteristics (i.e. fruit size, weight and dry matter concentration).

1.1.3.4 Assessment of kiwifruit seedlings from specific crosses and their responses to gibberellins

In kiwifruit, little information is available about the initial selection of genotype or phenotype in relation to growth or vigour characteristics. For that reason, kiwifruit seedlings from six specific crosses of *A.chinensis* showing a wide range of vigour were assessed in terms of their architecture and response to exogenous gibberellins (GA) application. Vattiprolu (2012) has found that kiwifruit is really responsive to GA application. Applying GA immediately after bud break can stimulate the growth of kiwifruit shoots to continue growing and produce more nodes (Vattiprolu, 2012), indicating that the shoot architecture of kiwifruit can be manipulated by exogenous application of GA. The reason for using exogenous applications of GA was to test whether the phenotype of kiwifruit can be altered or manipulated by the plant growth regulator (**Hypothesis V**). Therefore, the objective of this study was to evaluate early architectural traits in relation to vigour and their response to exogenous GA application.

Therefore, the major research objectives in this thesis included:

i. To evaluate the inter-specific hybrid kiwifruit rootstocks as a potential vigour controlling rootstock in kiwifruit and their effects on shoot architecture, flowering and possibly fruiting.
ii. To elucidate how rootstock vigour and their ability are correlated with the physiological and morphological characteristics, and the hormonal relationship of the grafted scions.
iii. To evaluate the mechanism (s) of bark inserts/grafting in manipulating vigour and regulating fruit quality in kiwifruit.
iv. To assess early architectural traits of kiwifruit seedlings in relation to vigour characteristics and their response to application of gibberellins.

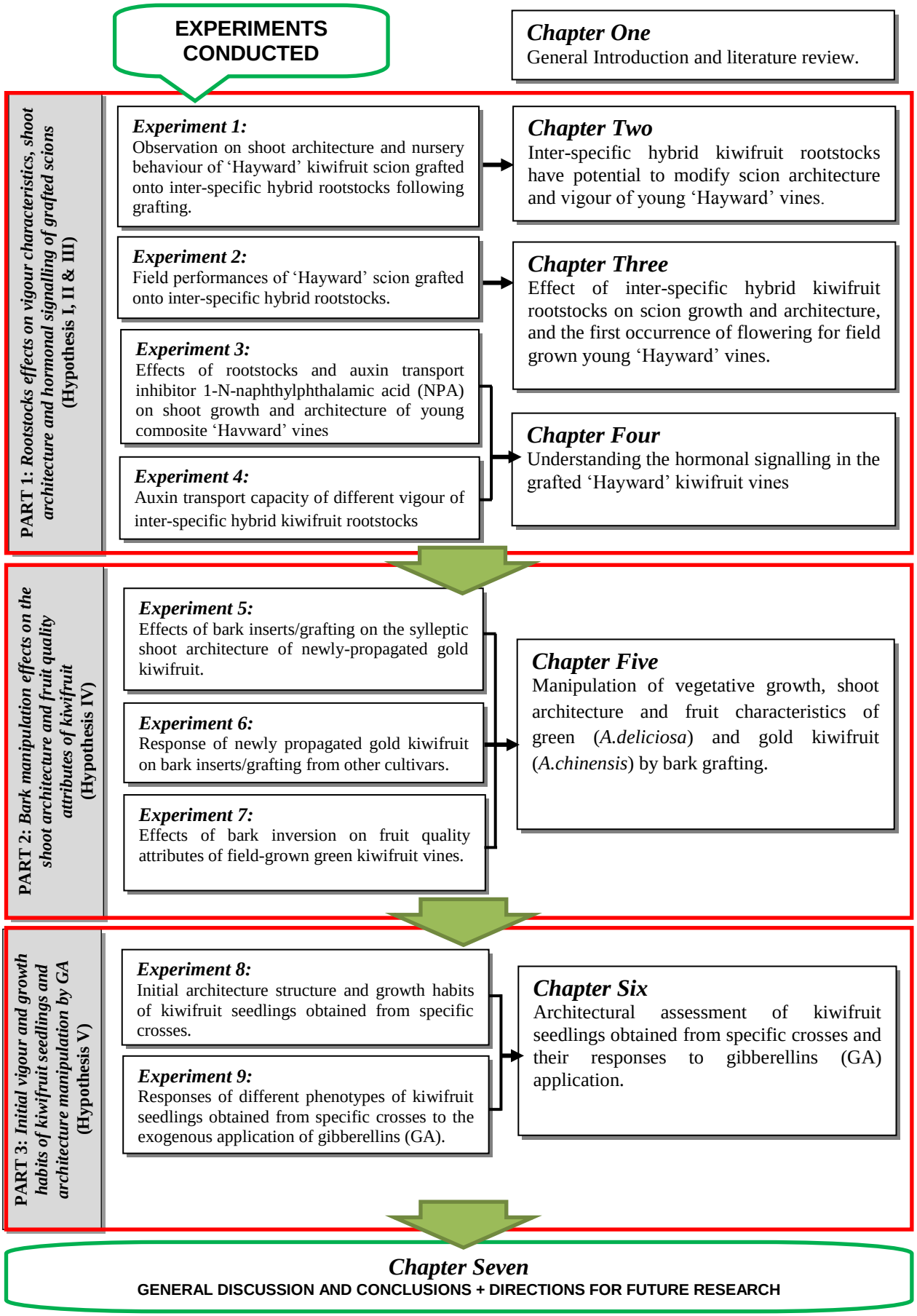

Figure 1.2. Conducted experiments and arrangement of chapters in this thesis.

1.2. Literature Review

1.2.1 The general botany of kiwifruit

Kiwifruit is placed in the *Actinidiaceae* family under the genus of *Actinidi*a. According to Ferguson (1990), more than 50 species were found that belong to the genus *Actinidia*. These species can be found in temperate forests, hills or mountains of southern-western China and from Siberia to Indonesia. Almost all the *Actinidia* species are woody and perennial climbing plants. They are vigorous and can be described as a perennial climbing or straggling plants reaching almost 30 feet (9 meters). Kiwifruit can be categorised as subtropical plants. Their growth and fruiting habit are very similar to grapevines. Some *Actinidia* species such as *A. venosa* have strong-growing vines and others species such as *A. kolomikta* have been categorised as less vigorous.

Kiwifruit shoots of the current season come from axillary buds of the previous season's growth (Ferguson, 1990). The shoots and young leaves are either hairless or covered with red hairs. The bark of mature shoots is usually brown or grey to chestnut brown with short and linear lenticels. The leaves are simple and in an alternate position with long petioles. The mature leaves are hairless on the upper surface and a dark green colour. The fruit is spherical, either ovoid or cylindrical depending on the species. The fruit contains many hundreds of small dark seeds that arranged in a circle about the inner pericarp. The fruits skin are covered with short or stiff brown hairs. The flesh is firm when it ripens, juicy and luscious with bright green colour but sometimes yellow or brownish. The fruit taste or flavour is quite acidic. Ferguson (1990) reported there is some variation in the range of flesh colour of kiwifruit. *A. deliciosa* had a bright-green flesh and *A. chinensis* had a range of colours from yellow and orange to red. In addition, shape, size, hairiness, skin, taste and chemical composition are different according to the species or types.

1.2.2 The origin and history of kiwifruit in New Zealand

Kiwifruit is a native plant of China. The species originated in the province of Hupeh, Szechuan, Kiangsi and Fukien in Yangtze Valley, in the northern China and also the province of Zhejiang on the coast of eastern China (Morton, 1987). Wild species have been cultivated on a small scale at China for more than 300 years ago and are still cultivated today. Chinese botanists have called the kiwifruit "*Yang Tao"* meaning "strawberry peach" or "*Mihoutau"* meaning "monkey peach". The "*Mihoutau"* name was believed to has been used since at least the *Tang Dynasty* around 618-907 A.D. (Ferguson, 1990; 2004). Ichang or Yichang is one of the Chinese cities that played an important role in the distribution and domestication of kiwifruit. The city was a starting point for transit into western China and also a centre for missionary activities. Most of the kiwifruit plants or seeds left China from Iching to Great Britain, New Zealand and United States (Ferguson, 1990). In 1847, Robert Fortune, the agent of the Royal Horticultural Society in London collected and described from dried specimens, *Actinidia chinensis*. After that, in 1890, E.H. Wilson sent kiwifruit seeds from Hupeh to England for planting as ornamental plants.

Kiwifruit was introduced and domesticated in New Zealand by missionaries in 1904 (Ferguson, 2004). It was believed that in the early 20 century, one of the New Zealand missionary, Miss Isabel Frasier had visiting Ichang. She brought the kiwifruit seeds back to Wanganui, New Zealand in January 1904, and gave to Thomas Allison who was a Wanganui solicitor and orchardist. He then passed down the kiwifruit seeds to his older brother, Alexander Allison who was interested in plants and trees. They were probably the first kiwifruit plants grown in New Zealand. For a long time, the kiwifruit was known by the binomial *Actinidia chinensis*. The early botanical description of *Actinidia chinensis* was based on flower specimens of a male plant (Ferguson, 1990). Subsequently, early plant collectors and botanists identified that there at least two variants of *Actinidia chinensis*. The obvious characters of these two variants of *Actinidia chinensis* are in the hairiness and size of the fruits as shown in Figure 1.3 (Ferguson, 1990). Finally, these two variants were classified as distinct species by (Liang & Ferguson, 1984). According to them, the species or variant that have smooth skinned and hairless fruit belongs to *Actinidia chinensis* and hairy-fruited species or

variant belongs to *Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson. Besides that, chromosome number, geographic distribution, flower size and leaf shape also differ between these two species (Ferguson, 1990). The details of distinguishing characters of *Actinidia chinensis* and *Actinidia deliciosa* have been described previously (Ferguson, 1990). According to Ferguson (1984), he mentioned that there were two reasons for changing of the botanical nomenclature in the kiwifruit, (1) "the discovery that an earlier valid name had been overlooked and application of priority rule meant that the epithet *deliciosa* should be used"; (2) "based on the conclusion that the two varieties within a species were better treated as two separate species".

Figure 1.3. The fruits of *Actinidia deliciosa* (left) and *Actinidia chinensis* (right). The most obvious character was in the hairiness of fruit skins. (Source of pictures from Ferguson (2004).

1.2.3 Rootstocks in kiwifruit.

1.2.3.1 Seedling rootstocks

For many years, kiwifruit growers in New Zealand have used, and are still using, *A. deliciosa* cv. 'Bruno' seedlings as a rootstock (Clearwater et al., 2007a; Clearwater, Lowe, et al., 2007b; Lawes, 1990; Warrington, 2000) because they are vigorous, easily established as seedlings and had high grafting success with 'Hayward' and 'Hort16A' scions. However, field performances of 'Hayward' scions on 'Bruno' seedling rootstocks are not as good as on 'Hayward' on their own roots and other clonal rootstocks in terms of flowering and yield (Cruz-Castillo et al., 1991; Wang et al., 1994b). Furthermore, with 'Hort16A' as a scion, 'Bruno' seedlings caused delayed budburst, reduction in floral budburst and flowering compared with 'Hort16A' on its

own roots or on 'Kaimai' rootstocks (Thorp et al., 2003). In addition, trials in Italy showed that the 'Hayward' scions grafted onto 'Bruno' seedling rootstocks displayed the lowest cumulative yield compared with 'Hayward' on their own roots, after seven years of field planting (Monastra & Testoni, 1991). The fruits from vines grafted on 'Bruno' seedling rootstocks also had a lower soluble solid concentration and a higher acidic level at harvesting and storage. However, since only a few reports have been published on 'Bruno' rootstock seedlings, this makes the comparison with other rootstocks is less clear.

The 'Hort16A' seedlings sometimes were used as a rootstock but the performance is less known compared to 'Bruno' seedlings. According to Clearwater (2007a), there was a report from growers that vines from 'Hort16A' seedlings showing a symptom of the vascular disease associated with the roots, and this might be due to being less tolerant to water logging (Clearwater et al., 2007a). Another trial also suggested that 'Hort16A' seedlings with 'Hayward' as scions produced a similar yield to those on 'Bruno' seedlings (Clearwater et al., 2007a). Similar to problems with 'Bruno' seedlings, only a few trials with 'Hort16A' seedlings as a rootstock have been carried out and therefore, it is difficult to make a justification whether 'Hort16A' seedlings are suitable with 'Hayward' scions or not. Nevertheless, 'Hort16A' seedlings are commonly easy to establish and give high grafting success with 'Hort16A' and 'Hayward' scion (Clearwater et al., 2007a).

1.2.3.2 Clonal rootstocks

One of the registered clonal rootstocks in New Zealand was *Actinidia hemsleyana* cv. 'Kaimai' that was formerly known as a 'TR2' (Lowe, 1989, 1991). 'Kaimai' rootstocks gave advantages in terms of promoting flowers on 'Hayward' scions. In the early trial with 'Hayward' as a scion, 'Kaimai' rootstocks doubled the number of flowers compared with standard *A. deliciosa* clonal rootstocks in Te Puke, New Zealand without using Hydrogen Cynamide (Hi-Cane) (Lowe, 1989, 1991). Furthermore, fruits from vines on 'Kaimai' rootstocks had an early maturity and higher soluble solid concentration than the fruits on standard rootstocks, but the DMC was unaffected (Lowe, 1991). Later, Wang et al. (1994b) reported, a higher number of flowers per

shoot were obtained from vines grafted with 'Kaimai' rootstocks. This rootstock had improved the synchrony of spring bud break and decreasing floral abortion before anthesis. Similarly, with 'Hort16A' as scions, 'Kaimai' rootstocks also caused more synchronous in flowering, increased number of flowers, earlier bud break, and earlier in fruit maturity (Clearwater et al., 2007a). Nevertheless, thinning cost was increased because of the high number of flower promoted by 'Kaimai' rootstocks, including increased the number of misshapen and lateral fruit (Wang et al., 1994b). Nevertheless, 'Kaimai' rootstock is easily propagated by hardwood cuttings, but difficult to topwork in the field either with 'Hayward' or with 'Hort16A' scions. However, this problem can be overcome by bench grafting of hardwood cuttings or summer grafting using cool-stored wood. Although 'Kaimai' is believed less sensitive to water logging, this rootstock has a problem for being difficult to graft with 'Hort16A' or 'Hayward' due to sap exudation is copious (Clearwater et al., 2007a).

Self-rooted *A. deliciosa* cv. 'Hayward' propagated from cuttings sometimes has been used as clonal rootstocks in a few experimental trials (Cruz-Castillo et al., 1991; 1997; Wang et al., 1994b). It was believed that rootstocks from self-rooted 'Hayward' produced less fruit variability compared to 'Bruno' seedling. Previous studies showed that with 'Hayward' as scions, rootstocks from self-rooted *A. deliciosa* cv. 'Hayward' tended to reduce flower numbers (Cruz-Castillo et al., 1997; Wang et al., 1994b). Wang et al. (1994b) also found that the peak time of budbreak of 'Hayward' scion was slightly delayed if grafted onto *A. deliciosa* rootstocks. With 'Hort16A' as scions, clonal 'Hayward' rootstocks also delayed budbreak, reduced flower numbers per bud, produced fruit with low DMC compared with 'Kaimai' rootstocks (Wang et al., 1994b). However, higher yield was produced when self-rooted 'Hayward' had grown on their own roots compared with vines grafted onto 'Bruno' seedling rootstocks (Cruz-Castillo et al., 1991; Monastra & Testoni, 1991). The selection of 'Hayward' used as a rootstock can also affect growth, productivity and fruiting performance in kiwifruit (Cruz-Castillo et al., 1997). Studies by Cruz-Castillo et al. (1997) found root materials obtained from the good vines performances (i.e. high yield, large fruit) as a rootstock, tended to produce greater cropping performance than from poor source root materials (i.e. low yield, small fruit). Thus, it was shown that the orchard performance, on which the original rootstocks were selected, was correlated with their relative field performance.

Self-rooted 'Hort16A' also being used as a clonal rootstock and might believe producing poor vines growth and reduced flower numbers (Clearwater et al., 2007b). The self-rooted 'Hort16A' was also possibly sensitive to water logging indicating this rootstock might be not suitable for sites with heavy soils (Clearwater et al., 2007b). Wang et al. (1994b) reported, with 'Hayward' as scions, clonal 'Hort16A' rootstocks caused delayed bud-break and reduced flowering by 23%. Another clonal rootstock for kiwifruit is the Italian cultivar, 'D1' (Monastra & Testoni, 1991). According to Cleawater et al. (2002), 'D1' was selected from a population of open pollinated 'Bruno' seedlings and this rootstock is believed more suitable for water logged and calcareous soils (Viti et al., 1990). However, limited information is available for 'D1' rootstocks. Recently, Thorp et al., (2012) reported that one of the kiwifruit rootstock namely 'Bounty 71' has produced slightly less vigorous canopy for the commercial gold kiwifruit cultivars, 'Hort16A', G3 and G9. At the same time, this rootstock can increase flower numbers and fruit size compared with 'Hayward' and 'Kaimai' rootstocks. However, fruits grown from the 'Bounty 71' rootstock tended to get incidence of physiological pitting suggesting this rootstock might be less efficient in nutrient accumulation and uptake. In addition, leaf breakdown was also observed on the leaves from the vines grafted with 'Bounty 71' (Anon., 2012).

In summary, there is a strong need to develop vigour-controlling rootstocks in kiwifruit that can offer great advantages as proven in other fruit trees (Rom & Carlson, 1987; Webster, 1995a). None of the seedlings or clonal rootstocks in kiwifruit reported by previous researchers could control the vigour of grafted scions (Table 1.2). Only one clonal rootstock namely 'Bounty 71' has shown promising (Anon., 2012), but the vigour reduction is very limited. Furthermore, in flower-promoting rootstocks such as *A. hemsleyana* and *A. eriantha*, both of these cultivars did not suppress the vegetative growth of grafted scion (Wang et al., 1994a). Nevertheless, clonal rootstocks in kiwifruit can offer some advantages compared with seedling rootstocks (Table 1.2). To date, a few techniques have been proven to control excess vegetative growth in kiwifruit, i.e. girdling (Currie et al., 2008), 'zero-leaf pruning' (Gardiner & Max, 2005) and 'tip squeezing' (Max & Currie, 2005). However, all these techniques only offer a short-term solution in controlling vegetative growth in kiwifruit.

Table 1.2. Comparison between seedlings and clonal rootstocks in kiwifruit.

Rootstock clones	Rootstock types	Advantages	Disadvantages	Sources
'Bruno'	Seedlings	• Vigorous growth and easily established as a seedlings. • High grafting success with 'Hort16A' and 'Hayward'. • Tolerant to waterlogging.	• Had caused variation in vines and fruit quality traits (dry matter, size, maturity etc.). • Had delayed budbreak, reduced floral budbreak, reduced flower number per bud.	Clearwater et al. (2007a; b) Cruz-Castillo et al. (1997) Lawes (1990) Throp et al. (2003) Wang et al. (1994)
'Kaimai'	Clonal	• Easily propagate from hardwood cuttings. • Tolerant to waterlogging. • Increased flower numbers without the use of HC on 'Hayward' scion. • Produced synchronous flowering, early fruit maturity without affecting dry matter on 'Hayward' scions.	• Low grafting success with 'Hort16A' and 'Hayward'. • Increased numbers of misshapen fruits. • Increased thinning cost due to high flower number and crop load. • Less effects on flowering with 'Hort16A' as a scion.	Lowe (1989; 1991) Throp et al. (2003) Wang et al. (1994b) Clearwater et al. (2007a)
Self-rooted Hayward	Clonal	• High grafting success with 'Hort16A'. • Less variation in fruit quality traits than 'Bruno' seedlings. • High yield when grown on their own roots.	• Had reduced flower numbers on 'Hayward' scion. • Had delayed budbreak, reduced flowers per bud, delayed maturity and lower dry matter concentration.	Cruz-Castillo et al. (1991; 1997) Monastra and Testoni (1991) Wang et al. (1994b)
'Hort16A'	Seedlings	• Easily established as a seedlings. • High grafting success with 'Hort16A' and 'Hayward'.	• Had caused variation in vines and fruit quality traits (dry matter, size, maturity etc.). • Produced lowest flower numbers, least synchronous budburst on 'Hayward' scion. • Less tolerant to water logging.	Clearwater et al. (2007a)
'D1'	Clonal	• Produced high fruit numbers and yield. • Tolerant to waterlogging. • Suitable for calcareous soils.	• Not available in New Zealand. • Less information about this rootstock.	Monastra and Testoni (1991) Viti et al. (1990)
'Bounty 71'	Clonal	• Promoted high flower numbers, larger fruit size and high dry matter. • Produced slightly less vigorous canopy or moderate reduced vigour. • Easy to propagate either from semi or hardwood cuttings.	• Low grafting success during winter. • Less trials and reports about this rootstock. • Not tolerant to PSA.	Anon. (2012) Thorp et al., (2013) M. Clearwater (personal communication, Jun 27, 2016)

1.2.4 The physiological mechanisms of vigour control by rootstock

A number of theories have been postulated in attempts to explain the mechanism (s) how rootstocks may control the scions vigour. These includes; i) different in anatomical characteristics between rootstocks and scions, ii) alteration in endogenous hormones translocation or other substances such as phenolics, from root to shoot or *vice versa*, iii) restriction in the movement of mineral nutrients and iv) restriction in water supply (review by Gregory et al., 2013). In addition, it has been suggested that 'graft union' between rootstock and scion was also involved in the dwarfing mechanisms in composite trees. The physiological mechanisms of vigour control by rootstock were discussed below and also have been briefly summarised in Figure 1.4.

1.2.4.1 Anatomical characteristics

Anatomical characteristics of rootstocks have been shown to be correlated with the vigour of the scions. Anatomy of stem, root, and leaf have been used as a technique for selecting dwarfing characteristics on the rootstock of fruit trees such as apple (Beakbane, 1941, 1967; Beakbane & Majumder, 1975; Beakbane & Thompson, 1939), mango (Kurian & Iyer, 1992; Majumdar et al., 1969), avocado (Jimenez & Priego, 1987), citrus (Saeed et al., 2010) and cherry (Végvári et al., 2008). For example, studies on apple showed that stem and root of dwarfing rootstock, M.9 had higher xylem to phloem ratio (Beakbane & Thompson, 1939, 1947). Vigorous and dwarfing apple rootstocks have a bark: wood ratios of 0.61-0.98 and 1-2.3, respectively. In addition, xylem to phloem ratio in the stem of low vigour apple trees such as 'Delicious' and 'Golden Delicious' was higher than high vigour apple trees (Jaumien & Faust, 1984). Furthermore, dwarfing M.9 apple rootstock had significantly smaller vessel diameter, more xylem vessels, fibers, parenchyma and rays compared with M.12 vigorous rootstock (Beakbane, 1956; Beakbane & Thompson, 1947). Simon and Chu (1984) also found outer bark of vigorous apple dwarfing rootstocks (EMLA 27 and Ottawa 3) was significantly thicker compared with semi-dwarfing rootstock MM.106, and almost 60% of this bark consisted of non-functioning phloem tissue.

Stomatal density also can be related to the vigour of the rootstocks. It has been used to classify the rootstocks into different vigour group on the basis of stomata counts. For example, in apple, a strong correlation was found between stomata density (the number of stomata per unit leaf area of leaf) and rootstock vigour (Beakbane & Majumder, 1975; Pathak et al., 1976). The stomatal density was highest in the vigorous rootstocks such as M.25 and MM.111, and the lowest in the dwarfing rootstocks such as M.9 and M.27. In contrast, Barrientos-Pérez and Sánchez-Colín (1983) found that the stomata density of Colin V-33 of dwarfing avocado rootstocks was almost two times higher than the normal rootstock. However, less studies have been conducted on the basis of this characteristic to determine potential dwarfing rootstocks. Recently, anatomical features of roots, stems and leaves have also been used in the selection of potential vigour controlling rootstock in citrus (Saeed et al., 2010). Less vigorous rootstocks such as Flying Dragon (*P.trifoliata*) have higher proportions of bark (phloem) in the roots and stems, as well as smaller vessel elements in the xylem compared with vigorous rootstocks. Xylem anatomical characteristics of roots and shoots of have been shown to be related to the vigour of selected inter-specific hybrid peach rootstocks (Tombesi et al., 2011; 2010). Tombesi and co-workers (2011; 2010) found that more vigorous rootstocks such as 'HBOK 50' had higher numbers of larger vessels and fewer small vessels than dwarfing rootstocks.

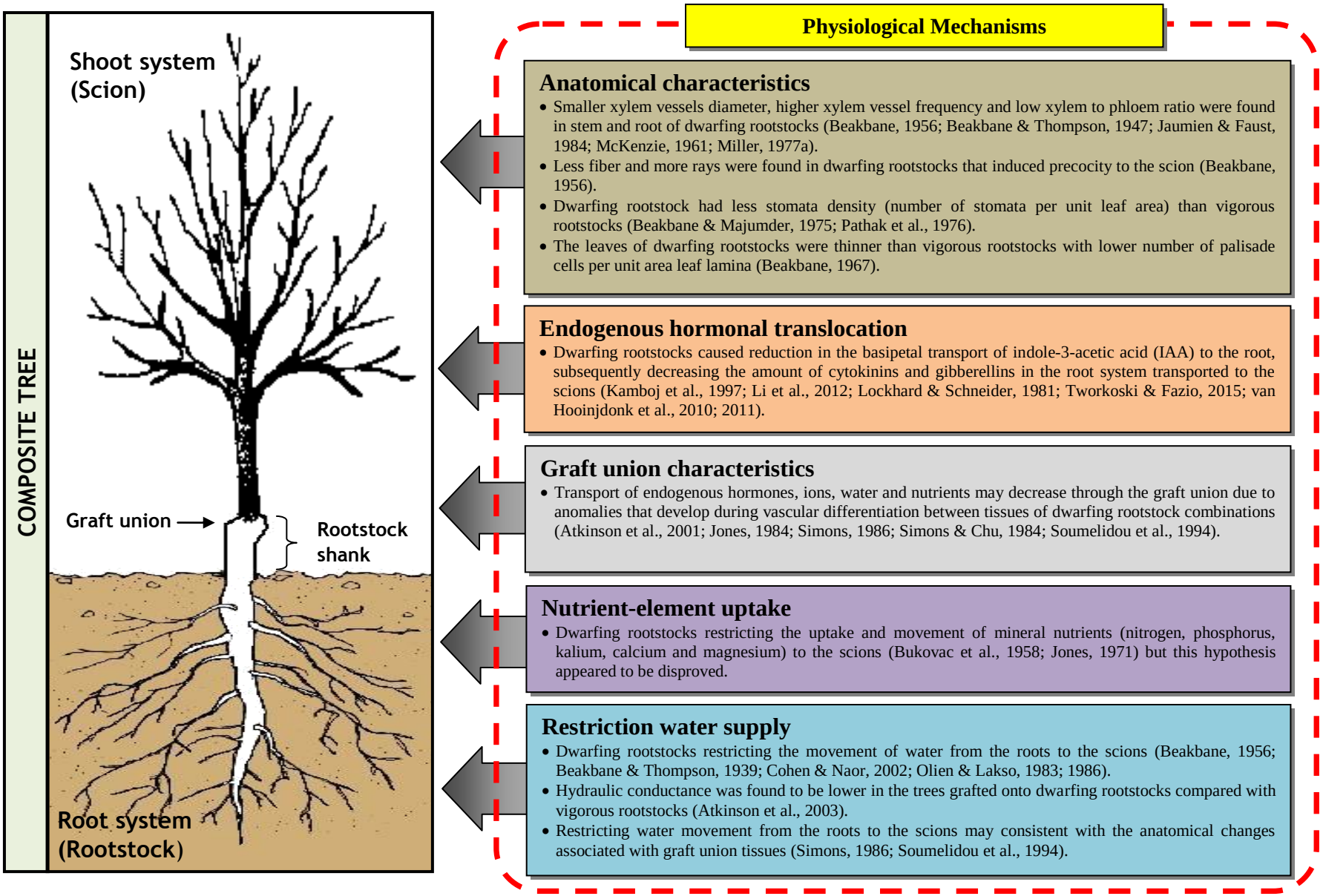

Figure 1.4. The summary of the hypotheses on the physiological mechanism of dwarfing rootstocks on composite trees.

1.2.4.2 Restriction of water uptake

An early anatomical study in apple by Beakbane and Thompson (1939) led to the hypothesis that dwarfing rootstock may reduce scion vigour by restricting the transport of water from the rootstock to the scion. Supports for this hypothesis came from the observation that the stem and leaf water potentials of scions grafted on dwarfing rootstocks were lowered than the scions grafted on vigorous rootstocks, and thus may cause a reduction in plant vegetative growth. Xylem water potential is likely to be related to xylem hydraulic conductivity (Kramer & Boyer, 1995). Previous study has shown that stem water potential of 'Empire' scions was reduced when grafted on M.9 and M.26 dwarfing rootstocks compared with MM.104 and MM.106 vigorous rootstocks (Olien & Lakso, 1986). Another study found that the leaf-specific hydraulic conductivity of 'Golden Delicious' scion grafted onto M.9 dwarfing rootstock was lower than MM.106 vigorous rootstock when expressed on a leaf area basis, but did not significantly differ between these two rootstocks (Cohen & Naor, 2002).

Further study by Atkinson et al. (2003) found that the root hydraulic conductivity from two dwarfing rootstocks, M.9 and M.27 was lower than vigorous MM.106 rootstocks. Similarly, Nardini et al. (2006) studied the hydraulic architecture of young olive (*Olea europea* L.) trees grafted on three rootstocks with contrasting vigour controlling ability. They found root hydraulic conductivity of 'Leccino Dwarf' dwarfing rootstocks was lower than 'Leccino Minerva' vigorous rootstocks, and they concluded that low root hydraulic conductivity is one of the main factors that caused a reduction in the vegetative growth of the scion olive trees (Nardini et al., 2006). In peach (*Prunus persica* L.), 'Flavorcrest' scions on size-controlling rootstocks namely 'Hiawatha' and 'K-146-43' have been reported to have lower midday stem water potential than the scions on the vigorous rootstocks 'Nemaguard' (Basile et al., 2003). Basile et al. (2003) also found that the root hydraulic conductivity of 'Crimson Lady' scions was significantly lower when grafted on 'K-146-43' compared with scions grafted on 'Nemaguard' rootstocks when expressed per unit leaf area. Moreover, scions of 'Mayfire' grafted on 'K-146-43' rootstocks also had lower root hydraulic conductance (rootstock), when measured with both the evaporative flow and high-pressure methods (Solari et al., 2006). In kiwifruit, the effects of rootstocks on water status of scions have been described in Section 1.2.5 of this chapter.

1.2.4.3 Nutrients translocation and uptake

It was hypothesised that dwarfing rootstocks may influence the uptake of minerals and this is partly responsible for their dwarfing influence upon scion. A previous study by Bukovac et al. (1958) found a higher reduction in transport of radioactivity of ^{32}P and ^{45}Ca from the root to the scion of 'McIntosh' apple grafted into M.9 compared with M.16. Similarly, Jones (1971) also found that the nutrient elements which are nitrogen, potassium and phosphorus in the xylem sap of apple scions were reduced when grafted onto M.7 rootstocks compared to the MM.104 rootstocks. Thus, it can be suggested that the reduction of nutrients uptake may be due to the smaller root system of dwarfing rootstocks. However, this hypothesis is not well accepted due to lack of supporting evidence. For example, in apple, dwarfing apple rootstock M.9 can impose many beneficial characteristics (i.e. control scion vigour, increased flower precocity and fruit quality) and this could not be explained by this hypothesis. Besides that, Atkinson and Else (2001) found that the transport of mineral ions and solutes (i.e. potassium, magnesium, calcium and hydrogen) were greater in M.9 rootstock than from MM.106 rootstock when the effects of root mass and leaf area were taken into account.

1.2.4.4 Alteration of endogenous hormones translocation

It was proposed that dwarfing rootstocks may reduce the basipetal transport of indole-3-acetic acid (IAA) to the root, subsequently decreasing the amount of CK and GA in the root system transported to the scions (Kamboj et al., 1997; Lockhard & Schneider, 1981; Soumelidou et al., 1994; van Hooinjdonk et al., 2010; 2011; Webster 2004). Previously, Lockhard and Schneider (1981) postulated that the bark of dwarfing apple rootstocks (such as M.9) had lower in capability to transport IAA than vigorous rootstocks. In addition, they also postulated that less amount of IAA from scion transported to the root system of dwarfing rootstocks would affect the CK production and root growth, subsequently limiting shoot extension growth of the scions. Studies in apple have shown that dwarfing rootstocks such as M.9 and M.27 had lower basipetal transport of radio-labelled IAA in apical shoots (Kamboj et al., 1997; Soumelidou et al., 1994). Furthermore, transport of IAA from the leaves of 'Fiesta' scions to the roots of M.9 and M.27 dwarfing rootstocks was lower than vigorous rootstocks (Kamboj et al.,

1997). In addition, Soumelidou et al. (1994) reported lower transport of IAA in M.9 dwarfing rootstock was due to lower capacity to support polar auxin transport compared to shoots from the vigorous rootstocks. Similarly, in peach, the IAA transport in 'Armking' scions was significantly reduced when grafted on Mr.S.2/5, dwarfing rootstocks (Sorce et al., 2002).

Reduced basipetal transport of IAA from the scion to root will decrease root growth, consequently the amount of CK transported to the scion, therefore reducing scion vigour. Kamboj et al. (1999) reported that CK concentration in root pressure exudate and in scion xylem sap was lower for dwarfing apple rootstocks than for vigorous rootstocks. In addition, a lower total concentration of zeatin plus zeatin riboside was found in the 'Fiesta' scion grafted onto M.9 dwarfing rootstock (Kamboj et al., 1999). A study by Sorce et al. (2008) in peach also found that zeatin riboside in 'Armking' scion grafted on Mr.S.2/5 dwarfing rootstock was lower compared with scion on GF 677 vigorous rootstock. A few studies also suggest that GA may be involved in the hormonal mechanisms in dwarfing rootstock (Tworkoski & Fazio, 2015; van Hooijdonk et al., 2010, 2011). There was an evidence that GA is transported within the xylem sap of apple trees (Ibrahim & Dana, 1971), suggesting that the root of apple might synthesise and transport GA to the scion (Jones & Lacey, 1968). Kamboj et al. (1999) also reported that bark of dwarfing rootstocks had higher concentrations of abscisic acid than vigorous rootstocks and speculated that abscisic acid may have an important role in polar auxin transport.

Recently, studies by van Hooijdonk et al. (2010, 2011) on apple rootstocks have provided a new evidence that 'endogenous signalling mechanism' between IAA, CK and GA are involved in rootstock-scion interactions. By applying auxin transport inhibitor, 1-N-naphthylphthalamic acid (NPA) to the stem of vigorous rootstocks such as MM.106 and M.793, they found that the scions architecture of 'Royal Gala' were closely similar to those scions grafted on M.9 dwarfing rootstocks (van Hooijdonk et al., 2010). The mean total shoot length, node number and the number of secondary shoots of scions were decreased on the trees treated with NPA or grafted on M.9 dwarfing rootstocks. However, application of benzylaminopurine-BAP (synthetic CK) and GA (GA_{4+7}) to the scions treated with NPA or grafted on M.9 dwarfing rootstocks has reinstated the formation of secondary shoots and reduced the proportion of primary

and secondary shoots that terminated growth early. Thus, van Hooijdonk et al., (2010) hypothesised that dwarfing rootstocks may decrease the basipetal transport of IAA to the root system, subsequently reducing the amount of CK and GA transported from the root to scion.

Later, van Hooijdonk et al. (2011) examined the level of endogenous hormones in the xylem sap of 'Royal Gala' scion grafted on M.9, MM.106, M.793 and 'Royal Gala' rootstocks. They found that the rate of IAA diffusing in the xylem sap from the primary shoot apex was decreased from January to April, irrespective of rootstock. However, at the same times, the concentration of zeatin riboside (CK component) increased and this occurrence appeared to coincide with the increases in the axillary growth on the scions. In February, scions on M.9 rootstocks had a higher concentration of zeatin riboside than 'Royal Gala' rootstocks, but the primary shoot of scions on M.9 rootstocks had less axillary bud growth, suggesting that other endogenous hormones may also be involved in controlling shoot branching. By March, the concentration of GA (GA_{19}) in the xylem sap of scions on M.9 was significantly decreased compared with scion on 'Royal Gala'. These results indicate that the dwarfing apple rootstocks may also limit the production of GA (especially GA_{19}) in the root system supplied to the scions (van Hooijdonk et al., 2011). Further study by Li et al. (2012) also demonstrated that the IAA and CK were involved in regulating scions growth by the apple rootstocks. They also evaluated the gene expression that associated in regulating IAA and CK during the development of scions growth. As noted, *P1N1* and *IPT3* genes play an important role in regulating IAA and CK for biosynthesis of IAA (Paponov et al., 2005) and CK (Takei et al., 2001), respectively. They found that the transport of IAA in 'Red Fuji' scions was reduced when grafted onto M.9 dwarfing rootstocks compared to similar scions grafted onto *Malus* x *Micromalus* Makino rootstocks. These events appeared to coincide with the reduction in the level of expression of the *PIN1* gene in leaves, barks, and roots of scions grafted onto M.9 than the scions on vigorous rootstocks. Reduction in the transport of IAA from scions also had caused a reduction in the content of t-Z (a component of CK) in roots, as well as a reduction the amount of t-Z transported to scions (Li et al., 2012). These effects also had caused a reduction in the level of expression of the *IPT3* gene in scions grafted on M.9 rootstocks compared to scions on vigorous rootstocks.

Even though recent study by Tworkoski and Fazio (2015) mentioned that they did not find any correlations between hormone concentrations and growth parameters such as height, node number and stem diameter; nevertheless, they did found that under the greenhouse condition, the abscisic acid (ABA) and ABA conjugate, were significantly higher in the root and the rootstock shank of scions grafted on M.9 than MM.111 rootstocks. Besides that, ABA and related compounds were considerable higher in scions grafted onto M.9 than MM.111 rootstocks. Furthermore, when the ABA level was measured in the reciprocal grafting between M.9 and MM.111, ABA and related compound were almost higher in all scions grafted onto M.9 than MM.111 rootstocks (Tworkoski & Fazio, 2015). They also found that scions of M.9 and MM.111 on M.9 rootstocks may contain a higher level of GA_{3+4}. Moreover, the GA_{19} were higher in the root and exudate of 'Gala' scions when grafted onto MM.111 than M.9 rootstocks, indicating that M.9 rootstocks may have a higher capacity for GA degradation than MM.111 rootstocks (Tworkoski & Fazio, 2015).

Other interesting findings in Tworkoski and Fazio (2015) study on the greenhouse experiment, particular scions on dwarfing rootstocks such as M.7 and M.9 tended to be taller than scions grafted onto MM.111 vigorous rootstocks, and this trend was reversed when the similar combination was planted in the field. Therefore, they suggest that the possibilities of abundant of resources (i.e. nutrients, irrigation, pest and disease control etc.) and restriction of root growth by pots during the greenhouse planting may have influenced the mechanisms of vigour control by the roostocks (Tworkoski & Fazio, 2015). Besides above findings, they also found scions of M.9 and M.27 were still considerably shorter compared with other scions regardless of rootstocks vigour, suggesting the vigour controlling in composite trees was stem-related and was not solely associated with the root or the graft-union rootstocks (Tworkoski & Fazio, 2015). Therefore, it would be reasonable to suggest that the stem part of the rootstocks (i.e. the rootstock shank) is responsible for hormonal alterations in the scions grafted onto dwarfing rootstocks. However, all these aspects are still not fully understood in kiwifruit vines.

1.2.4.5 Graft-union

It was hypothesized that transport of endogenous hormones, ions, water and nutrients may decrease through the graft-union due to anomalies that develop during vascular differentiation between tissues of rootstock combinations (Atkinson et al., 2001; Jones, 1984; Simons, 1986; Simons & Chu, 1984; Soumelidou et al., 1994). The reduction was positively correlated with the dwarfing effects of the rootstocks. Jones (1971) found the concentration of N, P and K in the xylem sap of 'Cox' apple scion was decreased in dwarfing rootstock, M.7 and M.9 compared with the xylem sap from the vigorous rootstock, MM.104. In addition, total solute in xylem sap collected from above the graft union on M.9 rootstock was 30 to 50% lower than that the total solute from the lower graft-union (Jones, 1971; 1984). Thus, Jones (1971; 1984) postulated that graft union is the site that contributed to dwarfing effect in the grafted trees. Further investigation on graft-union indicated that significant differences were found between the anatomical structure of the rootstocks and scions combinations, and most of the vascular tissue developed at graft-union associated with malfunctions (Simons, 1986; Simons & Chu, 1984). Vascular tissues that developed at graft-union showed abnormal anatomical features and were arranged in a swirling pattern, thus became necrotic during growth of the plant (Simons, 1986). Extreme dwarfing rootstocks such as EMLA.27 and Ottawa 3 had excessive non-conducting phloem, as much as 60% of the bark.

Later study by Soumelidou et al. (1994) found in apple M.9 dwarfing rootstock, the xylem tissues near to graft-union between scion and rootstock contains smaller and fewer vessels than in the vigorous MM.106. This could be the factor for the reduction in water supply and nutrient to the scion, thereby contributing to the dwarfing effects. In the first year after grafting, the diameters of xylem vessels of M.9 near to graft union were larger than MM.106, indicating lower IAA level reaching these tissues. However, in the second year, the smaller diameter of xylem vessel in M.9 was observed, suggesting higher IAA level accumulates near to graft union because of difficulty in crossing graft interface. Atkinson et al. (2003) suggested that graft-union is a site that had low hydraulic conductivity and this may affect the movement of substances in the xylem. They found calculated hydraulic conductivity of the graft tissue of scion of 'Queen Cox' grafted on M.27 and M.9 dwarfing rootstock was lower than the scion grafted on vigorous rootstock MM.106 (Atkinson et al., 2003) due to anatomical

disfunction of xylem in graft tissue (Soumelidou et al., 1994). A study by Cohen et al. (1985) found hydraulic resistance between the root and stem of 'Liberty' apple scion grafted on M.9 was higher than scion on vigorous MM.106 and MM.111 rootstocks, implicating that the graft-union as the site of increased resistances.

However, graft union is not consistently reported to be a factor contributed to the dwarfing of grafted trees. For example, Jones (1971; 1984) has pointed out that graft-union had no effect on water transport. In contrast, Soumelidou et al. (1994) reported a difference in xylem formation due to low IAA level at graft union which is responsible for restricting water supply to scion, subsequently reduction in scion growth. Contrasting results are probably due to the differences in the method of collecting xylem sap. In addition, the difference in age of scions or rootstocks that has been used could lead to the differences in the results. Basile et al. (2003) reported that the graft-union in peach had little effect on water transport through the stem of the dwarfing and vigorous rootstocks. They found hydraulic conductance of graft union of one year old of 'Crimson Lady' scions was not significantly differed between trees grafted on dwarfing rootstock 'K-146-44' and vigorous rootstock 'Nemaguard'. Similarly, in one-year-old grafted olive trees, hydraulic resistance of graft union only contributed 1 to 3% from the overall hydraulic resistance of the plants regardless on dwarfing or vigorous rootstock (Nardini et al., 2006), indicating that the vascular system between scion and rootstock was completely re-instated one year after grafting. Therefore, it could be argued whether graft-union was contributed to the dwarfing effects in grafted trees, although it could initiate the decrease in the transport of IAA (Soumelidou et al., 1994).

1.2.5 Effects of rootstocks on kiwifruit vines

In kiwifruit, little work has been done to elucidate the effects of rootstocks on the plant architecture, yield and fruit quality. Some study on anatomy in flower-promoting rootstocks in kiwifruit was done by Wang et al. (1994a). However, flower-promoting rootstocks such as *A. eriatha* and *A. hemsleyana* did not have the ability to control the vegetative growth of scions (Wang et al., 1994a). Study on the effect of rootstock on hydraulic conductivity in kiwifruit has shown contrasting results to what have been

found on other fruit crops. Clearwater et al. (2004) investigated the hydraulic architecture of four different vigour-controlling *Actinidia* clonal rootstocks grafted with *Actinidia chinensis* cv. 'Hort16A' as a scion. Four *Actinidia* species namely *A. polygama*, *A. kolomikta*, *A. macrosperma* and *A. hemsleyana* were selected as rootstocks. Based on their leaf area and shoot growth, *A. macrosperma* and *A. hemsleyana* are high-vigour rootstocks and *A. polygama* and *A. kolomikta* are low-vigour rootstock (Clearwater et al., 2004).

Clearwater et al. (2004) found that the leaf-specific hydraulic conductance of *A. chinensis* scions was higher in low-vigour rootstocks (*A. polygama* and *A. kolomikta*) than in high-vigour rootstocks (*A. macrosperma* and *A. hemsleyana*), which is the opposite pattern from what have been found in the other fruit trees. Therefore, they concluded that the plant hydraulic characteristics do not provide a clear explanation in terms of the effects of rootstocks on scion vigour in kiwifruit. However, leaf area index and crown size of scion grafted on the low-vigour rootstocks were reduced compared with high-vigour rootstocks. In a later study, they found that effects of rootstocks on scion vigour in kiwifruit were clearly observed during the initial period of shoot development, immediately after budburst (Clearwater et al., 2006). Observation on *A. chinensis* scions grafted onto eight clonal rootstocks over two growing seasons has shown that the leaf area index of scions on the low-vigour rootstocks was reduced compared to scions on the high-vigour rootstocks. Further investigation by Clearwater et al. (2007) found that scion vigour on different vigour controlling rootstocks was correlated with the spring root pressure. Under consistent irrigation in spring, high root pressure was observed before and during scion budburst in scion grafted on high vigour rootstocks such as *A. deliciosa*, *A. hemsleyana* and *A. macrosperma*. In contrast, low-vigour rootstocks such as *A. polygama* and *A. kolomikta* exhibited their root pressure after scion budburst and the scion shoots showed the signs of water stress. Therefore, they proposed that the effects of rootstocks on shoot vigour in kiwifruit were caused by the differences in the phenology between the rootstocks and scions (Clearwater et al., 2007).

1.2.6 The relationship between vigour and plant immune system

Kiwifruit is facing a new disease problem, a bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae*, commonly known as PSA (Vanneste et al., 2011). This bacterium has caused a huge damage to the New Zealand kiwifruit industry including other countries such as Japan, Korea and Italy. Previous studies have shown that auxin is related to plant disease mechanism and plays an important role in plant disease susceptibility pathways (Sequeira, 1963; Yamada, 1993). It was notable that most of the *Pseudomonas syringae* strains can affect the plant (i.e. host) either by manipulating host auxin biosynthesis or produce auxin themselves to impede the host physiological processes (Glickmann et al., 1998; Kazan & Manners, 2009; Yamada, 1993). It was also suggested that production of auxin by *Pseudomonas* strains could inhibit plant defence mechanisms (Robinette & Matthysse, 1990). As a response mechanism, the plants will produce salicylic acid as a defence mechanism against pathogens (Dempsey et al., 1999). Interestingly, recent evidence indicated that salicylic acid may inhibit pathogen growth especially *Pseudomonas syringae* strains through suppressing auxin signalling pathway (Navarro et al., 2006; Wang et al., 2007; Zhang et al., 2007). Based on the evidence from literature above, it could be hypothesised that the reduction in the basipetal transport of auxin could increase plant immune system as a protective mechanism against pathogens. Therefore, theoretically, reducing the vigour of kiwifruit vines (i.e. reducing auxin transport), may possibly increase the resistance or tolerance of vines to the disease problems. This would be one of the possible approaches to encounter the PSA problem in kiwifruit.

1.2.7 Vigour control of tree growth by bark inversion

Early studies on bark inversion were carried out in the 1950s by Karl Sax, who was the horticulturist at Arnold Arboretum, Harvard University. Early observation by Sax (1950) on one-year-old apple trees whips that have been grafted upside down (inverted position), and he found that the lateral branches start growing towards the ground. The branches on this type of trees were slowly extended upward and the canopy spread evenly with wide and unbreakable crotch angle (Sax, 1950). In the later studies, Sax and

Dickson (1954; 1956) hypothesised that the dwarfing effects caused by apple rootstocks were due to a retardation of phloem transport. By inverting the single ring bark of one and two years old apple trees, the dwarfing effect clearly observed during early growth stage, but after several years the effects were diminished (Sax, 1954; 1956). In addition, there was a swelling of the stem above the inverted bark and regeneration of new tissues around bark inverted area. Thus, they concluded that the dwarfing effect by single inverted bark was only temporary. After the renewal of tissues, the polarity of phloem transport is reoriented again and permits normal phloem transport downward (Sax & Dickson, 1956). Brase and Way (1959) also found that grafted of the two-inch wide ring of bark in an inverted position on one-year-old apple trees, has caused severe stunting for almost two years. However, after two years, the normal growth was resumed, indicating dwarfing effect has been diminished (Brase & Way, 1959).

In order to get a more permanent dwarfing effect, Sax and Dickson (1956) grafted inverted two rings (double bark inversion) of 'Cortland' apple, and they found a reduction in shoot growth and also no growth on trunk diameter. However, the dwarfing effects could not be sustained from this technique due to lateral diffusion of nutrient sap soon results in a lateral orientation of new phloem and xylem forming normally polarised tissues regenerated at the seams of two inverted rings of bark. Nevertheless, inversion the ring of bark promoted the axillary buds outgrowth below the bark graft-union area, indicating auxin is suppressed and apical dominance is released. Thus, they summarised that by reversing the polarity through inverting the ring of bark may prevent a renewal of phloem elements, presumably by preventing sufficient movement of auxins and nutrients into the inverted phloem cells (Sax & Dickson, 1956). Using radioactive tracer, Dickson and Samuel (1956) applied radioactive phosphorus into young 'Baldwin' apple trees through the leaves, which had been dwarfed by inverting two rings of bark the previous year. They found a high accumulation of the radioactive phosphorus above the inverted ring of bark compared with below (Dickson & Samuels, 1956), indicating the transport of radioactive phosphorus was restricted at the bark graft-union area and cannot be transported downward to the root system.

Further investigation on the bark inversion technique on apple as a method for controlling vigour have been carried out by later studies (Gastol & Poniedzialek, 2007; Lockhard & Schneider, 1981; Poniedzialek et al., 2004), including in avocado (Köhne & Schutte, 1993) and mango (Gaskin, 1963). However, only the study by Lockard and Schneider (1981) demonstrated a strong evidence of a dwarfing effect on apple trees through a bark inversion technique. The bark of two apple varieties namely 'Gravenstien' or MM.111 and M.26 (dwarfing apple type), were used in their study. A strip of bark from MM.111 or M.26 was inverted and grafted back onto the MM.111 scions. They found that a clear dwarfing effect on 'Gravenstien' or MM.111 apple scion resulted from the insertion of M.26 bark grafts. However, more dwarfing effects appeared when the bark of MM.111 and M.26 bark was grafted back in an inverted orientation (Lockhard & Schneider, 1981). Similarly, Arakawa et al. (1984) also found a reduction in growth rate of 'Fuji' and 'Hokuto' apple when the bark was inverted and grafted back to the scion. Bark grafting in the inverted position reduced mean shoot length and trunk cross-section area of 'Melrose' (Gastol & Poniedzialek, 2007) and 'Jonica' apple (Poniedzialek et al., 2004). Recently, Watson (2009) found that growth rate was reduced by the insertion of M.9 bark into the stem but not when the bark was in an inverted position.

In summary, previous studies on the effects of bark inversion are difficult to compare, because in some studies there was no shoot growth data presented to support their hypotheses (i.e., Sax, 1950; 1954; Arakawa et al., 1997; Lockard and Schneider, 1981). In addition, no standardised method was used to evaluate the growth even when the same species was used in the experimental trials. This also might be the reason why the previous studies on bark grafting or bark inversion reporting the inconsistent results. Furthermore, most of the authors did not state clearly whether the bark grafted was made on the scion or rootstock. This makes the comparison is more complicated and unclear. Several authors may have reported that the bark inversion may cause severe damages on the trees (e.g. Gaskins, 1963; Köhne & Schutte, 1993). This could be the reason why this technique is not used in practice compared with dwarfing rootstocks, interstocks or chemical application. Nevertheless, it was decided that experiments using bark inserts/grafting were warranted in our study on kiwifruit.

1.2.8 Control of branching/axillary outgrowth by apical dominance

1.2.8.1 Proposed theories of mechanisms in apical dominance

Apical dominance has been defined as; a) complete or nearly complete control of lateral buds by the apex, b) dominance of one growing shoot over another, and c) the apex influence on the orientation of branches and leaves (Martin, 1987). It has been also described as control exerted by the shoot apex over lateral bud outgrowth (Cline, 1991, 1997) and termed as "correlative inhibition" by Hillman (1984). Lang et al. (1987) classified apical dominance according to dormant states or growth inhibited as; i) *paradormancy* which is the inhibition of growth by distal organs, ii) *endodormancy* which is the inhibition of growth by distal internal bud signals, iii) *ecodormancy* which is the inhibition of growth by temporary unfavourable environment condition (Figure 1.5).

Apical dominance has been studied starting in the early 1900's and mostly focusing on the hormones or nutrient hypothesis/theories. The "Nutritive Theory" initiated by Loeb (1918) suggested that the apical meristems will be the strongest sink for nutrient and water within the plant, limit availability to axillary buds below the critical level. However, this theory was not accepted as a direct addition of nutrient to inactive lateral bud does not cause them to grow out. For example, Cutter (1972) reported that direct nutrient supply to the dormant buds did not cause bud outgrowth. Furthermore, Leaky and Longman (1986) found the application of nitrogen, phosphorus and potassium had no effect on the sprouting of the decapitated shoot of *Triplochiton scleroxylon*. In contrast, the treatment promotes elongation of those shoots, which did sprout. Early classical study on the involvement of hormones in apical dominance was proposed by Thimann and Skoog (1934) on the herbaceous plant, *Vicia faba* L. They found that auxin was an early hormone to control plant processes and growth at that time. This hormone was extracted from the cultures of the fungus *Rhizopus suinus* L. and applied to the surface decapitated shoot of the plants through the agar block. They found that auxin can inhibit lateral bud outgrowth. Later, they found that removal of shoot apex could stimulate lateral buds growth that previously was not growing from the plant that producing a dominant central stem leader (Thimann & Skoog, 1934).

They hypothesised that terminal bud had produced an excess of auxin and some of the auxin has transferred down to inhibit lateral bud outgrowth. Further evidence against this theory was that radio-labelled auxin applied to the decapitated stump did not accumulate in the axillary buds (Hall & Hillman, 1975). However, auxin is known can move basipetally in stems and accordingly not much of it can move acropetally into a lateral bud.

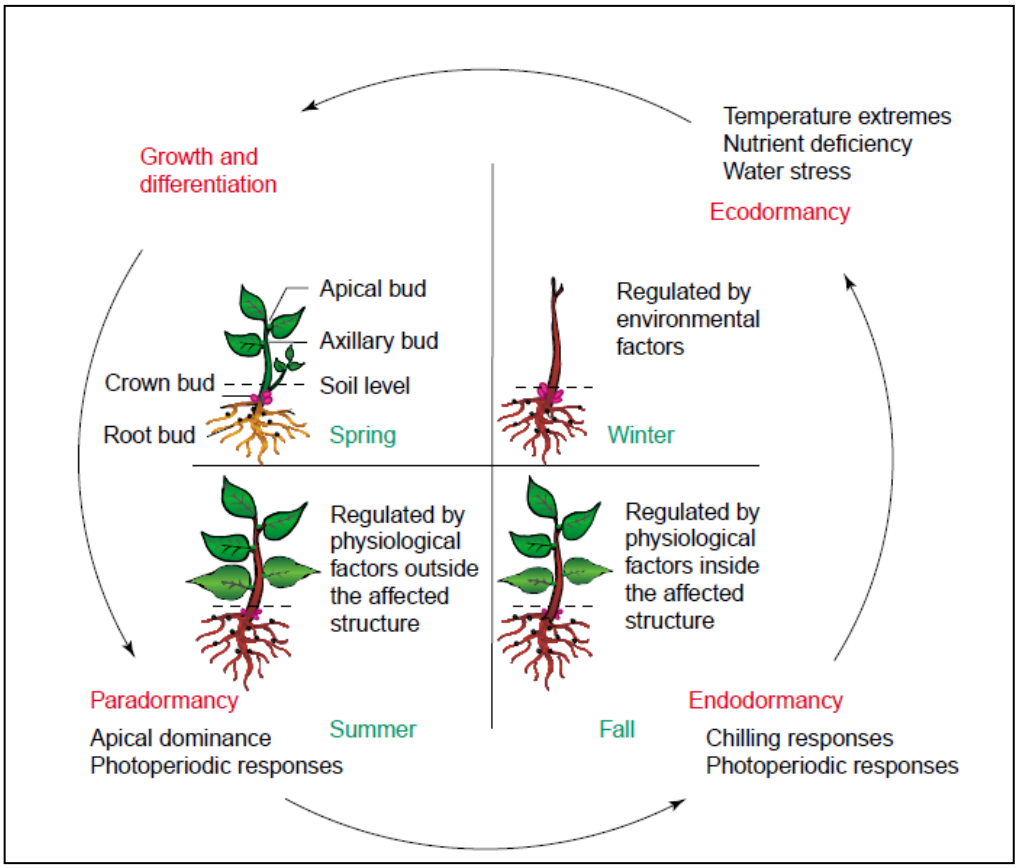

Figure 1.5. Classification of apical dominance according to the definition by Lang et al. (1987).

1.2.8.2 Hormonal control of axillary bud outgrowth/shoot branching

It was known that axillary bud activity is regulated by the activity of plant hormones (Ongaro & Leyser, 2008) and modulated by the environment condition such as light quality and nutrient (Cline, 1991). Auxin (i.e. IAA) was the early hormone to be related to the controlling shoots branching. It was abundantly synthesised in the shoot tips and young expending leaves (Ljung et al., 2001). IAA is transported basipetally down the shoot in a polar manner by active transport in the polar transport stream in the vascular parenchyma (Blakeslee et al., 2005; Lomax et al., 2010). Thus, removal of shoot tips

will remove the source of auxin (i.e. IAA), therefore axillary bud will growth. Although it is clear the IAA plays an important role in the inhibition of axillary outgrowth (branching), the actual mechanism of IAA in controlling branching is still unclear. For example, Brown et al. (1979) reported that application of IAA directly onto the bud does not inhibit outgrowth. In addition, Prasad et al. (1993) found radiolabelled IAA that was applied apically does not enter the bud in any quantity. Therefore, the IAA moving in the stem must act indirectly. This indirect action probably involves interaction with CK.

CK are synthesised in both shoots and roots and transported from the root to the shoot (Cline, 1991). It also has been found to promote branching (Cline, 1991; Nordström et al., 2004). For example, application of exogenous CK directly will cause buds outgrowth (Sachs & Thimann, 1967) and high level of CK was found in the active buds (Dann et al., 1985). In addition, reducing apically derived IAA by decapitation can increase levels of endogenous CK in the xylem sap (Bangerth, 1994; Shimizu-Sato et al., 2009). The role of CK in control of branching is in contrast to IAA and there is an evidence that IAA can regulate cytokinin biosynthesis. A few studies have reported that auxin can inhibit CK biosynthesis in the stem nodes and root, subsequently inhibit axillary buds outgrowth (Nordström et al., 2004; Tanaka et al., 2006). IAA may also reduce CK level by stimulating CK oxidase activity that can cause oxidative breakdown of CK (Zhang et al., 1995). However, there was no evidence that IAA level could be repressed by CK (Nordström et al., 2004). For example, CK was found to enhance the production and transport of IAA out of the shoot apex (Bangerth, 1994). Furthermore, CK could also enhance auxin biosynthesis and thereby forms a homeostatic feedback regulatory loop of IAA and CK synthesis (Jones et al., 2010).

A few studies have also found that GA is also important in controlling branching, as found in sweet cherry (Elfving et al., 2011), *Jatropha curcas* (Ni et al., 2015), and possibly kiwifruit (Vattiprolu, 2012). Application of GA_3, GA_4 and combination of GA_{4+7} to the lateral bud of one-year-old wood of sweet cherry trees can stimulate the branching. In addition, application of GA_{4+7} alone was almost as effective as the application of synthetic CK (6-benzyladenine) in stimulating branching (Elfving et al., 2011). A study in *Jatropha curcas*, Ni et al. (2015) reported that lateral branch outgrowth could be effectively stimulated by the application of GA_3 or the 6-

benzyladenine (synthetic CK). However, more stimulatory effects of lateral branching of *Jatropha curcas* have been observed when both of these hormones were applied together, even in lower concentration (i.e. 10 μM of each hormone) (Ni et al., 2015). In kiwifruit, application of GA_3+GA_{4+7} as foliar spraying increased the number of sylleptic axillary shoots in both potted 'Hayward' and 'Hort16A' young vines (Vattiprolu, 2012). Furthermore, combine application of GA_3+GA_{4+7} at the onset of bud break of the field-grown 'Hayward' vines also had increased the number of shoots, indicating the GA had stimulated both apical and sub-apical meristems. Therefore, based on the literature above, GA may also have a significant role in regulating shoot branching in particular plant species, including kiwifruit. However, the actual mode of action of GA in controlling shoot branching is still largely unknown and need further investigation, especially in kiwifruit vines.

1.2.8.3 'Strigolactones'- A novel branching hormone

A classic series of experiments indicated that movement of auxin basipetally (downward) in the vascular cambium might trigger the production of a second inhibitor, that can move acropetally (upward) through the xylem to inhibit axillary buds outgrowth (Snow, 1931, 1937). Thus, Snow (1931) proposed "secondary inhibiting effect" that moved up into buds through the transpiration stream. Almost seventy years after Snow's pioneery work grafting studies and hormone measurements in mutants that display increased branching, such as *rms* in pea and *max* in *arabidopsis*, revealed the involvement of a long-distance branch inhibiting signal that could move acropetally from the lower part of the stems and roots and inhibit shoot branching (Brewer et al., 2009; Dun et al., 2009; Gomez-Roldan et al., 2008). This branch inhibiting signal is referred as 'Shoot Multiplication Signal' (SMS) that moves acropetally in shoots and inhibit lateral bud outgrowth (Foo et al., 2001). Studies have indicated that SMS could be identified as a carotenoid-derived compound that can control other biological processes. Recently, scientists have now identified that a new group of compounds or plant hormone called 'strigolactones'. Strigolactones (SL) are compounds derived from carotenoid-derived terpenoid lactone, which may be involved in the inhibition of shoot branching (Gomez-Roldan et al., 2008; Umehara et al., 2008; Xie & Yoneyama, 2010).

According to Matusova et al. (2005), SL are produced by the action of 'Carotenoid Cleavage Dioxygenase' genes (CCD7 and CCD8). CCD genes have been shown to play an important role in the controlling branch development in the experimental plants such as pea, *arabidopsis*, petunia and rice. SL also triggers germination of parasitic weeds such as *Striga* spp. and *Orobanche* (Goldwasser et al., 2008; Matusova et al., 2005) (Figure 1.6). These parasitic weeds utilised SL as a plant-derived signal to promote seed germination (Figure 1.6). SL also function as a communication chemical in symbiotic arbuscular mycorrhizal fungi that ease nutrient uptake (Akiyama & Hayashi, 2006). If the plant is under the nutrient-deficient condition, strigolactones will be used as a signal in molecules form to suppress shoot branching. According to Yoneyama et al. (2009), there are three main biological activities of SL which are; i) germination stimulation, ii) hyphal branching and iii) shoot branching inhibition.

Early observation on SL by Gomez-Roldan et al. (2008) and Umehara et al. (2008), found a significant reduction in SL levels in roots of enhanced shoot branching of *Arabidopsis* mutants (*max/rms/dwarf*). There was also evidence that endogenous and exogenous application of SL to pea plants can inhibit shoot branching. For example, germination assay of *Orobanche* seeds treated with exudates of CCD8 and CCD7 mutant pea was decreased compared with wild types exudates, indicating low SL level in mutant pea (Gomez-Roldan et al., 2008). However, it is not clear which SL, or downstream product related to SL, is the putative branch-inhibiting hormone (D.J. Woolley, personal communication, February 15, 2012). Furthermore, Gomez-Roldan et al. (2008) found CCD8 shoot branching mutants of pea are low in SL level and application of SL can preserve wild-type branching phenotype of pea to CCD8. Later, Umehara et al. (2008) studied the effect of SL treatment on rice *d* mutants by using hydroponic culture system and found application of synthetic SL (GR24) in the media can inhibit tiller bud outgrowth of two-week old rice *d* mutants seedling at low dose 1 μM of SL. Similar effect was also observed when naturally occurring SL were used. These results showed the function of SL in inhibiting tiller bud outgrowth and in branching inhibitor pathway in rice *d* mutants (Umehara et al., 2008). Overall, based on the provided literature below, branching mutants in plants that are lacking CCD7 or CCD8 have reduced concentrations of SL, and applications of synthetic SL can restore the wild-type branching phenotype to the mutant.

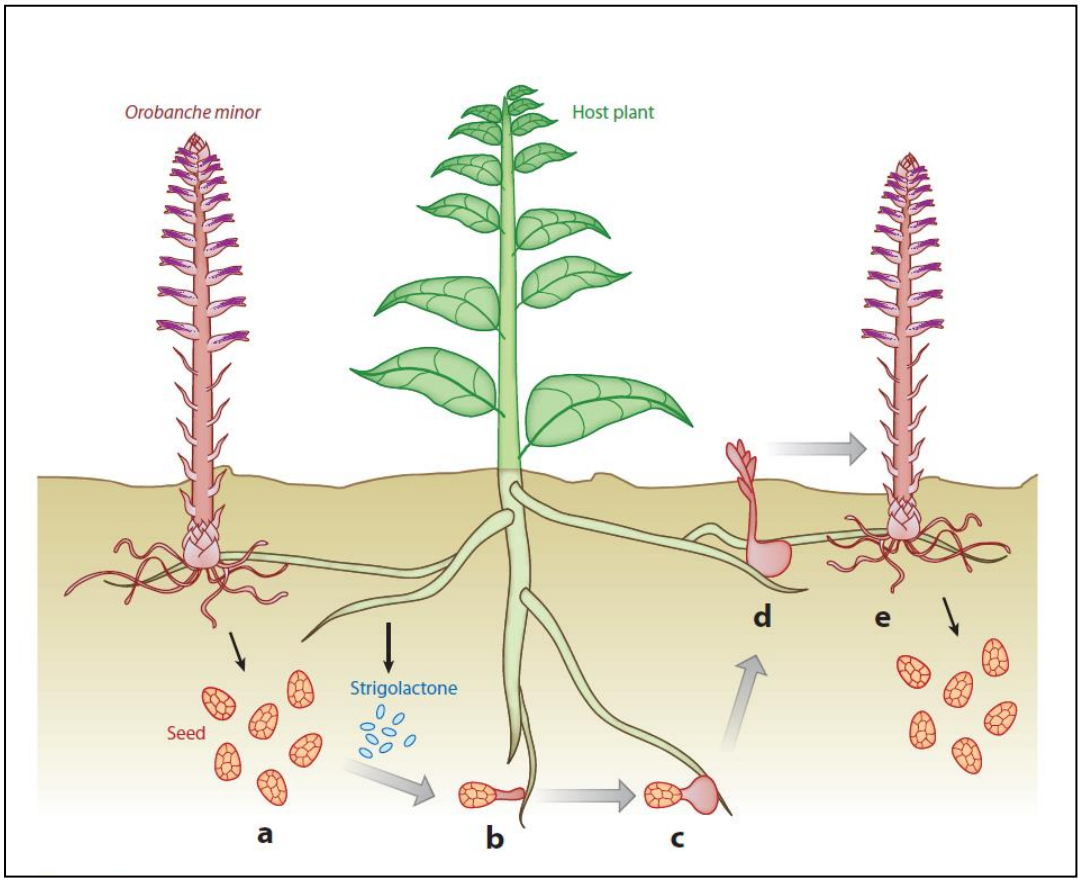

Figure 1.6. Life cycle of *Orobanche minor*, a root parasitic plant: a) seed germination is elicited by host-derived stimulants (strigolactones), b) seedling attaches to host root with haustoria, c and d) parasite tubercles grow underground for several weeks or months before emergence of the flowering shoots, and e) The parasite produces a large number of seeds, which remain viable for many years in soil. (source Xie and Yoneyama(2002))

In kiwifruit, there were clear evidence that rootstocks can influence shoot architecture and yield (Clearwater et al., 2004; 2006; 1997; Cruz-Castillo et al., 1991; Wang et al., 1994b), suggesting that the root-derived signals, such as SL, may be important for controlling kiwifruit branching. A recent study showed that high expression of AcCCD7 and AcCCD8 genes were found in the roots of kiwifruit and this is associated with MAX3 and MAX4 genes (orthologous encoding of plastid-targeted CCDs) (Ledger et al., 2010). More expression of the AcCCD7 and AcCCD8 genes was found in the young fruit and seeds of wild-type kiwifruit. However, in transgenic kiwifruit, there was a reduction in AcCCD8 gene expression and this correlated with an increase in the total number of branches produced by the plants (Ledger et al., 2010). Furthermore, Honda et

al. (2011) found the number of axillary shoots was increased when grafted transgenic kiwifruit plants onto wild-type kiwifruit seedlings due to overproduction of CK.

In addition, grafted transgenic kiwifruit had shorter internode length and smaller leaves compared with wild-type kiwifruit (Honda et al., 2011). Studies by Vattiprolu et al. (2012) showed that applying the auxin transport inhibitor, N-1-naphthylphthalamic acid (NPA) to the stem of primary shoot of kiwifruit increased the number of secondary shoots (more branching occur), even though the length of primary shoot was reduced; possibly reduced apically derived auxin, reduces the amount of SL in roots of kiwifruit, as well as increasing cytokinin level in roots (Bangerth, 1994) and the result of these two hormonal signals, i.e. reduction in SL and increase in CK, would explain the increased branching. Thus, it may be that hormonal signalling from the root system (rootstock) plays an important role in developing shoot architecture (scion) of kiwifruit. However, the definite mechanisms of SL and CK, together on interactions with other hormones such as IAA and GA in controlling shoot branching of kiwifruit are not fully understood and not well studied.

1.2.9 The importance of architectural traits in fruit trees

Tree architecture has been described as the organization of different plant structural components (Costes et al., 2006a). The most important part in fruit crops architecture is largely determined by their branching habits. Shoot architectural structures and growth habits can have an important impact on crop management, intensification, and orchard design (Costes et al., 2006a; Lauri et al., 2008). Many of the potential architectural traits or characteristics could be identified as early as the seedling stages. Seedling trees usually show diverse physiological and morphological characteristics especially in their architectural form, and these characteristics have been utilized by the plant breeders to explore the potential of these traits. In fruit trees, the growth of young seedlings has been related to the length of the juvenile period (Hartmann & Engelhorn, Hackett, 1985; 1990; Thompson & Grauke, 2003). Generally, the juvenile period and seedling vigour are used in fruit breeding programmes as a pre-selection criterion of the plants. Tree seedlings with short juvenile periods are desirable because not only does it shorten the

breeding cycle, it also can be used to reduce the vegetative period of composite trees when used as rootstocks (Hartmann & Engelhorn, 1990). It has been suggested that seedling vigour could be used as a main pre-selection criteria in the fruit breeding programme, for example in olive trees (De la Rosa et al., 2006). It is likely that vigorous seedlings may have a shorter juvenile period than low-vigour seedlings. Previous studies have shown that the seedlings must reach certain sizes and node number before they can produce flowers, and the vigorous seedlings are likely to produce early flowers than low-vigour ones (Hackett, 1985; Visser et al., 1976). Studies in other fruit crops such as apple (Visser, 1965), pear (Visser et al., 1976), plum (Hartmann & Engelhorn, 1990) and olive (De la Rosa et al., 2006; Santos-Antunes et al., 2005) found that vigour of seedlings may have a significant influence on the percentage of flowering in the first three years of growth. For example, Visser (1965) found that there was a significant correlation between the juvenile period and the flowering of apple seedlings, and they also found that the juvenile period is strongly related to the initial vigour of the seedlings (Visser et al., 1976). Similarly in plum, by selecting vigorous seedlings with traits that have many thorns and large leaves, Hartmann &Engelhorn (1990) found that seedlings with these traits had better cropping, with larger fruit size. In olive, the vigour of seedlings, measured by trunk girth, was significantly lower for non-flowering seedlings compared to seedlings that flowered for the first time (Santos-Antunes et al., 2005). In the case of kiwifruit, there has also been a tendency to select for high-vigour, possibly to reduce labour time costs in the breeding programme, so low-vigour seedlings may have been discarded or removed during breeding selections, due to the probable long juvenile period. In this assessment, we are interested in kiwifruit seedlings that may have dwarfing abilities or low-vigour traits. These vigour traits could be used as potential vigour-controlling rootstocks and/or as low-vigour scions.

Recently, architectural traits based on the branching pattern have been used to predict the agronomic importance of fruit trees. Branching at the early stage of tree development is important and is considered as an advantage for early light interception and establishment of orchards (Robinson, 2004; Van Oosten, 1976). Since branching mainly develops during the early stage of trees growth (Costes & Guédon, 2002), this trait is expected to be a potential early selection criteria, as it could influence agronomic behaviour during the mature phase (Costes et al., 2006a; Lauri et al., 2008). Previous studies have found great variability in the architectural traits and branching patterns in

apple (De Wit et al., 2002; Segura et al., 2006) and olive seedlings (Hammami et al., 2011; 2012). In kiwifruit, little information is available on the selection of genotype and/or phenotype in relation to the potential growth habits or vigour characteristics. Most kiwifruit breeders have focused on the development of new cultivars with unique flavours, improvement of eating quality, the increase of nutritional components levels. However, other growth characteristics or traits that may be desirable in kiwifruit management are low-vigour or dwarfing characteristics. These traits are valuable in the development of vigour-controlling rootstocks or they can be used as low-vigour scions. However, little attention was given to these characteristics during the early selection of kiwifruit seedlings in the breeding programme. Variability in the shoot architecture and growth habits of kiwifruit seedlings obtained from the breeding programme or crosses warrant further scientific evaluation, not only for fruit quality purposes, but possibly for vigour characteristics and potential architectural traits as well. In addition, pre-selection of potential rootstocks in kiwifruit with special preferable characteristics need to be done as early as possible, starting with the seedlings stage. The terminology we developed to characterise different phenotypes is described in Section 6.2.1.2.

1.2.10 Modification of plant architecture by gibberellin

Many important processes in horticultural crops are regulated by endogenous hormonal mechanisms and the use of bio-regulators. The use of bio-regulators has been shown to alter long-distance signalling between the shoots and roots system that may change the physiological processes of the trees (review by Mallahi and Burns, 2007). Many studies have found involvement of endogenous hormones in the long-distance signalling between shoot and root systems (review by Baluška, 2013). However, in kiwifruit, there is not much information available on the effects of endogenous hormones or the use of bio-regulators on physiological aspects. As noted elsewhere, kiwifruit vines produce excessive vegetative growth, which reduces the light penetration inside the canopy, which is important for floral bud development and fruit quality (Miller et al., 2001). Therefore, information on how kiwifruit shoots respond to the application of bio-regulators is important in order to improve our understanding on the role of endogenous hormones in regulating the shoot architecture in kiwifruit and may provide a way to

temporarily change the vigour of a plant while the crop canopy is being established. Previous studies that focus on the endogenous hormones have shown that vigour of seedling trees could be related with the deficiency in gibberellins levels (Cristoferi & Filiti, 1981; Gotô, 1978; Phinney, 1983), including in composite plants such as pea (Lockard & Grunwald, 1970), apple (Bulley et al., 2005; van Hooijdonk et al., 2010), citrus (Fagoaga et al., 2007) and plum (El-Sharkawy et al., 2012). However, other than a few attemps to reduce vigour with growth retardants (anti-gibberellins), few detailed studies have been conducted and reported on the role of gibberellins in kiwifruit (D. J. Woolley, personal communication, July 17, 2013).

1.2.11 Summary of literature

Development of vigour controlling rootstocks has the potential to greatly improve production and orchard management of kiwifruit. However, to date lacks vigour-controlling rootstocks for commercial use in kiwifruit management. Therefore, there is a strong need to breed new kiwifruit rootstocks that can control excessive vigour and improve fruit quality, as well as resistant to diseases such as PSA. Despite recent advances in the genetic studies of kiwifruit, the basic understandings of rootstock effects on scion growth are poorly understood, particularly in kiwifruit. In addition, there is little-published information regarding vigour-controlling rootstocks in kiwifruit. Thus, understanding the physiological mechanisms on how rootstock can affect the scion vigour could be the first step for developing desirable rootstock candidate in kiwifruit. The involvement of endogenous hormones in regulating shoot branching would also provide an important information on branching mechanism in kiwifruit. Overall, findings from this study, hopefully could improve understanding of physiological mechanisms on vigour control in kiwifruit.

Chapter Two

2. Inter-specific hybrid kiwifruit rootstocks have potential to modify scion architecture and vigour of young 'Hayward' vines

2.1 Introduction

Rootstocks have been extensively used in many fruit tree crops, such as apple, pear, plum and peach (Rom & Carlson, 1987; Webster, 1996). In apple, the availability of Malling 9 (M.9) dwarfing rootstocks has allowed the development of high density orchards, and has brought significant benefits in orchard management, costs and productivity. In order to manage excessive vegetative growth in kiwifruit, growers depend on horticultural manipulation (i.e. pruning, shoot tipping, girdling, root pruning), as there are no rootstocks that strongly reduce scion vigour, as there are for many other fruit crops (Palmer, 2007; Warrington, 2000). In fruit trees, knowledge of plant architecture is widely used to improve crop management and optimise production (Costes et al., 2006a). Plant architecture has also a major agronomic importance because it strongly influences tree efficiency and final yield. Over the years, modification of the architectural structures has led to increases in the crop establishment and productivity. Characteristics such as size, shape, branching habit, position of leaves and flower organs, as well as fruiting pattern, are useful for determining tree growth and development. Two main factors can regulate tree architecture; the first is the scion genotype (Lauri et al., 1995; Lespinasse & Delort, 1986; Quinlan & Tobutt, 1990) and the second is the rootstock (Mudge et al., 2009; Webster, 1995a). Rootstocks are generally propagated vegetatively from potential rootstock cultivars (Hartmann et al., 2011; Webster et al., 1995b) comprising two parts, i) the stem shank and ii) the root system (Figure 1.4, Chapter One).

In some fruit species (e.g. apple, pear and grape), rootstocks assist orchard management, for example, controlling tree or vine vigour, pest and disease resistance, increasing

yield, fruit quality and adaptability of trees (Gregory et al., 2013; Koepke & Dhingra, 2013; Rom & Carlson, 1987). However, kiwifruit canopy management is difficult because of extremely strong vegetative vigour of the vine, but success in developing good vigour-reducing rootstocks has been very limited (Anon., 2012). In a compound fruit tree, the architectural structure and branching habit of the scion is modified by the genetics of the rootstock, as early as the nursery stage. In apple, Seleznyova et al. (2007) reported that scions on M.9 dwarfing rootstocks may grow similarly to scions on vigorous rootstocks in the first year of growth following grafting, but scion dwarfing was evident in the second year following grafting after increased flowering. It was suggested, greater flowering induced by dwarfing rootstock in the second year of tree growth had significant effect on scion architecture (Seleznyova et al., 2008).

However, a growing body of evidence also suggests that the effects of rootstock mediated vigour control can be measured in the first season of growth in a range of fruit crops. For example, across a range of apple scions, the number of sylleptic shoots (Volz et al., 1994), and their length and node number (Costes et al., 2001; van Hooijdonk et al., 2010) were reduced when grafted on dwarfing M.9 rootstock, often in the first year. Study by Fazio and Robinson (2008) found that the dwarfing rootstock, G.16 as well as semi-dwarfing rootstocks, G.935 and CG.4213, had the ability to increase number of sylleptic shoots (branching) of 'Fuji' scion when compared to vigorous rootstocks such as CG.7037 and CG.8534. In other fruit trees such as pear, rootstocks can affect the node neo-formation of primary and secondary shoots of the scion, contributing to the differences in the proportions of annual shoot types (Watson et al., 2012). For grape vine crops, the effect of rootstock on initial scion growth can be observed within a few months following grafting (Cookson et al., 2012; Tandonnet et al., 2010). Tandonnet et al. (2010) found that grape rootstocks varied in their ability to stimulate axillary shoot development of the scion. In addition, Cookson et al. (2012) reported that stem biomass of the scion (i.e. stem dry weight) was significantly affected by the rootstock genotype in first year of the growth cycle. Therefore, it can be concluded that important changes in the scion architectural structures by the rootstock may occur in the first and second year of growth following grafting. In kiwifruit, no studies have described the modification of scion architecture by the rootstocks, during the initial stage of vine growth. We hypothesised that rootstock effects in grafted kiwifruit vines can be expressed during the early stage of vines growth, then will affect the initial architecture

of grafted scion (**Hypothesis I**). For this reason, the main objective of this chapter was to identify the modification by the kiwifruit rootstocks on scion vigour soon after grafting, and to describe the architectural changes involved in growth modifications by hybrid kiwifruit rootstocks. Only limited information is available about rootstocks for kiwifruit (Section 1.2.3). Nevertheless, previous assessments on mature vines have provided useful insights on modification of scion architecture by the kiwifruit rootstocks. For example, a study by Clearwater et al. (2004) on different species of kiwifruit rootstocks showed that leaf area and crown size of gold kiwifruit (*A. chinensis* cv. 'Hort16A') scions were reduced when grafted onto low vigour rootstocks, such as *A. kolomikta*. The reduction in growth was clearly observed during the initial period of shoot growth immediately after bud burst (Clearwater et al., 2006). Other examples of clonal rootstocks in kiwifruit such as *A. eriantha* and *A. hemsleyana* were only suitable as a flower-promoting rootstock and not suitable for vigour controlling rootstocks, as they did not suppress vegetative growth of grafted scions (Wang et al., 1994b). Recently, the rootstock 'Bounty 71' has been reported as a promising clonal rootstock for kiwifruit (Anon., 2012). Despite promoting higher flower number and producing larger fruit size on 'Hort16A' scions, vine vigour and growth imparted by this rootstock were greatly reduced (Anon., 2012). Therefore, less canopy management required due to very minimal pruning during winter. Based on the information available, we can conclude that in kiwifruit, only a few clonal rootstocks are available, many of which are not commercially propagated.

Inter-specific hybrid rootstocks have been widely used for vigour control, increased precocity and fruit quality in fruit crops such as apple (Cummins & Aldwinckle, 1983), *Prunus* (Gruppe, 1984), cherry (Callesen, 1997; Lang, 2000) and citrus (Grosser et al., 1995). Therefore, the use of inter-specific hybrid kiwifruit rootstocks could help to produce potential rootstocks that maximise the potential of new cultivars in a particular environment (Clearwater et al., 2002). In a trial at Plant and Food Motueka Research Station, Nelson, New Zealand in 2006, the vigour of *A. chinensis* cv. 'Hort16A' (gold kiwifruit) scion, based on trunk cross-sectional area (CSA) was affected by inter-specific hybrid rootstocks (Friend et al., 2014). However, no trials have been done on the effect of inter-specific hybrid rootstocks on *A. deliciosa* cv. 'Hayward' (green kiwifruit). In the present study, thirteen inter-specific hybrid kiwifruit rootstocks were assessed in terms of their effects on scion growth and architecture of 'Hayward'

kiwifruit. The experiment described in this chapter was designed to evaluate the modification of scion architecture by the kiwifruit rootstocks, and to assess whether the alteration of scion vigour could be measured in the first or second year of growth following grafting.

2.2 Materials and Methods

2.2.1 Study site and establishment of plant material

The experiment was performed during the 2011 and 2012 growing season at the Plant Growth Unit (PGU), Massey University, Palmerston North, New Zealand. Green kiwifruit cv. 'Hayward' scions were cleft grafted onto thirteen inter-specific hybrid rootstocks (see Section 1.2, Chapter One) in August 2010 at Plant and Food, Motueka Reseach Station, Nelson. The grafted plants were grown in 1L polybags and after establishment they transferred to Palmerston North in April 2011 on 2 x 2 m pallets. The grafted plants were placed at the standing out area at PGU in order to monitor the growth and development of the plants.

2.2.2 Propagation and growing medium for grafted materials

2.2.2.1 Grafting of inter-specific hybrid rootstocks

Grafting of inter-specific hybrid rootstocks was conducted during winter (dormant plants). A 'Hayward' scion (green kiwifruit) was grafted onto inter-specific hybrid rootstock stems, using a cleft-graft cut produced by a grafting machine (Ragget Industries, Gisbone, New Zealand) (Figure 2.1). Rootstock stems, consisting of three to four nodes, and scions, consisting of two nodes, were selected for the grafting materials. During grafting, the length of rootstocks and scions was standardized to approximately 20 cm and 12 cm, respectively. The stem diameter of rootstocks and scions were matched carefully in order to increase grafting success. The graft-union was sealed with grafting tape and the bottom of the rootstocks was scored using a sharp knife before dipping in 200 mg/L of rooting hormone solution, Indole-3-Butyric-Acid (IBA), for 30

seconds. The top of cuttings were sealed with pruning paste (Bacseal®Super, Bayer CropScience, NZ).

2.2.2.2 Growing conditions of experimental plants

After grafting, plants were initially grown under mist and then transferred into 1L polythene bags containing four parts of composted bark to one part coarse river sand, plus 5.5 kg/m^3 8-9 month Osmocote, 1.5 kg/m^3 trace element fertiliser and 2.5 kg/m^3 lime. A single shoot was grown in the first season, which was trimmed at an approximate height of 1 metre midway through summer. After the first season, the plants were potted into 4L polythene bags. The plants were grown outside throughout the trial and irrigation was provided to the plants daily using drippers.

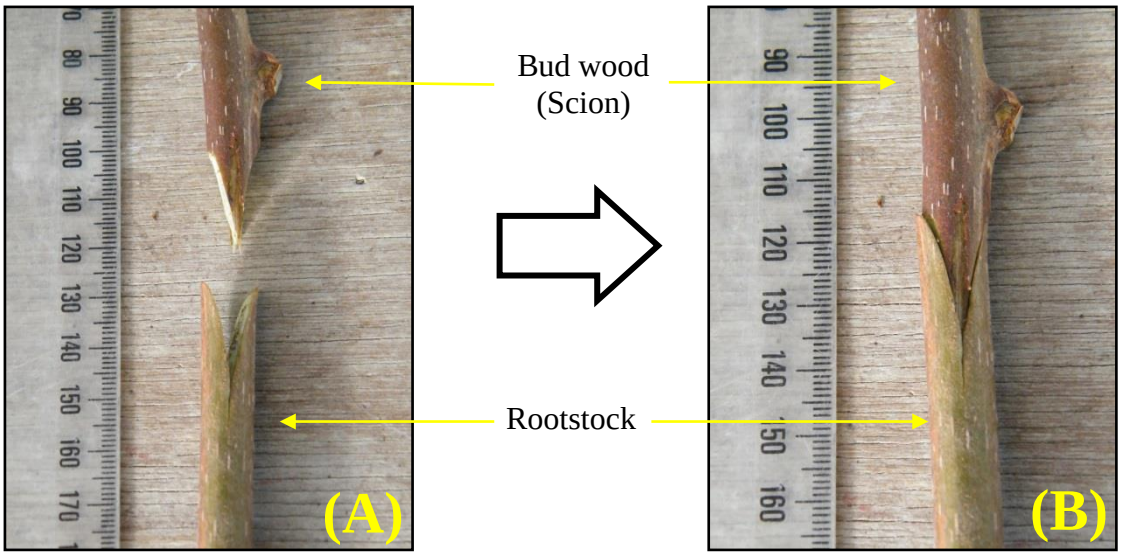

Figure 2.1. An example of cleft-graft of a 'Hayward' scion grafted onto the inter-specific hybrid rootstock stem. (A) Cleft cut made using grafting machine, and (B) matching the wedge cut of scion stem into the stem of rootstock.

2.2.3 Measurements of shoot architecture and growth characteristics

An initial assessment of the rootstock and scion architecture was carried out on the one-year-old plants during late autumn (May 2011), approximately 8 months following grafting. The diameter (mm) of the rootstocks and scions (original grafted wood)were measured 30 mm above the growing medium and graft-union (respectively) using

digital callipers (Mitutoyo, Japan); diameter of primary shoot (new scion growth) was measured 10 mm above the base of the primary shoot (Figure 2.2); all diameters measured were converted into cross-sectional areas (CSA). The length of primary shoots was measured using flexible tape, beginning at the shoot base and finishing at the shoot tip. However, the initial length of primary shoot was not reported because the plants had previously been headed back to a similar length (red-dotted line, Figure 2.2). The number of nodes were manually counted and recorded. Mean internode length of the primary shoots was calculated by dividing shoot length by the number of nodes. In early spring 2011 (4/10/2011), bud break on the primary shoot was manually counted and the proportion breaking calculated by dividing the total number of buds that broke by the number of nodes per primary shoot and expressed as a percentage. After 14 months, the second assessment of shoot architecture was performed during summer 2012 (5/1/2012). The diameters of rootstocks and the initial primary shoot of each scion were again measured to obtain cross-sectional areas. Proleptic axillary shoots produced from the primary shoot of the scion were classified as short, medium or long according to the description of kiwifruit architecture developed by Seleznyova et al., (2002) and summarised in Figure 2.2. The length and stem cross-sectional area of each different proleptic axillary shoots on the primary shoot were also measured and recorded. The proportion of different shoot types (%) was calculated by dividing number of shoots (i.e. long, medium and short) by total number of shoots x 100. Two dominant non-terminated proleptic shoots were also selected in order to develop permanent cordons for vines to be transplanted to the field (Chapter Three).

2.2.4 Experimental design and statistical analysis

The experiment was a Completely Randomised Design, located at the standing out area at Plant Growth Unit, Massey University; with up to 12 replicates for the first assessment. For the second assessment, only 6 replicates of vines per rootstock were evaluated because half of them being used for another set of experiment (Chapter Four). Data were manually entered into a Microsoft Excel spreadsheet, and summarised using Pivot Table functions. Data were checked for homogeneity, and Analysis of Variance (ANOVA, *F*-test) was performed using the GLM procedure of SAS (9.1, SAS Institute

Inc., Cary, NC USA). If necessary, the data were subjected to square root or logarithmic transformation before subjecting to ANOVA.

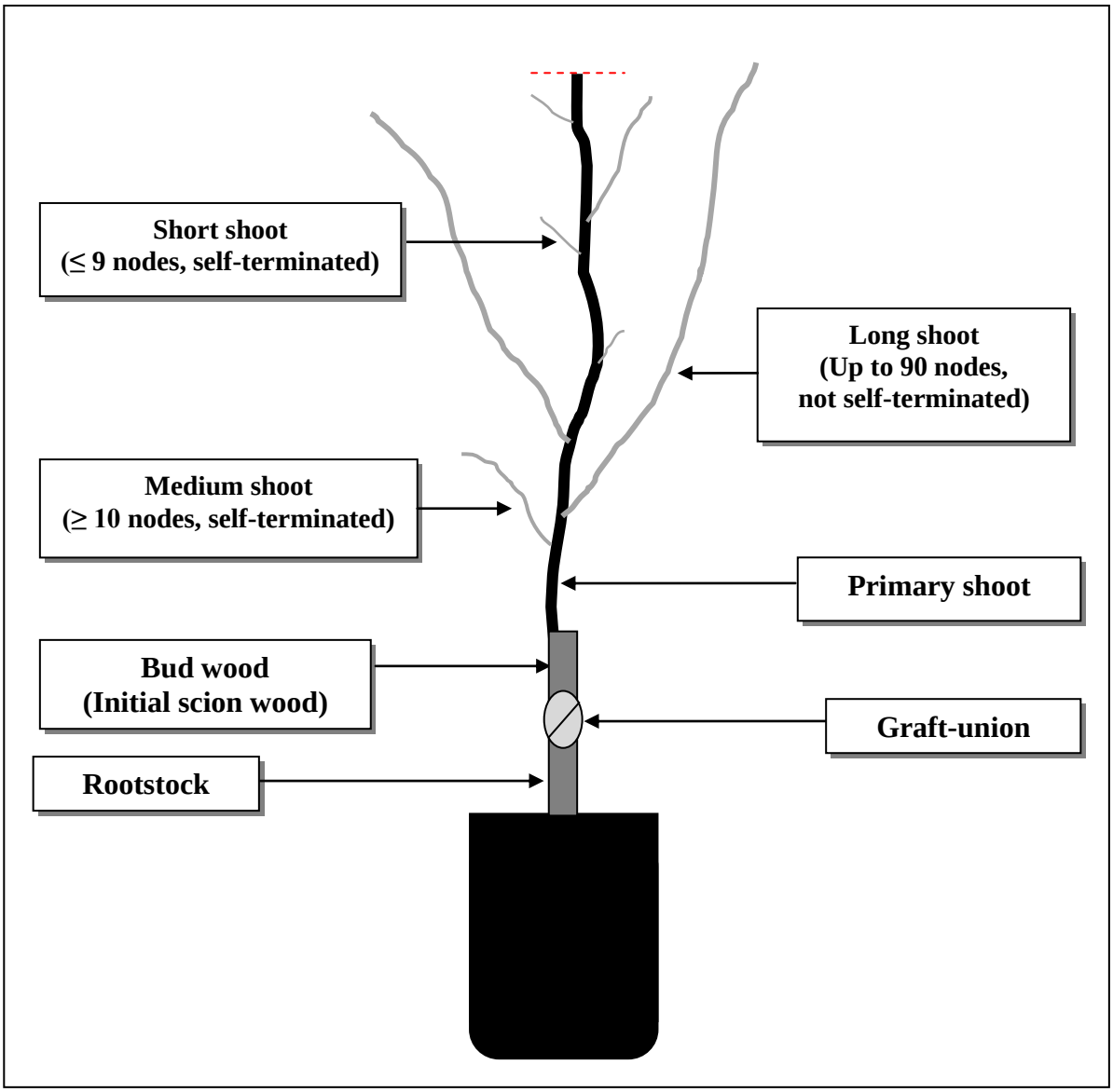

Figure 2.2. Schematic representation of the architecture in the second season of growth of *Actinidia deliciosa* cv. 'Hayward' on inter-specific hybrid rootstocks (14 months after grafting). Classification of shoot types produced on the primary shoot of the scion, as per Seleznyova et al. (2002).

2.3 Results

2.3.1 Trunk cross-sectional area (CSA) of rootstocks and scion bud wood

At eight months following grafting, the mean trunk CSA of inter-specific hybrid rootstocks were significantly different (P=0.0001) (Table 2.1). The highest mean trunk CSA of rootstocks were that of rootstocks No.18 (largest), No.84 and No.87 with a range between 80 mm^2 and 100 mm^2, while rootstocks No.8 and No.45 had the lowest mean trunk CSA with 55.0 mm^2 and 53.0 mm^2, respectively. Other rootstocks had an intermediate range of trunk CSA between 60 mm^2 and 80 mm^2 (Table 2.1). The difference in trunk CSA between the largest and smallest rootstocks was almost 50%. The trunk CSA of bud wood (initial scion wood) was also different (P<0.0001) between inter-specific hybrid rootstocks (Table 2.1). After eight months, the trunk CSA of the bud wood was still positively correlated with the trunk CSA of the rootstock (Figure 2.3A).

After fourteen months, the mean trunk CSA of rootstocks was still significantly different (P=0.001) between inter-specific hybrid rootstocks (Table 2.1). Selection No.18, No.84 and No.87 still produced the largest trunk CSA of rootstocks, whilst rootstocks No.8, No.21 and No.45 had the smallest trunk CSA with less than 70 mm^2. Inter-specific hybrid rootstocks also had a significant affect (P=0.006) on the mean trunk CSA of bud woods (Table 2.1). By this time, most of the rootstocks produced larger stem CSA of the bud wood, except for rootstocks No.8, No.55 and No.21, with less than 83 mm^2. In general, rootstocks with a small scion trunk CSA area at eight months also had small trunk CSA area at fourteen months. However, the differences in trunk CSA of scion bud wood between eight and fourteen months were extremely noticeable in rootstocks No.45, No.87 and No.100, in contrast with rootstock No.55 and No.71 that only produced small gain in trunk CSA (Table 2.1).

Table 2.1. The mean trunk CSA of initial grafted rootstocks and 'Hayward' bud wood measured after 8 and 14 months following grafting.

Rootstock selection‡	Parentage of rootstocks	Mean trunk CSA (mm^2)				Difference in the mean trunk CSA of scion bud wood between 14 and 8 months (mm^2)
		After 8 months		After 14 months		
		Rootstock	Scion bud wood	Rootstock	Scion bud wood	
No.45	*A. polygama* x *A. chinensis*	53.0 (± 2.3)	49.2 (± 3.6)	63.4 (± 5.3)	95.9 (± 7.1)	**46.7** (Large gain)
No.8	*A. chinensis* x *A. macrosperma*	55.0 (± 7.6)	51.7 (± 7.7)	64.5 (± 18.5)	79.2 (± 24.3)	27.5
No.21	*A. chinensis* x *A. macrosperma*	60.7 (± 5.4)	59.1 (± 5.3)	66.6 (± 9.6)	82.9 (± 10.2)	23.8
No.19	*A. chinensis* x *A. macrosperma*	64.2 (± 2.9)	72.6 (± 4.8)	86.3 (± 6.1)	99.9 (± 8.8)	27.3
No.86	*A. macrosperma*	65.4 (± 5.2)	76.2 (± 6.8)	72.0 (± 8.6)	98.4 (± 6.1)	22.2
No.55	*A. polygama* x *A. chinensis*	70.5 (± 4.3)	63.7 (± 4.8)	72.5 (± 4.7)	76.5 (± 5.9)	**12.8** (Small gain)
No.100	*A. macrosperma* x *A. melanandra*	73.5 (± 9.2)	68.8 (± 5.4)	69.7 (± 11.4)	103.1 (± 12.6)	**34.3** (Large gain)
No.71	*A. polygama* x *A. chinensis*	75.2 (± 11.2)	67.6 (± 7.1)	72.4 (± 12.0)	83.9 (± 7.1)	**16.3**
No.101	*A. macrosperma* x *A. melanandra*	76.8 (± 3.5)	73.5 (± 4.5)	78.9 (± 8.1)	97.2 (± 9.1)	23.7
No.85	*A. macrosperma*	77.9 (± 8.8)	83.4 (± 8.5)	87.1 (± 10.7)	106.3 (± 12.4)	22.9
No.87	*A. polygama*	83.5 (± 10.1)	84.8 (± 13.2)	95.4 (± 14.9)	121.1 (± 16.3)	**36.3** (Large gain)
No.84	*A. polygama*	84.1 (± 5.7)	87.4 (± 5.8)	108.1 (± 8.0)	113.6 (± 4.5)	26.2
No.18	*A. chinensis* x *A. macrosperma*	100.6 (± 12.6)	104.2 (± 14.5)	123.6 (± 11.2)	134.0 (± 10.5)	29.8
$LSD_{0.05}$		18.8	19.3	28.5	29.2	
P-value		*P*=0.0001	*P*<0.0001	*P*=0.001	*P*=0.006	

Numbers in parentheses are standard error of means (±).

‡Rootstocks are ranked from smallest to largest based on trunk CSA at 8 months.

2.3.2 Characteristics of primary shoot of grafted scion

The mean trunk CSA of primary shoots were affected (*P*=0.03) by the inter-specific hybrid rootstocks (Table 2.2). There was a 40% difference between the smallest and the largest trunk CSA of primary shoots. The smallest trunk CSA of primary shoots were recorded for scions grafted onto rootstock No.8 (29.6 mm^2) and the largest was recorded for rootstocks No.18 and No.71 with trunk CSA of almost 50 mm^2. The mean internode length of the primary shoots were significantly affected (*P*=0.003) by the inter-specific hybrid rootstocks (Table 2.2). Rootstocks No.19, No.85 and No.86 had the longest internode lengths with 77.6 mm, 75.9 mm and 74.2 mm, respectively. However, the primary shoot of rootstocks No.8 and No.45 had the shortest internode length with less than 40 mm (Table 2.2).

2.3.3 Effect of rootstocks on spring bud break of primary shoots

Bud break along the primary shoot of the scion was assessed in early spring 2011 (4/10/2011). The percentage of bud break (%) of primary shoots of scions were significantly affected (*P*=0.0007) by the inter-specific hybrid rootstocks (Table 2.2). Most of rootstocks produced more than 40% bud break but rootstocks No.8 and No.86 produced 29.0% and 31.7% bud break, respectively. The highest mean bud break was recorded for the scion on rootstocks No.18, No.84 and No.87, with more than 50% bud break.

Table 2.2. The mean trunk CSA and mean internode length of the primary shoot after season one, and percentage bud break of primary shoot after season two, for 'Hayward' scions grafted onto inter-specific hybrid rootstocks.

[z]Rootstock selection	Mean trunk CSA of primary shoots (mm^2)	Mean internode length of primary shoots (mm)	Mean proportion of spring bud break (%)
No.45	36.4 (± 1.7)	37.4 (± 7.1)	44.6 (± 2.3)
No.8	29.6 (± 4.3)	30.8 (± 11.0)	29.0 (± 3.5)
No.21	39.4 (± 2.8)	60.9 (± 11.6)	40.2 (± 4.9)
No.19	45.9 (± 1.8)	77.6 (± 6.6)	47.0 (± 5.4)
No.86	46.9 (± 4.7)	74.2 (± 9.0)	31.7 (± 2.7)
No.55	40.4 (± 4.3)	43.6 (± 7.4)	46.6 (± 4.5)
No.100	38.2 (± 4.4)	50.5 (± 11.0)	42.1 (± 4.5)
No.71	49.8 (± 3.7)	59.7 (± 7.6)	44.8 (± 4.2)
No.101	41.7 (± 3.2)	60.9 (± 8.5)	41.5 (± 6.5)
No.85	46.5 (± 4.8)	75.9 (± 11.3)	48.6 (± 4.5)
No.87	46.4 (± 7.2)	53.3 (± 8.0)	52.5 (± 7.7)
No.84	45.6 (± 2.3)	67.9 (± 4.8)	52.5 (± 4.5)
No.18	49.7 (± 7.7)	44.9 (± 8.2)	51.4 (± 2.8)
$LSD_{0.05}$	1.08	27.3	12.9
P-value	*P*=0.03	*P*=0.003	*P*=0.0007

Numbers in parentheses are standard error of means (±).
[z]Rootstocks are ranked from smallest to largest based on the initial trunk CSA at 8 months.

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2.3.4 Relationship between trunk cross-sectional area of rootstock, bud wood and primary shoot (scion)

The trunk CSA of bud wood was strongly correlated (R^2=0.74, *P*<0.0001) with the stem trunk CSA of rootstocks at 8 months following grafting (Figure 2.3A). A similar relationship was also found 14 months following grafting (Figure 2.3B) with a R^2 of 0.64, (*P*<0.0001). Therefore, there was a general trend that rootstocks with a smaller stem CSA produced a smaller trunk CSA of the bud wood in both growing season, because initially the smallest rootstocks would have been grafted with thinner scion woods (see Figure 2.1). In contrast, the trunk CSA of primary shoots was not well correlated (R^2=0.33) with the trunk CSA of the rootstocks, with only a weak positive trend that trunk CSA of rootstocks may have an influence on trunk CSA of primary shoot of scions (Figure 2.3C).

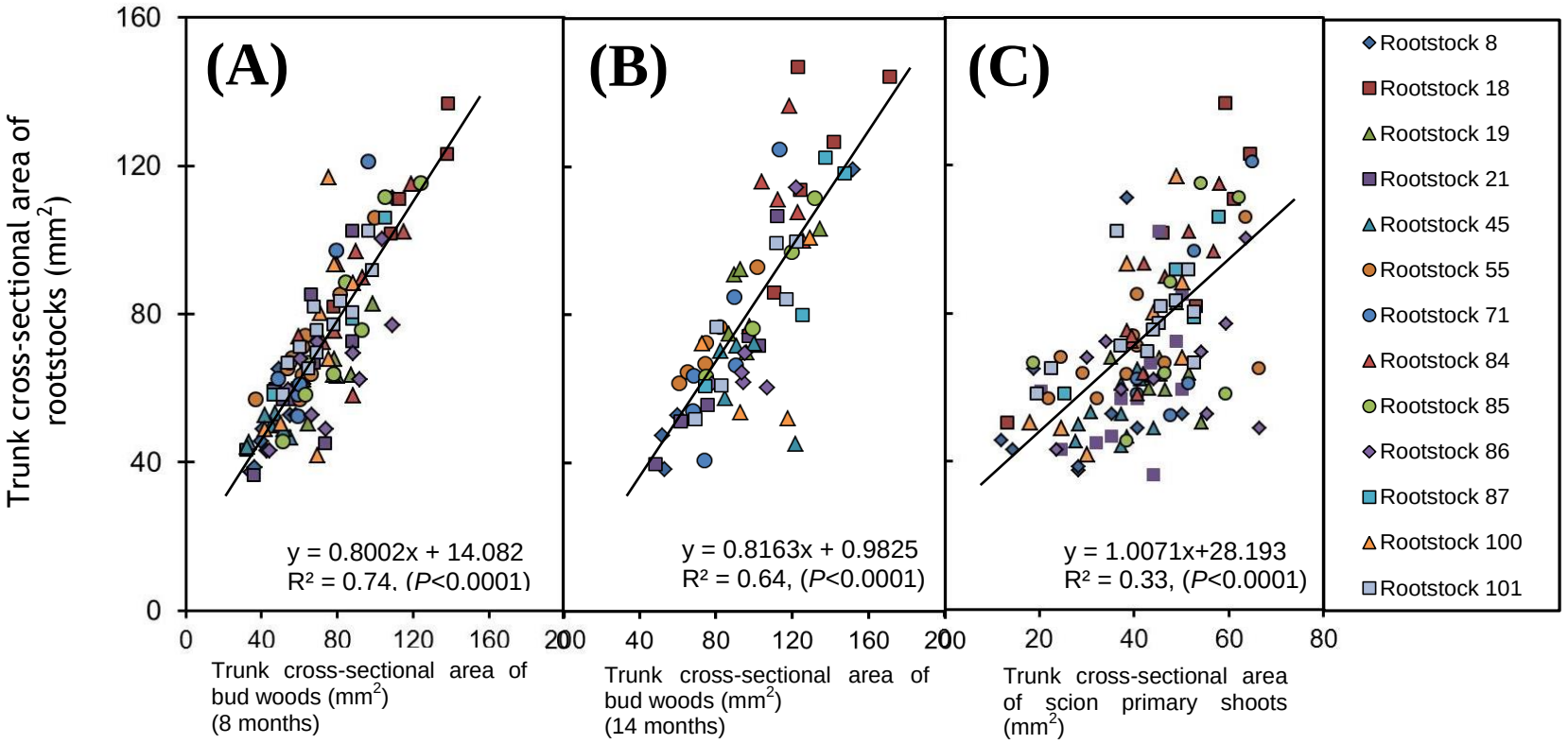


Figure 2.3. The relationship between trunk CSA of rootstocks (mm^2) and trunk CSA of the bud wood (mm^2) at; (A) 8 months, (B) 14 months, and (C) trunk CSA of scion primary shoots (mm^2) at 14 months. Noted here, the relationship between trunk CSA of rootstocks and bud wood, including trunk CSA of scion primary shoots was getting weaker from 8 months to 14 months.

2.3.5 Effect of rootstocks on total number of proleptic axillary shoots

There was a strong trend (*P*=0.06) that inter-specific hybrid kiwifruit rootstocks influenced the total number of proleptic axillary shoots that developed on the scion (Table 2.3). The lowest mean total number of proleptic axillary shoots were recorded for rootstock No.55 and No.85. In contrast, rootstocks such as No.18, No.45, No.84 and No.100 had six or more proleptic axillary shoots. After normalised the number of rootstocks (*n*=4), the total length of proleptic axillary shoots of scions also varied between inter-specific hybrid kiwifruit rootstocks with rootstock No.8 producing the lowest total length compared with other rootstocks (Figure 2.4). The highest total length of proleptic axillary shoots was recorded for rootstocks No.45 and No.55 with nearly 5000 mm (Figure 2.4).

Table 2.3. The mean total number of proleptic axillary shoots on primary shoot of 'Hayward' scions grafted onto inter-specific hybrid rootstocks during spring 2011-2012 growing season.

Rootstock selection*	**Mean total number of proleptic axillary shoots**	
No.45	2.4‡	**6.0 (± 0.4)**x
No.8	2.2	**5.0 (± 1.4)**
No.21	2.3	**5.5 (± 1.0)**
No.19	2.4	**5.8 (± 0.7)**
No.86	2.3	**5.2 (± 0.5)**
No.55	1.7	**3.0 (± 0.6)**
No.100	2.4	**6.0 (± 0.7)**
No.71	2.3	**5.3 (± 0.9)**
No.101	2.2	**5.0 (± 0.7)**
No.85	1.9	**4.0 (± 1.1)**
No.87	2.3	**5.5 (± 1.2)**
No.84	2.6	**6.8 (± 0.7)**
No.18	2.5	**6.2 (± 0.4)**
$LSD_{0.05}$		0.51
P-value		*P*=0.06

‡Data were transformed using square root transformation for ANOVA.
xNumbers in bold are means of raw data (± standard error of means).
*Rootstocks are ranked from smallest to largest based on the initial trunk CSA at 8 months.

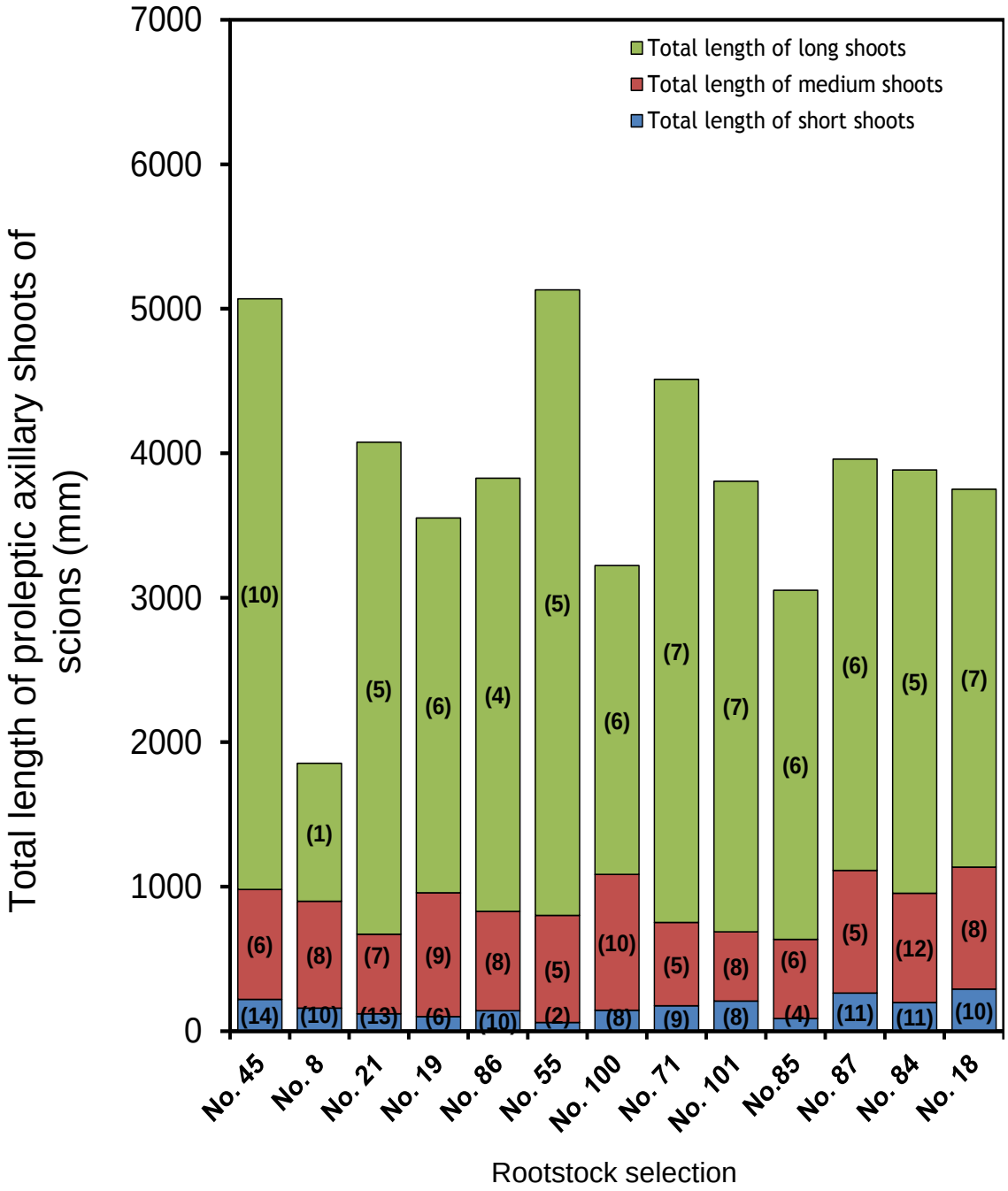


Figure 2.4. Total shoot length of proleptic axillary shoot (short, medium and long) of 'Hayward' scions. Rootstocks are ranked from smallest to largest based on the initial trunk CSA at 8 months (see Table 2.2). Numbers in parentheses within bars are the total number of proleptic axillary shoots for each shoot type (short, medium and long). Number of rootstocks was normalised to four for each rootstock (*n*=4). Refer Table 2.3 and Table 2.6 for the statistical analysis.

2.3.6 Effect of rootstocks on the proportion of different shoot types of scion

Inter-specific hybrid rootstocks did not significantly affect the mean proportion of short and medium shoots (P=0.28 and P=0.40, respectively). However, the mean proportion of long shoots was affected (P=0.04) by the inter-specific hybrid rootstocks (Table 2.4 and Figure 2.4). Rootstock No.55 tended to have a higher proportion of long shoots (Table 2.4) and a substantially lower proportion of medium and short shoots compared to other rootstocks. In contrast, a lower proportion of long shoots was recorded for rootstock No.8, and this rootstock had a higher proportion of medium and short shoots (Table 2.4).

Table 2.4. The mean proportion of different shoot types (long, medium and short) of 'Hayward' scions grafted onto inter-specific hybrid rootstocks during summer 2011-2012 growing season.

Rootstock selection*	Mean proportion of different shoot types					
	Long		Medium		Short	
No.45	0.57ǂ	**0.3 (± 0.05)**ˣ	0.44ǂ	**0.2 (± 0.03)**ˣ	0.68ǂ	**0.5 (± 0.05)**ˣ
No.8	0.14	**0.1 (± 0.08)**	0.61	**0.4 (± 0.06)**	0.72	**0.5 (± 0.09)**
No.21	0.52	**0.3 (± 0.07)**	0.41	**0.3 (± 0.10)**	0.54	**0.4 (± 0.15)**
No.19	0.43	**0.3 (± 0.09)**	0.65	**0.4 (± 0.04)**	0.44	**0.3 (± 0.14)**
No.86	0.46	**0.3 (± 0.07)**	0.64	**0.4 (± 0.06)**	0.44	**0.3 (± 0.16)**
No.55	0.74	**0.6 (± 0.11)**	0.45	**0.3 (± 0.10)**	0.19	**0.1 (± 0.07)**
No.100	0.45	**0.3 (± 0.13)**	0.63	**0.4 (± 0.12)**	0.47	**0.3 (± 0.12)**
No.71	0.57	**0.4 (± 0.10)**	0.39	**0.2 (± 0.09)**	0.57	**0.4 (± 0.06)**
No.101	0.59	**0.4 (± 0.06)**	0.57	**0.4 (± 0.11)**	0.34	**0.2 (± 0.12)**
No.85	0.46	**0.3 (± 0.12)**	0.38	**0.3 (± 0.17)**	0.53	**0.4 (± 0.21)**
No.87	0.55	**0.3 (± 0.11)**	0.37	**0.2 (± 0.08)**	0.69	**0.5 (± 0.05)**
No.84	0.37	**0.2 (± 0.04)**	0.64	**0.4 (± 0.05)**	0.64	**0.4 (± 0.03)**
No.18	0.57	**0.3 (± 0.06)**	0.60	**0.4 (± 0.04)**	0.49	**0.3 (± 0.09)**
$LSD_{0.05}$	0.26		0.32		0.39	
P-value	P=0.04		P=0.40		P=0.28	

ǂData were transformed using square root transformation for ANOVA.
ˣNumbers in bold are means of raw data (± standard error of means).
*Rootstocks are ranked from smallest to largest based on the initial trunk CSA at 8 months.

The data on the proportion of different type of proleptic axillary shoots produced from scion primary shoots were also calculated in terms of shoot termination (i.e. terminated or non-terminated shoots) (Table 2.5). The short and medium kiwifruit shoots were classified as terminated shoots, whereas long shoots as non-terminated shoots (Seleznyova et al., 2002). Rootstocks significantly affected the mean proportion of terminated shoots (P=0.04), as well as non-terminated shoots (P=0.04). In particular, there was higher proportion of non-terminated shoots and a low proportion of terminated shoots recorded for rootstock No.55 (Table 2.5). Conversely, rootstock No. 8 produced a lower proportion of non-terminated shoots and a higher proportion of terminated shoots than other rootstocks (Table 2.5).

Table 2.5. The mean proportion of terminated (short and medium shoots) and non-terminated (long shoots) shoot of 'Hayward' scions grafted onto inter-specific hybrid rootstocks during summer 2011-2012 growing season.

Rootstock selection*	**Terminated shoots**		**Non-terminated shoots**	
No.45	0.81ǂ	**0.7 (± 0.05)**ˣ	0.57ǂ	**0.3 (± 0.05)**ˣ
No.8	0.95	**0.9 (± 0.08)**	0.14	**0.1 (± 0.08)**
No.21	0.84	**0.7 (± 0.07)**	0.52	**0.3 (± 0.07)**
No.19	0.86	**0.8 (± 0.09)**	0.43	**0.2 (± 0.09)**
No.86	0.85	**0.7 (± 0.07)**	0.46	**0.3 (± 0.07)**
No.55	0.58	**0.4 (± 0.11)**	0.74	**0.6 (± 0.11)**
No.100	0.84	**0.7 (± 0.13)**	0.45	**0.3 (± 0.13)**
No.71	0.79	**0.6 (± 0.10)**	0.57	**0.4 (± 0.10)**
No.101	0.80	**0.6 (± 0.06)**	0.59	**0.4 (± 0.06)**
No.85	0.83	**0.7 (± 0.12)**	0.46	**0.3 (± 0.12)**
No.87	0.81	**0.7 (± 0.11)**	0.55	**0.3 (± 0.11)**
No.84	0.91	**0.8 (± 0.11)**	0.37	**0.2 (± 0.11)**
No.18	0.82	**0.7 (± 0.06)**	0.57	**0.3 (± 0.06)**
$LSD_{0.05}$	0.18		0.28	
P-value	P=0.04		P=0.04	

ǂData were transformed using square root transformation for ANOVA.
ˣNumbers in bold are means of raw data (± standard error of means).
*Rootstocks are ranked from smallest to largest based on the initial trunk CSA at 8 months.

2.3.7 Effect of rootstocks on the final length and total length of proleptic axillary shoots

Generally, the characteristics of different shoot types (short, medium and long) in terms of length were not significantly affected by the rootstocks (Table 2.6). However, there were trends that some rootstocks may influence the length of short and long shoots, as *P*-values were almost significant (*P*=0.10 and *P*=0.07, respectively). For short shoots, the longest length was recorded for the scions grafted onto rootstocks No.18, No.101 and No.87 with more than 60 mm. In contrast, the length of short shoots from the scion grafted onto rootstocks No. 21 and No.8 had the shortest with 39.2 mm and 39.8 mm, respectively (Table 2.6). This was almost 46% shorter than the length of the short shoot produced by rootstock No.18.

Inter-specific hybrid rootstocks had no significant effect (*P*=0.77) on the length of medium shoots (Table 2.6). Even so, the mean lengths of medium shoots from rootstocks No.8, No.21, No.45, No.71, No.85, No.86 and No.101 were found to be less than 200 mm, whilst other rootstocks such as No.18, No.84, No.19, No.87, No.55 and No.100 mostly had the length of medium shoots more of than 200 mm. For long shoots, there was a strong trend (*P*=0.07) that rootstocks may affect the length of long shoots. Out of four replicates, the scion grafted onto rootstock No.8 only produced one long shoot (Table 2.6 and Figure 2.4). The length of long shoots was shown to differ between rootstocks with a range from 600 mm to 1020 mm (Table 2.6). The shortest length of long shoots was recorded on the scion grafted onto rootstock No.18 and No.19 with 637.3 mm and 648.8 mm, respectively. Scions on rootstock No.55 had the longest length of long shoots (1010.8 mm), almost 40% longer than those on rootstock No.18 (637.3 mm).

In addition, regardless of shoot types (i.e. short, medium and long shoots), there was a substantial difference (*P*=0.0003, data not shown) in terms of the average length of proleptic axillary shoots of scions produced from the inter-specific hybrid kiwifruit rootstocks. Rootstock No.55 produced 50% greater in average length (477.5 mm) of proleptic axillary compared with other rootstocks (data not shown) because this rootstock had the greatest proportion of long non-terminated shoots (Table 2.4 and

Table 2.5). Inter-specific hybrid rootstocks also significantly differed (P=0.04) in the mean total length of proleptic axillary shoots (Table 2.6). Rootstocks No.45, No.55 and No.71 produced greater mean total length of proleptic axillary shoots with more than 1000 mm, whilst rootstock No.8 had the lowest mean total length of proleptic axillary shoots with 463.6 mm, approximately 45 to 60% lower than rootstocks No.45, No.55 and No.71 (Table 2.6).

Table 2.6. The mean length of short, medium and long of proleptic axillary shoots of 'Hayward' scions grafted onto inter-specific hybrid rootstocks.

Rootstock selection*	Mean length of proleptic shoots (mm)						Mean total length of proleptic axillary shoots (mm)‡	
	Short		Medium		Long			
No.45	43.9	(± 8.4)[x]	152.0	(± 13.8)	818.0	(± 86.8)	1013.9	(± 92.4)[x]
No.8	39.8	(± 11.7)	184.7	(± 13.2)	956.0	[y]	463.6	(± 221.4)
No.21	39.2	(± 8.5)	174.1	(± 15.4)	767.5	(± 70.1)	909.7	(± 78.2)
No.19	50.3	(± 8.7)	208.2	(± 11.7)	648.8	(± 52.5)	757.4	(± 131.6)
No.86	48.7	(± 6.1)	196.5	(± 19.9)	721.0	(± 126.6)	829.7	(± 153.6)
No.55	42.5	(± 17.5)	217.9	(± 36.2)	1010.8	(± 89.1)	1245.3	(± 96.9)
No.100	48.2	(± 8.9)	235.1	(± 36.7)	712.7	(± 43.7)	805.8	(± 187.5)
No.71	54.2	(± 6.4)	199.8	(± 17.1)	891.7	(± 55.1)	1069.9	(± 62.3)
No.101	69.4	(± 4.7)	157.2	(± 16.8)	807.9	(± 42.4)	973.6	(± 34.4)
No.85	43.8	(± 1.3)	182.4	(± 42.8)	805.6	(± 134.6)	762.8	(± 215.0)
No.87	65.7	(± 2.1)	212.5	(± 81.7)	711.9	(± 74.1)	990.0	(± 99.6)
No.84	52.2	(± 8.1)	206.8	(± 24.6)	704.0	(± 59.7)	837.0	(± 121.8)
No.18	72.9	(± 8.4)	204.0	(± 15.4)	637.3	(± 99.7)	899.7	(± 100.6)
$LSD_{0.05}$	25.2		70.8		270.9		355.3	
P-value	*P*=0.10		*P*=0.77		*P*=0.07		*P*=0.04	

[y]Raw data, only one shoot available.

[x]Numbers in parentheses are standard error of means (±).

*Rootstocks are ranked from smallest to largest based on the initial trunk CSA at 8 months.

‡The average sum of all shoot length present per scion.

2.3.8 Effect of rootstocks on the stem CSA of proleptic axillary shoots

The CSA of short, medium and long shoots (Table 2.7) were not significantly affected by the inter-specific hybrid rootstocks (*P*=0.25, *P*=0.84 and *P*=0.42, respectively). Nevertheless, for short shoots, rootstocks No.8, No.85, No.86 and No.87 had the largest shoot CSA with more than 20 mm^2, whilst other rootstocks mostly produced less than 20 mm^2. The CSA of medium shoots was found to be less than 30 mm^2, except for rootstock No.100 (Table 2.7). For long shoots, the shoot CSA from rootstocks No.18, No.21, No.71 and No.84 tended to have smaller shoot CSA than other rootstocks. The largest and smallest CSA of long shoots were recorded for rootstocks No.45 and No.100 being 45.8 mm^2 and 26.1 mm^2, respectively.

Table 2.7. The mean CSA of short, medium and long shoots of proleptic axillary shoots of 'Hayward' scions grafted onto inter-specific hybrid rootstocks.

Rootstock selection*	Mean CSA of proleptic axillary shoots (mm^2)		
	Short	Medium	Long
No.45	17.0 (± 2.8)	24.3 (± 1.0)	45.8 (± 11.2)
No.8	20.7 (± 3.1)	27.3 (± 0.5)	26.2 [y]
No.21	15.7 (± 2.3)	23.5 (± 1.1)	35.8 (± 2.9)
No.19	19.1 (± 2.3)	27.0 (± 2.0)	43.6 (± 4.9)
No.86	23.6 (± 2.4)	27.6 (± 2.5)	41.9 (± 2.7)
No.55	15.4 (± 3.0)	27.8 (± 1.8)	39.9 (± 3.7)
No.100	16.4 (± 2.0)	34.6 (± 11.0)	26.1 (± 6.7)
No.71	15.3 (± 1.3)	24.6 (± 0.5)	38.6 (± 2.8)
No.101	17.8 (± 1.4)	24.2 (± 1.9)	41.3 (± 2.6)
No.85	23.5 (± 1.0)	28.3 (± 1.5)	39.7 (± 2.1)
No.87	20.3 (± 1.5)	23.7 (± 8.2)	41.4 (± 6.2)
No.84	17.9 (± 2.1)	25.8 (± 0.7)	37.5 (± 3.5)
No.18	19.2 (± 1.9)	28.4 (± 1.9)	37.5 (± 1.8)
$LSD_{0.05}$	7.2	10.7	15.8
P-value	*P*=0.27	*P*=0.83	*P*=0.57

[y] Raw data and only one shoot available.

* Rootstocks are ranked from smallest to largest based on the initial trunk CSA at 8 months.

2.3.9 Relationships between trunk cross-sectional area of rootstock and branching of scions

The trunk cross-sectional area (CSA) of inter-specific hybrid kiwifruit rootstocks and scion primary shoots were compared with the branching and total length of proleptic axillary shoots. There was no correlation (R^2=0.20, *P*=0.16) between the trunk CSA of rootstocks and the total number of proleptic axillary shoots (Figure 2.5A). Similarly, the trunk CSA of rootstocks were not correlated at all (R^2=0.0004, *P*=0.87) with the total length of proleptic axillary shoots of scions (Figure 2.5B). In addition, the trunk CSA of scions primary shoots was also not correlated with the total number (R^2=0.05, *P*=0.06) or total length (R^2=0.006, *P*=0.52) of proleptic axillary shoots (Figure 2.6A and Figure 2.6B). Overall, no significant relationships were found between the trunk CSA of rootstocks and scion primary shoots with the number of shoots (i.e. branching), and the total length of proleptic axillary shoots (Figure 2.5 and Figure 2.6).

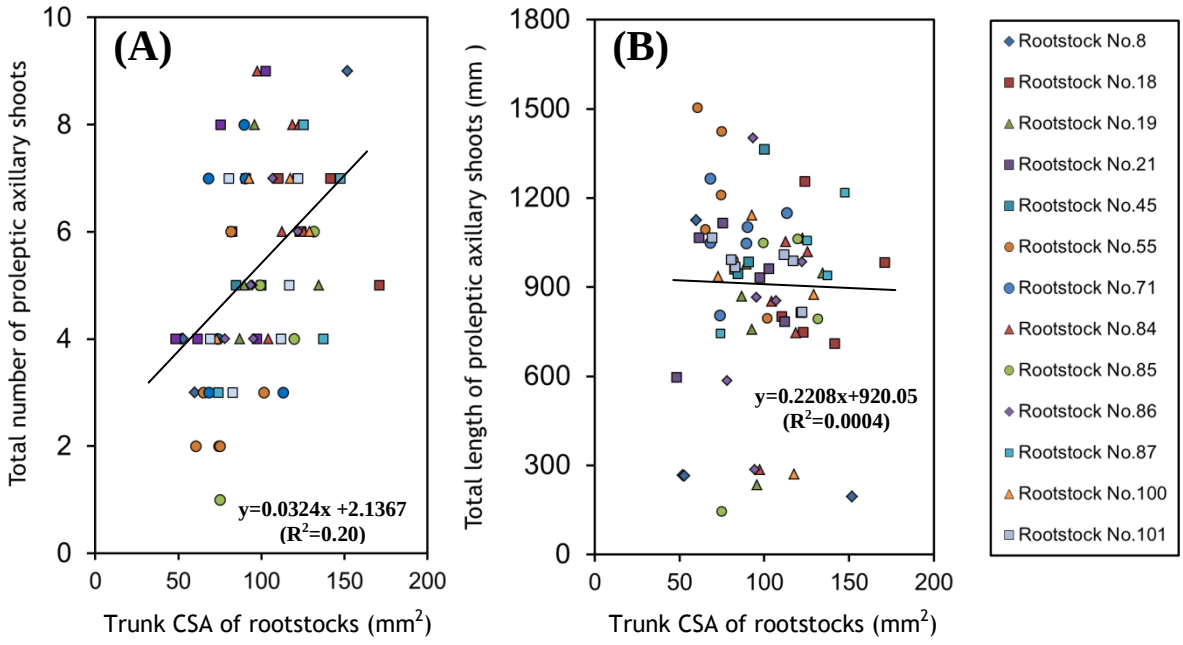


Figure 2.5. The relationship between trunk CSA of rootstocks (mm^2) and; (A) total number, and (B) total length of proleptic axillary shoots of scions (mm).

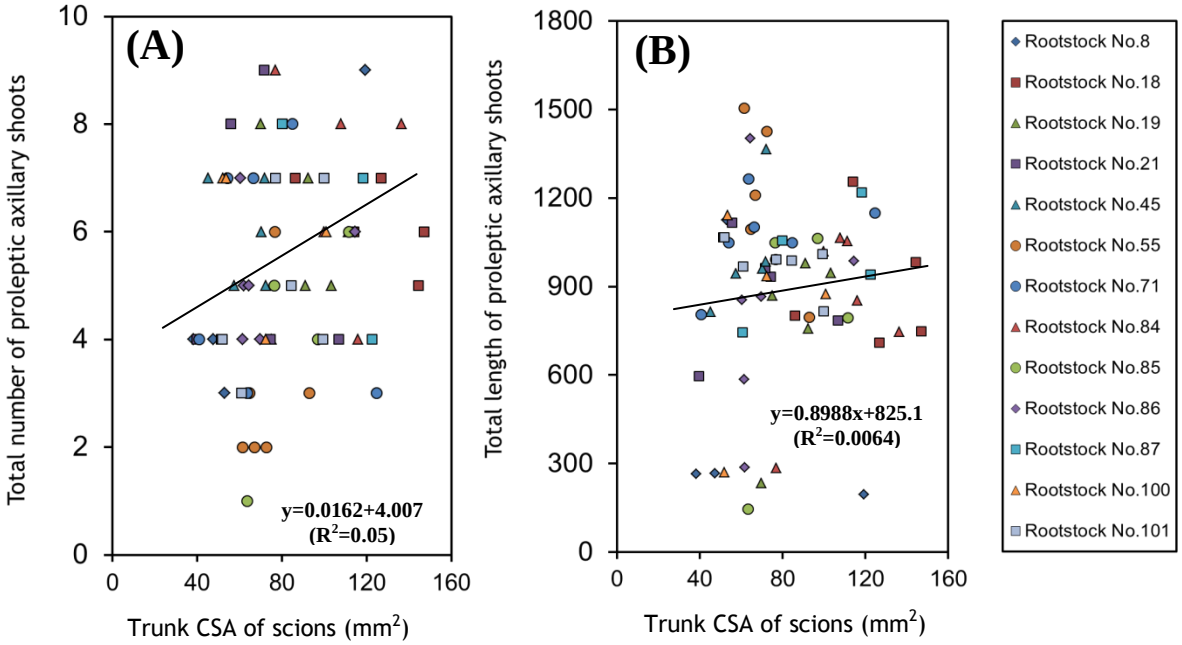


Figure 2.6. The relationship between trunk CSA of scions (mm^2) and; (A) total number, and (B) total length of proleptic axillary shoots of scions.

2.3.10 The summary of vigour rating of rootstocks by the end of second season following grafting

Based on the final results on the main trunk CSA of primary shoots (Table 2.2), mean proportion of different shoot types (Table 2.4 and Table 2.5) and mean total shoot length of proleptic axillary shoots (Table 2.6 and Figure 2.4), the new vigour raking of inter-specific hybrid kiwifruit rootstocks by the end of second growing season are proposed as shown in Table 2.8. Rootstocks No.8, No.19, No.100 and 85 were grouped as low-vigour rootstocks. Meanwhile, No.86, No.101, No.87, No.84 and No.21 could be grouped as intermediate vigour, and No.45, No.55 and No.71 can be grouped as high-vigour rootstocks.

Table 2.8. Vigour rating of inter-specific hybrid kiwifruit rootstocks with the original parentage by the end of the second growing season.

Rootstock Selections*	Parentage of rootstocks	Vigour group*	Mean trunk CSA of primary shoots (mm^2)	Mean total shoot length of proleptic shoots (mm)
No.8	*A. chinensis* x *A. macrosperma*		29.6	463.6
No.19	*A. chinensis* x *A. macrosperma*	Low-vigour	45.9	757.4
No.100	*A. macrosperma* x *A. melanandra*		38.2	805.8
No.85	*A. macrosperma*		46.5	762.8
No.86	*A. macrosperma*		46.9	829.7
No.101	*A. macrosperma* x *A. melanandra*		41.7	973.6
No.87	*A. polygama*	Intermediate vigour	46.4	990.0
No.84	*A. polygama*		45.6	837.0
No.18	*A. chinensis* x *A. macrosperma*		49.7	899.7
No.21	*A. chinensis* x *A. macrosperma*		39.4	909.7
No.45	*A. polygama* x *A. chinensis*		36.4	1013.9
No.55	*A. polygama* x *A. chinensis*	High-vigour	40.4	1245.3
No.71	*A. polygama* x *A. chinensis*		49.8	1069.9

*Rootstocks are grouped according to vigour, based on the final results on the main trunk CSA of primary shoots (Table 2.2), mean proportion of different shoot types (Table 2.4 and Table 2.5) and mean total shoot length of proleptic axillary shoots (Table 2.6 and Figure 2.4).

Refer Table 2.1 for the initial vigour rating of inter-specific hybrid kiwifruit rootstocks for comparison.

2.4 Discussion

Evidence of the earliest expression of scion dwarfing by rootstocks in other fruit crops such as apple (Costes et al., 2001; Seleznyova et al., 2007; van Hooijdonk et al., 2010), pear (Watson et al., 2012) and grape (Cookson et al., 2012; 2013; Tandonnet et al., 2010) are well documented by many authors. However, in kiwifruit, no one has yet studied on how rootstocks may modify scion growth and architecture at the early stage of vine growth. Therefore, the main objectives of this chapter were; (i) to quantify the architectural modification of scions by inter-specific hybrid kiwifruit rootstocks, and (ii) whether the dwarfing expression imposed by kiwifruit rootstocks could be measured in the early stage of vine growth and development (in the first or second year of growth). A further objective of this chapter was: (iii) to evaluate the modifications imposed by the kiwifruit rootstocks on the overall architecture of the scions, and whether these modifications were similar or different from that found in other fruit trees such as apple, pear and grape. We hypothesise that any modifications by kiwifruit rootstocks during the early scion development may have a critical impact on the future of vine structure, as found in other fruit crops (van Hooijdonk et al., 2010).

A key finding of this chapter was that the inter-specific hybrid kiwifruit rootstocks appeared to modify the initial vigour of 'Hayward' scions at the early stage of vine growth (in both years 2011 and 2012 growing season). This was clearly demonstrated by the significant differences in the trunk CSA of scion primary shoots in the first growing season (Table 2.1), the scion bud break and the proportion of proleptic shoots in second growing season (Table 2.2 and Table 2.4), which may have modified the total shoot length of scions (Table 2.6 and Figure 2.4). However, the vigour ranking of scions based on trunk CSA of inter-specific hybrid kiwifruit rootstocks in the first growing season did not appear to continue into the second year of vine growth. Our results after two seasons of growth (Table 2.8) indicate that the rootstocks No.8, No.19, No.100 and No.85 could be grouped as low-vigour, No.86, No.101, No.87, No.84, No.18 and No.21 could be grouped as an intermediate vigour, whereas No.45, No.55 and No.71 could be grouped as high-vigour. This vigour ranking based on the overall growth (i.e. total shoot length) of grafted scions (Table 2.6 and Figure 2.4) thus depends on the number of different types of shoots (either long, medium and short) (Table 2.3, Table 2.4 and

Table 2.5) (Clearwater et al., 2006; Seleznyova et al., 2002). Even though in this study, we found that the total length of proleptic shoots was the factor contributing most to the vigour of scions, it was not sufficient to represent the whole vigour of the vines. For the future study, we suggest the measurement of whole plant dry mass would be more meaningful in interpreting the overall vigour of grafted vines. In this study, the destructive sampling could not be conducted because the vines were needed for the field evaluation (Chapter Three). The changes in vigour rating of rootstocks were clearly observed between the first season (Table 2.1) and the second seasons of growth (Table 2.8), even though there was no correlation with parentage. Nevertheless, particular parentage of inter-specific hybrid rootstocks tended to give high-vigour for example the hybrid that came from crosses between *A. polygama* and *A. chinensis* (Table 2.8). Meanwhile, hybrid from crosses between *A. chinensis* and *A. macrosperma* tended to produce the rootstocks that had low- and intermediate- vigour ability (respectively) of the grafted scions (Table 2.8).

2.4.1 Rootstock influence on growth of grafted scions

Generally, rootstocks with a small trunk CSA also had small bud wood trunk CSA in the both growing seasons. It was also observed, rootstocks that limited the growth of the scion bud wood also having smaller primary shoot CSA (Table 2.2). A 43% difference in trunk CSA of bud wood was found between the extremes in rootstocks. The trunk CSA of the scion primary shoots that grew from the grafted bud wood also varied between the inter-specific hybrid rootstocks, indicating the trunk CSA of scion primary shoots could be influenced by rootstocks genotypes (Table 2.1 and Table 2.2). In mature composite kiwifruit vines, Friend et al. (2014) reported that inter-specific rootstocks are also capable of affecting trunk CSA of *A. chinensis* 'Hort16A' scions. While the diameter of the rootstock shank is not a good predictor of plant size and vigour (Fazio & Robinson, 2008), trunk CSA is correlated with tree volume (Khatamian & Hilton, 1977; Webster, 1995a) and is often used as a proxy measure for the total growth of a plant over its life. In fruit trees, initial stem calliper in nursery stage also has been thought to be correlated with the tree growth in the orchard (Reighard, 1990); for example in peach (*Prunus persica* L.), there is a correlation between stem calliper of tree in the

greenhouse and planted in orchard (Reighard, 1990). In contrast, Iwasaki et al. (2011) reported that trunk calliper of 5-month-old seedling rootstocks of Satsuma mandarin were not correlated with the calliper of 7-years-old grafted trees. It is unlikely that these discrepancies are probably due to different grafting techniques, timing of measurement or the age of planting materials. However, the interpretation of the effect of rootstocks on trunk CSA in young plant could be misleading if the plant has been headed such as in case of our study (Section 2.2.3 and Figure 2.2), due to heading of shoots may stimulate much secondary thickening of trunks. Our results showed that rootstock No.45 that produced the smallest trunk CSA in the first year of growth (Table 2.1), but appeared to produce among the highest in total shoot length in the second growing season (Figure 2.4), suggesting that trunk CSA of rootstocks during early stage of vine growth did not provide a good indication of scion vigour. Even though our preliminary data have shown that there was a correlation between the calliper of kiwifruit rootstocks and scions in the first and second year following grafting (Figure 2.3), but this correlation became weaker and weaker over time and other parameters may provide a clearer early indication of vigour. Therefore, it is suggested that the use of whole tree dry mass is the most meaningful measure of plant or vine vigour especially when related to dwarfing characteristic of plants.

In the present study, the actual data on the initial length and node number of scion primary shoots are not reported since the primary shoots were headed back to similar length (Figure 2.2). Nevertheless, the node numbers and length that present on scion primary shoots were recorded in order to obtain internode length. Generally, in ungrafted *Actinida* species, a strong relationship between mean internode length and final node number exists (Seleznyova et al., 2002), as well as the number of leaves (Foster et al., 2007); this is associated with the rate of shoot growth and growth cessation. Even though our result indicated that the mean internode length of the primary shoots of 'Hayward' scions was significantly different between inter-specific hybrid rootstocks (Table 2.2), it should be noted that any alteration of internode length was not directly affected by rootstock (review by Webster, 2004), as found in other fruit trees such as pear (Seleznyova et al., 2013; Watson et al., 2012) and apple (Seleznyova et al., 2003; van Hooijdonk et al., 2011). Studies for the above fruit trees have demonstrated that the length of a shoot increased with its node number, which means the influence of rootstock on node neoformation indirectly affected any treatment mean

internode length. When mean internode length of a shoot was plotted as a function of the shoot node number, it was clear that internode length depended on the shoot node number where shoots with fewer nodes had shorter internodes. Therefore, no direct effect of the rootstock was found in the process of extension of individual internodes. We believe that similar result may be demonstrated for kiwifruit. Considerable effort has been made to plot the relationship between the length and node number of scion primary shoots but unfortunately, the actual relationship could not be found probably due to incomplete data on scion primary shoots in our study (Appendix 1).

2.4.2 Rootstock effects on spring bud break of scions

In early spring of the second year of growth, the proportion of bud break (%) on the primary shoots of 'Hayward' scion significantly differed between inter-specific hybrid rootstocks (Table 2.2), but was not correlated with the trunk CSA of rootstocks (Table 2.1). Therefore, our result also indicates that the genotype of inter-specific hybrid rootstocks may have an influence on release of axillary buds from dormancy (bud break). This finding is consistent with results for other kiwifruit rootstock trials. For example, comparing bud break of 'Hayward' on rootstocks of different species, Wang et al., (1994) found reduced bud break when grafted to *A. chinensis* and increased bud break when grafted to *A. hemsleyana*. Recent finding also reported that the bud break of 'Hort16A' scion differed when grafted onto 'Bounty71', Kaimai and 'Hayward' rootstocks (Anon., 2012). In contrast with other fruit trees, rootstock did not affect the proportion of axillary bud break in apple (Costes et al., 2001; Seleznyova et al., 2003) and pear (Watson et al., 2012), regardless of rootstock vigour. Kiwifruit rootstocks are thought to have an influence on scion bud break by altering the vine carbohydrate level (Wang et al., 1994b). Besides that, bud break along the primary shoots of scions may be controlled by, or associated with the growth rate of the main axis (Costes & Guédon, 2002). Vigorous canes may have a propensity to produce higher bud break (Volz et al., 1994) and vigorous rootstocks tend to produced more vigorous canes than less vigorous rootstocks (Clearwater et al., 2006). In kiwifruit, bud break has significant influence on the orchard productivity (McPherson et al., 1994). Therefore, the development of rootstocks that can confer synchronise bud break would be advantageous in kiwifruit

production and could be used as an alternative for chemical application (i.e. hydrogen cyanamide). Even though finding in this study has shown that inter-specific hybrid rootstocks can produce higher scion bud break (more than 40%), further trials under field condition are still needed to evaluate the consistency of rootstock effect on bud break (Chapter Three), because particular rootstocks such as No.8 and No.86 from low and intermediate vigour tended to produce lower scion bud break (Table 2.2). Generally, we are aiming for the particular rootstocks that can produce high and compact bud break, in order to have many of fruiting shoots (i.e. medium- and short-terminated shoots) and fewer high-vigour shoots (i.e. long non-terminated shoots), which gives high yields of large fruit (considering with sufficient Leaf Area Index).

2.4.3 Rootstock effects on the characteristics and production of proleptic axillary shoots (branching) of scions

The shoots that formed from the scion primary shoots were termed as 'proleptic axillary shoots' since the shoots came from the buds of previous year growing season and have a winter resting period (dormant) (Foster et al., 2007; Seleznyova et al., 2002). These shoots (proleptic axillary shoots) produced in early spring of the second-year of growth were allowed to grow until the summer season. We have classified all the proleptic shoots that were produced along the scion primary shoots according to the architectural description made by Seleznyova et al. (2002) to identify whether rootstocks may modify the proportion and characteristic of different shoot types. In the present study, the characteristics of shoots (in terms of length) of proleptic long and short shoots were affected by the inter-specific hybrid rootstocks, but not medium shoots (Table 2.6). However, rootstocks did not affect the shoot CSA of any type of proleptic shoots (Table 2.7). Differences in the characteristics of proleptic axillary imparted by the kiwifruit rootstocks also have contributed to the differences in the number of shoots and total shoot length of grafted 'Hayward' scions. For example, rootstocks from low-vigour (e.g. No.8, No.19 and No.100) and intermediate vigour group (e.g. No.18, No.84 and No.87) produced the shortest length of long proleptic shoots of scions (Table 2.6). According to Hirst and Ferree (1995b), shoot morphological characteristics (e.g. length and diameter) may be one way in which rootstock influence productivity by affecting

the individual length of shoots, and this can influence tree branching habits (Webster, 1995a). In kiwifruit, the canopy architecture of vines is the combined result of the shoot characteristics (e.g. length and diameter of shoots) and the size of leaf area, as well as vine management (i.e. training and pruning system) (Clearwater et al., 2006; Thorp et al., 2003). However, kiwifruit vine requires careful canopy management to maintain high yield and producing high fruit quality while controlling canopy vigour in order to minimise competition for resources between rapidly growing shoots and fruit development.

In apple, the length of the 2-year-old branch section of 'Starkspur Supreme Delicious' apple scion was affected by the rootstock, and this correlated with the scion trunk CSA (Hirst & Ferree, 1995b). In addition, Lauri et al., (2006) had shown that M.9 rootstock reduced mean length of second-year axillary annual shoots in apple trees. Later study on the same rootstock also had found that the size of annual shoot in terms of diameter was not significantly affected by the M.9 rootstock (Costes & García-Villanueva, 2007). In the present study, although the rootstock effects on the shoot characteristics was not as pronounced as the effects reported in apple, there was an indication that kiwifruit rootstocks may have some influence on the characteristic of scion proleptic shoots (Table 2.6). The overall architecture of scions in terms of total length of proleptic axillary shoots (Table 2.6 and Figure 2.4) were significantly affected by the inter-specific hybrid rootstocks, due to reduction in the mean length of long and short proleptic axillary shoots (Table 2.6). In addition, there was a substantial difference in the mean average total length of proleptic shoots between rootstocks (Table 2.6). For instance, even though rootstock No.55 (high-vigour) had the lowest mean number of shoots (Table 2.3), most of the shoots produced were non-terminated long shoots (Table 2.5), and this has contributed to the highest mean of total shoot length in this rootstock (Table 2.6). Therefore, a change in the average length of shoots between the different inter-specific hybrid kiwifruit rootstocks may also reflect a change in the proportion of different shoot types of scions (Table 2.4 and Table 2.5).

In the present study, even though the proportion of short and medium proleptic axillary shoots was not affected by the inter-specific hybrid rootstocks, rootstocks significantly affected the proportion of long proleptic axillary shoots (Table 2.4). Therefore, these effects have contributed to the significant differences in the proportion of non-

terminated and terminated shoots of scions (Table 2.5). In our study, particular rootstock such as No.8 and No.84 (from low and intermediate vigour group) produced amongst the highest proportion of terminated shoots (i.e. medium and short shoots), in contrast with rootstock such as No.55 that came from the high vigour group (Table 2.5). These results indicate that the inter-specific hybrid kiwifruit rootstocks are capable to alter the proportion of different shoot types of grafted scions, by affecting the transition from the preformed to the neoformed nodes. According to Seleznyova et al. (2002), short and medium shoots of kiwifruit are self-terminated and had only preformed nodes, whilst long shoots are non-terminated and had a number of neoformed nodes. It was suggested that the fate of kiwifruit shoots whether can be short or medium shoots, stopping growth after budbreak, or long shoots, continuing growth until the end of the season could be regulated by the rootstock during the early stage of shoot development immediately after bud break (Clearwater et al., 2006). Besides that, it is also believe that kiwifruit rootstock may modify the shoots node preformation or neoformation of grafted scion by affecting the competition of shoots for availability of carbohydrates reserves (Piller et al., 1998) and root-derived hormones such as auxin, CK and gibberellins (van Hooijdonk et al., 2010, 2011). These multiple factors that affecting kiwifruit shoot extension and shoot growth cessation warrant further investigation (Chapter 3, Chapter 4 and Chapter 6).

The ability of kiwifruit rootstocks to alter the proportion of different shoot types of scions are well documented in mature composite kiwifruit vines (e.g. Clearwater et al., 2006; Anon., 2012). 'Hort16A' scions when grafted onto low-vigour rootstocks such as *A. kolomikta* and *A. polygama* (Clearwater et al., 2006) and 'Bounty71' (Anon., 2012) may produce shoots that have high proportion of terminated shoots possibly due to less shoots that have neoformed nodes. It was noted in our study that only rootstock No.87 (intermediate vigour) came from *A. polygama* selection that is known as low-vigour as reported by Clearwater et al. (2006) (Table 2.8). In other fruit trees such as apple and pear, an important evidence of scion dwarfing by rootstocks was increasing the proportion of shoots that terminated early in the growing season due to slower shoot growth (van Hooijdonk et al., 2010; Watson et al., 2012). Besides that, studies for the above fruit trees have also demonstrated that rootstocks may also influence the rate and timing of termination of active extension growth and decrease the node neoformation. In kiwifruit, Clearwater et al. (2006) reported that terminated shoots produced from

scions grafted onto low-vigour rootstocks grew more slowly and terminated earlier than terminated shoots of scions grafted onto high-vigour rootstocks, indicating the probability of shoot termination after budbreak is related to its shoot growth rate (Clearwater et al., 2006). However, their study did not produce credible information on shoot growth rate to support their hypothesis. In this thesis, considerable effort has been made to elucidate the effect of kiwifruit rootstock on shoot growth rate of grafted scions (Chapter 3). Based on our preliminary results (Table 2.4, Table 2.5 and Table 2.6), it is postulated that particular inter-specific hybrid kiwifruit rootstocks such as No.8, No.19 and No.85 (*A. chinensis* x *A. macrosperma*- low-vigour) and No.55 and No.71 (*A. polygama* x *A. chinensis*- high-vigour) may have potential to affect the scion shoot termination during early vine growth (Table 2.5), and this effect could be similar to what has been found in apple (van Hooijdonk, 2009).

Generally, branching of trees is believed to be correlated with vigour, larger trees will produce more branching than smaller trees (Fazio & Robinson, 2008). However, in apple, Fazio and Robinson (2008) has demonstrated that vigour of rootstocks (i.e. dwarfing or vigorous) may not correlate with the branching of grafted scions, as they found that dwarfing rootstocks that have larger scion diameter can produce more branching than scion from vigorous rootstocks. Our finding indicates that early vigour of kiwifruit vines based on branching was not uniformly indicated by the trunk CSA. For example, the shoot number of scions was not greatly different (Table 2.3) between rootstock No.45 and No.18 that had the lowest and largest trunk CSA, respectively in the first growing season (Table 2.1). The early vigour of kiwifruit scions and rootstocks (i.e. trunk CSA) was also not correlated with the number, or the total length of proleptic axillary shoots (Figure 2.5 and Figure 2.6). It would be reasonable to suggest that scion branching in kiwifruit is not necessarily correlated with trunk CSA of rootstocks and scions, which supports the finding in apple (Fazio & Robinson, 2008). Furthermore, the inter-specific hybrid rootstocks used in our study came from the hybridazation between *A. chinensis*, *A. macrosperma*, *A. polygama* and *A. melanandra* (Table 2.8), each of which conferred different characteristics. Therefore, in grafted kiwifruit, it seems that other factors such as the hormonal status and the genetic background of rootstock may have more influence on scion branching, rather than stem size of rootstocks (Quinlan & Tobutt, 1990). In other vine species such as grape, there was also evidence that

particular rootstocks may also have an influence on the lateral stem development (i.e. branching) of scions during the first year of growth cycle (Tandonnet et al., 2010).

Grafting two different genotypes together may also regulate the differential genetic expression and modification of genetic transcription between rootstocks and scions. In the present study, 'Hayward' scion was grafted onto different selection of rootstocks that came from the crosses between four kiwifruit species (Section 1.1, Chapter One). In apples, scions grafted on vigorous rootstocks have been associated with an increase in the number of up-regulated gene expression in the scions (Jensen et al., 2010). Recent studies in grape which involved the grafting of scions on different rootstock genotypes, a number of genes were triggered that are related to the up- and down-regulated endogenous hormones (i.e. auxin, CK and gibberellins) (Cookson & Ollat, 2013). However, we know little about genes expression and their implication on endogenous hormones in grafted kiwifruit. Besides auxin, CK and gibberellins, it should be realised that other hormonal signals are involved in controlling branching in plants. For example, a recent study found that a new group of compounds or plant hormone called 'strigolactones' (SLs) are important in branching. SLs are compounds derived from carotenoid-derived terpenoid lactone produced in roots, which may be involved in the inhibition of shoot branching (Gomez-Roldan et al., 2008).

Evidence in the present study may have shown that kiwifruit rootstocks can influence shoot architecture, suggesting that root-derived signals such as SLs, may be important in controlling scion branching. SLs are produced by the action of 'Carotenoid Cleavage Dioxygenase' genes (CCD7 and CCD8), and these genes have been shown to play an important role in controlling branching of model plants such as pea, *Arabidopsis*, *Petunia* sp. and rice (Matusova et al., 2005). In kiwifruit, high expression of CCD genes were found in roots of wild-type kiwifruit (Ledger et al., 2010). However, in transgenic *A. chinensis* kiwifruit plants, Ledger et al. (2010) found that there was a reduction in the expression of CCD genes, and this correlated with an increase in the total number of axillary shoots produced by the plants (Ledger et al., 2010). Interestingly, increases in the number of axillary buds was observed when transgenic kiwifruit plants were grafted onto wild-type kiwifruit (Honda et al., 2011), suggesting that the SLs from the roots might be involved in controlling axillary buds growth. Therefore, this growing evidence opens new possibilities that branching in kiwifruit may not be solely regulated by the interactions of auxin, CK and gibberellins.

It was notable that scions on dwarfing apple rootstocks may grow vigorously similar to the scion grafted onto vigorous rootstocks while in the nursery stage (Faust, 1989). Therefore, this present study was continued in the field trial to identify which rootstock may confer dwarfing or invigorating to the grafted scion (Chapter Three). The present chapter provided new information regarding how the growth and architecture of 'Hayward' scions have been modified by the inter-specific hybrid kiwifruit rootstocks, during the first- and second-year of growth following grafting. The inter-specific hybrid kiwifruit rootstocks were able to alter the trunk CSA of the scion primary shoots in year one (2011) and bud break in year two (2012), but the early vigour of 'Hayward' scion may not be correlated with trunk CSA. Besides that, inter-specific hybrid kiwifruit rootstocks were also capable of modifying the proportion of terminated (short and medium shoots) and non-terminated shoots (long shoots) produced from the grafted scions, thus modifying the overall architecture (in terms of total length of shoots) of scions on grafted vines. Based on the present study, our results indicate (in order of increasing vigour) that rootstock No.8, No.85, No.100 and No.19 could be grouped as low-vigour, No.86, No.101, No.87, No.84 and No.18 could be grouped as an intermediate vigour, whereas No.71, No.45 and No.55 could be grouped as high-vigour. Even though, there was no clear correlation between vigour and parentage of inter-specific hybrid rootstocks, particular parentage tended to give low- or intermediate-vigour, for example the hybrid that came from crosses between *A. chinensis* and *A. macrosperma*. However, the hybrid from *A. polygama* and *A. chinensis* tended to produce rootstocks that had high-vigour ability of the grafted scions (Table 2.8). Overall, we strongly believe that inter-specific hybrid *Actinidia* rootstocks have the potential to modify scion architecture and vigour of the scion during early stage of vine development.

2.5 Summary

In order to improve understanding on how kiwifruit rootstocks may affect scion growth, 'Hayward' scions were grafted onto thirteen inter-specific hybrid kiwifruit rootstocks. The effects of these rootstocks on the initial scion vigour and architecture were assessed during the first (2011) and second-year (2012) following grafting. Early assessment at eight months following grafting, the vigour of grafted scion was based on the trunk CSA of rootstocks. In the following order of increasing vigour, No.45 (lowest), followed by No.8, No.21, No.19, No.86, No.55, No.100, No.71, No.101, No.85, No.87, No.84 and No.18 (highest). In the first year of growth following grafting (2011), the trunk CSA of the rootstocks, scion bud wood and scion primary shoots were significantly different between inter-specific hybrid kiwifruit rootstocks. No correlation was found between trunk CSA or primary shoots and trunk CSA of rootstocks, but trunk CSA of rootstocks were highly correlated with the trunk CSA of bud wood in both growing seasons. The trunk CSA of rootstocks during early stage of growth did not provide a good indication of scion vigour, because particular rootstocks such as No.45 that had the smallest trunk CSA in the first growing season produced among the highest total shoot length of scion in the second growing season. Even though there was a correlation between the trunk CSA of kiwifruit rootstocks and scions in the first and second year following grafting, but this correlation became weaker and weaker over time and other parameters may provide a clearer early indication of vigour. Even though internode length of scion primary shoots was significantly different between inter-specific hybrid kiwifruit rootstocks, it was only indirect effect of kiwifruit rootstock on grafted scions.

In the second-year of growth following grafting (2012), the percentage of spring bud break of the scion primary shoots significantly differed between inter-specific hybrid rootstocks. There was a trend that the inter-specific hybrid rootstocks may modify the length of proleptic axillary long and short shoots. However, the trunk CSA of long, medium and short shoots was not affected. The total length of proleptic axillary shoots of scion significantly differed between the inter-specific hybrid rootstocks due to changes in the length and proportion of short and long proleptic axillary shoots. The early vigour of kiwifruit scions and rootstocks (based on trunk CSA) was not correlated

with the number, or the total length of proleptic axillary shoots, suggesting that scion branching (i.e. proleptic shoots) in kiwifruit is not necessarily correlated with vigour of rootstocks or scions. The vigour ranking of scion based on trunk CSA of inter-specific hybrid kiwifruit rootstocks in the first growing season did not appear to continue into the second years of vine growth. Our preliminary results indicate that the selection No.8, No.19, No.100 and No.85 could be grouped as low-vigour rootstocks, No.86, No.101, No.87, No.84 and No.18 could be grouped as intermediate vigour rootstocks, whereas No.45, No.55 and No.71 could be grouped as high-vigour rootstocks. Particular parentage of rootstocks hybrid tended to give low- or high-vigour ability to the grafted scions, for example crosses between *A. polygama* and *A. chinensis*, and between *A. chinensis* and *A. macrosperma*, respectively. Overall, in this chapter, there were strong evidence that the inter-specific hybrid kiwifruit rootstocks have the potential to modify scion architecture and vigour of the scion during early stage of vine development.

Chapter Three

3. Effect of inter-specific hybrid rootstocks on scion growth and architecture, and the first occurrence of flowering for field grown young 'Hayward' vines

3.1 Introduction

In Chapter Two, it was shown that inter-specific hybrid kiwifruit rootstocks may have affected the early growth and altered the initial shoot architectural structures of the 'Hayward' scions. In general, the scion primary shoot cross-sectional area and the amount of spring budbreak were significantly affected by the rootstocks. There was also a trend for rootstocks to alter the proportion of proleptic axillary shoot types, where scions on particular rootstocks had fewer long proleptic shoots. The lengths and numbers of long and short proleptic shoots of scions from particular rootstocks were also slightly modified, thus reducing the total length of proleptic shoots on grafted scions. Therefore, these results have shown that there was promising evidence that kiwifruit rootstocks have potential to modify scion architecture and vigour of young 'Hayward' scions during initial vine development. In order to further evaluate rootstock effects on scion growth and architecture, including flowering, the 'Hayward' scions that had been previously grafted onto inter-specific hybrid kiwifruit rootstocks (Chapter Two) were planted into the field and their growth was monitored for two growing seasons. In previous studies, clonal kiwifruit rootstocks may have affected the grafted scions by altering bud break pattern and flowering (Anon., 2012; Cruz-Castillo et al., 1991; Lowe, 1989, 1991; Wang et al., 1994b), trunk cross-sectional area (CSA) (Friend et al., 2014) and also shoot architectural structures, as well as leaf size of scions (Clearwater et al., 2006). It was reported that the kiwifruit rootstocks may affect nutrient uptake, which may affect the nutrients status in the leaves and the quality of fruits of the grafted scions (Anon., 2012; Thorp et al., 2007).

One of the clear examples of rootstock effects on scion growth in kiwifruit was from the study by Clearwater et al. (2006). They monitored shoot development and architectural characteristics of 'Hort16A' scions grafted onto eight different clonal *Actinidia* rootstocks of different vigour. They found that the low-vigour rootstocks species such as *A. polygama* and *A. kolomikta* produced a higher proportion of terminated shoots (i.e. short and medium shoots) than non-terminated shoots (i.e. long shoots) on the grafted scions (Clearwater et al., 2006). In addition, higher proportions of slower growing shoots were found when grafted onto these low-vigour rootstocks and the slower growing shoots of scions on low-vigour rootstocks tended to terminate earlier than shoots grafted onto high-vigour rootstocks (Clearwater et al., 2006). Our findings in Chapter Two demonstrated that particular parentages of inter-specific hybrid kiwifruit rootstocks tended to give low- or high-vigour ability to the grafted 'Hayward' scions, especially from the crosses between *A. polygama* and *A. chinensis*, and between *A. chinensis* and *A. macrosperma*, respectively.

Therefore, based on the literature described above and findings in Chapter Two, there is evidence that kiwifruit rootstocks can influence scion growth and architecture similar to the changes described by Clearwater et al. (2006) using non-hybrid *Actinidia* rootstocks. However, since the inter-specific hybrid kiwifruit rootstocks used in this study are relatively new and of different parentage, the information on the scion vigour control and precocity induced by these rootstocks when planted in field condition is unknown. It was hypothesised that the vigour and architecture structure of 'Hayward' scions can be influenced by the hybrid kiwifruit rootstocks when planted in the field and the vigour of grafted scion can be related to the parentage of rootstock (**Hypothesis II**).Therefore, inter-specific hybrid kiwifruit rootstocks were evaluated in a field trial over two consecutive growing seasons. We suggest that the effect of inter-specific hybrid rootstocks on the architecture of 'Hayward' scions will be more expressed when planted in the field. In this chapter, the modifications imposed by the inter-specific hybrid kiwifruit rootstocks on the grafted scions were determined to compare whether the modifications are similar or different from those reported previously for other kiwifruit rootstocks.

3.2 Materials and methods

3.2.1 Experimental site

The experiment was conducted during 2012 – 2014 growing seasons at the Fruit Crops Unit, Massey University, Palmerston North, New Zealand. The soil type was a Manawatu fine sandy loam as described by van Hooijdonk (2009) having an upper layer of 500 mm of fine sandy loam underlain by 400 mm of fine sand and gravelly coarse sand below 900 mm. The experimental site had been previously planted with apple trees. On 21/9/2011, the soil was levelled and ploughed, then harrowed to provide a fine tilth for planting.

3.2.2 Establishment of planting materials

Grafted plants were prepared as previously described in Chapter Two (Section 2.2.2.1). The plants were established outside in a sheltered area and hardened off before planting into the orchard. On the 30/11/2011, the grafted plants of 'Hayward' on thirteen inter-specific hybrid kiwifruit rootstocks, including 'Hayward' (*Actinidia deliciosa*) propagated from cuttings (GN; self-rooted control) were planted in the field at a spacing of 4 m between rows and 1.7 within rows (Figure 3.1A and Figure 3.1 B). Vigour rating of scion was determined according to the results reported in Chapter Two. Planting height was standardised to leave 150 mm of rootstock stem above the soil level. Before planting, 0.5 m x 0.5 m width and 0.5 m depth planting holes were made using a spade and 2 kg of compost was added into each planting hole. The grafted plants were carefully taken out from the polybag and some of the roots were lightly trimmed and teased out before transplanting into the ground. Immediately after planting, 2 L of water was given manually to each plant to minimise transplanting stress. All the plants were trained using a pergola support structure consisting of 3 to 4 m high solid wooden post (16 by 16 cm thickness) rammed into the ground (Figure 3.1A). High-tensile galvanised wires were strung along the post and the ends of wires were attached to well-anchored end posts. During the first growing season in the field, a few long non-terminated shoots were

allowed to grow straight up along the support string. The growing shoots along the string were supported and tied using a hand-tying machine. In order to develop permanent cordons, two strong growing shoots were selected and trained along the string that were tied at a 45-degree angle, resulting in a Y-shaped crotch structure (Figure 3.1C). The remaining shoots below the main trunk were removed throughout the growing season to promote trunk and cordon development. After the growing shoots had reached the top of the strings they were headed back at 7 mm stem thickness and laid down along the support wires (Figure 3.1D). The irrigation system consisted of 19 mm polytube connected to under-vine mini sprinklers. Each sprinkler sprayed water in a 6 foot diameter circle under the canopy of vines. The sprinkler ran for 2 minutes, 3 times per day at 4 L hr^{-1}. No growth regulators were used to promote bud break or flowering in this trial. Other orchard management practices were typical of those for commercial kiwifruit production in New Zealand.

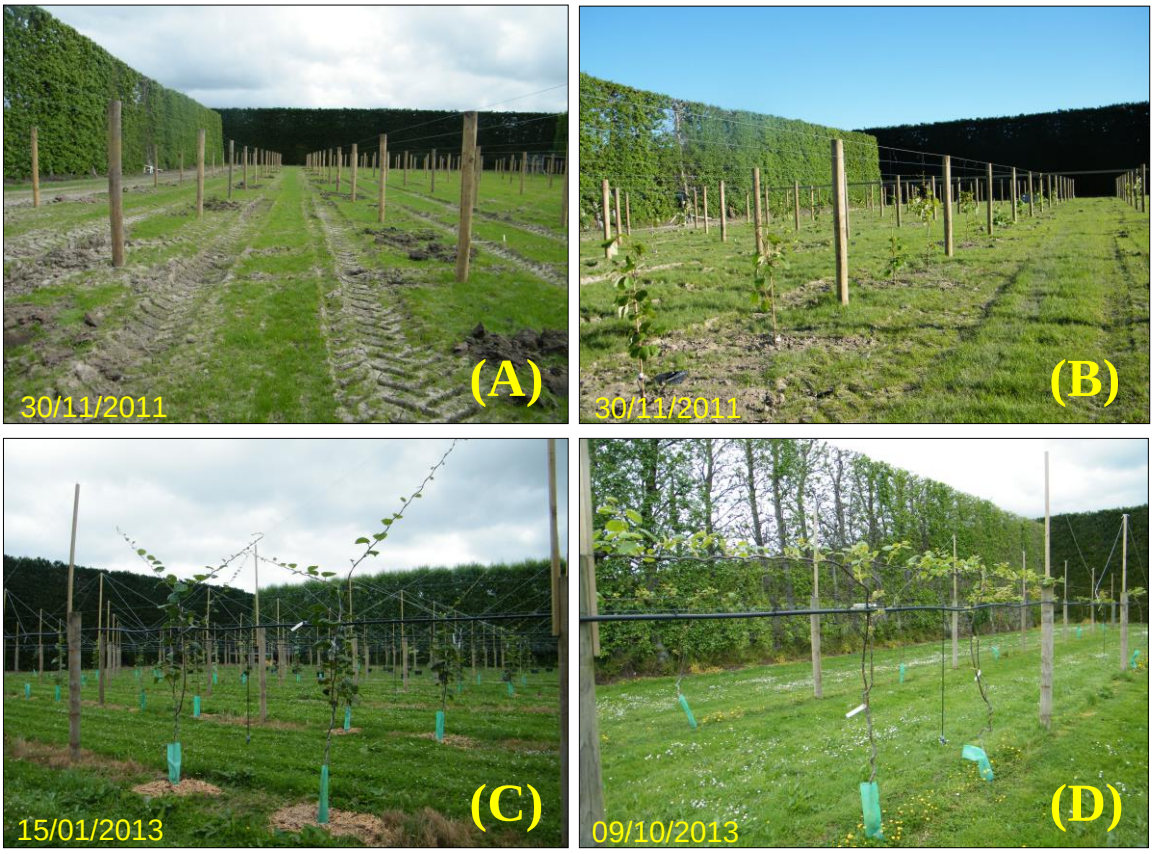


Figure 3.1. (A) Example of pergola structure used in this study, (B) the experimental plants were planted and trained using support strings (30/11/2011), (C) two strong actively growing shoots were trained Y-shaped structure (15/01/2013), and (D) the shoots were laid down to become permanent cordons (09/10/2013).

3.2.3 Measurements of scion growth

Measurement of scion growth was conducted throughout the growing season as previously described in Chapter Two (Section 2.2.3). During spring (16/9/2012), the proportion of scion bud break were calculated by dividing number of buds that broke with total number of buds per scion, expressed as a percentage (%). Three weeks after that, the number of flowers per lateral shoot was counted. In mid-December 2012, the shoots present on each scion were identified and classified as previously described (Figure 2.2, Chapter Two). In mid-December, all the proleptic axillary shoots were classified as short, medium and long shoots. Long shoots were defined as non-terminated, medium shoots as terminated with 10 or more nodes and short shoots as terminated with 9 or less nodes (Seleznyova et al., 2002). In mid-summer (16/2/2013) before laying down the cordons along the support wires, the characteristics of cordons in terms of length, node number and shoot cross-sectional area were recorded. In addition, the pruning weight (fresh and dry weight) of the removed shoots was recorded. All shoots were dried in an oven at 70 °C until constant weight was reached. In early summer 2013, the trunk diameter of scions and rootstocks was also measured using digital calliper (Mitutoyo, Japan) and converted into cross-sectional area (CSA).

In spring 2013, the bud break patterns of 'Hayward' scions were recorded for each cordon. Starting from 9/9/2013 (day 1 of bud break), the buds that burst during each observational time were recorded for each rootstock at 5 day intervals until 16/10/2013. In addition, relative bud break was also calculated and expressed as: the percentage of buds that opened during each observational time relative to the total bud number which opened, following the method by Wang et al., (1994b). To measure the growth rate of shoots, the length of shoots was recorded starting at approximately 50% bud break. Six shoots emerging from cordon were randomly selected and tagged (approximately 300 shoots in total). Starting on 1/10/2013, the length of each tagged shoot was measured with a tape measure (from the base to shoot apex) at weekly intervals until the final measurement on 29/11/2013 and the growth rate of shoots (mm per day^{-1}) calculated. In mid-November 2013, all the shoots from each rootstock were classified again as described before. The number of

flowers and fruits for the whole vine were manually counted and recorded again. The shoot CSA of proleptic axillary shoots (i.e. long, medium and short shoots) was measured in the middle of January 2014. The rootstocks were ranked (in order of increasing vigour) according to the results in Chapter Two. In order to organise the parentages of inter-specific hybrid kiwifruit rootstocks used in this study (refer Table 1.1, Chapter One), hereafter each original crosses parentages (in presented result tables) will be referred and simplified to by the code names. Four different species were used; *A. chinensis, A. macrosperma, A. polygama* and *A. melanandra*; these species are preferred to the short name **'*A. chi*'**, **'*A. mac*'**, **'*A. poly*'** and **'*A. mel*'**, respectively. In particular tables, no data were reported for rootstocks No.21, No.71 and self-rooted GN due to replanting and some vines still small.

3.2.4 Statistical analysis

The experimental plants were arranged in randomised completed block design (RCBD) with one grafted plant of each rootstock randomly arranged within each of four blocks. All data were manually entered into an Excel Spreadsheet and summarised using the pivot table function. The data were analysed using GLM procedure of SAS (version 9.2, SAS Institute Inc. NC USA). Before that, the data were checked for normality using Shapiro-Wilk procedure. If the data were not normally distributed, transformation using logarithm or square root was used before ANOVA analysis. If both transformations did not improve the normality of data, the non-parametric ANOVA (Kruskal-Wallis test) was used and mean comparisons were made using Least Significant Difference (LSD) test. For non-parametric ANOVA, the data were ranked before subjecting them to ANOVA by sorting the data into order and replacing each value by its relative position in the order using SAS command; PROC RANKS=DATA. As a result of ranking the data, larger ranks are associated with larger value and smaller ranks are associated with smaller values. In addition, selected group of rootstocks (according to the the current results on shoot growth rate) were subjected to the Contrast Analysis procedure of SAS (version 9.2, SAS Institute Inc. NC USA) in order to compare the vigour of rootstocks.

3.3 Results

3.3.1 Rootstock effects on spring bud break and floral precocity of scions

3.3.1.1 Growing season 2012-2013

In the growing season of 2012, there was a significant difference (P=0.03) in the mean proportion of bud break (%) on 'Hayward' scions grafted onto different inter-specific hybrid rootstocks (Table 3.1). Scions on rootstocks No.18 and No.19 produced the lowest mean bud break and the highest were recorded on rootstocks No.8, No.55 and No.86. The mean flower number on 'Hayward' scions was significantly different between inter-specific hybrid rootstocks (P<0.0001). Rootstocks No.8, No.18 and No.19 produced considerably high flower number per vine. It was noted that despite having a lower bud break, the scion grafted onto rootstock No.18 produced significantly higher flower number per vine compared to scions on other rootstocks (Table 3.1).

Table 3.1. Effects of inter-specific hybrid rootstocks on the mean bud break (%) and mean number of flowers (floral precocity) on 'Hayward' kiwifruit scions during 2012-2013 growing season.

Rootstock selection*	Parentages	Mean bud break (%)	Total number of buds	Mean number of flowers per vine
No.8	***A. chi* x *A. mac***	43.4 (± 4.4) ^{a}	**88**	1.4 (± 0.5) ^{b}
No.19	***A. chi* x *A. mac***	25.3 (± 2.7) ^{c}	**121**	1.3 (± 0.5) ^{bc}
No.100	***A. mac* x *A. mel***	34.5 (± 3.4) ^{abc}	**155**	0.3 (± 0.2) ^{cde}
No.85	***A. mac***	35.8 (± 3.9) ^{abc}	**127**	0.0 (± 0.0) ^{e}
No.86	***A. mac***	40.6 (± 4.6) ^{a}	**167**	0.0 (± 0.0) ^{e}
No.101	***A. mac* x *A. mel***	36.0 (± 3.2) ^{ab}	**137**	0.8 (± 0.5) ^{cde}
No.87	***A. poly***	34.7 (± 4.1) ^{bc}	**108**	0.1 (± 0.1) ^{de}
No.84	***A. poly***	27.9 (± 2.9) ^{abc}	**135**	0.8 (± 0.3) ^{bcd}
No.18	***A. chi* x *A. mac***	27.0 (± 2.1) ^{c}	**187**	3.3 (± 0.4) ^{a}
No.45	***A. poly* x *A. chi***	34.9 (± 3.9) ^{abc}	**108**	0.0 (± 0.0) ^{e}
No.55	***A. poly* x *A. chi***	40.3 (± 4.3) ^{abc}	**150**	0.4 (± 0.3) ^{cde}
	***P*-value‡**	P=0.03	-	P<0.0001

Numbers in the parentheses are standard error of means (±).
‡The non-parametric ANOVA (Kruskal-Wallis Test).
Means sharing the same letters are not significantly different at P=0.05 according to $LSD_{0.05}$ test.
No data were recorded for rootstocks number 21, 71 and self-rooted control (GN) due to replanting and the vines still small.
*In order of increasing vigour according to the results in Chapter Two.

3.3.1.2 Growing season 2013-2014

In the growing season of 2013, the inter-specific hybrid rootstocks may have affected the duration and compactness of bud break of 'Hayward' scions (Figure 3.2 and Figure 3.3). For the first two weeks (starting on 9/9/2013 until 19/9/2013), no significant difference was recorded on the mean percentage of scion bud break (P=0.21, P=0.61 and P=0.46, respectively). Only scions grafted onto rootstocks No.18, No.19, No.87, No.100 and No.101 including control vines (GN) produced more than 20% of bud break. However, the mean percentage of bud break was significantly different between rootstocks starting on 24/9/2013 onwards. On 16/10/2013, the final mean bud break of 'Hayward' scions (Figure 3.4) was significantly different between inter-specific hybrid rootstocks (P=0.02). Except for rootstock No.71, the range of bud break produced by inter-specific hybrid kiwifruit rootstocks was between 40 to 60 % (Figure 3.4). No significant differences were found on the mean final bud break (%) from scions grafted onto inter-specific hybrid kiwifruit rootstocks compared to control vines (GN), except for rootstock No.71 (data not shown, Figure 3.4). Nevertheless, the final mean scion bud break (%) on rootstocks No.18, No.45 and No.87 was considerably higher and almost comparable with GN.

Although no significant difference was found between inter-specific hybrid kiwifruit rootstocks (data not shown), the peak times of bud break of scions differed to some extent between rootstocks. The peak time of bud break was highest between day 16th until day 20th (approximately from 1st until 3rd weeks) on all rootstocks, including control vines, except for rootstock No.21, No.71 and No.100 (Figure 3.3). Similar patterns of peak time of bud break of scions were observed among rootstocks combination No.8, No.18 and No.19. Besides that, the scions on rootstocks No.45, No.55 and No.84 also exhibited the similar pattern of peak time and compactness of bud break (Figure 3.3). In addition to this result, the time period over approximately 70% of scion bud break for rootstocks No.45, No.84, No.87, No.100 and No.101 occurred over a period of 14 days. Meanwhile, for rootstocks No.18, No.19, No.55, No.71, No.85, No.86 and GN occurred over a period of 19 days. The longest time period of scion bud break was recorded on rootstocks No.21 and No.8 with 24 and 29 days, respectively (Figure 3.3, highlighted in red).

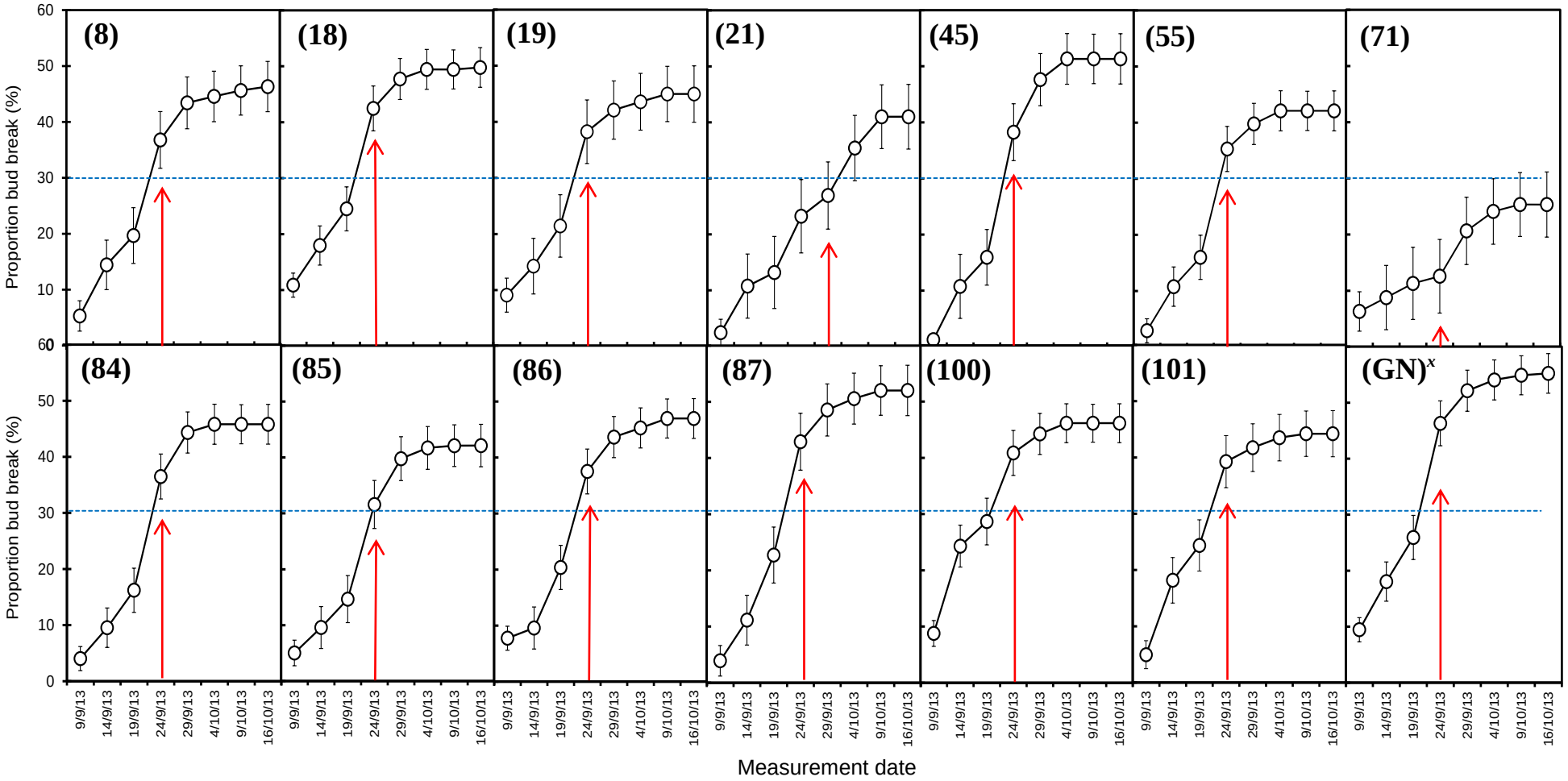


Figure 3.2. Time course for bud break of 'Hayward' scion grafted onto inter-specific hybrid rootstocks during spring 2013 season. No significant differences on the bud break were observed on the date of 9/9/13, 14/9/13 and 19/9/13. Red solid arrows indicate the date when bud break differed significantly between rootstocks. Significant differences in percentage bud break between rootstocks were observed starting the date of 24/9/13 onward with *P*=0.02, *P*=0.006, *P*=0.02, *P*=0.02 and *P*=0.02, respectively. [x]GN- self rooted control. Blue lines were drawn for guideline. Numbers in the parenthesis are rootstock selection.

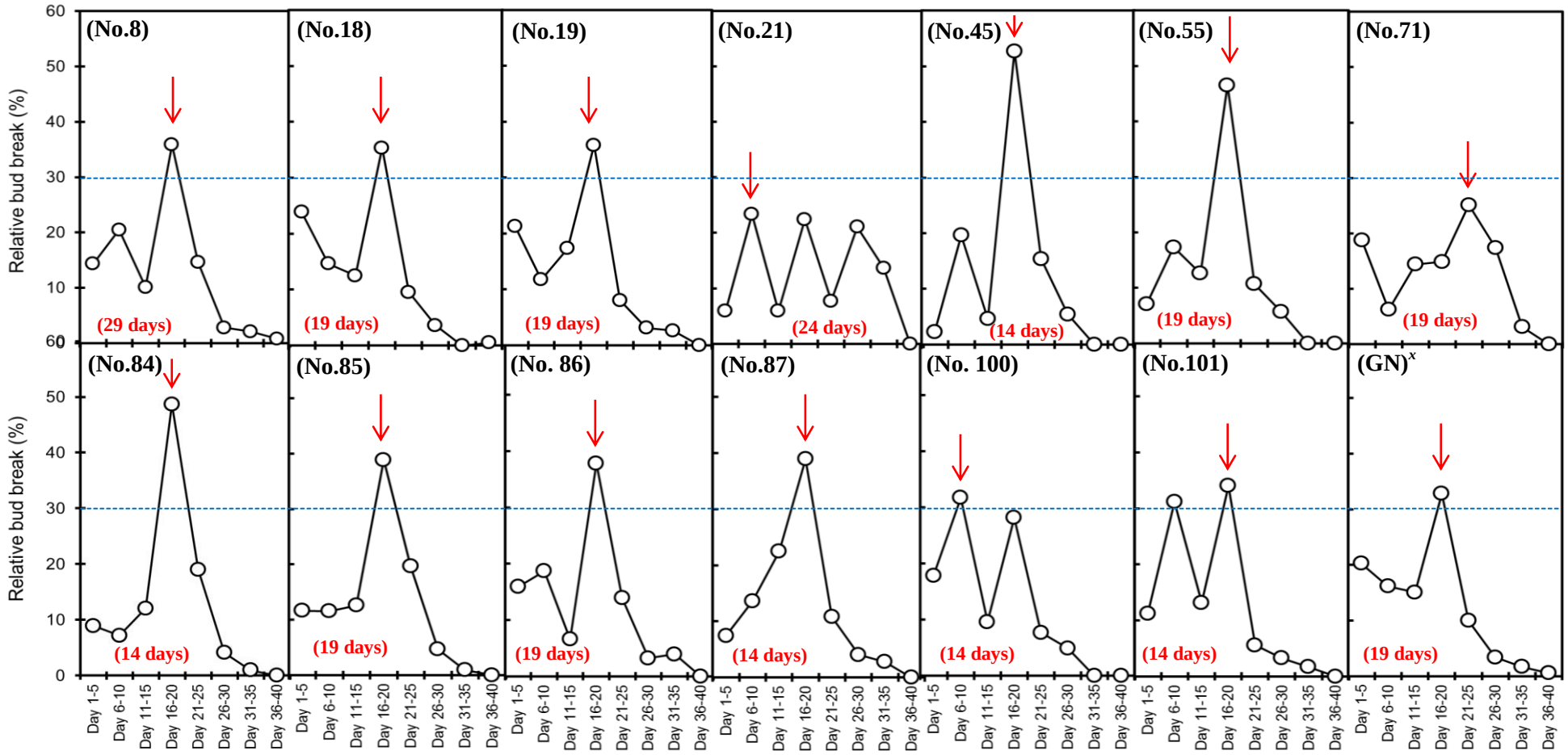


Figure 3.3. The mean relative bud break of 'Hayward' scion grafted onto inter-specific hybrid rootstocks during spring 2013 season. Red solid arrows indicate the date when the highest or peak times of mean bud break were recorded for each rootstock. Relative bud break is expressed as the percentage of buds that was opened during each observational time relative to the total bud which opened (Wang et al., 1994). ^{x}GN - self-rooted control. Blue lines were drawn for guideline. Numbers in the parenthesis are rootstock selections. Numbers in the parenthesis that highlighted in red are the days period over 70% bud break.

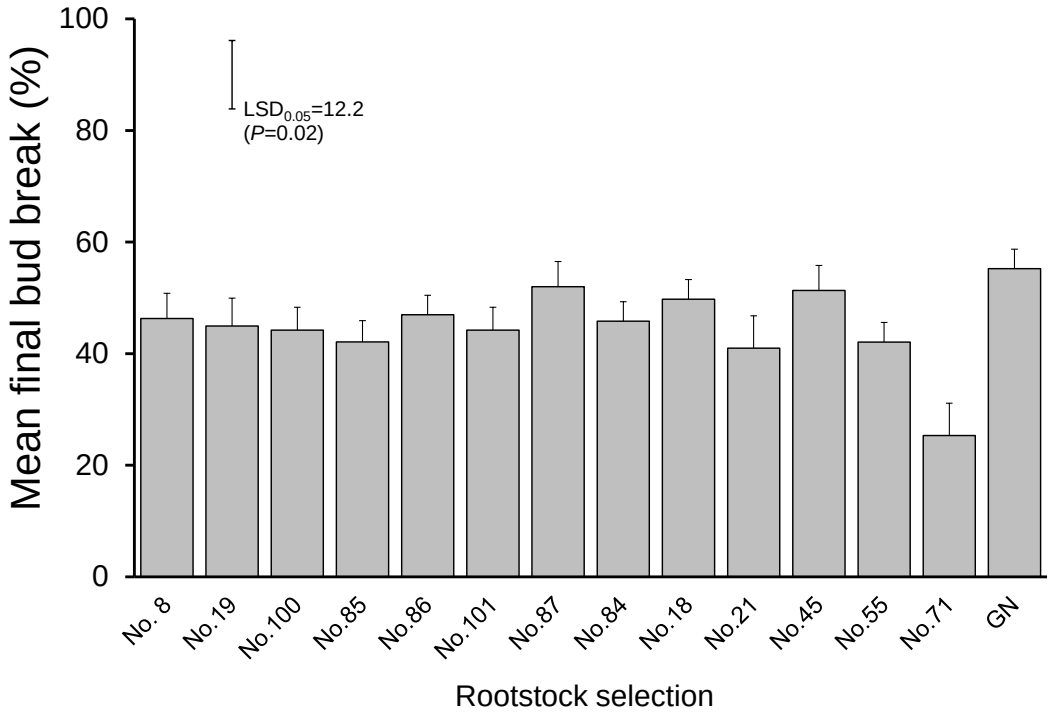


Figure 3.4. The mean final bud break (%) of 'Hayward' scion grafted onto inter-specific hybrid rootstocks during spring 2013 season. [x]GN - self-rooted control. In order of increasing vigour according to the results in Chapter Two. Vertical bars indicate standard error of means.

3.3.2 Rootstock effects on spring growth, shoot production and termination of axillary shoots

3.3.2.1 Growing season 2012-2013

3.3.2.1.1 The proportion of proleptic axillary shoots in summer 2012-2013 season

In summer 2012, the mean proportion of proleptic long shoots of 'Hayward' scions was not affected (P=0.54) by inter-specific hybrid rootstocks (Table 3.2). Nevertheless, the proportion of long shoots on rootstocks No.8, No.18, No.85 and No.87 appear lower compared to other rootstocks. No significant difference (P=0.59) was also found for the mean proportion of medium shoots. In rootstocks No.8, No.18, No.19, No.45, No.55 and GN, there was a trend that the mean proportion of medium shoots was lowered and some of them did not produce medium shoots. However, even though not significant, higher proportions of medium shoots were found on rootstocks No.85 and No.87 (Table 3.2). Inter-specific hybrid kiwifruit rootstocks also did not affect the mean proportion of short shoots (P=0.33) of 'Hayward' scions. Even so, rootstocks No.8 and No.87 tended to have higher proportions of short shoots (Table 3.2). The contrast detail tests were conducted on different rootstock groups such as low (*LV*), intermediate (*IV*) and high (*HV*) vigour rootstock groups, based on the current results in Figure 3.8 (shoot growth rate of scions). The mean proportion of short shoots did not differ significantly among rootstock groups (Table 3.2). However, rootstock group was significantly different in the mean proportion of long and medium shoots, except between *LV* and *IV* groups (Table 3.2). There was also a trend (P=0.10) that the mean proportion of medium shoots may be varied between *IV* and *HV* rootstock groups.

The proportion of shoots was further calculated as terminated (medium and short shoots) or non-terminated (long shoots). There were no significant differences either in the mean proportion of non-terminated (P=0.16) or terminated shoots (P=0.41) as shown in Table 3.3. Nevertheless, there was a trend that some rootstocks tended to increase the proportion of non-terminated shoots. For example, rootstocks No.19,

No.45, No.55 and GN had higher mean proportion of non-terminated shoots (Table 3.3) and substantially lower mean proportion of terminated shoots. Besides that, other rootstocks such as No.8, No.18, No.85 and No.87 produced higher proportion of terminated shoots and considerably lower proportion of non-terminated shoots (Table 3.3). Contrast analysis indicated that the mean proportion of non-terminated shoots differed significantly between rootstock groups (Table 3.3). There was also a trend (P=0.13) that the proportion of non-terminated shoots may be different between *LV* and *HV* rootstock groups. The mean proportion of terminated shoots between *HV* and other rootstock groups (i.e. *LV* and *IV* groups) was significant (P=0.01 and P=0.04, respectively). However, no significant difference was found on the mean proportion of terminated shoots between *LV* and *IV* rootstocks groups.

3.3.2.1.2 The number of proleptic axillary shoots in summer 2012-2013 season

In summer 2012-2013, the mean number of long, medium and short shoots were not significantly different between inter-specific hybrid kiwifruit rootstocks (P=0.61, P=0.60 and P=0.42, respectively) (Table 3.4). Nevertheless, it was noted that fewer medium and long shoot types were produced compared to short shoots. In addition, the mean total number of shoots was also not significantly different (P=0.69) between inter-specific hybrid kiwifruit rootstocks (Table 3.4). Even though higher mean total numbers of shoots were recorded on the rootstock No.100, No. 84, No.87 and GN, no significant differences were recorded from these rootstocks compared with other rootstocks (Table 3.4). Contrast analysis showed that the mean number of medium and short shoots was not significantly different between rootstock groups (Table 3.4). However, there was a trend (P=0.09) that the mean number of long shoots may be varied between *LV* and *HV* rootstock groups. Similar results were also found when comparing between the very low-vigour (*VLV*) rootstock groups (i.e. No.18, No.87 and No.100) and high-vigour (*HV*) rootstock groups (i.e. GN and No.101). The mean number of long shoots also did not differ significantly between *IV* and *LV* or *HV* rootstock groups. The difference in the mean total number of shoots between *HV* and *LV* or *VLV* rootstock groups only approached significance (P=0.06 and P=0.07, respectively).

Table 3.2. Effect of rootstocks on the mean proportion of different shoot types of 'Hayward' scions at early summer 2012.

Rootstock selection*	Parentages	Shoot types		
		Long	Medium	Short
No.8	***A. chi* x *A. mac***	0.77 (± 0.1) [a]	0.00 (± 0.0) [a]	0.23 (± 0.1) [ab]
No.19	***A. chi* x *A. mac***	0.83 (± 0.2) [a]	0.06 (± 0.1) [a]	0.11 (± 0.1) [ab]
No.100	***A. mac* x *A. mel***	0.84 (± 0.1) [a]	0.16 (± 0.1) [a]	0.00 (± 0.0) [b]
No.85	***A. mac***	0.70 (± 0.1) [a]	0.25 (± 0.2) [a]	0.05 (± 0.1) [ab]
No.86	***A. mac***	0.84 (± 0.1) [a]	0.13 (± 0.1) [a]	0.03 (± 0.1) [ab]
No.101	***A. mac* x *A. mel***	0.88 (± 0.1) [a]	0.12 (± 0.1) [a]	0.00 (± 0.0) [b]
No.87	***A. poly***	0.63 (± 0.2) [a]	0.25 (± 0.1) [a]	0.12 (± 0.1) [ab]
No.84	***A. poly***	0.85 (± 0.2) [a]	0.15 (± 0.2) [a]	0.00 (± 0.0) [b]
No.18	***A. chi* x *A. mac***	0.81 (± 0.1) [a]	0.00 (± 0.0) [a]	0.19 (± 0.1) [a]
No.45	***A. poly* x *A. chi***	0.95 (± 0.1) [a]	0.05 (± 0.1) [a]	0.00 (± 0.0) [b]
No.55	***A. poly* x *A. chi***	0.90 (± 0.1) [a]	0.00 (± 0.0) [a]	0.10 (± 0.1) [ab]
GN	***A. deliciosa***	0.89 (± 0.1) [a]	0.05 (± 0.1) [a]	0.06 (± 0.1) [ab]
	***P*-value‡**	*P*=0.54	*P*=0.59	*P*=0.33

***Contrast Analysis*x**			Pr > F		
			Long shoot	Medium shoot	Short shoot
All rootstocks	***vs***	**GN (Control)**	*P*=0.001	*P*=0.0017	ns
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.101 & GN** (*HV group*)	*P*=0.004	*P*=0.01	ns
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.45, No.55, No.84, No.85, No.86** (*IV group*)	ns	ns	ns
No.45, No.55, No.84, No.85, No.86 (*IV group*)	***vs***	**No.101 & GN** (*HV group*)	*P*=0.004	ns (*P*=0.10)	ns
No.18, No.87, No.100 (*VLV group*)	***vs***	**No.45, No.55, No.84, No.85, No.86** (*IV group*)	*P*=0.02	ns	ns

Numbers in the parentheses are standard error of means (±).

‡The non-parametric ANOVA (Kruskal-Wallis Test).

Means sharing the same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. ns – Non-significant according to *Contrast Analysis* SAS.

No data were recorded for rootstocks number 21 and 71 due to replanting and the vines still small.

Green cuttings (GN) - Self-rooted control.

*In order of increasing vigour according to the results in Chapter Two.

xVery low-vigour (*VLV*), Low vigour (*LV*), Intermediate vigour (*IV*) and High vigour (*HV*) groups according to the results on *shoot growth rate* in Figure 3.8.

Table 3.3. Effect of rootstocks on the mean proportion of terminated or non-terminated of 'Hayward' scions at early summer 2012-2013.

Rootstock selection*	Parentages	Non-Terminated shoots	Terminated shoots
No.8	***A. chi* x *A. mac***	0.77 (± 0.1) [a]	0.23 (± 0.1) [ab]
No.19	***A. chi* x *A. mac***	0.83 (± 0.2) [a]	0.17 (± 0.2) [ab]
No.100	***A. mac* x *A. mel***	0.84 (± 0.1) [a]	0.16 (± 0.1) [b]
No.85	***A. mac***	0.70 (± 0.1) [a]	0.30 (± 0.1) [ab]
No.86	***A. mac***	0.84 (± 0.1) [a]	0.16 (± 0.1) [ab]
No.101	***A. mac* x *A. mel***	0.88 (± 0.1) [a]	0.12 (± 0.1) [b]
No.87	***A. poly***	0.63 (± 0.2) [a]	0.37 (± 0.2) [ab]
No.84	***A. poly***	0.85 (± 0.2) [a]	0.15 (± 0.2) [b]
No.18	***A. chi* x *A. mac***	0.81 (± 0.1) [a]	0.19 (± 0.1) [a]
No.45	***A. poly* x *A. chi***	0.90 (± 0.1) [a]	0.10 (± 0.1) [ab]
No.55	***A. poly* x *A. chi***	0.95 (± 0.1) [a]	0.05 (± 0.1) [b]
GN	***A. deliciosa***	0.89 (± 0.1) [a]	0.11 (± 0.1) [ab]
	***P*-value‡**	*P*=0.16	*P*=0.41

Contrast Analysis[x]			Pr > F	
			Non- Terminated shoot	**Terminated shoot**
All rootstocks	***vs***	**GN (Control)**	*P*=0.008	*P*=0.03
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.101 & GN** (*HV group*)	ns (*P*=0.13)	*P*=0.01
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.45, No.55, No.84, No.85, No.86** (*IV group*)	*P*=0.002	ns
No.45, No.55, No.84, No.85, No.86 (*IV group*)	***vs***	**No.101 & GN** (*HV group*)	*P*=0.0005	*P*=0.04
No.18, No.87, No.100 (*VLV group*)	***vs***	**No.45, No.55, No.84, No.85, No.86** (*IV group*)	*P*=0.0005	ns

Numbers in the parentheses are standard error of means (±).

‡The non-parametric ANOVA (Kruskal-Wallis Test).

Means sharing the same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. ns – Non-significant according to *Contrast Analysis* SAS.

No data were recorded for rootstocks number 21 and 71 due to replanting and the vines still small.

Green cuttings (GN) - Self-rooted control.

*In order of increasing vigour according to the results in Chapter Two.

[x]Very low-vigour (*VLV*), Low vigour (*LV*), Intermediate vigour (*IV*) and High vigour (*HV*) groups according to the results on *shoot growth rate* in Figure 3.8.

Table 3.4. The mean number of long, medium and short proleptic axillary shoots of 'Hayward' scions grafted onto inter-specific hybrid kiwifruit rootstocks at end of summer 2012-2013 growing season.

Rootstock selections*	Parentages	Means number of different shoot types			Mean total number of shoots
		Long	Medium	Short	
No.8	***A. chi* x *A. mac***	2.3 (± 1.0) [a]	0.0 (± 0.0) [a]	0.5 (± 0.3) [a]	2.8 (± 1.1) [a]
No.19	***A. chi* x *A. mac***	1.8 (± 0.6) [a]	0.3 (± 0.3) [a]	0.5 (± 0.5) [a]	2.5 (± 0.6) [a]
No.100	***A. mac* x *A. mel***	3.0 (± 0.6) [a]	1.0 (± 0.7) [a]	0.0 (± 0.0) [a]	4.0 (± 1.2) [a]
No.85	***A. mac***	2.3 (± 0.6) [a]	0.8 (± 0.5) [a]	0.3 (± 0.3) [a]	3.3 (± 0.6) [a]
No.86	***A. mac***	3.5 (± 0.6) [a]	0.8 (± 0.5) [a]	0.3 (± 0.3) [a]	4.5 (± 1.3) [a]
No.101	***A. mac* x *A. mel***	2.5 (± 0.5) [a]	0.5 (± 0.5) [a]	0.0 (± 0.0) [a]	3.0 (± 1.0) [a]
No.87	***A. poly***	3.3 (± 1.4) [a]	0.3 (± 0.8) [a]	0.8 (± 0.8) [a]	5.3 (± 1.8) [a]
No.84	***A. poly***	2.8 (± 0.5) [a]	0.8 (± 0.8) [a]	0.0 (± 0.0) [a]	3.5 (± 0.6) [a]
No.18	***A. chi* x *A. mac***	2.8 (± 0.3) [a]	0.0 (± 0.0) [a]	0.8 (± 0.3) [a]	3.5 (± 0.5) [a]
No.45	***A. poly* x *A. chi***	1.8 (± 0.5) [a]	0.0 (± 0.0) [a]	0.5 (± 0.5) [a]	2.3 (± 0.9) [a]
No.55	***A. poly* x *A. chi***	3.0 (± 0.4) [a]	0.3 (± 0.3) [a]	0.0 (± 0.0) [a]	3.3 (± 0.6) [a]
GN	***A. deliciosa***	4.0 (± 3.0) [a]	0.5 (± 0.5) [a]	0.5 (± 0.5) [a]	5.0 (± 4.0) [a]
	***P*-value**[ǂ]	*P*=0.42	*P*=0.60	*P*=0.61	*P*=0.69

Contrast Analysis[x]			Pr > F			
			Long shoot	Medium shoot	Short shoot	Mean total number of shoots
All rootstocks	***vs***	**GN (Control)**	ns	ns	ns	ns
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.101 & GN** (*HV group*)	ns (*P*=0.09)	ns	ns	ns (*P*=0.07)
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.45, No.55, No.84, No.85, No.86** (*IV group*)	ns	ns	ns	ns
No.45, No.55, No.84, No.85, No.86 (*IV group*)	***vs***	**No.101 & GN** (*HV group*)	ns	ns	ns	ns (*P*=0.10)
No.18, No.87, No.100 (*VLV group*)	***vs***	**No.101 & GN** (*HV group*)	ns (*P*=0.09)	ns	ns	ns (*P*=0.06)

Numbers in the parentheses are standard error of means (±). [ǂ]The non-parametric ANOVA (Kruskal-Wallis Test).
Means sharing the same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. ns – Non-significant according to *Contrast Analysis* SAS.
No data were recorded for rootstocks number 21 and 71 due to replanting and the vines still small.
Green cuttings (GN) - Self-rooted control. *In order of increasing vigour according to the results in Chapter Two.
[x]Very low-vigour (*VLV*), Low vigour (*LV*), Intermediate vigour (*IV*) and High vigour (*HV*) groups according to the results on *shoot growth rate* in Figure 3.8.

3.3.2.1.3 The characteristics of trained cordons at the end of summer 2013

At the end of summer 2013, the final mean length of long proleptic axillary shoots that have been trained to be permanent cordons (Figure 3.1C) were significantly affected (P=0.004) by the inter-specific hybrid rootstocks (Table 3.5). The mean final length of long shoots (cordons) on rootstock No.45 was significantly longer (3575.0 mm) compared with other rootstocks. In contrast, the final mean length of cordons of the first-three rootstocks (No.8, No.18 and No.19) and also rootstocks No.84 and No.100 was significantly shorter than rootstock No.45. The mean node number of long shoots was also significantly affected (P=0.01) by inter-specific hybrid rootstocks. The mean internode length was also significantly affected (P=0.008) by the inter-specific hybrid rootstocks with a range of 58 mm to 76 mm. Similarly, the mean shoot CSA was significantly affected (P=0.004) by inter-specific hybrid rootstocks and rootstock No.85 had a significantly larger shoot CSA compared with other rootstocks (Table 3.5). Comparing between the length and the node number, there was a strong positive linear relationship between the final length and the node number of long proleptic axillary shoots that have been trained to be permanent cordons (Figure 3.5). A high regression coefficient was obtained (R^2=0.84, P<0.0001) between the length and node numbers of these shoots.

Table 3.5. Effect of rootstocks on the characteristics of long proleptic axillary that have been trained as cordons in summer 2013 (Early February 2013).

Rootstock selection*	Parentages	Mean length (mm)	Mean node number	Mean internode length (mm)	Mean shoot CSA
No.8	***A. chi* x *A. mac***	1946.0 (± 221.0) ^{cd}	31.8 (± 2.9) ^{cd}	60.9 (± 3.3) ^{de}	114.5 (± 14.0) ^{cd}
No.19	***A. chi* x *A. mac***	1715.0 (± 248.2) ^{d}	28.8 (± 2.9) ^{d}	58.7 (± 4.8) ^{e}	91.9 (± 15.2) ^{d}
No.100	***A. mac* x *A. mel***	2295.0 (± 120.5) ^{bcd}	33.5 (± 1.9) ^{cd}	69.0 (± 3.0) ^{abcd}	126.4 (± 12.1) ^{cd}
No.85	***A. mac***	3045.7 (± 179.3) ^{ab}	43.7 (± 2.9) ^{ab}	70.2 (± 2.9) ^{bcde}	196.9 (± 18.4) ^{a}
No.86	***A. mac***	2790.0 (± 253.2) ^{abc}	38.7 (± 3.2) ^{bcd}	71.8 (± 1.1) ^{ab}	139.0 (± 13.6) ^{bcd}
No.101	***A. mac* x *A. mel***	2700.0 (± 631.9) ^{bc}	36.8 (± 5.2) ^{bcd}	72.1 (± 9.1) ^{ab}	147.5 (± 13.3) ^{abc}
No.87	***A. poly***	2582.5 (± 611.0) ^{bc}	36.8 (± 6.4) ^{bcd}	68.2 (± 4.0) ^{abcde}	157.7 (± 29.5) ^{abc}
No.84	***A. poly***	2005.0 (± 285.0) ^{cd}	32.9 (± 3.4) ^{cd}	59.9 (± 3.1) ^{de}	110.4 (± 14.8) ^{cd}
No.18	***A. chi* x *A. mac***	2261.3 (± 253.4) ^{bcd}	36.1 (± 2.9) ^{bcd}	61.5 (± 3.0) ^{cde}	132.6 (± 14.9) ^{bcd}
No.45	***A. poly* x *A. chi***	3575.0 (± 257.6) ^{a}	50.0 (± 3.1) ^{a}	71.6 (± 3.5) ^{abc}	178.4 (± 25.7) ^{ab}
No.55	***A. poly* x *A. chi***	2490.0 (± 301.7) ^{bcd}	38.8 (± 3.7) ^{bcd}	63.2 (± 2.6) ^{bcde}	136.8 (± 13.4) ^{bcd}
GN	***A. deliciosa***	3045.0 (± 208.4) ^{ab}	39.5 (± 1.8) ^{bc}	76.9 (± 2.5) ^{a}	137.8 (± 27.8) ^{bcd}
	***P*-value**	*P*=0.004	*P*=0.01	*P*=0.008	*P*=0.004

Data in the parenthesis are standard error of means (±).
Means sharing same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
No data were recorded for rootstocks number 21 and 71 due to replanting.
Green cuttings (GN) - Self-rooted control.
*In order of increasing vigour according to the results in Chapter Two.

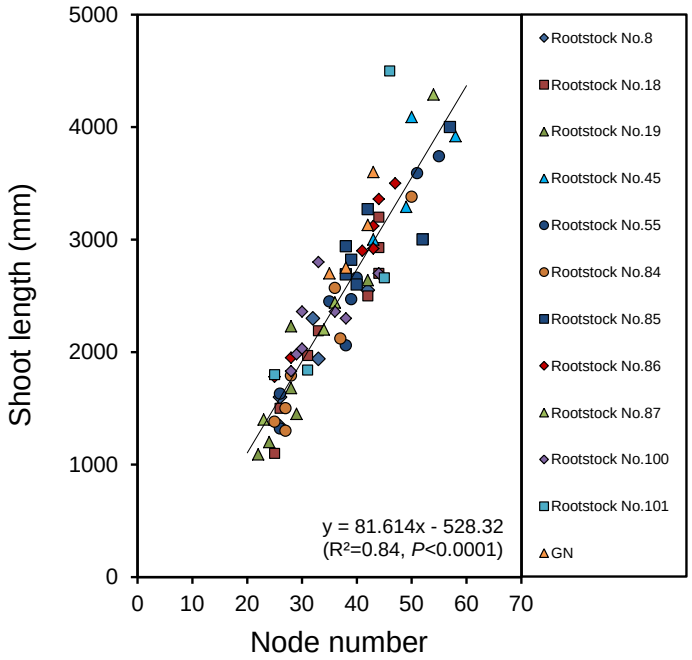


Figure 3.5. The relationship between the final length and node number of long proleptic shoots that have been trained to be permanent cordons at summer 2013 (early February 2013).

3.3.2.1.3 Pruning weight of scions in growing season 2012-2013

Inter-specific hybrid kiwifruit rootstocks did not significantly differ (P=0.86) in the mean pruning fresh weight (g) of the scion shoots (Table 3.6). Nevertheless, based on the raw data available (see number in parenthesis and bold, Table 3.6), there was a trend that rootstocks No.45, No.55, No.85, No.86 and No.87 increased the pruning fresh weight. The highest mean pruning fresh weight (g) was recorded on rootstock No.55 with 677.2 g. In contrast, rootstock No.101 produced the lowest in the pruning fresh weight with 243.8 g, and this was almost three times lower than rootstock No.55. The mean dry weight of pruned shoots also did not significantly differ (P=0.24) between inter-specific hybrid rootstocks (Table 3.6). Despite the scions on rootstock No.18 having lower pruning fresh weight, this rootstock produced considerable higher dry weight of pruned shoots (Table 3.6). Besides that, rootstocks No.86, No.87, No.100 and GN also produced higher dry weight of pruned shoots. The mean dry matter of pruning weight (%) was significantly affected by the kiwifruit rootstocks (P=0.04). Rootstocks No.18, No.84, No.87 and No.100 produced the highest mean dry matter of pruning weight with more than 35% (Table 3.6).

Table 3.6. Effect of inter-specific hybrid rootstocks on the mean pruning weight of 'Hayward' kiwifruit scions at end of summer 2013 growing season.

Rootstock selection*	Parentages	Mean fresh weight (g)		Mean dry weight (g)		Mean dry matter (%)	
No.8	***A. chi* x *A. mac***	324.2 (± 117.1)	ǂ**5.6** a	91.3 (± 28.7)	ǂ**4.4** a	32.8 (± 5.8)	ǂ**5.7** ab
No.19	***A. chi* x *A. mac***	381.8 (± 147.4)	**5.7** a	61.8 (± 17.7)	**4.0** a	19.4 (± 2.6)	**4.3** bc
No.100	***A. mac* x *A. mel***	471.3 (± 169.2)	**6.0** a	140.5 (± 23.6)	**4.9** a	35.3 (± 4.3)	**5.9** ab
No.85	***A. mac***	728.4 (± 399.9)	**6.2** a	125.5 (± 60.7)	**4.5** a	20.1 (± 1.4)	**4.5** bc
No.86	***A. mac***	625.7 (± 176.7)	**6.2** a	182.0 (± 51.2)	**4.8** a	28.0 (± 5.4)	**5.2** ab
No.101	***A. mac* x *A. mel***	243.8 (± 81.4)	**5.4** a	73.3 (± 12.6)	**4.2** a	30.3 (± 5.7)	**5.5** ab
No.87	***A. poly***	635.8 (± 166.6)	**6.0** a	162.0 (± 34.1)	**4.8** a	34.2 (± 6.0)	**5.7** ab
No.84	***A. poly***	383.3 (± 218.5)	**5.6** a	100.1 (± 14.9)	**4.1** a	40.8 (± 5.8)	**6.0** ab
No.18	***A. chi* x *A. mac***	271.4 (± 37.2)	**5.6** a	100.5 (± 12.6)	**4.6** a	40.0 (± 5.7)	**6.2** a
No.45	***A. poly* x *A. chi***	671.0 (± 175.9)	**6.3** a	50.6 (± 2.3)	**3.9** a	12.1 (± 6.1)	**3.2** c
No.55	***A. poly* x *A. chi***	677.2 (± 240.5)	**6.3** a	137.1 (± 49.4)	**4.7** a	24.9 (± 4.3)	**5.0** abc
GN	***A. deliciosa***	499.8 (± 179.5)	**6.1** a	143.9 (± 61.6)	**4.8** a	27.2 (± 4.1)	**5.2** ab
	***P*-value**	*P*=0.86		*P*=0.24		*P*=0.04	

Data are means of raw data (±standard error of means).
ǂData in bold were transformed means using log transformation.
Means sharing same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
[x] No data were recorded for rootstocks number 21 and71 due to replanting and the vines still small.
Green cuttings (GN) - Self-rooted control.
* In order of increasing vigour according to the results in Chapter Two.

3.3.2.2 Growing season 2013-2014

3.3.2.2.1 Spring growth of proleptic axillary shoots in 2013 growing season

Irrespective of rootstock, the differences in the shoot growth of 'Hayward' between long, medium and short shoots measurable during the first month after bud break (Figure 3.6). Long shoots grew faster than short and medium shoots for the first-four to five weeks after bud break due to abortion of the apices of short shoots (termination) occurring earlier than long shoots (non-terminated shoots). By six weeks after bud break, most of the long shoots reached more than 900 mm in length except for the long shoots produced from rootstocks No.18, No.71 and No.84. Nevertheless, there was a trend that the growth of long shoots from rootstock No.8, No.18 and No.19 slowed growth for the final three weeks of observation (Figure 3.6). Medium shoots grew much slower than long shoots in the first three to four weeks after bud break. Medium shoots of scions on rootstocks No.45 and No.101 reached more than 600 mm in length by the six weeks after bud break and grew slightly faster than medium shoots from other rootstocks (Figure 3.6). For short shoots, regardless of rootstocks, the slow growth of apical shoots was clearly

observed between weeks three and four after bud break (Figure 3.6). In summary, differences in the patterns of shoot growth were observed on the long, medium and short shoots of 'Hayward' scion when grafted onto inter-specific hybrid rootstocks. Additionally, in order to identify which rootstock may have induced slower growth rate on grafted 'Hayward' scion, the shoot growth rate per day (mm per day^{-1}) was calculated and plotted as shown in Figure 3.7.

3.3.2.2.2 The growth rate of proleptic axillary shoots in 2013 growing season

The growth of long shoots grafted onto rootstocks No.18 and No.87 showed the slowest growth rate pattern (Figure 3.7). The growth rate of long shoots from rootstock No.18 only reached a maximum of 28.9 mm week^{-1} at week five after bud break (Figure 3.7, No.18), showing a steady decline thereafter. Similar pattern was found on the shoot growth rate of scion on rootstock No.87 (Figure 3.7, No.87). However, the growth rate of long shoots from rootstocks No.87 showed two distinct maximum times with 28.3 mm week^{-1} at week three and 31.9 mm week^{-1} at week six, respectively (Figure 3.7, No.87), before declining. In addition, the growth rate of long shoots from rootstocks No. 8, No.19 and No.100 only reached maximum of 40 mm week^{-1} at week six. Nevertheless, the growth rates of long shoots from all these rootstocks (No.18, No.87, No.100, No.8 and No.19) were still considerable slower compared to other rootstocks. The growth rate of rootstocks No.45, No.55, No.71 reached a maximum 45 mm day^{-1} at week six. Similar observation was also recorded for rootstocks No.84, No.85 and No.86 (Figure 3.7). However, at week six, the long shoots of scions on rootstocks No. 21, No.101 and GN reached the highest maximum growth rate with more than 50 mm day^{-1} compared with other rootstocks. Overall, measurement of shoot growth rate (Figure 3.7) revealed that particular rootstocks such as No.18, No.87 and No.100 had slowed the growth rate of long shoots of 'Hayward' scions that might contribute to the highest proportion of terminated shoots. If rootstocks No.18, No.87 and No.100 are the low-vigour rootstocks, we might expect a higher proportion of terminated shoots than non-terminated shoots from these rootstocks in summer 2013 season (Table 3.7 and Table 3.8).

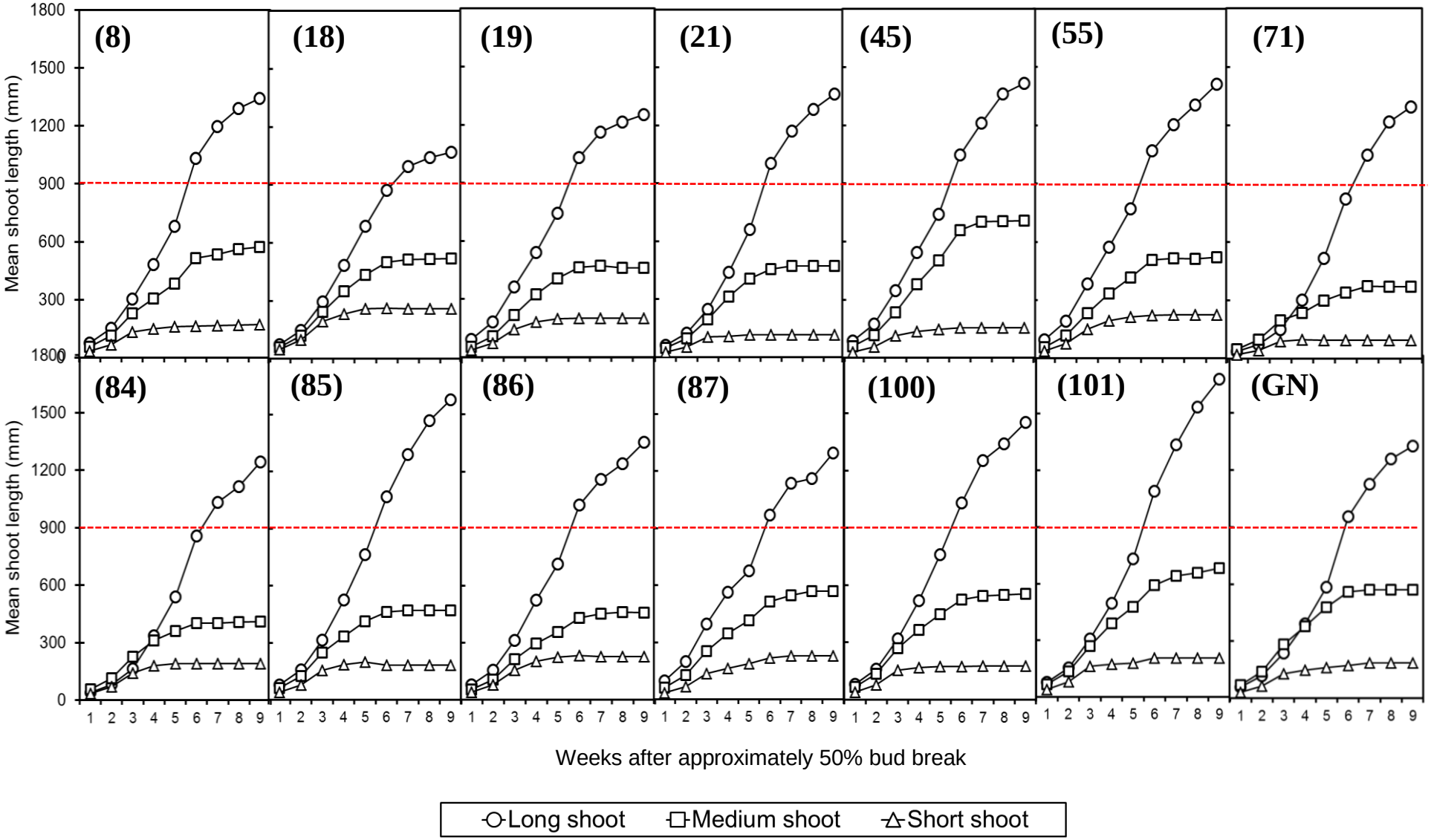


Figure 3.6. Growth and development (i.e shoot length) of long (○), medium (□) and short (Δ) shoots of the 'Hayward' scions growing on inter-specific hybrid rootstocks during spring 2013 season. The first date of measurement was on October 1st, 2013. Red lines were drawn for the guidelines. Numbers in the parentheses are rootstock selections.

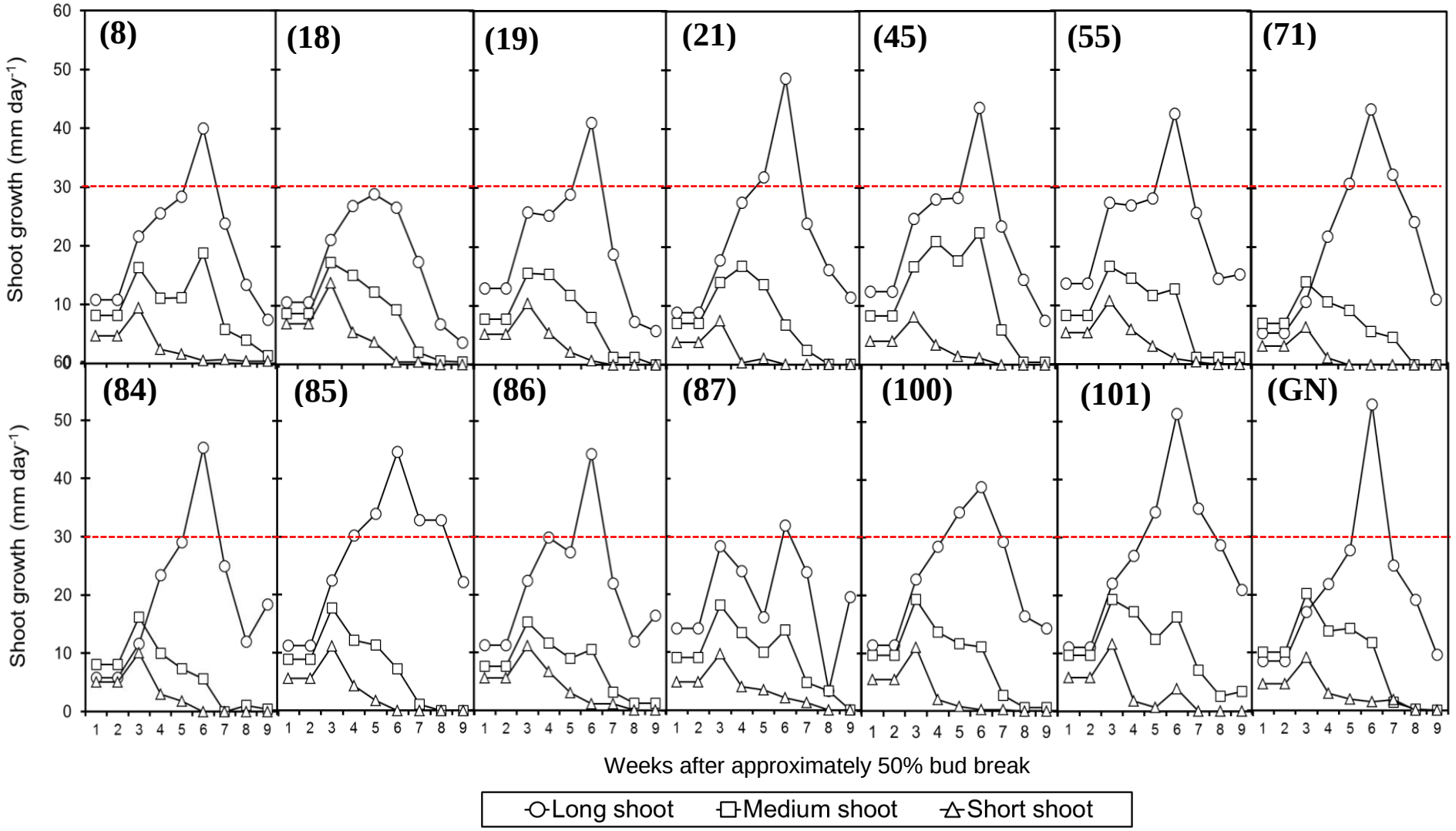


Figure 3.7. Shoot growth rate (mm day^{-1}) of long (○), medium (□) and short (Δ) shoots of the 'Hayward' scion growing on inter-specific hybrid rootstock during spring 2013 season. The first date of measurement was October 1st, 2013. Red lines were drawn for the guidelines. Numbers in the parentheses are rootstock selections.

3.3.2.2.3 The proportion of proleptic axillary shoots in 2013-2014 growing season

The mean proportion of different shoot types (long, medium and short) produced from 'Hayward' scions did not vary among rootstocks (Table 3.7). No significant differences were found in the mean proportion of long, medium and short of 'Hayward' scions (Table 3.7) when grafted onto inter-specific hybrid rootstocks (P=0.11, P=0.44 and P=0.84, respectively). However, there was a strong trend (P=0.11) that the mean proportion of long shoots may be affected by the inter-specific hybrid rootstocks. Although not statistically significant, rootstock selections No.18, No.87 and No.100 produced the lowest mean proportion of long shoots (Table 3.7) and these rootstocks also produced the highest mean proportion of short shoots. This may reflect the slower growth rate of long shoots of scions on the rootstocks No.18, No.87 and No.100 (Figure 3.7), possibly due to the termination of long shoots occurred earlier on these rootstocks (Figure 3.8). Other rootstocks such as No.55 also produced a lower mean proportion of long shoots. However, the mean proportions of medium and short shoots of these rootstocks were mostly equal with 0.40 and 0.41, respectively (Table 3.7). Although rootstock No. 21 and No.71 produced the highest mean proportion of long shoots, this could not be confirmed due to only two replicated vines being available for these rootstocks. According to contrast analysis, no significant differences were recorded on the mean proportion of long, medium and short shoots between rootstock groups, except for the long shoots between *IV* group and *HV* rootstock groups (significance at the P=0.02, Table 3.7). Even so, there were weak trends toward significance that the mean proportion of long and medium may be different between *LV* and *IV* group rootstock groups (P=0.12 and P=0.11, respectively).

The proportions of different shoot types (long, medium and short) were calculated in terms of shoot termination (i.e. terminated or non-terminated shoots) as shown in Table 3.8. Interestingly, the mean proportion of terminated and non-terminated shoots was significantly affected by the rootstocks (P=0.04 for both, respectively). The highest mean proportions of terminated shoots were recorded on rootstocks No.18, No.87 and No.100 with 0.88, 0.83 and 0.82, respectively (Table 3.8). In addition, a substantially lower mean proportion of non-terminated shoots was also recorded on the same rootstocks. This reflected the result found in Figure 3.8 that these rootstocks have a slow growth rate for long shoots. However, other rootstocks such as No.45, No.55, No.84

and No.86 including GN also produced considerable higher mean proportion of terminated shoots even though their shoots did not exhibit slower growth compared to rootstocks No.18, No.87 and No.100 (Figure 3.8). Contrast analysis indicated that significant differences in the mean proportion of non-terminated and terminated shoots (P=0.007, for both) between *IV* and *HV* rootstock groups (Table 3.8). Additionally, there were trends (P=0.06 and P=0.07) that the mean proportion of non-terminated and terminated shoots may be varied between *IV* and *LV* rootstock groups. Besides that, contrast analysis also indicated weak trends toward significance (P=0.12, for both) for the mean proportion of non-terminated and terminated shoots between *IV* and *HV* rootstock groups (Table 3.8).

3.3.2.2.4 The number of proleptic shoots at the end 2013-2014 growing season

Inter-specific hybrid kiwifruit rootstocks did not affect the mean number of long shoots (P=0.81). Although not significant, the number of long shoots of scions was highest on rootstock No.8 and GN, with almost three-fold higher than rootstocks No.18, No.71, No.84 and No.100 (Table 3.9). The mean number of medium shoots was also not significantly different (P=0.18) between inter-specific hybrid kiwifruit rootstocks. However, rootstocks No.85, No.86 and No.55 produced the highest mean number of medium shoots compared with other rootstocks (Table 3.9). There was a trend (P=0.13) that the mean number of short shoots was effected by the inter-specific hybrid kiwifruit rootstocks (Table 3.9). Although not significant, rootstocks No.100, No.84 and No.18 produced the highest mean number of short shoots compared to other rootstocks. The mean total number of shoots was also not significantly different (P=0.52) between inter-specific hybrid kiwifruit rootstocks. Nevertheless, there was a trend that rootstocks No.8, No.18 and No.86 produced the highest mean total number of proleptic shoots of scions (Table 3.9). Contrast analysis showed that rootstock groups did not significantly differ in the mean number of long and short shoots (Table 3.9). The mean number of medium shoots was significantly different (P=0.03) between *LV* and *IV* rootstock group. There was a strong trend toward significance (P=0.08) that the mean number of medium shoots may be different between *IV* and *HV* rootstock groups. No significant difference was recorded in the mean total number of proleptic shoots among rootstock groups (Table 3.9, contrast analysis).

Table 3.7. The mean proportion of long, medium and short proleptic axillary shoots of 'Hayward' scions grafted onto inter-specific hybrid kiwifruit rootstocks (2013-2014).

Rootstock selections*	Parentages	Means proportion of different shoot types		
		Long	Medium	Short
No.8	***A. chi* x *A. mac***	0.38 (± 0.05) ^{abc}	0.22 (± 0.05) ^{b}	0.40 (± 0.09) ^{a}
No.19	***A. chi* x *A. mac***	0.27 (± 0.19) ^{bcd}	0.25 (± 0.09) ^{ab}	0.48 (± 0.19) ^{a}
No.100	***A. mac* x *A. mel***	0.18 (± 0.02) ^{cd}	0.24 (± 0.03) ^{ab}	0.58 (± 0.05) ^{a}
No.85	***A. mac***	0.34 (± 0.05) ^{abc}	0.48 (± 0.03) ^{a}	0.18 (± 0.14) ^{a}
No.86	***A. mac***	0.25 (± 0.01) ^{abcd}	0.45 (± 0.10) ^{ab}	0.30 (± 0.12) ^{a}
No.101	***A. mac* x *A. mel***	0.31 (± 0.07) ^{abcd}	0.42 (± 0.06) ^{ab}	0.27 (± 0.14) ^{a}
No.87	***A. poly***	0.18 (± 0.10) ^{cd}	0.34 (±0 .05) ^{ab}	0.48 (± 0.06) ^{a}
No.84	***A. poly***	0.24 (± 0.17) ^{bcd}	0.32 (± 0.12) ^{ab}	0.44 (± 0.12) ^{a}
No.18	***A. chi* x *A. mac***	0.12 (± 0.03) ^{d}	0.26 (± 0.09) ^{ab}	0.62 (± 0.09) ^{a}
No.21 (n=2)	***A. chi* x *A. mac***	0.42 (± 0.02) ^{ab}	0.18 (± 0.07) ^{b}	0.40 (± 0.22) ^{a}
No.45	***A. poly* x *A. chi***	0.31 (± 0.10) ^{abcd}	0.32 (± 0.16) ^{ab}	0.37 (± 0.23) ^{a}
No.55	***A. poly* x *A. chi***	0.19 (± 0.06) ^{bcd}	0.41 (± 0.11) ^{ab}	0.40 (± 0.06) ^{a}
No.71 (n=2)	***A. poly* x *A. chi***	0.60 (± 0.20) ^{a}	0.20 (± 0.20) ^{ab}	0.20 (± 0.13) ^{a}
GN	***A. deliciosa***	0.28 (± 0.18) ^{abcd}	0.29 (± 0.04) ^{ab}	0.43 (± 0.10) ^{a}
	***P*-value‡**	*P*=0.11	*P*=0.44	*P*=0.84

*Contrast Analysis*x			Pr > F		
			Long shoot	Medium shoot	Short shoot
All rootstocks	***vs***	**GN (Control)**	ns	ns	ns
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.21, No.71, No.101 & GN** (*HV group*)	ns	ns	ns
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.45, No.55, No.84, No.85, No.86** (*IV group*)	ns	ns (*P*=0.11)	ns
No.45, No.55, No.84, No.85, No.86 (*IV group*)	***vs***	**No.21, No.71, No.101 & GN** (*HV group*)	*P*=0.02	ns	ns

Numbers in the parentheses are standard error of means (±).
‡The non-parametric ANOVA (Kruskal-Wallis Test).
Means sharing the same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. ns – Non-significant according to *Contrast Analysis* SAS.
Green cuttings (GN) - Self-rooted control.
*In order of increasing vigour according to the results in Chapter Two.
xLow vigour (*LV*), Intermediate vigour (*IV*) and High vigour (*HV*) according to the results on *shoot growth rate* in Figure 3.8.

Table 3.8. The mean proportion of terminated (short and medium shoots) and non-terminated (long shoots) of proleptic axillary shoots of 'Hayward' scions grafted onto inter-specific hybrid kiwifruit rootstocks (2013-2014).

Rootstock selections*	Parentages	Non-terminated	Terminated
No.8	***A. chi* x *A. mac***	0.38 (± 0.05) ^{abc}	0.62 (± 0.05) ^{cde}
No.19	***A. chi* x *A. mac***	0.33 (± 0.15) ^{abcde}	0.67 (± 0.15) ^{abcde}
No.100	***A. mac* x *A. mel***	0.18 (± 0.02) ^{de}	0.82 (± 0.02) ^{abc}
No.85	***A. mac***	0.34 (± 0.05) ^{abcd}	0.66 (± 0.05) ^{bcde}
No.101	***A. mac***	0.42 (± 0.16) ^{abcd}	0.58 (± 0.16) ^{bcde}
No.86	***A. mac* x *A. mel***	0.27 (± 0.03) ^{abcde}	0.73 (± 0.03) ^{abcde}
No.87	***A. poly***	0.17 (± 0.10) ^{de}	0.83 (± 0.10) ^{ab}
No.84	***A. poly***	0.24 (± 0.17) ^{bcde}	0.76 (± 0.17) ^{abc}
No.18	***A. chi* x *A. mac***	0.12 (± 0.03) ^{e}	0.88 (± 0.03) ^{a}
No.21 (n=2)	***A. chi* x *A. mac***	0.42 (± 0.02) ^{ab}	0.58 (± 0.02) ^{de}
No.45	***A. poly* x *A. chi***	0.21 (± 0.10) ^{abcde}	0.79 (± 0.32) ^{ab}
No.55	***A. poly* x *A. chi***	0.20 (± 0.05) ^{bcde}	0.80 (± 0.05) ^{abcd}
No.71(n=2)	***A. poly* x *A. chi***	0.60 (± 0.20) ^{a}	0.40 (± 0.20) ^{e}
GN	***A. deliciosa***	0.28 (± 0.18) ^{bcde}	0.72 (± 0.18) ^{abcd}
	***P*-value‡**	P=0.04	P=0.04

*Contrast Analysis*x			Pr > F	
			Non- terminated shoot	Terminated shoot
All rootstocks	***vs***	**GN (Control)**	ns	ns
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.21, No.71, No.101 & GN** (*HV group*)	ns (P=0.12)	ns (P=0.12)
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.45, No.55, No.84, No.85, No.86** (*IV group*)	ns (P=0.06)	ns (P=0.07)
No.45, No.55, No.84, No.85, No.86 (*IV group*)	***vs***	**No.21, No.71, No.101 & GN** (*HV group*)	P=0.007	P=0.007

Numbers in the parentheses are standard error of means (±).
‡The non-parametric ANOVA (Kruskal-Wallis Test).
Means sharing the same letters are not significantly different at P=0.05 according to $LSD_{0.05}$ test. ns – Non-significant according to *Contrast Analysis* SAS.
Green cuttings (GN) - Self-rooted control.
*In order of increasing vigour according to the results in Chapter Two.
xLow vigour (*LV*), Intermediate vigour (*IV*) and High vigour (*HV*) according to the results on *shoot growth rate* in Figure 3.8.

Table 3.9. The mean number of long, medium and short proleptic axillary shoots of 'Hayward' scions grafted onto inter-specific hybrid kiwifruit rootstocks at end of summer 2013-2014 growing season.

Rootstock selections*	Parentages	Means number of different shoot types						Mean total number of shoots
		Long		Medium		Short		
No.8	***A. chi* x *A. mac***	9.7 (± 3.5)	ǂ**3.0** a	6.3 (± 3.4)	ǂ**2.3** ab	9.3 (± 2.4)	ǂ**3.0** abc	25.3 (± 9.0) a
No.19	***A. chi* x *A. mac***	3.7 (± 2.0)	**1.5** a	2.3 (± 0.3)	**1.5** bc	7.7 (± 5.7)	**2.4** abcd	13.7 (± 5.9) ab
No.100	***A. mac* x *A. mel***	3.5 (± 0.3)	**1.9** a	4.8 (± 0.6)	**2.2** abc	11.8 (± 1.5)	**3.4** ab	20.0 (± 1.5) ab
No.85	***A. mac***	6.3 (± 0.6)	**2.5** a	9.5 (± 1.6)	**3.0** a	3.8 (± 1.0)	**1.9** bcd	19.5 (± 2.2) ab
No.86	***A. mac***	5.8 (± 0.5)	**2.4** a	9.8 (± 1.5)	**3.1** a	7.8 (± 3.5)	**2.6** abcd	23.3 (± 2.6) a
No.101	***A. mac* x *A. mel***	5.3 (± 0.3)	**2.3** a	8.3 (± 2.6)	**2.8** ab	5.3 (± 2.0)	**2.2** abcd	19.0 (± 4.0) ab
No.87	***A. poly***	4.7 (± 2.9)	**1.7** a	7.0 (± 1.7)	**2.6** ab	9.7 (± 1.9)	**3.1** abc	21.3 (± 5.7) a
No.84	***A. poly***	3.3 (± 1.9)	**1.7** a	7.7 (± 3.0)	**2.6** ab	13.0 (± 8.1)	**3.3** abc	24.0 (± 7.9) a
No.18	***A. chi* x *A. mac***	3.0 (± 0.9)	**1.7** a	6.0 (± 1.9)	**2.3** ab	15.8 (± 3.1)	**3.9** a	24.8 (± 1.5) a
No.21 (n=2)	***A. chi* x *A. mac***	6.0 (± 2.0)	**2.4** a	3.0 (± 2.0)	**1.6** bc	5.5 (± 1.5)	**2.3** abcd	14.5 (± 5.5) ab
No.45	***A. poly* x *A. chi***	3.7 (± 1.5)	**1.8** a	5.7 (± 2.8)	**1.9** abc	4.0 (± 2.3)	**1.6** cd	13.3 (± 6.9) ab
No.55	***A. poly* x *A. chi***	4.5 (± 2.3)	**1.9** a	10.0 (± 4.0)	**3.0** ab	2.7 (± 3.2)	**2.7** abcd	22.5 (± 5.5) a
No.71(n=2)	***A. poly* x *A. chi***	3.0 (± 1.0)	**1.7** a	1.0 (± 1.0)	**0.7** c	1.0 (± 0.0)	**1.0** cd	5.0 (± 0.0) b
GN	***A. deliciosa***	8.0 (± 7.0)	**2.2** a	5.3 (± 1.7)	**2.2** ab	7.3 (± 3.0)	**2.6** abcd	20.7 (± 8.4) a
	P-value	*P*=0.81		*P*=0.18		*P*=0.13		*P*=0.52

*Contrast Analysis*x			Pr > F			
			Long shoot	Medium shoot	Short shoot	Mean total number of shoots
All rootstocks	***vs***	**GN (Control)**	ns	ns	ns	ns
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.21, No.71, No.101 & GN** (*HV group*)	ns	ns	ns	ns
No.8, No.18, No.19, No.87, No.100 (*LV group*)	***vs***	**No.45, No.55, No.84, No.85, No.86** (*IV group*)	ns	*P*=0.03	ns	ns
No.45, No.55, No.84, No.85, No.86 (*IV group*)	***vs***	**No.21, No.71, No.101 & GN** (*HV group*)	ns	ns (*P*=0.08)	ns	ns

Data are means of raw data (±standard error of means).

ǂData in bold were transformed means using log transformation.

Means sharing same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. ns – Non-significant according to *Contrast Analysis* SAS.

xMissing data, Green cuttings (GN)- Self-rooted control.

*In order of increasing vigour according to the results in Chapter Two.

xLow vigour (*LV*), Intermediate vigour (*IV*) and High vigour (*HV*) according to the results on *shoot growth rate* in Figure 3.8.

3.3.2.2.5 The mean length, node number and internode length of proleptic axillary shoots at 2013-2014 growing season

The mean length, node number and internode length of long proleptic axillary shoots of scions was not significantly affected by the rootstocks (Table 3.10). Although not statistically different, the mean length of long shoots of scions from low vigour rootstock, for example rootstock No.18, had the shortest mean shoot length (1068.0 mm) compared to other rootstocks. In addition, rootstock No.101 which imparted the fastest growth rate of long shoots (Figure 3.7) had the longest mean shoot length with 1681.7 mm (Table 3.10). Variability in the mean node number and internode length were also observed on the long proleptic axillary shoots produced from all rootstocks. There were no significant differences in the mean length, node number and internode length of medium shoots (Table 3.10). However, the mean length and internode length of medium shoots from rootstock No.87 (that has been thought to be in the low-vigour rootstock class) were slightly reduced compared to other rootstocks. For short shoots, there was a strong tendency (P=0.06) that inter-specific hybrid rootstocks may affect the mean length of this shoot, even though most of the rootstocks produced similar mean node number. The differences in the mean length of short shoots may contribute to the significant differences in the mean internode length of short shoots (P=0.02). Rootstocks No.45, No.55 and green-cuttings (GN) produced the lowest mean internode length compared to other rootstocks. In summary, the mean length, node number and internode length of scion proleptic shoots were varied between inter-specific hybrid rootstocks. There was a strong positive linear relationship (R^2=0.91) between the final length and node number of long and medium shoots, while for short shoots, a quadratic relationship (R^2=0.66) was found between final length and node number of short proleptic axillary shoots (Figure 3.9). Thus, the final lengths of proleptic axillary shoots were correlated well with the final node number of shoots, suggesting that at any individual shoot length, the node number is similar regardless of inter-specific hybrid kiwifruit rootstocks. Therefore, the internode length of long, medium and short proleptic shoots was not affected by the inter-specific hybrid kiwifruit rootstocks. The shoot CSA of long and short shoots of scions (Table 3.11) was significantly affected by the inter-specific hybrid rootstocks (P=0.02 and P<0.0001, respectively). However, inter-specific hybrid rootstocks did not affect (P=0.38) the shoot cross-sectional area of medium shoots of scions (Table 3.11).

Table 3.10. The characteristics of different proleptic shoots (long, medium and short) of 'Hayward' scions grafted onto inter-specific hybrid kiwifruit rootstocks.

Rootstock selections	Parentages	Mean length (mm)	Mean node number	Mean internode length (mm)
Long shoots (non-terminated)				
No.8	***A. chi* x *A. mac***	1343.3 (± 127.4) ^{ab}	22.8 (± 1.7) ^{a}	58.5 (± 2.6) ^{a}
No.19	***A. chi* x *A. mac***	1252.5 (± 226.1) ^{abc}	24.0 (± 2.9) ^{a}	51.0 (± 3.7) ^{abc}
No.100	***A. mac* x *A. mel***	1453.3 (± 117.8) ^{ab}	27.0 (± 1.8) ^{a}	53.8 (± 2.7) ^{abc}
No.85	***A. mac***	1577.5 (± 141.3) ^{ab}	27.5 (± 2.6) ^{a}	57.8 (± 2.6) ^{a}
No.86	***A. mac***	1355.6 (± 106.3) ^{ab}	23.9 (± 1.4) ^{a}	56.6 (± 2.3) ^{abc}
No.101	***A. mac* x *A. mel***	1681.7 (± 103.7) ^{a}	28.3 (± 0.8) ^{a}	59.2 (± 2.7) ^{a}
No.87	***A. poly***	1222.5 (± 264.2) ^{ab}	25.3 (± 4.7) ^{a}	48.0 (± 6.5) ^{c}
No.84	***A. poly***	1244.3 (± 148.0) ^{abc}	21.4 (± 1.5) ^{a}	57.4 (± 3.5) ^{ab}
No.18	***A. chi* x *A. mac***	872.5 (± 134.8) ^{c}	21.4 (± 2.9) ^{a}	48.3 (± 3.5) ^{bc}
No.21	***A. chi* x *A. mac***	1362.5 (± 196.6) ^{ab}	25.3 (± 2.5) ^{a}	53.3 (± 3.5) ^{abc}
No.45	***A. poly* x *A. chi***	1415.0 (± 158.7) ^{ab}	25.8 (± 1.9) ^{a}	54.5 (± 2.0) ^{abc}
No.55	***A. poly* x *A. chi***	1411.7 (± 175.7) ^{ab}	26.5 (± 2.7) ^{a}	52.7 (± 2.8) ^{abc}
No.71	***A. poly* x *A. chi***	1295.0 (± 189.9) ^{abc}	25.0 (± 1.1) ^{a}	51.1 (± 5.2) ^{abc}
GN	***A. deliciosa***	1325.0 (± 112.8) ^{ab}	26.3 (± 1.3) ^{a}	50.0 (± 2.8) ^{abc}
***P*-value**		***P*=0.24**	***P*=0.33**	***P*=0.14**
Medium shoots (terminated)				
No.8	***A. chi* x *A. mac***	571.9 (± 56.1) ^{a}	16.3 (± 1.2) ^{abc}	35.0 (± 2.0) ^{a}
No.19	***A. chi* x *A. mac***	462.5 (± 74.7) ^{a}	12.5 (± 1.8) ^{bc}	36.5 (± 4.5) ^{a}
No.100	***A. mac* x *A. mel***	552.9 (± 73.8) ^{a}	14.4 (± 1.1) ^{abc}	38.2 (± 2.3) ^{a}
No.85	***A. mac***	469.4 (± 35.0) ^{a}	13.2 (± 0.7) ^{bc}	35.3 (± 1.1) ^{a}
No.86	***A. mac***	458.1 (± 49.5) ^{a}	13.8 (± 1.1) ^{bc}	33.0 (± 1.6) ^{a}
No.101	***A. mac* x *A. mel***	681.7 (± 63.0) ^{a}	16.5 (± 1.2) ^{ab}	39.1 (± 2.0) ^{a}
No.87	***A. poly***	677.5 (± 85.0) ^{a}	18.5 (± 1.5) ^{a}	24.1 (± 3.4) ^{a}
No.84	***A. poly***	410.7 (± 37.9) ^{a}	13.3 (± 0.7) ^{bc}	30.8 (± 2.5) ^{a}
No.18	***A. chi* x *A. mac***	483.6 (± 67.4) ^{a}	14.0 (± 1.2) ^{abc}	35.5 (± 1.8) ^{a}
No.21	***A. chi* x *A. mac***	472.0 (± 64.3) ^{a}	12.6 (± 0.7) ^{bc}	36.9 (± 4.0) ^{a}
No.45	***A. poly* x *A. chi***	660.0 (± 63.8) ^{a}	16.8 (± 1.6) ^{ab}	35.6 (± 5.4) ^{a}
No.55	***A. poly* x *A. chi***	521.0 (± 57.4) ^{a}	14.2 (± 0.8) ^{abc}	35.8 (± 2.5) ^{a}
No.71	***A. poly* x *A. chi***	370.0 (± 30.0) ^{a}	11.5 (± 0.5) ^{c}	32.1 (± 1.2) ^{a}
GN	***A. deliciosa***	604.2 (± 63.2) ^{a}	15.7 (± 1.3) ^{ab}	35.9 (± 1.6) ^{a}
***P*-value**		***P*=0.24**	***P*=0.15**	***P*=0.22**
Short shoots (terminated)				
No.8	***A. chi* x *A. mac***	161.4 (± 18.3) ^{abcd}	8.9 (± 0.6) ^{bc}	21.5 (± 1.9) ^{abc}
No.19	***A. chi* x *A. mac***	202.5 (± 25.0) ^{ab}	9.0 (± 0.4) ^{ab}	22.4 (± 2.4) ^{ab}
No.100	***A. mac* x *A. mel***	175.0 (± 10.9) ^{abcd}	8.0 (± 0.3) ^{abc}	21.9 (± 1.0) ^{abc}
No.85	***A. mac***	182.5 (± 35.7) ^{abc}	8.3 (± 0.9) ^{abc}	21.6 (± 2.2) ^{abc}
No.86	***A. mac***	215.0 (± 23.3) ^{a}	9.0 (± 0.5) ^{a}	22.9 (± 3.0) ^{a}
No.101	***A. mac* x *A. mel***	183.3 (± 18.6) ^{abc}	8.3 (± 0.7) ^{abc}	22.1 (± 1.7) ^{abc}
No.87	***A. poly***	207.8 (± 19.8) ^{a}	8.9 (± 0.3) ^{ab}	23.2 (± 1.9) ^{a}
No.84	***A. poly***	191.7 (± 21.5) ^{ab}	8.8 (± 0.6) ^{ab}	21.7 (± 1.8) ^{abc}
No.18	***A. chi* x *A. mac***	212.5 (± 24.2) ^{a}	9.0 (± 0.4) ^{ab}	23.1 (± 1.7) ^{a}
No.21	***A. chi* x *A. mac***	115.0 (± 15.0) ^{cd}	7.0 (± 1.0) ^{c}	16.5 (± 0.2) ^{abc}
No.45	***A. poly* x *A. chi***	135.0 (± 21.2) ^{bcd}	8.2 (± 0.7) ^{abc}	16.2 (± 1.4) ^{cd}
No.55	***A. poly* x *A. chi***	206.7 (± 27.8) ^{ab}	8.6 (± 0.5) ^{abc}	23.3 (± 2.2) ^{a}
No.71	***A. poly* x *A. chi***	95.0 (± 30.1) ^{d}	8.0 (± 2.7) ^{abc}	12.0 (± 2.6) ^{d}
GN	***A. deliciosa***	178.2 (± 13.2) ^{abc}	8.9 (± 0.5) ^{ab}	19.9 (± 0.9) ^{abc}
***P*-value**		***P*=0.06**	***P*=0.37**	***P*=0.02**

Numbers in the parentheses are standard error of means.

Means sharing same letters are not significantly different at P=0.05 according to $LSD_{0.05}$ test.

*In order of increasing vigour according to the results in Chapter Two.

Table 3.11. The mean shoot CSA (mm^2) of long, medium and short proleptic axillary shoots of 'Hayward' scions grafted onto inter-specific kiwifruit hybrid rootstocks in summer 2014.

Rootstock selection*	Parentages	Mean shoot cross-sectional area (mm^2)		
		Long	Medium	Short
No.8	***A. chi* x *A. mac***	113.5 (± 9.7) ǂ**4.7** cd	62.8 (± 4.1) ǂ**4.1** a	27.3 (± 2.2) ǂ**3.3** ab
No.19	***A. chi* x *A. mac***	107.4 (± 14.0) **4.6** d	54.1 (± 5.4) **4.0** ab	33.1 (± 5.2) **3.4** a
No.100	***A. mac* x *A. mel***	131.5 (± 8.0) **4.9** abcd	51.7 (± 3.7) **3.9** ab	33.5 (± 3.1) **3.5** a
No.85	***A. mac***	166.5 (± 16.5) **5.0** ab	57.0 (± 4.7) **4.0** ab	27.5 (± 2.4) **3.3** ab
No.86	***A. mac***	156.5 (± 13.9) **5.0** abc	54.9 (± 2.2) **4.0** ab	30.2 (± 2.5) **3.4** ab
No.101	***A. mac* x *A. mel***	134.9 (± 15.3) **4.8** abcd	47.8 (± 3.3) **3.9** b	27.7 (± 1.4) **3.3** ab
No.87	***A. poly***	118.5 (± 17.2) **4.7** bcd	48.2 (± 3.8) **3.9** ab	33.0 (± 5.1) **3.5** a
No.84	***A. poly***	121.0 (± 14.7) **4.8** bcd	53.2 (± 6.0) **3.9** ab	24.3 (± 1.7) **3.2** b
No.18	***A. chi* x *A. mac***	112.1 (± 9.7) **4.7** cd	46.5 (± 2.9) **3.8** b	18.8 (± 1.3) **2.9** c
No.21 (n=2)	***A. chi* x *A. mac***	114.4 (± 12.9) **4.7** cd	57.7 (± 5.4) **4.0** ab	26.7 (± 1.6) **3.3** ab
No.45	***A. poly* x *A. chi***	141.0 (± 17.0) **4.9** abcd	50.7 (± 6.5) **3.9** ab	26.7 (± 1.8) **3.3** ab
No.55	***A. poly* x *A. chi***	162.9 (± 10.6) **5.1** a	52.5 (± 3.5) **3.9** ab	30.0 (± 2.4) **3.4** ab
No.71 (n=2)	***A. poly* x *A. chi***	106.7 (± 13.6) **4.6** d	58.7 (± 2.2) **4.1** ab	-[x] -[x]
GN	***A. deliciosa***	123.8 (± 8.4) **4.8** abcd	50.1 (± 3.2) **3.8** b	30.6 (± 2.0) **3.4** ab
***P*-value**		*P*=0.02	*P*=0.38	*P*<0.0001

Data are means of raw data (±standard error of means).
ǂData in bold were transformed means using log transformation.
Means sharing same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
[x]Missing data, Green cuttings (GN)- Self-rooted control.
*In order of increasing vigour according to the results in Chapter Two.

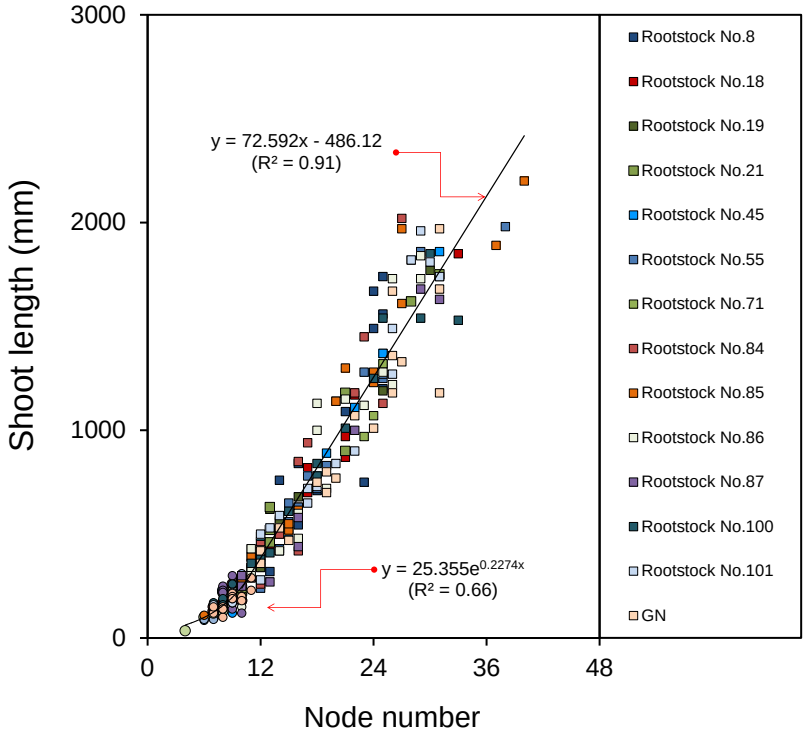


Figure 3.8. The relationship between final length and node number of proleptic axillary shoots of 'Hayward' scions. The length and node number of proleptic axillary long, medium and short shoots are pooled in the same graph.

3.3.3 Rootstock effects on the total node number and node number distribution of proleptic axillary shoots

3.3.3.1 The total node number of shoots in 2012-2013 growing season

At the end 2012-2013 growing season, the mean total node number of proleptic shoots was not significantly affected (P=0.45) by the inter-specific hybrid kiwifruit rootstocks (Figure 3.9A). Nevertheless, rootstocks No.55, No.85 and No.86 produced the highest mean total node number of shoots than other rootstocks (Figure 3.9A). Conversely, scions on rootstocks No.100, No.101 and also GN produced significantly lower mean total node number compared to rootstock No.85. Unfortunately, no data were recorded for rootstocks No.21 and No.71 because some of the vines still small due to re-planting (Figure 3.9A).

3.3.3.2 The total node number of shoots in 2013-2014 growing season

No significant difference was recorded (P=0.38) on the mean total node number of shoots at the end of growing season of 2013-2014 (Figure 3.9B). Although not significant, based on the raw data, variation in the mean node number of scion shoots was observed between inter-specific hybrid kiwifruit rootstocks (Figure 3.9B). Scions on rootstocks No.8, No.85, No.86 and GN had the highest mean total node number compared to other rootstocks. In contrast, the lowest mean total node number was recorded on rootstocks No.18, No.19, No.87 and No.100. Even though scions on rootstocks No.21 and No.71 also produced the lowest mean total node number, it could not be confirmed because only two replicates available for these rootstocks (Figure 3.9B). Although no significant difference was detected (P=0.34), the combined mean total node number for both growing seasons varied between inter-specific hybrid kiwifruit rootstocks (Figure 3.9C). Scions on rootstocks No.85, No.86, No.55 and No.8 had the higher mean total node number than scions on other rootstocks. In contrast, scions on rootstock No.19 had the lowest mean total node number with almost 35 to 40 % lower than rootstocks No.85, No.86, No.55 and No.8 (Figure 3.9C).

3.3.3.3 The node number distribution in 2013-2014 growing season

The distribution of the node number of proleptic axillary shoots in summer 2013-2014 growing season varied between inter-specific hybrid kiwifruit rootstocks, especially the proportion of shoots that have 6 to 10 nodes (Figure 3.10). The increased mean proportion of terminated shoots of the scions on rootstocks No.18, No.87, No.100 and also No. 84 (Table 3.7) were a result of increase in the proportion of shoots with 15 or less nodes, and reduction in proportion of shoots with more than 15 nodes (Figure 3.10). The scion on rootstock No.19 also had a higher proportion of shoots with 10 or less nodes and lower proportion of shoots with 11 to 15 nodes (Figure 3.10). GN also had a higher proportion of shoots with 6 to 15 nodes. However, less proportion of shoots with 1 to 5 nodes and substantially increased proportion of shoots with more than 15 nodes were recorded on GN vines (Figure 3.10). Other inter-specific hybrid kiwifruit rootstocks produced a considerably lower proportion of shoots with 15 or less nodes, and increased proportion of shoots with more than 15 nodes, especially on rootstocks No.45, No.55, No.85 and No.101 (Figure 3.10).

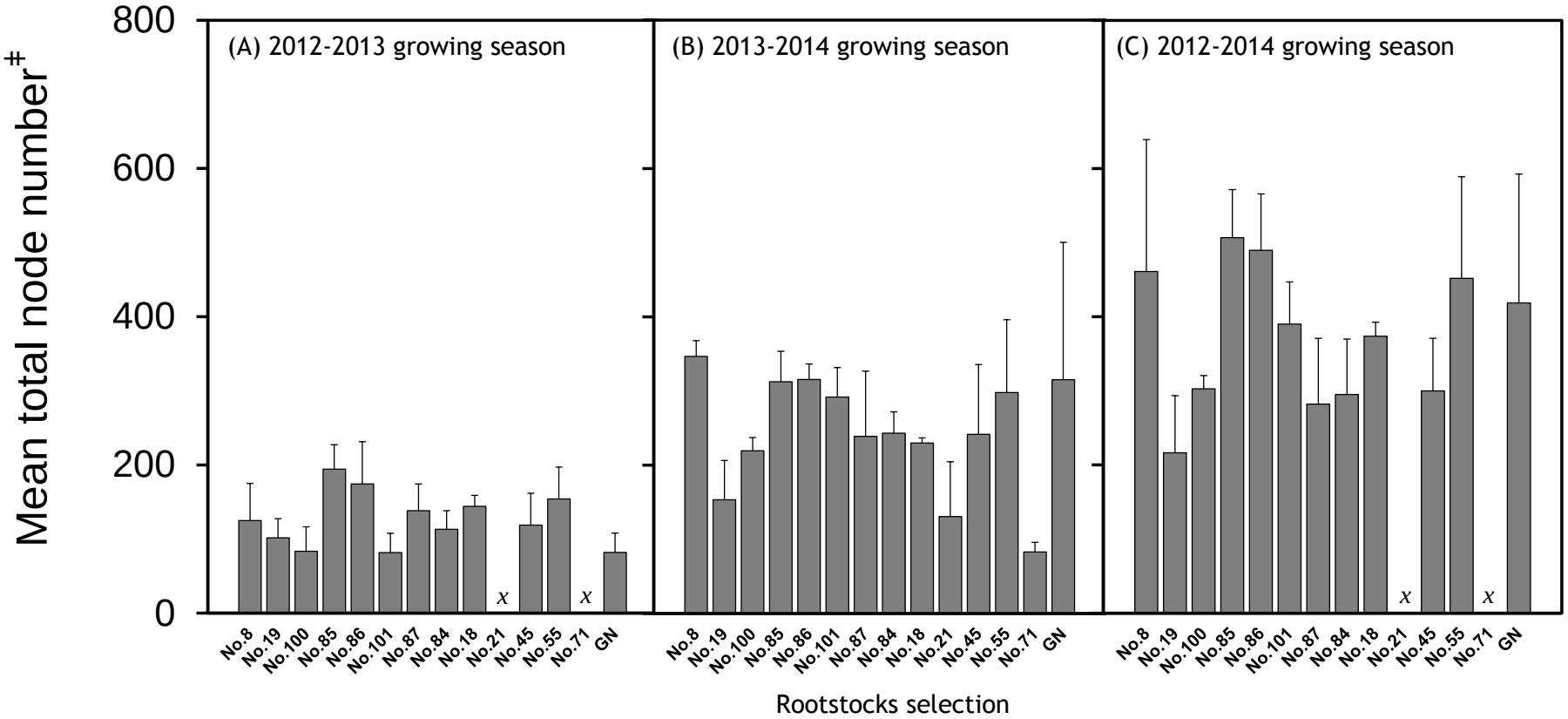


Figure 3.9. ‡Mean total node number of 'Hayward' scions during growing season of 2012-2014. Vertical bars indicate standard error of means. [x] No data were recorded for rootstocks number 21 and 71 due to replanting in 2012-2013 growing season, and excluded in graph C.

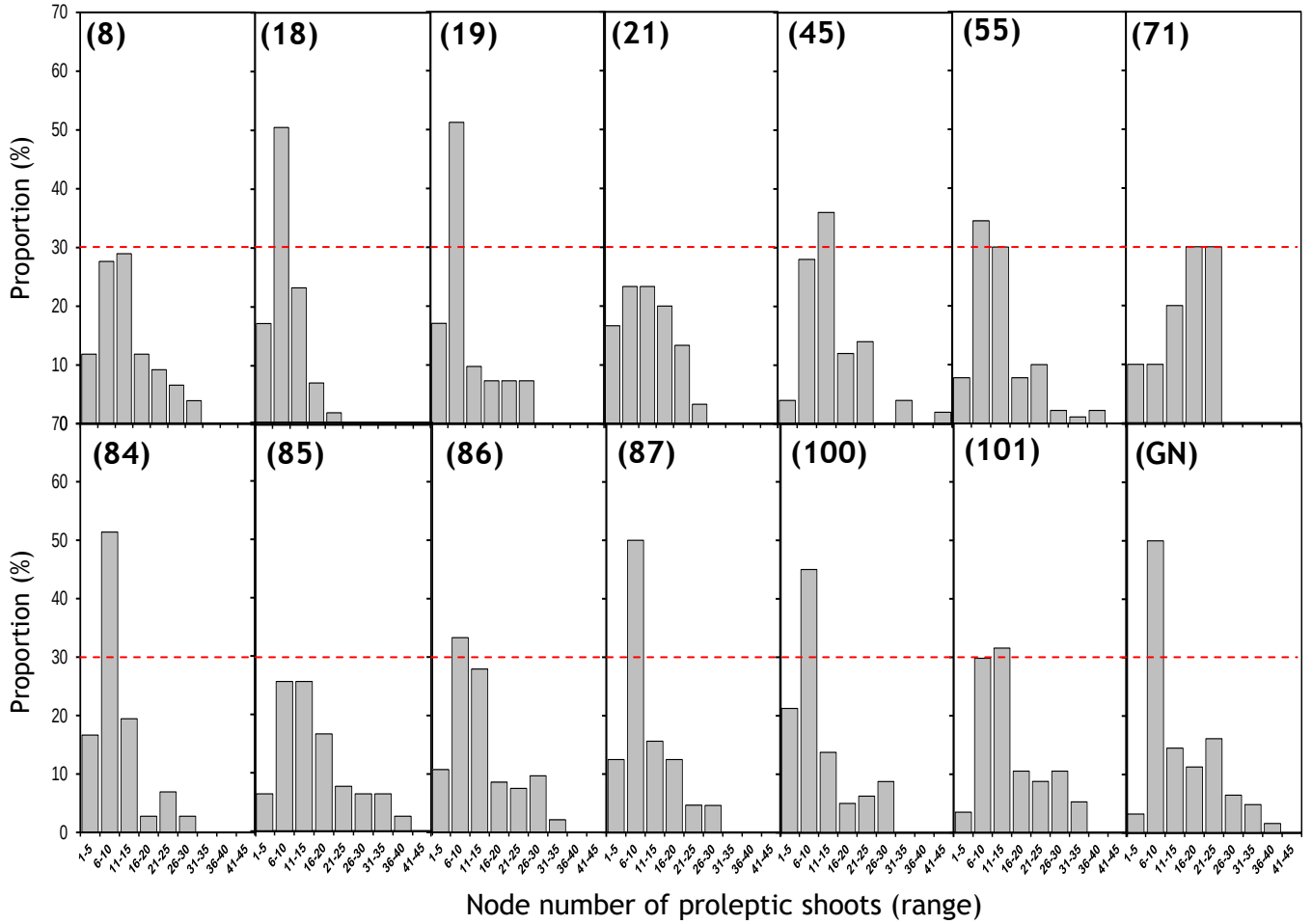


Figure 3.10. The node number distributions of the proleptic axillary shoots at the end of 2013-2014 growing season for each rootstock. Numbers in the parentheses are rootstock selections.

3.3.4 Trunk CSA of scions and rootstocks in 2012-2013 growing season

The mean trunk CSA of rootstocks was significantly different between rootstocks (*P*=0.01, Table 3.12). The mean trunk CSA of rootstocks of No.19 and No.101 was significantly smaller compared with control vines (i.e. cuttings) with 164.5 mm^2 and 188.3 mm^2, respectively. However, the mean trunk CSA of other rootstocks did not significantly differ compared to the control vines (GN), even though some of the rootstocks had slightly smaller trunk CSA, for example in rootstocks No.8 and No.18. The largest mean trunk CSA was recorded for rootstocks No.55 (374.7 mm^2). Rootstocks also significantly differed in the mean trunk CSA of scions (*P*=0.009, Table 3.12). Scions on rootstocks No.19 and No.101 exhibited the smallest mean trunk CSA with 221.7 mm^2 and 249.0 mm^2, respectively. This was approximately two times smaller than other rootstocks (Table 3.12). The largest scion trunk CSA was on rootstock No.55. There was a positive linear relationship (R^2=0.67, *P*<0.0001) between the trunk CSA of rootstocks and the trunk CSA of grafted scions (Figure 3.11). Therefore, the rootstocks that have smaller trunk CSA tended to have smaller trunk CSA of scions.

Table 3.12. Trunk CSA of kiwifruit rootstocks and scions (mm^2) in summer 2013 (early February 2013).

Rootstock selections*	Parentages	Rootstock CSA (mm^2)	Scion CSA (mm^2)
No.8	***A. chi* x *A. mac***	207.3 (± 38.3) abc	244.6 (± 31.1) cd
No.19	***A. chi* x *A. mac***	164.5 (± 19.3) c	221.7 (± 13.5) d
No.100	***A. mac* x *A. mel***	300.2 (± 40.3) abc	417.7 (± 68.7) abcd
No.85	***A. mac***	348.2 (± 64.7) ab	555.3 (± 103.4) a
No.86	***A. mac***	284.3 (± 16.6) abc	424.4 (± 25.9) abc
No.101	***A. mac* x *A. mel***	188.3 (± 42.2) bc	249.0 (± 45.4) cd
No.87	***A. poly***	328.7 (± 15.0) abc	446.9 (± 12.4) ab
No.84	***A. poly***	341.0 (± 77.8) ab	371.6 (± 55.7) abcd
No.18	***A. chi* x *A. mac***	247.4 (± 8.3) abc	294.3 (± 16.0) bcd
No.45	***A. poly* x *A. chi***	361.3 (± 149.0) ab	384.0 (± 137.0) abcd
No.55	***A. poly* x *A. chi***	374.7 (± 21.6) a	545.6 (± 55.6) a
GN	***A. deliciosa***	370.0 (± 87.3) a	370.0 (± 87.3) abcd
***P*-value**		*P*=0.01	*P*=0.009

Means sharing same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
Numbers in parentheses are standard error of means (±).
No data were recorded for rootstocks number 21 and 71 due to replanting.
Green cuttings (GN)- Self-rooted control.
*In order of increasing vigour according to the results in Chapter Two.

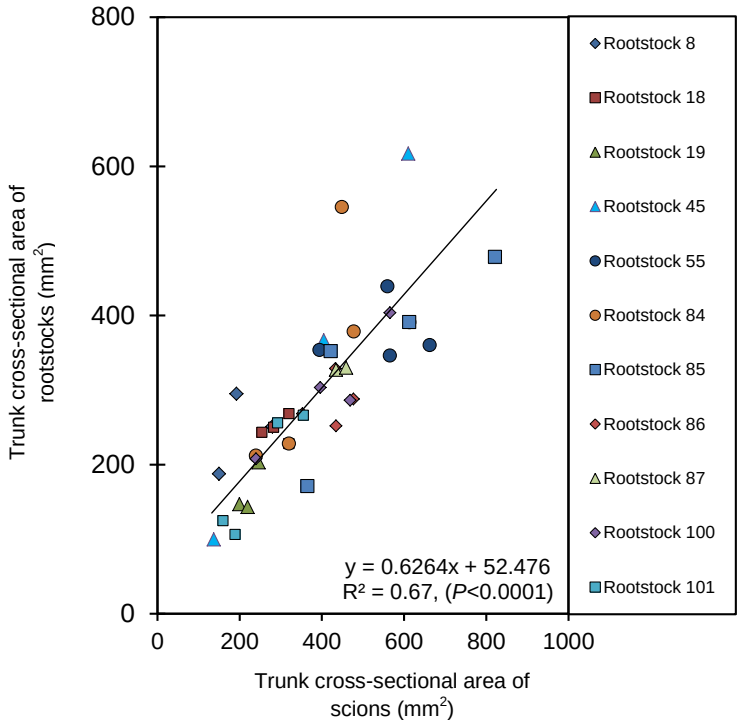


Figure 3.11. The relationship between trunk cross-sectional area of rootstocks (mm^2) and trunk cross-sectional area of scions (mm^2) in summer 2013 (early February 2013).

3.3.5 Rootstock effects on the proportions of flowering proleptic shoots and flowering in 2013-2014 growing season

The mean proportion of flowering and non-flowering shoots did not significantly differ between rootstocks (P=0.70 and P=0.75, respectively) (Table 3.13). However, there was a trend that some rootstocks may have increased the proportion of flowering shoots such as rootstocks No.19, No.85, No.86 and No.101. Since few flowers were produced from these young vines, some of which still do not have well-developed canopies, only the mean total flower and fruit number per vines were reported. The mean flower number per vine, fruit number per vine and fruit setting (%) of 'Hayward' scions were also not significantly affected by the rootstocks (P=0.36, P=0.20 and P=0.23, respectively) (Table 3.14). Similarly, the mean flower number per lateral shoot and flower number per winter bud also did not significantly different between inter-specific hybrid kiwifruit rootstocks (P=0.28 and P=0.18, respectively).

However, there was a trend that rootstocks No.18, No.100 and No.85 may have increased the mean number of flowers per winter bud (Table 3.14). Unfortunately, after flowering assessment, three vines from rootstock No.87 died (sudden death) without knowing the cause. Therefore, these three vines were removed immediately to avoid any disease or virus infections to other vines. Data on number of fruits and fruit setting could not be collected and reported from this rootstock (i.e. rootstock No.87, Table 3.14).

Table 3.13. The mean proportion of flowering and non-flowering shoots of 'Hayward' scions grafted onto inter-specific hybrid kiwifruit rootstocks.

Rootstock selections*	**Parentages**	**Mean proportion of flowering shoots**	**Mean proportion of non-flowering shoots**
No.8	***A. chi* x *A. mac***	0.68 (± 0.10) ^{ab}	0.32 (± 0.10) ^{ab}
No.19	***A. chi* x *A. mac***	0.71 (± 0.15) ^{ab}	0.29 (± 0.15) ^{ab}
No.100	***A. mac* x *A. mel***	0.65 (± 0.05) ^{ab}	0.35 (± 0.05) ^{ab}
No.85	***A. mac***	0.80 (± 0.09) ^{a}	0.20 (± 0.09) ^{ab}
No.86	***A. mac***	0.70 (± 0.14) ^{ab}	0.30 (± 0.14) ^{ab}
No.101	***A. mac* x *A. mel***	0.72 (± 0.07) ^{a}	0.28 (± 0.07) ^{ab}
No.87	***A. poly***	0.60 (± 0.02) ^{ab}	0.40 (± 0.02) ^{a}
No.84	***A. poly***	0.49 (± 0.21) ^{ab}	0.51 (± 0.21) ^{a}
No.18	***A. chi* x *A. mac***	0.68 (± 0.04) ^{a}	0.32 (± 0.04) ^{ab}
No.21 (n=2)	***A. chi* x *A. mac***	0.66 (± 0.01) ^{ab}	0.34 (± 0.01) ^{ab}
No.45	***A. poly* x *A. chi***	0.63 (± 0.15) ^{ab}	0.37 (± 0.15) ^{a}
No.55	***A. poly* x *A. chi***	0.57 (± 0.17) ^{ab}	0.43 (± 0.17) ^{ab}
No.71 (n=2)	***A. poly* x *A. chi***	0.00 (± 0.00) ^{b}	0.00 (± 0.00) ^{b}
GN	***A. deliciosa***	0.51 (± 0.11) ^{ab}	0.49 (± 0.11) ^{a}
***P*-value‡**		*P*=0.70	*P*=0.75

Numbers in the parentheses are standard error of means (±).
‡The non-parametric ANOVA (Kruskal-Wallis Test).
Means sharing the same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
*In order of increasing vigour according to the results in Chapter Two.

Table 3.14. The mean number of flowers per vine, fruit number per vine, fruit setting (%), flower number per lateral shoot and flower number per winter bud of 'Hayward' scions grafted onto inter-specific hybrid kiwifruit rootstocks.

Rootstock selection*	Parentages	Mean number of flowers/vine	Mean number of fruits/vine	Mean fruit setting (%)	Mean flower number/lateral shoots	Mean flower number/winter buds
No.8	***A. chi* x *A. mac***	40.3 (± 17.6) ^{a}	22.7 (± 13.3) ^{abc}	53.8 (± 11.0) ^{ab}	1.5 (± 0.41) ^{ab}	0.1 (± 0.02) ^{ab}
No.19	***A. chi* x *A. mac***	21.0 (± 8.7) ^{a}	11.3 (± 6.1) ^{abc}	44.7 (± 12.1) ^{ab}	1.6 (± 0.70) ^{abc}	0.1 (± 0.02) ^{a}
No.100	***A. mac* x *A. mel***	34.3 (± 5.4) ^{a}	30.5 (± 7.4) ^{ab}	86.6 (± 10.1) ^{a}	1.8 (± 0.35) ^{ab}	0.2 (± 0.01) ^{a}
No.85	***A. mac***	49.0 (± 11.7) ^{a}	42.3 (± 9.7) ^{a}	88.9 (± 5.2) ^{a}	2.4 (± 0.45) ^{a}	0.2 (± 0.03) ^{a}
No.86	***A. mac***	42.6 (± 11.1) ^{a}	34.6 (± 7.8) ^{ab}	84.6 (± 5.0) ^{a}	1.8 (± 0.46) ^{ab}	0.1 (± 0.03) ^{a}
No.101	***A. mac* x *A. mel***	29.7 (± 5.6) ^{ab}	21.0 (± 10.1) ^{abc}	60.9 (± 27.8) ^{a}	1.6 (± 0.09) ^{ab}	0.1 (± 0.01) ^{ab}
No.87	***A. poly***	44.0 ^{x}	33.0 ^{x}	- ^{x}	- ^{x}	- ^{x}
No.84	***A. poly***	32.0 (± 15.0) ^{ab}	10.0 (± 4.0) ^{bc}	42.5 (± 16.6) ^{ab}	1.0 (± 0.41) ^{abc}	0.1 (± 0.05) ^{ab}
No.18	***A. chi* x *A. mac***	34.8 (± 2.4) ^{a}	24.8 (± 5.9) ^{ab}	71.2 (± 17.3) ^{ab}	1.4 (± 0.12) ^{ab}	0.2 (± 0.01) ^{a}
No.21 (n=2)	***A. chi* x *A. mac***	25.0 (± 14.0) ^{ab}	16.5 (± 14.5) ^{abc}	48.8 (± 30.7) ^{ab}	1.1 (± 0.57) ^{abc}	0.1 (± 0.04) ^{ab}
No.45	***A. poly* x *A. chi***	22.7 (± 11.5) ^{ab}	18.0 (± 10.7) ^{abc}	51.6 (± 28.9) ^{ab}	1.1 (± 0.58) ^{abc}	0.1 (± 0.03) ^{ab}
No.55	***A. poly* x *A. chi***	42.8 (± 17.6) ^{a}	35.7 (± 13.2) ^{ab}	66.0 (± 22.6) ^{a}	1.7 (± 0.56) ^{ab}	0.1 (± 0.04) ^{a}
No.71 (n=2) ^{x}	***A. poly* x *A. chi***	0.0 (± 0.0) ^{b}	0.0 (± 0.0) ^{c}	0.0 (± 0.0) ^{b}	0.0 (± 0.00) ^{b}	0.0 (± 0.00) ^{b}
GN	***A. deliciosa***	17.0 (± 8.5) ^{ab}	14.0 (± 7.1) ^{abc}	55.4 (± 28.7) ^{ab}	1.0 (± 0.34) ^{bc}	0.1 (± 0.04) ^{ab}
***P*-value**‡		*P*=0.36	*P*=0.20	*P*=0.23	*P*=0.28	*P*=0.18

Numbers in the parentheses are standard error of means (±).
‡The non-parametric ANOVA (Kruskal-Wallis Test).
^{x}Data not available or raw data.
Means sharing the same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
*In order of increasing vigour according to the results in Chapter Two.

3.4 Discussion

The objectives of this trial, carried out over two seasons, were to evaluate the extent to which the selected rootstocks planted in the field affected scion vigour, over-all architecture and stability of the differences between rootstock effects on scion growth noted in Chapter Two. A key finding of this chapter was the initial vigour ranking of 'Hayward' scion in Chapter Two did not appear to continue into the next following season when the vines were planted in the field. A new vigour ranking was proposed based on the findings in this chapter.

3.4.1 Effects of rootstocks on growth and shoot architectural structures of 'Hayward' scions

3.4.1.1 Shoot growth of proleptic axillary shoots

In fruit trees, rootstocks may influence the scion growth by affecting the onset of shoot extension in the spring, and the growth rates of shoot extension (Rom, 1996; Webster, 1995a; Webster, 2004). In addition, several reports also indicate that vigour-controlling rootstocks markedly reduce scion shoot growth as found in apple (Rom, 1996; Webster, 1995a) and peach (Weibel et al., 2003). Similar to kiwifruit, Clearwater et al. (2006) demonstrated that low-vigour rootstocks produced higher proportions of slower-growing shoots on grafted 'Hort16A' scions. Therefore, in our study, the shoot development of all types of shoot (long, medium and short shoots) in terms of their length (mm) were measured and recorded. During spring 2013, after approximately 50% of bud break (21 days after initial bud break), the length of each selected shoot that developed from the cordons was measured weekly (Figure 3.7). At the third week (19/9/2013), the differences between long, medium and short shoots were still unknown because all shoots appeared similar in their morphology. The results in the present study showed, regardless of rootstocks that differences in the shoot extension for all shoot types could clearly be observed in the first month after the buds were released from dormancy (Figure 3.7). This result was consistent with the previous study by Clearwater et al., (2006) on 'Hort16A' scions when grafted onto *A. hemsleyana, A. macrosperma,*

A. polygama and *A. kolomikta* rootstocks. They reported that the differences between long, medium and short shoot of 'Hort16A' scions were detectable during the first month of scion growth (Clearwater et al., 2006).

It was also observed that long shoots grew faster than medium shoots, and short shoots grew slower than medium shoots (Figure 3.7), similar to results reported by Clearwater et al. (2006). It was interesting to note that towards to the end of the measurement dates (week 6 until 9), most of the long shoots of scion on rootstocks No.8, No.18, No.19, No.87 and No.100 grew much slower compared to the long shoots from other rootstocks (Figure 3.7). Therefore, these results indicate that particular inter-specific hybrid kiwifruit rootstocks may influence shoot extension, especially of long shoots of 'Hayward' scions, but less markedly influence other type of shoots (i.e. medium and short shoots). Similar to the previous study, scions on low-vigour kiwifruit rootstocks (i.e. *A. polygama* and *A. kolomikta*) also had a higher proportion of slower-growing shoots compared with scions on higher-vigour rootstocks (i.e. *A. hemsleyana* and *A. macrosperma*) (Clearwater et al., 2004; 2006). It was notable in our study that scions grafted onto hybrid No.87 that came from the selection of *A. polygama* species imparting the low-vigour ability. Meanwhile, vines on rootstock hybrids No.8, No.18, No.19 and No.100 that came from the crosses between *A. chinensis* and *A. macrosperma* also had shown low-vigour habit and grew much slower compared to other hybrid rootstocks (Figure 3.7).

Differences in the final length of long shoots (Table 3.10), imparted by inter-specific kiwifruit rootstocks could be due to early termination (Figure 3.7) and/or slower growth rate during development (Figure 3.8). Although long shoot of kiwifruit was defined as non-terminated (Seleznyova et al., 2002), but the growth of long shoot from particular rootstocks such as No.18, No.19, No.87 and No.100 was slowed down and almost stop. Both a slower early growth rate and more rapid slowing of growth (Figure 3.7 and Figure 3.8) could lead to shorter and less vigorous of long shoots. The patterns of shoot development of scions were further calculated in terms of the growth rates (mm $week^{-1}$) for every shoot for each scion on the inter-specific hybrid kiwifruit rootstocks (Figure 3.8). This data enabled us to identify which rootstocks may have imparted the slowest shoot growth rate of grafted 'Hayward' scions. Even though Clearwater et al., (2006) reported that low-vigour kiwifruit rootstocks had slower-growing shoots, however, their

study did not report the actual growth rates of different shoot types. In our study, we found that there were noticeable differences in the shoot growth rates of scions among inter-specific hybrid kiwifruit rootstocks (Figure 3.8). Our result clearly showed that the long shoots of rootstocks No.18 (*A. chinensis* x *A. macrosperma*) and No.87 (*A. polygama* selection) had the slowest shoot growth rates (mm week^{-1}) compared to other rootstocks, while rootstocks No.8, No.19 (*A. chinensis* x *A. macrosperma*) and No.100 (*A. macrosperma* x *A. melanandra*) also had slower growth rates (Figure 3.8). Conversely, the highest shoot growth rates of long shoots were of scions grafted onto rootstocks No.21 (*A. chinensis* x *A. macrosperma*), No.101 (*A. macrosperma* x *A. melanandra*) including 'Hayward' self-rooted control cuttings (GN). Therefore, it was suggested that rootstocks No.18, No.19, No.87 and No.100 could be classified as low-vigour rootstocks based on the slower growth rate imparted to the grafted 'Hayward' scions. It was also noted that termination of long shoots could be defined as the rapid decrease in growth rate to near zero (Figure 3.8), but may not involve abortion of apical. Thus, a long shoot of kiwifruit may more or less stop growing although the bud has not aborted.

Growth rates of the shoots have been used as a fundamental characteristic of rootstock vigour effects on the scion. For example, scions on apple dwarfing rootstock such as M.9 and M.27 also had the slowest growth rates compared with the scions on vigorous rootstocks such as MM.106 (Rom, 1996; Webster, 1995a). Furthermore, recent study also found that M.9 dwarfing rootstock decreased the growth rate of primary shoots of 'Royal Gala' scions (van Hooijdonk, 2009). In peach, selected scions on dwarfing rootstocks (e.g. K-146-44 and K-146-43) had the slowest growth rates than vigorous rootstocks (e.g. Nemaguard) (Weibel et al., 2003). Further observation on the results had also found that the peak of growth rate of non-terminated long shoots on rootstock No.18 occurred one week early (at five weeks after 50 % bud break) than other rootstocks. Therefore, in the present study, regardless of rootstocks, the peak times of growth rate of kiwifruit shoots occurred between the fifth and sixth weeks (Figure 3.8). In contrast with apple, the peak growth rate of shoots of 'Delicious' scions on various rootstocks occurred two to four weeks after bud break (Rom, 1996). Differences in the timing of growth rate may be caused by the differences in the growth habit between the tree and vine crops, considering that kiwifruit vine had a longer period of vegetative growth than apple trees (Palmer, 2007).

3.4.1.2 The proportion of proleptic axillary shoots

According to Clearwater et al. (2006), the probability of shoot termination in kiwifruit after bud break was correlated with their growth rate. Generally after bud break, shoots that were pre-determinated to terminate grew more slowly than non-terminated shoots (Clearwater et al., 2006). In the present study, the abortion of apical shoots (i.e. termination) occurred earlier on the most slower-growing shoots on low-vigour kiwifruit rootstocks as demonstrated by our findings (Figure 3.7 and Figure 3.8). These results are similar to those obtained by Clearwater et al. (2006) with non-hybrid rootstocks. In further detail of our results, the proportions of different shoot types of scions were classified according to description made by Seleznyova et al. (2002). Even though lack of statistical significance on the effect of rootstocks on the proportions of long, medium and short shoots (Table 3.2 and Table 3.7), contrast analyses on the selected rootstock groups based on their vigour classes (i.e. low, intermediate and high vigour) indicate that the proportion of proleptic axillary shoots (especially long and medium shoots) may be affected by inter-specific hybrid rootstocks (contrast analyses in Table 3.2 and Table 3.7). It was also observed that the proportions of long proleptic axillary shoots of scions were lower in rootstocks No.18, No.19, No.87 and No.100, and higher proportions of short shoots were recorded on the same rootstocks (Table 3.7). Contrast analyses on the selected rootstock groups indicate that inter-specific hybrid kiwifruit rootstocks may also affect the proportion on terminated and non-terminated shoots (Table 3.3 and Table 3.8).

In growing season 2013-2014, rootstocks No.18, No.87 and No.100 including rootstocks No.19 and No.84 produced higher proportions of terminated shoots (i.e. short and medium shoots), and substantially lower proportions of non-terminated shoots (i.e. long shoots) (Table 3.7). These effects have contributed to the larger differences in the proportion of terminated and non-terminated shoots in kiwifruit (Table 3.8). Most of the low-vigour kiwifruit rootstocks produced higher proportion of terminated shoots on the grafted scions. In addition, these results could be also due to the modifications in the number of preformed and neoformed nodes of scions by the rootstocks (Seleznyova et al., 2002), as demonstrated with the different node number distribution (Figure 3.11). Higher proportion of shoots with 15 or less nodes (i.e. preformed nodes) and reduced the proportion of shoots with more than 15 nodes were recorded in scion on No.18,

No.87 and No.100. It was also noted that the final mean node number of proleptic axillary shoots slightly lower in these rootstocks compared with other rootstocks and GN control (Figure 3.9C). Although GN control vines also produced higher proportion of shoots with 15 or less nodes, but it also had higher proportion of shoots with more than 15 nodes (i.e. neoformed nodes) (Figure 3.10). These results support the previous study on the effects of different vigour of kiwifruit rootstocks on 'Hort16A' scions that slower-growing shoots of scions on low-vigour rootstocks tended to have higher proportions of terminated shoots than non-terminated shoots (Clearwater et al., 2006). Studies on other fruit trees such as apple have reported similar findings that vigour-controlling rootstocks (i.e. dwarfing rootstocks) may affect the scions growth by affecting the shoot growth rates, and influence the time of termination of shoots, subsequently altering the shoot types. For example, in apple, M.9 dwarfing rootstock increased the proportion of short shoots than long shoots whether in its first (Costes et al., 2001) or second growing season (Seleznyova et al., 2003, 2008). Based on our findings, therefore, it is suggested that inter-specific hybrid kiwifruit rootstocks are capable to alter the shoot growth rates of scions, and this effect has contributed to the differences in the proportions of shoots types of scions.

3.4.1.3 The characteristics of proleptic axillary shoots

In apple, rootstocks may modify the characteristics of shoots of grafted scions (Hirst & Ferree, 1995b). For example, the 'Starkpur Supreme Delicious' scions had the shortest average shoots length when grafted onto P.22 dwarfing rootstock (Hirst & Ferree, 1995b). Similarly, peach scions of 'Flavorcrest' and 'Loadel' produced the shortest average shoot length when grafted onto 'K-146-43' and 'K-146-44' dwarfing rootstocks (Weibel et al., 2003). In the present study, during the 2012 -2013 growing season, the characteristics of long shoots that had been trained to become a permanent cordon were significantly affected by the inter-specific hybrid kiwifruit rootstocks (Table 3.5). In the next growing season (2013-2014), there were trends that inter-specific hybrid kiwifruit rootstocks may have affected the characteristics (i.e. length, node number, internode length and shoot CSA) of proleptic axillary shoots (Table 3.10 and Table 3.11). For example, in long proleptic shoots, the mean length, node and internode length were not significantly different between rootstocks. However, the long shoots on rootstock No.18

that have slowest growth rate (Figure 3.8) had the shortest length and internode length, as well as the lowest node number (Table 3.10). Contrasting results were found on rootstock No.101 that imparted highest shoot growth rate. In short shoots, inter-specific hybrid rootstocks significantly affected the length and internode length but not node number (Table 3.10). However, inter-specific hybrid rootstocks did not affect the length, node and internode length of medium shoots. Therefore, our results show that the variability in the characteristics of proleptic axillary shoots of 'Hayward' scions may partly due to the differences in the shoot growth rate imparted by the different vigour of rootstocks (Figure 3.8), which resulted in the differences in their final length, node, internode length and shoot CSA (Table 3.10 and Table 3.11). Besides that, it is believed that high proportions of short and medium shoots of scions on low-vigour kiwifruit rootstocks such as No.18, No.19, No.87 and No.100 (Table 3.7 and Table 3.8) may contribute to the reduction of crown volume of scions, therefore reducing the canopy size of vines (Figure 3.10B and C).

In kiwifruit, terminated shoots (i.e. short and medium shoots) on low-vigour rootstocks usually had a higher proportion of budbreak, which produced high flower numbers, because they previously were well exposed to sunlight during the final growing season (Clearwater et al., 2004). Meanwhile, Thorp et al. (2003) reported that the ability to attract carbohydrates and minerals is slightly different between the short and long kiwifruit shoots. Long kiwifruit shoots tend to bear fruits that have higher dry matter concentration and calcium than fruits found on short shoots (Thorp et al., 2003). Besides that, Thorp et al. (2003) also found that large diameter shoots growing on vigorous kiwifruit vines can be very floriferous relative to their bud number as they are more likely to be growing in positions exposed to sunlight compared to small diameter shoots, that may be more shaded. It therefore seems that these discrepancies are probably due to differences in the growing stage of shoots and differences in canopy position of fruits that might have contributed to the variation in the quality of the kiwifruit (Miles et al., 1996; Smith et al., 1994; Thorp et al., 2003). However, our aim is to develop kiwifruit vine with less vigorous vegetative growth, thus there would be less intra-canopy shading and as a result fruit quality would be improved.

In this present study, regardless of rootstocks, the length of different shoot types correlated well to the total number of nodes (Figure 3.6 and Figure 3.9). In long and medium shoots, the regression model is linear, but for the short shoots, the quadratic model is the most suitable. This result is consistent with the shoot architectural description of kiwifruit reported by Seleznyova et al. (2002) on 'Hort16A' cultivar. These results also indicate that the shoots that grew longer during the spring and summer season had more node number and neo-formed nodes, which may have contributed to a higher leaf area per shoot. In this study, it has been shown that the shoots (especially long non-terminated shoots) from particular inter-specific hybrid kiwifruit rootstocks (e.g. No. 45, No.86 and No.101) tended to a have higher shoot growth rate and grew longer than shoots on other rootstocks (e.g. No.18, No.87 and No.100). As found in study by Clearwater et al. (2006), the total leaf area of the first 20 nodes of long shoots on high-vigour kiwifruit rootstocks was significantly higher than low-vigour rootstocks (Clearwater et al., 2006). This effect contributed to higher total leaf area of grafted scions per vine in high-vigour rootstocks. We believe that the inter-specific hybrid kiwifruit rootstocks may also have a similar influence on the size and total leaf area per shoot, though this aspect was not studied.

Endogenous hormones such as auxin (IAA), gibberellins and cytokinins have been implicated in the meristematic activity (Sachs, 1965; Sassi & Vernoux, 2013). Long shoots of kiwifruit were classified as non-terminated shoots and had a number of neo-formed nodes (Seleznyova et al., 2002). According to Foster et al. (2007), the long shoots are the shoots that are still actively growing with a gradient of new leaves at apical shoot. This shoot is believed to have served as a potential site of endogenous IAA due to gradient of new leaves can be found in this shoots (Ljung et al., 2001). Therefore, any growth alteration in kiwifruit vines either by vigour-controlling rootstocks (Clearwater et al., 2007) or auxin transport inhibitor (Vattiprolu, 2012) will largely affect the long shoots, instead of affecting the medium or short shoots. Even though means number of total shoots was not significantly different amongst hybrid rootstocks (Table 3.4 and Table 3.9), there was a trend that number of total shoots was increased by low-vigour group, especially in 2012-2013 growing season (contrast analysis, Table 3.4).

3.4.1.4 Trunk cross-sectional area (CSA) of rootstocks and scions

In our study, the trunk CSA was significantly different between inter-specific hybrid rootstocks, with a range of 165 mm^2 to 375 mm^2 (Table 3.12). Similarly, the trunk CSA of scions also varied between rootstocks ranging from 222 mm^2 to 546 mm^2 and the first-three rootstocks (i.e. No.8, No.18 and No.19) that came from the selection of *A. chinensis* and *A. macrosperma* produced the smallest trunk CSA of scions (Table 3.12). It was also notable in previous chapter that trunk CSA of rootstocks and scions was significantly different in the first and second growing seasons (Chapter Two). The trunk CSA of rootstocks was correlated well with the CSA of scions, indicating there was a trend that rootstocks with smaller trunk CSA may produce smaller trunk CSA of scions, presumably scion budwood that were initially thick were grafted with thick rootstocks (Chapter Two). However, it was clear in this chapter that by year-three, particular scion budwoods that were producing a thick trunk were not related to the initial trunk thickness of hybrid rootstocks. The result in this chapter have demonstrated that the inter-specific hybrid kiwifruit rootstocks still had significant influence on the trunk size of 'Hayward' scions when planted in the field conditions. Trial at Motueka, New Zealand also found that the vigour of 'Hort16A' scions based on trunk CSA was also affected by the inter-specific hybrid kiwifruit rootstocks (Friend et al., 2014). According to Vasconcellos & Castle (1994), rootstocks can influence the growth of scion trunk by affecting size of the vessel elements. Thus, in kiwifruit, the radial growth of grafted scions may be controlled or restricted by the growth of rootstocks based on their trunk development. In many fruit trees, tree growth and yield have been positively correlated with the trunk size at orchard level (Hirst & Ferree, 1995b; Strong & Azarenko, 2000). Similarly in kiwifruit, the trunk size of the vines has been correlated with the fruiting performances such as yield and fruit size (Cruz-Castillo et al., 1997; Monastra & Testoni, 1991). For example, 'Hayward' scions grafted onto 'D1' rootstocks had shown greater productivity and highest yield value, even though they had the smallest trunk cross-sectional area compared with scion on 'Bruno' rootstocks (Monastra & Testoni, 1991). Cruz-Castillo et al. (1997) also demonstrated that trunk size of kiwifruit vines is the influential factor in separating vigour of scions between rootstocks, as they also found that vines developed larger trunk size in the early stage of vine growth, generally had a larger mean fruit size (Cruz-Castillo et al., 1997). However, in the present study, we did not observe the correlation between the trunk size

and fruiting performances. Due to insufficient yield data in the present study, therefore, the correlation between growth performance and yield warrants further observations in the future. Besides that, it is relatively unknown if kiwifruit vines with larger trunk size may have the capability to synthesis endogenous hormones or have the ability to metabolise more carbohydrate sources that can influence fruiting performances and hydraulic properties.

3.4.1.5 Pruning weight of scions

The pruning weight is an approximate measure of vegetative growth and also as an indicator of plant vigour because it relates with labour activities. In kiwifruit, excessive vegetative growth has increased production cost in terms of pruning labours. Therefore, controlling vegetative vigour of vines by rootstock is the ultimate goal in kiwifruit orchard management to reduce labour cost. A few studies on other fruit trees has demonstrated that pruning weight of scions was affected by the rootstocks, as found recently in grape (Souza et al., 2015) and peach (Giorgi et al., 2005; Weibel et al., 2003). For example in peach, the pruning weight of scions was highest in trees on vigorous rootstock 'Nemaguard' than trees on dwarfing rootstocks such as 'K-146-44' and 'K-146-43' (Weibel et al., 2003). Similar to grape, pruning weight of 'Cabernet Sauvignon' scions was reduced when grafted onto '101-14' low-vigour rootstocks (Souza et al., 2015). However, in kiwifruit, limited information is available regarding rootstock effects on pruning weight. One of the studies conducted in Italy reported that the pruning weight of 'Hayward' scions tended to reduce when grafted onto 'Bruno' and 'D1' rootstocks than onto ungrafted vines (Monastra & Testoni, 1991). In the present study, even though there were significant differences in the shoot growth and the proportion of different shoot types of scions between inter-specific hybrid rootstocks, the differences in the pruning weight (i.e. fresh and dry weight) between rootstocks could not be found (Table 3.6). The reason for the lack of significance is unknown, perhaps due to insufficient replication in our study. Hence, further evaluation with a large number of replication is needed in the future. We expect that a greater difference in pruning weight of scions between inter-specific hybrid kiwifruit rootstocks (particularly the cumulative pruning weight) as the vines age.

3.4.2 Effects of rootstocks on bud break, precocity and flowering of 'Hayward' scions

3.4.2.1 Spring bud break

In our study, we found that inter-specific hybrid kiwifruit rootstocks can affect the bud break of 'Hayward' scions by affecting the duration, compactness and timing of peak scion bud break (Figure 3.2 and Figure 3.3). During the 2012 spring season, the overall proportion of bud break of grafted 'Hayward' scions was significantly affected by the inter-specific hybrid rootstocks (Table 3.1). Unfortunately, the comparison between inter-specific hybrid kiwifruit rootstocks and GN as a control vines could not be made because GN vines were still small due to re-planting. In the next spring season of year 2013, the inter-specific hybrid rootstocks still had significant influence on the mean percentage of bud break of scions (Figure 3.4). However, the bud break of scions was not consistent with the vigour that was found in Chapter Two. However, the most notable effect of inter-specific hybrid kiwifruit rootstocks was clearly found on the timing of peak bud break of 'Hayward' scions. Results in this chapter have supported the previous findings in Chapter Two that inter-specific hybrid kiwifruit rootstocks consistently affected scion bud break in the next following season.

Furthermore, a few consistent differences were also recorded between inter-specific hybrid rootstocks on the timing of peak scion bud break. When bud break was calculated as relative bud break (i.e. the percentage of buds that was opened during each observational time relative to the total bud which has opened), the peak time of bud break varied between inter-specific hybrid rootstocks (Figure 3.3). For example, 'Hayward' scions on rootstocks No.8, No.18 and No.19 exhibited a similar peak time (35%) of bud break, and these rootstocks came from the same crosses or from the same parentage, between *A. chinensis* and *A. macrosperma*. Similar patterns were also found for rootstock combinations of No.45 and No.55 because they also came from the same parentage (*A. polygama* and *A. chinensis*). Other rootstock combinations such as No.100 and No.101 also had shown similar peak time patterns of bud break, because they also came from the same crosses (i.e. between *A. macrosperma* and *A. melanandra*). Another interesting finding that found from these results was the parentage of the inter-

specific hybrid kiwifruit rootstocks may have an influence on the number of days spread or compactness of 'Hayward' scion bud break (Figure 3.3). For example, the time period over which 70% scion bud break occurred on rootstocks No.8, No.18, No.19 and No.21 that came from the crosses *A. chinensis* and *A. macrosperma* was between 19 to 29 days. Meanwhile, the number of days spread for rootstocks No.45, No.55 and No.71 (crosses between *A. polygama* and *A. chinensis*) was between 14 to 19 days. For the rootstocks No.84 and No.87 (*A. polygama* selection), the time period over which 70% scion bud break occurred was 14 days. A similar number of days was recorded on rootstocks No.100 and No.101 that came from the crosses between *A. macrosperma* and *A. melanandra*. However, 19 days was required to get 70% of scion bud break on rootstocks No.85 and No.86 that came from *A. macrosperma* selection. Therefore, our findings strongly demonstrated that the genetic origin of rootstocks from the original parentage may have significant influence on the 'Hayward' scion bud break.

In other fruit trees, bud break of scions is not clearly affected by the rootstock as reported for pear (Watson et al., 2012) and apple (Costes et al., 2001; Seleznyova et al., 2003). In contrast with vine species such as grape, Nikolaou et al. (2000) found that the rootstock has a significant influence on the amount of scion bud break, with the vigorous rootstock 110R imparting relatively high bud break. Similarly in kiwifruit, Wang et al. (1994) reported that the percentage of bud break of 'Hayward' scions was highest when grafted onto rootstocks from *A. hemsleyana* clone. Even though recent study found that the bud break of 'Hort16A' scions did not differ significantly between 'Bounty 71' and 'Hayward' or 'Kaimai' rootstocks, the percentage of scion bud break varied between the rootstocks (Anon., 2012). Therefore, the results in the present study suggest that inter-specific hybrid kiwifruit rootstocks have significant influence on the duration, compactness and peak time of bud break of grafted 'Hayward' scion (Figure 3.2 and Figure 3.3), thus support and extend those of Wang et al. (1994). Our findings have also highlighted the importance of rootstock selections in kiwifruit because it may have influenced the productivity (McPherson et al., 1994), as the degree and synchrony of bud break were highly correlated with the reproductive organ (i.e. flowering) in kiwifruit (Wang et al., 1994b).

In Chapter Two, it was noted that 'Hayward' scions on inter-specific hybrid kiwifruit rootstocks produced up to 50 % bud break (Chapter Two). However, in this chapter, during early spring 2012 after transplanting, the mean proportion of scion bud break on inter-specific hybrid kiwifruit rootstocks was slightly reduced and was only up to 40% (Table 3.1). Therefore, there were differences in the proportions of scion bud break on inter-specific hybrid kiwifruit rootstocks between the experiments in Chapter Two and this chapter. These results indicate that there was a variation between years, presumably due to the differences in the cultural practices (nursery and field planting) and climatic effects. It was also observed that the growth of all the experimental vines was slower when they were transplanted in field conditions. This occurred immediately after the transplant of the experimental vines into the field on 30/11/2012, presumably due to 'transplanting shock'. Although the vines were well watered immediately after the transplanting, the roots of inter-specific hybrid kiwifruit rootstocks may be particularly sensitive to any disturbance. Van Hooijdonk (2009) also reported the same evidence in his study on apple rootstocks. Therefore, for future studies, it is suggested that transplanting needs to occur during winter dormancy to prevent any confounding effects on the experimental vines. Nevertheless, it was observed that all the vines had resumed normal growth in the next following season. Even though in our study, the ranges of bud break of scions induced by inter-specific hybrid rootstocks were quite low (i.e. between 40 to 50%); nevertheless, there was a strong trend that the particular inter-specific hybrid rootstocks such as rootstocks No.18, No.45 and No.87 can consistently induce high bud break on grafted scions (Table 3.1 and Figure 3.4).

In the present study, there were trends that particular rootstocks such as No.8, No.18, No.19 and No.87 including No.100 produced a considerably higher percentage of bud break in the first week of bud outgrowth (Figure 3.2). These rootstocks also had a higher relative bud break (i.e. approximately 20%) in the same week (Figure 3.3). Even though rootstock No.71 also had higher relative bud break (Figure 3.3), this could not be confirmed due to only two replicated vines being available. The variation in scion bud break between inter-specific hybrid kiwifruit rootstocks may also indicate that rootstocks may have an effect on carbohydrate status of scion shoots (Wang et al., 1994a), by creating a competition among buds for limited resources (e.g. nitrogen and carbon) (Austin et al., 2002). Besides that, Clearwater et al. (2006) suggested that variation in the bud break of kiwifruit shoots is also correlated with the local carbon

availability, for example the amount of sun exposure in the previous growing season. In addition, the roots of flower-promoting rootstocks such as *A. hemsleyana* and *A. eriantha* contain more starch and complex mucilage-containing crystalline idioblast cells, leading Wang et al. (1994a) to speculate that reserve mobilization (possibly carbon and nitrogen) and plant water status in early spring bud break had important effects on scion flower production.

Bud break in kiwifruit can be also affected by the different type of shoots (i.e. terminated or non-terminated). Thorp et al. (2003) reported that larger diameter, non-terminated canes (i.e. long shoots) are more productive than small diameter canes (i.e. short and medium shoots) due to higher percentage bud break. Whereas, Clearwater et al. (2004) reported that terminated shoots well exposed to sun, may also promote high proportion of bud break and flowering in the following season. However, terminated shoots are usually shaded in a vigorous canopy by longer shoots and the higher overall Leaf Area Index (Clearwater et al., 2006). Both of these shoot types can be affected by using different vigour of kiwifruit rootstocks (Clearwater et al., 2004). Other studies found that shaded shoots or canes during growing stage had lower meristem sucrose contents during winter than un-shaded shoots or canes (e.g. Richardson et al., 2010). Therefore, it is believed that inter-specific hybrid kiwifruit rootstocks may influence the carbohydrate status of scions by affecting the availability of resources from the roots. However, to our knowledge, it is still largely unknown whether the effect of rootstocks on the carbohydrate allocation to the scions has an influence on the scion growth, because no extensive literature is available regarding this matter.

3.4.2.2 Precocity and flowering

In this study, the flowers produced during the season of 2012 were the first occurrence of flowering imparted by the inter-specific hybrid rootstocks on 'Hayward' scions. The mean flower number of 'Hayward' scions was significantly different between inter-specific hybrid rootstocks (Table 3.1). It was interesting to note that a few rootstocks such as No.18, No.8 and No. 19 produced considerably higher flower number in the first growing season after transplanting in the field (Table 3.1). More interestingly, rootstock No.18 produced significantly higher mean flower number per vine compared to other

rootstocks. 'Hayward' scions on this rootstock produced twice the number of flowers, in contrast with some of the rootstocks that still did not produce any flower at this stage (Table 3.1). Therefore, this result indicates that selected inter-specific hybrid kiwifruit rootstocks may have an effect on the ability to induce fruitfulness (i.e. precocity) of the scions. These results are interesting and could be the first evidence showing that kiwifruit rootstocks have the ability to affect the precocity of scions similar to apple (Hirst & Ferree, 1995a; 1995b) and sweet cherry rootstocks (Lang, 2000). In addition to these results, it was notable in our study that precocious kiwifruit rootstocks such as No.18, No.84 and No.87 also had the highest dry matter of shoots (Table 3.6), indicating that the rootstocks may induce changes in partitioning of dry matter and carbohydrate content of 'Hayward' scions, thereby affecting the potential bearing shoots. These results are consistent with the study in plum that found different vigour of rootstocks may induce different in the dry matter and starch content of bearing shoots of scions (Milošević & Milošević, 2012). However, the interactions between rootstocks and scions in regulating carbohydrates partitioning of kiwifruit vines are complex and need further work.

It was also noted in the 2013-2014 growing season that rootstock No.18 produced the slowest growing shoots (Figure 3.7 and Figure 3.8) and had the highest proportion of terminated shoots (Table 3.8). It seems that there was an existing relationship between floral precocity and vigour in the vines of this rootstock. Nevertheless, it still remain unclear whether this result could indicate a relationship between the scion vigour and the ability of rootstock to induce early flowering as reported in apple (Seleznyova et al., 2008). Besides that, it has also been suggested that early termination of shoot growth may led to increase flower initiation (Luckwill, 1970). As found in apple, floral transition induced by dwarfing rootstock M.9 in the first year of tree growth had significant influence on the scion vigour in the following growing season by reducing the number of extension annual shoots (Seleznyova et al., 2008). In kiwifruit, although previous study has found that the slower growing shoots of scions that terminated earlier occurred on low-vigour rootstocks (Clearwater et al., 2006); no detailed studies have been conducted to elucidate the link between growth reduction and floral transition by kiwifruit rootstocks. Therefore, with these new hybrid rootstock selections of kiwifruit, it would be interesting to further study whether there is a strong relationship between precocity of flowering, amount of flowering and differences in vigour of scions

imparted by rootstocks. In addition, it would be useful to include self-rooted 'Hayward' rootstock to help quantify the hybrid rootstock effect on precocity of grafted scion.

In the growing season of 2013, inter-specific hybrid kiwifruit rootstocks did not significantly affect the proportion of flowering shoots of scions (Table 3.13) even though there was an indication that scion flowering may vary between rootstocks. Similarly, the mean flower and fruit number, as well as fruit setting was not significantly differed between inter-specific hybrid rootstocks (Table 3.14). As stated in many reviews, rootstock does affect the flowering of grafted scion (Atkinson & Else, 2001; Lang, 2000; Webster, 1995a). In kiwifruit, there was much evidence that rootstocks can affect flowering (Anon., 2012; Cruz-Castillo et al., 1997; Lowe, 1989, 1991; Wang et al., 1994b). Previous studies reported *A. hemsleyana* cv. "Kaimai' rootstocks can promote flowering of 'Hayward' scions and double flower number compared to standard clonal *A. deliciosa* rootstocks (Lowe, 1989, 1991; Wang et al., 1994b). Furthermore, a recent study on 'Bounty 71' rootstocks found this rootstock can also promote flower number up to 50 % on 'Hort16A' scions (Anon., 2012). However, excessive flowers that are produced by particular kiwifruit rootstocks may cause an increase in thinning cost for the growers. Therefore, long-term evaluation on the scion flowering is still needed for these inter-specific hybrid kiwifruit rootstocks.

In our study, the lack of significant differences between rootstocks during this stage is somewhat surprising (Table 3.14), since rootstock effects on flowering and fruiting have been well documented in kiwifruit (Anon., 2012; Cruz-Castillo et al., 1997; Lowe, 1989, 1991; Wang et al., 1994b). Explanation of why there was a lack of significant among inter-specific hybrid rootstocks during this growing season (2013) was probably due to variability in our flowering data. In future studies, it would be interesting to include 'Bruno' and self-rooted control rootstocks as comparison. Nevertheless, it worthwhile to note that particular rootstocks such as No.8, No.18, No.55, No.84, No.85, No.86 and No.100 produced two to three-fold higher in flower number per vine and flower number per winter bud compared with GN control (Table 3.14). Therefore, it would be reasonable to suggest that inter-specific hybrid kiwifruit rootstocks have potential to affect the flowering ability of 'Hayward' scions. Besides that, it is believed that the scions flowering during this time may be controlled or influenced by other factors such as winter temperature (Linsley-Noakes & Allan, 1987; Warrington &

Stanley, 1985). In kiwifruit, the amount of winter chilling may have an influence on flowering, and different cultivars may need different chilling requirement (Linsley-Noakes & Allan, 1987). In our study, we also believe that the effects of inter-specific hybrid kiwifruit rootstocks on flowering may be more apparent later, after the canopies of scions are well developed, because different amounts of shading in the canopies imparted by the rootstocks. Overall, it can be suggested that inter-specific hybrid kiwifruit rootstocks have the ability to modify the architectural structures of 'Hayward' scions. However, the initial vigour ranking that was found in Chapter Two did not appear to continue in the next following season when planted in field-conditions. Nevertheless, the 'dwarfing expression' on grafted scion imparted by the inter-specific hybrid kiwifruit rootstocks can be clearly observed and identified in this chapter. Therefore, a new vigour ranking was proposed (in order of increasing vigour); kiwifruit hybrids No.18, No.87, No.100, No.8 and No.19 were classified as low-vigour rootstocks, hybrids No.45, No.55, No.84, No.85, No.86 and No.71 were classified as intermediate vigour rootstocks, and hybrids No.21 and No.101 including self-rooted green-cuttings can be grouped as high-vigour rootstocks (Table 3.15). Our results supported the finding from previous study (Clearwater et al., 2006) that kiwifruit rootstocks may have an effect on the shoot growth of scions, and affecting the time of shoots termination, subsequently altering the proportion of different shoot types of scions similar to the previous study. In addition, the results of the current study also extended our knowledge on the modification of kiwifruit rootstocks on grafted scions, as we found that rootstock effects are more apparent on the duration and timing of bud break, and also growth rate of proleptic axillary shoots. Thus, in order to further extent our results, experiments on the endogenous hormonal transport are needed and were extended in the next chapter (Chapter Four).

Table 3.15. The characteristics of inter-specific hybrid kiwifruit rootstocks and their vigour classification.

[†]Vigour rating	Rootstocks	Classification of vigour	Characteristics
1 2 3 4 5	No.18[y] No.87 No.100 No.8[y] No.19[y]	Low vigour	- Imparted the slowest shoot growth rate of scions (especially long shoots). - Produced considerable high proportion of terminated shoots (>80%) and lower proportion of non-terminated shoots of scions (<20%) especially No.18, No.87 and No.100.
6 7 8 9 10 11	No.45[x] No.55 No.84 No.85 [x] No.86 [x] No.71	Intermediate vigour	- Imparted the moderate shoot growth rate of scions (especially long shoots). - Produced high to moderate proportion of terminated shoots (60-80%) and proportion of non-terminated shoots of scions between ranges 20-30%.
12 13 14	No.21 No.101 Green-cuttings (self-rooted control)	High vigour	- Imparted the fastest shoot growth rate of scions (especially long shoots). - Produced low proportion of terminated shoots (<60%) and high proportion of non-terminated shoots of scions between ranges 40-60%.

[†]In order of increasing vigour.
[x]Rootstocks that not producing flowers in the first growing season.
[y]Rootstocks that producing high flower numbers in the first growing season (precocity).

3.5 Summary

Assessments on the inter-specific hybrid kiwifruit rootstocks on the growth and architectural structures of 'Hayward' scions were conducted in the field trial. The modification (s) of 'Hayward' scions by the inter-specific hybrid kiwifruit rootstocks plus self-rooted vines were observed during the 2012 - 2014 growing season. In the 2012 and 2013 growing season, inter-specific hybrid kiwifruit rootstocks affected the overall bud break of 'Hayward' scions. The most notable effect of inter-specific hybrid kiwifruit rootstocks was on the duration and compactness of scion bud break. The relative scion bud break and the time period (days) over which 70% scion bud break occurred were differed between rootstocks and this pattern could be related to the parentage of the rootstocks. The average flower numbers of 'Hayward' scions was significantly different between inter-specific hybrid kiwifruit rootstocks. Scions on rootstocks such as No.8, No.18 and No.19 produced considerably higher flower number per vine with the highest was recorded on rootstock No.18. All these rootstocks could be considered as low-vigour rootstocks due to imparting slower shoot growth rate on grafted scions. However, in the next 2013 growing season, the mean proportion of flowering shoots, mean flower and fruit number, as well as mean fruit setting of 'Hayward' scions did not differ kiwifruit rootstocks. There were trends that particular rootstocks hybrid may produce two to three-fold higher in flower number per vine and flower number per winter bud compared with GN control.

Regardless of rootstocks, the differences in shoot development and extension for long, medium and short shoots of grafted scions were clearly observed in the first month after buds release from dormancy (i.e. bud break). The long shoots of scions from rootstock No.8, No.18, No.19, No.87 and No.100 grew much slower than other inter-specific hybrid kiwifruit rootstocks. The long shoots of scions grafted onto rootstocks No.8, No.18, No.19, No.87 and No.100 produced the slowest growth rate compared with other rootstocks, with scions on rootstock No.18 and No.87 had the slowest shoot growth rates. However, the highest shoot growth rate were found on the long shoots of scions from rootstocks No.21 and No.101. Contrast analyses on the selected rootstock groups based on their vigour classes (i.e. low, intermediate and high-vigour) indicate that the proportion of proleptic axillary shoots (especially long and medium shoots) may be

affected by inter-specific hybrid rootstocks. Rootstocks No.18, No.87 and No.100 had the lowest proportions of long shoots, and substantially higher proportions of short shoots. Similar rootstocks produced higher proportions of terminated shoots and lower proportions of non-terminated shoots on grafted scions. Therefore, hybrid kiwifruit rootstocks are capable of affecting the scion shoot growth rate, and influencing the time of shoot termination, subsequently altering the shoot types of scions similar to what have been found on other species of kiwifruit rootstocks. The final mean length, node number, internode length and shoot CSA of the long shoots of 'Hayward' scions that have been trained to become cordons were significantly different between hybrid kiwifruit rootstocks. In next growing season 2013-2014, there were trends that inter-specific hybrid kiwifruit rootstocks may affect the length, node and internode length of long proleptic axillary shoots. The long shoots of scions from rootstock No.18 (low-vigour) that imparted the slowest shoot growth rate had the shortest length, internode length and node number, whereas contrasting results found in rootstock No.101 (high-vigour) that imparted the highest shoot growth rate. Hybrid kiwifruit rootstocks also had significant influence on the length, internode length and CSA of short shoots. Based on these results, variability in the characteristics of proleptic axillary shoots of 'Hayward' scions may reflect the differences in the shoot growth rate imparted by the inter-specific hybrid kiwifruit rootstocks, which results in the alterations in their final length, node number and internode length.

The trunk CSA of rootstocks and scions was significantly different between the rootstock hybrids with the smallest were recorded on the rootstocks No.8, No.18 and No.19. There was also a trend that rootstocks with smaller trunk CSA may produce smaller trunk CSA of scions. Although the pruning weight of scions varied slightly, no significant difference was found between inter-specific hybrid kiwifruit rootstocks. Therefore, based on this study, a new vigour ranking was proposed (Table 3.15) based on the shoot growth rate (in order of increasing vigour); hybrid kiwifruit rootstocks No.18, No.87, No.100, No.8 and No.19 were classified as low-vigour rootstocks, hybrids No.45, No.55, No.84, No.85, No.86 and No.71 were classified as intermediate vigour rootstocks, and hybrids No.21 and No.101 including self-rooted green-cuttings can be grouped as high-vigour rootstocks.

Chapter Four

4. Understanding the hormonal signalling in the grafted 'Hayward' kiwifruit vines

4.1 Introduction

In Chapter Three, it was shown that inter-specific hybrid kiwifruit rootstocks may have different abilities to control the vigour of the grafted 'Hayward' scions. The ability to control the vigour of grafted scions by rootstocks could be related to endogenous hormonal signalling between the shoot and root systems (Li et al., 2012; Tworkoski & Fazio, 2015; van Hooijdonk et al., 2010; 2011). Therefore, it would be interesting to further study the hormonal mechanisms in these new selection of kiwifruit rootstocks. It was well accepted that physiological understanding of the endogenous hormonal signalling is a key in manipulating tree growth for better canopy form and yield in fruit tree species, but in kiwifruit, information on this aspect is limited. Understanding hormonal mechanisms operating in kiwifruit could contribute to the future improvement of vigour control in kiwifruit. Interactions between auxin, cytokinin (CK) and gibberellins (GA) are important in controlling tree growth (Gregory et al., 2013; Li et al., 2012; Lockhard & Schneider, 1981; Tworkoski & Fazio, 2015; van Hooijdonk et al., 2010; 2011). To explain how rootstocks control scion vigour, the main concept focuses on the hormonal signalling from shoot to root, or *vice versa* and this mechanism has been recently revealed in studies with apple rootstocks (e.g. Li et al., 2012; Tworkoski & Fazio, 2015; van Hooijdonk, 2009; van Hooijdonk et al., 2010, 2011).

Recent studies by van Hooijdonk et al. (2010; 2011) examined the architectural changes of scions on apple dwarfing rootstocks treated with combinations of plant growth regulator (i.e. CK and GA) and an auxin transport inhibitor. By applying the auxin transport inhibitor, 1-N-naphthylphthalamic acid (NPA) in a ring around the graft-union region of three vigorous rootstocks (MM.106, M.793 and Royal Gala as control), the 'Royal Gala' scions showed reduced shoot growth, node production, decreased branching, and early shoot termination, irrespective of the vigour of the rootstocks

compared to no NPA (van Hooijdonk et al., 2010). The architectural changes on scions imposed by the NPA were similar to the effects when 'Royal Gala' scions are grafted onto M.9 dwarfing rootstocks. However, the effects of auxin inhibition by NPA could be reversed with exogenous application of GA and CK. For scions on M.9 dwarfing rootstocks or NPA-treated rootstocks, application of CK (benzylaminopurine) re-instated the formation of seceondary shoots, whereas GA (GA_{4+7}) had reduced the proportion of primary and secondary shoots that terminated growth early. Thus, van Hooijdonk (2010) proposed that reduced basipetal transport of IAA from the shoot to the root may reduce the production of GA and CK transported from root (rootstock) and shoot (scion) and clearly showed that the modification of endogenous hormones from the shoot to root system, or the root to shoot by rootstocks may affect the overall architecture of grafted scions (van Hooijdonk et al., 2010). However, in kiwifruit, until now, there is only limited information as to whether this type of hormonal mechanism could be involved in regulating kiwifruit vigour.

In an attempt to elucidate hormonal signalling in kiwifruit, Vattiprolu (2012) applied NPA to stem cuttings of *A.chinensis* replicating the trial that have been conducted in apple (van Hooijdonk et al., 2010). In contrast to apple, restricting auxin transport from the shoot to root by applying NPA to the stem cuttings had no significant effects on the total growth of kiwifruit, even though reduction in the length and leaf area of primary shoots were observed (Vattiprolu, 2012). This may not be surprising because these experiments utilised rooted cuttings, so no rootstock (root system plus stem) was involved. In contrast to these results, direct spraying of NPA to the foliage significantly reduced shoot growth and caused early termination (Vattiprolu, 2012). Interestingly, it was also found that application of GA (GA_3 and GA_{4+7}) by foliar sprayings immediately after bud break increased vigour and transformed all potential terminated short and medium shoots into non-terminated long shoots (Vattiprolu, 2012). Thus, Vattiprolu (2012) concluded that GA had a significant role in stimulating both the apical and sub-apical meristems in kiwifruit. Therefore, it seems likely that, as in apple, GA have a major role in kiwifruit vigour, but the role of auxin transport to the root system in affecting endogenous GA is less clear.

Auxin transport has been implicated in the growth coordination between scions and rootstocks. Lockard and Schneider (1981) proposed that the dwarfing effect of grafted trees could be due to the restriction of auxin transport in the stem bark of dwarfing rootstocks. Later studies by Soumelidou et al. (1994) and Kamboj et al. (1997) found that the transport and uptake of ^{14}C- and ^{3}H-labelled indole-3-acetic acid was higher in vigorous rootstocks compared to dwarfing rootstocks (Kamboj et al., 1997; Soumelidou et al., 1994). A recent study by van Hooijdonk et al. (2011) has also revealed that there was a relationship between shoot-produced auxin (i.e. IAA) and endogenous root-produced hormones such as CK and GA in controlling shoot growth of composite apple trees during the growing season. By measuring the level of IAA diffusing from the apex of 'Royal Gala' primary shoots, they found that the mean rate of IAA in shoots gradually declined from February onwards irrespective of rootstock vigour (van Hooijdonk et al., 2011). In the same period of growth, the mean concentration of CK (i.e. zeatin riboside) in the xylem sap increased, and these events appeared to coincide with increases in the number of axillary shoots formed on the scions. On the other hand, the xylem sap of primary shoots on M.9 dwarfing rootstock had a greater concentration of ZR during February than the primary shoots on 'Royal Gala' rootstocks, but the scions on M.9 did not produce more axillary shoots, suggesting that other endogenous hormonal signal in regulating scion branching. During March, the level of GA (i.e. GA_{19}) in the xylem sap of scions on M.9 were significantly lower than in the xylem sap of scions on 'Royal Gala' rootstocks (van Hooijdonk et al., 2011).

Based on the literature described above, there was sufficient evidence that IAA and other endogenous hormones (i.e. CK and GA) are involved in controlling scion vigour in grafted apple trees. However, to date, no detailed study has been conducted to evaluate endogenous hormones in kiwifruit. We hypothesis that decreased IAA supply to roots by applying NPA to the stem junction between hybrid kiwifruit rootstock and scion may influence other hormones such as CK and GA, thus modifying the overall architecture of 'Hayward' scion (**Hypothesis III**). Therefore, this study was initiated to evaluate the involvement of endogenous hormonal signalling, focusing on IAA transport in composite kiwifruit vines, and to assess how the mechanisms of auxin transport in the new selection of inter-specific hybrid kiwifruit rootstocks relates to their vigour ability imparted to the grafted 'Hayward' scions. The information generated from this study could assist further studies in revealing the actual hormonal mechanisms

that are involved in regulating growth in kiwifruit. In kiwifruit, excessive vegetative vigour is still a major constraint to kiwifruit growers. In order to control kiwifruit growth, pruning is required to maintain vines in a manageable state, and this has increased production cost for kiwifruit growers. The lack of potential vigour controlling rootstocks may be a limiting factor for kiwifruit vines management (Clearwater et al., 2006), and this has created a huge productivity gap in terms of harvest index between kiwifruit and other fruit crops, such as apple (Palmer, 2007). Therefore, information on hormonal regulation in grafted kiwifruit vines could provide valuable information of physiological understanding on how the endogenous signalling between shoot and root may interact. Indeed, it is important to understand endogenous hormonal signalling mechanism in grafted kiwifruit, and this could be the basis in developing a controlling technique in kiwifruit vines. In addition, the effect of rootstock and auxin transport inhibitor (NPA) on shoot development and the growth characteristics of grafted kiwifruit vines have not been tested experimentally, even though some works have been done on non-grafted vines (Vattiprolu, 2012). Therefore, this chapter was designed in order to improve our physiological understanding on the hormonal interaction in composite kiwifruit vines. The specific objectives were to:

(1) Elucidate the hormonal signalling from shoot to root (i.e. decreasing IAA supply to roots by applying NPA) and effects on vigour (i.e. shoot growth and architectural structures) of young composite 'Hayward' kiwifruit vines.
(2) Assess IAA transport in inter-specific hybrid rootstocks and its relationship to the vigour of grafted 'Hayward' scions thereon.

4.2 Materials and methods

In this chapter, two major experiments were conducted in order to achieve the specific objectives described in section 4.1. Briefly, the characteristics and vigour classification of inter-specific hybrid kiwifruit used in this chapter is shown in Table 4.1. In Experiment 1, the effects of NPA on shoot architectural structures were assessed on composite 'Hayward' vines following the method developed by van Hooijdonk (2009) and Vattiprolu (2012). 'Hayward' scions grafted onto the inter-specific hybrid kiwifruit rootstocks No.19, No.55, No.84, No.86 and No.87, plus self-rooted 'Hayward' (GN) as a control, were evaluated for their responses to NPA. In Experiment 2, measurement of auxin (i.e. IAA) transport was conducted on stem cuttings of rootstocks No.18, No.45, No.55, No.86, No.87, No.100 and No.101. In addition, rootstock namely 'Bounty 71' from intermediate vigour class was included for the comparison (Anon., 2012). The methods of auxin transport system were modified from the method by Soumelidou et al., (1994) and Kamboj et al. (1997) to suit the kiwifruit stem segments for this experiment. Noted here, due to limited number of samples available for particular rootstocks, both of the experiments were used different rootstock materials. Nevertheless, most of the rootstock hybrids represent the overall vigour classes (low, intermediate and high) based on the results reported in Chapter Three (Table 4.1).

Table 4.1. The characteristics and vigour classification of inter-specific hybrid kiwifruit rootstocks used in Experiment 1 and Experiment 2.

[†]Vigour rating	Rootstocks selection	Parentage of rootstocks	Vigour group	Experiment One	Experiment Two
1	**No.18**	*A. chinensis* x *A. macrosperma*			✓
2	**No.87**	*A. polygama*		✓	✓
3	**No.100**	*A. macrosperma* x *A. melanandra*	Low	✓	✓
4	**No.8**	*A. chinensis* x *A. macrosperma*			
5	**No.19**	*A. chinensis* x *A. macrosperma*		✓	
6	**Bounty 71**	-[x]			✓
7	**No.45**	*A. polygama* x *A. chinensis*			✓
8	**No.55**	*A. polygama* x *A. chinensis*	Intermediate	✓	✓
9	**No.84**	*A. polygama*		✓	
10	**No.86**	*A. macrosperma*		✓	✓
11	**No.101**	*A. macrosperma* x *A. melanandra*			✓
12	**Bruno**	*A. deliciosa* cv. 'Bruno'	High	✓	
13	**GN***	*A. deliciosa* cv. 'Hayward'		✓	

[†]In order of increasing vigour according to the findings in Chapter Three.
*Self-rooted 'Hayward' cuttings as control.
[x]Produced slightly less vigorous (Anon., 2012) or moderate reduced vigour when grafted with 'Hort16A' scions (M. Clearwater, personal communication, Jun 27, 2016). Unknown parentage from open pollinated of *A. macrosperma*.

4.2.1 Experiment 1: Effects of rootstock and auxin transport inhibitor, 1-N-naphthylphthalamic acid (NPA) on scion shoot growth and architecture of young composite 'Hayward' vines

4.2.1.1 Study site and establishment of experimental plant materials

The experiment was conducted at Plant Growth Unit, Massey University, Palmerston North, New Zealand. The inter-specific hybrid kiwifruit rootstocks stated above were selected for this experiment. The grafted plants were prepared as previously described in Chapter Two (Section 2.2.2). All the plants were established outside in a sheltered area to harden off before being transferred to the standing out area (Figure 4.1). The experimental plants were planted in 50 L easy-lift bags containing standard Dalton^{TM} growing medium (C.A.N fines A grade 50%, fiber 30%, pumice 7 mm 20% and Supertine Super 1kg/M^3). In addition, the growing medium contained slow release fertilisers with trace elements, 150 g of Dolomite (Osmocote 200 g for 8-9 months and Osmocote 100 g for 3-4 months) per 100 L of growing media. The composite plants consisted of 150 mm of rootstock stem above the potting mix with one primary shoot approximately 250 mm long. The irrigation system consisted of 19 mm polytube line placed under the plant row. For each bag (40 L), a 4 L hr^{-1} pressure compensator dripper was used. To each compensator, a 1.5 m length of flexible PVC line (3 mm internal diameter) was connected and a stake drip emitter was attached to the end of the PVC line. A single stake drip emitter was placed into the medium near the centre of each polythene growing bag. Irrigation was scheduled daily for one hour in the morning and evening using an automated irrigation controller (Hunter, Smart Valve Controller, USA).

4.2.1.2 Synthesis of 1-N-naphthylphthalamic acid (NPA)

The synthesis of 1-N-naphthylphthalamic acid (NPA) was carried out in the laboratory at the Institute of Agriculture and Environment, Massey University, Palmerston North, New Zealand. The NPA was synthesised following the method of Thomson et al., (1973). Briefly, 1-Naphthylamine (5 g) was dissolved in 500 mL of toluene, and phthalic anhydride (5 g) was dissolved in a further 500 mL of hot toluene. The two-toluene solutions were mixed and then stored at 25°C for 24 hr to enable precipitation. Precipitated NPA was then filtered through filter papers (Whatmans No. 1). The precipitate was washed five times in clean toluene to remove any unreacted reagents (Currie, 1997). Toluene was removed from the NPA by evaporation within a fume hood.

4.2.1.3 Testing of the biological efficacy of synthesised 1-N-naphthylphthalamic acid using a lettuce root growth bioassay

The biological efficacy of the synthesised NPA was tested and measured using lettuce root bioassay. Lettuce seedlings cultivar 'Butter Crunch' were germinated for 12 hr at 24°C in distilled water to promote radical emergence from the seed coat. Upon radical emergence, 12 lettuce seedlings per petri-dish (50 mm in diameter) were grown in 2 ml of NPA solution, with each petri-dish containing a different mol L^{-1} concentration of NPA dissolved as ammonium salt. All petri-dish were arranged within an incubator maintained at 25°C and the seedlings grown 500 mm beneath fluorescent light (4 x 18 W Cool White Tubes, 80 μmol sec^{-1} m^{-2}). After 72 hr, the length of the root was measured with a ruler. Normal root growth and development were observed when the seedlings were germinated in distilled water (control) and similar results were found when germinated in 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10} mol L^{-1} of NPA (Table 4.2, Figure 4.2A and B) but root length was significantly decreased at higher concentration and at 10^{-4}, 10^{-5} and 10^{-6} mol L^{-1} of NPA roots grew upwards because of loss of gravitropism due to inhibition of auxin transport (Figure 4.1A and Figure 4.1B). The seedlings germinated in 10^{-3} mol L^{-1} of NPA had the shortest root length compared to the other treatments (Table 4.2 and Figure 4.2B).

4.2.1.4 Application of NPA to kiwifruit vines

NPA was dissolved in a few drops of ammonium hydroxide (NH_4OH) and the solution was dried in a rotary evaporator until the solution became crystallised (i.e. to form ammonium salt). Then, 100 mL of distilled water was added to dissolve the crystallised NPA and the NPA solution was added to the melted lanolin at 40 °C. The mixture was stirred slowly until the NPA solution and lanolin were mixed together. The mixture of NPA and lanolin was kept in a beaker and covered with tin-foil until used after one day. In the present study, 10 mg/mL^{-1} of NPA per plant was used followed the previous trial by Vattiprolu (2012), as she found this concentration reduced the length of the primary shoot of 'Hort16A' kiwifruit much more compared to 1 and 25 mg/mL^{-1} of NPA. To apply NPA at the graft-union region, first, the grafting tape was carefully removed from the graft-union. A 30 mm length of epidermis (15 mm each of rootstock and scion) was scraped with a sharp scalpel. The NPA dissolved in pre-heated lanolin (1 mL of hydrated lanolin containing 10 mg/mL^{-1} of NPA per plant) was then applied to the scraped portion of the graft-union using a syringe. The portion of the stem where NPA was applied was immediately wrapped with tin-foil to keep the lanolin in place and to maintain the activity of NPA for as long as possible. The NPA was re-applied fortnightly starting from 30/1/2013 until 16/3/2013. The NPA was applied again fortnightly starting at approximately two weeks before the expected spring bud break, from 28/8/2013 until 22/9/2013.

4.2.1.5 Measurement of shoot growth and architecture of 'Hayward' vines

The measurements of scion growth and architecture were conducted throughout the experimental period as described in Chapter Two (Section 2.2.3). After the application of NPA, starting from 6/2/2013, the length of primary shoots was measured fortnightly until 25/6/2013. The primary shoot length was measured from the first node at the base of the primary shoot to the youngest unfurled leaf. In early July, during the winter season, the final length (mm), node number, and internode length (mm) of primary shoots were recorded. The stem diameter (mm) of rootstock and scion were measured 30 mm below and above the graft-union (respectively) where the NPA was applied; converted to cross-sectional area (CSA). In March 2012, before leaf fall, all the leaves

of primary shoots were stripped to determine the leaf area (cm^2) using a Leaf Area Meter (LI-3100, LI-COR, Nebraska, USA).

In addition, the characteristics of the leaves such as fresh weight (g), dry weight (g) and leaf mass per area (LMA) (g/m^2) were measured and recorded using a digital balance (GW6202, Sartorius). All the leaves were dried using a drying oven at 70°C until the leaves reached a constant dry weight, and measured within 30 second of removal from the oven. LMA (g/m^2) was obtained by dividing the leaf dry weight (g) with leaf area (m^2). In the early spring 2013 season, the bud break of the scion primary shoots was recorded. Starting from 10/9/2013 (first day buds burst were visible), the buds that burst were recorded at five days intervals until 10/10/2013. The relative bud break was also recorded similar as describe in a previous chapter (Section 3.2.3, Chapter Three). In addition, the type of proleptic axillary shoots produced along the primary shoots after bud break were recorded using the classification of Seleznyova et al., (2002) similar to the description in Chapter Two (Figure 2.2, Section 2.2.3). The proportion of terminated and non-terminated shoots was calculated and the leaf area of proleptic axillary shoots was recorded as previously described.

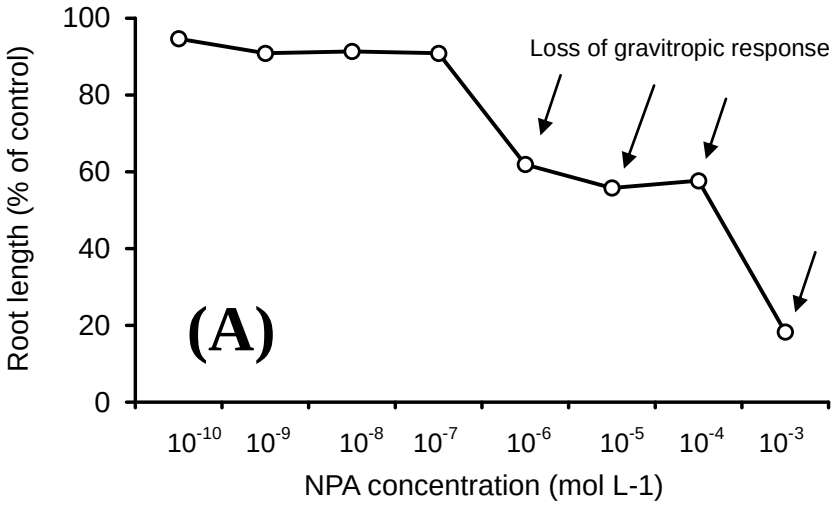
4.2.1.6 Experimental design and statistical analysis

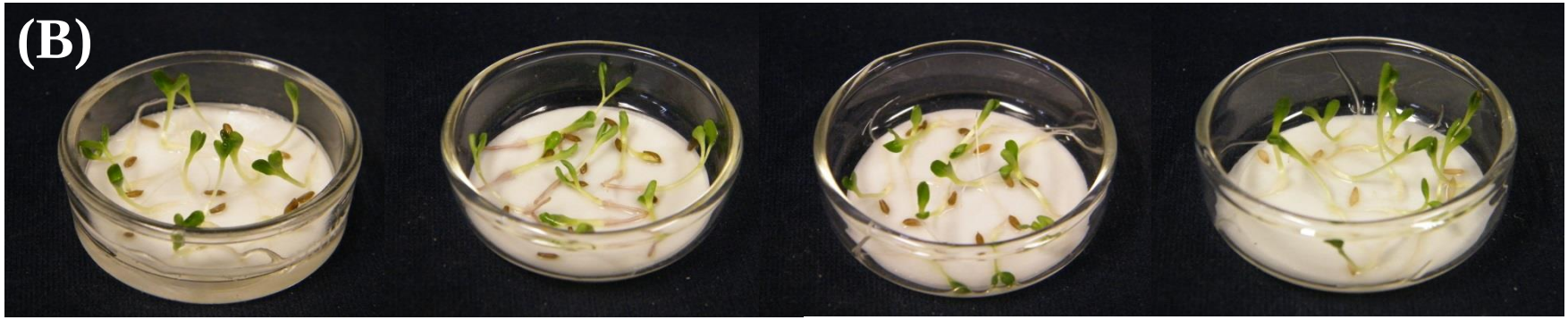
The experimental vines were arranged at a standing out area at Plant Growth Unit (Figure 4.1). The experiment design was a Randomised Complete Block Design (RCBD) with a factorial arrangement of treatments. The 'Hayward' scions were grafted onto No.19, No.55, No.84, No.86, No.87, No.100 and 'Bruno' rootstock. Self-rooted cuttings (GN) were treated as control vines. Each treatment was replicated ten times with half of the vines on each rootstock type including GN were treated with NPA (± NPA). However, because of wind damage, only two replicates of No. 84 and 'Bruno' were available for each treatment. Data for the main effects (rootstock and NPA) and interactions were analysed using the GLM procedure of SAS (9.1, SAS Institute Inc., NC USA). In addition, the pre-planned comparison of the effects of rootstocks and NPA were analysed using Least Square Means Test (lsmeans).

Figure 4.1. The arrangement of the experimental vines at the standing out area, Plant Growth Unit, Massey University, Palmerston North. The primary shoots of 'Hayward' scions were trained and supported using string attached to the galvanised iron post.

Table 4.2. The root characteristics of lettuce seedlings cv. 'Butter Crunch' after being treated with different concentration of NPA.

Concentration of NPA	Root characteristics
Control	Normal root growth
10^{-3}	Short root length
10^{-4}	Loss of gravitropic response and root growth upward
10^{-5}	Loss of gravitropic response and root growth upward
10^{-6}	Loss of gravitropic response and root growth upward
10^{-7}	Normal root growth
10^{-8}	Normal root growth
10^{-9}	Normal root growth
10^{-10}	Normal root growth





(CONTROL) **(10^{-3})** **(10^{-4})** **(10^{-5})**

Figure 4.2. Effects of different concentrations of 1-N-naphthylphthalamic acid (NPA) on: (A) the mean length of lettuce cv. 'Butter Crunch' roots expressed as a percentage of mean root length for control seedlings, and; (B) morphological characteristics of the root after treated with NPA.

4.2.2 Experiment 2: Auxin transport capacity of inter-specific hybrid kiwifruit rootstocks with different vigour

4.2.2.1 Study site and establishment of experimental plant materials

Selected inter-specific hybrid kiwifruit rootstocks (i.e. No.18, No.45, No.55, No.86, No.87, No.100, No.101 and Bounty 71) planted in 3 L polybags provided material for this study (Figure 4.3B). The cuttings of inter-specific hybrid kiwifruit rootstocks were supplied by Plant and Food Research, Motueka, Nelson, New Zealand. The cuttings were propagated during the 2011 winter season at the Plant Growth Unit, Massey University, Palmerston North from semi-hard and hard-wood winter cuttings (Figure 4.3A). During propagation, the bottom of cuttings were scored using sharp knives and treated with a rooting hormone solution of 500 mg L^{-1} IBA dissolved in 25% ethanol. The cuttings were planted in 6 cm x 6 cm plug-trays containing the growth medium (Dalton^{TM}) as described in Section 4.2.2.1. The cuttings were arranged in a temperature control room, with a bench heating system to maintain the temperature of the growing medium at 20 to 22 °C cuttings (Figure 4.3A). After the cuttings rooted, they were transferred in the 10 L pots cuttings (Figure 4.3B) containing the growing medium as described in Section 4.2.2.1.

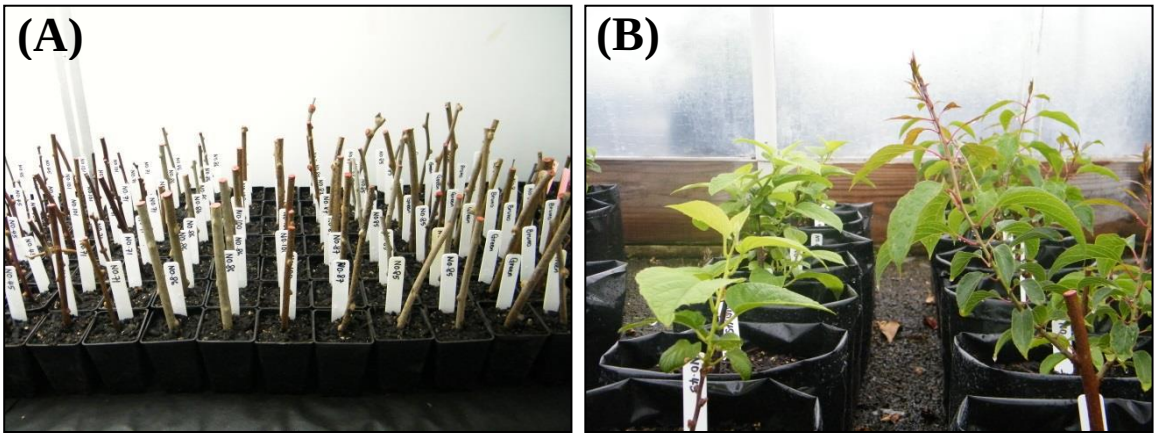


Figure 4.3. (A) Inter-specific hybrid kiwifruit rootstocks propagated from cuttings, and; (B) The cuttings of inter-specific hybrid kiwifruit rootstocks two months after transplanted into 3 L polybags.

4.2.2.2 Transport of IAA in rootstocks using agar-donor receiver transport system and [^{14}C]-IAA

The experiment was conducted in the laboratory of The Institute of Agriculture and Environment at Massey University, Palmerston North, New Zealand. The stems of primary shoots (proleptic) that were 100 mm in length were taken from three years-old kiwifruit rootstocks and wrapped in tin foil to prevent dehydration. The stem segments were then placed in zip-lock bags that were placed in a cool-box and immediately brought back to the laboratory for further processing. The transport of [^{14}C]-IAA was studied using 60 mm-long explants from the kiwifruit rootstocks. The basal end of each section was cut obliquely (Figure 4.4A) to identify it as such. Similar method to those of Kamboj (1997) and Soumelidou et al. (1994) were used, but the transport system was slightly modified to suit the kiwifruit stem segments for this study (Figure 4.4A, B and C). The apical ends of the stem segments were immersed in a buffer of 0.2 M disodium phosphate and 0.1 M citric acid (pH 5.0) in an auto-sampler vial containing a solution of [^{14}C]-IAA with a depth of 10 mm (Soumelidou et al., 1994). The basal end of the segments was embedded in 1 ml agar contained in a test tube (Figure 4.4A). The junction of the auto-vial sampler was sealed using parafilm (Parafilm M, BEMIS Flexible Packaging®, PM-996, WI 54956, USA).

The [^{14}C]-IAA transport experiment was conducted in late autumn of 2013-2014 (May 2014) and early summer of 2014-2015 growing season (December 2014) for period of 24 hr and 48 hr at 22-24^{o}C. After this transport period, the stem segments were removed from the system. The whole segments were rinsed with distilled water and tissue paper was used to remove excess water on the segments. The diameter of apical end of stem the segments was then measured using a digital calliper. After that, the stem segments were cut into three sections, each 20 mm in length. The sections were termed apical, middle or basal sections and weighted using a digital balance (Entris WL-1019, Sartorius). Radioactivity was extracted from these segments and receiver agar blocks by adding 1 ml of ethanol overnight at room temperature in the dark at 4^{o}C. The cocktail plus radioactive rootstock segments were placed in small glass vials before loaded for counting. Radioactivity was counted using a liquid scintillation system (PerkinElmer's Liquid Scintillation Systems) and expressed as either total activity (dpm) or specific activity (dpm mm^{-3} cross section area in contact with donor solution).

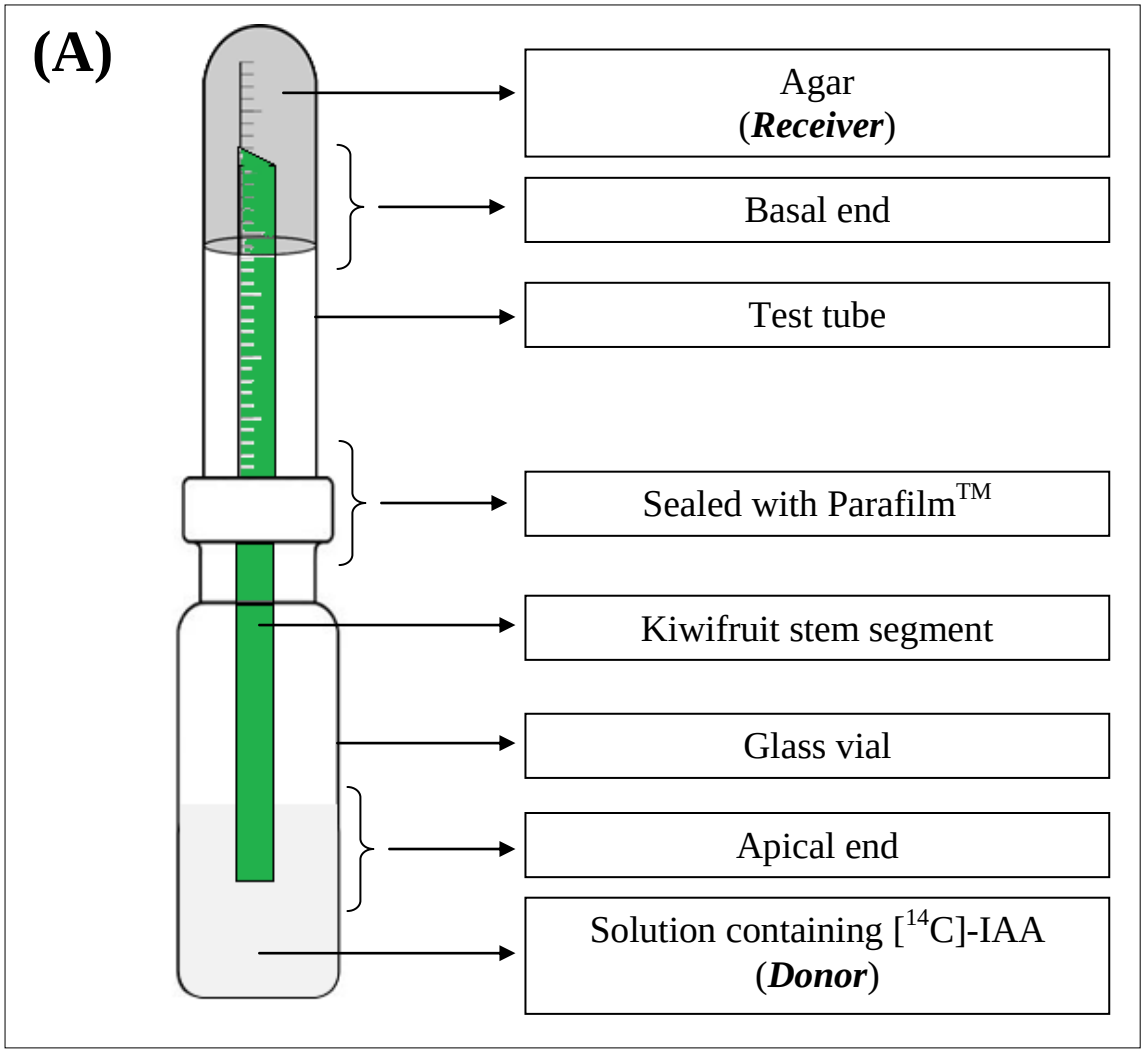


Figure 4.4. (A) A schematic diagram of agar-donor receiver transport system of [^{14}C]-IAA in stem segment of kiwifruit rootstocks (modified from Soumelidau et al. (1994b) and Kamboj (1996)); (B) Arrangement of transport system of [^{14}C]-IAA of kiwifruit stem segments of inter-specific hybrid kiwifruit rootstocks tested in laboratory; (C) Close-up of [^{14}C]-IAA transport system used in Experiment Two.

4.2.2.3 Experimental design and statistical analysis

The experiment was a completely randomised block design and blocking was conducted within incubation time. There were eight rootstocks used for this experiment, which were No.18, No.45, No.55, No.86, No.87, No.100, No.101 and 'Bounty 71'. Each rootstock was replicated ten times and subjected to the transport system for either 24hr or 48hr. Data collected from the Scintillation Counter (PerkinElmer's Liquid Scintillation Systems) were transferred to Excel Pivot Table. Since the data were not normally distributed, logarithm transformation was used before subjecting the data for analysis using the GLM procedure of SAS (9.1, SAS Institute Inc. NC USA). Due to the large variation in the size of apical segments, the treatment means were compared using Tukey's test after Analysis of Covariance (ANVOCA) adjustment using cross-sectional area of apical as covariate. Data presented was expressed as either total activity (log dpm) or specific activity (log dpm).

4.3 Results

4.3.1 Experiment 1

Two days after application of NPA, the apex and leaves of primary shoots of 'Hayward' scions showed epinasty (Figure 4.5A). The obvious symptom was a bending down of the apical shoot apex, which was observed in all primary shoots of NPA-treated vines (Figure 4.5A). In addition, two weeks after the application of NPA, axillary outgrowth was observed on the rootstock stem below the site of NPA application (Figure 4.5B).

Figure 4.5. (A) The apical (yellow arrow) and leaves of primary shoots of 'Hayward' scion exhibiting epinasty two day after application of NPA, and (B) the axillary outgrowth on the rootstock stem two week after application of NPA. No axillary outgrowth was observed on the rootstock stem without NPA (data not shown).

4.3.1.1 Main effects of rootstocks and NPA treatment on the characteristics of primary shoots of 'Hayward' scions

4.3.1.1.1 The length of primary shoots of scions

The primary shoot of 'Hayward' scions on all rootstocks treated with NPA, including the self-rooted control terminated earlier than untreated vines (Figure 4.6). It was observed that the termination of primary shoots started about two weeks after NPA application. However, the termination of primary shoot growth of 'Hayward' scions grafted onto low-vigour rootstock No.19 (*A. chinensis* x *A. macrosperma*) and high-vigour rootstock Bruno (*A. deliciosa*) were similar between NPA-treated and untreated vines (Figure 4.6). No significant effect was found on rootstock x NPA treatment interactions on the final mean length of primary shoots of grafted scions (P=0.61) (Table 4.3). However, there was a significant main effect of rootstocks (P=0.03) and NPA treatment (P=0.0007) on the final mean length of primary shoots (Table 4.3). For the main effect of rootstocks, the final mean length of primary shoots was significantly longer in GN (*A. deliciosa*, 'Hayward' cuttings) compared to the primary shoots of 'Hayward' scions grafted onto intermediate vigour rootstocks No.84 (*A. polygama*) and No.86 (*A. macrosperma*), as well as low-vigour rootstock No.100 (*A. macrosperma* x *A. melanandra*) with rootstock No.84 produced the shortest mean length (Table 4.3).

4.3.1.1.2 Node number of primary shoots of scions

Rootstock x NPA treatment interaction was not significant for the final mean node number of primary shoots (P=0.60). There were significant main effects for both rootstocks (P=0.03) and NPA treatment (P=0.0009) on the mean node number of primary shoots of 'Hayward' scions (Table 4.3). For the main effect of rootstocks, the final mean node number of primary shoots of 'Hayward' scions was significantly reduced in an intermediate vigour rootstocks No.55 (*A. polygama* x *A.chinensis*), No.84 (*A. polygama*,) and No.86 (*A. macrosperma*), plus low-vigour rootstock No.100 (*A. macrosperma* x *A. melanandra*) compared to GN (*A. deliciosa* cv. 'Hayward') (Table 4.3). The NPA treatment also significantly reduced the final mean node for primary shoots of 'Hayward' scions.

4.3.1.1.3 Internode length of primary shoots of scions

Rootstock x NPA treatment interaction was not significant (P=0.99) for the final mean internode length of primary shoots of grafted scions. There was also no significant main effect either rootstock (P=0.69) or NPA treatment (P=0.16) for the final mean internode length of primary shoots of 'Hayward' scions (Table 4.3). Even though not significant, the main effect of NPA treatment may indicate some reduction in the final mean internode length of primary shoots of 'Hayward' scions (Table 4.3).

4.3.1.1.4 The trunk cross-sectional area of primary shoots of scions

No significant difference (P=0.26) was found for the rootstock x NPA treatment interaction (Table 4.3). The mean trunk cross-sectional area (CSA) of primary shoots of scions was significantly affected by the main effect of rootstocks and NPA treatment at P=0.0003 and P=0.02, respectively (Table 4.2). Self-rooted GN (*A. deliciosa* cv. 'Hayward') produced significantly larger in the trunk CSA of primary shoots. The trunk CSA of primary shoots of 'Hayward' scions on intermediate vigour rootstock No.84 (*A. polygama*) and high-vigour rootstock Bruno (*A. deliciosa* cv. 'Bruno') was the smallest compared to other rootstocks. NPA treatment also significantly reduced the trunk CSA of primary shoots of 'Hayward' scions (Table 4.3). Overall, the NPA treatment (+NPA) on the particular inter-specific hybrid kiwifruit rootstocks reduced the final mean length, node number and trunk CSA of the primary shoots of 'Hayward' scions compared to the untreated (-NPA) vines. However, the internode length of primary scion shoots was not affected by the rootstock and NPA treatment.

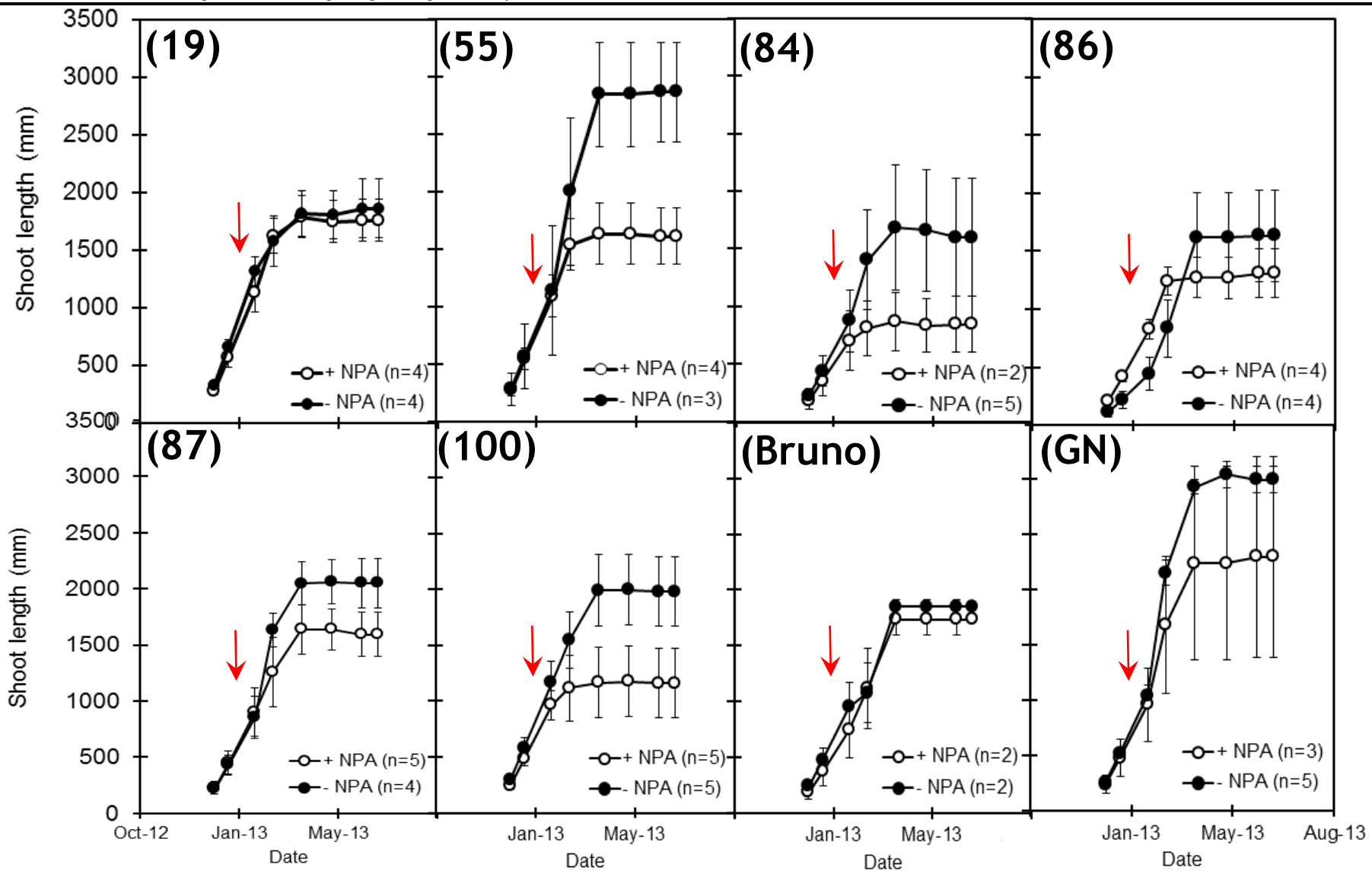


Figure 4.6. Effect of rootstocks and auxin transport inhibitor (±NPA) on the mean primary shoots length of 'Hayward' scions. The applications of NPA were given four times (Jan 30th, Feb 15th, Feb 28th and Mar 16th) and the red solid arrow represents the starting date of NPA application. Letters in parentheses are rootstock number, and GN is for green cutting (own-root).

Table 4.3. Main effect of rootstock and auxin transport inhibitor (±NPA) on characteristics of the primary shoots (length, node, internode length and shoot CSA of 'Hayward' scions at the end of first growing season (June 2013).

Main effects		The characteristics of primary shoots of 'Hayward' scions			
		Mean shoot length (mm)	**Mean node number**	**Mean internode length (mm)**	**Shoot CSA (mm^2)**
Rootstock (R)ǂ (including parentages)					
No.87	*A. polygama*	1820.0 ^{abc}	28.3 ^{ab}	64.1 ^{a}	91.5 ^{bc}
No.100	*A. macrosperma* x *A. melanandra*	1506.7 ^{bc}	23.2 ^{b}	65.3 ^{a}	86.7 ^{bc}
No.19	*A. chinensis* x *A. macrosperma*	1811.3 ^{abc}	28.3 ^{ab}	66.6 ^{a}	103.2 ^{b}
No.55	*A. polygama* x *A.chinensis*	2151.4 ^{ab}	32.4 ^{a}	64.9 ^{a}	103.6 ^{b}
No.84	*A. polygama*	1315.0 ^{c}	21.4 ^{b}	62.5 ^{a}	79.4 ^{c}
No.86	*A. macrosperma*	1595.7 ^{bc}	23.3 ^{b}	68.0 ^{a}	95.5 ^{bc}
Bruno	*A. deliciosa* cv. 'Bruno'	1790.0 ^{abc}	28.8 ^{ab}	62.3 ^{a}	78.5 ^{c}
GN	*A. deliciosa* cv. 'Hayward'	2460.0 ^{a}	34.0 ^{a}	73.1 ^{a}	124.0 ^{a}
$LSD_{0.05}$		698.3	8.8	11.3	20.2
P-value		*P*=0.03	*P*=0.03	*P*=0.69	*P*=0.0003
NPA treatment (T)					
+ NPA		1504.4 ^{b}	23.8 ^{b}	63.7 ^{a}	90.5 ^{b}
- NPA		2116.9 ^{a}	31.2 ^{a}	68.2 ^{a}	102.0 ^{a}
$LSD_{0.05}$		345.0	4.3	5.6	9.9
P-value		*P*=0.0007	*P*=0.0009	*P*=0.16	*P*=0.02
Interaction (R x T)		*P*=0.61	*P*=0.60	*P*=0.99	*P*=0.26

Means sharing the same letters within a column are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
ǂInitial vigour ranking of scion on each rootstock determined according to results in Chapter Three. In order of increasing vigour.
GN – Self-rooted control.

4.3.1.2 The relationship between shoot length and node number of primary shoots of 'Hayward' scions

There was a strong positive relationship ($R^2 > 0.60$) between shoot length and node number of scion primary shoots when the data from NPA treatment (±NPA) were pooled and plotted together for each rootstocks (Figure 4.7). This shows that the length of primary shoot increased as the node number increased. For each treatment (with or without NPA), the relationships for individual rootstock are presented in Appendix 2.

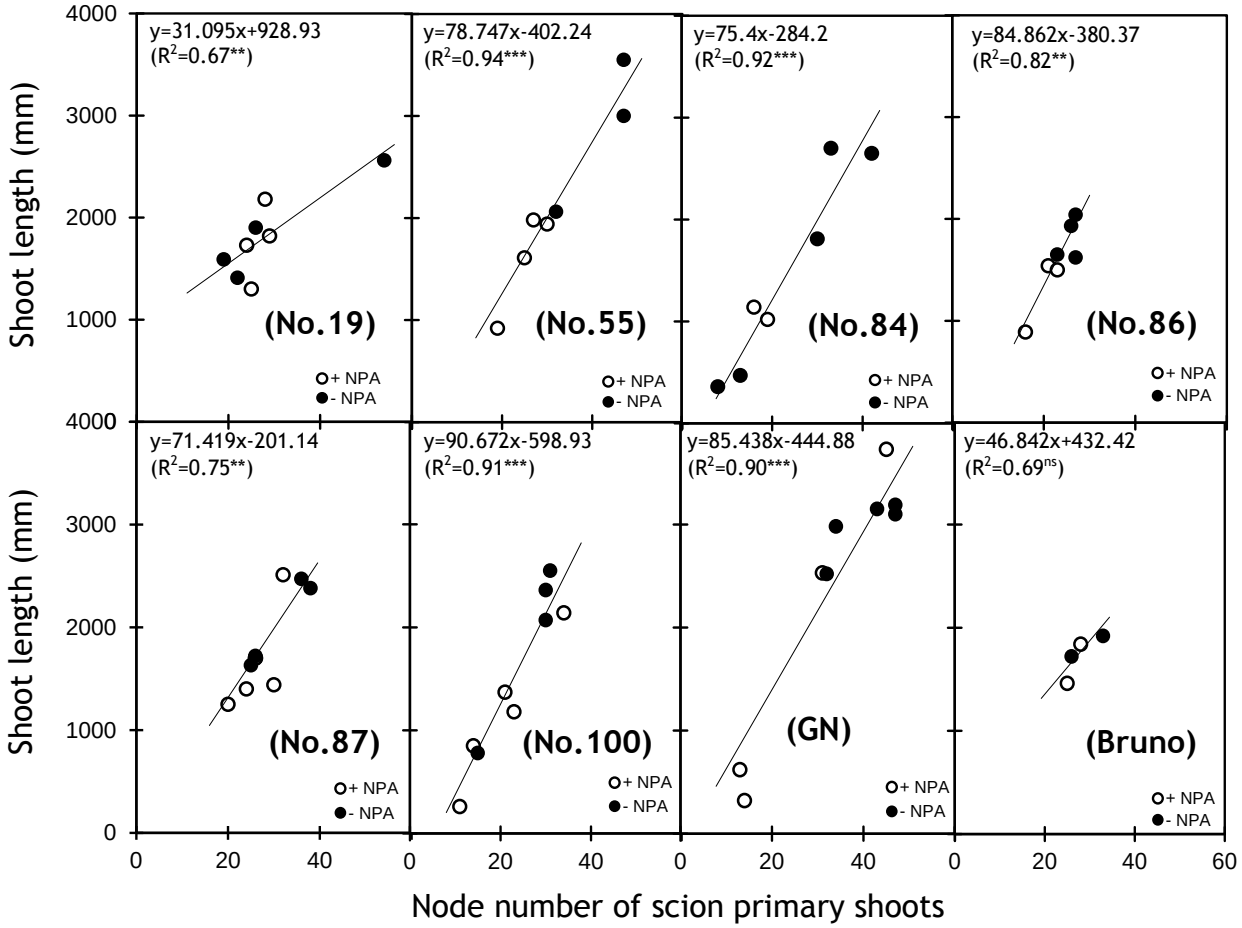


Figure 4.7. The relationship between the length and node number of the primary shoots 'Hayward' scions with (○) and without NPA (●) application (data were pooled together). For individual NPA treatment (± NPA) on every each rootstock, see Appendix 2. ns, *, **, * non-significant or significant at *P*≤0.05, 0.01 and 0.001, respectively.**

4.3.1.3 Main effects of rootstocks and NPA treatment on the leaf characteristics of primary shoots of 'Hayward' scions

4.3.1.3.1 Leaf area (LA) of primary shoots

The rootstock x NPA treatment interaction was not significant (*P*=0.92) for the mean LA (cm^2) of primary shoots of 'Hayward' scions (Table 4.4). There were significant main effects of rootstocks (*P*=0.02) and NPA treatment (*P*=0.01) on the final mean LA (cm^2) of primary shoots of 'Hayward' scions (Table 4.4). For the main effect of rootstocks, the mean LA (cm^2) of primary shoots increased significantly in Bruno rootstock when compared to the mean LA of primary shoots on rootstocks No.84, No.86 and No.100 (Table 4.4). For the main effect of NPA treatment, the final mean LA (cm^2) of scion primary shoots reduced significantly with NPA treatment.

4.3.1.3.2 Leaf fresh (FW) and dry weight (DW) of primary shoots

Rootstock x NPA treatment interactions were not statistically significant for the leaf FW and DW (g) of primary shoots of 'Hayward' scions (*P*=0.92 and *P*=0.81, respectively) (Table 4.4). For the total mean leaf FW and DW of leaves (g per shoot), only rootstocks had significant effect on the mean leaf DW (g) of primary shoots (*P*=0.05). However, the mean leaf FW (g) almost approached significance at *P*=0.06 (Table 4.4). For main effect of rootstocks, the mean leaf FW of primary shoots was significantly higher in high-vigour rootstock Bruno compared to the intermediate vigour rootstocks No.84 (*A. polygama*) and No.86 (*A. macrosperma*), plus low-vigour rootstock No.100 (*A. macrosperma* x *A. melanandra*). In addition, the mean leaf DW (g) of primary shoots was significantly reduced when grafted onto intermediate vigour rootstock No.84 and No.86 when compared to the other rootstocks (Table 4.4). Significantly higher mean leaf DW (g) of scion primary shoots was recorded on low-vigour rootstock No.19 (*A. chinensis* x *A. macrosperma*) compared to intermediate vigour rootstock No.84 (*A. polygama*) and No.86 (*A. macrosperma*). The main effect of NPA treatment did not significantly affect the mean leaf FW and DW (g) of primary shoots (*P*=0.12 and *P*=0.81, respectively). However, there was a general trend (*P*=0.12) that NPA treatment reduced the mean final leaf FW of primary shoots (Table 4.4).

4.3.1.3.3 Leaf mass per area (LMA) of primary shoots

There was no significant influence of rootstock x NPA treatment interaction (*P*=0.32) on the mean LMA (g/m^2) of primary shoots of 'Hayward' scions (Table 4.4). The main effect of rootstocks was significant for the LMA of scion primary shoots (*P*=0.05) and close to significance for NPA treatment (*P*=0.08) (Table 4.4). For the main effect of rootstocks, the mean LMA of scion primary shoots on intermediate vigour rootstocks No.84 (*A. polygama*) was significantly reduced compared to the primary shoots on low-vigour rootstocks No.19 (*A. chinensis* x *A. macrosperma*) and No.100 (*A. macrosperma* x *A. melanandra*), and high-vigour rootstock Bruno (Table 4.4). For the main effect of NPA treatment, there was a trend (*P*=0.08) that LMA of primary shoots had increased with NPA treatment compared to the untreated vines (Table 4.4).

Table 4.4. Main effects of rootstocks and auxin transport inhibitor (±NPA) on the final mean leaf characteristics (leaf area, leaf fresh weight, leaf dry weight and leaf mass per area) of 'Hayward' scions at the end of first growing season (June 2013).

Main effects		The leaf characteristics of primary shoots of 'Hayward' scions			
		Mean leaf area (cm^2)	**Mean leaf fresh weight (g)**	**Mean leaf dry weight (g)**	**Mean LMA (g/m^2)**
Rootstock (R)[ǂ] (including parentages)					
No.87	*A. polygama*	3380.4 ^{abc}	189.6 ^{abc}	52.3 ^{abc}	13.7 ^{ab}
No.100	*A. macrosperma* x *A. melanandra*	2757.3 ^{c}	159.7 ^{bc}	42.8 ^{abc}	15.8 ^{a}
No.19	*A. chinensis* x *A. macrosperma*	3733.8 ^{abc}	215.4 ^{abc}	59.6 ^{a}	15.8 ^{a}
No.55	*A. polygama* x *A. chinensis*	4022.2 ^{ab}	219.5 ^{ab}	55.7 ^{ab}	13.7 ^{ab}
No.84	*A. polygama*	2926.3 ^{bc}	150.0 ^{c}	34.8 ^{c}	12.4 ^{b}
No.86	*A. macrosperma*	2751.2 ^{c}	154.1 ^{bc}	39.5 ^{bc}	13.5 ^{ab}
Bruno	*A. deliciosa* cv. 'Bruno'	4514.7 ^{a}	228.4 ^{a}	52.4 ^{abc}	16.1 ^{a}
GN	*A. deliciosa* cv. 'Hayward'	3511.9 ^{abc}	212.7 ^{abc}	53.1 ^{ab}	11.9 ^{ab}
$LSD_{0.05}$		1217.8	67.2	17.9	3.2
P-value		*P*=0.02	*P*=0.06	*P*=0.05	*P*=0.05
NPA treatment (T)					
+ NPA		3039.0 ^{b}	177.8 ^{a}	47.2 ^{a}	15.0 ^{a}
- NPA		3810.7 ^{a}	201.7 ^{a}	50.2 ^{a}	13.2 ^{a}
$LSD_{0.05}$		1217.8	33.0	8.8	1.6
P-value		*P*=0.01	*P*=0.12	*P*=0.32	*P*=0.08
Interactions (R x T)		*P*=0.92	*P*=0.91	*P*=0.81	*P*=0.32

Means sharing the same letters within a column are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
[ǂ]Initial vigour ranking of scion on each rootstock determined according to results in Chapter Three. In order of increasing vigour.
GN – Self-rooted control.

4.3.1.4 The pattern of leaf area along the primary shoots of 'Hayward' scions

Figure 4.8 shows the individual LA of primary shoots of 'Hayward' scions as a function of nodal positions for the first 20 similar nodes. Generally, the individual LA for the first 20 nodes was similar up to about Node 5, and gradually decreases until Node 15 or Node 20 (Figure 4.8). However, for the rootstocks No.55, No.84, No.86, No.87, Bruno, and GN, the primary shoot of NPA treated vines (+NPA) relatively had smaller LA starting from Node 5 until Node 15 or Node 20. In contrast, the individual LA along the node of primary shoots of 'Hayward' scions on low-vigour rootstocks No.19 (*A. chinensis* x *A. macrosperma*) and No.100 (*A. macrosperma* x *A. melanandra*) were almost similar between NPA treated and untreated (Figure 4.8). Slight reduction trends on the individual LA were found on the primary shoots of rootstocks No.84 and Bruno; however, only two replicates were available for these rootstocks (Figure 4.8).

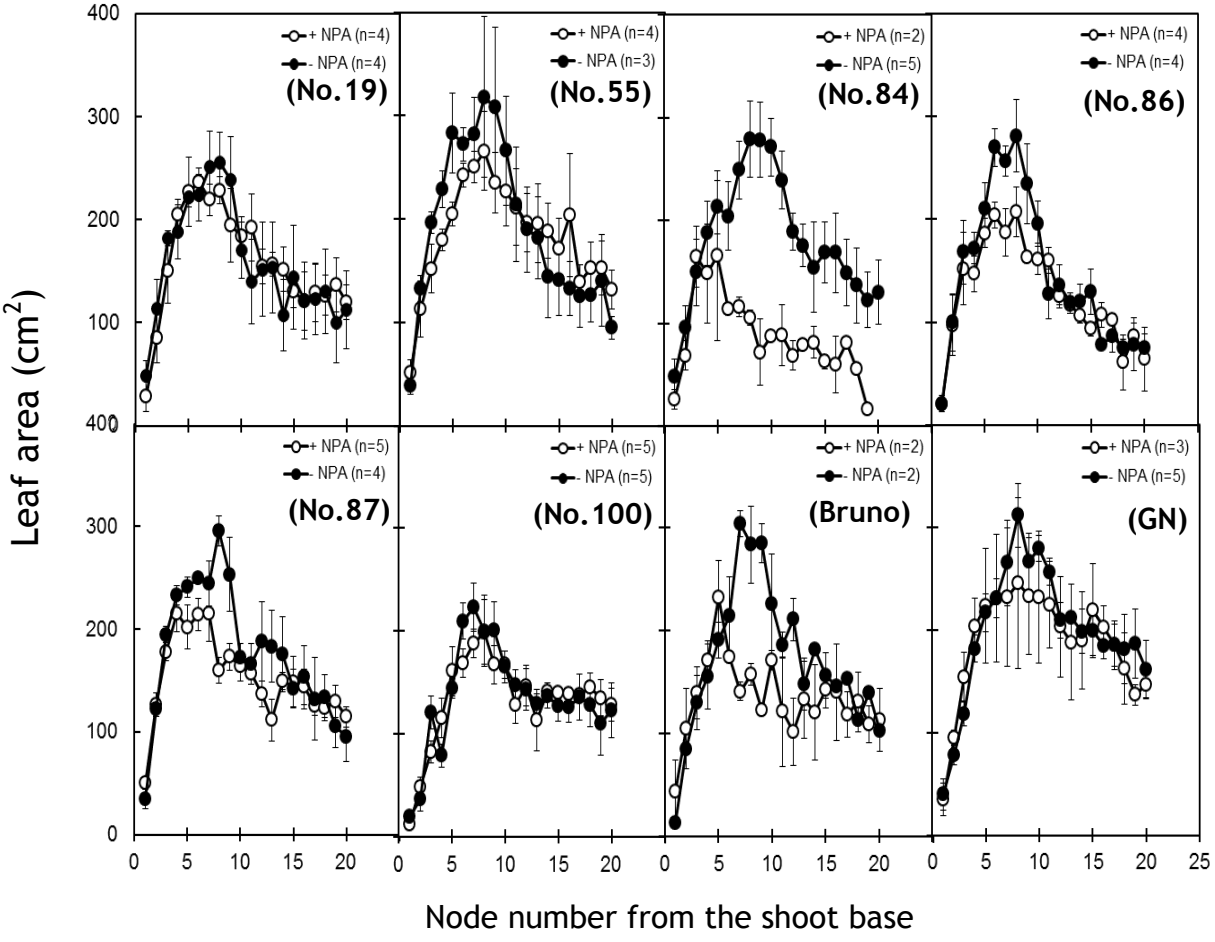


Figure 4.8. Effect of rootstocks and auxin transport inhibitor (±NPA) on the individual leaf size (cm^2) along the node of primary shoots of 'Hayward' scions.

4.3.1.5 The final trunk cross-sectional area (CSA) of rootstocks and budwood

At the winter season of 2013, the rootstock x NPA treatment interactions for the final trunk CSA of rootstocks and scion budwoods were not significant at the end of growing seasons (P=0.68 and P=0.93, respectively) (Table 4.5). Highly significant differences were found on the main effects of rootstocks and scion bud woods on trunk CSA (P=0.0002 and P<0.0001, respectively) (Table 4.5). However, the main effects of NPA treatment (±NPA) were not significant (P=0.25 and P=0.66, respectively). For the main effect of rootstocks, the final mean trunk CSA of rootstocks was significantly larger on intermediate vigour rootstock No.86 (*A. macrosperma*) compared to low-vigour rootstock No.19 (*A. chinensis* x *A. macrosperma*) and high-vigour rootstock No.55 (*A. polygama* x *A. chinensis*). Similarly, the final mean trunk CSA of budwoods were also larger in rootstock No.86 compared with the rootstock No.19 that had the smallest mean trunk CSA of scions (Table 4.5).

Table 4.5. Main effect of rootstocks and auxin transport inhibitor (±NPA) on the final mean trunk cross-sectional area (CSA) of rootstocks and budwoods at the end of first growing season (June 2013).

Main effect		**Mean trunk cross-sectional area (CSA) (mm^2)**	
		Rootstock	**Budwood**
Rootstock (R)[ǂ] (including parentages)			
No.87	*A. polygama*	129.1 [ab]	159.3 [ab]
No.100	*A. macrosperma* x *A. melanandra*	130.9 [ab]	168.2 [ab]
No.19	*A. chinensis* x *A. macrosperma*	82.0 [c]	120.6 [cd]
No.55	*A. polygama* x *A. chinensis*	111.5 [b]	162.6 [ab]
No.84	*A. polygama*	124.1 [ab]	148.5 [bc]
No.86	*A. macrosperma*	150.5 [a]	187.5 [a]
Bruno	*A. deliciosa* cv. 'Bruno'	152.4 [a]	177.5 [ab]
GN[x]	*A. deliciosa* cv. 'Hayward'	134.9 [ab]	110.0 [d]
$LSD_{0.05}$		29.3	31.6
P-value		*P*=0.0002	*P*<0.0001
NPA treatment (T)			
+ NPA		121.7 [a]	150.5 [a]
- NPA		129.3 [a]	154.7 [a]
$LSD_{0.05}$		14.2	15.4
P-value		*P*=0.25	*P*=0.66
Interaction (R x T)		*P*=0.68	*P*=0.93

Means sharing the same letters within a column are not significantly different at P=0.05 according to $LSD_{0.05}$ test.
[ǂ]Initial vigour ranking of scion on each rootstock determined according to the results in Chapter Three. In order of increasing vigour.
GN – Self-rooted control.
[x]For GN, the trunk CSA was measured above and below site of NPA application.

4.3.1.6 Spring bud break of primary shoots of 'Hayward' scions

There was no significant effect of rootstocks x NPA treatment interaction on the mean percentage of scions bud break (*P*=0.51) in the early spring seasons (Table 4.6). Similarly, the main effect of rootstocks was not significant (*P*=0.13) for the percentage of spring bud break (Table 4.6). The highest percentage bud break was recorded on intermediate vigour rootstock No.84 (*A. macrosperma*), followed by low-vigour rootstocks No.100 (*A. macrosperma* x *A. melanandra*) and No.87 (*A. polygama*), then high-vigour rootstocks No.55 (*A. polygama* x *A. chinensis*) and 'Bruno' rootstock. The lowest percentage of spring bud break was recorded on GN with less than 25% (Table 4.6). Intermediate vigour rootstock No.84 (*A. polygama*) also produced significantly higher in the mean percentage of scions bud break compared to rootstocks No.19, No.86 and GN (Table 4.6). The main effect of NPA treatment did not significantly affect the mean percentage of bud break of 'Hayward' scions (*P*=0.96) (Table 4.6).

Table 4.6. Main effect of rootstock and auxin transport inhibitor (±NPA) on the mean proportion of bud break of 'Hayward' scions in the spring growing season (October 2013).

Main effects		**Mean percentage of spring budbreak (%)**	
Rootstock (R)[‡] (including parentages)			
No.87	*A. polygama*	28.2 [ab]	**(41)**
No.100	*A. macrosperma* x *A. melanandra*	28.6 [ab]	**(37)**
No.19	*A. chinensis* x *A. macrosperma*	24.8 [b]	**(37)**
No.55	*A. polygama* x *A. chinensis*	28.0 [ab]	**(40)**
No.84	*A. polygama*	30.8 [a]	**(34)**
No.86	*A. macrosperma*	25.0 [b]	**(29)**
Bruno	*A. deliciosa* cv. 'Bruno'	28.0 [ab]	**(27)**
GN	*A. deliciosa* cv. 'Hayward'	23.8 [b]	**(34)**
$LSD_{0.05}$		5.6	
P-value		*P*=0.13	
NPA treatment (T)			
+ NPA		27.4 [a]	
- NPA		26.9 [a]	
$LSD_{0.05}$		2.7	
P-value		*P*=0.96	
Interaction (R x T)		*P*=0.51	

Means sharing the same letters within a column are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
Number in parentheses and bold are means the number of buds per shoot.
[‡]Initial vigour ranking of scion on each rootstock determined according to the results in Chapter Three. In order of increasing vigour.
GN – Self-rooted control.

4.3.1.7 Main effect of rootstocks and NPA on the total length, total node number and number of shoots of proleptic axillary shoots of 'Hayward' scions

The rootstocks x NPA treatment interactions were not significant for the mean total length, mean total node number, and mean total number of proleptic axillary shoots (P=0.75, P=0.29 and P=0.22, respectively) (Table 4.7). However, the main effect of rootstocks was significant (P=0.01) for the mean total length of the proleptic axillary shoots (Table 4.7). For the main effect of rootstocks, the mean total shoot length of proleptic axillary shoots was significantly higher in GN (*A. deliciosa* cv. 'Hayward') compared to low-vigour rootstocks No.19 (*A. chinensis* x *A. macrosperma*) and No.100 (*A. macrosperma* x *A. melanandra*), as well as 'Bruno'. The mean total node number and mean number of the proleptic axillary shoots of scions were also affected significantly by the rootstocks (P=0.004 and P=0.007, respectively). Scions on the low-vigour rootstock No.87 (*A. polygama*) had significantly higher mean total node number of proleptic axillary shoots compared to other rootstocks, except for intermediate vigour rootstock No.84 (*A. polygama*) and high-vigour rootstock No.55 (*A. polygama* x *A. chinensis*) (Table 4.7). Scions on low-vigour rootstock No.87 also produced significantly higher mean number of proleptic axillary shoots (Table 4.7). The NPA treatment had significant influence on the mean total length (P<0.0001) and the mean total node number (P=0.008) of scion proleptic axillary shoots (Table 4.7). There was also a general trend (P=0.11) that the mean number of proleptic axillary shoots was affected by NPA treatment (Table 4.7). With NPA treatment, the mean total length and node number, and possibly number of proleptic shoots were reduced.

4.3.1.8 Main effect of rootstocks and NPA on the total leaf area of proleptic axillary shoots

There was no significant rootstock x NPA treatments interaction (P=0.80) on the mean total LA (cm^2) of proleptic axillary shoots (Table 4.7). Rootstocks had a significant effect (P=0.001) on the mean total LA (cm^2) of proleptic axillary shoots of scions (Table 4.7). NPA treatment also significantly reduced (P<0.0001) the mean total LA (cm^2) of proleptic axillary shoots.

Table 4.7. Main effects of rootstocks and auxin transport inhibitor (±NPA) on the mean total length, node number and number of proleptic shoots of 'Hayward' scions at the end spring growing season (October 2013).

Main effects		Mean total length (mm)[x]		Mean total node number	Mean number of shoots	Mean total leaf area (cm^2)
Rootstock (R)[ǂ] (including parentages)						
No.87	*A. polygama*	43.8 [abc]	**(2044.9)**	93.4 [a]	11.6 [a]	3456.5 [a]
No.100	*A. macrosperma* x *A. melanandra*	39.2 [bcd]	**(1616.2)**	68.3 [bcd]	9.4 [abc]	2209.3 [cd]
No.19	*A. chinensis* x *A. macrosperma*	38.1 [cd]	**(1701.4)**	55.8 [d]	6.9 [dc]	2079.8 [cd]
No.55	*A. polygama* x *A. chinensis*	47.9 [ab]	**(2335.7)**	76.6 [abc]	10.0 [ab]	3277.7 [ab]
No.84	*A. polygama*	45.8 [abc]	**(2285.1)**	86.0 [ab]	9.8 [ab]	2862.9 [abc]
No.86	*A. macrosperma*	44.3 [abc]	**(2063.7)**	65.4 [bcd]	7.6 [bcd]	2447.9 [bcd]
Bruno	*A. deliciosa* cv. 'Bruno'	31.7 [d]	**(1120.0)**	53.0 [d]	6.2 [d]	1872.6 [d]
GN	*A. deliciosa* cv. 'Hayward'	51.2 [a]	**(2762.6)**	85.2 [ab]	9.8 [ab]	3399.5 [a]
$LSD_{0.05}$		8.7		23.3	2.8	893.4
P-value		*P*=0.01		*P*=0.004	*P*=0.007	*P*=0.001
NPA Treatment (T)						
+ NPA		36.0 [b]	**(1506.0)**	64.7 [b]	8.3 [a]	2182.3 [b]
- NPA		49.8 [a]	**(2574.5)**	81.3 [a]	9.5 [a]	3294.4 [a]
$LSD_{0.05}$		4.3		11.4	1.4	435.9
P-value		*P*<0.0001		*P*=0.008	*P*=0.11	*P*<0.0001
Interaction (R x T)		*P*=0.75		*P*=0.29	*P*=0.22	*P*=0.80

Means sharing the same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
[x]Data were transformed to square root to facilitate ANOVA.
[ǂ]Initial vigour ranking of scion on each rootstock determined according to the results in Chapter Three. In order of increasing vigour.
Numbers in parentheses and bold are raw data.
GN – Self-rooted control.

4.3.2 Experiment 2

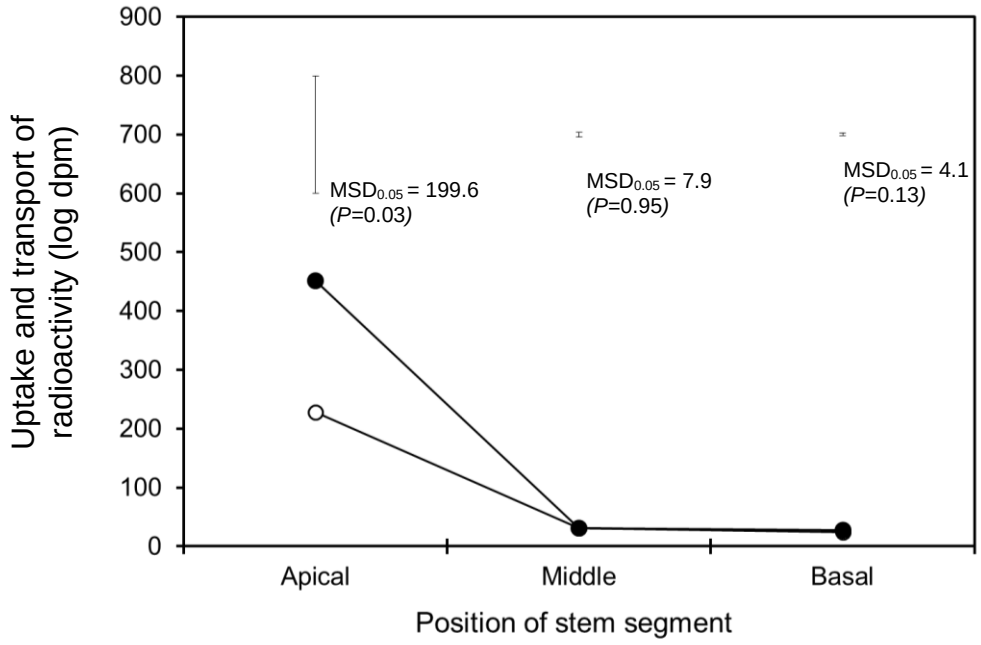
4.3.2.1 Uptake and transport of radioactivity in late autumn season

In late autumn (May 2014), no significant difference was found (P=0.49) on the movement of radioactivity from the stem segments to the agar receptors between low-vigour rootstock No.18 (*A. chinensis* x *A. macrosperma*) and high-vigour rootstock No.101 (*A. macrosperma* x *A. melanandra*) (Table 4.8). However, the total uptake and transport of radioactivity in all segments and agar were significantly different (P=0.04) (Table 4.8). The total radioactivity in the apical segments of low-vigour rootstock No.18 was significantly lower (P=0.03) than the activity in the apical segments of high-vigour rootstock No.101 (Figure 4.9). However, no significant difference (P=0.95) was found in the middle segments between these two rootstocks (Figure 4.9). For the basal segments (Figure 4.9), there was a trend that radioactivity in low-vigour rootstock No.18 (*A. chinensis* x *A. macrosperma*) were lower compared to high-vigour rootstock No.101 (*A. macrosperma* x *A. melanandra*). Overall, there was significantly higher uptake and transport of radioactivity in the high-vigour rootstocks compared to the low-vigour rootstock (Table 4.8).

Table 4.8. Total uptake and transport of radioactivity in low-vigour rootstock No.18 (*A. chinensis* x *A. macrosperma*) and high-vigour rootstock No.101 (*A. macrosperma* x *A. melanandra*) during late autumn season (May 2014).

Rootstock[ǂ] (including parentages)		**Uptake and transport of radioactivity recovered in agar (log dpm)**	**Total uptake and transport of radioactivity[x] (log dpm)**
No. 18	*A. chinensis* x *A. macrosperma*	24.3 [a]	316.0 [b]
No. 101	*A. macrosperma* x *A. melanandra*	24.6 [a]	534.6 [a]
$MSD_{0.05}$		7.4	203.9
***P*-value**		P=0.49	P=0.04

Means sharing same letters within a ***column*** are not significantly different at P=0.05 according to Tukey's test, after ANCOVA adjustment using cross-sectional area of apical as covariate.
[ǂ]Initial vigour ranking of scion on each rootstock determined according to the results in Chapter Three.
MSD - minimum significant difference at P=0.05 according to the Tukey's test.
[x]Sum of activity in all the segments and agar.



Rootstock	***P*=0.02**
Position	***P*<0.0001**
Rootstock x Position	***P*=0.005**

Figure 4.9. Distribution pattern of radioactivity in the three apical, middle and basal stem segment No.18 (low-vigour, ○) and No.101 (high-vigour, ●) rootstocks. Rootstocks were classified according to vigour from previous experiment (Chapter Three). Vertical bars represent the minimum significant difference (MSD) at *P*=0.05 according to the Tukey's test.

4.3.2.2 Uptake and transport of radioactivity in the early summer season of 2014

4.3.2.2.1 Total uptake and transport

There were significant main effects of rootstocks (P=0.002) on the total uptake and transport of radioactivity (Table 4.9). There were also significant main effects of time (P<0.0001) and interaction between rootstock and time (P=0.002). After 24h, uptake and transport of radioactivity were significantly higher in intermediate-vigour rootstock No.55 (*A. polygama* x *A. chinensis*) compared with rootstocks No.18, No.100, No.86, No.101, and 'Bounty 71' (Table 4.9), but did not differ significantly from rootstocks No.87 and No.45. The lowest level of radioactivity recorded in high-vigour rootstock No.101 (*A. macrosperma* x *A. melanandra*). At 48h, the radioactivity was significantly higher in rootstock No.45 (*A. polygama* x *A. chinensis*) compared to other rootstocks (Table 4.9) and the lowest were recorded for rootstocks No.18, No.100, No.86, and No.101. In addition, radioactivity did not significantly differ between rootstock No.55 and 'Bounty 71' (Table 4.9).

Table 4.9. Total uptake and transport of radioactivity recovered (sum of activity in all the segments and agar) after 24h and 48h. Data were log-transformed for statistical analysis (log dpm).

Rootstock[ǂ]	Parentages	Vigour	24h (log dpm)	48h (log dpm)
No.18	*A. chinensis* x *A. macrosperma*	Low	8.69 ^{bc}	8.81 ^{d}
No.87	*A. polygama*	Low	8.83 ^{ab}	9.37 ^{b}
No.100	*A. macrosperma* x *A. melanandra*	Low	8.54 ^{bc}	8.72 ^{d}
Bounty 71	-[x]	Intermediate	8.79 ^{bc}	8.95 ^{cd}
No.45	*A. polygama* x *A. chinensis*	Intermediate	8.87 ^{ab}	9.89 ^{a}
No.55	*A. polygama* x *A. chinensis*	Intermediate	9.28 ^{a}	9.32 ^{bc}
No.86	*A. macrosperma*	Intermediate	8.60 ^{bc}	8.65 ^{d}
No.101	*A. macrosperma* x *A. melanandra*	High	8.34 ^{c}	8.84 ^{d}

Rootstock	P=0.002
Hour	P<0.0001
Rootstock x Hour	P=0.002

Means sharing same letters within a ***column*** are not significantly different at P=0.05 according to Tukey's test, after ANCOVA adjustment using cross-sectional area of apical as covariate.

[ǂ]Initial vigour ranking of scion on each rootstock determined according to the results in Chapter Three. In order or increasing vigour.

[x]Slightly less vigorous or moderate reduced vigour according to (Anon., 2012) and M. Clearwater (personal communication, June 27, 2016), respectively.

[x]Unknown parentage from open pollinated of *A. macrosperma*.

In rootstocks No.87, No.45, and No.101, uptake and transport of radioactivity in 48h were significantly higher than 24h, but no significant differences were in other rootstocks (Figure 4.10). For 24h, there was a general trend that the uptake and transport of radioactivity increased with increasing rootstock vigour, but only up to rootstock No.55. However, for 48h, the trend was not consistent for all rootstocks (Figure 4.10).

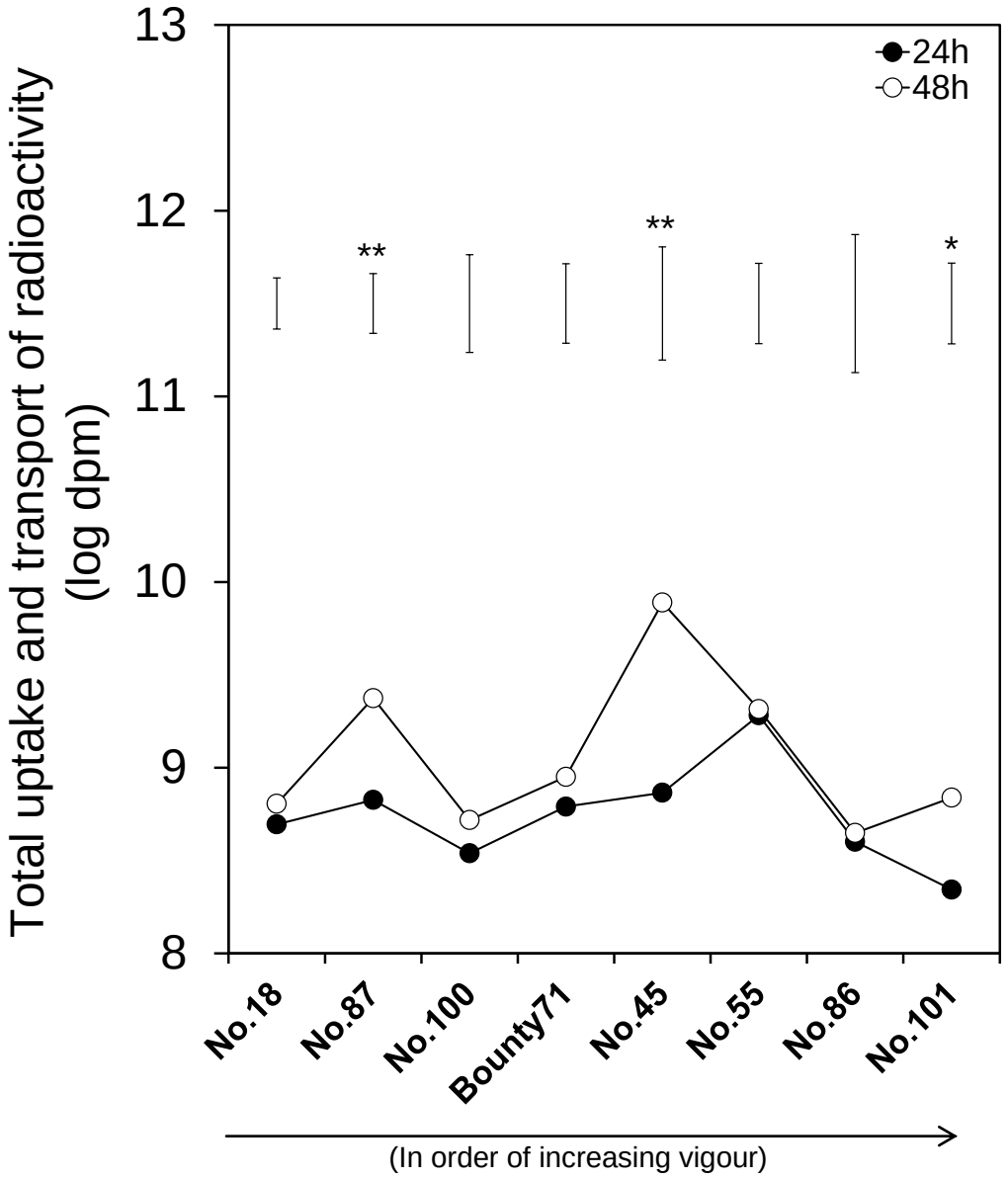


Figure 4.10. Comparison in the total uptake and transport of radioactivity between 24h (●) and 48h (○). Data were log-transformed for statistical analysis. Rootstocks were classified according to vigour from previous experiment (Chapter Three). Bars indicate MSD at 5%. * and ** significant at *P*≤0.05 and 0.01, respectively according to Tukey's test.

4.3.2.2.2 Radioactivity in stem segments and agar from rootstocks of differing vigour

In both hours (24h and 48h), rootstock had significant effect (P<0.0001) on the uptake and transport of radioactivity (Table 4.10 and Table 4.11). Similarly, position (P<0.0001) and rootstock x position interaction (P<0.0001) were also significant (Table 4.10 and Table 4.11). There were also significant effects of interaction between rootstock, hour, and position (P<0.002) (Table 4.12). In 24h, apical segments of intermediate vigour rootstock No.55 (*A. polygama* x *A. chinensis*) accumulated significantly higher level of radioactivity than rootstocks No.18, No.100, No.86, and 101, but did not significantly differ with rootstocks No.87, No.45 and Bounty 71 (Table 4.10). The lowest uptake and activity of [^{14}C]-IAA were recorded on high-vigour rootstock No.101 (*A. macrosperma* x *A. melanandra*) (Table 4.10). The uptake and transport of radioactivity in the middle and basal segments were also significantly higher for rootstock No.55 than other rootstocks. Similarly, in agar, significantly higher uptake and radioactivity were also recorded in intermediate rootstock No.55 (*A. polygama* x *A. chinensis*) than other rootstocks (Table 4.10).

For 48h, significantly higher uptake and transport of radioactivity in apical segments were recorded on intermediate vigour rootstock No.45 (*A. polygama* x *A. chinensis*) than other rootstocks, but did not significantly different between rootstock No.55 and low-vigour rootstock No.87 (*A. polygama*) (Table 4.11). No significant differences were recorded on the uptake and transport of radioactivity in the apical segments between rootstocks No.18, No.100, No.86, No.101 and 'Bounty 71' (Table 4.11). In the middle segments, the uptake and transport of radioactivity were significantly reduced in rootstocks No.18 and No.100 than other rootstocks. In basal segments, intermediate vigour rootstock No.45 (*A. polygama* x *A. chinensis*) accumulated significantly higher uptake and transport of radioactivity, but low-vigour rootstock No.18 (*A. chinensis* x *A. macrosperma*) had the lowest. In agar, rootstock No.45 and No.55 had significantly higher in uptake and radioactivity than other rootstocks (Table 4.11).

Table 4.10. Uptake and transport of radioactivity in different position of kiwifruit rootstock stem segments (apical, middle, and basal) after 24h. Data were log-transformed for statistical analysis. Specific activity was expressed as log dpm mm^{-2} cross-sectional area of apical.

Rootstock[‡]	Parentages	Stem segment (Position)			
		Apical	Middle	Basal	Agar
No.18	*A. chinensis* x *A. macrosperma*	8.67 ^{bc}	4.77 ^{bc}	3.42 ^{e}	3.34 ^{d}
No.87	*A. polygama*	8.77 ^{abc}	4.68 ^{bcd}	5.08 ^{bc}	3.58 ^{cd}
No.100	*A. macrosperma* x *A. melanandra*	8.50 ^{bc}	3.99 ^{d}	4.86 ^{bcd}	3.91 ^{bc}
Bounty71	- [x]	8.75 ^{abc}	4.52 ^{bcd}	4.87 ^{bcd}	3.97 ^{bc}
No.45	*A. polygama* x *A. chinensis*	8.80 ^{ab}	4.92 ^{b}	5.58 ^{ab}	4.22 ^{b}
No.55	*A. polygama* x *A. chinensis*	9.19 ^{a}	6.25 ^{a}	6.00 ^{a}	4.71 ^{a}
No.86	*A. macrosperma*	8.57 ^{bc}	4.05 ^{cd}	4.64 ^{cd}	3.64 ^{cd}
No.101	*A. macrosperma* x *A. melanandra*	8.31 ^{c}	4.26 ^{bcd}	3.97 ^{de}	3.35 ^{d}
Rootstock		$P < 0.0001$			
Position		$P < 0.0001$			
Rootstock x Position		$P < 0.0001$			

Means sharing same letters within a ***column*** are not significantly different at P=0.05 according to Tukey's test, after ANCOVA adjustment using cross-sectional area of apical as covariate.
[‡]Initial vigour ranking of scion on each rootstock determined according to the results in Chapter Three. In order or increasing vigour.
[x]Slightly less vigorous or moderate reduced vigour according to (Anon., 2012) and M. Clearwater (personal communication, June 27, 2016), respectively. [x]Unknown parentage from open pollinated *A. macrosperma.*

Table 4.11. Uptake and transport of radioactivity in different position of kiwifruit rootstock stem segments (apical, middle, and basal) after 48h. Data were log-transformed for statistical analysis. Specific activity was expressed as log dpm mm^{-2} cross-sectional area of apical.

Rootstock[‡]	Parentages	Stem segment (Position)			
		Apical	Middle	Basal	Agar
No.18	*A. chinensis* x *A. macrosperma*	8.79 ^{cd}	3.84 ^{c}	3.45 ^{d}	3.37 ^{d}
No.87	*A. polygama*	9.24 ^{bc}	4.85 ^{ab}	5.59 ^{b}	4.38 ^{b}
No.100	*A. macrosperma* x *A. melanandra*	8.69 ^{d}	3.74 ^{c}	4.53 ^{c}	4.03 ^{bc}
Bounty71	- [x]	8.70 ^{d}	4.46 ^{b}	4.74 ^{c}	3.82 ^{cd}
No.45	*A. polygama* x *A. chinensis*	9.82 ^{a}	5.10 ^{a}	6.73 ^{a}	5.10 ^{a}
No.55	*A. polygama* x *A. chinensis*	9.25 ^{b}	4.56 ^{b}	5.95 ^{b}	5.00 ^{a}
No.86	*A. macrosperma*	8.58 ^{d}	4.45 ^{b}	3.84 ^{c}	4.00 ^{bc}
No.101	*A. macrosperma* x *A. melanandra*	8.51 ^{d}	4.75 ^{ab}	4.50 ^{c}	3.45 ^{d}
Rootstock		$P < 0.0001$			
Position		$P < 0.0001$			
Rootstock x Position		$P < 0.0001$			

Means sharing same letters within a ***column*** are not significantly different at P=0.05 according to Tukey's test, after ANCOVA adjustment using cross-sectional area of apical as covariate.
[‡]Initial vigour ranking of scion on each rootstock determined according to the results in Chapter Three. In order or increasing vigour.
[x]Slightly less vigorous or moderate reduced vigour according to (Anon., 2012) and M. Clearwater (personal communication, June 27, 2016), respectively. [x]Unknown parentage from open pollinated of *A. macrosperma.*

Table 4.12. Interaction between rootstock, hour and stem position (apical, middle, and basal) on total activity, uptake and transport of radioactivity after 24h and 48h. Data were log-transformed for statistical analysis. Specific activity was expressed as log dpm mm^{-2} cross-sectional area of apical.

Rootstock[ǂ]	Parentages		Stem segment (Position)		
		Hour	Apical	Middle	Basal
No.18	*A. chinensis* x *A. macrosperma*	**24h**	8.67 [a]	4.77 [b]	3.42 [c]
		48h	8.79 [a]	3.84 [b]	3.45 [c]
No.87	*A. polygama*	**24h**	8.77 [a]	4.68 [b]	5.08 [b]
		48h	9.24 [a]	4.85 [c]	5.59 [b]
No.100	*A. macrosperma* x *A. melanandra*	**24h**	8.50 [a]	3.99 [c]	4.86 [b]
		48h	8.67 [a]	3.74 [c]	4.53 [b]
Bounty71	- [x]	**24h**	8.75 [a]	4.52 [b]	4.87 [b]
		48h	8.70 [a]	4.46 [b]	4.74 [b]
No.45	*A. polygama* x *A. chinensis*	**24h**	8.80 [a]	4.92 [b]	5.58 [b]
		48h	9.82 [a]	5.10 [c]	6.73 [b]
No.55	*A. polygama* x *A. chinensis*	**24h**	9.19 [a]	6.25 [b]	6.00 [b]
		48h	9.25 [a]	4.56 [c]	5.95 [b]
No.86	*A. macrosperma*	**24h**	8.57 [a]	4.05 [b]	4.64 [b]
		48h	8.58 [a]	4.45 [b]	3.84 [b]
No.101	*A. macrosperma* x *A. melanandra*	**24h**	8.31 [a]	4.26 [b]	3.97 [b]
		48h	8.51 [a]	4.75 [b]	4.50 [b]

Rootstock	$P < 0.0001$
Hour	$P = 0.002$
Position	$P < 0.0001$
Rootstock x Hour	$P < 0.0001$
Rootstock x Position	$P < 0.0001$
Hour x Position	$P = 0.007$
Rootstock x Hour x Position	$P = 0.002$

Means sharing same letters within a ***row*** are not significantly different at P=0.05 according to Tukey's test, after ANCOVA adjustment using cross-sectional area of apical as covariate.

[ǂ]Initial vigour ranking of scions on each rootstock determined according to the results in Chapter Three. In order or increasing vigour.

[x]Slightly less vigorous or moderate reduced vigour according to (Anon., 2012) and M. Clearwater (personal communication, June 27, 2016), respectively. [x]Unknown parentage from open pollinated of *A. macrosperma*.

4.3.2.2.3 Distribution patterns of radioactivity in the stem segments

The distribution patterns of radioactivity at the different part of stem segments (i.e. apical, middle, and basal) for selected inter-specific hybrid kiwifruit rootstocks are shown in Figure 4.11. The apical segments had higher uptake and radioactivity than middle and basal segments in all rootstocks (Figure 4.11). Generally, there were decreasing patterns of uptake and radioactivity from the apical to the basal segments in all rootstocks. However, in particular rootstocks, for example in low-vigour rootstocks No.87 (*A. polygama*) and No. 100 (*A. macrosperma* x *A. melanandra*), plus intermediate vigour rootstock No.45 (*A. polygama* x *A. chinensis*), there were increasing patterns from middle to the basal segments (Figure 4.11). The uptake and transport of radioactivity in all segments were almost similar in all rootstocks between 24h and 48h. There were significant differences in the uptake and transport of radioactivity between apical and middle segments (Figure 4.11). Significant differences in the radioactivity between middle and basal segments were also noted in some rootstocks except for rootstocks No.86, No.101, and 'Bounty 71'.

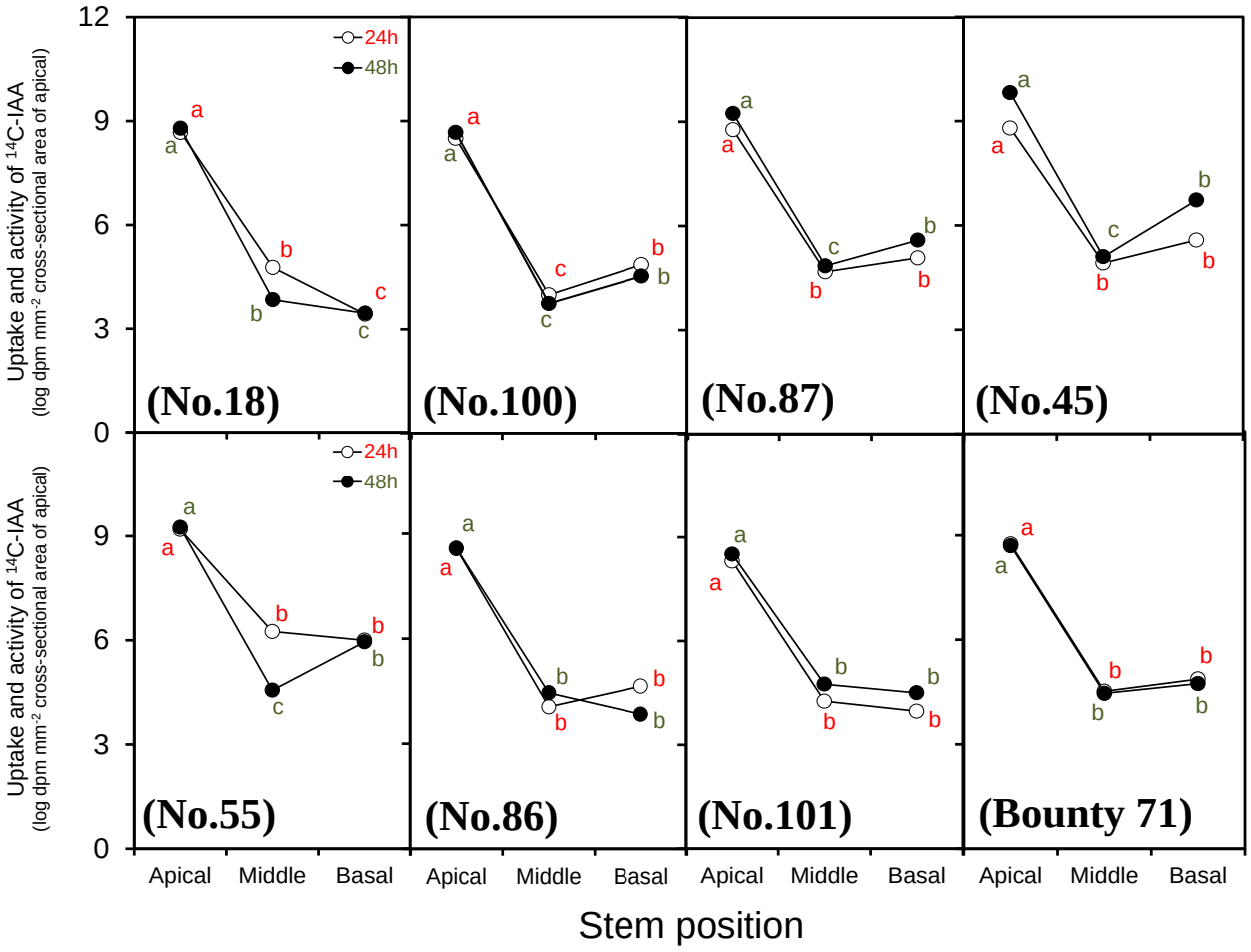


Figure 4.11. Distribution patterns of radioactivity in the three different stem segments (apical, middle and basal) of kiwifruit rootstocks during the summer season of 2014. Rootstocks were classified according to vigour from previous experiment (Chapter Three). Means sharing the same letters and colour are not significantly different at *P*=0.05 according to Tukey's test, after ANCOVA adjustment using cross-sectional area of apical as covariate.

4.4 Discussion

4.4.1 Experiment 1

The first objective of this chapter was to elucidate the hormonal signalling in composite 'Hayward' vines. In order to achieve this objective, we implemented similar methods from the previous studies in grafted apple (van Hooijdonk et al., 2010) and kiwifruit cuttings (Vattiprolu et al., 2011b). By inhibiting the auxin transport system using NPA from the scion (i.e. shoot system) to the rootstock (i.e. root system), the modification (s) on the architectural structures of young composite 'Hayward' kiwifruit vines could be assessed and evaluated. In shoots, indole-3-acetic acid (IAA), the predominant naturally occurring auxin is involved in the meristematic activity of apical shoots, and also has been implicated in many processes, ranging from cell expansion and cell division to cell differentiation (Bartel, 1997). However, in kiwifruit, the mechanisms of IAA transport and its relations with other hormones such as CK and GA are still not fully understood compared with other fruit trees (e.g. apple).

Studies in apple have proposed that the dwarfing M.9 rootstocks reduced basipetal transport of IAA to roots, subsequently reducing the amount of root produced CK (Li et al., 2012; Lockhard & Schneider, 1981; van Hooijdonk et al., 2010) and GA transported to the scions (Tworkoski & Fazio, 2015; van Hooijdonk et al., 2010). By applying NPA on the stem of vigorous apple rootstocks (i.e. MM.106 and MM.793), the primary shoot of 'Royal Gala' scions terminated earlier, which decreased the final mean length and node number of primary shoots. These responses were similar to the effects of M.9 dwarfing rootstocks on the primary shoots of similar scions (van Hooijdonk et al., 2010). Similarly, Li et al. (2012) also found that the level of IAA content in 'Red Fuji' scions was lower when grafted onto M.9 dwarfing rootstocks. It appears that the initial dwarfing mechanism lies in the IAA supply and transport from the shoot to root system. Therefore, by understanding the mechanism of this hormone, it could be the first step in developing a growth control method for kiwifruit vines. In the present study, two days after NPA treatment, the leaves of 'Hayward' scions from all rootstocks regardless of vigour classes showed epinasty, as well as distorted shape of scion leaves was also observed (Figure 4.5A). The apical shoots of scions were bending downwards,

suggesting the loss of gravity responses (Figure 4.5A). In addition, two weeks after that, new axillary outgrowth were observed on rootstock stems below the NPA application area (Figure 4.5B), indicating that the basipetal IAA transport has been restricted by the NPA treatment. These responses are similar to the observation found in apple (Grochowska et al., 1994; van Hooijdonk, 2009) and kiwifruit cuttings (Vattiprolu, 2012) when TIBA or NPA was used to restrict IAA transport. These responses also supported the concept of the release of apical dominance as mentioned by Domagalska and Leyser (2011).

Although there was no overall significant interactions (Tables 4.3, 4.4 and 4.7), there were significant differences between NPA treated (+NPA) and untreated (-NPA) vines on the particular hybrid kiwifruit rootstocks. In this study (Experiment 1), restricting basipetal IAA transport by applying NPA to the rootstock stem of 'Hayward' vines growing on an intermediate- and high-vigour rootstock groups such as No.55 (*A. polygama* x *A. chinensis*) and GN cuttings (respectively), decreased slightly the growth (Figure 4.6 and Table 4.3) and leaf area (Table 4.4 and Figure 4.8) of primary shoot of 'Hayward' scions. The spring bud break of scions of all rootstocks appeared to be unaffected by the NPA application (Table 4.6), suggesting that the auxin (i.e. IAA) did not involve in regulating bud outgrowth in composite kiwifruit vines. Remarkably, the overall growth and vigour of 'Hayward' scions in terms of total shoot length, total node number and total leaf area were significantly reduced when IAA transport from shoot to root system was inhibited by the NPA application, regardless rootstock vigour (Table 4.7). However, the ability of NPA to decrease scion vigour and leaf size significantly for the intermediate-vigour (i.e. No.55, No.84 and No.86) and high-vigour rootstock group ('Bruno' and self-rooted GN cuttings) may indicate higher level of IAA was substantially reduced in these rootstock groups (Figure 4.6, Tables 4.3, 4.4 and 4.7).

Meanwhile, inhibition of IAA by NPA application to the stem of 'Hayward' scions that have been grafted onto low-vigour rootstock group (i.e. No.87, No.100 and No.19) only imparted a small reduction in growth and vigour (Figure 4.6, Tables 4.3, 4.4 and 4.7), suggesting the level of IAA transported from shoot to root system in these rootstocks could be lower compared with other vigour classes of inter-specific hybrid kiwifruit rootstocks. Therefore, our results may indicate that low-vigour kiwifruit rootstocks may have decreased basipetal transport of IAA from scion-to-root, thus reducing scion

growth and vigour. Even though no correlation between vigour and the parentages of inter-specific hybrid kiwifruit rootstocks, we postulate that the level of IAA transported could be more related to the genetic origin of the rootstocks (Experiment 2).

4.4.1.1 Effects of rootstocks and NPA on the growth of primary shoots

In many plant species, the formation of new nodes and internode extension are regulated by the activity of the apical and sub-apical regions of the shoot apical meristem (SAM), (Sachs, 1965; Sassi & Vernoux, 2013). However, the role of endogenous hormones, particularly IAA, CK and GA in regulating apical and sub-apical regions of kiwifruit are largely unknown. In our study, we found that the growth of primary shoots of 'Hayward' scions on particular rootstocks were reduced when NPA was applied to the rootstock stem, except for the primary shoots on rootstocks No.19 and 'Bruno' (Figure 4.6 and Table 4.3). It was observed that the length of primary shoots of scions decreased after the first application of NPA and almost fully terminated two weeks after the NPA treatment (Figure 4.6). These results indicate that the apical meristem of primary shoots reduced its growth due to restriction of IAA transport. However, for untreated vines, the primary shoots of scions were continuously growing, indicating sufficient supply of IAA in the apical meristem of scion primary shoots. In addition to these results, both rootstock and NPA treatment had significant influence on the final length, node number, and shoot cross-sectional area (CSA) of primary shoots of 'Hayward' scions (Table 4.3). It therefore seems that IAA is involved in regulating SAM and production of node neoformation of primary shoots of kiwifruit scions, similar to what have been found in apple (van Hooijdonk et al., 2010). Exogenous application of NPA to the primary shoots of 'Hayward' cuttings that are actively growing can also reduce their length; however, the meristematic activity of primary shoots of 'Hayward' kiwifruit scions can be prolonged by the exogenous application of GA (Vattiprolu, 2012).

It has been demonstrated in apple, restriction of IAA by NPA application to the stem region of vigorous rootstocks of MM.106, MM.793, and 'Royal Gala' reduced the length and node number of the scion primary shoots, because the node neoformation by the SAM temporarily was slowed and/or ceased early (van Hooijdonk et al., 2010). These effects were similar to the scions when grafted onto dwarfing M.9 rootstocks (van

Hooijdonk et al., 2010). However, contrasting with the present study, NPA treatment on the low- and high-vigour rootstocks such as No.19 (*A. chinensis* x *A. macrosperma*) and 'Bruno' (*A. deliciosa*) (respectively) did not cause the primary shoots of scions to terminate earlier (Figure 4.6). These results may suggest that the restriction of IAA transport on these kiwifruit rootstocks may not reduce the level of IAA transported from shoot to root, and to influence the meristematic activity (i.e. SAM) of grafted 'Hayward' scions. The reason of why the NPA did not affect the growth of scion primary shoots is relatively unknown, presumably the rootstocks of No.19 and 'Bruno' may have had sufficient amount of IAA in shoot system to keep the SAM of scions active and/or the level of IAA transported was actually lower in these rootstocks. Besides that, our results (Figure 4.6 and Tables 4.3) may also suggest that various responses of kiwifruit rootstocks to NPA probably due to differences in the tissues sensitivity of rootstocks to IAA transport inhibitor (i.e. NPA).

The amount of IAA transported from shoot to root could also affect the size of the root system of rootstocks as reported in grafted apple (van Hooijdonk, 2009). van Hooijdonk (2009) found that the application of NPA to the rootstock stems of MM.106, MM.793, and 'Royal Gala' decreased the final mean dry weight of the root system when compared to untreated trees. Similarly, the M.9 dwarfing rootstock also significantly reduced the total growth of the root system when compared to vigorous rootstocks (van Hooijdonk, 2009). These findings suggest that shoot-produced IAA is needed for the root growth, and inhibition of IAA transport from scion to root may be an important mechanism by which dwarfing M.9 rootstock decreased root growth compared to vigorous MM.106, MM.793 and 'Royal Gala' rootstocks (van Hooijdonk, 2009). Our results also found that the growth responses of scions grafted onto hybrid No.19 (*A. chinensis* x *A. macrosperma*), 'Bruno' and possibly hybrid No.86 (*A. macrosperma*) were similar between NPA treated and non-treated (Figure 4.6). This result may suggest that the IAA from the root of these rootstocks could not be transported in sufficient amounts to keep the SAM activity of scions active, indicating that the root-to-shoot signalling is also important in regulating shoot growth (Dodd, 2005). In our study, even though no rooting assessment was conducted, we believe that the endogenous level of IAA in roots of hybrid rootstock No.19, 'Bruno' and possibly hybrid No.86 may be different due to the difference in the size of root system (Appendix 12). Therefore, additional assessment on the root systems would be required to confirm this suggestion.

As noted in many literatures, the restriction of IAA transport either by decapitation or NPA application may also affect the transport of other hormones such as GA (Ross, 1998; Ross et al., 2003; van Hooijdonk et al., 2010, 2011). Indeed, GA is required for IAA synthesis at the shoot apex. In pea plant, both IAA and GA are required for internode extension by the sub-apical zone of the SAM. The bioactive GA, GA_1 may be the only GA that is related to the elongation of internodes (Ross et al., 2003). IAA is needed to maintain the level of GA_1 by promoting GA_1 biosynthesis and by inhibiting GA_1 deactivation. More importantly, GA_1 stimulated elongation is normally associated with an increase in IAA level (Ross et al., 2003). Removal of the shoot apex, or restriction of the basipetal IAA transport, decreased the concentration of endogenous GA_1 in subtending elongating internodes, which resulted from decreased GA 3-oxidase activity, thereby decreasing the conversion of GA_{20} into bioactive GA_1 (Ross et al., 2003). In the present study, restriction of IAA by the NPA application did not affect the internode length of primary shoots of 'Hayward' scions in all rootstocks (Table 4.3). Therefore, this result indicates that the restriction of IAA transport from the shoots either by the NPA application and/or possibly the inter-specific hybrid kiwifruit rootstocks did not affect the level of GA_1 in the shoot system (i.e. scion). This result is in agreement with a previous study in wild peas that found the restriction of IAA by NPA application to elongating internodes did not affect the level of GA_1 above the application site (Ross, 1998). However, the level of GA_1 in the root system of the inter-specific hybrid kiwifruit rootstocks may be affected by the NPA application, as Ross (1998) found reduction in GA_1 and IAA level below the site of NPA application (i.e. root system).

4.4.1.2 Effects of rootstocks and NPA on the bud break of scion primary shoots

Bud break and subsequent branch outgrowth are dynamically controlled by the endogenous hormonal signalling, particularly IAA that is produced in the shoots and CK produced in the roots (Campoy et al., 2011; Costes & Guédon, 2002; Faust et al., 1997). It has been known for many decades that auxin (i.e. IAA) from the apical shoot is the primary signal that is directly or indirectly responsible for inhibiting bud outgrowth, while CK was implicated in breaking the dormancy or initiation of bud outgrowth (Cline, 1994). Therefore, the NPA was re-applied again to the stem region two weeks before expected bud break. This enables us to assess whether the restriction

of IAA may have an effect on the initiation of buds outgrowth of the scion primary shoots. The application of NPA in early spring did not affect the patterns (Appendix 3, 4 and 5) and overall bud break of 'Hayward' scions, regardless of rootstock vigour (Table 4.6). Therefore, the restriction of endogenous IAA transport on the composite kiwifruit vines at the earliest possible stage did not affect the initiation of axillary meristem (i.e. bud break) (Table 4.6). In a similar manner, exogenous application of 1-Naphthaleneacetic acid (NAA) to the two different cuttings kiwifruit cultivars, 'Hort16A' (*A. chinensis*) and 'Hayward' (*A. deliciosa*) also did not affect the bud break in early spring (Vattiprolu, 2012). This result might not be surprising because for almost as long as it has been known, auxin (i.e. IAA) does not act directly to suppress bud growth (Brewer et al., 2009). It has been demonstrated in peas (*Pisum sativum*) that the application of NPA directly to the buds had no effect on the initial bud outgrowth and the NPA-treated buds grew normally for a few days after the initial application (Brewer et al., 2009). Thus, our results may indicate that IAA is not involved in the initiation of bud outgrowth in grafted kiwifruit. It would also be reasonable to suggest that the level of IAA transported from shoot to root during early spring may have not been affected by the NPA application. It was also found in apple that the rate of IAA diffusion from the apical primary shoots was not affected by the different vigour of rootstocks throughout the growing season (i.e. from summer until winter) (van Hooijdonk, 2009). Therefore, in our study, these could be the reasons why the restriction of IAA by the NPA did not significantly affect the bud break of kiwifruit (Table 4.6).

However, when the main effect of rootstocks is taken into consideration, our results (Table 4.6) indicate that the inter-specific hybrid kiwifruit rootstocks may have influence on the spring bud break of scion primary shoots. This result supports our earlier findings in Chapter Two and Three of this thesis that root system of kiwifruit (i.e. rootstock) may have an effect on the scion bud break (Table 4.6). It was also noted that the bud break of scions varied among the inter-specific hybrid kiwifruit rootstocks (Table 4.6). Even though no distinct patterns were found on the bud break between the inter-specific hybrid kiwifruit rootstocks and the control vines (Appendix 3, 4 and 5), the bud break of scion primary shoots on inter-specific hybrid kiwifruit rootstocks was slightly higher compared to the self-rooted GN control vines (Table 4.8). In kiwifruit, the ability of rootstocks to influence scion bud break have been demonstrated by numbers of studies (Anon., 2012; Wang et al., 1994b), and other vine species such as

grape (Nikolaou et al., 2000). It was believed that the genetic origin of rootstocks may have a strong influence on the scion bud break (Wang et al., 1994b). For example Wang et al. (1994) found that the bud break of *A. deliciosa* cv. 'Hayward' scions was increased when grafted with *A. hemsleyana* rootstocks, but reduced when grafted onto *A. chinensis* rootstocks. Kiwifruit rootstocks also have been thought to influence scion bud break by altering the vine carbohydrate level (Wang et al., 1994b). Meanwhile, in contrast with other fruit tree species, the scion bud break was not significantly affected by the rootstocks as reported for apple (Costes et al., 2001; Seleznyova et al., 2003) and pear (Watson et al., 2012). Even though the effect of inter-specific hybrid kiwifruit rootstocks in this chapter was not as pronounced as found in Chapter Two and Chapter Three, there were consistent trends that the inter-specific hybrid kiwifruit rootstocks are capable of influencing 'Hayward' scions bud break.

Bud break is thought to be regulated by the root-produced CK (Jones, 1973; Torrey, 1976). In apple, a few studies have found that CK in shoots was involved in the sylleptic bud break (Cook et al., 2001; Cutting et al., 1991; Tworkoski & Miller, 2007). Higher bud break in early spring appeared to coincide with the high concentration of CK in shoots of 'Granny Smith' and 'Braeburn' apples (Cook et al., 2001; Cutting et al., 1991). Tworkoski and Miller (2007) reported that IAA to CK ratio could be the factor in regulating bud break of apple scions, and the level of these two hormones in scions was influenced by the different vigour of rootstocks. In grape, the CK level in xylem exudates of 'Thompson' scions grafted onto different vigour of rootstocks was found to be positively correlated with the spring (proleptic) bud break (Nikolaou, 2000). NPA treatment on the stem of rooted kiwifruit cuttings resulted in a temporary elevation of CK level in root xylem exudate in a similar manner to girdling (Currie, 1997). Decapitated apical buds by shoot tipping, mimicking the removal of sources of IAA, may also cause an increase in the percentage of shoot bud break (van Hooijdonk et al., 2014). However, in apple trees, van Hooijdonk et al., (2012) found that application of NPA to the graft-union of composite 'Royal Gala' reduced CK level after 48 hours in xylem sap. Therefore, there was a transicient increase of endogenous CK before the level of CK in xylem sap decreased. Based on our findings and literature above, there were sufficient evidence that the bud break of kiwifruit could be affected by the endogenous level of CK from the root system (i.e. rootstock), because exogenous application of CK (6-Benzyladenine) did not affect spring bud break of kiwifruit

(Vattiprolu, 2012). In the present study, the level of endogenous CK in grafted 'Hayward' scions was not measured and quantified. Hence, assessment on the endogenous CK level during pre- and post-bud break of scions grafted onto inter-specific hybrid kiwifruit rootstocks is required and warranted in future observations. Other study reported that gibberellins (GA) may be required to enhance the rate of bud break in kiwifruit (Lionakis & Schwabe, 1984), but the role and involvement of GA in release of proleptic axillary bud outgrowth (as opposed to subsequent growth) in kiwifruit are still unclear. Nevertheless, several studies have reported that GA may also involve in the release of sylleptic axillary shoots (Lawes, 1979; Vattiprolu, 2012). Therefore, it was suggested in grafted kiwifruit, auxin, CK and GA may have a prominent role in axillary bud release, and this might be attributed the hormonal status of rootstocks. Further study on the endogenous hormones is required to elucidate whether the release of kiwifruit buds from dormancy (bud break) of scions is regulated solely by auxin, CK and GA, or an interaction among these hormones.

4.4.1.3 Effects of rootstocks and NPA on the number of proleptic axillary shoots (i.e. branching)

The buds that broke on the primary shoot of scions (i.e. bud break) were allowed to grow and develop. These shoots are termed as proleptic axillary shoots or secondary shoots as they came from buds of previous growing season and having a winter rest. At this time, we continuously applied NPA fortnightly to determine if the reduction in IAA transport from the shoots to roots system may have had an influence on the growth of secondary shoots (i.e. proleptic branching). Results showed that the inter-specific hybrid kiwifruit rootstocks had a significant effect (P=0.007) on the branching of the scion primary shoots (Table 4.7). Furthermore, there was a trend (P=0.11) that restriction of IAA transport by NPA treatment may have an influence on the number of proleptic axillary shoots (Table 4.7). In apple, restriction of IAA by NPA or the stem tissue of M.9 dwarfing apple rootstock may affect the mean number of secondary shoots (i.e. branching) that formed on the primary shoots of 'Royal Gala' scions (van Hooijdonk et al., 2010). Therefore, changes in branching architecture of scions on dwarfing rootstock M.9 was due to the reduction in the basipetal transport of IAA from shoot to root, thereby reducing the amount of root-produced CK and GA transported to scion (van Hooijdonk et al., 2010, 2011).

Study on sylleptic branching of kiwifruit, Vattiprolu (2012) reported that application of IAA below the NPA site on the stem may reduce the number of sylleptic axillary shoots in self-rooted 'Hort16A' kiwifruit cuttings, possibly due to reduction in CK levels as Currie (1997) also found that decapitation followed by NAA application to the cut surface of cuttings decreased CK levels. Studies *in vitro* has also shown that exogenous application of CK in tissue culture medium is required to induce sylleptic axillary shoot formation in kiwifruit explants (Standardi, 1983) and this was influenced by the concentration and type of CK used (Akbaş et al., 2007; Arigita et al., 2005; Moncaleán et al., 2003b). For example, Moncaleán et al. (2003b) reported that the level of endogenous IAA at the end of the multiplication phase of kiwifruit explants was influenced by the presence of CK in the culture medium. Endogenous IAA level recorded was at least three-fold higher in the leaves from micro-shoots cultured treated with CK than non-treated, and these micro-shoots showing the greatest growth and development (Moncaleán et al., 2003b). Adding the gibberellins (GA_3) into the culture medium may enhance the growth of axillary shoot of kiwifruit explants, as well as increase the shoot length because of the high uptakes of GA_3 by these kiwifruit explants was observed (Moncaleán et al., 2003a). Besides that, exogenous application of GAs (GA_3+GA_{4+7}) also increased the formation of sylleptic axillary shoots primary shoots of self-rooted 'Hayward' cuttings (Vattiprolu, 2012). Another synthetic CK compound, N-(2-chloro-4-pyridyl)-N-phenylurea (CPPU) also has been reported to induce axillary shoot formation of kiwifruit explants (Caboni et al., 2009). Therefore, it seems that branching in kiwifruit may require certain level of CK for axillary outgrowth and GA for elongation of axillary shoots. However, it is questionable whether the mechanism (s) of hormonal signalling of proleptic branching exhibits the similar responses in those of sylleptic branching in kiwifruit. In the present study, it is also reasonable to suggest that different genotype of kiwifruit rootstocks may have had different capability to transport CK (important for scion branching) from the root to shoot as found in apple (Kamboj et al., 1999).

Recently, it was discovered that auxin (i.e. IAA) regulations act as a feedback mechanism for strigolactones (SLs) synthesis (Hayward et al., 2009). SLs are new group of hormones that are produced in plant roots, which are involved in the inhibition of shoot branching in plants (Gomez-Roldan et al., 2008). Therefore, the evidence from previous studies (Clearwater et al., 2004; 2006; Wang et al., 1994b), and current

findings in this chapter, as well as findings on other chapters of this thesis (Chapter Two and Chapter Three) have shown that the inter-specific hybrid kiwifruit rootstocks can influence the shoot architecture and vigour of grafted scions. Furthermore, these evidence may also suggest that 'root-derived signals' from rootstocks such as SLs may be involved in controlling shoot branching of kiwifruit scions. SLs produced by the action of 'Carotenoid Cleavage Dioxygenase' genes (*CCD7* and *CCD8*), and these genes may have significant influence in controlling branching of model plants (e.g. *Arabidopsis*, pea, rice and petunia) (Matusova et al., 2005). It was also found that highly branching of wild-type kiwifruit had high expression of *CCD* genes, contrasting with transgenic kiwifruit vines with less branching had lower expression of *CCD* genes (Ledger et al., 2010). However, when transgenic kiwifruit was grafted onto wild-type kiwifruit, the branching of transgenic kiwifruit vines was increased; indicating SLs from the root system (i.e. rootstock) may be involved in controlling the branching of shoots (i.e. scion). Therefore, the presence of SLs and its relationship with the branching mechanisms of kiwifruit could not be ignored and warrant further observation (Appendix 11). The variability in the number of proleptic axillary shoots of scions produced between NPA treated and untreated vines (Table 4.6) may indicate that some rootstock genotypes may have not responded consistently to restriction of IAA. We suggest that the differences in the genetic material of the inter-specific hybrid kiwifruit rootstocks may have affected the branching ability of the 'Hayward' scions. It should be noted that the branching processes in plants are regulated by the genetic signals (Leyser, 2003) and it is also can be dependent on the 'graft-transmissible signals' in root and/or shoot that is regulated by the specific genes, as demonstrated in model pea plants (*Pisum sativum* L.) (Beveridge et al., 2000). Besides that, branching can also be influenced by the environmental condition, endogenous hormonal signalling and growth manipulation (e.g. pruning) (Evers et al., 2011). Indeed, shoot branching is highly plastic and can be influenced by environmental conditions (Evers et al., 2011).

4.4.1.4 Effects of rootstocks and NPA on the leaf growth of scions

It was interesting to note that both of rootstocks and NPA treatment had significant effects on the leaf growth of grafted 'Hayward' scions (Table 4.4 and Figure 4.8). Furthermore, the restriction of IAA transport by NPA treatment significantly reduced the mean LA of scion primary shoots (Table 4.4) and total LA in the second growing season (Table 4.7). Besides that, there were trends that the restriction of IAA may have reduced the leaf characteristics (i.e. FW, DW and SLW) of scion primary shoots (Table 4.7). A previous study by Vattiprolu (2012) also found reduction in the LA of primary shoots of 'Hort16A' stem cuttings when treated with NPA, even though no significant reduction in total growth of vines was observed. In model plant *Arabidopsis*, reduction in the leaf growth of NPA treated vines in growing medium appeared to coincide with the reduction in the IAA content of the leaves (Ljung et al., 2001). In kiwifruit, the individual LA of long non-terminating shoots of 'Hort16A' scions for the first 20 nodes were also reduced when grafted onto low-vigour rootstocks such as *A. polygama* and *A. kolomikta*, and this effect has contributed to the reduction in total LA per scion (Clearwater et al., 2006).

Therefore, in our study, there was a similarity in the modification by NPA and low-vigour rootstocks on the leaf growth of scions as reported by Clearwater et al. (2006), because we also found there were general trends that the LA of scion primary shoots for the first 15 nodes on particular rootstocks treated with NPA was reduced compared to the untreated vines (Figure 4.8). Thus, it would be reasonable to suggest that the sufficient amount of IAA in the shoot system is important for the leaf growth and development of kiwifruit. It should be noted that IAA is required in many activities of vascular tissue development, such as in initiation of new leaves of tomato plants (Reinhardt et al., 2000), in the cell division phase of leaf development (Keller et al., 2004; Ljung et al., 2001) and in leaf vascular development (Mattsson et al., 2003; Sieburth, 1999). However, in kiwifruit, the involvement of IAA in leaf growth is still poorly understood and need to be further studied.

4.4.1.5 Effects of rootstocks and NPA on the total growth of grafted 'Hayward' scions

NPA treatment on the particular inter-specific hybrid kiwifruit rootstocks decreased the overall growth of the grafted 'Hayward' scions (Table 4.7). In the first growing season, the length and node number of scion primary shoots on the rootstocks No.55, No.84, No.86, No.87, No.100 and GN were reduced slightly with the application of NPA (Table 4.3 and Figure 4.6). The reduction in the length, node number, and CSA of the scion primary shoots were 30%, 24%, and 11%, respectively compared to the scion primary shoots of untreated vines. Remarkably, there were 28% and 20% reduction in the mean total length and node number of proleptic axillary shoots (respectively) of NPA-treated vines compared to the untreated vines in the second growing season (Table 4.7). Reduction in the mean total length and mean node number of proleptic axillary shoots may have contributed to the reduction in the leaf area of grafted 'Hayward' scions. Although the reductions in the growth of scions for NPA-treated vines (Table 4.3 and Figure 4.6), particularly in the mean number of proleptic axillary shoots were small (Table 4.7), they had a pronounced effect on the overall growth and architecture of 'Hayward' scions compared to the untreated vines. Although the effects were not as pronounced as reported for apple (e.g. van Hooijdonk, 2009; van Hooijdonk et al., 2010), these findings have revealed the important role of IAA signalling in regulating shoot architecture of scions by the kiwifruit rootstocks.

The actual data on the endogenous hormones (especially IAA, CK and GA) are not available, because we did not measure and quantify the level of these hormones. So, the available data for this aspect do not allow us to make any specific conclusions about the actual level of IAA for the inter-specific hybrid kiwifruit rootstocks used in this study (Experiment Two). Nevertheless, between studies in Chapter Three and in this chapter, there were general similarities in the results regarding to the vigour of the particular inter-specific hybrid kiwifruit rootstocks and their effects on the grafted 'Hayward' scions. As found in Chapter Three, rootstocks No.87 (*A. polygama*), No.100 (*A. macrosperma* x *A. melanandra*), and No.19 (*A. chinensis* x *A. macrosperma*) were grouped as low-vigour rootstocks. Meanwhile, rootstock No.55 (*A. polygama* x *A. chinensis*) and GN cuttings (i.e. self-rooted) can be grouped as high-vigour rootstocks or vines. Comparing the results in this chapter with the results in Chapter Three,

particularly on the mean total length of secondary shoots (i.e. proleptic axillary shoots), rootstock No.87, No.100, and No.19 still produced the lowest mean total length of secondary shoots compared to rootstock No.55, while GN produced the highest mean total length of secondary shoots than scion on other inter-specific hybrid kiwifruit rootstocks (Table 4.3 and Figure 4.6). Hence, it seems there was a correlation between the vigour of rootstocks found in Chapter Three with the data found in this chapter, as the scions growth was still limited when grafted onto low-vigour rootstocks of No.87, No.100 and No.19.

Nevertheless, the unexpected result found in our study was 'Hayward' scion on *A. deliciosa* cv. 'Bruno' rootstock reduced the total length, node number, and number of proleptic axillary shoots on 'Hayward' scion when compared to the other inter-specific hybrid kiwifruit rootstocks (Figure 4.6, Tables 4.3 and 4.7). Thus, this effect has contributed to the reduction in the overall growth of scions on this rootstock. This result is contrasting with the report from the previous study that found 'Bruno' rootstock did not completely reduce the growth of grafted scions and can be considered as a vigorous rootstock in trials at other places in New Zealand (Clearwater et al., 2007a). The reason for this is unknown, but could be related to the rootstock genotype x environment interactions. Nevertheless, it should be noted that the growth of scions need to be observed for a few years before final size of the composite vines is realised, as some vigorous plants do not perform vigorously during their initial growth. In our study, we believed that the vigour of grafted kiwifruit scion may be controlled by the multiple interactions between the genetic and hormonal level of rootstocks, together with the interaction with environmental condition. Therefore, further experimentation using a controlled environment and/or trial at multiple locations (Foster et al., 2016) would provide an interesting information on the effects of kiwifruit rootstocks in controlling shoot growth of grafted scions, especially on the hormonal aspects.

4.4.2 Experiment 2

The second objective of this chapter was to assess the IAA transport in the shoot explants in relation to the vigour of inter-specific hybrid kiwifruit rootstocks. Many studies have demonstrated that IAA is one of the hormones that have been implicated in regulating the scion growth by dwarfing rootstocks (Kamboj et al., 1997; Li et al., 2012; Soumelidou et al., 1994; van Hooijdonk et al., 2010), but fewer studies were conducted to elucidate the involvement of endogenous IAA in vigour control of kiwifruit scions. A recent study conducted on kiwifruit cuttings was attempted to reveal the hormonal relationship between the shoot and root system (e.g. Vattiprolu, 2012); however, the actual involvement of IAA is still remains unclear. We believed that the hormonal mechanism (s) in stem of kiwifruit cuttings could be different with the composite kiwifruit vines. As found in our first experiment, the results suggested that the IAA may be involved in regulating the meristematic activity of 'Hayward' scions. Restriction of IAA transport by NPA treatment applied to the stem junction between the shoot and root may cause reduction in the growth of scions compared to the untreated vines (Experiment One, Section 4.4). However, particular inter-specific hybrid kiwifruit rootstocks did not respond to the NPA treatment, as the shoot growth of 'Hayward' scions from NPA treatment was similar to the untreated vines (Figure 4.6), indicating that the differences in genetic origin of particular hybrid kiwifruit rootstocks exhibit differences in stem tissue sensitivity. We suggest that the meristematic activity of 'Hayward' scion was influenced by the basipetal transport of IAA from the shoot, and this could be controlled by the amount of IAA reached to the rootstock. Our results may also suggest that the level of endogenous IAA transported in the stem varied between inter-specific hybrid kiwifruit rootstocks, and could be due to differences in genetic origin. Based on the findings in Section 4.4, it is worthy to study the involvement of auxin transport and its contributions to the vigour control in the inter-specific hybrid kiwifruit rootstocks.

Many evidence demonstrated that the intercellular auxin (i.e. IAA) movement depends on a number of auxin transporting mechanisms, including either active or passive processes that transport auxin over long or short distances. During plant development, the major mechanism for controlling auxin distribution appears to be the active

directional cell-to-cell movement of auxin that is mediated by plasma membrane-based in influx and exflux carriers (review by Petrášek & Friml, 2009). According to Prasad and Dhonukshe (2013), phloem vessels may act as the highways for long-distance auxin transport; however, for short-distance auxin transport, a unique system of cell-to-cell polar transport is exploited. As auxin can enter the cell either with the assistance of influx carrier, or in a passive manner and as auxin requires efflux carriers for its exit out of the cell, the efflux carrier bears critical rate-limiting and directional influence on auxin transport (Prasad & Dhonukshe, 2013). Auxin transport (i.e. IAA) has been associated with the vigour of the rootstocks in many fruit tree species, as reported for apple (Kamboj et al., 1997; Soumelidou et al., 1994; van Hooijdonk et al., 2010) and peach (Sorce et al., 2002). However, the information on the relationship between auxin transport and vigour is still lacking in kiwifruit.

At the end of the growing season (late autumn 2014), the radioactivity in the apical segments of high-vigour kiwifruit rootstock No.101 (*A. macrosperma* x *A. melanandra*) were significantly higher compared to apical segments of low-vigour kiwifruit rootstock No.18 (*A. chinensis* x *A. macrosperma*) (Figure 4.9). In addition, the difference in the total radioactivity was significant between low- and high-vigour kiwifruit rootstocks (Table 4.8). High-vigour rootstock No.101 had significantly greater total uptake and transport of radioactivity compared to low-vigour rootstock No.18 (Table 4.8). These results may suggest that low-vigour kiwifruit rootstocks No.18 may have lower basipetal transport of IAA in the stem tissues at the end of the growing season. Besides that, our results may also reflect the influence of IAA transport on the growing stage and timing of shoot termination. During this time, it was observed that the shoots of rootstocks No.100 growth almost ceased the growth or terminated (data not shown). Unfortunately, detailed shoot growth data were not collected in this experiment to relate the level of IAA and vigour of these kiwifruit rootstocks. Therefore, supportive data on shoot growth is important and need to be presented in future studies. As shown in apple, the shoot of low-vigour rootstock (i.e. M.9 dwarfing rootstock) ceased growth (i.e. terminated) much earlier than the shoots of vigorous rootstocks, and this appears to coincide with the reduction in the basipetal transport of IAA toward the end of the growing season (Kamboj et al., 1997; van Hooijdonk et al., 2011). It should be noted that the level of IAA transported in the grafted scions could be influenced by the vigour of the rootstocks (van Hooijdonk et al., 2011). Besides that, the IAA level transported in

the stems may have influenced the meristematic activity and termination of scions, and this could be controlled by the vigour of the rootstocks. In other fruit trees such as apple, high proportions of terminated shoots were found when scions grafted onto M.9 dwarfing rootstocks, due to M.9 rootstocks promote early termination on grafted scions. Similarly, the restriction of IAA transport by NPA to the stem of vigorous apple rootstocks (i.e. MM.106, M.793, and Royal Gala) also promoted early termination of shoot growth (van Hooijdonk et al., 2010).

Therefore, the general similarities in the architectural changes imposed by the stem of M.9 dwarfing apple rootstocks and NPA application had proven that the reduction in the basipetal transport of IAA from shoot to root is essential in the physiological process regulating scion vigour of grafted trees on dwarfing rootstocks (Lockhard & Schneider, 1981; van Hooijdonk et al., 2010). In kiwifruit, it was also notable that scions on low vigour rootstocks may have higher proportion of terminated shoots (i.e. short and medium shoots), and less non-terminated long shoots (Clearwater et al., 2006). Similar to our study, the apical shoot of 'Hayward' scions on particular inter-specific hybrid kiwifruit rootstocks also tended to terminate earlier when IAA was restricted by NPA application (Section 4.4). This result indicates that the meristematic activity and termination of kiwifruit shoots may be under the control of IAA transport. Thus, it is reasonable to suggest that low-vigour kiwifruit rootstocks such as hybrid No.87, No.100 and No.18 (in order of increasing vigour) may have a tendency to lower auxin transport in their stem barks compared to the high vigour kiwifruit rootstocks similar to what have been found in dwarfing apple rootstocks (Kamboj et al., 1997; Lockhard & Schneider, 1981; van Hooijdonk et al., 2010). However, quantitative hormonal measurements are still necessary in the future in order to identify the actual involvement of IAA in the kiwifruit rootstock stems as reported for apple (Li et al., 2012; Tworkoski & Fazio, 2015; van Hooijdonk et al., 2011). Besides that, limited data available (i.e. comparison made only between two rootstocks) do not allow us to make any specific conclusion until the whole range of vigour of inter-specific hybrid kiwifruit rootstocks are used for assessment.

The assessments on the uptake and transport of IAA in the stem segment of inter-specific hybrid kiwifruit rootstocks were continued during the summer 2014 season. In order to extend our knowledge on the IAA transport in other kiwifruit rootstocks, the

inter-specific hybrid kiwifruit rootstocks such as No.100 and No.87 (low-vigour), No.45, No.55 and No.86 (intermediate-vigour), including 'Bounty 71' were included for further assessment. In this assessment, the movement of radioactivity in the stem segments of inter-specific hybrid kiwifruit rootstocks were evaluated at two different hours (i.e. 24h and 48h). This enables us to find out whether there is a difference in the time period of radioactivity between the inter-specific hybrid kiwifruit rootstocks. As reported in apple, Soumelidou et al., (1994) conducted a similar IAA transport experiment within three hours and they did not find any differences in the uptake and radioactivity of IAA between vigorous and dwarfing apple rootstocks. However, in later a study, Kamboj et al., (1997) tested the IAA uptake activities for a longer time (i.e. 12h), and they found that the apple rootstocks actually differed in the uptake and transport of IAA. Therefore, it is worthy for us to evaluate the differences in uptake and transport of radioactivity at two different times, since this is the first study with an attempt to evaluate the IAA transport for the kiwifruit rootstock.

In the present study, the rootstock, time and rootstock x time interactions had significant effects on the total uptake and transport of radioactivity (Table 4.10, Table 4.11 and Table 4.12). Similarly, the transport of radioactivity into agar receptor was also significant between the inter-specific hybrid kiwifruit rootstocks and time (Table 4.12). These results indicate that the inter-specific hybrid kiwifruit rootstocks may have different ability to uptake and transport of IAA in their stem tissues similar to what have been found in apple rootstocks (Kamboj et al., 1997; Soumelidou et al., 1994). Our results showed that there was a general trend that transport of radioactivity in the stem could be related to the vigour of inter-specific hybrid kiwifruit rootstocks although the trend was not consistent for both hours (Figure 4.10). It was noted that the total uptake and transport of radioactivity were increased with increasing the vigour of the inter-specific hybrid kiwifruit rootstocks especially for 24h incubation. However, the trend was not consistent or 48h incubation (Figure 4.10). The increasing pattern could only be seen from rootstock No.18 (low-vigour) to rootstock No.55 (intermediate-vigour), but, after that, the total uptake and transport of radioactivity were reduced in more vigorous kiwifruit rootstocks of No.86 and No.101 (Figure 4.10).

These results were unexpected because many studies have found that the level of IAA in the stem is increased with increase of the rootstocks or plant vigour (Kamboj et al., 1997; Sorce et al., 2002; Soumelidou et al., 1994; van Hooijdonk et al., 2010). An early study in apple found that higher amounts of auxin in apple bark tissues of vigorous than dwarfing rootstocks (Martin & Stahly, 1967). Furthermore, the shoot extracts from normal 'Cortland' and 'Golden Delicious' plants were found to contain higher levels of auxin at all stages of growth than those from dwarf mutants (Jindal et al., 1974). Besides that, the stem of dwarfing apple rootstocks (e.g. M.9 and M.27) reduced the basipetal transport of ^{14}C-[carboxy]-labelled IAA (Soumelidou et al., 1994). Kamboj et al., (1997) also found that the dwarfing apple rootstocks reduced the transport of [^{3}H]-IAA when compared to vigorous rootstocks (e.g. MM.111 and MM.104), while another study also reported that the dwarfing apple rootstocks had reduced the concentration of endogenous IAA than vigorous rootstocks (Michalczuk, 2002). In other fruit trees such as peach, Sorce et al., (2002) found that less vigorous rootstocks transported less IAA in the xylem sap, and concluded that auxin may have a positive correlation with the vigour of the rootstocks. Similarly in mango, a study found that the less vigorous seedling cultivars may contain lower endogenous level of IAA in leaves compared to vigorous cultivars (Murti et al., 2000). Therefore, it is possible that many factors may have affected the level of IAA transport in the kiwifruit rootstocks observed in our study, especially for an intermediate- and high-vigour rootstock hybrids No.55, No.86, and No.101. For example, in the nursery condition, the plentiful resources such as nutrient, water, and pest and disease control may have influenced the vigour of the rootstocks. Besides that, we also believed that the restriction of the root system by pots may have limited the root growth, subsequently may have had an influence on the IAA transport in the stems, and thereby may have affected the vigour of these rootstocks (Appendix 12). The root of vigorous rootstocks could be more restricted by pots than less vigorous rootstocks, probably due to larger root system in vigorous rootstocks (Appendix 12). It is likely that multiple factors could have influenced the transport and uptake of IAA in the kiwifruit stems, therefore these factors need to be considered in future studies.

In general, higher uptake and transport of radioactivity were found in the apical segments compared to the middle and basal segments regardless of rootstock vigour (Table 4.12 and Figure 4.11), where the segments were supplied with [^{14}C]-IAA (i.e. apical). After that, the radioactivities were gradually decreased from the apical to

middle segments and from the middle to basal segments (Figure 4.11). However, the uptake and transport of radioactivity were not much different between the middle and basal segments for all inter-specific hybrid kiwifruit rootstocks (Figure 4.11). These patterns found in our results were similar to the pattern reported in studies with apple rootstocks (Kamboj et al., 1997; Soumelidou et al., 1994), Japanese pear (*Pyrus pyrifolia*) (Ito et al., 2001) and bean (*Pheseolus vulgaris*) (Lim & Tamas, 1989). According to Kamboj et al. (1997), the higher uptake and activity at the apical segments in contact with donor solution could be partly explained in terms of IAA uptake in excess amount moved by the polar transport system. The higher uptake in the apical could result from a loading of the intercellular spaces and apoplast close to the cut surface of the apical segments (Kamboj et al., 1997). However, we did not determine whether the higher uptake and transport of radioactivity were widely distributed in the whole apical segments, or whether it was limited to the surface area that is in contact with the donor solution. Therefore, to identify this, the apical segments need to be segmented. However, in this study, we were facing a difficulty to do this, due to the stem segments of kiwifruit rootstocks was really hard to cut by using ordinary laboratory knives. Therefore, it was not possible for us to determine the distribution and activity of IAA in the whole apical segments.

In addition, it was also noted that different rootstocks may have different in their intrinsic ability to transport IAA in the stem tissues. As found in apple, the dwarfing rootstock shoot explants exhibited a lower velocity of IAA transport than vigorous rootstock explants (Kamboj et al., 1997; Soumelidou et al., 1994). In this study, even though no consistent trends were found with the rootstock vigour (Figure 4.10), we strongly believe that inter-specific hybrid kiwifruit rootstocks also may have different ability to transport IAA, thereby exhibiting a different velocity of IAA transport in the stem tissues. The mechanism by which the tissue of the kiwifruit rootstock stem decreases the basipetal transport of IAA is largely unknown. However, studies in other fruit trees such as apple indicate that the bark of dwarfing rootstocks exhibited an increased capacity to destroy IAA (Gur & Samish, 1968) and contained higher concentrations of growth inhibiting phenols and lower concentrations of growth promoting phenols (Martin & Stahly, 1967) that may act to enhance or suppress the oxidative decarboxylation of IAA, respectively (Lockhard & Schneider, 1981). Besides that, dwarfing of trees is usually associated with either higher IAA oxidase content

and/or IAA oxidase activity (Chong & Andrew, 2006; Jindal et al., 1974; Martin & Stahly, 1967). In sweet cherry rootstocks, the least vigorous rootstocks such as Gil48/1 and Gil48/8 had significantly higher IAA oxidase content and activity than standard Mazzard rootstocks (Chong & Andrew, 2006). However, the most accepted theory of dwarfing on fruit trees were pointing to the endogenous level of IAA in the stem tissues of the rootstocks (Kamboj et al., 1997; Li et al., 2012; Soumelidou et al., 1994; van Hooijdonk et al., 2010, 2011).

In this study, the data gathered so far indicate that auxin (i.e. IAA) may have been involved in regulating kiwifruit scion growth and vigour (Experiment 1). Besides that, our data in this chapter also demonstrated that the level of auxin in the stem tissues of inter-specific hybrid kiwifruit rootstocks maybe different depending on the vigour and cultivar (Experiment 2). However, the proportions of ^{14}C in the root system that were still associated with IAA and the metabolic fate of [^{14}C]–IAA in the stem segments (Kamboj et al., 1997) were still not determined for these kiwifruit rootstocks. Therefore, these will allow new studies in the future. Nevertheless, this study has contributed to the additional evidences on the role of endogenous auxin (i.e. IAA) in the stem tissues of kiwifruit rootstocks that are involved in regulating the meristematic activity of scions, similar to what have been found in other fruit trees.

4.5 Summary

In this chapter, two major studies were conducted to improve our physiological understanding on the endogenous hormonal signalling mechanism (s) in kiwifruit rootstocks focusing on the auxin (i.e. IAA). In the first study (Experiment 1), the newly grafted 'Hayward' scions onto different vigour of inter-specific hybrid kiwifruit rootstocks, including 'Bruno' and 'Hayward' self-rooted control (GN), were evaluated in terms of their hormonal signalling between the shoot to root or *vice versa* by using auxin transport inhibitor, 1-N-naphthylphthalamic acid (NPA) applied to the stem junction at graft-union. Two days after NPA application, there was an absence of epinastic curvatures in leaves of scion primary shoots. The apical shoots of scions on the NPA treated vines were bending downwards, together with the production of axillary outgrowth below the NPA application sites, indicating that the basipetal IAA transport in the stem tissues of scion primary shoots was restricted and inhibited.

The meristematic activities of scion primary shoots on low-vigour (No.18 and No.100), intermediate-vigour (No.55 and No.84) rootstocks plus self-rooted 'Hayward' (GN) were affected by the NPA application. The shoot growth in terms of length of scion primary shoots was decreased slightly and fully terminated two weeks after the first application of NPA, but the primary shoot of untreated vines was still continuously growing. The final length, node number and trunk CSA of primary shoots were also reduced with NPA treatment. In contrast, NPA treatment did not affect the shoot growth and final shoot characteristics of 'Hayward' scions when grafted onto low- and high-vigour rootstocks No.19 and 'Bruno' (respectively). NPA treatment did not affect the internode length of scion primary shoots regardless of rootstocks vigour. Even though not significant, the bud break of scion primary shoots on the hybrid kiwifruit rootstocks was slightly higher compared to the control vines (i.e. own rooted cuttings), indicating that there were consistent trends that the inter-specific hybrid kiwifruit may have the ability to influence scion bud break as found in previous chapters. The number of proleptic axillary shoots (i.e. branching) was significantly affected by the inter-specific hybrid kiwifruit rootstocks and possibly NPA treatment. NPA treatment significantly reduced the LA, FW, and DW of scion primary shoots, but opposite pattern was found on LMA of scion primary shoots. The final mean total LA of proleptic axillary shoots

was also significantly reduced with the NPA application in the second growing season. Restriction of IAA on the particular inter-specific hybrid kiwifruit rootstocks may have decreased the overall growth in terms of final mean total length, mean total node number, and mean LA of proleptic axillary shoots of 'Hayward' scions.

The second objective of this study was to assess the IAA transport in different vigour of inter-specific hybrid kiwifruit rootstocks (Experiment 2). By using 60 mm-long shoot explants of selected hybrid kiwifruit rootstocks, the stem segments were subjected to an agar-donor receiver transport system. The uptake and transport of radioactivity were measured at three different stem segments (i.e. apical, middle, and basal). Preliminary observation between rootstock No.18 (low-vigour) and No.101 (high-vigour) conducted in late autumn season of 2014 found that the uptake and transport of radioactivity in middle and basal segments did not differ between rootstocks. However, the uptake and transport of radioactivity were significantly lower in the apical segments of rootstock No.18, and it was two times lower than the apical segments of rootstock No.101. In summer of 2014, the uptake and transport of radioactivity of kiwifruit rootstock hybrids including 'Bounty 71' were evaluated at two different hours (i.e. 24h and 48h). Results indicate that the total uptake and transport of radioactivity in all stem segments (apical, middle and basal) and agar were significantly different between low-vigour (No.18) and high-vigour rootstock (No.101). The total radioactivity in the apical segments of low-vigour rootstocks was significantly lower than high-vigour rootstocks. Rootstock, time and rootstock x time interactions had significant influence on the total uptake and transport of radioactivity. Even though the total amount of radioactivity in all of the segments showed no consistent pattern, the total activity recovered in agar showed a trend to increase vigour from low- to high-vigour rootstock (No.18 to No.55, respectively). The unexpected result was that the uptake and transport of radioactivity were reduced in the stem of high-vigour rootstock No.55, No.86 and No.101. Overall, the data gathered in this chapter indicate that auxin (i.e. IAA) signalling may have a significant role in regulating growth and vigour of grafted 'Hayward' scions (Experiment 1), and the level of IAA in the stem tissues of inter-specific hybrid kiwifruit rootstocks may differ depending on the vigour and cultivars of the kiwifruit rootstocks (Experiment 2). Even though no correlation between vigour and the parentages of inter-specific hybrid kiwifruit rootstocks, we postulate that the level of IAA transported could be more related to the original genetic of the rootstocks.

Chapter Five

5. Manipulation of vegetative growth, shoot architecture and fruit characteristics of green *(A.deliciosa)* and gold kiwifruit *(A.chinensis)* by bark grafting

5.1 Introduction

Generally, dwarfing rootstocks are widely used to control vegetative growth in many fruit trees (Rom & Carlson, 1987; Webster, 1995a). Currently, there is a lack of dwarfing or vigour controlling rootstocks for kiwifruit (Palmer, 2007; Warrington, 2000). A few growth manipulation techniques such as pruning, girdling, shoot tipping and root pruning have been adopted for managing excessive vigour of kiwifruit vines. Kiwifruit has vigorous vegetative growth habits (Ferguson, 1984) and has long periods of vegetative growth (Palmer, 2007). These problems have caused dense canopies (Lancaster & MacRae, 2000) and reduced fruit quality, in particular dry matter concentration (DMC) due to insufficient light exposure in the canopy (Biasi et al., 1995); controlling excess vegetative growth leads to an increase in the production cost of kiwifruit management. In order to manage excessive vegetative growth in kiwifruit, summer pruning is applied and this technique has been adopted as a strategy for kiwifruit canopy management known as 'leader pruning' (Miller et al., 2001). The objective of summer pruning is to reduce canopy density and, at the same time to promote the development of less vigorous canes to carry the next season crops. However, since pruning increases the production cost, other control techniques are still needed to improve canopy management in kiwifruit. Normally, excess vegetative growth in kiwifruit during early spring can be reduced by girdling techniques (Currie et al., 2005). A study on gold kiwifruit 'Hort16A', showed that vines girdled on main trunks or leaders usually have smaller leaves, produce an abundance of flowers and have higher proportion of less vigorous shoots (i.e. terminated shoots) than on non-

girdled vines (Currie et al., 2005). Other studies also found that girdling on kiwifruit vines can reduce leaf photosynthesis (Black et al., 2012). In terms of fruit growth manipulation, applying girdling during late summer can increase fruit DMC (Boyd & Barnett, 2011; Patterson & Currie, 2011).

Another technique for manipulating growth and vigour in kiwifruit that has been developed is 'zero-leaf pruning' (Gardiner & Max, 2005). This technique involves selective pruning on strong actively growing long shoots (non-terminated) in proximity to the last fruit. As a result, no new growth will develop; therefore, there is no need to prune again on these shoots. 'Tip squeezing' technique have also been shown to be promising in controlling summer canopy growth (Max & Currie, 2005). This technique requires damaging the tip of non-terminated or actively growing shoots by squeezing the tip. This is similar to the removal of the tips but the tips will still be intact and survive. Squeezing the tips is more less similar to the 1-Naphthaleneacetic acid (NAA) gel application, but it is believed that fruits produced from this technique will have low dry matter concentration especially on 'Hort16A' cultivar (Max & Currie, 2005). Although all the techniques mentioned above have shown promise in controlling vigour of kiwifruit, there is still a need for permanent techniques which can give sustaining effects, such as vigour controlling rootstocks or more-effective low labour cost management techniques.

In order to improve understanding of vigour control of fruit trees, growth manipulation by blocking auxin transport was designed and implemented. For example, Lockhard and Schneider (1981) used the bark grafting technique to demonstrate the involvement of auxin in controlling the growth and vigour of apple trees. They found that by grafting a completed ring of bark in an inverted orientation on the main stem (i.e. reverse polarity of phloem cells), the growth and vigour of apple trees was reduced (Lockhard & Schneider, 1981). Therefore, they hypothesize that the reduction in tree growth by bark grafting was a result of the reduction in auxin transported from shoot to root and this may also reduce cytokinins transport from the root to shoot. Other growth manipulation using auxin transport inhibitor using, 1-N-Naphthylphthalamic acid (NPA) has also been used to elucidate the cause of dwarfing by the M.9 rootstock imparted to the grafted scion (van Hooijdonk, 2009). However, Vattiprolu (2009) found that the application of NPA to young kiwifruit vines did not significantly reduce the growth of

kiwifruit and she hypothesized that the hormonal signalling mechanism in kiwifruit could be different from that of apple. Therefore, in order to further evaluate the mechanism of blocking auxin transport in kiwifruit, and to investigate the practicality of bark insertions, growth manipulation by bark grafting was tested and implemented.

There is still lack information on aspects of hormonal control mechanism for kiwifruit as compared to other fruit trees. Although the basis for hormonal control of dwarfing in fruit trees has recently been revealed (e.g. apple, van Hooijdonk, 2009), this aspect is poorly understood in kiwifruit vines. Besides that, limited information on growth manipulation is available and only a few trials have been conducted on kiwifruit. Therefore, to improve our physiological understanding of vigour control in kiwifruit, the bark grafting technique was tested with the aim to manipulate the polarity of phloem cells of kiwifruit bark. This technique also mimics the blocking of auxin transport by NPA (see Chapter Four) or girdling. It was hypothesis that by inverting a ring of bark of kiwifruit, the auxin transported from the shoot to root system will be reduced, thus limiting the vine growth (**Hypothesis IV**). This chapter was designed to evaluate bark grafting as a technique to manipulate growth and vigour, as well as fruit characteristics in kiwifruit. Even though some of the authors reported that a lack of practicality in this technique (e.g. Brase & Way, 1956; Gaskin, 1963), nevertheless, it is worth investigating, since kiwifruit is a crop that still requires considerable vigour manipulation, unlike other fruit crop such as apple that have good dwarfing rootstocks. Information generated from this study can also improve our physiological understanding on the restriction of auxin transport in kiwifruit. The specific objectives of this study are highlighted below and the methods are detailed in the next section (Section 5.2):

(1) To evaluate how changes in vegetative growth and shoot architectural structures of young kiwifruit vines are affected after the vines are subjected to bark grafting in various orientations (*Growth Manipulation 1,GM*1).

(2) To assess whether grafting a ring of bark from other kiwifruit cultivars may have an influence on the vegetative growth and shoot architectural structures of young kiwifruit vines (*Growth Manipulation 2, GM*2).

(3) To evaluate bark grafting as a technique to regulate fruit characteristics (in terms of fruit weight, size, DMC etc.) of field-grown kiwifruit vines (*Growth Manipulation 3, GM*3).

5.2 Materials and methods

5.2.1 Effects of bark grafting on vegetative growth and shoot architecture of young, potted 'Hort16A' kiwifruit vines (*Growth Manipulation* 1 and 2)

5.2.1.1 Study site and establishment of experimental plant material

The experiment was performed over three growing seasons at the Plant Growth Unit (PGU), Massey University, Palmerston North, New Zealand. One-year-old self-rooted gold kiwifruit 'Hort16A' cuttings were used as experimental plants. The plants were placed in a tunnel house and standing out area at the PGU to monitor their growth and development. Irrigation was given three times a day using a drip irrigation system.

5.2.1.2 Propagation and growing medium

The cuttings were propagated from semi-hardwood cuttings (Vattiprolu, 2012). Briefly, cuttings with three buds and approximately 20 to 25 cm in length were taken from kiwifruit vines at Fruit and Crops Unit, Massey University. During propagation, kiwifruit cuttings were treated with a rooting hormone solution (500 mg L-1 IBA dissolved in 25% ethanol) for five seconds, following the quick dip method. Before dipping into the hormone solution, the bottom of the cuttings was scored by using sharp knives to enhance the absorption of the rooting hormone. The cuttings were immediately planted in 6 cm x 6 cm plug-tray containing Daltons™ growing medium (C.A.N fines A grade 50%, fiber 30%, pumice 7 mm 20% and Sepertine Super 1kg/M^3). The planting trays were arranged under a mist sprinkler system in the glass house, together with a bench heating system to maintain the medium temperature at 20 to 22 °C. After four to six weeks, rooted cuttings with uniform growth were transferred into 45 L polybags with standard growing medium containing slow release fertilisers with trace elements, 150 g of Dolomite (Osmocote 200 g for 8-9 months and Osmocote 100 g for 3-4 months) per 100 L of growing media.

5.2.1.3 Bark grafting treatment on the kiwifruit stem

*GM*1 and *GM*2 were conducted from early summer (December) until winter (June). Due to lack of young 'Hayward' vines, the bark grafting treatment was tested on young 'Hort16A' vines (Figure 5.1A). Firstly, the stem of a young vine was headed to a single stem. The main stem of young 'Hort16A' kiwifruit vines were subjected to a bark grafting treatment by removing a 15-mm width strip of bark using a specially designed tool (Figure 5.1B) and sharp knives, leaving approximately three to four nodes above and below the graft-union. For the first trial (*GM*1), only one insertion of bark, 15 mm in width was used. The orientation of bark was marked with colour pen by an arrow to reduce error during insertion or grafting later. The strip of removed bark was grafted back to the main stem either in normal or in inverted orientation (Figure 5.2). During this time, attention was given to the arrow mark (Figure 5.2) on the bark to ensure the right bark orientation (normal or inverted). For the second trial (*GM*2), three strips of bark from new selected kiwifruit cultivars that have been thought tolerant to *Pseudomonas syringae* (PSA) namely G3, G9 and G14 were grafted back to the stem of gold kiwifruit cv. Hort16A similarly to *GM*1. The inserts were then tightly held by wrapping grafting tape many times around and the graft-union was sealed with pruning paste (Bacseal®Super, Bayer CropScience, NZ) to avoid dehydration of the graft-union. Vines without any bark grafting treatment were used as control vines. The experimental plants and graft-unions were monitored weekly throughout the experimental period.

5.2.1.4 Measurements of shoot architecture and vegetative growth

At the end of March, all sylleptic axillary shoots from the control and treated vines were classified according to the description made by Seleznyova et al., (2002). All the shoot architectural measurements on the different types of shoots were made similar to the Section 2.2.3 (Chapter Two). At the end of the summer season before leaf fall, all the leaves of every shoot types were stripped to determine the leaf areas using a Leaf Area Meter (LI-3100, LI-COR, Nebraska, USA). In *GM*2, additional data on the individual leaf area were also recorded, by numbering the nodes starting from the base up to 15th node.

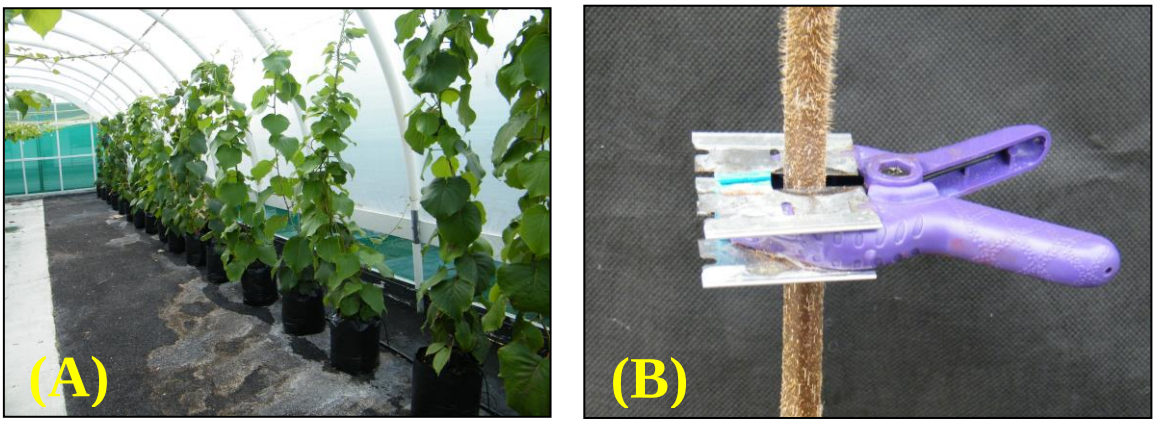


Figure 5.1. (A) Self-rooted gold kiwifruit 'Hort16A' cuttings planted in 45 L polybag, and (B) a specially designed tool used for bark grafting.

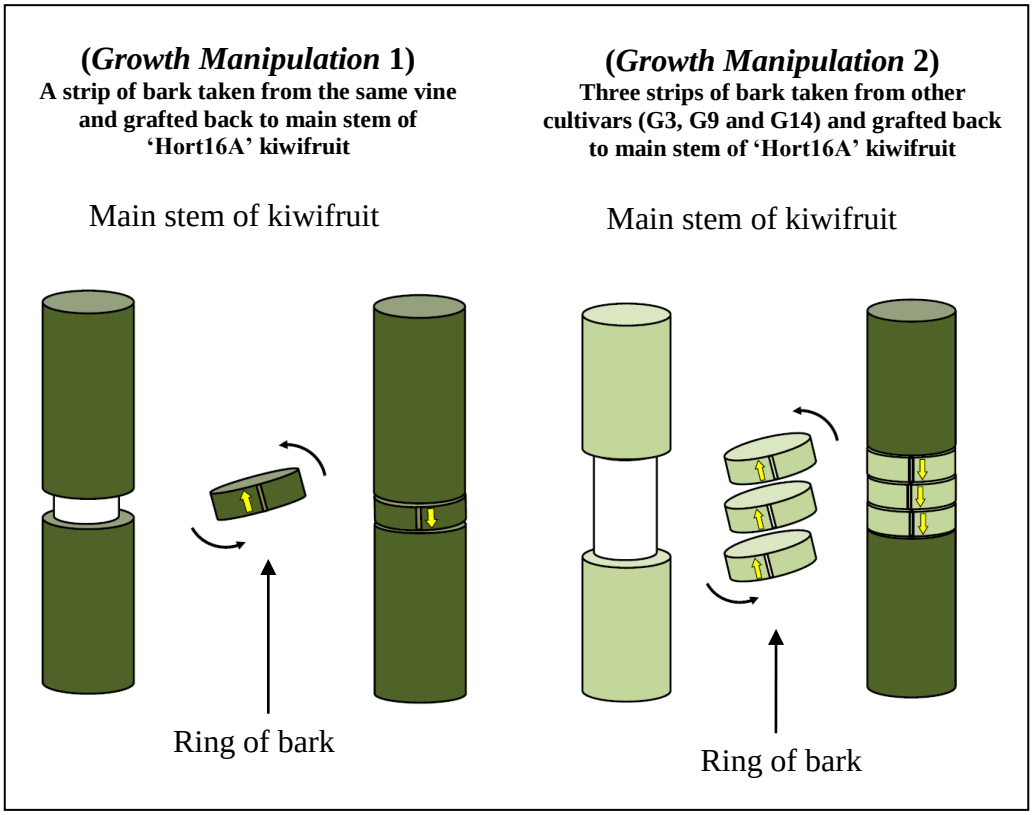


Figure 5.2. Schematic diagrams of the method of bark grafting on stem of young, potted 'Hort16A' kiwifruit vines. Noted here, in *Growth Manipulation* 1, only one strip of bark was taken and grafted back on the same vines either in normal or an inverted orientation. In *Growth Manipulation* 2, three strips of barks were taken from other kiwifruit cultivars (G3, G9 and G14) and grafted back on the main stem of 'Hort16A' vines either in normal or an inverted orientation.

5.2.2 Effects of bark grafting on the fruit characteristics of green kiwifruit cv. 'Hayward' vines (*Growth Manipulation* 3)

5.2.2.1 Study site and experimental plant materials

This study was performed at the kiwifruit orchard, Fruit and Crop Unit, Massey University, Palmerston North, New Zealand using mature green kiwifruit, *Actinidia deliciosa* cv. 'Hayward' grown on T-Bar trellis and managed under standard commercial agricultural practices for kiwifruit. The vines were planted with 1:5, male to female ratios. Irrigation was given twice daily with the Dan-Modlar micro sprinklers system, running on 19-mm lateral tubing. Vines with uniform tree size were selected for this study which consisted of three treatments; i) bark grafting in inverted orientation, ii) bark grafting in normal orientation and iii) girdling as control. All the treatments were conducted during two different seasons, early summer (December) and late summer (March). The effects of bark grafting and girdling were monitored over two fruiting seasons to identify any carry-over effect of all treatments on fruit characteristics. All treatments were only applied once in the first year of fruiting.

5.2.2.2 Bark grafting treatment on the kiwifruit vines

The steps of the bark grafting are shown in Figure 5.3. Firstly, thick cardboard paper, 10 cm in width, was placed on the stem of kiwifruit vines as a guide and a straight line drawn around the stem bark using a marker pen (Figure 5.3A). After that, the phloem bark was cut using a hacksaw until the xylem layer was reached (Figure 5.3B). Arrows were drawn on the bark to indicate orientation (Figure 5.3C). The bark/phloem layer was removed using sharp knives (Figure 5.3D) and inserted back onto main stem in inverted or normal orientation. For both treatments, attention was given to the arrow mark on the bark insert to ensure the correct orientation. The inserted bark was then tightly held by applying grafting tape a few times around the stem (Figure 5.3E) and covered with pruning paste to avoid dehydration of the insert (Figure 5.3F). All the experimental vines were monitored.

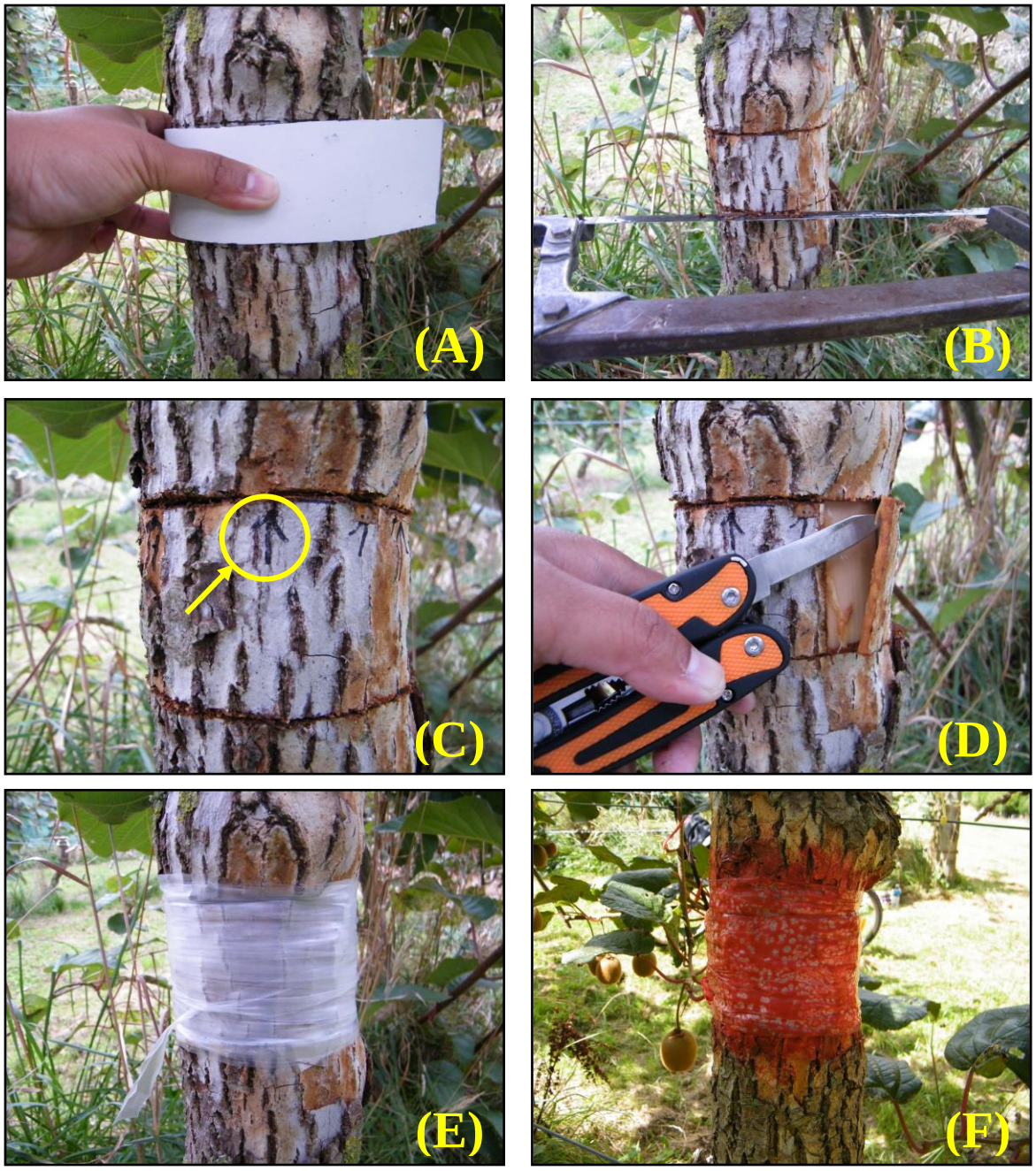


Figure 5.3 (A-F). The steps for bark inversion on green kiwifruit cultivar 'Hayward' vines. Yellow arrow indicates the bark orientation (inverted or normal).

5.2.2.3 Measurements of fruit characteristics and dry matter concentration

All measurements on fruit characteristics and dry matter concentration (DMC) were carried out immediately after harvest. The first harvesting of fruits was conducted over two seasons, during May similar to the normal harvesting time for kiwifruit in the Manawatu region. All fruits from the treated and control vines were harvested and counted to obtain the fruit number (i.e. crop load) for every vine. Sixty fruits were randomly selected and kept inside a snap-lock bag to avoid dehydration. The fruits were immediately brought back to the laboratory for analysis. Measurement of fruit characteristics (Figure 5.4) followed the method described by Currie (1997). The length (L) and width of fruits (W_1 and W_2) were measured using digital calliper (Mitotuyo, Digimatic, Japan) and the fruit size was obtained by multiplying L, W_1 and W_2. The fruit fresh (FW) and dry weights (DW) were measured using a digital balance (GW6202, Satorius). To measure the fruit dry weight (g), the whole fruit was sliced, put in a brown envelope and placed in a drying oven at 70°C until the dry weight was constant and DMC was calculated using the standard formula [(fruit DW/fruit FW) x 100%]. In order to obtain a correlation between FW with DMC at the end of study period, whole fruits were used rather than taking equatorial slices measurements.

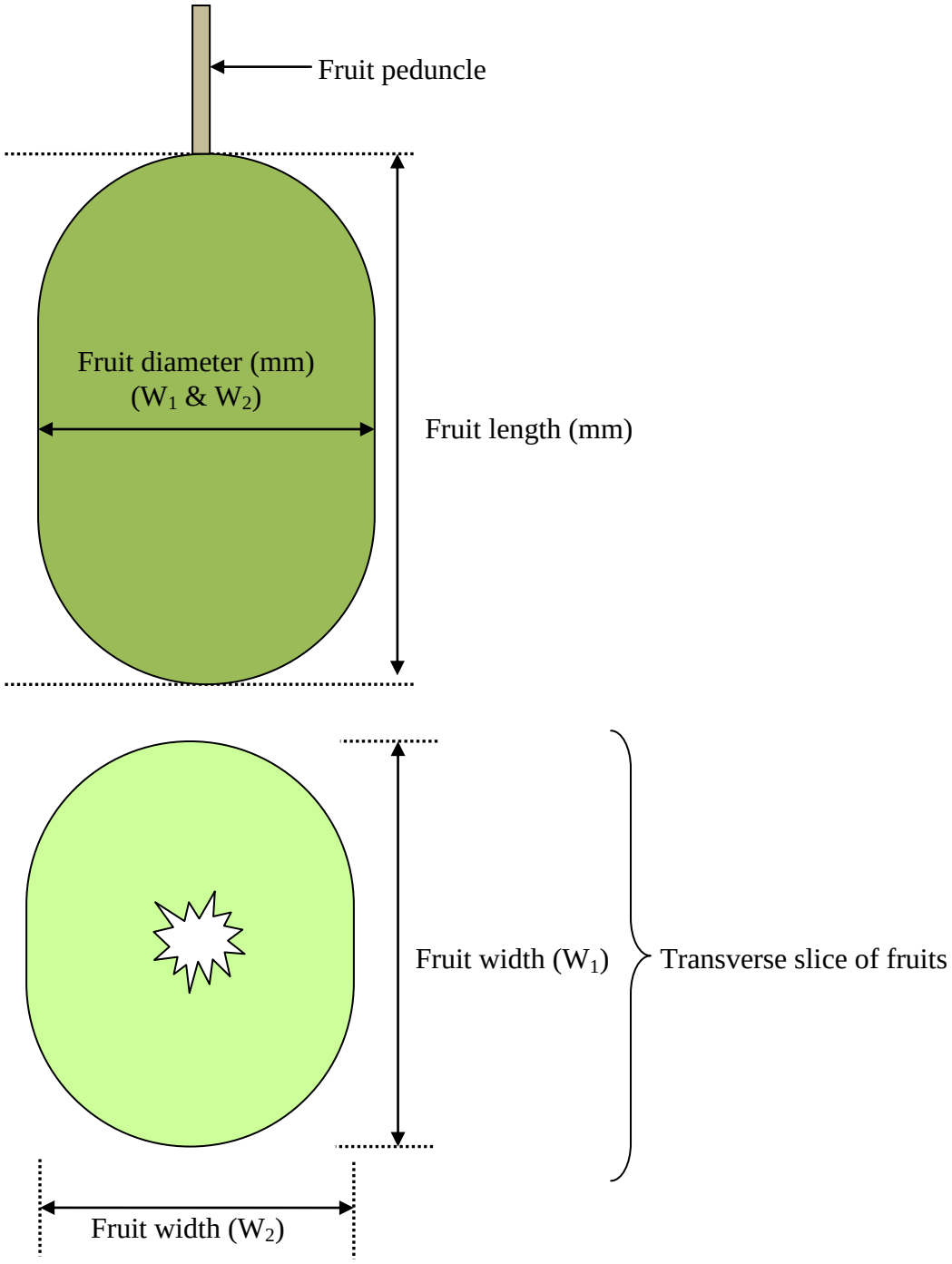


Figure 5.4. Schematic diagram of fruit characteristics measurements made on green kiwifruit (*Actinidia deliciosa* cv. 'Hayward').

5.2.3 Experimental design and statistical analysis

All the data were manually entered in an Excel spreedsheet and summarised using Microsoft Excel Pivot Tables. Before conducting analyses using the GLM procedure of SAS (9.1, SAS Institute Inc., NC USA), data were checked for outliers and homogeneity of variance using Goodness of Fit Test (Shapiro-Wilk). All data were normally distributed so no transformation was carried out on the raw data.

5.2.3.1 Bark grafting treatment on young 'Hort16A' potted vines

For *GM*1, the experiment was conducted as a completely randomised design (CRD) with six single vine replicates. Treatments were; i) no bark grafting treated as control, ii) bark grafting in an inverted orientation, and iii) bark grafting in normal orientation. For *GM*2, the experiment was conducted as a Randomised Complete Block Design (RCBD) with factorial arrangement of bark grafting treatments (3x2). There were two bark grafting treatments (normal and inverted orientation) and the insertion of barks from the three different kiwifruit cultivars (G3, G9 and G14). Vines without bark grafting were used as control vines. Each treatment was replicated five times. ANOVA *F*-test was used to determine significant level and mean separation were compared by using Least Significant Difference (LSD) Test at $P \leq 0.05$.

5.2.3.2 Bark grafting on field-grown of 'Hayward' vines

All the treated vines were arranged in a randomized complete block design (RCBD) with factorial arrangement. Each treatment was replicated three times with single vine replicate within three blocks per season. Three treatments were tested: i) standard girdling treated as control, ii) bark grafting in an inverted orientation, and iii) bark grafting in normal orientation. In addition, treatments were imposed at two times of year, early summer (early December) and late summer (late February). The effects of the treatment on fruit characteristics at harvest were assessed by Analysis of Covariance (ANCOVA), where the number of fruits per vine was used as covariate, since crop load

per vine was not adjusted. The effects of bark grafting treatment was observed in two consecutive years, to identify any carry over treatment effect on the vines. Two analyses were performed separately on both years to evaluate the effects of treatments during the two different times of year (early and late summer). Differences between means were compared using Tukey's Honest Significant Difference (HSD) Test at P=0.05.

5.3 Results

5.3.1 Growth Manipulation 1 (*GM*1)

5.3.1.1 Early responses of kiwifruit vines to bark grafting

Three weeks following bark grafting treatments, there was axillary shoots developed below the area of graft-union (Appendix 6). In addition, the production of axillary out-growth below the grafting union was significantly higher in the vines grafted with bark in an inverted orientation than in normal orientation (Appendix 7B). However, control vines did not produce any axillary bud outgrowth (Appendix 7A). These axillary shoots were removed to avoid confounding effects on the grafted vines. Nevertheless, the pruning weight of these shoots including suckers was weighed and recorded. There was a significant difference (P=0.0002) in the mean of pruning weight (kg) among the treatments (Appendix 7B). The mean pruning weight (kg) of axillary growth and suckers below graft-union on the vines grafted in inverted orientation was significantly greater compared to the vines grafted in normal orientation and control (Appendix 7B). Swelling and thickening of bark above the graft-union area were also apparent in the inverted treatments (Figure 5.5A), and less obvious in treatments of normal orientation (Figure 5.5B).

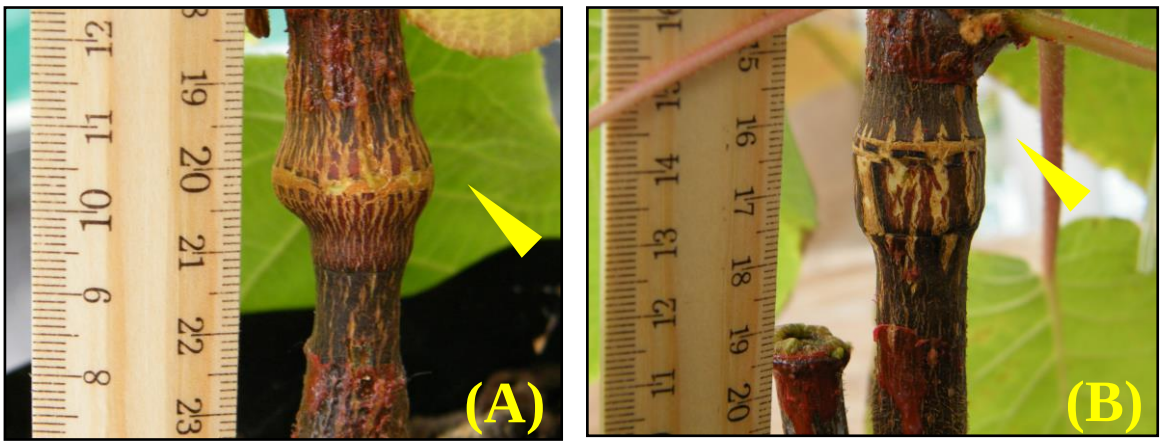


Figure 5.5. Yellow arrows indicate; (A) bark swelling and thickening above the bark graft-union area in inverted orientation, and (B) swelling and thickening of bark were less obvious on the graft-union in normal orientation.

5.3.1.2 Effects of bark grafting on the characteristics of long sylleptic shoots

The final length of long sylleptic shoots from vines that have been bark grafted in an inverted orientation may have been reduced (P=0.17) compared with vines grafting in normal orientation and the control (Figure 5.6A). There was a significant reduction (P=0.01) in the mean node number of long shoots in the vines treated with bark grafting treatment compared to control vines (Figure 5.6B). The differences between bark grafting treatments and control only approached significance (P=0.05) for mean internode length. The vines treated with bark grafting in inverted orientation had significantly longer mean internode length compared with the vines from control (Figure 5.6C). There was a significant difference (P=0.04) for the mean cross-sectional area (CSA) of long shoots (mm^2) between the vines treated with bark grafting and the control (Figure 5.6D). The mean CSA of long shoots from vines grafted with bark grafting in normal orientation was significantly smaller than the control (Figure 5.6D). The final mean leaf area (cm^2) of long shoots on the vines treated with bark grafting in inverted orientation may have been slightly reduced (P=0.15) when compared to the control vines (Figure 5.6E). However, the mean leaf area (cm^2) from vines treated with bark grafting in an inverted orientation was significantly reduced compared to the control vines (Figure 5.6E). There was a trend that bark grafting may have an effect (P=0.13) on the mean number of leaves of long shoots (Figure 5.6F). The final mean number of leaves was significantly reduced in the vines treated with inverted bark grafting compared to the control, but not in the vines grafted in normal orientation (Figure 5.6F).

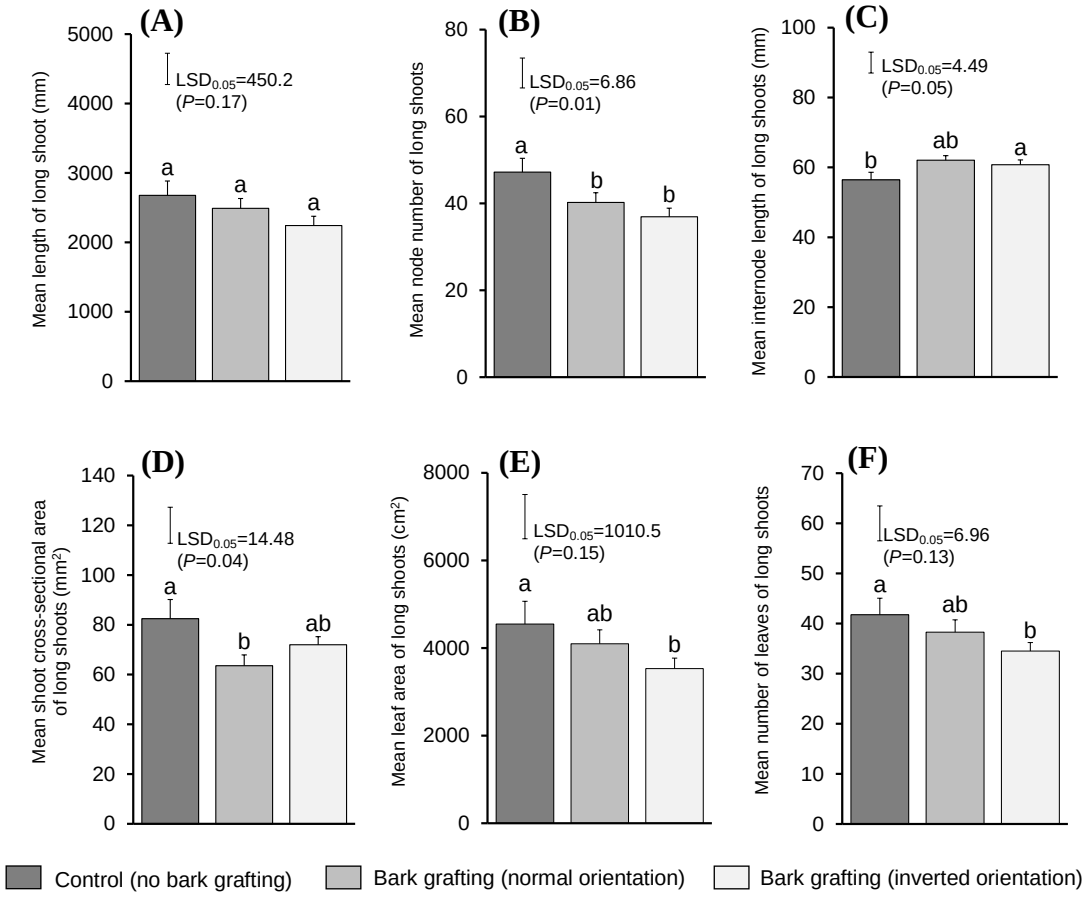


Figure 5.6. Effects of bark grafting on the mean final; (A) length, (B) node number, (C) internode length, (D) stem cross-sectional area, (E) leaf area, and (F) number of leaves of long sylleptic axillary shoots of young gold kiwifruit cv. 'Hort16A' vines. Means sharing same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. Bars denote the LSD at *P*=0.05.

5.3.1.3 Effects of bark grafting on the characteristics of medium sylleptic shoots

The characteristics of medium shoots (length, node number, internode length, shoot CSA, number of leaves and leaf area) were not significantly affected by the bark grafting treatments (Figures 5.7A-F).

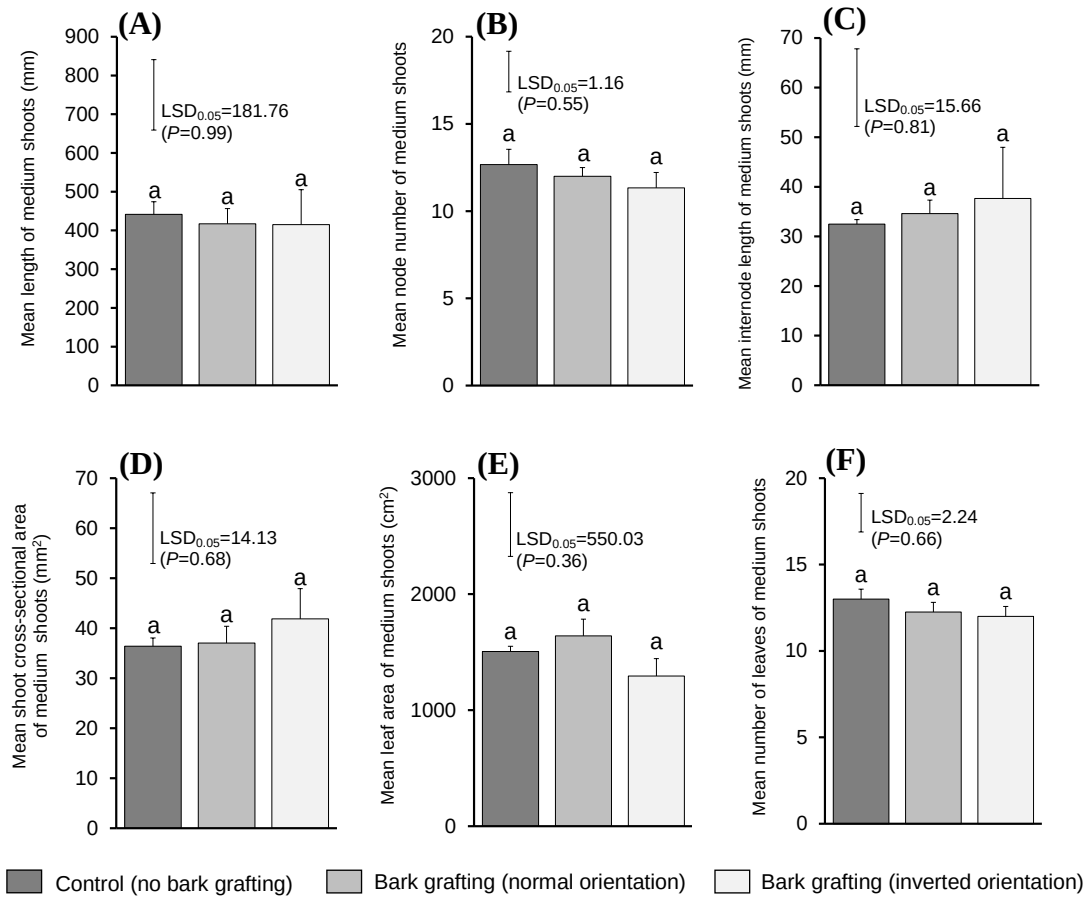


Figure 5.7. Effects of bark grafting on the mean final; (A) length, (B) node number, (C) internode length, (D) stem cross-sectional area, (E) leaf area, and (F) number of leaves of medium sylleptic axillary shoots of young gold kiwifruit cv. 'Hort16A' vines. Means sharing same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. Bars denote the LSD at *P*=0.05.

5.3.1.4 Effects of bark grafting on the characteristics of short sylleptic shoots

Bark grafting in both orientations did not significantly affect the final characteristics (length, node number, internode length, shoot CSA, number of leaves and leaf area) of short shoots (Figure 5.8A-F).

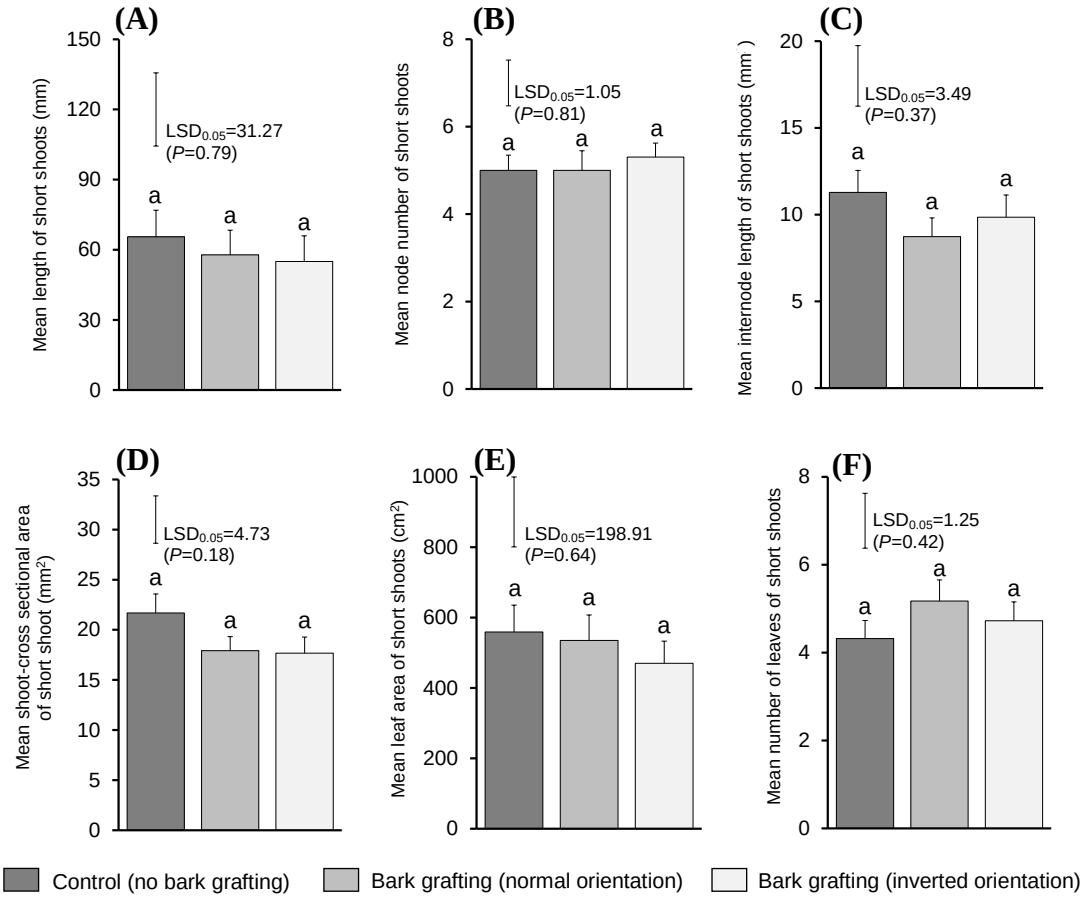


Figure 5.8. Effects of bark grafting on the mean final; (A) length, (B) node number, (C) internode length, (D) stem cross-sectional area, (E) leaf area, and (F) number of leaves of short sylleptic axillary shoots of young gold kiwifruit cv. 'Hort16A' vines. Means sharing same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. Bars denote the LSD at *P*=0.05.

5.3.1.5 Effects of bark grafting on the total length, leaf area and node number of sylleptic axillary shoots

The final mean total length (mm), mean total node number and mean total leaf area (cm^2) of sylleptic axillary shoots (short, medium and long shoots) were not significantly different between bark grafting treatments and control vines (*P*=0.66, *P*=0.42 and *P*=0.45, respectively) (Table 5.1).

Table 5.1. Effects of bark grafting on the mean total shoot length (mm), mean total leaf area (cm^2) and mean total node number of sylleptic axillary shoots of young 'Hort16A' vines.

Bark grafting treatment	Mean total shoot length/vine‡ (mm)	Mean total leaf area/vine‡ (cm)	Mean total node number/vine‡
Control *(no bark grafting)*	13,961[a] (± 1369.5)	26,430[a] (± 3294.0)	268.6[a] (± 26.8)
Bark grafting *(normal orientation)*	13,216[a] (± 1518.2)	24,728[a] (± 2611.7)	237.3[a] (± 26.5)
Bark grafting *(inverted orientation)*	12,071[a] (± 1358.0)	21,169[a] (± 2850.0)	220.3[a] (± 21.3)
$LSD_{0.05}$	4350.2	8816.2	75.7
P-value	*P*=0.66	*P*=0.45	*P*=0.42

Means sharing same letters within a column are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. Numbers in parenthesis are standard error of means (±).
‡Combine total length, total leaf area and total node number of long, medium and short sylleptic shoots.

5.3.1.6 Effects of bark grafting on the mean proportion and mean total number of sylleptic axillary shoots

The mean proportions of long, medium and short sylleptic axillary shoots (Table 5.2) were not significantly different between bark grafting and the control vines (*P*=0.98, *P*=0.31 and *P*=0.69, respectively). Similarly, bark grafting treatment also did not significantly affect (*P*=0.85) the mean total number of sylleptic axillary shoots (i.e. short, medium and long) (Table 5.2).

Table 5.2. Effects of bark grafting on the mean proportion and mean total number of sylleptic axillary shoots (branching) of young 'Hort16A' vines.

Bark grafting treatment	Mean proportion of sylleptic shoot types			
	Long	**Medium**	**Short**	**Mean total number of shoots**
Control *(no bark grafting)*	0.53^{a} (± 0.08)	0.05^{a} (± 0.03)	0.42^{a} (± 0.13)	10.60^{a} (± 1.43)
Bark grafting *(normal orientation)*	0.55^{a} (± 0.07)	0.13^{a} (± 0.06)	0.32^{a} (± 0.09)	9.97^{a} (± 1.08)
Bark grafting *(inverted orientation)*	0.54^{a} (± 0.14)	0.04^{a} (± 0.02)	0.42^{a} (± 0.07)	10.50^{a} (± 1.38)
$LSD_{0.05}$	0.28	0.13	0.28	3.96
P-value	*P*=0.98	*P*=0.31	*P*=0.69	*P*=0.85

Means sharing same letters within a column are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. Numbers in parenthesis are standard error of means (±).

5.3.1.7 The relationship between final shoot length and node number of sylleptic axillary shoots

Bark grafting (Figure 5.9B and C) and control (Figure 5.9A) vines had very similar relationships between the final length and node number for each shoot type (long, medium and short shoots). For long and medium shoots (Figure 5.9A, B and C, large graphs), there were strong positive linear relationships between final length and node number with regression coefficients (R^2) more than 80% for both treated and control vines. However, for short shoots, a quadratic relationship was more suitable (Seleznyova et al., 2002) and closely fitted the relationship between length and node number (Figure 5.9D, E and F, small graphs). A similar linear relationship was also found in short shoots; there was a strong positive relationship (R^2>0.80) between the final length and node number (Figure 5.9D, E and F, small graphs). These results suggest that there were strong trends for all treatments that increasing node number is associated with increased shoot length. Therefore, the internode length of shoots was not affected by the bark grafting treatment. It also should be noted, by comparing the point values for long and medium shoots on the scatter graph between bark grafting and control vines, the point values from the bark grafting vines were much more compact than the point values from the control vines, especially the point values from vines grafted in inverted orientation (Figure 5.9A, B and C).

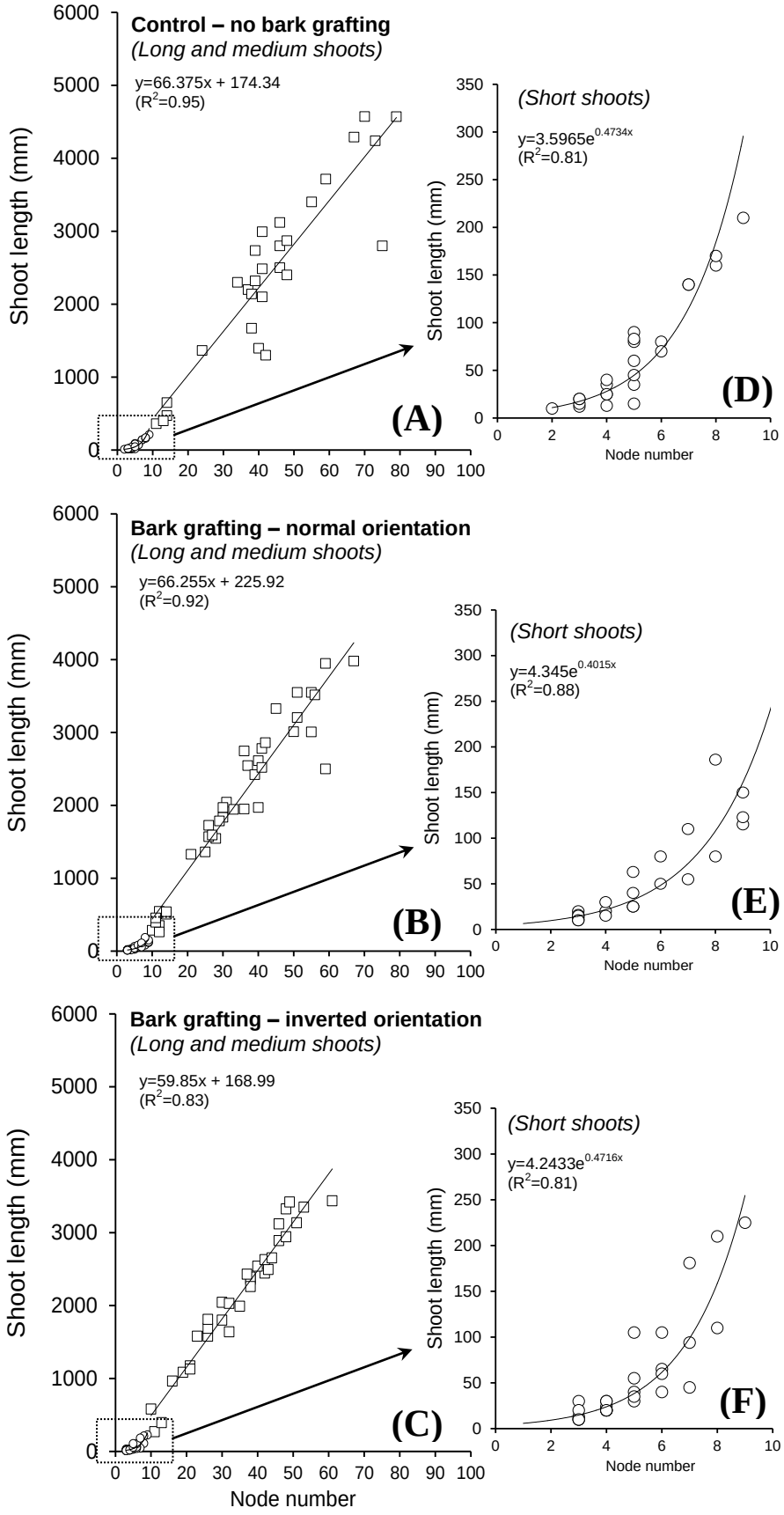


Figure 5.9. The relationship between the final length and node number of young 'Hort16A' vines at the end of summer season. The length and node number of long, medium and short shoots for each treatment are pooled in the same graph (large graphs).

5.3.2 Growth Manipulation 2 (*GM*2)

5.3.2.1 Early responses of kiwifruit vines to bark grafting from other cultivars

Two weeks after the bark grafting treatments, there were development of shoot axillary outgrowths below the graft-union area regardless of bark grafting orientation and insertion of bark from different cultivars (Figure 5.10A). The buds below the bark grafting site broke and developed into shoots. Nevertheless, these axillary shoot outgrowths were removed to avoid any confounding effects to the shoot growth of vines. After one month following bark grafting, the grafting tape was removed to examine the graft-union. There was an obvious swelling and thickening of stem above the bark graft union in the vines grafted in an inverted orientation (Figure 5.10B), but this effect was not observed in vines grafted in normal orientation (Figure 5.10C). Overall, most of the vines were successfully grafted and survived until end of experimental period.

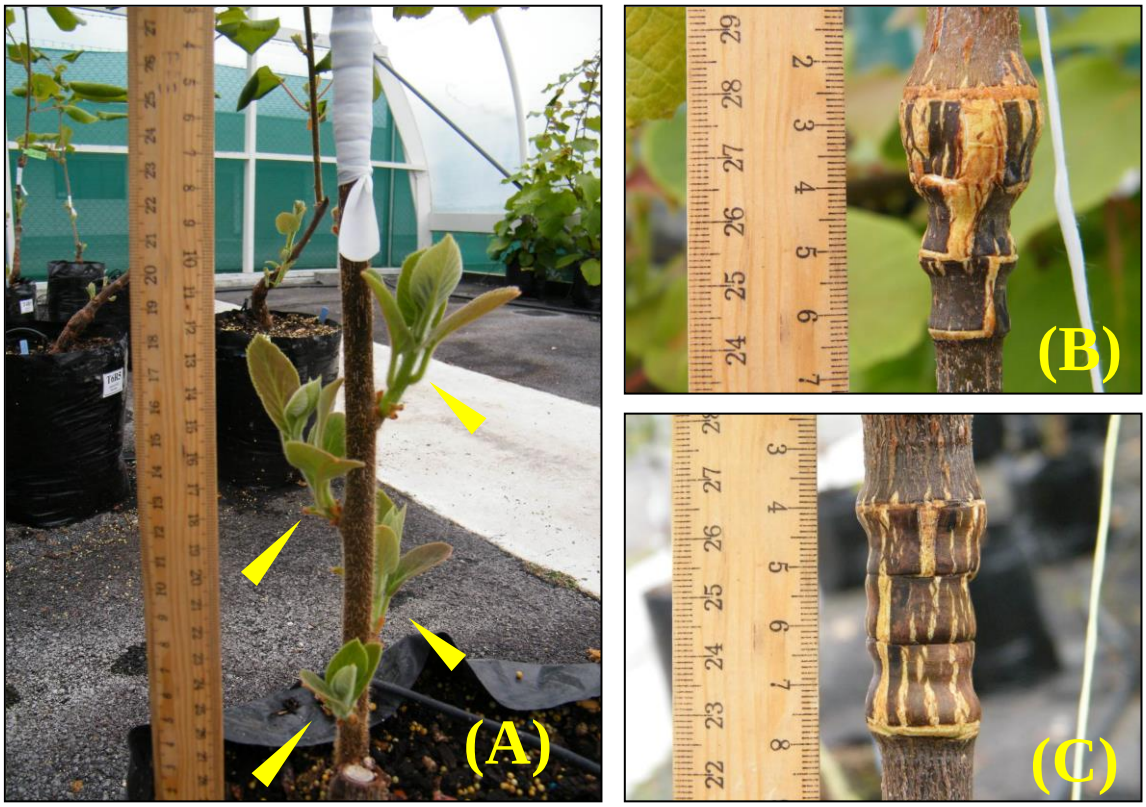


Figure 5.10. (A) Yellow arrows indicate the development of axillary outgrowth below the bark grafting area; (B) bark swelling and thickening on the top of graft-union in inverted bark grafting and; (C) no swelling and thickening of bark was observed in the graft-union of normal orientation.

5.3.2.2 The monthly production of sylleptic axillary shoots between bark grafting and control vines

The bark grafting treatment was performed in the early summer season (middle December). Starting the first month following bark grafting, the length of each sylleptic axillary shoots (mm) was measured and recorded. The measurements continued until late summer (early April). No significant difference was found in the mean total shoot length between bark grafting in both orientation and the control vines from the first until the fourth month following bark grafting in vines grafted with bark from G3 cultivar (Figure 5.11A). However, it was observed that the mean total shoot length of sylleptic axillary shoots may have been slightly reduced in the vines grafted in inverted orientation compared with vines in normal orientation and the control. The reduction in the mean total shoot length of vines grafted in inverted orientation was more clearly observed in the second, third and fourth month following bark grafting (Figure 5.11A).

There was a significant difference in the mean total length of sylleptic axillary shoots starting from the first until the fourth month following bark grafting in the vines grafted with bark from G9 cultivar (Figure 5.11B). In the first and second month following bark grafting, the mean total length of sylleptic axillary shoots from vines grafted with bark of G9 cultivar in inverted orientation was significantly reduced compared with control, but was not significantly different from vines grafted in normal orientation (Figure 5.11B). However, in the third and fourth month following bark grafting, vines with bark grafted from G9 in inverted orientation were significantly reduced in mean total length of sylleptic axillary shoots compared to the control and normal orientation (Figure 5.11B). In vines grafted with bark from G14 cultivar, no significant difference was found on the mean total length of sylleptic axillary shoots from the first until the fourth month following bark grafting (Figure 5.11C). However, the bark grafting vines may have been slightly reduced in the mean total length of sylleptic axillary shoots compared to control vines. Overall, comparing between vines grafted from bark of G3, G9 or G14, the reduction in mean total length of sylleptic axillary shoots was markedly observed in vines grafted in inverted orientation with bark from G9 cultivar (Figure 5.11B).

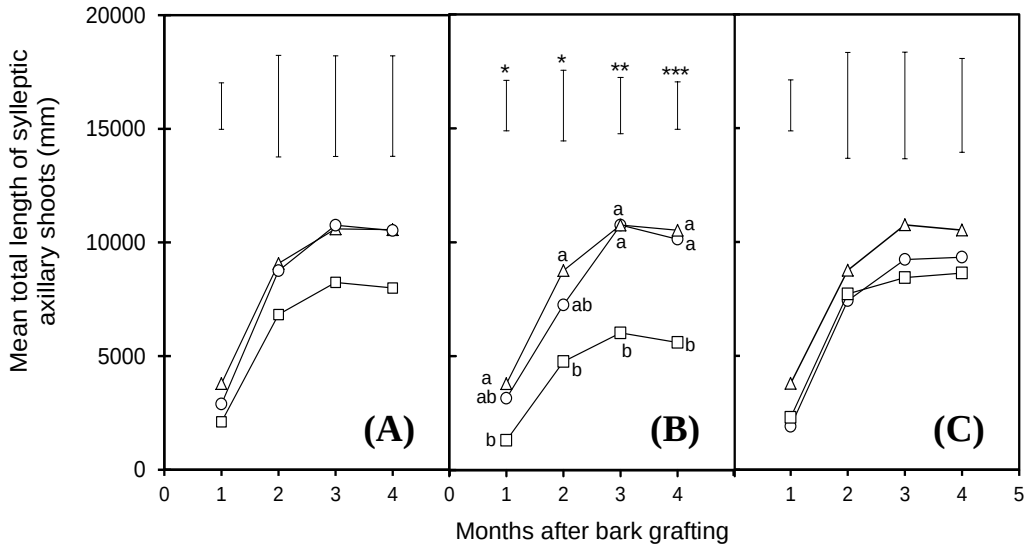


Figure 5.11. Mean total length of sylleptic axillary shoots (mm) of young 'Hort16A' vines during the first-four months following bark grafting. Insertion of bark from; (A) G3 cultivar, (B) G9 cultivar, and (C) G14 cultivar. Symbols indicate, (∆) control-no bark grafting, (○) bark grafting-normal orientation, and (□) bark grafting-inverted orientation.
,**, significant at *P*≤0.05, 0.01 and 0.001, respectively according to ANOVA *F*-test. Bars denote the LSD at *P*=0.05.**

5.3.2.3 Effect of bark grafting treatment and insertion of bark of various cultivars (G3, G9 and G14) on the final mean total length and mean total node number of sylleptic axillary shoots

Two-way ANOVA was performed to assess whether any interaction between bark grafting in different orientation and the insertion of bark from different cultivars on the final total shoot length (mm) of sylleptic axillary shoots of 'Hort16A' kiwifruit. There were no significant effects of bark grafting treatments x cultivar interactions on the final mean total length (mm) (*P*=0.57) and final mean total node number (*P*=0.19) of sylleptic axillary shoots of young 'Hort16A' vines (data not shown). Therefore, only the main effects are reported here (bark grafting treatments and cultivars) and shown in Figure 5.12. There was a significant main effect of bark grafting treatments (*P*=0.05) on the mean final total length (mm) of sylleptic axillary shoots (Figure 5.12A). The mean total length (mm) of sylleptic axillary shoots was significantly reduced in vines grafted with inverted bark orientation compared to control vines (Figure 5.12A). The main effect of the insertion bark of cultivars (G3, G9 and G14) was not significant (*P*=0.58) for the mean final total length (mm) of sylleptic axillary shoots (Figure 5.12B). The main effect of bark grafting treatments was significant for the mean total node number

of sylleptic axillary shoots (*P*=0.05). The mean total node number of vines grafted in inverted orientation was significantly reduced compared with control vines (Figure 5.12C). The reduction in mean total node number in vines grafted with inverted orientation was almost 38% lower than control vines. The insertion of bark of G3, G9 and G14 cultivars had no significant effect (*P*=0.76) on the final mean total node number of sylleptic axillary shoots (Figure 5.12D). Therefore, the reduction in the mean total length of sylleptic axillary shoots in vines grafted in an inverted orientation (Figure 5.12A) mainly resulted from a reduction in the mean total node number (Figure 5.12C), whereas the insertions of bark of different cultivars did not affect the mean final total length nor the mean final total node number of sylleptic axillary shoots (Figure 5.12B and D).

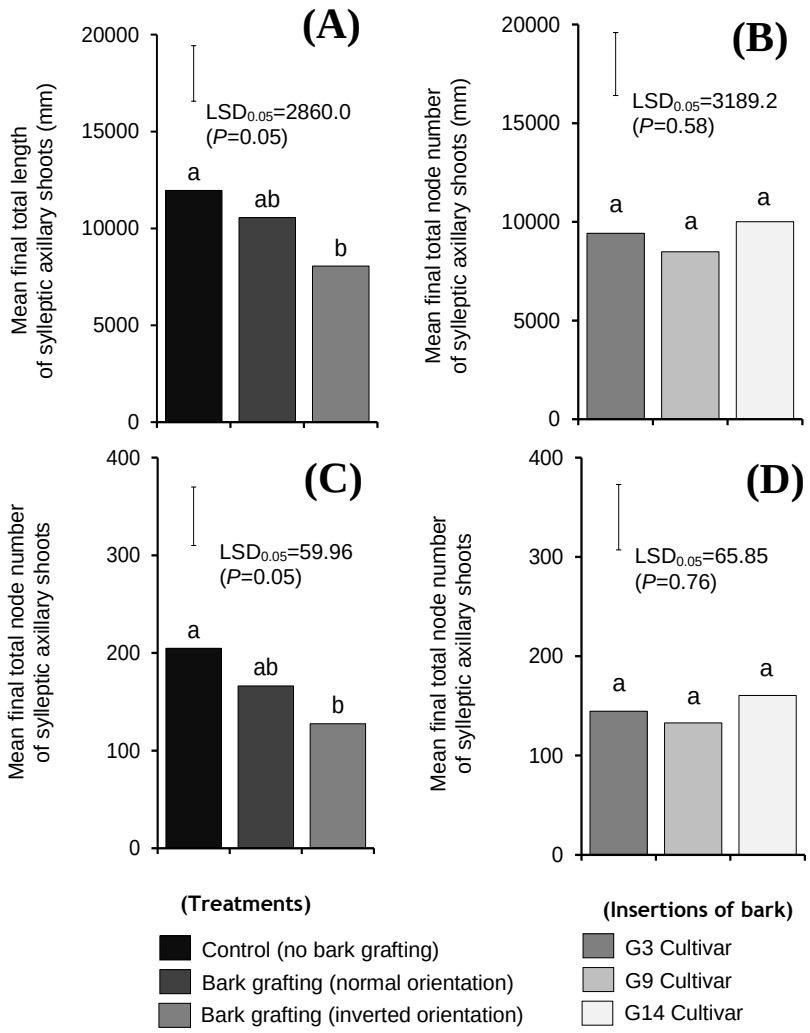


Figure 5.12. Main effects of bark grafting treatment and insertion of cultivars on the mean final total length (Panel A and B) and mean total node number (Panel C and D) of sylleptic axillary shoots (mm) of young 'Hort16A' vines. Means sharing same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. Bars denote the LSD at *P*=0.05.

5.3.2.4 Effect of bark grafting treatment and insertion of bark of various cultivars (G3, G9 and G14) on the characteristics of long sylleptic axillary shoots

The assessment on the characteristics of sylleptic axillary shoots could only be made on the long shoots due to small proportions of medium and short shoots that were found in this trial (data not shown). Only the characteristics of long shoots (i.e. length, node number, internode length and shoot cross-sectional area) are described here and the results are shown in Table 5.3. Overall, there were no significant interactions between bark grafting treatments x cultivars, or the main effects of bark grafting treatments and insertion of barks on the characteristic of long sylleptic axillary shoots (Table 5.3).

Table 5.3. Effects of bark grafting on the characteristics of long sylleptic axillary shoots (length, node number, internode length and shoot cross-sectional area) of young 'Hort16A' vines.

Main effects	Mean shoot length (mm)	Mean node number	Mean internode length (mm)	Mean shoot cross-sectional area (mm^2)
	Long sylleptic axillary shoots			
Bark grafting treatments (T)				
Control *(no bark grafting)*	2555.6 [a]	61.2 [a]	41.7 [a]	286.2 [a]
Bark grafting *(normal orientation)*	2618.3 [a]	60.8 [a]	43.3 [a]	295.1 [a]
Bark grafting *(inverted orientation)*	2418.6 [a]	59.4 [a]	40.8 [a]	294.7 [a]
$LSD_{0.05}$	420.8	4.4	6.0	50.4
P-value	*P*=0.32	*P*=0.48	*P*=0.38	*P*=0.93
Insertion of barks (IB)				
G3	2636.0 [a]	60.2 [a]	43.5 [a]	295.3 [a]
G9	2349.5 [a]	60.4 [a]	39.4 [a]	282.1 [a]
G14	2581.4 [a]	59.6 [a]	43.5 [a]	309.2 [a]
$LSD_{0.05}$	467.4	4.9	6.7	56.0
P-value	*P*=0.43	*P*=0.87	*P*=0.34	*P*=0.60
Interactions (T x IB)	*P*=0.81	*P*=0.40	*P*=0.93	*P*=0.60

Means sharing same letters within a column are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
The characteristics of short and medium shoots are not reported here due insufficient samples.

5.3.2.5 Effect bark grafting treatment and insertion of bark of various cultivars (G3, G9 and G14) on the leaf area of long shoots and total leaf area of sylleptic axillary shoots (long, medium and short shoots)

5.3.2.5.1 Mean leaf area of long sylleptic axillary shoots

Due to the reasons stated in the Section 5.3.2.4, only leaf area (cm^2) of long sylleptic axillary shoots is reported here. There was no significant interaction (*P*=0.26) between bark grafting treatments and cultivars on the mean leaf area (cm^2) of long sylleptic axillary shoots (Table 5.4). For the main effects, the mean leaf area of long sylleptic axillary shoots was significantly affected (*P*=0.001) by the bark grafting treatments (Table 5.4). The mean leaf area (cm^2) of long sylleptic axillary shoots in vines with bark grafted in normal orientation was significantly higher compared to the control and vines grafted with bark in an inverted orientation (Table 5.4). The insertion of bark of various cultivars (G3, G9 and G14) also had significant effect (*P*=0.04) on the mean leaf area (cm^2) of long sylleptic axillary shoots. Vines with the insertion of bark from G9 cultivar had significant lower mean leaf area (2677.1 cm^2), and vines with the insertion of bark from G3 and G14 cultivars had almost comparable mean leaf area with 3340.9 and 3363.5 cm^2, respectively.

5.3.2.5.2 Mean total leaf area of sylleptic axillary shoots

There were no significant cultivars or bark grafting treatments x cultivars interactions (*P*=0.64) on the mean total leaf area per vine of the sylleptic axillary shoots (Table 5.4). However, there was a significant main effect (*P*=0.03) of bark grafting treatments. Regardless of orientation, the bark grafting treatments significantly reduced the mean total leaf area (cm^2) of sylleptic axillary shoots. However, no significant difference was recorded (*P*=0.50) on the insertions of bark of various cultivars (G3, G9 and G14) on the mean total leaf area of sylleptic axillary shoots (long, medium and short shoots) (Table 5.4).

Table 5.4. Effects of bark grafting on the final leaf area (cm^2) of long sylleptic axillary shoots and mean total leaf area (cm^2) per vine of young 'Hort16A' vines.

Main effects	Mean leaf area (cm^2) of long sylleptic axillary shoots	Mean total leaf area (cm^2) per vine[‡]
Bark grafting treatments (T)		
Control (*no bark grafting*)	2843.8 [b]	15653 [a]
Bark grafting (*normal orientation*)	3486.3 [a]	10682 [b]
Bark grafting (*inverted orientation*)	2672.6 [b]	9102 [b]
$LSD_{0.05}$	528.8	4405.6
P-value	*P*=0.001	*P*=0.03
Insertion of barks (IB)		
G3	3340.9 [a]	8779 [a]
G9	2677.1 [b]	9620 [a]
G14	3363.5 [a]	11514 [a]
$LSD_{0.05}$	585.8	4794.3
P-value	*P*=0.04	*P*=0.50
Interactions (T x IB)	*P*=0.26	*P*=0.64

Means sharing same letters within a column are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
[‡]Mean total leaf area (cm^2) for all sylleptic axillary shoot types (long, medium and short shoots).
The characteristics of short and medium shoots are not reported here due insufficient samples.

5.3.2.5.3 Leaf area of long sylleptic axillary shoots for the first 15 nodes

Nodes were number from the base and leaf size was measured and recorded up to the 15th node. In general, regardless of the insertions of bark from different cultivars, the individual leaf area of long sylleptic axillary shoots (cm^2) starting from the 1st node until 15th node was smaller for the vines grafted with bark in inverted orientation (Figure 5.13) regardless of insertions of bark of various cultivars (G3, G9 and G14). The leaf area gradually increased starting from 1st node until approximately 4th or 5th node, and thereafter progressively decreased until 15th node. In addition, the vines grafted with bark of G9 (Figure 5.13B) and G14 (Figure 5.13C) cultivars in inverted orientation had a clear reduction in individual leaf area (cm^2) compared to vines grafted with bark of G3 in similar orientation (Figure 5.13A). Nevertheless, the individual leaf area of long sylleptic axillary shoots for the first 15 nodes from vines grafted with bark of G3 cultivar in inverted orientation was still considerably smaller compared with vines grafted in normal orientation (Figure 5.13A).

There was no significant difference (*P*=0.25) on the mean total leaf area of long sylleptic axillary shoots for the first 15 nodes between normal and inverted orientation in vines grafted with bark from G3 cultivar (Appendix 8). There was a trend (*P*=0.15) that the mean total leaf area of long sylleptic axillary shoots for the first 15 nodes may have been reduced with the insertion of bark of G14 cultivar in inverted orientation (Appendix 8). However, the mean total leaf area of long sylleptic axillary shoots for the first 15 nodes was significantly reduced (*P*=0.004) in the vines with bark grafted from G9 cultivar in an inverted orientation (Appendix 8). Overall, the mean total leaf area of long sylleptic axillary shoots for the first 15 nodes may have been slightly reduced in vines grafted with bark in inverted orientation especially from the insertion of bark from G9 cultivar (Figure 5.13B and Appendix 8).

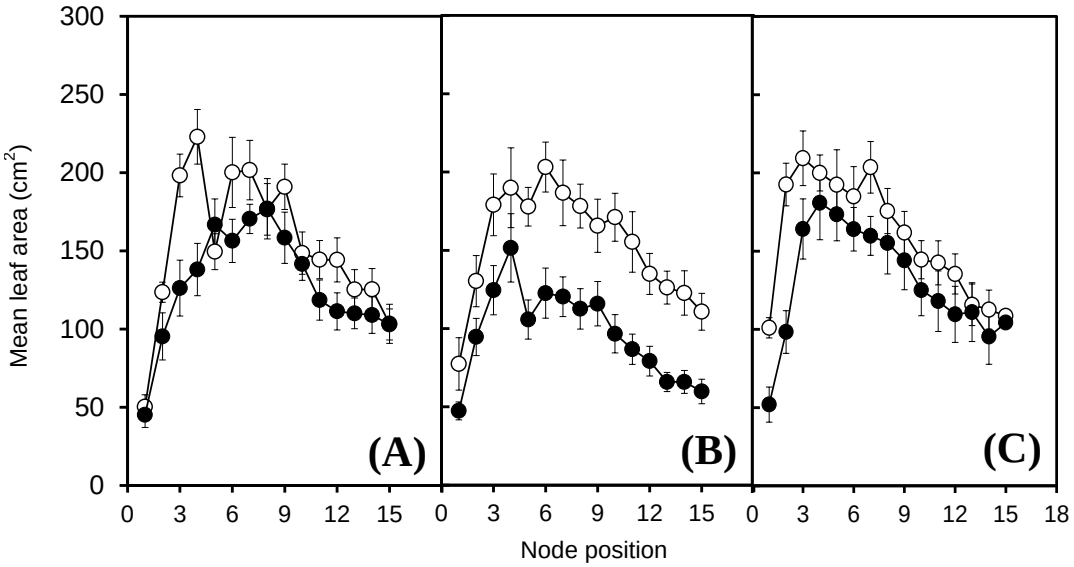


Figure 5.13. Mean leaf area as a function of node position of long sylleptic axillary shoots of young 'Hort16A' vines (cm^2) at the end of growing season (April 2012), means value (± standard error). (A) Bark grafting from G3 cultivar, (B) bark grafting from G9 cultivar, and (C) bark grafting from G14 cultivar. Symbols indicate, bark grafting-normal orientation (○), and bark grafting-inverted orientation (●). Nodes are numbered from the shoot base and *n*=6-9 for each node position.

5.3.2.6 The relationship between final shoot length and node number of sylleptic axillary shoots

Figure 5.14 shows the relationship between the final shoot length (mm) and node number of sylleptic axillary shoot of 'Hort16A' vines that have been bark grafted from other kiwifruit cultivars (G3, G9 and G14). Similar to Section 5.3.1.5, the data for shoot length and node number of all type of shoots were pooled together in the one graph. All the bark grafting treatments and cultivars showed an almost identical relationship between final shoot length and node number of sylleptic axillary shoots (Figure 5.14). There was a strong linear positive relationship (R^2>0.80) between final shoot length and node number of long and medium sylleptic axillary shoots for all bark grafting treatments and insertion of bark from different cultivars (Figure 5.14, large graphs). For short sylleptic axillary shoots, unfortunately, correlations between final shoot length and node number for bark grafting of G9 cultivar could not be made due to unavailability of shoots. For bark grafting of G3 and G14 cultivars, there was a strong positive quadratic relationship between final shoot length and node number of short sylleptic axillary shoots with R^2=0.83 and R^2=0.91, respectively (Figure 5.14, small graphs).

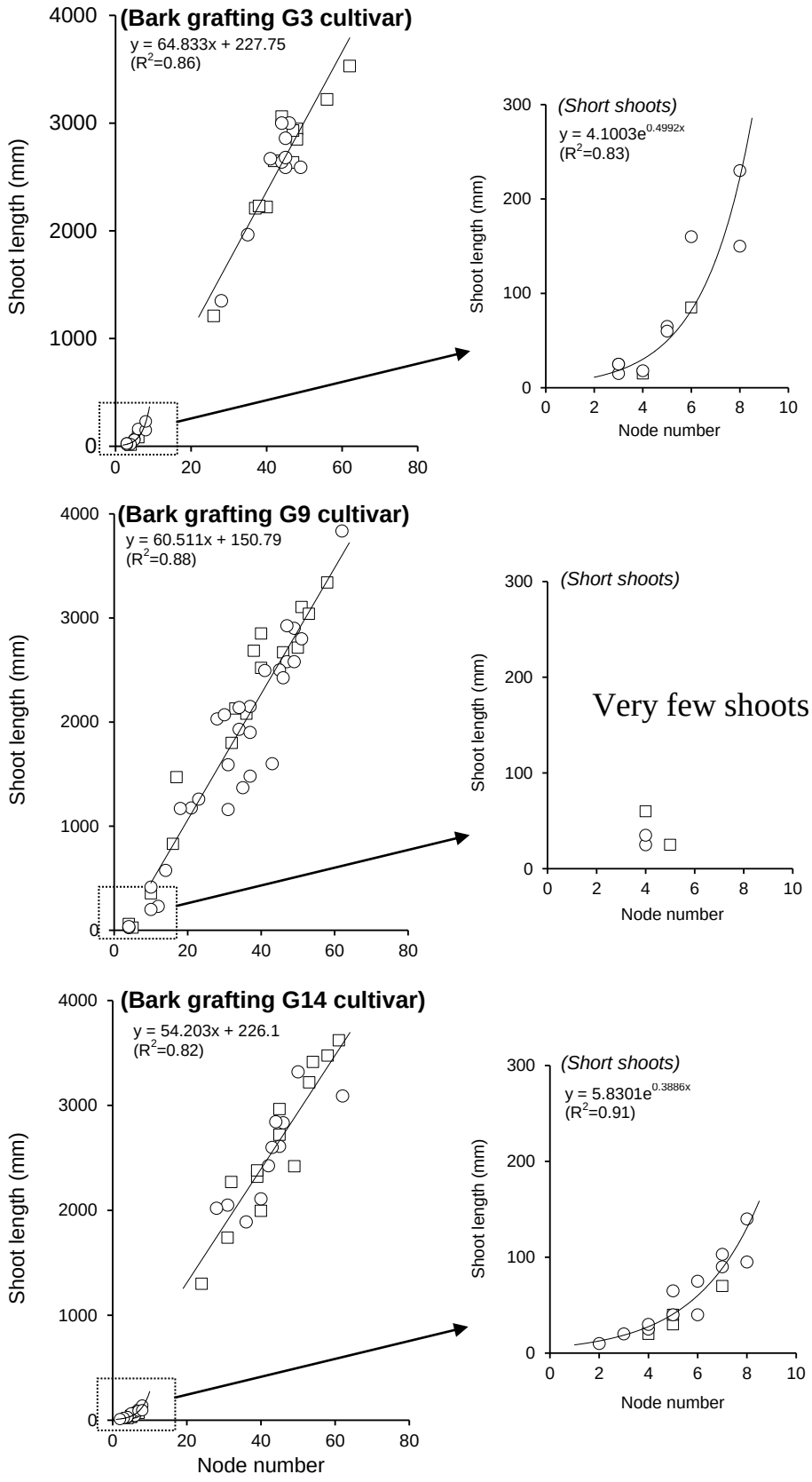


Figure 5.14. The relationship between the final length and node number of young 'Hort16A' vines at the end of summer season. Symbols indicate; (□) bark grafting in normal orientation and (○) bark grafting in inverted orientation. The length and node number of long, medium and short shoots for each treatment were pooled together in the same graph (large graphs).

5.3.2.7 Effects of bark grafting and insertion of bark of various cultivar (G3, G9 and G14) on the total number of sylleptic axillary shoots (i.e. branching).

There was no significant interaction effect (*P*=0.30) between bark grafting treatments and insertion of cultivars on the mean total number of sylleptic axillary shoots (Table 5.5). Similarly, the main effects of bark grafting treatments and insertions of bark were not significant (*P*=0.13 and P=0.14, respectively) for the mean total number of sylleptic axillary shoots of bark grafting vines (Table 5.5).

5.3.2.8 Effects of bark grafting and insertion of bark of various cultivar (G3, G9 and G14) on the spring bud break of young 'Hort16A' vines.

In the following spring, the percentage of bud break (%) was calculated by dividing the buds that broke with the node number. Noted here, the shoots that produced from the buds are proleptic shoots because the buds had a resting winter period. No significant interaction was found (*P*=0.50) between the bark grafting treatments and the insertions of bark of various cultivars on the mean percentage of spring bud break (Table 5.5). The main effect of bark grafting treatment was not significant (*P*=0.13) for the mean percentage of spring bud break (Table 5.5). However, there was a trend that the mean spring bud break (%) of vines was affected (*P*=0.06) by the insertion of bark of different cultivars (G3, G9 and G14) (Table 5.5). The mean bud break of 'Hort16A' vines was significantly reduced in the vines with the insertions of bark of G3 compared to vines grafted with bark of G14 cultivar.

Table 5.5. Effect of insertions of bark of various cultivars (G3, G9 and G14) on the total number of sylleptic axillary shoots (i.e. branching) and the percentage of spring bud break (%) of young 'Hort16A' vines.

Main effects	Mean total number of sylleptic axillary shoots	Mean percentage of spring bud break[ǂ] (%)
Bark grafting treatments (T)		
Control (*no bark grafting*)	6.6 [a]	56.0 [a]
Bark grafting (*normal orientation*)	4.7 [a]	56.6 [a]
Bark grafting (*inverted orientation*)	4.7 [a]	50.5 [a]
$LSD_{0.05}$	2.0	8.5
P-value	*P*=0.13	*P*=0.13
Insertions of bark (IB)		
G3	3.7 [a]	46.8 [b]
G9	4.6 [a]	54.7 [ab]
G14	5.7 [a]	57.8 [a]
$LSD_{0.05}$	2.3	9.3
P-value	*P*=0.14	*P*=0.06
Interactions (T x IB)	*P*=0.30	*P*=0.50

Means sharing same letters within a column are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test.
[ǂ]Buds that broke to form proleptic axillary shoots.

5.3.3 Growth Manipulation 3 (*GM*3)

Over a period of two years, the fruit characteristics from bark grafting and girdling were analysed using Analysis of Covariance (ANCOVA) using fruit numbers as the covariate. The data were analysed separately for both harvesting years (See Section 5.2.3.2).

5.3.3.1 Bark grafting effects on the fruit fresh and dry weight, and dry matter concentration (DMC) during the first harvesting year.

In the first harvesting year, for the main effect of treatments, the mean fruit FW (g) of kiwifruit was significantly different (P=0.01) between vines with bark grafting treatments and girdling (Table 5.6). Inverted bark grafting and girdling treatments significantly increased mean fruit FW (g) compared to vines with bark grafted in normal orientation. However, no significant difference was found between treatments applied in early and late summer season (P=0.39). Similarly, no significant interactions (P=0.67) were found between treatments and seasons on the mean fruit FW (g) of kiwifruit (Table 5.6). There was a trend that bark grafting treatment may have influenced the mean fruit DW (g) (P=0.10), while the difference between treatments applied in early and late summer only approached significance (P=0.05). Nevertheless, the mean separation analysis showed that the mean fruit DW (g) of kiwifruit was significantly lower in vines grafted with bark in normal orientation compared to the other treatments (Table 5.6). In addition, no significant interaction was found (P=0.26) between treatments and time of year on the mean fruit DW (g) of kiwifruit. The mean DMC of fruits did not significantly differ (P=0.74) between bark grafting and girdling vines. However, there was a significant time of year effect (P=0.04) on the mean DMC of fruits, with fruits from treatment conducted in early season significantly higher than late season (Table 5.6). However, no significant interactions were found (P=0.73) between bark grafting treatments and time of year on the mean DMC of fruits.

5.3.3.2 Bark grafting effects on the fruit fresh and dry weight, and dry matter concentration (DMC) during the second harvesting year.

There were no significant treatment effects (P=0.19) or treatment x time of year interactions (P=0.64) on the mean fruit FW (g) in the second following year. However, there was a noticeable significant main effect of time of year (P=0.05) on the mean fruit FW (g). Treatment applied in early summer season had significantly greater mean fruit FW (g) compared to vines treated in late summer. There were no significance of either bark grafting treatment (P=0.90) or time of year effect (P=0.58) on the mean fruit DW (g). Similarly, there were no significant interactions (P=0.89) between treatments and time of year on the mean fruit DW (g). The mean DMC of fruits was not significantly affected by the bark grafting treatment, but there was a trend close to significant (P=0.10) that the treatment applied in the first year (early and late summer) may have an influence on the mean DMC of kiwifruit in the current year (Table 5.6). However, there was no significant bark grafting treatment x time of year interactions (P=0.59) on the mean DMC of fruits.

Table 5.6. Effect of bark grafting (normal and inverted orientation) and girdling on the mean fruit fresh weight (g), dry weight (g) and dry matter concentration (DMC) of 'Hayward' kiwifruit in the first and second harvesting year.

Main effects	Mean fruit fresh weight (FW) (g)		Mean fruit dry weight (DW) (g)		Dry matter concentration (DMC) (%)	
	First Year[ǂ]	Second Year[ǂ]	First Year[ǂ]	Second Year[ǂ]	First Year[ǂ]	Second Year[ǂ]
Treatment						
Bark grafting (*inverted orientation*)	109.7 a[x]	95.8 a	18.3 a	17.1 a	16.3 a	16.8 a
Bark grafting (*normal orientation*)	100.4 b	98.7 a	16.0 b	17.2 a	16.3 a	16.7 a
Girdling	110.3 a	103.3 a	18.0 a	16.9 a	16.0 a	16.3 a
Time of year						
Early summer	105.4 a	103.4 a	16.8 b	17.2 a	16.8 a	17.0 a
Late summer	108.1 a	95.3 b	18.1 a	16.9 a	15.6 b	16.2 a
Treatment	**	ns	ns (P=0.10)	ns	ns	ns
Time of year	ns	*	*	ns	*	ns (P=0.10)
Treatment x Time of year	ns	ns	ns	ns	ns	ns

ns, *, **, *** non-significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively according to ANOVA *F-test*.
[x]Means sharing same letters within a column are not significantly different at P=0.05 according to Tukey's (HSD) test.
[ǂ]Year of harvesting.

5.3.3.3 Bark grafting effects on the fruit length, diameter and size in the first harvesting year.

Dimensional measurements were made using digital callipers (Mitotuyo, Digimatic, Japan) to estimate the size of kiwifruit from the treated vines. Briefly, the length of fruits was measured from the top to the base of the fruits (L), and two measurements were made for width of fruits, maximum (W_1) and minimum width (W_2). Estimated fruit size was made by multiplying L x W_1 x W_2 (see Section 5.2.2.3 for explanation).

There was a significant treatment effect (*P*=0.02) on the mean fruit length (mm). However, no significance were recorded on time of year (*P*=0.97) and treatments x time of year interaction (*P*=0.56) for the mean fruit length (mm) of kiwifruit (Table 5.7). The mean fruit diameter either maximum (W_1) or minimum (W_2) was significantly affected by the treatments (both, *P*=0.0008). The fruits from vines grafted with bark in inverted orientation and girdling were significantly larger in diameter compared to fruits from vines grafted in normal orientation (Table 5.7). However, the main effect of time of year were not significant for the mean maximum (W_1) and minimum (W_2) fruit diameter (*P*=0.86 and *P*=0.93, respectively). Similarly, no significance in the treatments x time of year interactions were found on the mean maximum (W_1) and minimum (W_2) diameter of fruits (*P*=0.45 and *P*=0.13, respectively). There was a strong trend (*P*=0.06) that the mean fruit size (L x W_1 x W_2) was affected by the bark grafting and girdling treatments (Table 5.7). The mean fruit size from vines grafted with inverted orientation produced fruit nearly four to ten percent larger compared to the other treatments. However, there were no significant time of year effects (*P*=0.91) or treatments x time of year interactions (*P*=0.46) on the mean fruit size of kiwifruit (Table 5.7).

5.3.3.4 Bark grafting effects on the fruit length, diameter and size in the second harvesting year.

There were no significant treatment (*P*=0.37) and time of year effects (*P*=0.29), or treatment x time of year interactions (*P*=0.57) on the mean fruit length (mm) of kiwifruit (Table 5.7). Similarly, no significant interactions between treatment and time of year were found on the mean maximum (W_1), minimum (W_2) fruit diameter and mean fruit size (L x W_1 x W_2) (*P*=0.66, *P*=0.15 and *P*=0.59, respectively). Treatment effects were only significant for mean fruit length (*P*=0.02), mean W_1 (*P*=0.02) and mean W_2 (*P*=0.01), as well as mean fruit size (*P*=0.06) the in the first harvesting year, but not in the second harvesting year (Table 5.7). The only significant effects were a reduction in fruit diameter (*P*=0.02 and *P*=0.001), by grafting or girdling late in the summer season. There was also a trend (*P*=0.10) that overall mean fruit size (L xW_1xW_2) was reduced in the late summer (Table 5.7).

Table 5.7. Effect of bark grafting (normal and inverted orientation) and girdling on the mean fruit length (mm), fruit diameter (mm) and fruit size of 'Hayward' kiwifruit in the first and second harvesting year.

Main effects	Mean fruit length-L (mm)		Mean fruit diameter-W_1 (mm)		Mean fruit diameter-W_2 (mm)		Mean fruit size (LxW_1xW_2)	
	First Year[ǂ]	Second Year[ǂ]	First Year[ǂ]	Second Year[ǂ]	First Year[ǂ]	Second Year[ǂ]	First Year[ǂ]	Second Year[ǂ]
Treatment								
Bark grafting (*inverted orientation*)	64.9 a[x]	58.7 a	54.3 a	54.0 a	50.3 ab	48.5 a	182375 a	156253 a
Bark grafting (*normal orientation*)	61.0 b	60.1 a	52.8 b	54.2 a	49.7 b	48.9 a	166067 a	160698 a
Girdling	63.7 ab	60.8 a	54.6 a	55.3 a	51.3 a	49.6 a	176380 a	167667 a
Time of year								
Early summer	63.3 a	60.7 a	53.9 a	55.4 a	50.4 a	49.9 a	175556 a	168853 a
Late summer	63.1 a	59.0 a	54.0 a	53.4 b	50.5 a	48.1 b	176325 a	154225 a
Treatment	*	ns	**	ns	**	ns	ns (P=0.06)	ns
Time of year	ns	ns	ns	*	ns	***	ns	ns (P=0.10)
Treatment x Time of year	ns	ns	ns	ns	ns	ns	ns	ns

ns, *, **, *** non-significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively according to two-ways ANOVA *F-test*.
[x]Means sharing same letters within a column are not significantly different at P=0.05 according to Tukey's (HSD) test.
[ǂ]Year of harvesting.

5.3.3.5 Bark grafting and girdling effects on fruit weight distribution.

The fruit weight (g) distribution from each treatment (bark grafting and girdling) and season (early and late summer) for the first and second harvesting year were compared (Figures 5.15 to 5.18). The distributions of the fruit weight were analysed using Kolmogorov-Smirnov at the 95% significance level, following a previous study by Woodward (2006) on the same cultivar. For the fruits from the vines treated with bark grafting or girdling in early (Figure 5.15A, B and C) and late summer (Figures 5.16A and C) in the first harvesting season, the majority of fruit weight distribution could be approximated by a normal distribution (P>0.05), except for the distribution of fruits from vines grafted in normal orientation in late summer (Figure 5.16B) where the P-value was significant (P=0.03). In the second harvesting season, distributions of fruit weight also approximated to the normal distribution (P>0.05) for all the treatments, even though the distributions of fruit weight from few treatments had shown skewedness (for example Figures 5.17C, 5.18A and B). In order to support results in Figure 5.15 until Figure 5.18, assessment of the skewedness of fruit weight distribution was conducted using box-plot whisker as shown in Appendix 9.

In summary, girdling and bark grafting treatment in an inverted orientation tended to produce higher mean fruit weight (g) compared to treatment with bark grafting in normal orientation. However, the treatment effects were only observed in the first harvesting year and less profound effects were observed in the second harvesting year.

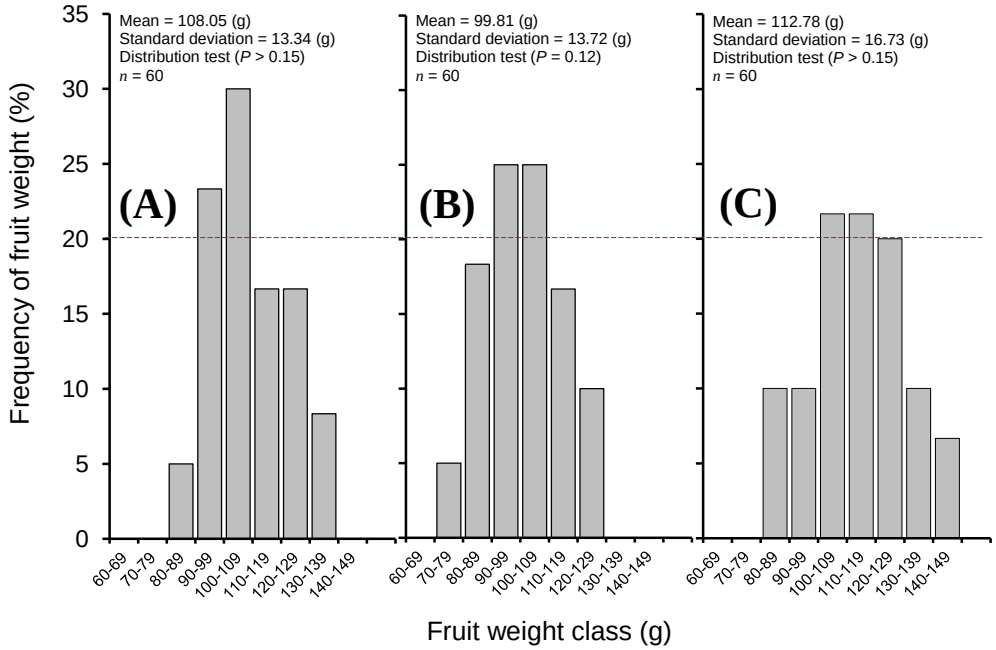


Figure 5.15. Frequency of fruit weight distribution (%) from the treatments; (A) Bark grafting – inverted orientation, (B) Bark grafting – normal orientation, and (C) girdling conducted in the first harvesting year (early summer, December).

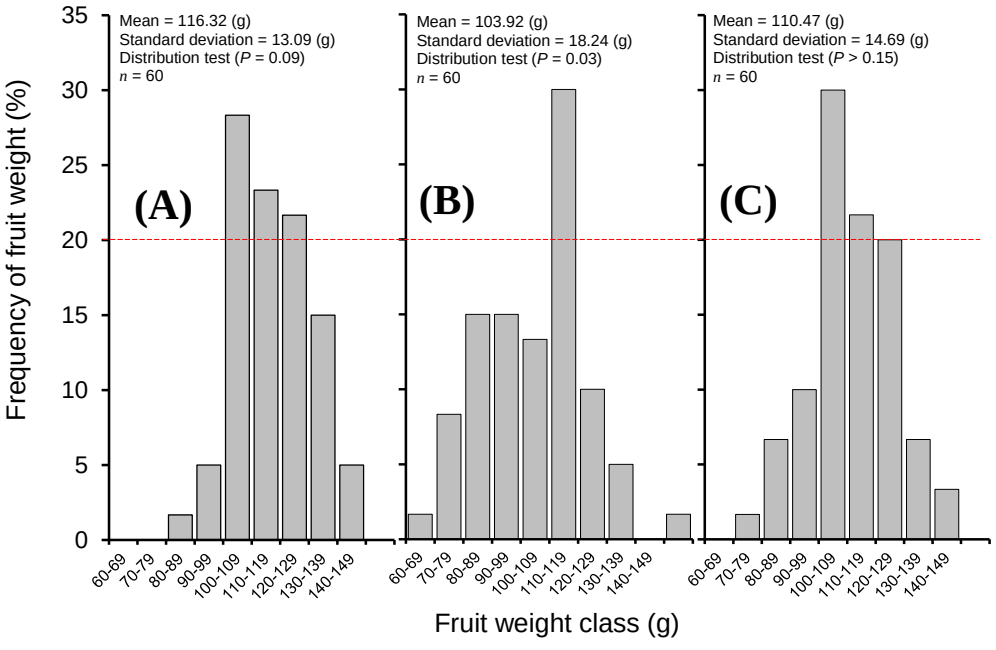


Figure 5.16. Frequency of fruit weight distribution (%) from the treatments; (A) Bark grafting – inverted orientation, (B) Bark grafting – normal orientation, and (C) girdling conducted in the first harvesting year (late summer, February).

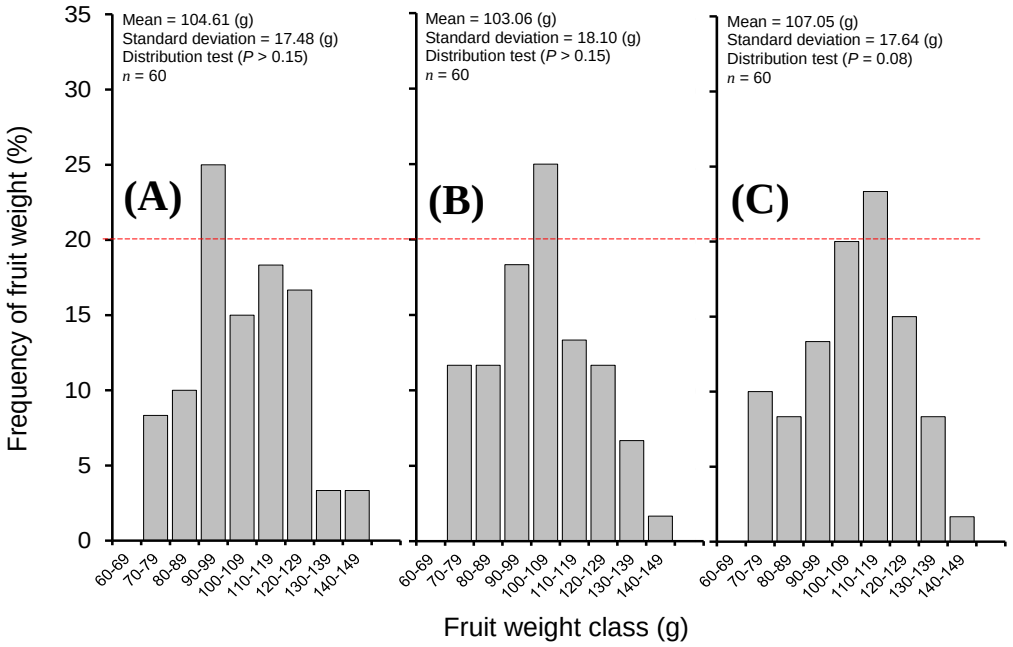


Figure 5.17. Frequency of fruit weight distribution (%) from the treatments; (A) Bark grafting – inverted orientation, (B) Bark grafting – normal orientation, and (C) girdling conducted in the second harvesting year (early summer, December).

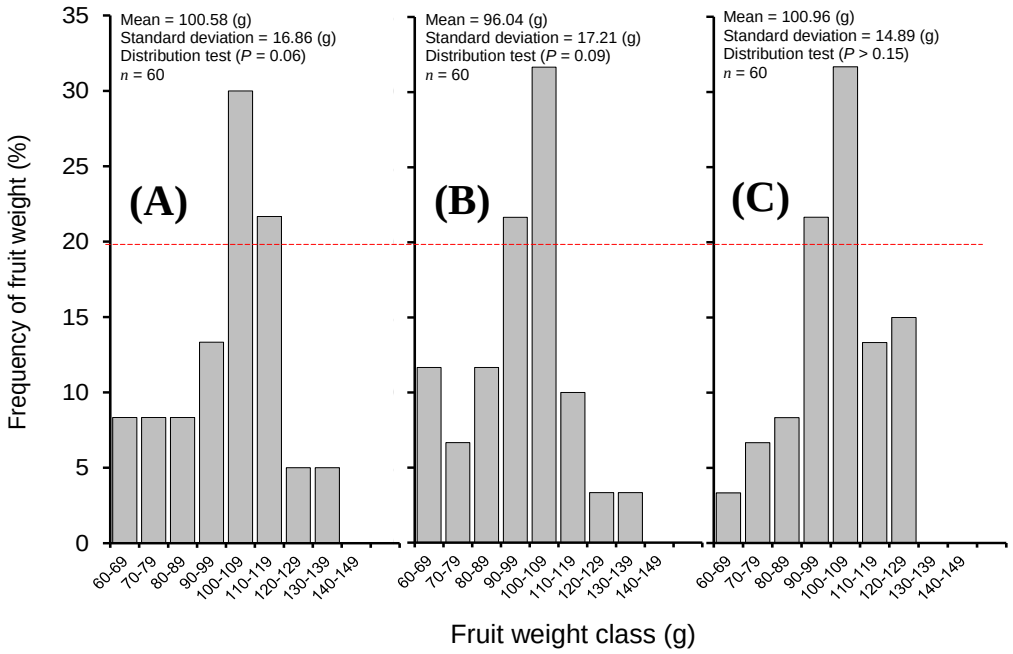


Figure 5.18. Frequency of fruit weight distribution (%) from the treatments; (A) Bark grafting – inverted orientation, (B) Bark grafting – normal orientation, and (C) girdling conducted in the second harvesting year (late summer, February).

5.4 Discussion

The first objective of these experiments was to evaluate the changes in vegetative growth and shoot architecture of young kiwifruit vines by bark grafting in different orientations. In kiwifruit, little is known about the hormonal mechanisms that regulate vigour of the vines. Therefore, it is worth studying the responses of kiwifruit vines to the inhibition of auxin transport by using bark grafting. Further objective of this study was to evaluate bark grafting as a vigour manipulation technique and also to improve physiological understanding on the responses of young kiwifruit vines to the restriction of auxin transport. Besides that, bark grafting was also evaluated as a technique to regulate fruit characteristics and quality in mature kiwifruit vines.

The idea for this study came from the observation made by previous researchers who found that grafting inverted ring of barks (i.e. bark grafting) on the main tree stem, reduced the growth and vigour of a few fruit crops, such as apples (Lockhard & Schneider, 1981; Poniedziałek et al., 2000; Sax, 1954; Sax & Dickson, 1956), as well as regulating fruit growth (Arakawa et al., 1998; Brase & Way, 1959; Gastol & Poniedzialek, 2007; Poniedziałek et al., 2000), in avocado (Köhne & Schutte, 1993) and mango (Gaskin, 1963). However, this technique is not widely used as compared to dwarfing rootstock and interstock, or chemical applications (e.g. prohexadione-Ca and paclobutrazol) due to several reasons. For example, several authors found that bark grafting may cause severe damages to the tree as reported in apple (Brase & Way, 1959; Lockhard & Schneider, 1981) and mango (Gaskin, 1963). This could be the reason why this technique is not practically used as compared to girdling; the procedure is also more time consuming. In this study, nevertheless, the kiwifruit vines that were treated with bark grafting have shown interesting growth effects and appeared healthy in the next growing season.

The concept of hormonal control of plant growth has been widely accepted. Plant hormones such as auxin (IAA), cytokinin (CK) and gibberellins (GA) are the major endogenous hormones that are involved in the mechanisms of controlling the plant growth. Alterations of these hormones may alter the coordination between the shoot and root system of plants. As noted in many studies, the polarity of auxin translocations may

be a primary factor in controlling plant growth (review by Teale et al., 2006). In the shoot system, auxin is transported from cell to cell, and indole-3-acetic acid (IAA) is the predominant naturally occurring auxin that moves basipetally from the shoot apex (i.e. shoot tip) to the base (Lomax et al., 1995). Since the polarity of cells can be retained regardless of orientations (Antoszewski et al., 1978; Sheldrake, 1973) by reversing the polarity of cells, the translocation of IAA in cells may be reduced and subsequently may alter vigour (Lockhard & Schneider, 1981; Sax, 1954; van Hooijdonk, 2009) and fruit growth (Brenner et al., 1989; Goren et al., 2004; Ozga & Reinecke, 2003) of fruit tree crops.

5.4.1 Growth Manipulation 1 and 2

5.4.1.1 The indication of reduction in auxin transport

5.4.1.1.1 Release of axillary buds or new shoot growth below bark graft-union

In the *GM*1, a ring of phloem bark was grafted back onto the main stem in either normal or inverted orientation. Early observations showed that the development of axillary buds outgrowth occurred at nodes below the graft-union area after three weeks following bark grafting treatments regardless of bark orientations (Appendix 6). A similar response was also found in the *GM*2, where all the active axillary buds below the bark graft-union area were broken and developed into sylleptic axillary shoots (Figure 5.12A). Other studies have also reported similar evidence of restriction of auxin transport either by bark grafting (Dickson & Samuels, 1956; Sax, 1954) or by using the auxin transport inhibitor, 1-N-Naphthylphthalamic acid (NPA) (Domagalska & Leyser, 2011; van Hooijdonk, 2009; Vattiprolu, 2012). In a study with 'Hort16A' kiwifruit cultivar, application of NPA to the stem portion below the first node of the primary shoots had stimulated the axillary bud below the application site (Vattiprolu, 2012). Similar results were also obtained on the grafted apple trees when NPA was applied at the junction of graft-union (van Hooijdonk, 2009). Inhibition of auxin transport on the stem of young citrus trees by girdling may also cause the development of axillary buds below the girdled area (Cimò et al., 2013). Nevertheless, in the present study, all the axillary outgrowth below the bark graft-union site were removed, because it may affect

the growth of upper shoots if were allowed to develop, since IAA is believed to be synthesized in young growing regions especially in the shoot apex and young expanding leaf (Ljung et al., 2001). In our study, therefore, it is suggested that the basipetal transport of IAA had been restricted and did not pass through in sufficient amount when the bark was grafted in an inverted orientation, as previously suggested (Lockhard & Schneider, 1981). This response also supports the concept of release of apical dominance in plants, since application of auxin transport inhibitors may also lead the bud outgrowth at nodes below the application site (reviewed by Domagalska & Leyser, 2011). Further supporting evidence was that the amount of pruning weight of these axillary buds outgrowth was significantly greater in the vines with bark grafting in inverted orientation than normal orientation (Appendix 7). Therefore, it would be reasonable to suggest that the basipetal transport of IAA was more restricted in inverted bark grafting than normal orientation. As noted in previous studies, the bark of phloem cells can retain their original polarity regardless of their grafted position (Antoszewski et al., 1978; Sheldrake, 1973; Thair & Steeves, 1976). Therefore, by inverting the ring of barks had caused the phloem cells to be re-orientated, thus impeding the downward basipetal transport of IAA.

5.4.1.1.2 The swelling of stems above the bark graft-union

Further evidence to support the suggestion that auxin was restricted is the swelling of bark above the graft-union site in both the growth manipulation (Figure 5.5 and Figure 5.10). The swelling of the stem was more apparent in the vines treated with inverted bark (Figure 5.5A and Figure 5.10B) and was not clearly observed in the vines treated with bark grafting in normal orientation (Figure 5.5B and Figure 5.10C). This feature of stem swelling may be the result of accumulation of IAA above the bark-graft site. Similar evidence was also reported in apple trees when a ring of bark was grafted back to main stem in inverted orientation (Lockhard & Schneider, 1981; Sax, 1954). The authors suggested that the swelling above the bark graft-union were due to the accumulation of IAA, including nutrients and carbohydrate compounds (Dickson & Samuels, 1956; Lockhard & Schneider, 1981; Sax, 1954). In a previous study on 'Baldwin' apple trees that have been dwarfed by inverting two rings of bark, Dickson and Samuels (1956) found higher concentration of radioactive isotope phosphorus accumulated above the inverted bark-graft union, and the concentration was remarkably reduced below the graft-union. They concluded that the flow of organic nutrients was

significantly retarded by the inverted ring of bark. Recent study in *Arabidopsis* by Ferguson and Beveridge (2009) also has demonstrated that stem-swelling is due to the accumulation of IAA when the stem was girdled. Nevertheless, in the present study, no hormonal measurements were conducted to identify the level of IAA on the bark-grafted vines. Therefore, it would be interesting to study the level of endogenous IAA in the bark grafting kiwifruit vines and this could help to identify whether swelling above the bark grafting, particularly in inverted orientation was due to the accumulation of IAA.

5.4.1.2 Effect of bark grafting on the characteristics of sylleptic axillary shoots

An objective for grafting the ring of barks in an inverted orientation was to reduce the transport of IAA from the shoot to root system, which may reduce the vigour of kiwifruit shoots. In *GM*1, only one single ring of bark was removed and grafted back to main stem either in normal or inverted orientation (Figure 5.2). Even though the reduction in growth and vigour by bark grafting was very limited, nevertheless some interesting findings were discovered in this study. In general, the bark grafting did not significantly reduce the length of long sylleptic axillary shoots (Figure 5.6A). However, there were trends that length, node number, internode length and CSA of long sylleptic axillary shoots were reduced (Figure 5.6). It was noted that reduction in the length of long shoots in bark grafting vines was due to reduction in node number (Figure 5.6A and B). For medium and short shoots, no significant effects of bark grafting were found on the characteristics of these shoots (Figure 5.7 and Figure 5.8). It seems that the bark grafting treatment (especially in inverted orientation) may have affected the characteristics of long sylleptic axillary shoots, but not medium and short sylleptic shoots.

Our findings indicate that the transport of IAA from the shoots would have been restricted by the bark grafting, particularly when the bark was grafted in an inverted orientation, presumably because the phloem cells had been re-oriented. The auxin (IAA) is one of the plant hormones that has been implicated in meristematic activity (Sachs, 1965; Sassi & Vernoux, 2013). In kiwifruit, long shoots were characterised as non-terminating shoot and had a number of neoformed nodes (Seleznyova et al., 2002). The long shoots of kiwifruit were also the shoots that were still actively growing with a

gradient of new leaves at shoot apex (Foster et al., 2007). Therefore, any alteration of IAA transport in the kiwifruit stems would be expected to affect the characteristics of long shoots rather than affecting medium and short shoots, as found in this study. In addition, the restriction of IAA by bark insert/grafting may influence the availability of IAA level in long shoots of kiwifruit, since the gradient of new expanding leaves can be found in this shoot, and may serve as a potential site of endogenous IAA (Ljung et al., 2001). Our results are also in agreement with the previous study on the similar kiwifruit cultivar that also found the length of primary shoots that were actively growing (i.e. long shoots) were reduced by the application NPA below the first node of shoots (Vattiprolu, 2012). In apple, the restriction of basipetal IAA transport from the shoot to root system reduced GA supplied from root to shoot, thereby decreasing primary neoformation of nodes and thus primary shoot length (van Hooijdonk, 2009).

In *GM*2, three rings of bark were taken from other cultivars (G3, G9 and G14) and then grafted to the main stem of young gold kiwifruit vines with different orientation similar to *GM*1 (Figure 5.2). The reason for grafting three rings of bark was to increase the dwarfing effect more permanently rather than using one single ring of bark. Furthermore, we could also evaluate whether there are any architectural modifications that might be induced by grafting of bark from other kiwifruit cultivars. In this part, assessments on the characteristics of sylleptic axillary shoots can only be made on the long shoots (Table 5.3), due to small proportions of the short and medium shoots from each treatment (data not shown) were found. The reason why there was a smaller proportion of medium and short shoots (i.e. terminated) compared to long shoots (i.e. non-terminated) is unknown, but may be due to interaction between IAA and CK in root system. It is worthy to note that restriction of IAA supply from shoot to root of kiwifruit may have cause transient built up of CK as reported by Currie (1997), which results in release of axillary buds to form new shoots. The new shoots with gradient of young developing leaves are believed to function as an important source of IAA, to keep the shoot apical meristem active and development into long non-terminated shoots. However, the main effects of bark grafting treatment and insertion of barks from other kiwifruit cultivars (G3, G9 and G14) did not affect the characteristics of long shoots (Table 5.3).

5.4.1.3 Effect of bark grafting on the total growth of sylleptic axillary shoots

In *GM*1, at the end of the growing season, no significant differences were recorded on the mean total length and node number of sylleptic axillary shoots between bark grafting and the control vines (Table 5.1), when only one single ring of bark was grafted back to main stem of kiwifruit in either normal or inverted orientation. Vattiprolu (2012) also did not find any reduction in mean total length of sylleptic axillary shoots when NPA was applied below the first node of main stem to restrict auxin transport. The reason for this may be due to the similarity of planting material used in both studies (i.e. tissues cultured plants), since tissue cultured plants are more vigorous than stem cuttings of kiwifruit (Loreti et al., 1991). Other reasons for the lack of significant reductions could be due to the regeneration of new phloem tissues under the bark graft (Lockhard & Schneider, 1981; Sax & Dickson, 1956), resulting in new translocation of normal polarity, since only 15mm in length of bark was taken and grafted back in either normal or inverted orientation.

In the *GM*2, three rings of bark were used, which means that the combined total length of grafted bark used is longer than in the *GM*1 (45 mm in total length). We also tested whether grafting a complete ring of bark from other cultivars may have an influence on the shoot architecture of 'Hort16A' kiwifruit similar as found in apple (Brase & Way, 1959; Dickson & Samuels, 1956; Lockhard & Schneider, 1981; Sax, 1954; Sax & Dickson, 1956) and peach (Mosse, 1960). When three barks were used, the monthly amount of shoots produced (i.e. in terms of total length of shoots) differed between bark grafting and the control (Figure 5.11). Similarly, a previous study also reported that the cumulative length of one year old 'Jonagold' apple tree was reduced when the ring of bark from M.9 dwarfing rootstock was bark grafted onto the main trunk (Poniedziałek et al., 2000). It was notable that the insertion of bark from other cultivars (G3, G9 and G14) may have an effect on the vigour of 'Hort16A' vines, particularly when grafted in an inverted orientation (Figures 5.11, 5.12 and Table 5.4). Grafting three rings of bark in an inverted orientation increased the dwarfing effect compared to the normal orientation, when G3 and G9 were used, but G14 was not as effective (Figure 5.11A and B). Therefore, there was an indication that the insertion of bark from some other cultivars may also have an influence on the overall production of sylleptic axillary shoots in kiwifruit (Figure 5.11). The reason for lack of significant interactions may be

due to insufficient replicates in *GM*2. Therefore, further study with a larger number of replicates is required to confirm this result. There was a noticeable difference in the results between trial in *GM*1 and *GM*2, since the length of barks used was also different. Therefore, the dwarfing effect can be increased by increasing the bark length with the use of three rings of barks, since the longer barks length used may result more dwarfing effect than shorter barks (Lockhard & Schneider, 1981). Hence, increasing bark length may also increase the dwarfing effect in the bark grafted kiwifruit vines. Similarly, as found in apple, the insertion of 20 cm of M 26 bark of composite 'Gravenstein' trees imparted more dwarfing effects than a 10 cm bark graft (Lockhard & Schneider, 1981).

Our results also have strengthen the previous hypothesis that the levels of IAA transport in the phloem tissues may be controlled by the genetic of the bark from the original plants, since the level of IAA transported in the phloem may be different between cultivars and species (Lockhard & Schneider, 1981). For example, dwarfing apple rootstocks had thicker bark than the bark from vigorous rootstocks (Beakbane, 1941). Besides that, the bark of dwarfing apple rootstocks contained a low level of IAA in the stem tissues (Rogers & Beakbane, 1957). Furthermore, the bark of dwarfing apple rootstocks also exhibited an increase ability to destroy IAA (Gur & Samish, 1968). Even though quantification of endogenous hormonal level was not carried out in this study, it is reasonable to suggest that the bark of the different kiwifruit cultivars (i.e. different genetic composition), may contain or possibly may control different amount of IAA passing basipetally through their phloem cells, since the insertions of barks from different cultivars had an effect on the total length and total node number of sylleptic axillary kiwifruit shoots (*GM*2), and this was not observed in *GM*1 (Figure 5.11).

5.4.1.4 Effect of bark grafting on leaf growth of sylleptic axillary shoots

In *GM*1, restriction of IAA transport by using a single bark insert, only imparted a small reduction in the mean total leaf area of young 'Hort16A' kiwifruit. However, the vines grafted with bark in inverted orientation had 20% reduced in mean total leaf area compared to control vines, possibly due to reduction in the mean total node number (Table 5.1). Interestingly, in *GM*2, with the presence of bark from other cultivars, the mean total leaf area of sylleptic axillary shoots of bark grafted vines was significantly

reduced compared to control vines (Table 5.4). The reduction of mean total leaf area in *GM*2 was approximately 42% and the reduction was almost double that found in *GM*1. We conducted a further assessment on the leaves of long sylleptic axillary shoots to identify whether the bark grafting treatment and insertion of barks from other cultivars may be affecting the individual leaf size along each node position followed the method by Clearwater et al. (2006). Unfortunately, only the leaf samples from long sylleptic axillary shoots available for further analysis. We found that the individual leaf size (cm^2) of the first 15 nodes of long sylleptic axillary shoots was smaller especially in the bark grafting vines in inverted orientation (Figure 5.13). In addition, the insertion of bark from cultivar G9 and G14 clearly showed the reduction in the individual leaf size compared to insertion of bark from G3 cultivar. Therefore, we believed that the reduction in the individual leaf size of each node (Figure 5.13) had contributed to the reduction in the mean total leaf area of long sylleptic axillary shoots for the first 15 nodes in the *GM*2 (Appendix 8). This effect may also have contributed to the reduction in overall leaf canopy of bark grafting vines. Our result is almost similar to the effect of kiwifruit rootstocks on the individual leaf area of long shoots of 'Hort16A' scions (Clearwater et al., 2006). In a study on 'Hort16A' kiwifruit cultivar, Clearwater et al. (2006) found that the total leaf area of the first 20 nodes of long non-terminated shoots was significantly lower when grafted onto low-vigour rootstocks such as *A. polygama* and *A. kolomikta*. Besides that, the individual leaf size starting at node 7 or higher was smaller on low-vigour rootstocks (Clearwater et al., 2006). Therefore, grafting ring of barks from other kiwifruit cultivars significantly decreased the leaf size (Table 5.4), especially when bark was grafted in inverted orientation, and these modifications of leaf sizes were most similar to those occurred when the vines were grafted onto low-vigour kiwifruit rootstocks (Clearwater et al., 2006).

As found in apple trees, the coordination of plant growth may involve endogenous hormonal signalling mechanisms between the shoot and root system. It has been proposed that the dwarfing apple rootstocks such as M.9 reduced the basipetal transport of IAA to root system, thus reducing the amount of CK in root, as well as GA transported to the shoot system (van Hooijdonk et al., 2010). Therefore, the reduction in plant growth lies on the decreasing of IAA transport from the shoot to the root system (van Hooijdonk et al., 2010, 2011). Furthermore, this effect may also involve the interaction between other hormones such as CK and GA. It was also found in this thesis

that the shoot growth rate of *A. deliciosa* cv. 'Hayward' scion on low-vigour kiwifruit rootstocks was slower than the scion on high-vigour rootstocks (Chapter Three). This could be due to the ability of these low-vigour rootstocks to transport less IAA from the shoot to the root system (^{14}C-IAA experiments in Chapter Four). Therefore, it would be reasonable to suggest that low-vigour kiwifruit rootstocks could possess a similar 'vigour-controlling ability' with dwarfing apple rootstocks, as we found a similarity between the mode of actions or mechanisms in dwarfing apple rootstocks and low-vigour kiwifruit rootstocks.

However, previous study in kiwifruit by Vattiprolu (2012) suggested that the growth of kiwifruit was not affected by the reduction of IAA from shoot to root system (Vattiprolu, 2012), because application of NPA to the kiwifruit stem did not reduce the length and the branching of the secondary shoots. Only small reduction in the length of primary shoots and leaf area was found in the NPA-treated vines (Vattiprolu, 2012). Thus, she concluded that the reduction of IAA from shoot to root system did not completely reduce the shoot growth of kiwifruit, except reduction in the main primary shoot. This statement could be disputed since auxin (i.e. IAA) not only involve in regulating the shoot growth (i.e. total shoot length), but it also involved in the development of other aerial parts of the plant (i.e. leaf growth) (Tables 5.1, 5.4, Figure 5.13 and Appendix 8). Although speculative, it seems in kiwifruit that the restriction of IAA transport from the shoot to root system is more likely to affect leaf growth rather than shoot growth, as indicated from using NPA (Vattiprolu, 2012), low-vigour rootstocks (Clearwater et al., 2006) or bark grafting (especially in inverted orientation) as shown in this Chapter.

Hormonal control of leaf growth and development is still poorly understood in kiwifruit, but studies on the effect of auxin (i.e. IAA) on vegetative growth in other plants have provided a good insight about the involvement of IAA in regulating the lateral organ development, especially leaf growth (Shani et al., 2006; Vernoux et al., 2010). IAA has been suggested to stimulate the cell division phase of leaf enlargement (Keller et al., 2004; Ljung et al., 2001) and leaf vascular development (Mattsson et al., 2003; Sieburth, 1999) in *Arabidopsis* plants, as well as in the initiation of new leaves in tomato (*Lycopersicon esculentum*) plants (Reinhardt et al., 2000). Growing *Arabidopsis* seedlings in medium containing NPA caused reduction in IAA content of the leaves and

the size of leaves was also reduced (Ljung et al., 2001). Similarly, application of NPA to the bean (*Phaseolus vulgaris* L.) leaf petioles inhibited leaf elongation and final leaf area (Keller et al., 2004).

In addition, evidence has been obtained from other species that some gibberellins are involved in controlling leaf growth, for examples in tomato (GA_3) (Jones, 1987) and sweet pea (GA_1, GA_{19} and GA_{20}) (Ross et al., 1993). It was also reported that dwarfing apple rootstocks had lower endogenous concentration of GA-like substances within leaves or shoots (Fontana-Degradi & Visai, 1978), with GA_{19} from the root system appeared to be an important signal regulating scion vigour (van Hooijdonk et al., 2011; Yadava & Lockard, 1977). Therefore, based on our results, it is suggested that restriction of IAA transport to the root system could be affecting leaf growth through an effect of root-produced GA (van Hooijdonk et al., 2010, 2011). However, in kiwifruit, the involvement of IAA transport and root-produced GA in regulating leaf growth and enlargement in kiwifruit is still unknown. Therefore, further research focusing on the endogenous signalling between IAA and GA in regulating leaf growth of kiwifruit is needed to elucidate this matter. Results in our study indicate that the reduction in leaf growth (Table 5.1, Table 5.4, Figure 5.13 and Appendix 8) may also contribute to the reduction in the vigour of kiwifruit vines (Table 5.1, Figures 5.11 and 5.12). In kiwifruit, vines with optimal Leaf Area Index (LAI) of about 3 (Antognozzi et al., 1991; Snelgar et al., 1998) and leaf to fruit ratio (Lai et al., 1989) are required for high productivity. Thus, by reducing the vigour of kiwifruit vines, this will allow the dry mass that would have gone into vegetative growth into fruit dry mass (i.e. reduced pruning cost and increased yield).

5.4.1.5 Effect of bark grafting on the production of sylleptic and proleptic axillary shoots

In *GM*1, bark grafting in normal and inverted orientations using a single ring of bark did not statistically cause a significant difference in the mean production of sylleptic axillary shoots (i.e. branching) (Table 5.2). However, in *GM*2, there was a trend that the main effect of bark grafting treatment and the insertion of bark from different cultivars may have influenced the mean total shoot number of sylleptic axillary shoots (Table

5.5). As stated in a previous section (Section 5.4.2), the amount of IAA in phloem tissues may be different among species, possibly due to differences in IAA metabolism and may be controlled by the genetics of the bark from the original plants (Lockhard & Schneider, 1981). Besides that, the barks of G3, G9 and G14 cultivars might be different in their ability to transport IAA through the phloem cells. As found in apple, the stem tissue of dwarfing rootstock M.9 had the ability to decrease IAA transport (Kamboj et al., 1997; Soumelidou et al., 1994). However, the ability stem tissues of these new kiwifruit rootstocks to transport IAA is unknown, as they are still relatively new. Therefore, it would be worthwhile to study the endogenous transport of IAA in the stem tissues of these new cultivars, plus other vigour controlling rootstocks for kiwifruit (Chapter Four).

Result from our study also showed that there was a trend that the number of sylleptic axillary shoots may be reduced by bark grafting compared with control vines (Table 5.5). Therefore, reduction of IAA transport from shoot to root system may be also affected the production of sylleptic axillary shoots in kiwifruit. However, our result appears to contradict those of Vattiprolu (2012). Vattiprolu (2012) found that the restriction of IAA transport by NPA did not affect the number of sylleptic axillary shoots, and she postulated that the hormonal control of shoot branching in kiwifruit might be different from what have been found in fruit trees such as apple (Vattiprolu, 2012). The reason for this discrepancy is unknown, but may be related to the different age and growth stage, as well as possibly the size of root systems of planting materials compared to that used in our study, since root system is major the contribution to the physiological processes in plants (review by Gregory et al., 2013). Nevertheless, as found in previous chapter, particular inter-specific hybrid kiwifruit rootstocks also tended to reduce the amount of branching of *A. deliciosa* cv. "Hayward' scions (Chapter Two). Although this effect was not as pronounce as reported for fruit trees, there was a similarity in our result to studies in apple. Reduction of IAA transport by dwarfing apple rootstock (i.e. M.9) or NPA treatment of the vigorous rootstock stems may also decrease the number of secondary shoots (i.e. branching) of scions (van Hooijdonk et al., 2010). It was also noted that there was a trend that bark grafting treatment may influence the proportion of bud break in the following spring (Table 5.5). Interestingly, the insertions of bark from other cultivars (G3, G9 and G14) may have an influence on the proportion of bud break as *P*-value was close to significant (*P*=0.06, Table 5.5).

These data may indicate that by altering the amount of IAA from the shoot to root system by grafting different bark of different genotypes, presumably may also have modified the amount of other endogenous hormones (i.e. CK and GA) transported from the root to the shoot systems (Lockhard & Schneider, 1981; van Hooijdonk et al., 2010, 2011), which may have altered the production of proleptic shoots and possibly production of sylleptic axillary shoots as well. As found in a previous study (Wang et al., 1994b) and findings in previous of this thesis (Chapters Two, Three and Four), the percentage of kiwifruit bud break can be influenced by the use of different rootstock genetics. Other studies also found that exogenous application of chemicals such as Hydrogen Cyanamide, or hormones such as Benzylaminopurine, can also affect the proportion of bud break in kiwifruit (Linsley & Noakes, 1989; Vattiprolu, 2012). Therefore, any alteration of endogenous hormonal transport may also affect the development of axillary shoots branching in kiwifruit vines.

5.4.2 Growth Manipulation 3

In *GM*3, bark grafting in different orientation (normal and inverted) was evaluated as a technique for regulating fruit characteristics and quality in mature green kiwifruit cv. 'Hayward'. As found in our previous study (Abdullah, 2011; Abdullah & Woolley, 2012), by inverting a 100 mm ring of bark of mature kiwifruit vines in late summer (early March), we found that the fruit size, fresh weight (FW) and dry matter concentration (DMC) were significantly increased compared with non-treated vines. We further evaluated the bark grafting treatment since we believed that the restriction of IAA transport by the bark grafting technique may also have influenced the fruit characteristics if applied during fruit growth stage. In addition, it is largely unknown whether the effect of temporary inhibition of IAA transport by the bark grafting may have a similar effect to the girdling technique. Therefore, further objective of this chapter was to compare the characteristics of fruits between the bark grafting and girdling vines. In this study, we tried to adopt the concept of blocking of phloem continuity as a means to prevent transport of carbohydrates and assimilate from the shoot to root system, a concept similar to the girdling technique (Goren et al., 2004; Theron & Steyn, 2007). An increase in the availability of assimilates and carbohydrates

to upper part of plants, caused by the bark grafting could be utilised by the fruits, a strong sink, during fruit growth and development. The bark grafting only involved the removal a single ring of phloem bark from the main stem and grafting back to the stem either in normal or inverted orientation without damaging the cambium and xylem (Figure 5.3). Blocking of phloem continuity by bark grafting may also restrict the IAA transport from the shoot to root system (Lockhard & Schneider, 1981), since IAA is also primarily transported by living tissues associated with the phloem (Hoad, 1995).

At the first harvesting year, the mean fruit fresh weight (FW) was significantly increased by the bark grafting in inverted orientation and girdling compared with bark grafting in normal orientation (Table 5.6). The difference in the FW between these treatments was almost 10%. In addition, there was a trend (P=0.10) that the mean fruit dry weight (DW) had increased in vines treated with bark grafting in inverted orientation and girdling. In the next harvesting year, however, the mean fruit FW and DW did not statistically significant between the bark grafting and girdling (Table 5.6). In addition, an unexpected result was that fruits from vines treated with bark grafting in inverted orientation produced the lowest mean fruit FW in this harvesting year (Table 5.6). The mean fruit DW and DMC did not significantly differ between bark grafting and girdling treatment in both years. These results suggest that the effects of bark grafting treatment or girdling on fruit FW and DW, as well as DMC of kiwifruit was only evident in the first harvesting year following the treatments, and the effects of treatments were lessened in the next following year. Our results also suggest that reduction in IAA and/or carbohydrates from the shoot to root system may have negative effects on fruit characteristics of kiwifruit in the second harvesting year following bark grafting or girdling treatments. It is not clear whether all mineral nutrients would be affected by both treatments. We also believe there might be short-term and long-term effects of bark grafting on the kiwifruit vines, especially on the root growth. Short-term changes; for example, Black (2011) found that root growth of girdled kiwifruit vines tended to be slower compared to intact vines. Yamane and Shibayama (2006) observed that root elongation in grapevines ceased for the two weeks following girdling, but resumed when the girdle healed. Longer-term effects of bark grafting and girdling might be related to the nutrient uptake and allocation to the shoot system. In kiwifruit, leaves from the girdled vines that were kept open around eight months had lower contents of mineral nutrients such as phosphorus (P), potassium (K), sulphur (S), calcium (Ca),

magnesium (Mg) and manganese (Mn) than leaves from ungirdled vines (Boyd, 2012). Besides that, study in apple found that concentration of K, Ca, Mg and P, but not zinc (Zn) and boron (B) were reduced in the sap of girdled trees (Bangerth, 2008). Therefore, it is reasonable to suggest that growth manipulation such as girdling and bark grafting treatments, which can affect root growth, could also affect nutrient uptake to some extent. It would be worthwhile for future study to identify whether bark grafting treatments may also affect root growth, which would suggest that uptake of nutrients might be affected.

Reduction in FW and size of fruits in the second harvesting year could be related to the depletion in nutrients uptake from the root to shoot system in the inverted bark and girdled vines (Tables 5.6 and 5.7). This would cause shortage of the nutrients in the upper part of vines, as mineral nutrients from the root system are also necessary for the fruit growth (Marcelle, 1993). As suggested before, the polarity of phloem cells may be involved in the reduction of nutrients from the root system (Antoszewski et al., 1978; Sheldrake, 1973). There would be two effects involved here; firstly, the root function may be reduced due to a lower supply of carbohydrate, and secondly, the inverted bark may directly reduce transport of nutrients from root to shoots. Study in apple found that the inverted bark grafting and girdling reduced the Ca of apple trees (Arakawa et al., 1996). Conversely, in a similar fruit crop, Gastol & Poniedzialek (2007) found calcium (Ca) content was not affected by bark grafting in inverted orientation, but reduction in nitrogen (N) content was observed compared with untreated trees. A girdling study on kiwifruit by Boyd and Barnett (2011) indicated that, after three years, fruit nutrient concentrations such as Ca, P and S were not affected and they also found higher DMC in all three years if the girdling treatment was kept open from late summer until winter (Boyd & Barnett, 2011). Based on our results, it is suggested that the use of bark grafting/inserts techniques may not commercially useable due to long-term effects on vines and production of kiwifruit. In addition, it was also found that this technique is more time-consuming to impose than girdling technique.

With girdling, it is expected that the effect of girdling treatment may be diminished in the following year, presumably due to healing or recovery of the girdled wound, and new connection of cells between the shoot and root system (Currie et al., 2005; Goren et al., 2004; Theron & Steyn, 2007). However, in the bark grafting treatments, the

reasonable assumption is that the healing and recovery processes would be different from girdling, because the phloem bark has still intact with cambium and xylem of the main stem. Comparing between bark grafting in inverted and normal orientation, it was noted that the FW and DW of fruits were slightly higher in the bark grafting in inverted than normal orientation in the first year after treatments. This result may suggest that in inverted bark grafting/inserts, the phloem cells retained their original polarity (Antoszewski et al., 1978; Lockhard & Schneider, 1981; Sheldrake, 1973). With a new reverse polarity formed, reduction in transport would be expected to persist for a longer duration than normal polarity. Therefore, the transport of assimilates and carbohydrates from the shoots may be impeded near to bark-graft union for a longer time, allowing the fruits to utilise the photoassimilates that would normally be diverted to the root system. In apple, it was reported that the reverse polarity in phloem cells could persist for more than 60 weeks (Antoszewski et al., 1978). However, in kiwifruit, it is unknown how long reverse polarity persists, particularly when bark is grafted in an inverted orientation. In girdling technique, the girdle area can heal sufficiently to resume the normal translocation of carbohydrate and hormones, whereas with bark grafting/inserts, the effect appears to be long-term, so that root growth and function is too severely affected.

It was also notable that the DMC of fruits was higher when bark grafting or girdling was conducted in early summer rather than late summer, regardless of growth manipulation treatments (Table 5.6). Even though the differences in DMC of fruits between early and late summer season are small, the differences may have an influence in terms of the quality and value of the fruits, as DMC is related to flavour acceptability and fruit taste (Burdon et al., 2004; Jaeger et al., 2003). According to Burdon et al. (2004), the range of DMC of 'Hayward' kiwifruit at harvest is 14 to 17 %, but can be vary according to season, harvesting time, canopy management and location. In our study, the range of fruit DMC was between 15.6 to 17 %, which is between the ranges reported by Burdon et al., (2004). The results found on DMC in this study are different from the response of summer trunk girdling reported by Currie et al. (2008). Summer trunk girdling conducted in late February and early March (late summer), could increase DMC sometimes, however, fruit size was not affected (Currie et al., 2008). Similarly, in our preliminary study, we also found that the fruit DMC and FW also increased when applied in early March, approximately late summer (Abdullah, 2011; Abdullah &

Woolley, 2012). This inconsistency of results may be caused by the differences in experimental location, temperature or differences in fruit development stage (Burdon et al., 2004; Goren et al., 2004; Snelgar et al., 2007). According to Snelgar et al. (2007), average DMC of kiwifruit differs substantially from year to year, and this variation in DMC is believed to be correlated with the changes in the temperatures during the growing season.

Bark grafting in an inverted orientation may have increased the fruit size (P=0.06) in the first harvesting year, but not in the second harvesting year (Table 5.7). This result indicates that the effect of bark grafting and girdling treatments on fruit size was only apparent in the year treatments were applied, and the effect diminished the following year. However, both treatments did not affect DMC in first or second harvesting year (Table 5.6). Study in apple tree, Arakawa et al. (1996) reported that fruit size of 'Fuji' apple was not influenced by the bark grafting treatment or girdling, except increased in soluble solid concentration and fruit firmness (Arakawa et al., 1996). Nevertheless, any inhibition of assimilate or carbohydrates flow by inverted bark grafting or girdling when fruits are in the starch accumulation phase has potential to increase DMC and fruit size, due to the additional assimilate available (Currie et al., 2005). In addition, since competition for carbohydrate immediately after flowering is high, with priority given to the developing fruitlets, we would expect any interruption of assimilate or carbohydrates flow at this time to result in higher fruit growth and subsequently an increase in fruit size (Buwalda & Smith, 1990).

Previous studies have demonstrated that any growth manipulation such as inverted bark grafting, girdling or using NPA may reduce IAA transported through phloem from shoot to root system (Goren et al., 2004; Lockhard & Schneider, 1981; van Hooijdonk et al., 2010), and the reduction of IAA transport may also influence the transport of other hormones such as CK and GA from root to shoot system (van Hooijdonk et al., 2010, 2011). The alteration of transport of these endogenous hormones may affect the coordination between shoot and root system or *vice versa*, subsequently may influence the plant growth. Without exception in fruit growth, any growth manipulation during various stages of fruit may also influence the translocation of endogenous hormones to the developing fruits (Brenner et al., 1989; Ozga & Reinecke, 2003). Also, endogenous hormones may enhance the partitioning of assimilates or carbohydrates to the

developing fruits (Brenner et al., 1989). In our study, it is also suggested that the temporary inhibition of IAA transport by bark grafting or girdling may cause accumulation of IAA in the upper part of kiwifruit vines (i.e. shoot system), since IAA could not be transported to the root system. Therefore, the availability of additional IAA may enhance the partitioning of assimilates or carbohydrates to be utilised by the fruits as stronger sinks for enhancing the cell enlargement and division (Brenner et al., 1989), subsequently increasing the fruit size of kiwifruit. In addition, it is also proposed that the IAA may be involved in the conversion of starch to more active carbohydrate and possibly enhanced nutrient translocation (Obata-Sasamato & Suzuki, 1973) which together with interaction with other hormones, may have increased the fruit size and DMC of kiwifruit.

In apple, as proposed by Lockhard and Schneider (1981), reduced transport of IAA from the shoot to root system may decrease CK biosynthesis. Recent study by van Hooijdonk et al. (2010; 2012) has strengten this long-held hypothesis and also found that reduced transport of IAA may also decreased GA from the root system. In kiwifruit, CK has been implicated to be involved in cell division during early stage of fruit growth (Lewis et al., 1996b; 1996a; Woolley et al., 1992). Girdling or decapication of kiwifruit shoots resulted in elevation of CK in root xylem exudates. Furthermore, application of NPA to the decapitated stem of kiwifruit vines initially increased CK levels in xylem sap (Currie, 1997), however this effect was temporary because after 72 hours, the level of endogenous CK was significantly lower than control (decapitated cuttings) (Currie, 1997). In agreement with Currie (1997), Vattiprolu (2012) also mentioned that inhibition of IAA transport by NPA or girdling on young kiwifruit vines may also increase CK level, as she found that the number of sylleptic axillary shoot increased. To link these literatures to our study is difficult, because previous studies did not observe the effect of IAA inhibition on fruit growth as we did. Nevertheless, it is resonable to suggest that the inhibition of IAA by girdling or bark grafting treatment (especially in inverted orientation) during fruit growth stage also may increase the level of CK (at least temporarily). Therefore, the temporary elevation of CK in upper part of vines might be involved in cell division of fruit, as Lewis (1996a) found that the level of CK was significantly higher during early fruit growth in kiwifruit. Therefore, we believed that increased CK supply in response to bark inserts or girdling, may be causal in increasing fruit size in kiwifruit (Table 5.7).

Fruit characteristics are generally found to be normally distributed within populations. In kiwifruit, the assumption of normality in fruit weight has been widely accepted for 'Hayward' kiwifruit (Burge et al.,1987; Judd et al., 1989; McAneney et al., 1989). An early study by Burge et al. (1987) found that the fruit weight distribution of kiwifruit from one harvesting season could be negetively skewed due to poor pollination (Burge et al., 1987). Another study examined the influence of water stress on the fruit weight distribution of 'Hayward' kiwifruit and found water stress reduced the mean fruit weight without affecting the distribution pattern (Judd et al., 1989). Similarly, McAneney et al., (1989) also found that the distribution of fruit weight of 'Hayward' scions grafted onto unknown seedling rootstocks had approximately close to normal distribution. However, a study by Woodward (2006) using large samples harvested from 96 commercial orchards at Te Puke, New Zealand found that the distribution of fruit weight of 'Hayward' was not normally distributed and mostly positively skewed. In our study, the distribution of fruit weight from each treatment and in different seasons were shown to be approximately normal (Figure 5.15 until Figure 5.18), even though some of them were slightly skewed (e.g. Figure 5.16A and B, Figure 5.19C, Figure 5.18A and B). Additional results in Appendix 9 also shown that the distributions of fruit weight from each treatment were highly variable between season and harvesting year. The reason for the variability in our results for both treatments and seasons is unknown. Thus, the available data in our study do not allow formulation of specific conclusions, especially since only 60 fruits from three replicated vines was used in our study. Therefore, for the future study, larger samples should be taken with more replicated vines per treatment.

It was found that the variation in fruit characteristics, especially FW, size and DMC in kiwifruit is known to be within vines rather than between vines (Miles et al., 1996; Smith et al., 1994; Woodward, 2006). Smith et al. (1994) found that different positions on the vine contributed highest variation in fruit characteristics, with the largest fruits were found at the apical ends of the lateral shoots and from early opening flowers. Furthermore, the fruit position within canopy also may influence the internal and external characteristic of fruits (Miles et al., 1996). Besides that, it was reported that the differences in growing site or location and differences in growing season also contributed to the variation in the fruit characteristics of kiwifruit (Woodward, 2006). It has been found that kiwifruit FW and size can be more easily regulated by cultural

practices than DW (Palmer, 2007). Similarly in this study, fruit FW and size were affected by the bark grafting or girdling treatment, even though the effects were only can be observed in the first harvesting year (Table 5.6 and Table 5.7). In contrast, neither bark grafting nor girdling influenced DMC (Table 5.6). However, there was a slight difference in the DMC between early and late summer season. Therefore, for further study, it is suggested that factors such as differences in fruit and canopy position, together with differences in fruit growth stage could be included as possible factors that affect fruit growth in any growth manipulation treatment.

Overall, our finding in *GM*3 suggests that the decrease in fruit FW, DW and size in second harvesting year may be due to reduced activities of particular nutrients and hormonal of the root system. Reduction in supply of carbohydrates and IAA to the root system by the bark grafting and girdling may have limited the root growth. However, we believe that there can be severe limitations in using bark grafting/inserts, rather than girdling, because girdling can be arranged so that the girdle heals sufficiently to supply the roots for part of the year. Girdling techniques are currently being adopted as a part of the annual vine management strategy for kiwifruit (Currie et al., 2005; 2008). However, to make useful recommendations to kiwifruit growers about bark grafting/inserts, other factors need to be considered. For example, re-doing the bark grafting/inserts each year would be labour intensive with little if any advantage over girdling, and using shorter inserts and replace each year may not be effective. Based on our findings in this chapter, we conclude that the use of bark grafting/inserts would not be commercially feasible for regulating fruit quality in kiwifruit. Nevertheless, these techniques may be useful to improve our understanding on physiological mechanism (s) of growth manipulation in kiwifruit vines.

5.5 Summary

In order to improve our physiological understanding of vigour control and regulation of fruit growth, the 'Growth Manipulation (*GM*)' by using bark inserts or grafting was conducted on young 'Hort16A' and mature 'Hayward' kiwifruit vines. These studies allowed us to improve our physiological understanding of the effect of restriction of auxin (IAA) and nutrient transport in kiwifruit vines. The main idea of this study was to manipulate cell polarity to restrict transport of IAA from the shoot to root system, as the polarity of cells should be retained regardless of cell orientation. In the first trial (*GM*1*)*, a complete ring of phloem bark with 15 mm in width was removed and grafted back to the main stem of 'Hort16A' kiwifruit either in normal (i.e. original polarity) or inverted orientation. Three weeks following bark grafting treatment, the active buds and suckers below the bark graft-union developed into new shoots indicating these buds have been released from apical dominance. There was also an appearance of bark swelling above the graft-union, and this appearance was more obvious in the vines with bark grafting with an inverted orientation. These evidence suggest that the basipetal transport of IAA from the upper part to the root system had been restricted and IAA did not pass through in sufficient amount to inhibit lower bud release when the bark was grafted in an inverted orientation. Even though individual characteristics of vegetative growth were often not significantly affected by the treatments, when the overall effect of small reductions in shoot length, node number, internode length, shoot CSA and, particularly leaf size (i.e. leaf area) were considered together, there was a reduction in vegetative growth, particularly when the bark was inverted.

In the second trial of bark grafting (*GM*2), three completed ring of phloem barks from other kiwifruit cultivars (either G3, G9 and G14) were taken and grafted onto main stems of young 'Hort16A' either in normal or inverted orientation, so that the total combine length of grafted barks used in the second trial was slightly longer (45 mm) than the length of bark in *GM*1 (15 mm). Results showed that the monthly total length of sylleptic axillary shoots differed between bark grafting and control vines. Regardless of the insertion of bark from different cultivars, the vines grafted with bark in an inverted orientation had the lowest length of sylleptic axillary shoots compared to other treatments. The insertion of G3 and G9 cultivars, especially in inverted orientation on

the main stem of young 'Hort16A' reduced the monthly production of sylleptic axillary shoots compared to the insertion of bark from G14 cultivar. In particular, the mean final total length and total node number of sylleptic axillary shoots were also reduced in the vines grafted in an inverted orientation than control, indicating that the insertion of bark from other kiwifruit cultivars (i.e. different genetic materials) may have an influence on the overall vigour of young 'Hort16' vines, presumably via the amount of auxin transported through the inserts to the root system. Similar to that found in *GM*1, the leaf area of young 'Hort16A' vines was also reduced by the bark grafting treatments. Overall, we found that increasing the length of bark also increased the dwarfing effect of the bark grafted vines. Overall, the results in *GM*2 suggest that the amount of IAA transport in the phloem tissues may be controlled by the genetics of the bark from the original plants, since the level of IAA transported in the phloem may be different between cultivars and species.

A further objective in this study was to evaluate the bark grafting (inverted and normal orientation) as a method for regulating fruit characteristics in mature green 'Hayward' kiwifruit vines (*GM*3). Comparing between treatment, season and year, the treatment effects on FW, DW, DMC and size were only evident in the first harvesting year, and the effects on were lessened or reversed in the following year, especially on FW and DMC. In the first harvesting year, vines treated with bark grafting in an inverted orientation and girdling had increased fruit FW and DW. However, the fruit FW was reduced in vines treated with bark grafting in inverted orientation in the following year compared with other treatments. Fruit DMC was not much effected by the treatment in both years. However, there was an indication that the treatment applied in early summer season may produce higher fruit DMC than applied in late summer season. Similarly, the fruit size also varied between season and harvesting year. In the first harvesting year, the fruit size was larger from vines treated with bark grafting in inverted orientation, but it became smaller in the following year. In summary for *GM*3, the bark grafting and girdling treatment did not consistently produce similar effects in each season and year. Overall, the use of bark grafting/inserts would not be commercially feasible for regulating fruit quality in kiwifruit, but these techniques may be useful to improve our understanding on physiological mechanism (s) of growth manipulation in kiwifruit vines.

Chapter Six

6. Architectural assessment of kiwifruit seedlings obtained from specific crosses and their responses to gibberellin application

6.1 Introduction

The search for potential vigour controlling rootstocks has turn out to be an important issue in kiwifruit orchard management. Several attempts have been made for many years to select kiwifruit cultivars that may be potentially used as vigour-controlling rootstocks (Anon., 2012; see Clearwater et al., 2002; 2007b; Thorp et al., 2013). However, most of the kiwifruit rootstocks did not completely reduce the excessive vegetative growth but, only imparted a small reduction in the vegetative growth of the grafted scions. It was demonstrated in previous chapters of this thesis (Chapter Two, Three and Four) that there were a few selected inter-specific hybrid kiwifruit rootstocks that could be further evaluated as potential vigour-controlling rootstocks. However, rootstock selections and breeding programmes are long-term research activities that include the selection of potential parents with dwarfing genes, hybridization, preliminary evaluation and selection for desired rootstock traits, assessment of scion genotypes grafted on the potential rootstocks, orchard evaluation, and scion-rootstock production trials with the promising rootstocks. Besides that, field evaluations of vigour-controlling rootstocks are costly, time and space consuming, involving field trials with vines composed of specific scions and rootstocks combinations. According to van Nocker and Gardiner (2014), conventional selection and breeding of woody perennial tree crops using phenotypic selection require five to seven years per cycle to complete. Therefore, another possible approach is taking the advantages of the initial architectural traits of kiwifruit seedlings from a breeding programme and explore their potential traits. Besides that, plant growth regulators could be used to manipulate the architecture of the plants.

A recent study by Vattiprolu et al. (2012) on *A. deliciosa* cv. 'Hayward' has found that the application of GA mixtures (GA_3 + GA_{4+7}) on kiwifruit shoots immediately after bud break stimulated the growth of apical and lateral axillary shoots. Usually, during the development of kiwifruit shoots, around 40% of them will develop into short shoots, 40% into medium shoots and only 20% will develop into long shoots (Sale & Williams, 1983). However, foliar spray of GA transformed all the potential short and medium shoots into long shoots by stimulating both apical and sub-apical meristem to produce more nodes and longer internode lengths (Vattiprolu, 2012). Thus, she concluded that the growth of kiwifruit shoots is dependent on a sufficient amount of bio-active gibberellins in order to regulate the apical and sub-apical meristem of kiwifruit shoots. Gibberellins also have been implicated in the dwarfing mechanism of composite apple trees (van Hooijdonk et al., 2010, 2011). For example, shoot termination is the most consistently expressed architectural trait modified by dwarfing apple rootstocks. 'Royal Gala' scions on M.9 dwarfing rootstock reduced node production, early termination and axillary bud activity. Similar effects were also found when the auxin transport inhibitor (i.e. N-1-Naphthylphthalamic Acid or NPA) was applied to rootstock stems, irrespective of the vigour of the apple rootstocks. However, these effects of restricting auxin transport could be reversed with shoot applied gibberellins, suggesting scions on dwarfing rootstocks gibberellins were deficient in gibberellins (van Hooijdonk et al., 2010; 2011). In this thesis, we also found that selected inter-specific hybrid kiwifruit rootstocks promoted early shoot termination of scions (Chapter Three), indicating that the particular kiwifruit rootstocks may also produce a deficiency in gibberellins.

Therefore, in this chapter, two experiments were conducted in order to elucidate; i) the early architectural traits of kiwifruit seedlings in relation to the vigour (Experiment 1) and, ii) their responses to exogenous application of gibberellins (GA) (Experiment 2). The overall objective of this chapter was to evaluate architectural traits including branching and shoot termination that may be modified by kiwifruit seedlings that could be useful traits for vigour-controlling rootstocks or low vigour scions. By using low-vigour scions, the 'dwarfing effect' can be maintained regardless of rootstock vigour, allowing better utilization of rootstock characteristics, such as adaptability for different environments and soil conditions, as well as disease resistance (Bulley et al., 2005). In addition, because the phenotype of plants can be manipulated by exogenous application of GA, it may be possible to manipulate the shoot architecture of kiwifruit (Vattiprolu,

2012). As demonstrated in a recent study (Vattiprolu, 2012), exogenous application of GA mixture can alter the apical and sub-apical meristem activities of kiwifruit shoots to become non-terminated long shoots, instead of terminated medium and/or short shoots. In this study, it was hypothesised that the differences in the initial architecture structure of kiwifruit seedlings may be attributed to the different level of GA and exogenous supply of GA may alter the growth and vigour of kiwifruit seedlings (**Hypothesis V**). Gibberellins could be useful to temporarily stimulate the potential cultivars with low-vigour abilities to quickly develop the desired canopy density in newly-planted kiwifruit orchards. After canopy establishment, application of GA could be stopped to increase carbohydrate allocation to fruit production.

6.2 Materials and methods

6.2.1 Experiment 1: Initial shoot architecture of kiwifruit seedlings obtained from specific crosses

6.2.1.1 Study site and establishment of experimental plant materials

This study was conducted during the 2012-2013 growing season at the Plant and Food Research (PFR), Palmerston North, New Zealand. In May 2012, 102 kiwifruit seedlings obtained from specific-crosses that had been germinated and planted into 3L pots were selected (Figure 6.1). Briefly, the seeds were collected from well ripened soft fruits from the breeding crosses. The fruits were than blended using a kitchen blender and the slurry was passed through a fine mesh sieve to collect the seeds. The seeds were then collected and dried in room temperature for a few days. To germinate the seeds, moist sand was used as a medium, placed in germination trays and arranged on a laboratory bench. After 10-14 days, the germinated seeds were planted into pots containing sterile, well drained standard potting mix. The seedling was placed inside a glasshouse for hardening-off and the shoots were supported using bamboo sticks. The standard growing medium was similar to that described in Chapter Two (Section 2.2.2).

Figure 6.1. The kiwifruit seedlings obtained from specific crosses planted in 3L pots hardening inside a glasshouse at PFR, Palmerston North, New Zealand.

6.2.1.2 The measurement of the vine architectural structures

In order to characterise the differences between the phenotypes of kiwifruit seedlings (Figure 6.2), we adopted the terminology 'main primary shoot' that refers to the first order of the main trunk as conducted in a previous study with apple (De Wit et al., 2002) and olive (Hammami et al., 2012). This simple visual observation based on the different main shoot categories (i.e. long and short shoots) can be used to define the early architectural traits based on the morphological criteria (Costes et al., 2006a). The kiwifruit seedlings were segregated according to the initial phenotype (i.e. single or multiple stem). After that, the seedlings were segregated again according to the primary shoot characteristics (i.e. long or short shoots) and number of stems produced per seedling. There were four different phenotypes that were identified from the seedling population; i) Long Single Stem (LS), ii) Short Single Stem (SS), iii) Long Multiple Stem (LMS), and iv) Short Multiple Stem (SMS) (Figure 6.2). The architectural characteristics of the primary shoots of these four phenotypes, such as the number of shoots, length (mm), node number, internode length (mm), and basal diameter (converted to cross-sectional area-mm^2 - shoot CSA), were measured and recorded as described in Chapter Two (Section 2.2.3). The measurements were conducted during the winter season of 2012 (at growth cessation in middle June 2012) after all the leaves had fallen and only the shoot structure remained. No pruning was performed in order to not interfere with the natural growth habit of the seedlings.

6.2.1.3 Experimental design and statistical analysis

The differences between phenotypes described in Section 6.2.1.2 were considered as treatment. The experiment was a completely randomised design with unequal replicates. Due to the data being highly skewed and transformation not improving it, non-parametric ANOVA was used for statistical analysis using Kruskal-Wallis test, followed by Bonferroni Multiple Comparisons Test for mean comparisons. Data were ranked before ANOVA by sorting the data into order and replacing each value by its relative position in the order. Besides that, the variables measured (i.e. number of shoots, length, node number, internode length, and shoot CSA) were submitted to Principal Component Analysis (PCA) using SAS (9.1, SAS Institute Inc., Cary, NC USA) to determine inter-correlation between these variables.

6.2.2 Experiment 2: Responses of different phenotypes of kiwifruit seedlings obtained from specific crosses to exogenous application of gibberellins

6.2.2.1 Study site and establishment of experimental plant materials

The experiment was conducted at the location stated in Experiment 1 (Section 6.2.1). The four different phenotypes (LMS, LS, SMS and SS) that had been identified earlier in Experiment 1 were used for this trial. Sixty vines per phenotype with uniform sizes were selected and planted in 30L polybags. The growing medium was similar to that described in a previous chapter (Section 4.2.1.1, Chapter Four). The experimental vines in polybags were arranged in a tunnel house according to the experimental design. The irrigation was supplied three times a day using an automated irrigation controller (Hunter, Smart Valve Controller, USA). In order to avoid confound effects of shoot bending, the actively growing shoots were trained upright using white nylon strings and fastened using a hand tying machine.

6.2.2.2 Application of gibberellin treatments

Gibberellins were sprayed onto the vines fortnightly, beginning at the onset of spring 2012 bud break (early October 2012) until middle January 2013. A mixture of GA_3 (Sigma product, No.48880-5G-F) and GA_{4+7} (NOVAGIB®, Fine Agrochemicals Ltd., Worcester, UK) were made using the method described by Vattiprolu et al., (2012). As reported previously, the shoot growth of kiwifruit can be stimulated at 500 ppm and 1000 ppm of GA mixtures without damaging shoots (Vattiprolu, 2012). Therefore, to avoid any damage to the shoot growth, the minimum level of 500 ppm of GA mixtures (GA_3 and GA_{4+7} at a 1:1 ratio). Briefly, aqueous solution of GA_3 (500 mg/L) and GA_{4+7} (500 mg/L) was prepared a day before application to the vines. Both of the gibberellin solutions were mixed together and sprayed to run-off using a hand spray. The control plants were just sprayed with distilled water. The foliar application of GA_3 and GA_{4+7} was carried out in the early morning (approximately 7.00 am until 8.30 am) or later after sunset (approximately 6.00 pm until 7.30 pm) to avoid photo-toxic re-action. Adjacent vines were covered with a large plastic sheet during spraying to prevent contamination from spray drift.

6.2.2.3 Measurement of vine architectural structures

The measurements of shoot architectural structures were conducted in the middle of February 2013, approximately one month after the final date of GA application. The shoots produced from the treated and control vines of all phenotypes were classified according to the architectural description developed by Seleznyova et al., (2002), and were further classified either as non-terminated or terminated shoots. The length, node number and base diameter (converted to shoot CSA) of the different lateral axillary shoots were measured and recorded similar to Section 6.2.1.2. The data on the total shoot length, total node number, and total number of lateral branching axillary shoots (proleptic and sylleptic) from treated and untreated vines were also recorded and presented. In this study, the treatment effects on the different proportions of terminated and non-terminated were reflected in similar changes in the data for the different proportions of shoots types (short, medium and long shoots). Therefore, the data for the proportions of long, medium and short shoots are not presented.

6.2.2.4 Experimental design and statistical analysis

The experiment was a Randomised Complete Block Design (RCBD) with a factorial arrangement of the treatments (4 x 2). There were four seedling phenotypes (LMS, LS, SMS and SS) and two GA treatments (with and without GA). Each treatment was replicated seven times. Data were manually entered in Microsoft Excel and summarised using Pivot Table procedures. Data for the main effects (seedling phenotypes and GA treatments) and interactions were analysed using the GLM procedure of SAS (9.1, SAS Institute INC. NC USA). Mean separations for the main effects were made using LSD test at P=0.05. For the interactions data, pre-planned comparisons were made using Least Square Means (lsmeans test). When the data were not normally distributed, the raw data were subjected to an appropriate transformation before being subjected to analysis of variance (ANOVA).

6.3 Results

6.3.1 Experiment 1

6.3.1.1 The proportion of the different phenotypes within a seedling population

The shoot morphology of four phenotypes are shown in Figure 6.2. Out of 102 kiwifruit seedlings that were assessed in this trial, there were 45.1% (46 seedlings) and 17.6% (18 seedlings) that can be grouped as SMS and LMS phenotypes respectively (Figure 6.3). In addition, the population of these kiwifruit seedlings consisted of 22.6% (23 seedlings) SS and 14.7% (15 seedlings) LS phenotypes. Therefore, there were 62.7% multiple stem (i.e. SMS and LMS) and 37.3% single stem (i.e. SS and LS) vines within the kiwifruit seedling population (Figure 6.3).

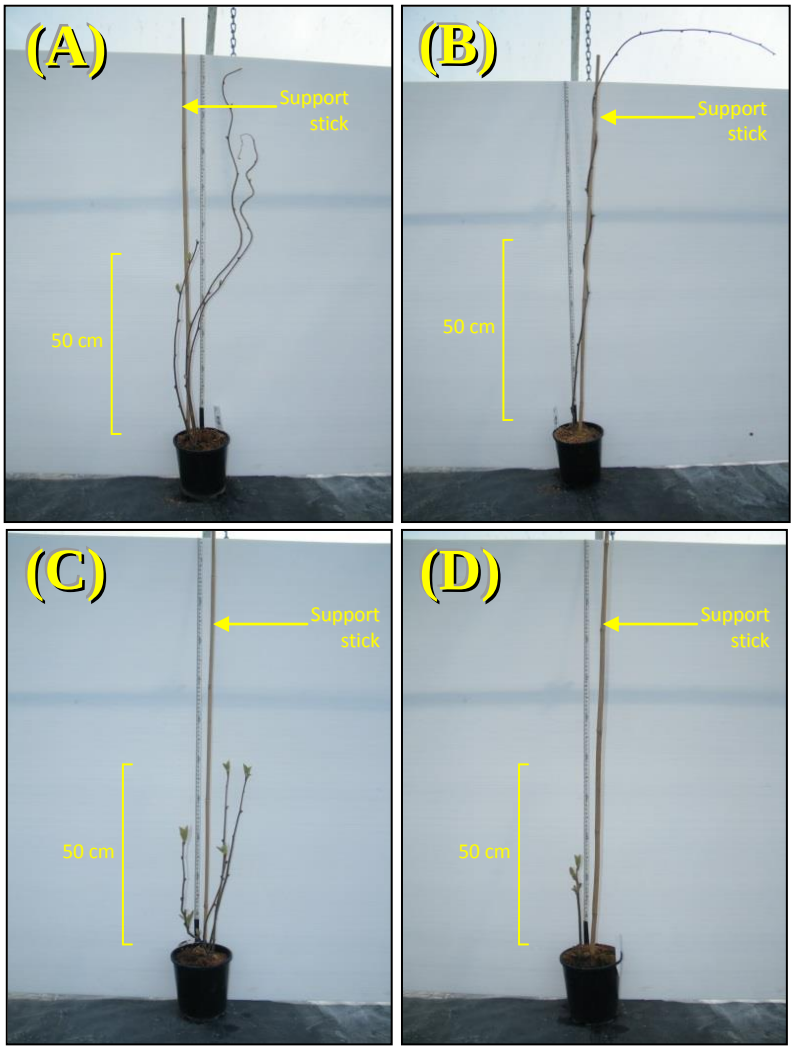


Figure 6.2. Four different phenotypes found within the population of kiwifruit seedlings; (A) Long Multiple Stems (LMS), (B) Long Single Stem (LS), (C) Short Multiple Stems (SMS), and (D) Short Single Stem (SS).

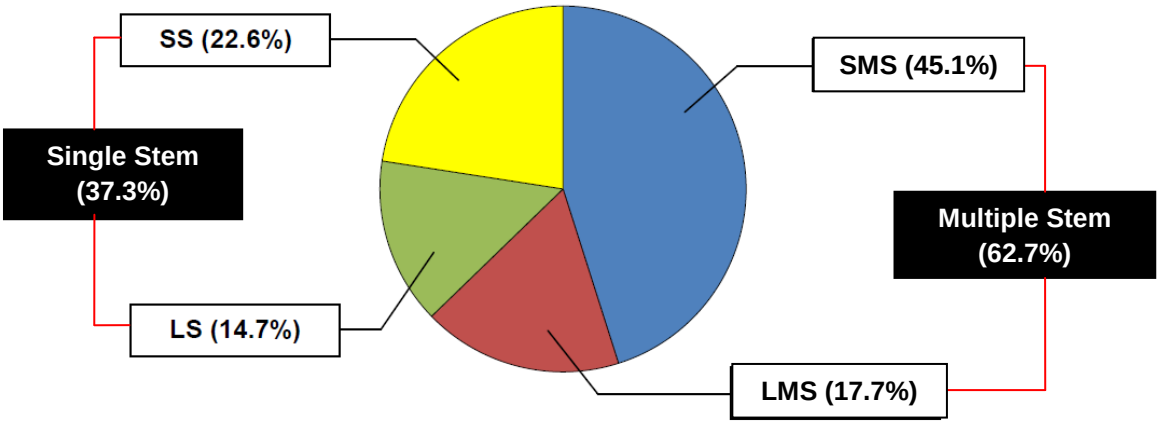


Figure 6.3. The proportions (%) of the different phenotypes (i.e. Single and multiple stem phenotypes) found from the 102 kiwifruit seedlings population. Long Multiple Stem (LMS), Long Single Stem (LS), Short Multiple Stem (SMS), and Short Single Stem (SS) (*n*=102).

6.3.1.2 The characteristics of the primary shoots among phenotypes

The analyses of the primary shoots characteristics among the phenotypes are shown in Table 6.1 and Table 6.2. There were highly significant differences ($P<0.0001$) for the mean number and the mean shoot length (mm) of primary shoots among the phenotypes (Table 6.1). Similarly, the mean number of nodes, the mean internode length, and the mean CSA (Table 6.1) of primary shoots were also significantly different ($P<0.0001$) among the phenotypes (Table 6.1). The mean number of primary shoots was highly significant between single and multiple stem phenotypes (Table 6.2). Number of primary shoots in SMS and LMS are almost three-fold higher than SS and LS phenotypes. LS phenotype produced significantly higher mean length of primary shoots, followed by LMS and SS phenotypes. However, SMS phenotype produced the shortest mean length of primary shoots (Table 6.2). Similar results were obtained for the mean node number of primary shoots with LS phenotype producing the highest mean node number of primary shoots compared to other phenotypes. Besides that, the mean internode length of primary shoots of SS and SMS phenotypes were significantly shorter compared to LS and LMS phenotypes (Table 6.2). In addition to these results, the mean CSA of primary shoots was significantly greater in LS phenotype compared to LMS and SMS phenotypes, but not significantly different compared to SS phenotype (Table 6.2).

Table 6.1. Non-parametric Analysis of Variance (Kruskal-Wallis ANOVA Test) of four different phenotypes, Long Multiple Stem (LMS), Long Single Stem (LS), Short Multiple Stem (SMS), and Short Single Stem (SS).

Primary shoot Characteristics	Mean rank	Standard error	Standard deviation	Chi-Square statistic	df	*P*-value
No. of primary shoots	2.2	0.1	1.0	80.3	3	$P<0.0001$
Length (mm)	640.0	50.3	508.2	75.3	3	$P<0.0001$
Number of nodes	13.1	0.6	6.3	69.8	3	$P<0.0001$
Internode length (mm)	43.3	1.5	14.7	64.3	3	$P<0.0001$
Shoot CSA (mm^2)	29.6	29.6	0.9	38.1	3	$P<0.0001$

Table 6.2. Comparison of the characteristics of the primary shoots between different phenotypes of kiwifruit seedlings obtained from specific crosses.

Primary shoot characteristics (Means)	Single Stem		Multiple Stem	
	Phenotypes			
	LMS (*n*=18)	**SMS (*n*=46)**	**LS (*n*=15)**	**SS (*n*=23)**
No. of primary shoots	2.9 (± 0.1)^{a}	2.9 (± 0.1)^{a}	1.0 (± 0.0)^{b}	1.0 (± 0.0)^{b}
Shoot length (mm)	842.7 (± 52.0)^{b}	311.5 (± 52.0)^{d}	1701.3 (± 69.4)^{a}	446.1 (± 26.5)^{c}
Number of nodes	14.5 (± 0.8)^{b}	9.0 (± 0.8)^{d}	26.3 (± 0.9)^{a}	11.7 (± 0.5)^{c}
Internode length (mm)	56.6 (± 1.8)^{b}	33.5 (± 1.3)^{c}	64.9 (± 1.6)^{a}	38.2 (± 1.9)^{c}
Shoot CSA (mm^2)	30.7 (± 1.9)^{b}	24.1 (± 0.7)^{c}	39.0 (± 3.1)^{a}	33.6 (± 2.0)^{ab}

Data in parenthesis are standard error of means (±).
Means sharing the same latters in a ***row*** are not significantly different at the $P \leq 0.05$ level according to Bonferroni Multiple-Comparison Test.

6.3.1.3 The correlation between the characteristics of the primary shoots among phenotypes

In order to understand the relationship between the characteristics of the primary shoots among phenotypes, the Pearson Correlation Coefficients Test was carried out on the characteristics of primary shoots (Table 6.3). The number of primary shoots were negatively correlated with the shoot length (R^2= -0.40), number of nodes (R^2= -0.48) and shoot CSA (R^2= -0.48). Besides that, the CSA of the primary shoots were slightly correlated with the number of nodes (R^2=0.52) and shoot length (R^2=0.48) of the primary shoots (Table 6.3). However, the CSA of the primary shoots was not correlated with the internode length of primary shoots (R^2=0.32). Similarly, the number of shoots was also not correlated with the internode length of primary shoots (R^2= -0.22) (Table 6.3).

Table 6.3. Correlations among parameters of shoot characteristics of kiwifruit seedlings obtained from specific crosses.

Primary shoot characteristics	No. of primary shoots	Shoot length (mm)	Number of nodes	Internode length (mm)	Shoot CSA
No. of primary shoots	-				
Shoot length (mm)	-0.40***	-			
Number of nodes	-0.48***	0.96***	-		
Internode length (mm)	-0.22*	0.85***	0.72***	-	
Shoot CSA (mm^2)	-0.48***	0.48***	0.52***	0.32***	-

ns, *, **, *** non-significant or significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively according to Pearson Correlation Coefficients Test.

6.3.1.4 The Principal Component Analysis (PCA) on the characteristics of primary shoots

According to the PCA analysis, the first and the second principal components (Figure 6.4A and Figure 6.4B, respectively) accounted for 84.9% of the total variance. All the characteristics of primary shoots (i.e. shoot length, number of nodes, internode length and shoot CSA) except the number of shoots were positively associated with the first Principal component (Figure 6.4A). PCA Loading Plot was used to interpret the relationship between the characteristics of primary shoots. According to the PCA Loading Plot (Figure 6.4A), a few characteristics such as the number of nodes and internode length of the primary shoots were most associated with the length of the primary shoots. However, the number of primary shoots (i.e. branching) seems to be negatively associated with these characteristics. Similarly, the shoot CSA of primary shoots seem to not be associated with the shoot length, number of nodes, and internode length of primary shoots (Figure 6.4A). Results in Figure 6.4A have confirmed the results obtained in Table 6.3, because there were similarities in the results obtained from both analyses. In addition to these results, the PCA Score Plot is presented in order to interpret the relationship among the phenotypes found in this trial. The Score Plot (Figure 6.4B) showed a wide variability of all seedlings, even though several groupings can be observed which were related to the different phenotypes (Figure 6.4B). According to PCA Score Plot in Figure 6.4B, the phenotypes of SMS are positioned and clearly observed on the left side of negative PC1 loadings (Figure 6.4A). In contrast, the phenotype of LS was more clearly positioned on the positive PC1 loadings (Figure 6.4A). Whereas, other phenotypes such as LMS and SS tended to spread in the middle of the Score Plot between PC1 and PC2, which showed wide phenotypic range in these phenotypes (Figure 6.4B).

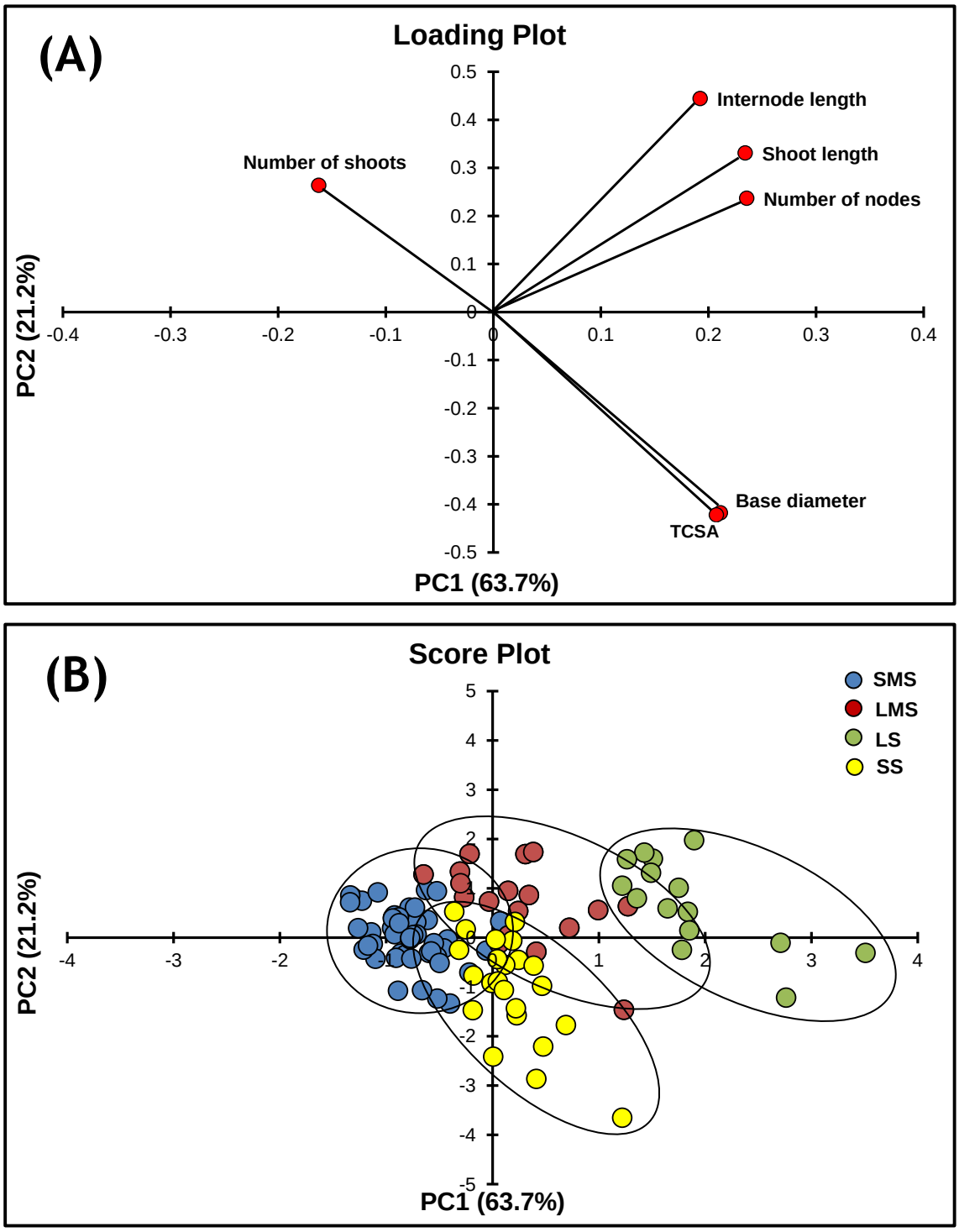


Figure 6.4. Principal Component Analysis for all parameters studied. (A) Loading Plot for interpreting relationship among parameters (shoot length, node number, internode length and shoot CSA. (B) Score Plot for interpreting relationship among kiwifruit seedling phenotypes (Long Multiple Stem-LMS, Long Single Stem-LS, Short Multiple Stem-SMS, and Short Single Stem-SS).

6.3.2 Experiment 2

6.3.2.1 The characteristics of long proleptic axillary shoots (non-terminated)

The seedling phenotypes x GA treatment interactions was significant for the mean CSA (P=0.008) and close to significant (P=0.08) for the mean length of long lateral axillary shoots (Table 6.4). However, no significant interactions were found on the mean node number and internode length (P=0.15 and P=0.51, respectively). For the main effect of seedling phenotypes, there was a trend towards significance (P=0.11) that the mean node number of long shoots was affected by seedling phenotypes and approached significance for the mean internode length (P=0.06) (Table 6.4). With GA, the mean node number and mean internode length were significantly reduced (P=0.04 and P=0.001, respectively) when compared to untreated vines (Table 6.4).

6.3.2.2 The characteristics of medium proleptic axillary shoots (terminated)

Comparison for the medium shoots (Table 6.4) can only be made between the phenotypes of LMS and SMS due to the fact that medium shoots were not present on the phenotypes of SS and LS. The phenotypes x GA treatment interactions were significant for the mean internode length (P=0.01) and close to significant for the mean length of medium shoots (P=0.08). However, no significant interactions were found between seedling phenotypes and GA for the mean node number (P=0.72) and mean CSA of medium shoots (P=0.77). For the main effect of seedling phenotypes, the mean internode length of medium shoots was significantly shorter (P=0.006) in SMS compared to LMS phenotypes (Table 6.4). The mean internode length was significantly longer (P=0.02) when treated with GA compared to untreated vines. The mean node number and mean CSA of medium shoots did not differ significantly between LMS and SMS phenotypes (P=0.89 and P=0.42, respectively). GA treatment also did not affect the mean node number and mean CSA of medium shoots (P=0.31 and P=0.15, respectively) (Table 6.4).

6.3.2.3 The characteristics of short proleptic axillary shoots (terminated)

The seedling phenotypes x GA treatment interactions were not significant for the mean length, node number, internode length and CSA of short shoots (P=0.28, P=0.97, P=0.55 and P=0.96, respectively) (Table 6.4). No significant main effects of seedling phenotypes were found on the mean length, node number and CSA of short shoots (P=0.64, P=0.55 and P=0.35, respectively) (Table 6.4). However, the mean node number of short shoots was significantly different (P=0.04) between the seedling phenotypes, with the highest and the lowest mean node number recorded on the SS and LMS phenotypes, respectively. The main effects of GA significantly increased the means length, node number, and internode length (P=0.002, P=0.0004 and P=0.02, respectively), but not affected the mean CSA of short shoots (P=0.87) (Table 6.4).

Table 6.4. Main effects of seedling phenotypes and the application of gibberellins (± GA) on the characteristics of proleptic axillary shoots.

Main effects	Mean shoot length (mm)	Mean node number	Mean internode length (mm)	Mean shoot CSA
Long proleptic axillary shoots				
Phenotypes				
LS	1844.9 [a]	32.8 [a]	56.43 [a]	70.04 [a]
SS	1817.9 [a]	31.5 [a]	58.31 [a]	71.14 [a]
LMS	1630.4 [a]	26.3 [a]	62.72 [a]	58.68 [b]
SMS	1645.4 [a]	29.6 [a]	55.82 [a]	62.34 [ab]
$LSD_{0.05}$	419.9	7.41	6.98	12.93
***P*-value**	*P*=0.39	*P*=0.11	*P*=0.06	*P*=0.04
GA treatment				
+ GA	1534.3 [b]	28.2 [b]	55.26 [b]	59.84 [b]
- GA	1967.1 [a]	32.4 [a]	61.59 [a]	72.85 [a]
$LSD_{0.05}$	224.1	3.95	3.72	6.91
***P*-value**	*P*=0.0003	*P*=0.04	*P*=0.001	*P*=0.0004
Interactions	*P*=0.08	*P*=0.15	*P*=0.51	*P*=0.008
Medium proleptic axillary shoots				
Phenotypes				
LS[‡]	-	-	-	-
SS[‡]	-	-	-	-
LMS	705.7 [a]	13.6 [a]	51.2 [a]	31.9 [a]
SMS	582.3 [a]	13.8 [a]	41.9 [b]	35.1 [a]
$LSD_{0.05}$	167.8	2.8	6.2	8.5
***P*-value**	*P*=0.14	*P*=0.89	*P*=0.006	*P*=0.42
GA treatment				
+ GA	702.8 [a]	14.2 [a]	49.8 [a]	31.2 [a]
- GA	551.6 [a]	12.9 [a]	41.4 [b]	37.3 [a]
$LSD_{0.05}$	172.9	8.5	6.4	8.7
***P*-value**	*P*=0.11	*P*=0.31	*P*=0.02	*P*=0.15
Interactions	*P*=0.08	*P*=0.72	*P*=0.01	*P*=0.77
Short proleptic axillary shoots				
Phenotypes				
LS	52.6 [a]	4.4 [ab]	3.2 [a]	27.5 [a]
SS	64.1 [a]	5.0 [a]	3.6 [a]	26.1 [a]
LMS	61.9 [a]	4.2 [b]	3.3 [a]	25.8 [a]
SMS	54.7 [a]	4.6 [ab]	3.5 [a]	20.9 [a]
$LSD_{0.05}$	28.4	0.8	0.8	10.2
***P*-value**	*P*=0.64	*P*=0.04	*P*=0.55	*P*=0.35
GA treatment				
+ GA	70.6 [a]	5.0 [a]	3.7 [a]	24.9 [a]
- GA	45.7 [b]	4.1 [b]	3.1 [b]	25.4 [a]
$LSD_{0.05}$	15.1	0.4	0.4	5.4
***P*-value**	*P*=0.002	*P*=0.0004	*P*=0.02	*P*=0.87
Interactions	*P*=0.28	*P*=0.97	*P*=0.55	*P*=0.96

Means sharing the same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ Test.
[‡]Medium shoots are not available in the phenotypes of long single shoots (LS) and short single shoots (SS).

6.3.2.4 The proportion of terminated and non-terminated axillary shoots

The proportions of terminated and non-terminated shoots were recorded regardless of whether they were proleptic or sylleptic. The seedling phenotypes x GA treatment interactions were not significant for the mean proportion of terminated shoots (P=0.19) and non-terminated axillary shoots (P=0.18) (Table 6.5). However, the main effects of seedling phenotypes and GA treatment significantly affected the mean proportions of non-terminated (i.e. long shoots) and terminated (i.e. medium and short shoots) lateral axillary shoots (P<0.0001 for both) (Table 6.5). For the main effect of phenotypes, the mean proportion of non-terminated axillary shoots was significantly higher in the phenotypes of SS compared to other phenotypes, whereas the LS phenotype had a significantly lower mean proportion of non-terminated axillary shoots. Opposite patterns were recorded on the mean proportion of terminated shoots. The LS phenotype had a significantly higher mean proportion of terminated shoots compared to other phenotypes (Table 6.5). In contrast, the mean proportion of terminated shoots was significantly reduced in SS phenotype. For the main effect of GA treatment, the mean proportion of non-terminated axillary shoots was significantly increased (P=0.04) with GA. There was a trend (P=0.10) that the mean proportion of terminated shoots was reduced with application of GA (Table 6.5).

Table 6.5. Main effects of seedling phenotypes and the application of gibberellins (± GA) on the proportions of terminated and non-terminated of axillary shoots.

Main effects	ǂMean proportion of non-terminated shoots		ǂMean proportion of terminated shoots	
Phenotypes				
LS	3.8 [c]	**(15.1)**	9.2 [a]	**(84.9)**
SS	6.5 [a]	**(42.9)**	7.5 [c]	**(57.1)**
LMS	5.2 [b]	**(29.4)**	8.4 [b]	**(70.6)**
SMS	5.5 [b]	**(31.5)**	8.2 [b]	**(68.5)**
$LSD_{0.05}$	1.3		0.7	
***P*-value**	P<0.0001		P<0.0001	
GA treatment				
+ GA	5.6 [a]	**(33.2)**	8.1 [a]	**(66.8)**
- GA	4.8 [b]	**(25.7)**	8.6 [a]	**(74.3)**
$LSD_{0.05}$	0.7		0.5	
***P*-value**	P=0.04		P=0.10	
Interactions	P=0.18		P=0.19	

Means sharing the same letters are not significantly different at P=0.05 according to $LSD_{0.05}$ Test.
ǂData were transformed to square root for analysis.
Numbers in parentheses and bold are means of raw data.

6.3.2.5 The total number of shoots, total length, and total node number of lateral axillary shoots

The seedling phenotypes x GA treatment interactions were not significant for the mean total number of axillary shoots, mean total length, and mean total node number of lateral axillary shoots (P=0.72, P=0.44 and P=0.60, respectively) (Table 6.6). However, the main effect of seedling phenotypes significantly affected (P=0.0005) the mean total number of lateral axillary shoots (Table 6.6). The LMS and LS phenotypes had significant higher mean total number of lateral axillary shoots compared to SS and SMS phenotypes, and the lowest mean total number of lateral axillary shoots was recorded in SS phenotype. There was a trend (P=0.10) that the mean total length of lateral axillary shoots could be affected by the seedling phenotypes. The mean total length of lateral axillary shoots of SS phenotypes may have had lowered than other phenotypes (Table 6.6). There was also a strong trend that the mean total node number of lateral axillary shoots was significantly different between the seedling phenotypes, as P-value was close to significance (P=0.07). In addition, the mean total node number of lateral axillary shoots of LMS phenotype was significantly greater compared with SS phenotype (Table 6.6). The main effects of GA treatment significantly affected the mean total number (P=0.0003), mean total length (P<0.0001) and mean total node number (P<0.0001) of lateral axillary shoots. The GA treatment increased the mean total number of lateral axillary shoots compared to untreated phenotypes (Table 6.6). Similarly, the mean total length and mean total node number of axillary shoots were significantly increased with GA treatment.

Table 6.6. Main effects of seedling phenotypes and the application of gibberellins (± GA) on the total number of shoots, total length and total node number of axillary shoots.

Main effects	‡Mean total number of lateral axillary shoots		‡Mean total length of lateral axillary shoots (mm)		‡Mean total node number of lateral axillary shoots	
Phenotypes						
LS	6.1 ^{ab}	**(39.5)**	133.5 ^{a}	**(19142)**	20.3 ^{ab}	**(444.0)**
SS	4.4 ^{c}	**(20.9)**	122.3 ^{a}	**(15734)**	17.5 ^{b}	**(325.8)**
LMS	6.7 ^{a}	**(46.4)**	150.2 ^{a}	**(23930)**	22.0 ^{a}	**(513.2)**
SMS	5.4 ^{b}	**(31.3)**	130.4 ^{a}	**(17461)**	19.5 ^{ab}	**(394.1)**
$LSD_{0.05}$	1.3		29.4		4.4	
***P*-value**	*P*=0.0005		*P*=0.10		*P*=0.07	
GA treatment						
+ GA	5.1 ^{a}	**(41.8)**	150.8 ^{a}	**(23563)**	22.6 ^{a}	**(529.4)**
- GA	4.3 ^{b}	**(25.5)**	114.8 ^{b}	**(13822)**	16.6 ^{b}	**(291.4)**
$LSD_{0.05}$	0.7		15.7		2.4	
***P*-value**	*P*=0.0003		*P*<0.0001		*P*<0.0001	
Interactions	*P*=0.72		*P*=0.44		*P*=0.60	

Means sharing the same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ Test.
‡Data were transformed to square root for analysis.
Numbers in parentheses and bold are means of raw data.

The interpretation of main effects in Tables 6.4 requires consideration of interactions present in the data. We further analysed the data using Least Square Means Test (lsmeans). Even though not highly significant for some variables, it is worthwhile presenting the details of interactions in this study (Table 6.7). In LMS, LS and SS phenotypes, the mean length of long shoots was significantly shorter when treated with GA (*P*=0.03, *P*=0.04 and *P*=0.008, respectively), but not in SMS phenotype (*P*=0.93) (Table 6.7). The mean node number of long shoots did not significantly differ between GA treated and untreated in the LMS, LS, and SMS phenotypes (*P*=0.49, *P*=0.17 and *P*=0.54, respectively). However, there was a trend (*P*=0.11) that mean node number was reduced in LS phenotype with GA treatment. In SS phenotype, GA treatment significantly increased (*P*=0.01) the mean node number of long shoots. With GA treatment, the mean internode length of long shoots in LMS phenotype was significantly longer (*P*=0.004) compared to untreated shoots. However, no significant differences were found on the mean internode length of long shoots in other phenotypes (*P*=0.17, *P*=0.16 and *P*=0.21, respectively). In addition, the mean shoot CSA of long shoots was significantly smaller in the phenotypes of LMS, LS, and SS when treated with GA (*P*=0.0007, *P*=0.003 and *P*=0.03, respectively), except for SMS phenotype (*P*=0.36).

For the medium lateral axillary shoots, only the data for the LMS and SMS phenotypes are presented, as LS and SS phenotypes did not produce this shoot type (Table 6.7). With GA treatment, the mean length of medium shoots was significantly longer (P=0.02) for LMS compared to untreated phenotypes. Whereas in SMS phenotype, the mean length of medium shoots was not significantly different between GA treated and untreated (P=0.98). The mean node number of medium shoots was not significantly affected by the GA treatment for LMS and SMS phenotypes (P=0.34 and P=0.62, respectively). The mean internode length of medium shoots was also not significantly affected (P=0.92) by the GA treatment for SMS phenotype. However, the mean internode length of medium shoots of LMS phenotype was significantly increased (P=0.002) with GA treatment. GA treatment did not significantly affect the mean shoot CSA of medium shoots for both phenotypes (P=0.22 and P=0.40) (Table 6.7).

For LMS phenotype, GA treatment significantly increased (P=0.001) the mean length of short lateral axillary shoots compared to untreated shoots (Table 6.7), but not on other phenotypes. With GA treatment, the mean node number of short shoots was significantly greater in phenotypes of LMS and SS (P=0.04 for both). Furthermore, there were trends that GA treatment may have increased the mean node number of short shoots in phenotypes of LS and SMS (P=0.07 and P=0.11, respectively). No significant differences were found on the mean internode length of short shoots between GA treated and untreated shoots for LS, SMS and SS phenotypes (P=0.33, P=0.49 and P=0.32), except for LMS phenotype (P=0.01). The mean CSA of short shoots did not greatly differ between GA treated and untreated for all phenotypes. Overall, the characteristics of long shoots were most affected by the GA treatment regardless of seedling phenotypes, and the medium and short shoots were affected less (Table 6.7).

Table 6.7. The Least Square Means Analysis for the effects of seedling phenotypes and gibberellins treatments (± GA) on the characteristics of the proleptic axillary shoots.

		The characteristics of proleptic axillary shoots			
		Mean shoot length (mm)	**Mean node number**	**Mean internode length (mm)**	**Mean shoot CSA (mm^2)**
Long shoots					
LMS	**- GA**	1904.9*	27.8^{ns}	69.1***	74.4***
	+ GA	1401.6	24.9	57.4	48.2
LS	**- GA**	2082.3*	35.9^{ns}	58.9^{ns}	81.2***
	+ GA	1607.0	29.7	53.8	60.6
SMS	**- GA**	1636.3^{ns}	28.4^{ns}	58.4^{ns}	59.4^{ns}
	+ GA	1482.6	30.9	53.2	65.2
SS	**- GA**	2287.3***	37.3**	61.1^{ns}	82.7*
	+ GA	1482.6	27.4	56.3	65.3
Medium shoots					
LMS	**- GA**	509.5*	12.3^{ns}	40.5**	36.4^{ns}
	+ GA	803.8	14.2	56.5	29.1
LS	**- GA**				
	+ GA		*- Medium shoots were not available -*		
SMS	**- GA**	583.1^{ns}	13.3^{ns}	42.1^{ns}	38.1^{ns}
	+ GA	581.6	14.2	41.7	33.2
SS	**- GA**				
	+ GA		*- Medium shoots were not available -*		
Short shoots					
LMS	**- GA**	34.9***	3.6*	ǂ2.7*	20.1^{ns}
	+ GA	84.5	4.6	3.8	21.5
LS	**- GA**	45.0^{ns}	4.0^{ns}	ǂ3.0^{ns}	28.2^{ns}
	+ GA	61.9	4.8	3.4	26.9
SMS	**- GA**	48.2^{ns}	4.2^{ns}	ǂ3.3^{ns}	25.5^{ns}
	+ GA	61.1	5.0	3.6	26.1
SS	**- GA**	54.8^{ns}	4.6*	ǂ3.4^{ns}	27.2^{ns}
	+ GA	71.8	5.4	3.8	25.1

*, **, *** and ns - significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.0001$ and not significant, respectively according to Least Square Means (lsmeans) analysis.
The comparison was made between each pair of GA (±GA) treatment for each seedling phenotypes.
ǂData were transformed to square root for analysis.

We also noticed that the effects of GA treatment on the production of the tertiary lateral shoot branching (i.e. sylleptic axillary shoots) (Figure 6.5). Regardless of phenotypes, lateral sylleptic axillary shoots (sylleptic shoots) formed from buds on the actively growing main proleptic stems, two or three weeks after GA application (Figure 6.5A). Continuous application of GA caused these lateral sylleptic axillary shoots to grow, and produce a few small leaves (Figure 6.5B). At the end of the experimental period, we observed that these lateral axillary shoots (sylleptic) had developed into complete shoots (Figure 6.5C). In addition to these results, we noticed that the initiation of lateral axillary shoots outgrowth also occurred at the apical part of proleptic axillary shoots (Figure 6.5D). All data for all shoot types are presented in Tables 6.5 and 6.6 as a combined data for total length, total node number, and total number of lateral proleptic and sylleptic axillary shoots. These data represent the overall vigour for all phenotypes either treated with GA or not, and our results indicate that the architectural structure of seedling phenotypes that initially low-vigour such as LS and SS can be altered by the application of GA.

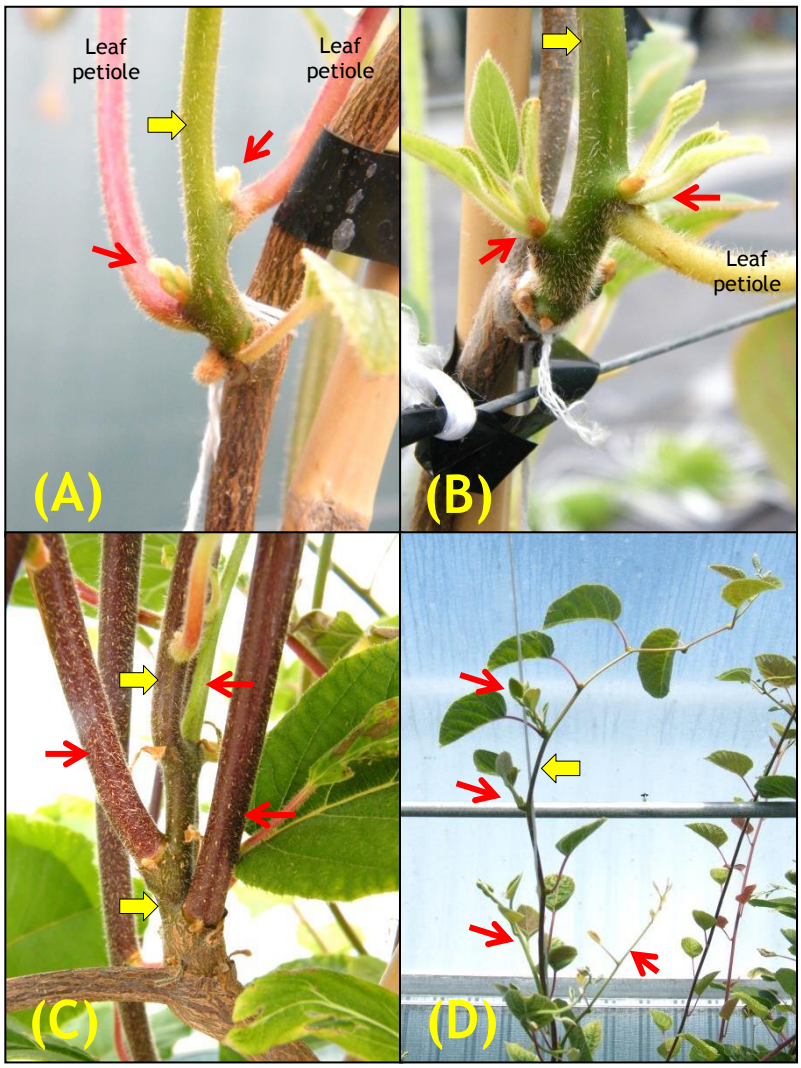


Figure 6.5. Responses of kiwifruit buds to the application of GA treatment (GA_3+GA_{4+7}) and GA stimulated lateral tertiary axillary shoots (i.e. sylleptic branching) in all phenotypes. (A) Lateral axillary buds breaking on the main stem of proleptic shoots to form sylleptic shoots. (B) Sylleptic lateral axillary shoots developed and formed a few leaves. (C) Sylleptic lateral axillary shoots developed into complete shoots at the end of experimental period. Pictures A until C: Axillary buds located at the base of the proleptic shoots. (D) Stimulation of lateral axillary shoots (sylleptic) on the buds located at the top of the apical proleptic shoots. *Yellow large arrows indicate the main stem of the proleptic shoots. Red small arrows indicate the stimulated lateral sylleptic axillary shoots.

6.3.2.6 The relationship between the final length and node number of axillary shoots for each phenotype

Figure 6.8 shows the effect of the seedling phenotypes and GA treatments on the relationship between the final length and node number of proleptic and sylleptic axillary shoots. The data for all shoot types (short, medium and long shoots) for each phenotype either treated (+GA) or untreated (-GA) were pooled together in one graph. All the seedling phenotypes from both treatments had displayed almost identical relationship between the final length and node number of axillary shoots. For the shoots that had 9 nodes or less (i.e. short shoots), the quadratic function is closely fitted to the plots as reported by Seleznyova et al., (2002) (Figure 6.6). However, for shoots that had 9 nodes or more (i.e. medium and long shoots), the linear regression is most suitable for the plots (Seleznyova et al., 2002). According to the regression analysis, there were strong positive quadratic relationships (R^2>0.60) between the lengths and node numbers for the shoots that had nine or less nodes for all phenotypes (Figure 6.6). Furthermore, strong positive linear relationships (R^2>0.70) were also found between the lengths and node numbers for each phenotype (Figure 6.6). Therefore, the results indicate that there were strong trends for all phenotypes that increasing node number is associated with increased shoot length, with gibberellin treatments (+GA) showing markedly similar relationships.

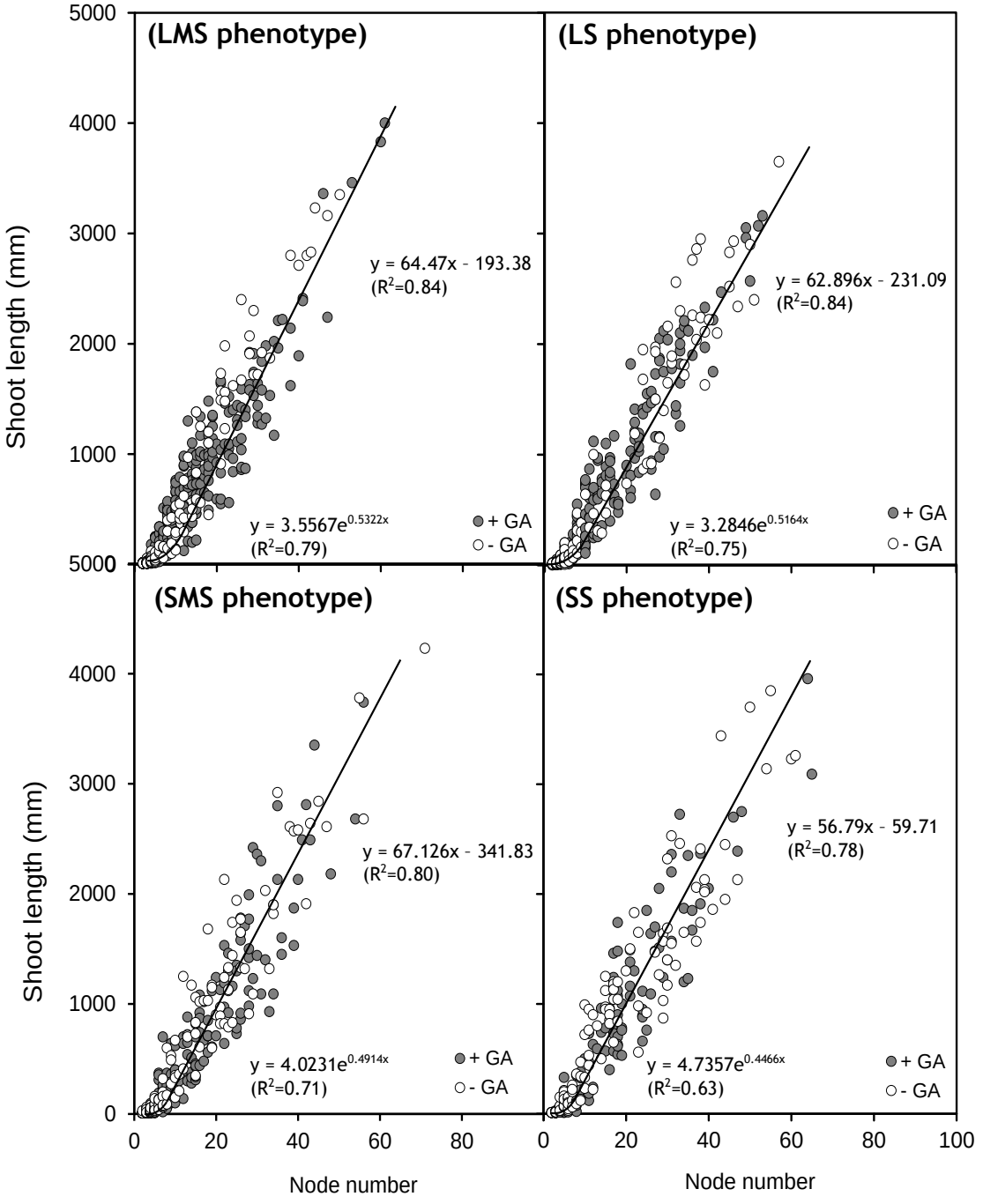


Figure 6.6. The relationships between the final length and node number of the axillary shoots (proleptic and sylleptic shoots) at the end of the experimental period from all seedling phenotypes and application of GA (±GA).

6.4 Discussion

6.4.1 Experiment 1

In fruit tree crops, the architecture and growth characteristics may have an important impact on the orchard management, orchard design, and future yield (Costes et al., 2006a; Lauri et al., 2008). Agronomic performances such as tree size and form, light penetration in the canopy, and flowering and fruit bearing habit could be affected by the architectural structures of the fruit trees (Costes et al., 2006a). Besides that, other characteristics or traits such as the variation in the branching habits and shoot types (i.e. long or short shoots), may also contribute to the differences in the architectural canopy of the fruit trees (Costes et al., 2006a), and are important for the early establishment of orchard (Lauri et al., 2008; Robinson, 2004; Van Oosten, 1976; Wertheim, 1978). Because these traits can be expressed in the early growth stage of trees (i.e. seedling stage), it should be evaluated as early as possible (Costes et al., 2006; Lauri et al., 2008). In kiwifruit, there is a lack of information on the selection of genotype and phenotype in relation to the vigour characteristics. Therefore, in order to extend our knowledge on the relationship between architectural traits and vigour of kiwifruit, the seedlings obtained from specific crosses were evaluated as early as the first growing season (Figure 6.1). The detailed architectural characteristics produced from the kiwifruit seedlings were presented and described in the results section (Section 6.3).

6.4.1.1 The phenotypic differences in the kiwifruit seedlings architecture

There were two different groups found in the kiwifruit seedlings population obtained from the specific-crosses (Figure 6.2 and Figure 6.3). The first group was the seedlings that have produced multiple shoots, either long or short shoots as their main primary shoots (i.e. multiple stem group) (Figure 6.2A and Figure 6.2C). Another group was the seedlings that have produced only one single shoot, either long or short shoots (i.e. single stem group) (Figure 6.2B and Figure 6.2D). The multiple stem groups represented the highest proportion in the seedling population with 62.8% compared to the single stem group with only 37.3% (Figure 6.3). These two groups can be further segregated into four distinct phenotypes based on their main primary shoots, which were; i) Long Multiple Stem (LMS) (Figure 6.2A), ii) Short Multiple Stem (SMS)

(Figure 6.2C), iii) Long Single Stem (LS) (Figure 6.2B), and iv) Short Single Stem (SS) (Figure 6.2D). These results indicate that there was a great variability in the initial architectural structure of kiwifruit seedlings. The variability in the seedling architecture in terms of the main primary shoots have been observed in other fruit trees such as apple (De Wit et al., 2002), olive (Hammami et al., 2011; 2012; Pritsa et al., 2003) and avocado (Barrientos-Pérez & Sánchez-Colín, 1983). In apple, De Wit et al. (2002) found similar groups based on the main primary shoot growth characteristics from the seedling population obtained from the crosses between 'Telamon' and 'Braeburn' apple, but they found that the non-branching group was larger than the branching group (De Wit et al., 2002). In other fruit trees such as olive, Prista et al. (2003) evaluated the seedlings from open pollinated crosses and they found that most of the seedlings can be divided into two different groups, seedlings with lateral shoots or single-stemmed seedlings. Further evaluation with a larger olive population, based on the main axis of primary shoots, found that the most frequent initial plant-form types for young olive seedlings can be grouped as a 'mono-axis' or single stem shoot rather than 'multi-axes' (Hammami et al., 2012). Therefore, there were similarities in our results (Figure 6.2 and Figure 6.3) on the grouping types of the seedlings obtained from breeding crosses with studies on other fruit crops (i.e. apple and olive).

6.4.1.2 The early architecture of kiwifruit seedlings based on the primary shoots characteristics

To facilitate the comparisons among the phenotypes, further measurements on the shoot length, node numbers, internode length and base diameter (converted into shoot CSA) were conducted. These characteristics may reflect the vigour of the kiwifruit seedlings evaluated in our study (Segura et al., 2006). There were highly significant differences in the main primary shoot characteristics between the phenotypes (Table 6.1). The main primary shoot characteristics in terms of length, node number, internode length, and shoot CSA varied between seedling phenotypes (Table 6.2). Most of the phenotypes that had long main primary shoots such as LS and LMS may have longer shoot length than other phenotypes (SMS and SS), with the mean values of shoot length for LS and LMS being 1701.3 mm and 842.7 mm, respectively. These values are almost two or three-times longer than the shoots from SS (446.1 mm) and SMS (311.5 mm) phenotypes (Table 6.2). The longer shoots length in LS and LMS are likely to be related to the

significant higher in the node number in these phenotypes. This also was confirmed with the correlation analysis, because we found a highly significant correlation (R^2=0.96) between the length and node number of the main primary shoots (Table 6.3). These results indicate that the initial vigour and growth habits of the seedlings are highly variable between the phenotypes.

The relationship between the architectural structures and the characteristics of kiwifruit shoots have been developed by Seleznyova and co-workers on mature vines of *Actinidia chinensis* (Seleznyova et al., 2002). Their study described the quantitative description of the different shoot types in kiwifruit vines based on the mode of node number distribution and also the presence or absence of neo-formed nodes (Seleznyova et al., 2002). There were three different shoot types found in kiwifruit; short, medium and long. The short shoots had nine or less nodes, and medium shoots had more than nine nodes. Both of these shoots were self-terminated. Long shoots were non-terminated and may have a total number of nodes up to 90 (Seleznyova et al., 2002). Comparing our data with the architectural description made by Seleznyova et al. (2002), we found that the primary stem of LS and LMS phenotypes could be classified as long shoots due being non-terminated and longer than the shoots from SS and LS phenotypes, even though the mean node number was less in LMS phenotype (Table 6.2). However, in our study, we may not expect the primary shoots of LMS and LS phenotypes to produce shoots of 90 nodes in its first few years until the vines are well established. Although the average shoots of SMS phenotype may have had only nine nodes and was supposed to be classified as short shoots, the average internode length was longer (>27 mm) than of that which were reported by Seleznyova et al., (2002). Therefore, for SS and SMS phenotypes, their main primary shoots may be classified as medium shoots.

Additionally, the growth habit parameters such as plant height (i.e. length), internode length, and stem size (i.e. diameter or stem cross-sectional area) are often used for estimating seedling vigour. Seedling vigour based on height and stem size of primary shoots have been widely used in practice in order to discard seedling plants with a long juvenile period. This practice needs to be done as early as possible, during nursery or greenhouse stage, rather than later in the field. In other fruit trees such as olive (De la Rosa et al., 2006; Pritsa et al., 2003; Santos-Antunes et al., 2005), plum (Hartmann & Engelhorn, 1990) and apple (De Wit et al., 2004; Lapins, 1969; Segura et al., 2006),

vigorous seedling growth is considered as an advantageous trait for obtaining a short juvenile period. For example, De la Rosa et al. (2006) observed that in olive seedlings, the height and diameter of shoots were highly correlated with the length of juvenile period. Our study also found that the height (i.e. length) of the main primary shoots was slightly correlated with the shoot CSA (Table 6.3), and this is similar to the previous study in olive, which also found similar evidence (Hammami et al., 2011). Other studies in olive (Santos-Antunes et al., 2005) and apple (Visser, 1970) reported that the vigour of seedlings based on the stem diameter and node number may have influenced the occurrence of the first flowering (i.e. on the length of the juvenile period). But, in some cases, there were contrasting results (e.g. Ariza et al., 2004; Pritsa et al., 2003). However, most of the previous studies did not find any correlation between juvenile period and yield. In kiwifruit, Cruz-Castillo et al. (1997) reported that the trunk size of kiwifruit vines may be an influential factor in separating vigour of scions and rootstocks, as they found that vines with a larger trunk size in the early stage of vines growth, generally had a larger mean fruit size (Cruz-Castillo et al., 1997). However, it should be noted, their study did not standardise the crop load of vines across the treatment. Besides that, there is little literature on the relationship between the early vigour and juvenility in kiwifruit.

6.4.1.3 The initial stem characteristics and its relation to vigour of kiwifruit seedlings

In this work, the variability in the main primary stems or shoots was clearly observed between kiwifruit seedling phenotypes (Figure 6.2). The number of main primary shoots of the kiwifruit seedlings was significantly different between phenotypes (Table 6.1). Comparing between phenotypes, it was noted that the LMS and SMS produced almost three-fold higher main primary shoots compared to LS and SS phenotypes (Table 6.2). However, our results also showed that the number of primary shoots of kiwifruit seedlings was negatively correlated with the length, node number and shoot CSA of main primary shoots and not correlated with the internode length of main primary shoots (Table 6.3). These results are consistent with numerous studies in apple (De Wit et al., 2002; Segura et al., 2006) and avocado (Barrientos-Pérez & Sánchez-Colín, 1983) that found within the branched plants, a weak correlation between the number of shoots and the characteristics of main primary shoots. Therefore, based on the results and the

evidence in previous studies, we also suggest that the branching in kiwifruit seedlings was not necessarily correlated with the vigour of the main primary shoots.

The above results supported the findings in Chapter Two of this thesis, and is in agreement with the study in apple by Fazio and Robinson (2008). Theoretically, branching processes is thought to be correlated with the vigour of the plants (Fazio & Robinson, 2008). However, as found in Chapter Two, the number of shoots produced from the primary shoots of 'Hayward' scions that have been grafted onto inter-specific hybrid kiwifruit rootstocks were not correlated with the vigour of the rootstocks and scions, as measured using shoot CSA. The results of the present study also showed that the differences in the number and length of shoots may be controlled by the differences in the degree and timing of shoots growth. These processes might be controlled by the genetic origin of the kiwifruit seedlings. According to Schmitz and Theres (1999), the initiation of axillary meristems (i.e. branching) and the pattern of shoot development may have been under the influenced of genes. The differences in the genetics of kiwifruit seedlings could be described by the highly significant differences in the characteristics of main primary shoots between the single stem and multiple stem groups (Tables 6.1 and 6.2). For example, phenotypes of SS and SMS had similar mean internode length, but the mean shoot length, node number, and shoot CSA differed significantly. Also, the phenotypes of LMS and LS produced shoots with similar type (i.e. long shoots), but they were different in terms of number of shoots (Table 6.2). Besides that, most of the multiple stem phenotypes displayed strong main shoot growth in terms of the characteristics of main primary shoots compared to single stem phenotypes (Table 6.2). These evidence in the present study were almost similar to the evidences found in the study with the seedlings from crosses between 'Telamon' and 'Braeburn' apple (De Wit et al., 2002). Furthermore, another study with seedlings obtained from the open pollinated of dwarf avocado 'Colin V-33', (Barrientos-Pérez & Sánchez-Colín, 1983) found that most of the multiple stem seedlings are shorter than those from single main primary shoot (Table 6.2). Our results are in agreement with this study, because most of the multiple stem phenotypes were significantly shorter than non-branched phenotypes (Table 6.2). Therefore, we suggest that the highly significant differences in the number of shoots and architectural structures among phenotypes may be attributed to the differences in the genetic components and/or origin of the kiwifruit seedlings. However, it is largely unknown whether factors such as environmental

condition and hormonal signalling during the seedling germination may have influenced the initial shoot production, as these factors are certainly affect the branching process (Evers et al., 2011; Kerstetter & Hake, 1997).

6.4.1.4 Parameter relevance related to the vigour of the kiwifruit seedlings

In this study, we tried to reveal the relevance of the initial shoot characteristics (i.e. branching, shoot length, node number, internode length and shoot CSA) in defining the growth habit and architecture of kiwifruit seedlings. Besides that, we used simple visual classification and described the normal morphological characteristics that can be found on the samples. Based on these simple approaches, the most significant characteristic with highest ability in describing the difference among the architecture of kiwifruit seedlings will be largely useful in future plant breeding programmes in developing specific cultivars. Based on the data collected and analysed, the characteristics such as the length, node number, internode length and shoot CSA were discriminated strongly among the phenotypes (Figure 6.4). The main primary shoot length was also strongly correlated with other characteristics such as node number and internode length (Table 6.3), as was confirmed by PCA analysis (Figure 6.4A). In kiwifruit, the length and node number are the most important variable in determining between different shoot types in kiwifruit and may contribute to the characteristics of plant form (either low- or high-vigour) (Saleznyova et al., Clearwater et al., 2006; 2002). Even though the shoot CSA seems to be less correlated with other characteristics, especially with the number of main primary shoots (Table 6.3), but some studies used shoot CSA to estimate vigour (e.g. Barden et al., 2002; Khatamian & Hilton, 1977). Studies on the rootstock effects in mature kiwifruit vines conducted in Motueka, New Zealand indicated that the scion growth may be affected by the vigour of rootstocks based on the stem or trunk size (i.e. trunk cross-sectional area) (Friend et al., 2014). Similar results were also found in Chapters Two and Three in this thesis, as we found that there were strong trends that vigour of grafted ‘Hayward’ scions, as measured by the trunk cross-sectional area may be correlated with the vigour of the inter-specific hybrid rootstocks. Although all these characteristics (i.e. plant height, node number, internode length and shoot CSA) are widely used indicators to estimate vigour of the seedlings (2012; e.g. Hammami et al., 2011; Segura et al., 2006; Seleznyova et al., 2002), they were not sufficient to evaluate vigour in this study. Indirect measurements such as plant height, node number,

internode length and shoot CSA did not represent the whole vigour or total growth and not satisfactory in describing the architecture of kiwifruit during the initial stage of seedlings growth. We suggest that the use of total plant dry matter could be more meaningful to describe the vigour of the seedling phenotypes. However, our data is limited with only one sample vine available per phenotype. Thus, destructive sampling could not be conducted in our study to measure the dry matter of the whole seedlings. As well as dry weight measurement, molecular markers linked to the dwarfing traits must first be identified and established, as reported in apple (Pilcher et al., 2008; Rusholme et al., 2003) and pear (Knäbel et al., 2015). This approach would enable the selection of desirable rootstocks at the seedling stage, thereby reducing the need for time-consuming and expensive field trials.

Even though initial shoot number varied significantly among phenotypes (Tables 6.1 and 6.2), it was negatively correlated with other characteristics, particularly with the internode length of main primary shoots (Table 6.3), and this was confirmed by the PCA analysis (Figure 6.4A). These results suggest that the initial shoot number of kiwifruit seedlings is not associated with the vigour of the seedlings, especially the growth of the main stem. Although the basis for the initial shoot production during initial seedling growth is not fully understood, it may have significant influence on the future physiological architecture and agronomic of the fruit tree crops that are related to the planting design, canopy light interception, tree size and flowering and fruiting habits (Costes et al., 2004). However, the relationship between initial vigour of kiwifruit seedlings and the productivity is has not been established and warrant further observation. The PCA plot in Figure 6.4B indicates that there was a large variability amongst kiwifruit seedlings tested in this study, even though some grouping (especially for SMS phenotypes) were quite large compared to the phenotypes of LMS, LS and SS (Figure 6.4B). This result may suggest that there is a huge variation in the genetic origin of these kiwifruit seedlings that may have had significant influence on the initial architecture of the seedlings. In fruit trees management, abundant lateral shoots or excessive branching is an undesirable trait due to the increased labour cost for pruning, as reported for olive (Santos-Antunes et al., 2005). Recent study also found that the use of a highly multi-stemmed parent is not recommended in olive breeding because of its tendency to transmit this characteristic to its descendants (Hammami et al., 2011). In kiwifruit, excessive vegetative growth that is caused by the fast-growing non-terminated

long shoots is problematic in kiwifruit management because it may cause dense vine canopies (Miller et al., 2001). High proportions of fruiting terminated shoots (i.e. short and medium shoots) are wanted in production of kiwifruit. It is largely unknown in kiwifruit whether the multiple stem genotypes or phenotypes may have inherited this characteristic to their descendants, as no detailed studies have been conducted to date. However, it has been shown that kiwifruit rootstocks can alter the proportions of axillary shoot types on the grafted scions (Clearwater et al., 2006), indicating that the choice of root systems may have had significant influence on the grafted scions. In kiwifruit, the low-vigour and terminated shoots are advantageous due to the higher proportion of bud break, which produced a high flower number because being previously well exposed to sunlight at the end of the growing season (Clearwater et al., 2004). Therefore, it would be reasonable to suggest that the selection of new cultivars that can confer these types of lateral branching traits would be beneficial to the kiwifruit management.

Differences amongst phenotypes are likely to have a hormonal basis. Even though no measurement of the endogenous hormonal level was conducted in our study, we suggest that the low-vigour phenotypes (i.e. SMS and SS) could have had lower level of particular endogenous hormones, especially GA. Our results indicate that SMS and SS phenotypes tended to produce terminated shoot types (i.e. medium shoots) rather than long non-terminated shoots (Table 6.3), suggesting that GA could be involved in shoot termination processes of these phenotypes. Vattiprolu (2011a) reported that GA were effective in stimulating apical and sub-apical meristems of kiwifruit shoots, as she found that exogenous application of GA to the 'Hayward' kiwifruit immediately after bud break had transformed the potential short and medium terminated shoots into long non-terminated shoots. It is generally known that dwarfing characters in plants were always related to the low level of endogenous GA. However, the information on the vigour aspect in relation to endogenous hormones is still lacking in kiwifruit vines. However, a number of classical studies in fruit trees have revealed that the genetic dwarf plants produced deficient amount of GA, as found in apple (Grochowska et al., 1984; Looney & Lane, 1984; Steffens & Hedden, 1992; Yadava & Lockard, 1977). Yadava and Lockhard (1977) found that the GA-like compounds were at the lowest level in the roots of the dwarfing apple rootstocks M.9 compared to vigorous rootstocks MM.111. Another study also found that the dwarf apple seedlings of 'McIntosh Wijcik'

only had one-third of the GA content compared to other apple seedlings (Looney & Lane, 1984). According to Faust (1989), there are four components that contributed to the genetic control of tree size; internode length, the growth rate of the trees, position of branching and branching angle. Similar to our results (Figure 6.2 and Table 6.2), the seedling phenotypes of SMS and SS had significantly smaller stature compared with the phenotypes of LMS and LS, and this may indicate that these phenotypes could be genetically dwarfed. However, it should be noted that the shoot growth could be influenced by environmental factors such as the season (Faust, 1989). Therefore, to identify whether the phenotypes of the kiwifruit seedlings used in this study is genetically low-vigour, these phenotypes need to be evaluated for a couple of seasons, together with the measurement of endogenous GA levels, and possibly other hormones in the future.

Costes et al. (2004) stated that studies in genetic and breeding for specific plant architecture require firstly, identification and selection of characteristics or traits that should be evaluated to identify diversity. In breeding practice, a search is always being made for characters which can be evaluated in the juvenile period, and are correlated to productivity in the mature trees or vines, in order to discard undesirable plants at an early stage of seedling development. For kiwifruit, in order to develop potential low-vigour vines (i.e. dwarfing), the important characteristic is shoot termination. In apple, this single trait is expressed by dwarfing rootstocks across different growing environments (Foster et al., 2016; van Hooijdonk, 2009) and also has been expressed by the kiwifruit rootstocks on the grafted vines (Clearwater et al., 2006). However, in kiwifruit, little attention has been given to the initial architecture and growth habit especially in selecting the potential genotypes or phenotypes in cultivar or rootstock breeding programmes. Therefore, assessments on the architectural characteristics and growth habit of kiwifruit in the seedlings stage are essential for the selection of new potential cultivar and rootstock in the future. We are interested in the seedling genotype or phenotype that has low-vigour ability. These types of seedlings may be valuable for the development of low-vigour scions or can be used as vigour-controlling rootstocks. Besides that, these seedlings may be suitable as a parent source for further breeding programmes. We also believe that these types of seedlings may have long juvenile period based on their initial growth characteristics. This might be the reason of why these seedlings were discarded during initial breeding selection. Therefore, these types

of seedlings and their potential traits such as branching type and shoot termination need to be further evaluated in field trial. Conventional breeding using phenotypic selection requires five to seven year per cycle to complete (van Nocker & Gardiner, 2014). Therefore, other methods such as 'marker-assisted selection' can be reliable approached for kiwifruit breeding to avoid any problems related to conventional plant breeding (review by Francia et al., 2005).

In conclusion, we observed distinct differences between phenotypes in the kiwifruit seedlings population, particularly the number of main shoots per phenotypes. The significant differences found in the shoot characteristics of the seedlings could be due to the differences in the shoot types produced by each phenotype. The phenotypes of LS and LMS produced long shoots, whereas phenotypes of SS and SMS produced medium shoots. There were inverse correlations between the number of main primary shoots and the characteristics of main primary shoots, suggesting the initial shoot development process in kiwifruit was independent to the vigour of the main shoots. We suggest that the differences in the shoot architectural structures among phenotypes may be attributed to the differences in the genetic components and/or origin of the kiwifruit seedlings. In this study, the parameters such as shoot length, node number, internode length and shoot CSA are useful attributes for the selection of kiwifruit seedlings. Even though number of primary shoots had a weak correlation with length, node number, internode length and shoot CSA, we strongly suggest that the 'multiple-stem trait' may have had significant influence on the future architecture of kiwifruit vines. Shaw (1988) mentioned that "the genetic correlation implies a relationship between the genes that conditioned two traits, whereas phenotypic correlation results from both shared genetic effects and a common response of independent genetic systems shared by the same environments. If the two types of correlation are similar to each other, the selection on phenotypes will also be a fairly 'accurate' selection of genotype" (Shaw, 1988). Therefore, it would be reasonable to suggest that the selection based on the phenotypic architecture of kiwifruit can also segregate the differences in the genotypic of kiwifruit seedlings as well. Besides that, it is also suggested that further study on marker-assisted selection is needed to identify the potential genes as another possible approach in breeding of a potential rootstock or low-vigour cultivar in kiwifruit.

6.4.2 Experiment 2

The objective of this experiment was to evaluate whether the phenotype of kiwifruit seedlings can be manipulated though the application of gibberellins in order to modify the vigour of the seedlings. In Experiment 1, there were four distinct phenotypes in the kiwifruit seedlings population that were obtained from the specific-crosses. We suggest that these phenotypes (Figure 6.2) may have had different levels of endogenous hormones, especially gibberellins (GA) that contributed to the different shoot architectural structures. We were interested in the phenotypes that have low-vigour ability (i.e. dwarfing effect) such as SMS and SS, and whether the application of GA treatment (GA_3+GA_{4+7}) can modify their architecture and morphology to become similar to the other phenotypes (i.e. LMS and LS). In theory, the main reason for this approach was firstly, to allow fulfilment of the desired canopy size by GA application, especially for low-vigour vines and then application of GA could then be stopped to channel a greater carbohydrate allocation to the reproductive stage instead of the vegetative stage. Secondly, by using low-vigour scions, the number of rootstocks available would be increased for other characteristics such as disease resistance, regardless of the rootstock vigour, as demonstrated in a study with apple (Bulley et al., 2005).

In kiwifruit, dwarfing or low vigour rootstocks are still lacking and growers still rely on horticultural manipulation, such as girdling, in order to control the excessive vegetative vigour (Palmer, 2007; Warrington, 2000). Therefore, any approaches that can give an advantage for kiwifruit management are worthy of investigation. Besides that, this approach may be useful to 'speed-up' the transition from juvenile phase to maturity phase in kiwifruit, because kiwifruit vines have a long period of vegetative growth (Palmer, 2007). The studies on the effects of exogenous GA on the shoot growth has been demonstrated in a wide range of fruit tree species such as peach (Tagliavini & Looney, 1991), avocado (Morales-Salazar et al., 1997) and citrus (le Roux & Barry, 2010; Monselise & Halevy, 1962). Most of the studies above used GA in order to reverse the effects of gibberellins-biosynthesis inhibitors such as paclobutrazol and prohexadione-calcium. Application of GA, either GA_3, GA_4, GA_7, and/or together with other chemical inductions such as BA (i.e. 6-benzylaminopurine or Promalin) has also been used to induce greater branch length in the nursery stage as reported for pear

(Palmer et al., 2011) and sweet cherry (Elfving et al., 2011). However, information on the application of GA on shoot growth and branching are still lacking in kiwifruit management, especially in the relation to vigour aspects, except for Vattiprolu (2012).

6.4.2.1 Effects of phenotypes and gibberellins on the characteristics and branching of lateral proleptic axillary shoots

6.4.2.1.1 The characteristics of lateral proleptic axillary shoots

In this study, application of GA immediately after the bud break had a significant influence on the characteristics of different proleptic axillary shoots in kiwifruit (i.e. long, medium and short shoots). In long proleptic axillary shoots, it was surprising that the length of the shoots was significantly shorter with GA treatment in all phenotypes, due to the reduction in the node number and internode length (Table 6.4 and Table 6.7). The results in the present study differed to a previous study by Vattiprolu (2012). They found that GA treated on the single shoot of *A. deliciosa* kiwifruit cultivars with similar concentration had increased the length, thus producing lateral proleptic axillary shoots that were slightly longer than untreated shoots (see Chapter 6, Vattiprolu, 2012). These discrepancies were probably due to the differences in the genotypes or cultivars between her study and our study, because Vattiprolu (2012) tested the GA on *A. deliciosa* cultivar. Therefore, the mechanisms of GA effects on the shoot growth must be different between kiwifruit genotypes or cultivars. Nevertheless, our results are in agreement with a study on sweet cherry branching, which also found that the mean length of GA treated shoots was slightly shorter than untreated shoots (Elfving et al., 2011).

In addition to these results, reductions in length, node number, internode length and shoot CSA of long lateral axillary shoots in GA treated vines could be due to the differences in the morphology between treated and untreated shoots (Table 6.4). Our results also demonstrated that the phenotypes x GA interactions were significant for shoot CSA and approached significance for length (Table 6.4 and Table 6.7), indicating that the length and size of long axillary shoots may be regulated and dependent on the amount GA supplied to the kiwifruit seedlings phenotypes. As observed in this study, regardless of phenotypes, most of the long lateral proleptic axillary shoots from GA treated vines became very thin and very slender compared to the long shoots from untreated vines, thus producing the shoots with slightly shorter and smaller shoot sizes

(Table 6.4). Shorter and smaller long proleptic axillary shoots from GA treated phenotypes (Table 6.4 and Table 6.7) were possibly due to limited supply of assimilates and nutrients to this shoot, since GA may have diverted the supply of assimilates and nutrients for developing of new vegetative organs to existing growth (i.e. new lateral shoots) (Iqbal et al., 2011), and these effects could be demonstrated by the increases in the total number of shoots for all phenotypes (Table 6.6).

Previous studies on the effects of GA tested on various plant species (Marth et al., 1956) and citrus seedlings (Monselise & Halevy, 1962) have also reported that GA treated shoots became thin and slender compared to untreated shoots. Even though Vattiprolu (2012) found opposite results with our study, but she did mention that the GA treated shoots became very thin and slender compared with untreated shoots. However, no credible data were reported to support their statements on the size of the lateral shoots. Our results in the present study may suggest that GA application may have altered assimilates allocation within the vines by diverting the carbohydrate and possibly nutrients to the new growing tip of the shoots (i.e. apical and/or sub-apical meristems). We believe this effect might change the proportion of dry matter of the whole vines between GA treated and untreated (Iqbal et al., 2011). Therefore, further research into optimising gibberellin concentrations to stimulate shoot growth without reducing shoot diameter excessively is required.

GA treatment on the phenotypes of LMS and SMS did not significantly affect the characteristics of axillary medium shoots, except for the internode length (Table 6.4). Nevertheless, we observed that the medium lateral axillary shoots in GA treated shoots were slightly longer, and this could be due to the longer internode length. It was interesting to note that only medium lateral axillary shoots (terminated shoots type) were available in phenotypes of LMS and SMS, and did not present for the phenotypes of SS and LS. The phenotypes of SS and LS only produced long non-terminated and short terminated axillary shoot types (Table 6.4 and Table 6.7). The reason why medium shoots were not available in the single stem phenotypes (i.e. LS and SS) probably due to GA having directly influenced the vegetative phase change of shoots either terminated or continue to growth. Our results indicate that SS and LS phenotypes tended to produce more number of long non-terminated shoots (Table 6.5), suggesting that SS and LS phenotypes may have more responsive to exogenous GA, causing conversion of

medium shoots (terminated type) to long shoots (non-terminated type). In addition, any growth alterations, for example, by using exogenous GA treatment during the active buds development, are likely to have influenced the activity of shoot apical meristem (SAM), and consequently the termination of shoots (van Hooijdonk et al., 2010). In kiwifruit, although all shoots eventually self-terminate, the difference between terminated and non-terminated shoots types is in the timing of growth termination (Seleznyova et al., 2002).

GA treatment may have had stimulated shoots to become non-terminated shoots by stimulating the SAM. This has been demonstrated in a previous study using similar GA type and concentration on 'Hayward' kiwifruit cultivar (Vattiprolu, 2012). Even though many kiwifruit shoots have potential to terminate soon after the bud break, some of the shoots will still produce leaves until the end of growing season (Seleznyova et al., 2002). It was also noted in our study that the seedling phenotypes x GA treatment interactions closed to significance for length (P=0.08) and were significant for internode length (P=0.01), suggesting that the regulation of the length and internode length of medium axillary shoots may be determined by the amount GA supplied to the LS and SS phenotypes (Table 6.4 and Table 6.7). Therefore, the fate of medium shoots could be influenced by the interaction between genotypes or phenotypes, and the level of GA supplied. It has been also noted in many literatures that the shoot fate of kiwifruit during an early stages of shoot development can be also affected by a few factors such as temperature (Seleznyova & Halligan, 2006), the root system (i.e. rootstock) (Clearwater et al., 2006), and growth manipulations (i.e. girdling or pruning) (Piller et al., 1998).

GA treatment increased the length of short axillary shoots by almost 50% compared with untreated short shoots, but the length did not differ statistically between the phenotypes (Tables 6.4 and 6.7). However, GA treatment did not affect the size of short (i.e. CSA), and the CSA of short shoots was almost comparable between the phenotypes. Interestingly, the GA treatment may have significant influence on the node number of short lateral axillary shoots, and it also varied between the phenotypes (Tables 6.4 and 6.7). Therefore, it seems that the GA treatment had a significant influence on the length of short lateral axillary shoots, and also caused increase in node number and longer internode length (Tables 6.4 and 6.7), presumably the short shoots were stimulated to grow for a slightly longer duration with GA treatment. These results

also suggest that the level and responses to GA may be expected to vary between seedling phenotypes. Our results suggest that GA significantly influenced the apical and sub-apical meristem and was important for the regulation of node neoformation in kiwifruit (Figure 6.6). GA treatment also had stimulated all types of shoots (i.e. short, medium and long shoots) to produce more nodes and elongated internode (Tables 6.6, 6.7, and Figure 6.6). It was interesting to note that the individual shoot length increased as the node number increased with a good correlation for all seedling phenotypes, regardless of GA treatments (Figure 6.6). These results indicate that GA stimulates apical and sub-apical meristem either to produce more nodes or direct effect on cell elongation (Table 6.7 and Figure 6.6).

The ability of GA to affect the apical and sub-apical of kiwifruit shoots could also be under the influence of other types of hormones such as auxin (IAA). It has been demonstrated in pea plants that both GA (especially GA_1) and IAA are required for internode extension by the sub-apical zone of SAM (Ross et al., 2003). Furthermore, GA_1 stimulated elongation is normally associated with an increase in IAA level (Ross et al., 2003). However, in kiwifruit, it is largely unknown how these hormones may interact in controlling the duration of extension growth of shoots, since some of the shoots were still terminated even after the application of GA. The result in this chapter hold promise for developing low-vigour kiwifruit vines that terminate shoot growth early, thereby providing assimilates to fruit sinks rather than vegetative growth. In other plants such as *Salix*, GA is believed to play an important role in the regulation of shoot tip abscission (i.e. termination) (Juntilla, 1976). According to Juntilla (1976), the shoot tip abscission or termination of shoots was believed to be due to the reduction in the level of GA_3 biosynthesis and GA_1, but this could be reversed by GA_3 application. Another study with composite apple trees, van Hooijdonk et al., (2010) reported that endogenous GA may have had an indirect effect on the axillary bud outgrowth by prolonging the duration of extension growth. In their study, they found that earlier shoot termination imposed by M.9 dwarfing rootstocks or auxin transport inhibitor (i.e. NPA) due to decreased in IAA transport from the scion to the root, could be reversed using GA application (GA_{4+7}) to the scion. Similarly, our results strongly support that the GA has significant influence in regulating SAM and has been also implicated in shoot termination of kiwifruit, supported the previous study in kiwifruit (Vattiprolu, 2012) and apple (van Hooijdonk et al., 2010). Based on our promising findings in this chapter,

it is therefore possible that low-vigour kiwifruit phenotypes may be contained low GA level, but exogenous GA could be used to stimulate early canopy development for fruiting production.

Even though our results showed that internode length of shoots (especially of long and medium lateral axillary shoots) was longer in GA treated vines regardless of phenotypes; indeed, the internode length of axillary shoots was not affected by the GA treatment (Figure 6.6). These figures had revealed an important finding in kiwifruit that the shoots of the same node number have a similar length irrespective of whether GA is applied. Therefore, the actual internode length was not modified by the GA treatment. Although the typical effects of GA on shoot growth are increased internode elongation (Brian, 1959) as reported in some fruit crops such as peach (Tagliavini & Looney, 1991) and citrus (le Roux & Barry, 2010) including kiwifruit (Vattiprolu, 2012), the interpretation in previous study of the GA effect on mean internode lengths can be completely misleading. Our results have demonstrated that GA treatment does not increase growth rate, but rather increases the duration of node formation for a larger proportion of shoots (Table 6.6 and Figure 6.5). Therefore, many shoots present will have more nodes and are longer. Shoots that have undergone a longer duration of node neo-formation irrespective of GA treatment will have more nodes and longer internodes than shoots that terminate earlier, which will have fewer nodes, be shorter, and have shorter internodes. Therefore, mean internode lengths are a completely misleading measurement. In kiwifruit, previous study has thought that the effect of GA on internode length of shoots resulted from the higher level of bio-active GA_1 (Vattiprolu, 2012). However, application of GA to kiwifruit shoots at high concentration may have had negative effects to the elongation and extension of SAM, due to supra-optimal level of GA. For example, Vattiprolu (2012) tested a particular range of GA (GA_3+GA_{4+7}) on kiwifruit shoots, and found GA at 800 mg L^{-1} did not stimulate shoot elongation and may reduce the node number, although the internode length of kiwifruit shoots was increased. In contrast, GA concentration at 500 mg L^{-1} could increase node number and internode length of kiwifruit shoots by regulating of both cell division and cell elongation (Vattiprolu, 2012). At 1000 mg L^{-1}, GA only stimulates internode elongation by activation of sub-apical meristem, increasing lateral shoot length, but not node production (Vattiprolu, 2012). Thus, in Vattiprolu study, it was assumed that GA at higher concentration may have damaged the apical meristem and only the young

internodes would be left to grow (Vattiprolu, 2012). However, at optimal level, GA promotes neoformation at the nodes (stimulation of the apical meristem) of kiwifruit and this is the dominant effect on shoot length, similar to apple (van Hooijdonk, 2009).

6.4.2.1.2 The branching of proleptic and sylleptic axillary shoots

In our study, GA concentration at 500 mg L^{-1} was chosen and used in order to obtain the maximum effects for all phenotypes without damaging the shoots, and also to stimulate both apical and sub-apical meristems, this concentration was based on a previous study testing the ability of GA to induce branching on two commercial kiwifruit cultivars, *A. chinensis* cv. 'Hort16A' and *A. deliciosa* cv. 'Hayward' (Vattiprolu, 2012). In our study, we used a similar GA concentration, but on the different kiwifruit seedling phenotypes. This enables us to assess the interactions between phenotypes and GA on the branching patterns of these seedlings (Section 6.4.2.2). We found that GA treatment on different kiwifruit phenotypes significantly increased the shoot branching of kiwifruit in term of total number of proleptic and syleptic axillary shoots for each phenotype (Table 6.6 and Figure 6.5). Interestingly, the low-vigour phenotypes (i.e. SS and SMS) still produced the lowest number of branching compared with other phenotypes (i.e. LS and LMS) (Table 6.6). This result may reflect the low-vigour ability of these phenotypes as we found less branching was produced, even after application of GA. GA treatment also caused a significantly greater mean total length and possibly the mean total node number of axillary shoots for all phenotypes (Table 6.6). It was interesting to note, that the SMS and SS phenotypes still exhibited low-vigour ability, because we found these phenotypes still had the lowest total final mean total number, length and node number of lateral axillary shoots (Table 6.6).

In this study, photographs of the GA effects on the kiwifruit shoots were taken in order to support our quantitative data (Figure 6.5). Regardless of phenotypes, we found that the GA treatment stimulated the buds at the base of the proleptic shoots to form sylleptic axillary shoots although the apex of proleptic axillary shoots were still actively growing (Figure 6.5A). These lateral sylleptic axillary shoots continued to develop and had a few small leaves after two or three weeks (Figure 6.5B). We observed that most of the buds located at the base of proleptic shoots are responsive to the GA treatment. Consistent supply of GA had caused these sylleptic lateral axillary shoots continue to grow and develop into normal shoots (Figure 6.5C). However, it was also observed that

these shoots were slightly thin and slender in shape (data not shown). Another interesting result found in this observation was some of the buds located at 15 nodes from the top of apical shoots also produced lateral axillary shoots even though the apical shoot were still actively growing, similar to the results found at the base of the shoots (Figure 6.5D). These results indicate that GA absorbed by the leaves can be transported to the rest of the plants via the phloem stream (Elliott et al., 2001), thus affecting the buds located on the top of apical shoots. According to Elliott et al., (2001) in their work with pea, gibberellin-biosynthesis is specially localised in plant organs such as young, actively growing buds, leaves, and upper internode. Therefore, any additional GA (for example application of exogenous GA) is likely to be utilised by these plant organs for enhancing growth. According to Elfving et al. (2011) in study with sweet cherry branching, the release of lateral axillary bud outgrowth by GA treatment could be due to the interaction with the apical-dominance control system in the plant, rather than any direct effect on cell elongation. Our results in Figure 6.5 are also similar to the study on *Jatropha curcas* branching (Ni et al., 2015). Study on *Jatropha curcas* found GA (especially GA_3) was highly effective at stimulating shoot branching compared to synthetic CK (6-benzyladenine) (Ni et al., 2015). Other previous studies have also reported similar evidences on the involvement of GA in stimulation of axillary bud outgrowth (i.e. branching), for example in *snapdragon* plants and citrus seedlings (Marth et al., 1956), sweet cherry (Elfving et al., 2011), avocado (Morales-Salazar et al., 1997) and forest trees (*Pinus sylvestris* and *Picea glauca*) (Little & McDonald, 2003).

Therefore, our results strongly indicated that GA plays an important role in the regulation of shoot branching in woody plant such as kiwifruit by affecting the apical and sub-apical bud meristem (Tables 6.4, 6.6 and Figure 6.5). The stimulation of apical and sub-apical buds to break and develop into lateral axillary shoots branching in perennial plants is also dynamically controlled by the other important hormonal signalling such as auxin (i.e. IAA) and CK (Campoy et al., 2011; Costes & Guédon, 2002; Faust et al., 1997). Certainly, GA is needed for IAA synthesis at the shoot apex and both of these hormones (especially GA_1) are required for internode extension by the sub-apical zone of the SAM (Ross et al., 2003). Furthermore, GA_1 stimulated elongation is normally associated with an increase in IAA level (Ross et al., 2003). Therefore, the involvement of IAA in regulating branching of kiwifruit should not be discounted. Another hormone, CK also plays a significant function in regulating bud outgrowth,

because the lateral bud outgrowth is thought to have closely correlated with CK level in the buds (Tanaka et al., 2006). However, it was found in kiwifruit that exogenous application of 6-Benzylaminopurine (BAP), a synthetic CK did not stimulate axillary shoots (i.e. branching) of kiwifruit, but GA alone appeared to increase the number of lateral axillary shoots of *A. chinensis* and *A. deliciosa* cultivars (Vattiprolu, 2012). It would be reasonable to suggest that the initial release of young kiwifruit buds may already be released by the CK but not be able to grow due to lack of GA. Another possibility is that the additional supply of GA may interact with CK, because high CK levels are also associated with increased branching (Beveridge et al., 1997). As demonstrated in a current study with *Jatropha curcas* seedlings (Ni et al., 2015), application of GA or synthetic CK (6-benzyladenine) to the buds only gave a very small effect in stimulating axillary outgrowth to form branching.

However, combined application of both hormones may have had significant 'promotive' effects to the bud outgrowth (Ni et al., 2015). Therefore, on the basis of the results of this study and previous literatures (e.g. Elfving et al., 2011; Ni et al., 2015; Vattiprolu, 2012), we suggest that the effectiveness of any hormones (e.g. GA, IAA and CK) in either inhibiting and/or releasing the bud outgrowth in kiwifruit, depended on the stage of growth of the shoots (Ali & Fletcher, 1970). Even though the role of CK for stimulating the apical meristem in kiwifruit is less clear, the extensive crosstalk between other hormones such as IAA and GA in regulation of meristem activity suggest that they are all very important in this process (Shani et al., 2006; Su et al., 2011). Besides GA, IAA and CK, a new group of hormones, strigolactones (SLs), produced in plant roots, has been found to be involved in the inhibition of shoot branching in plants (Gomez-Roldan et al., 2008). Even though some studies have demonstrated that the SLs may be involved in kiwifruit branching (e.g. Ledger et al., 2010), the actual involvement of SLs in kiwifruit and its effects on kiwifruit shoot branching is still elusive. Therefore, further studies on the role of SLs in regulating kiwifruit branching of different phenotypes are warranted in the future (Appendix 11).

6.4.2.2 Interaction between phenotypes and gibberellins in regulating overall vines architecture

In this study, the differences in the shoot types of kiwifruit (i.e. long, medium and short shoots) from both GA treated and untreated phenotypes were calculated in terms of proportion in non-terminated and terminated shoots (Table 6.5 and Figure 6.5). As reported previously, the non-terminated shoots were presented by the long shoots and the terminated shoots were represented by the short and medium shoots (Seleznyova et al., 2002). Similar terminology was used to evaluate the canopy architecture of *A. chinensis* cv. 'Hort16A' when grafted onto different vigour of kiwifruit rootstocks (Clearwater et al., 2006). In this study, application of GA treatment to all phenotypes is likely to affect the proportion of terminated and non-terminated shoots (Table 6.5). GA treatment had increased the mean proportion of non-terminated shoots and possibly may have reduced (P=0.10) the proportion of terminated shoots (Table 6.5). Interestingly, SS phenotype had a significant greater mean proportion of non-terminated shoots and lower proportion of terminated shoots compared to other phenotypes, but opposite pattern was recorded on LS phenotype (Table 6.5). In kiwifruit, potential phenotypes or genotypes with fewer long non-terminated shoots would be advantageous for the orchard management due to less pruning required, and this trait could be used as one of the indicator of low-vigour vines. A significant higher proportion of non-terminated shoots found in SS phenotype could be related to the level of GA in this type of seedlings. Generally, genetic dwarf plant stature can be related to the low level of endogenous GA (Grochowska et al., 1984; Looney & Lane, 1984; Steffens & Hedden, 1992; Yadava & Lockard, 1977). Therefore, it would be reasonable to suggest that SS phenotype may have a lower and/or insufficient level of endogenous GA. Therefore, application of exogenous GA could be functioning as an additional supply of GA to this phenotype. As a result, this additional GA may have activated both apical and sub-apical meristem, thereby increasing the proportion of non-terminated shoots of SS phenotype. Contrasting mechanisms may be found in LS phenotype. Additional exogenous GA could not be utilised by the LS phenotype, presumably this phenotype already have sufficient amount of endogenous GA. If LS phenotype have a higher level of endogenous gibberellin as suggested, then it is not surprising that they do not respond to additional gibberellin as dwarf mutants plants of many species respond to exogenous GA, whereas the tall genotype does not (Brian, 1959; Phinney, 1983).

The results of the present study are also similar to the findings with corn plants that also found that exogenous application of GA to the dwarf (*d1*) corn stimulated growth, but has little or no effect on tall wild-type corn plants (Phinney, 1983). For the future studies, assessment on endogenous hormones (IAA, CK and GA) on the different kiwifruit seedling phenotypes is required, whether the actual growth or vigour of each phenotype was related to endogenous hormones. As noted before, GA is believed in regulating shoot tip abscission (Juntilla, 1976; Powel, 1987) and is also involved in the extending the duration of shoot extension growth (van Hooijdonk et al., 2010). It would be interesting to find out, different seedling phenotypes (e.g. SS phenotypes) that have higher proportion of terminated shoots, whether they may have had the ability to transfer high proportion of terminated shoots if used as low-vigour scions or as vigour-controlling rootstocks. High proportion of terminated shoots is preferred because terminated shoots tended to produce high proportion of bud break and high flower numbers (Clearwater et al., 2004), and also contributes to the low-vigour in the canopy architecture of the vines (Clearwater et al., 2006). However, it can be affected by several factors such as pruning (Piller et al., 1998), rootstock (Clearwater et al., 2006) and temperature (Seleznyova & Halligan, 2006). Therefore, the development of promising kiwifruit cultivars that have tendency to promote high proportion of terminated that can produce high fruiting would be likely advantageous to kiwifruit management.

As a general conclusion for this section (Section 6.4.2, Experiment 2), we found that multi-stemmed phenotypes (i.e. LMS and SMS phenotypes) were less responsive to the exogenous application of GA. However, high responsiveness of single-stemmed phenotypes (i.e. LS and SS phenotypes) could be utilised to temporarily stimulate vegetative growth to establish full canopy. We have demonstrated in our study that the shoots and canopy architecture of the kiwifruit seedlings can be modified using the exogenous application of GA, thus changing the overall morphology and vigour of the vines. The kiwifruit seedlings were very responsive to the GA application supporting the previous findings in kiwifruit (Vattiprolu, 2012), apple (Lee & Looney, 1977), cherry (Oliveira & Browning, 1993) and a recent study in *Jatropha curcas* (Ni et al., 2015). Our results in this section have also contributed to the increase in our physiological understanding on hormonal influences on shoot branching in kiwifruit, as we found that GA acts a positive regulator for stimulating shoot branching in kiwifruit.

These results have strengthened the finding from a previous study that initially found GA involved in shoot branching in kiwifruit. Nevertheless, further study is still needed to prove the actual causality of branching in kiwifruit.

6.5 Summary

The kiwifruit seedlings obtained from the specific-crosses were evaluated in terms of their architectural traits and growth habit (Experiment 1). These seedlings were segregated according to their initial phenotype and no pruning was performed in order to not interfere with their natural growth habit. Two different seedling groups were observed; 1) the seedling groups that produced multiple stems, and 2) seedling groups that produced only one single stem. The multi-stemmed groups represented the highest proportion in the seedling population with 62.7% compared to the single-stemmed groups with only 37.3%. Further observation found that these two groups could be classified into four distinct phenotypes based on their main primary shoots which were; i) Long Multiple Stems (LMS), ii) Short Multiple Stems (SMS), iii) Long Single Stem (LS), and iv) Short Single Stem (SS). There were significant differences in the main primary shoot characteristics between the phenotypes in terms of length, node number, internode length and shoot CSA. Phenotypes that have long main primary shoots such as LS and LMS may have almost two or three-fold longer shoot length than other phenotypes (i.e. SMS and SS). The shoot length was positively correlated to the node number and internode length, but slightly correlated with shoot CSA. The primary stems of LS and LMS phenotypes could be classified as long shoots being non-terminated and longer than the shoots from SS and LS phenotypes, even though the mean node number was less in LMS phenotype. The primary stems of SMS and SS were classified as medium shoots, due to the average internode length was slightly longer than reported previously for short shoots. The number of shoots of kiwifruit seedlings was negatively correlated to the length, node number and shoot CSA of main primary shoots and not correlated to internode length of main primary shoots.

Application of GA immediately after the bud break had a significant influence on the characteristics of different proleptic axillary shoots in kiwifruit (i.e. long, medium and short shoots) (Experiment 2). The long shoots were significantly shorter with GA treatment in all phenotypes, due to the reduction in the node number and internode length. The phenotypes x GA interactions were significant for shoot CSA and approached significance for length, indicating that the length and size of long axillary shoots may be regulated and dependent on the amount GA supplied to the kiwifruit

seedlings phenotypes. Regardless of phenotypes, most of the long lateral axillary shoots from GA treated vines became very thin and very slender compared to the long shoots from untreated vines. Only medium lateral axillary shoots (terminated shoots type) were available in phenotypes of LMS and SMS when treated with GA. With GA treatment, SS and LS phenotypes tended to produce more number of long non-terminated shoots, suggesting these phenotypes may have more responsive to exogenous GA, causing conversion of medium shoots to long shoots. Regardless of phenotypes, GA treatment increased the length of short axillary shoots by almost 50% compared with untreated short shoots, by increasing node number and internode length. The internode length of axillary shoots was not affected by the GA treatment, as we found that the shoots of the same node number have a similar length irrespective of whether GA is applied. Therefore, the actual interpretation of the GA effect on internode lengths can be completely misleading and need to be discussed with caution.

The SS phenotype had a significant greater mean proportion of non-terminated shoots and lower proportion of terminated shoots compared to other phenotypes when treated with GA. GA treatment on kiwifruit seedlings phenotypes significantly increased the shoot branching of kiwifruit in term of total number of proleptic and syleptic axillary shoots for each phenotype. However, SS and SMS phenotypes still produced the lowest number of branching compared with other phenotypes even after GA application. This result may reflect the low-vigour ability of these phenotypes. We suggest that the effectiveness of any hormones (e.g. GA, IAA and CK) in either inhibiting and/or releasing the bud outgrowth in kiwifruit, depended on the growing stage of the shoots.

Overall, we found that multi-stemmed phenotypes (i.e. LMS and SMS phenotypes) were less responsive to the exogenous application of GA. However, high responsiveness of single-stemmed phenotypes (i.e. LS and SS phenotypes) could be utilised to temporarily stimulate vegetative growth to establish full canopy. Our study demonstrated that the shoots and canopy architecture of the kiwifruit seedlings can be modified using the exogenous application of GA, thus changing the overall shoot morphology and vigour of the vines. The findings in this chapter hold promise for developing low-vigour kiwifruit vines that terminate shoot growth early, thereby providing assimilates to fruit sinks rather than vegetative growth.

Chapter Seven

7. General discussion and conclusions

7.1 Introduction

An important objective of this thesis was to evaluate the vigour controlling mechanism (s) in relation to the aspect of the hormonal physiology, and the possible methods to manipulate the vigour of kiwifruit vines. Elucidation of this objective is essential to improve our physiological understanding of what is the actual mechanism(s) of vigour control in kiwifruit. Besides that, we believe that the knowledge of these aspects may contribute to a great improvement of the kiwifruit orchard management in the future.

Before we expand the discussion about the major findings of this thesis, we briefly summarise how we constructed all the studies to test the hypotheses and to achieve the major objective of this thesis (Section 1.1.3). The first part of this thesis has described the use of rootstocks as root system and their impact on the scions vigour and architecture of kiwifruit vines (Chapter Two and Chapter Three). In this study, thirteen inter-specific hybrid kiwifruit rootstocks were evaluated in terms of their potential as a vigour controlling rootstock in kiwifruit (Table 1.1, Chapter One). Modifications of the architecture structure of 'Hayward' scion imposed by inter-specific hybrid rootstocks were discussed in terms of the involvement of plant hormones auxin (IAA) that control the vigour of grafted scion (Chapter Four). The second part of this thesis has discussed the use of bark inserts/grafting as a method for growth manipulation in order to improve knowledge and understanding on vegetative vigour, and also the aspect of fruit growth in kiwifruit. The bark inserts/grafting in an inverted orientation was tested as a means to restrict the polar auxin transport (Chapter Five). Morphological and architectural changes on shoot architecture of young 'Hort16A' vines were observed and compared with bark inserts/grafting in normal orientation and non-treated vines. Besides that, the bark inserts/grafting was also tested as an alternative method for regulating fruit quality of 'Hayward' kiwifruit.

The third part of this thesis discussed the importance of the initial architectural traits and the possibilities to manipulate the vigour of kiwifruit seedlings without the rootstocks (Chapter Six). The main idea of this study was to evaluate the architectural traits of kiwifruit seedlings, which may be useful as vigour-controlling rootstocks or could be used as low-vigour scions. Breeding low-vigour scion cultivars is one option to control excessive vegetative growth, and this approach may allow better utilisation of rootstock available for different environments and soil conditions, as well as disease resistance. Because shoot phenotype can be manipulated by plant growth regulators (PGR), it may be possible to manipulate shoot architecture of kiwifruit by PGR applications. This approach would also be useful in developing newly planted low-vigour kiwifruit orchards. Theoretically, the application of PGR can be used to allow an earlier establishment of the desired canopies of potential low-vigour kiwifruit cultivars, and then the application of PGR could be stopped to allow carbohydrate allocation to the reproductive stage. The summaries of the major findings of this thesis are described and discussed in further sections.

7.1.1 Vigour control of kiwifruit vines: Why is it so important?

Vines that can produce optimal yield with high-quality fruits is a major target in kiwifruit orchard management. As noted, supplying markets with fruit of consistently high DMC, the better returns kiwifruit growers get (Burdon et al., 2004; Lancaster, 2002). Therefore, it requires proper canopy and crop load management based on the morphological and physiological understanding of how kiwifruit vines grow, and its relations to crop productivity. As noted by many literatures, excessive vegetative vigour in kiwifruit is the main constraint for achieving the major objective as stated above. This major problem has given a negative effect to the productivity of kiwifruit vines, because excessive vegetative vigour causes dense canopies that may affect fruit yield and quality by reducing dry mass into fruit (Snelgar et al., 1998), lowering harvest index (Friend et al., 2014), due to insufficient light exposure in the kiwifruit canopies (Biasi et al., 1995). Besides that, growers are finding excessive vegetative vigour in kiwifruit is difficult to control as the cultural practices were found to be expensive and may increase production cost, particularly for summer and winter pruning (Miller et al., 2001).

Controlling vegetative vigour is particularly relevant to kiwifruit orchard management that currently relies on horticultural manipulation (i.e. pruning and girdling) (Palmer, 2007). However, any form of pruning is an invigorating process (Wade & Westerfield, 2009), time-consuming and labour demanding, whereas girdling on kiwifruit vines did not completely reduce the excessive vegetative vigour (Currie et al., 2008). Besides that, unavailability of clonal dwarfing or vigour-controlling rootstocks in kiwifruit is still un-resolved issue. The control of kiwifruit vine vigour by potential rootstocks is therefore an essential key component of successful production systems and establishment of high-density orchards. For a few decades, the kiwifruit industry has had access to a very limited range of rootstocks, such as 'Bruno', 'Kaimai' and 'Hayward' for propagating the potential scions (Clearwater et al., 2007b; Lawes, 1990; Warrington, 2000). Recently, the introduction of clonal kiwifruit rootstock namely 'Bounty 71' has shown potential results. This rootstock has known to cause 'Hort16A' vines to produce slightly less vigorous (Anon., 2012) or moderate reduced vigour (M. Clearwater, personal communication, Jun 27, 2016), promoted higher flower number and larger fruit size (Anon., 2012) with slight tolerance to PSA (Thorp et al., 2013). However, to date, limited information is available on the effects of 'Bounty 71' rootstock on the grafted scions. Therefore, there is a strong need to develop potential vigour controlling rootstocks and/or other possible growth manipulation techniques in kiwifruit. In order to improve our knowledge, the physiological mechanism (s) of vigour control need to be understood first before any growth manipulations either by rootstocks and/or by other growth controlling techniques can be implemented in the kiwifruit orchard management.

In this chapter we briefly discussed the term of 'vigour' because it is often used to describe the growth performances of the trees or vines. This term has been widely used in many areas, especially in horticulture. Without exception in kiwifruit, this terminology is often used to represent the growth performance of the vines. There were many interpretations regarding the use of 'vigour' terms in plant growth. For example, Waring (1983) in his study of forest trees defined 'vigour' as relative to tree growth, expressed as the above-ground biomass increment per unit of photosynthetic tissue or growth efficiency. Whereas Nesme et al. (2005) stated that 'vigour' is defined as the intensity of vegetative growth because it is an important indicator for orchard management in fruit tree cropping systems. Faust (1989) interpreted that 'vigour' is the

rate of growth, and it can be determined genetically, but it can also be influenced by the cultural practices. Other studies in vine crop stated that 'vigour' is referring to the quality or condition that is expressed in a rapid growth of the parts of the vines (Dry & Loveys, 1998), that is referring to the rate of shoot growth or final shoot length (Lakso, 2013). However, indirect measurement of growth to interpret 'vigour' (e.g. shoot length, shoot growth rate etc.) can be misleading. Nevertheless, we suggest that the use of 'vigour' terminology is depending on the study objectives. For example, vigour in fruit trees can be predicted based on anatomical characteristics (e.g. ratio of phloem to xylem) (e.g. Beakbane & Thompson, 1939; Kurian & Iyer, 1992; Saeed et al., 2010; Tombesi et al., 2011), and vigour of trees based on morphological characteristics (e.g. shoot types and vegetative growth) (e.g. Clearwater et al., 2006; Gunckel et al., 1949; Nesme et al., 2005). Other studies have described the vigour based on the physiological characteristics (e.g. respiration, photosynthetic and water transport) (e.g. Clearwater et al., 2004; 2007; Gaudillère et al., 1992; Iwasaki et al., 2011; Way et al., 1983) and hormonal characteristics (e.g. Michalczuk, 2002; Sorce et al., 2007; Tworkoski & Fazio, 2015; van Hooijdonk, 2009). As outlined earlier in this thesis, we attempt to reveal the vigour characteristics of the kiwifruit vines that may be affected by the rootstock or other growth manipulations, and whether the vigour characteristics of kiwifruit can be manipulated in order to improve kiwifruit management. Whatever the reasons, we believed that the 'vigour' of kiwifruit vines may be described by a few characteristics and may not be solely regulated by one single characteristic. These aspects are described in details in further sections.

7.2 PART 1: Rootstock effects on scion vigour, vegetative growth and shoot architecture of kiwifruit vines

7.2.1 Initial modifications of scions architecture by rootstocks

In Chapter Two, the vigour of scions was initially ranked according to the trunk cross-sectional area (CSA) of inter-specific hybrid kiwifruit rootstocks. Results in this chapter have shown that the inter-specific hybrid kiwifruit rootstocks have the potential to modify scion vigour and architecture of scion during the early stage of vine growth. In

the first and second year of growth following grafting, the mean trunk CSA of rootstocks and scions were significantly different between inter-specific kiwifruit hybrids (Table 2.1). Our results indicate that rootstocks with small CSA also had small scion CSA in both growing seasons (Figure 2.3). In addition, differences in the mean CSA of scion primary shoots were recorded between inter-specific hybrid kiwifruit rootstocks (Table 2.1), with rootstocks that limited the growth of the scions also having smaller stem CSA of primary shoots (Figure 2.3). Even though several studies demonstrated that the vigour of trees could be measured by trunk diameter or CSA (Fazio & Robinson, 2008; Khatamian & Hilton, 1977; Reighard, 1990; Webster, 1995a; Westwood & Roberts, 1970), it should be noted that the measurement of trunk CSA is an indirect measure of tree growth and only can be used for estimating the vigour of trees. We suggest that the most meaningful measurement of tree vigour is plant dry mass (i.e. dry weight of trees), however, this may involve destructive sampling. The relationship between stem or trunk CSA and vigour of the kiwifruit vines was further discussed in detail in Section 7.2.5.

In the second growing season following grafting, the mean internode length of 'Hayward' scion primary shoots was significantly different from the inter-specific hybrid kiwifruit rootstocks, with the difference between the shortest and the longest internode length as much as factor of two (Table 2.2). Our finding on internode length was similar to that of the study on the rootstock effect on grape scions (Cookson et al., 2012). In kiwifruit, according to Foster et al. (2007), there is a strong relationship between mean internode length and final node number including a number of leaves, because this is associated with the shoot growth rate and shoot cessation (Seleznyova et al., 2002). However, it should be noted that many studies have demonstrated that rootstock did not affect internode length of grafted scion, as found on apple (Seleznyova et al., 2003; van Hooijdonk, 2009) and pear (Seleznyova et al., 2013; Watson et al., 2012). Even though in Chapter Two, the actual relationship between length and node number of scion primary shoots could not be presented due to insufficient data (Appendix 1), results from other chapters (Chapter Three and Chapter Four) indicate that the internode length of grafted scions was not modified by the kiwifruit rootstocks. Our results demonstrated that the length of a shoot increased with its node number, suggesting that no direct effect of the rootstock was found in the process of extension of individual internodes (see Figures 3.6, 3.9, Chapter Three and Figure 4.7, Chapter

Four). Unfortunately, detailed data on the node number of proleptic shoots were not recorded in our study (Chapter Two), because we just visually counted the nodes available on the shoots and classified them according to shoot types. This data also may provide valuable information on how kiwifruit rootstocks may regulate the shoot apical meristem (SAM) of lateral branching of scions. Another interesting result was that the bud break of the scion primary shoots in the second growing season following grafting was affected by the inter-specific hybrid kiwifruit rootstocks (Table 2.2) indicating that the bud break of scions also could be influenced by the kiwifruit rootstocks during the initial stage of vines growth. There were also strong trends that the inter-specific hybrid kiwifruit rootstocks may have affected the characteristics in terms of length and node number of long and short proleptic shoots (Table 2.6), but not for the medium shoots. However, the CSA of long, medium and short proleptic shoots of scions did not differ between the inter-specific hybrid kiwifruit rootstocks (Table 2.7). Even though the effect of inter-specific hybrid kiwifruit rootstocks on the shoot characteristics was not pronounced as reported in other fruit trees such as apple (Hirst & Ferree, 1995b), our results may suggest that there was an indication that kiwifruit rootstocks may have an influence on the initial shoot characteristics of grafted scions. Besides that, we believe that any early alteration on the characteristics of shoots by the kiwifruit rootstocks may largely affect the future vine growth in terms of canopy architecture and productivity as found on mature kiwifruit vines (Clearwater et al., 2006; Cruz-Castillo et al., 1991; 1997; Wang et al., 1994b) and apple (Hirst & Ferree, 1995b).

Inter-specific hybrid kiwifruit rootstocks may have also affected the different proportions of proleptic shoots of 'Hayward' scions, particularly long proleptic axillary shoots (Table 2.4). This effect has contributed to the differences in the proportion of non-terminated and terminated shoots (Table 2.5) during the initial stage of vine growth. These results were similar to the findings with mature composite kiwifruit vines that suggest kiwifruit rootstocks may alter the proportion of shoot types by affecting the shoot growth rate and shoot termination (Clearwater et al., 2006). Our results may also indicate that rootstocks influence the timing of shoot termination by affecting the node neo-formation in the early growing season as reported for apple (van Hooijdonk et al., 2010). It was notable that the total number (Table 2.3) and total length of proleptic axillary shoots (Table 2.6) were different between rootstocks. Therefore, our findings indicate that inter-specific hybrid kiwifruit rootstocks may have ability to modify the

scion branching, thus contributing to the difference in the initial canopy architecture of 'Hayward' scions. However, the branching of scion primary shoots was not correlated with trunk CSA of scions and rootstocks (Figure 2.5 and Figure 2.6), suggesting that branching of kiwifruit scions could be more influenced by the rootstock genetics (Fazio & Robinson, 2008; Quinlan & Tobutt, 1990) and probably the hormonal status of the rootstocks (van Hooijdonk et al., 2010).

Based on other studies and our results in this thesis (Chapter Two), the growth and architectural structure of the 'Hayward' scions following grafting in the nursery stage is largely influenced by the genetic of the kiwifruit rootstocks (Fazio & Robinson 2008; Quinlan & Tobutt, 1990). This chapter demonstrated the 'first evidence' that kiwifruit rootstocks may also have the ability to affect scion growth as early as in the first and second growing season following grafting, similar to the evidences found in other fruit trees such as apple (Costes et al., 2001; Fazio & Robinson 2008; Seleznyova et al., 2007; van Hooijdonk et al., 2010), pear (Watson et al., 2012) and other vine crops such as grape (Cookson et al., 2012; Tandonnet et al., 2010). Another key finding of this chapter was that the vigour ranking of scion based on trunk CSA of rootstocks in the first growing season did not appear to continue into the following growing season (Chapter Three). We also suggest that the use of trunk CSA is not actually true measure of tree or vine growth, and insufficient to describe the initial vigour of grafted 'Hayward' scions. Therefore, the use of plant dry mass (i.e. dry weight) is suggested for the future to measure the actual vine vigour. It should be noted that the data presented in our study provide evidence found under the experimental growing climate of Manawatu, New Zealand. Since this trail was the first conducted for kiwifruit; thus, the comparison only can be made with other fruit tree species, as no information is available for kiwifruit. A recent study in apple indicates that the rootstock expression can occur at different times in various locations, and the differences in temperature and growing climate may have a large influence on rootstock responses (Foster et al., 2016). Therefore, further trials at various locations are needed for the future studies.

7.2.2 The growth rate and termination of the shoots of grafted scions

7.2.2.1 Shoot growth rate of scions

In Chapter Three, the differences in shoot extension for the short, medium and long shoots of 'Hayward' scions were clearly observed in the first month of the shoot growth after the bud break, regardless of rootstocks vigour (Figure 3.6). The long shoots grew faster than the medium and short shoots, and short shoots grew much slower than medium shoots (Figure 3.6), indicating that the shoot growth rates were different between these shoots (Figure 3.7). Our results confirm the findings of an earlier study by Clearwater et al. (2006) that also found similar evidence on 'Hort16A' scions. It was interesting to note that the long shoots of scions on low-vigour rootstock group such as No.8, No.18, No.19, No.87 and No.100 grew much slower compared to the long shoots from other rootstocks (Figure 3.6 and Figure 3.7). However, the patterns of shoot development for medium and short shoots did not differ between inter-specific hybrid kiwifruit rootstocks (Figure 3.6). Our results are similar to those of previous studies on 'Hort16A' scions grafted onto a few selected *Actinidia* kiwifruit rootstocks, as Clearwater et al. (2004; 2006) found that scions on low-vigour rootstocks such as *A. polygamma* and *A. kolomikta* had higher slower-growing shoots than scions on high-vigour rootstocks, *A. hemsleyana* and *A. macrosperma*. However, their studies did not report the actual growth rate of shoots as we did (Figure 3.7).

Our results demonstrated that the shoot growth rates (mm per day) of different shoot types of scions (i.e. short, medium and long shoots) varied between the hybrid kiwifruit rootstocks (Figure 3.7). The non-terminated shoots (i.e. long shoots) showed an obviously different shoot growth rate than terminated shoot types (i.e. medium and short shoots). Differences in the shoot growth rate were noticeable especially for the long shoots on rootstocks No.8, No.18, No.19, No.87 and No.100 compared to other rootstocks (Figure 3.7). The long shoots on these rootstocks grew more slowly sooner than non-terminated shoots on other rootstocks such as No.21 and No.101 including GN cutting (self-rooted control). Therefore, these results have shown that the effect of kiwifruit rootstocks on the scions vigour is clearly observed through the shoot growth rate of scions, particularly the growth rate of long shoots.

It is important to note that the slowing of growth is part of the shoot termination or cessation process. According to Foster et al. (2007), shoot growth of kiwifruit appears to be affected by multiple factors such as environmental conditions, genotype and competition between shoots itself. These factors may also influence the final shoot fate whether the shoots will be long, continuing growth until the end of the season, or shoots will be short, stopping growth soon after bud break (Foster et al., 2007). Although there was considerable evidence in this study (Chapter Two and Chapter Three) to suggest that the inter-specific hybrid kiwifruit rootstocks affect the growth and termination of shoots, other factors as mentioned above need to be taken into consideration. Besides that, we also suggest that using a non-direct architectural approach or indirect measurements of growth (i.e. shoot characteristics) may not be sufficient to represent the actual vigour of shoots as well as vigour of vines.

Therefore, again, a growth type analysis approach by measuring dry mass gain of individual shoot types (actual growth) needs to be evaluated but it may involve destructive samplings. Since our assessment was conducted on young vines, it was impossible for us to implement this approach due to the limited number of shoot samples, and because the canopy of vines was still small. Furthermore, the effect of rootstock on the shoot termination process of scion at an individual shoots level needs to be further studied using high-resolution measurements (e.g. light and scanning electron microscopy studies) to understand better the nature of this process. Nevertheless, our findings in this thesis have provided new information and have strengthened previous findings (Clearwater et al., 2004; 2006) on how kiwifruit rootstocks affect the vigour of grafted scions (especially growth rate of shoots). Besides that, results provided here also supported the previous studies in other fruit trees such as apple (Rom, 1996; van Hooijdonk, 2009) and peach (Weibel et al., 2003), which found that the growth rate of scions was largely affected by the vigour of the rootstocks. In the future, it is suggested that studies on the effects of the kiwifruit rootstocks on scions shoot growth should be conducted comprehensively under a highly controlled environment, especially with a precise irrigation system.

7.2.2.2 The proportion of different shoot types of scions

In kiwifruit, the probability of shoot termination after bud break was correlated with their growth rate (Clearwater et al., 2006). Clearwater et al. (2006) has demonstrated that termination of long shoots of 'Hort16A' scions occurred earlier on the slower-growing shoots grafted onto low-vigour rootstocks, especially in the early spring. In our study, there was a strong trend that the mean proportion of long proleptic axillary shoots may be affected by the inter-specific hybrid kiwifruit rootstocks (Tables 3.2 and 3.7). When the differences in the shoot types were expressed in terms of shoot terminations (either non-terminated or terminated), we discovered that rootstocks No.18, No.87 and No.100 produced a higher proportion of terminated shoots (i.e. medium and short shoots) and a substantially lower proportion of non-terminated shoots (i.e. long shoots) (Tables 3.3 and 3.8). These effects could be due to the modification in the number of preformed and neoformed nodes of scions as demonstrated by the difference in the distribution of node numbers (Figure 3.10). Besides that, the growth rate of shoots of scions on low-vigour rootstocks No.18, No.87 and No.100 was much slower before termination than other rootstocks (Figure 3.7), thereby affecting the proportion of shoot types at the end of the growing season (Table 3.8). Our results are similar to the finding with the composite 'Hort61A' vines that low-vigour rootstocks such as *A. polygamma* and *A. kolomikta* tended to produce a higher proportion of terminated shoots (Clearwater et al., 2006). Other studies in apple also reported that rootstock such as M.9 may have the ability to alter the proportion of shoot types by increasing the proportion of short shoots relative to long shoots either in the first (Costes et al., 2001) or second growing season (Seleznyova et al., 2003; 2008).

The inter-specific hybrid kiwifruit rootstocks may have affected the characteristics of proleptic axillary shoots of scions in terms of length, node number, internode length and shoot CSA, especially for short and long shoots (Tables 3.5, 3.10 and 3.11). In the growing season 2012-2013, characteristics of long shoots that had been trained to become permanent cordons (Figure 3.1C) were significantly different between kiwifruit rootstocks (Table 3.5). In the next growing season (2013-2014), the kiwifruit rootstocks may have influenced the characteristics of proleptic short and long shoots, but less influence was found on medium shoots (Tables 3.10 and 3.11). Other fruit trees also reported that rootstocks can affect the characteristics of shoots of grafted scions, as

found in peach (Weibel et al., 2003) and apple (Hirst & Ferree, 1995b). We suggest that the changes in the characteristics of proleptic axillary shoots of 'Hayward' scions may partly be due to the differences in the shoot growth rate imparted by the kiwifruit rootstocks (Figure 3.7). The shoot growth of grafted scions on low-vigour rootstocks such as No.18, No.19, No.87 and No.100 was slower and then terminated earlier seasonally. These effects have contributed to the differences in the proportions of terminated and non-terminated shoots of scions (Tables 3.3 and 3.8), subsequently effecting canopy architecture of the grafted vines. Based on our results, we suggest that the final canopy architecture of 'Hayward' scions on the low-vigour rootstock group may have been reduced due to a high proportion of terminated shoots found on these rootstocks, in contrast to scions that grafted onto other rootstocks (e.g. No.101 and GN, self-rooted control). In kiwifruit, a high proportion of terminated shoots (medium and short shoots) is preferred because they tended to produce a high proportion of bud break and high flower numbers (Clearwater et al., 2004; 2006). However, it should be noted that rootstock would not be the sole factor that affects the termination of shoots in kiwifruit. Several factors such as temperature (Seleznyova & Halligan, 2006), plant hormone such as gibberellins (Juntilla, 1976; Powel, 1987; van Hooijdonk et al., 2010) and horticultural practices such as pruning (Clearwater et al., 2006; Piller et al., 1998) and girdling (Boyd, 2012) may also alter the shoot termination of kiwifruit vines.

Confusion arises in kiwifruit regarding on how the differences of shoot types may contribute to the canopy architecture and size of kiwifruit vines. For example, what are the differences between canopy architecture in vines that have many short shoots and vines that have a lesser number of long shoots? What are the implications of the number of shoots in terms of canopy sizes and vigour of vines? These questions have always been posed when we started this study. We suggest that these issues are likely to be due to the differences in the proportion of shoot types (i.e. short, medium and long) and their shoot characteristics, and possibly the branching habit of kiwifruit vines. These issues could also be associated with the different perspectives (Webster, Dry & Loveys, 1998; 1995a). For kiwifruit, we possibly should be looking at two different perspectives; i) at the whole vine level (Figure 7.1A) and, ii) at the single-shoot level (Figure 7.1B). For the first perspective, if the vines or trees that have many short and medium shoots (i.e. terminated shoots), they may have comparable vine weight or mass with vines that have less non-terminated long shoots (Webster, 1995a). However, the canopy volume or

architecture of those vines with many terminated shoots is very much reduced and smaller compared to vines that have less non-terminated long shoots as shown in Figure 7.1A. Normally, vines or trees with less tree mass and many short shoots but small canopy volume are considered low-vigour or dwarfed (Webster, 1995a). For the second perspective, the 'vigour' at the single-shoot level is relatively straightforward. Short shoots, thin in size, with small leaves are considered to be 'low-vigour' (Figure 7.1B), but contrasting with the long shoots that have relatively long internodes, thick stem and large leaves (Dry & Loveys, 1998). However, studies in kiwifruit found that larger size of shoots on vigorous vines can be more floriferous relative to bud number (see Thorp et al., 2003). Results of this thesis and findings of other studies in kiwifruit (Clearwater et al., 2004; 2006) clearly showed that kiwifruit rootstocks may have significant influences on both whole-vine and single-shoot levels (Figure 7.1), not only on the vigour aspects but on the productivity and quality aspects as well (Cruz-Castillo et al., 1991; 1997; Wang et al., 1994b).

In order to develop desirable canopy architecture and vigour in kiwifruit; therefore, we need to consider both perspectives to create a balance between vegetative and reproductive growth (i.e. low vigour vines with optimum yield). We suggest that kiwifruit vines may have wood or cane with very few buds but a high percentage ofbud break, and/or many buds, but a low percentage bud break. Both of these aspects may be considered as relatively low-vigour with respect to the overall amount of vegetative vigour (e.g. total amount of shoot growth). One might think that high bud break may suggest high-vigour, but in kiwifruit vines, we want rootstocks that can produce high scion bud break with slower growing shoots (i.e. many short and medium terminated shoots). Generally, pruning of kiwifruit vines is carried out during winter and 1-year-old long shoots are retained during winter to become replacement canes (Miller et al., 2001; Thorp, 2003). Even though less pruning work is required on vines grafted onto low-vigour rootstocks, kiwifruit growers may will hardly get replacement canes because vines on low-vigour rootstocks had less vegetative growth due to a lower proportion of long shoots. Therefore, a different pruning regime such as 'selective-pruning' or 'spur-pruning' can be implemented on low-vigour vines. However, implementation of these pruning regimes on low-vigour vines requires further research.

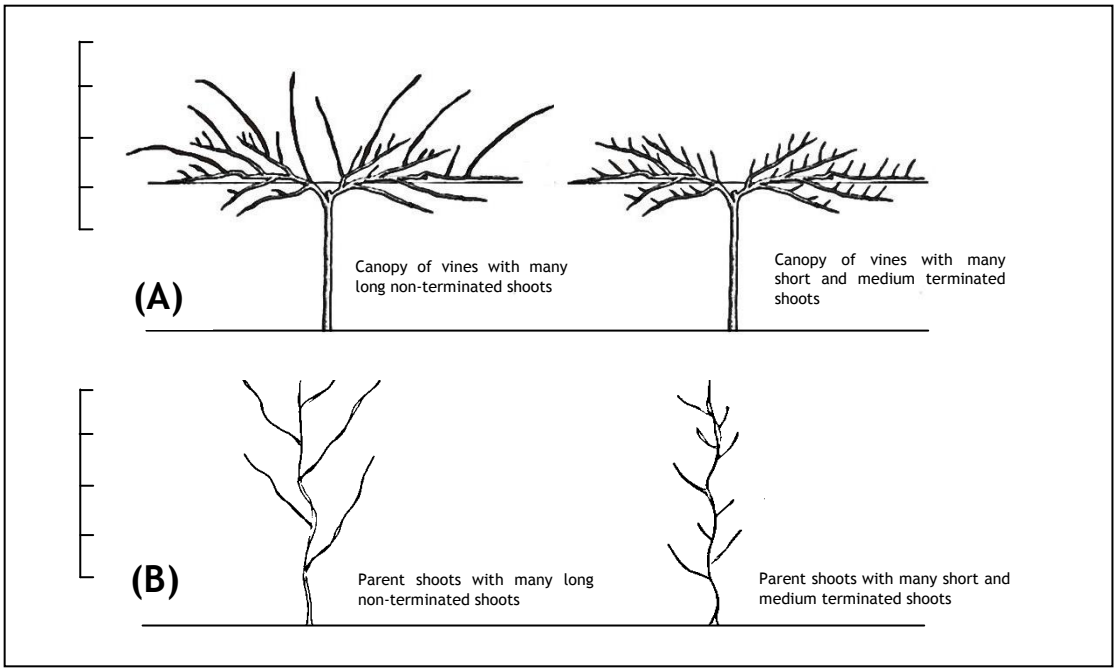


Figure 7.1. Schematic diagrams of the; (A) comparison of the canopy architectural structure of kiwifruit vines at the whole vine level, and (B) comparison of the shoot architectural structure of kiwifruit at the single shoot level.

7.2.3 The bud break of grafted scions

In Chapter Two, the initial percentage of scions bud break (%) was significantly affected by the inter-specific hybrid kiwifruit rootstocks in the second growing season following grafting. At the nursery stage, particular inter-specific hybrid kiwifruit rootstocks produced a higher initial percentage of scions bud break with more than 50% (Table 2.2). In Chapter Three, when they were first planted in the field condition, the scion bud break in the growing season of 2012-2013 and 2013-2014 was still affected by the inter-specific hybrid kiwifruit rootstocks (Table 3.1 and Figure 3.4). Remarkably, our results also have shown that the duration and timing of 'Hayward' scions bud break were significantly influenced by the inter-specific hybrid kiwifruit rootstocks (Figure 3.3 and Figure 3.5). When the scion bud break was expressed as relative bud break (i.e. the percentage of buds that was opened during each observational time relative to the total buds which opened), the patterns of peak time of scions bud break greatly differed between inter-specific hybrid kiwifruit rootstocks (Figure 3.3).

Our results in Chapter Two and Chapter Three imply that the bud breaks of kiwifruit from the dormancy were under the influence of the root system (i.e. rootstock), and thus support and extend those of Wang et al., (1994b). Remarkably, the effect of kiwifruit rootstocks can be observed as early as in the initial stage of young vines growth (Chapter Two). Normally, the influence of rootstocks on bud break was evidenced in mature kiwifruit vines (McPherson et al., 1994; Wang et al., 1994b), but in our study we found the effect of kiwifruit rootstocks can be observed as early as in the second growing season from grafting. However, our results are in contrast to the finding in pear (Watson et al., 2012), where rootstock did not affect the proportion of axillary bud break in the first or second year following grafting regardless of rootstock vigour. The peak time of scions bud break also varied between kiwifruit rootstocks, indicating that the duration and compactness of bud break could be under the influenced of genetic origin (i.e. parentage) of inter-specific hybrid kiwifruit rootstocks, because we found rootstocks with similar parentages exhibited similar peak times of bud break patterns on the grafted scions (Figure 3.3 of Chapter Three and Table 1.1 of Chapter One).

Overall, these results highlighted the importance of kiwifruit rootstocks in regulating scion bud break. In kiwifruit, a high proportion of bud break of the scions imparted by particular kiwifruit rootstocks would be likely considered as an advantage to kiwifruit management (McPherson et al., 1994), because the degree and synchrony of bud break were highly correlated with the reproductive organ in kiwifruit which that is flowering (Wang et al., 1994b). Besides, this may reduce our dependency to the PGR applications (e.g. Hydrogen Cyanamide) to increase the bud break. Nevertheless, we believe that the bud break of scions may be also under the influence of other factors such as scion genotype (Warrington et al., 1990; Watson et al., 2012), temperature (Austin et al., 2002), chilling requirement of vines (Linsley-Noakes, 1989) or rootstocks (Finetto, 2003; Webster, 1995a) and the hormonal status of vines (Vattiprolu, 2012). Therefore, besides rootstocks, these factors should be taken into consideration in the future studies.

7.2.4 Precocity and flowering of the grafted scions

In Chapter Three, the flower production during the season of 2012-2013 was the first occurrence of flowering imparted by the inter-specific hybrid kiwifruit rootstocks on the grafted 'Hayward' scions (Table 3.1). Our result showed a highly significant difference in mean flower number of scions between kiwifruit rootstocks, with scions on other particular rootstocks such as No.45, No.85 and No.86 did not produce any flower at all. However, scions on rootstocks No.8, No.18 and No.19 produced higher flower numbers compared to other rootstocks (Table 3.1), indicating that the particular inter-specific hybrid kiwifruit rootstocks may have an influence on the ability to induce fruitfulness (i.e. precocity) of the scions. In kiwifruit, many studies reported that kiwifruit rootstocks may affect flowering and fruiting of mature vines (Anon., 2012; Cruz-Castillo et al., 1997; Lowe, 1989, 1991; Wang et al., 1994b), but not their precocity. These results are really interesting, because this is the first evidence indicating that kiwifruit rootstock may also have an influence on the precocity of the grafted scions similar to the findings in other fruit trees such as apple (Hirst & Ferree, 1995a, 1995b) and sweet cherry rootstocks (Lang, 2000). However, the mechanism (s) of rootstocks induces precocity in fruit trees is still poorly understood, especially in kiwifruit. It was proposed that rootstocks influence precocity in young trees by affecting the partitioning of assimilates and endogenous hormones translocation (Webster, 1995a). Besides that, variation in scion bud break (Figure 3.2 until Figure 3.4) and flowering (Table 3.14) may indicate that kiwifruit rootstocks may have an effect on the uptake of carbohydrate and nitrogen level during early spring growth. This effect could be referring to the fact that roots of kiwifruit systems produce more starch and a lot of complex mucilage-containing crystalline idioblast cells, which could be an important source of nitrogen and carbon. Future studies on these aspects are needed and should be focusing on looking at mucilage output of various kiwifruit rootstocks.

The trend may exist that vigour of particular inter-specific hybrid kiwifruit rootstocks may correlate with the precocity of the scions. For example, rootstock No.18 that has the slowest shoot growth rate had higher flower numbers (Figure 3.8 and Table 3.1). In apple, there was a strong correlation between flowering and dwarfing induced by the rootstocks on the vigour of the grafted scions (Seleznyova et al., 2008). Floral transition induced by M.9 dwarfing rootstock in the first year of tree growth has a significant

influence on the scion vigour in the next growing season by reducing the annual shoot growth (Seleznyova et al., 2008). In our case, it is unknown whether these two effects are linked, as no detailed studies have been conducted in kiwifruit. However, some of the vigorous rootstocks in apple and sweet cherry can also induce precocious flowering and fruiting in the grafted scions (Webster, 1995a). A recent study in apple also found that 'Royal Gala' scion grafted onto own-root 'Royal Gala' produced high flower numbers in the second growing season following grafting (Vattiprolu, 2012). Therefore, it would be reasonable to suggest that dwarfing or low-vigour is not always associated with precocity. Nevertheless, precocity should be one of the factors that need to be considered when choosing the potential rootstock in kiwifruit. It should be noted that precocity and yield are dependent on the plant's ability for those flowers to set, retain and size fruits (Webster, 1995a). Even though we did not find any significant difference in the flowering number in the next growing season in field planting (Table 3.14), we believe that the effects of kiwifruit rootstocks of flowering and fruiting may be more apparent later, after the vines have fully developed canopies.

Therefore, the potential inter-specific hybrid kiwifruit rootstocks need to be evaluated for a few years more to get consistent data on the precocity and flowering of grafted scions. There is also growing evidence that the genes homologous to *FLOWERING LOCUS (FT)* have a key role in initiating floral transition in plants as reported for apple rootstocks (Foster et al., 2014). Recently, identification of genetic loci associated with dwarfing and precocity traits using quantitative trait loci (QTLs) analysis has been reported for pear rootstocks (Knäbel et al., 2015) and all these approaches may open a new research opportunity in the breeding programme for selecting potential rootstocks in kiwifruit.

7.2.5 Trunk cross-sectional area and vigour of kiwifruit vines

As outlined earlier in Section 7.2.1, the trunk or stem CSA of 'Hayward' scions and rootstocks significantly varied between inter-specific hybrid kiwifruit rootstocks in the first and second growing season following grafting (Table 2.1, Chapter Two). Furthermore, there were strong trends that the trunk CSA of 'Hayward' scions were significantly correlated with the trunk CSA of kiwifruit rootstocks in both growing seasons (Figure 2.3). Similarly, the trunk CSA of scion primary shoots also significantly correlated with the trunk CSA of rootstocks (Figure 2.3). Therefore, there was a trend that the rootstocks that have smaller trunk CSA may produce smaller trunk CSA of scions in both nursery and field planting. However, there were no correlations between the total length and number of proleptic shoots, with vigour based on trunk CSA of rootstocks and scions (Figure 2.5 and Figure 2.6). These results suggest that the trunk vigour of 'Hayward' scions may be dependent on the trunk vigour of rootstocks, but the vigour of scions as assessed by branching and total growth of scions was independent of the trunk vigour of rootstocks. In Chapter Three, there were significant differences in the trunk CSA of inter-specific hybrid kiwifruit rootstocks and 'Hayward' scions when they were transplanted in the field condition (Table 3.6). The trunk CSA of inter-specific hybrid kiwifruit rootstocks was also significantly correlated with the trunk CSA of 'Hayward' scions (Figure 3.3).

A recent study found that the vigour of 'Hayward' scions as measured by stem diameter was reduced when grafted onto 'Bounty 71' rootstocks (Anon., 2012), because scions on this rootstock exhibited a smaller stem diameter compared to scions on 'Hayward' rootstocks. Similar evidence was reported in a trial at Motueka, New Zealand, that the vigour of 'Hort16A' as assessed by the trunk CSA of scions was affected by the inter-specific hybrid kiwifruit rootstocks (Friend et al., 2014). In what way the kiwifruit rootstocks may control the development of scions trunk is still not fully understood, but according to Vasconcellos & Castle (1994), rootstocks may affect the growth of scions trunk by affecting the size of the vessel element of scions. Unfortunately, data on the relationship between the total growth of vines (i.e. total length, shoot number etc.) and the vigour of kiwifruit rootstocks and/or scions to elucidate the relationship between vigour of the rootstocks and scions trunk were not obtained in this study. Therefore, in

future studies, it would be of interest to clarify how the vigour of rootstocks and/or scions may correlate with the overall growth of grafted scions, especially on dry mass gain. Although trunk CSA is usually used as a vigour indicator, we found it was not sufficient to describe the vigour of kiwifruit vines in our study. In particular, trunk CSA was not related to the vigour of scions as assessed by the number of shoots (i.e. branching) and total shoot length of lateral shoots (Figure 2.5). We also found similar evidence during assessment of initial vigour of kiwifruit seedlings obtained from specific-crosses (Table 6.3), as initial vigour of seedlings as assessed by the length, node number, internode length and number of branching was not correlated with the stem CSA of the seedlings (Table 6.3).

Overall, rootstock genotypes of kiwifruit affect the initial growth and vigour of young 'Hayward' vines. We also suggest that the inter-specific hybrid kiwifruit rootstocks may in addition affect other vegetative characteristics such as leaf area and lateral branching of axillary shoots of the grafted scions. These effects could be mediated by hormonal signalling mechanism(s) between root and shoot system as reported in previous studies (van Hooijdonk, 2009; Vattiprolu, 2012) (next Section 7.3). Kiwifruit rootstocks have been thought to affect leaf area development in the early spring growth (Clearwater et al., 2006). However, due to time constraints in this study, we could not evaluate the effect of kiwifruit rootstocks on the leaf area of grafted scions. Nevertheless, considerable effort was made to study and develop an equation model for non-destructive leaf area estimation for 'Hayward' kiwifruit, as an initial approach for further assessing rootstocks effect on the leaf growth of 'Hayward' scions (Appendix 10). Results in Chapter Two and Chapter Three have shown that the scion growth and architecture of 'Hayward' may respond differently when grafted onto inter-specific hybrid kiwifruit rootstocks. For example, scions on rootstock No.87 that came from *A. polygama* selection still exhibited the low-vigour capability as reported previously on 'Hort16A' scions (Clearwater et al., 2004; 2006). Surprisingly, scions on rootstocks No.8, No.18, No.19 and No.100 that came from the selection between *A. chinensis* and *A. macrosperma* crosses also exhibited low-vigour, altough these selections were considered as high-vigour clones in previous studies (Clearwater et al., 2004; 2006). We believed these responses were probably due to the effects of rootstock-scion interaction.

7.3 PART 2: Hormonal effects on the vegetative vigour of kiwifruit

7.3.1 Auxin and shoot growth of kiwifruit

Hormonal control of shoot architecture in kiwifruit is still not fully understood. Therefore, a further objective of this thesis was to improve our physiological understanding on the hormonal control of grafted kiwifruit vines. The initial step was to understand the role of auxin in regulating shoot architecture of kiwifruit, since this is the first study attempted to reveal the hormonal signalling in grafted kiwifruit. It has been proposed, that M.9 dwarfing rootstocks in apple reduce the basipetal transport of auxin (IAA) to roots, subsequently reducing the amount of root produced cytokinins (CK) (Li et al., 2012; Lockhard & Schneider, 1981; van Hooijdonk et al., 2010) and gibberellins (GA) transported to scions (Tworkoski & Fazio, 2015; van Hooijdonk et al., 2010). In regulating shoot architecture, indole-3-acetic acid (IAA), predominantly naturally occurring auxin is involved in the activity of SAM, including many processes such as cell division and cell differentiation (Bartel, 1997). Therefore, as reported in the above studies, IAA is an important signal in regulating shoot growth. If the basipetal transport of IAA from shoots to roots is an important signal regulating kiwifruit scion vigour, then theoretically, an application of the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA) to the rootstock stem of composite kiwifruit should affect the shoot growth of scions as demonstrated in apple (van Hooijdonk et al., 2010).

In Chapter Four, we found that application of NPA to the stem of particular inter-specific hybrid kiwifruit rootstocks such as No.55, No.84, No.87, No.100 and 'Hayward' own-rooted cuttings (Table 4.1) had caused a reduction in the growth of scion primary shoots (Figure 4.6). It was observed that the growth of scion primary shoots was slowed after the first application of NPA and fully terminated approximately two weeks after NPA treatment (Figure 4.6), suggesting that the basipetal transport of IAA from shoot to root has been restricted. These effects were much similar to the study reported for self-rooted 'Hort16A' kiwifruit vines (Vattiprolu, 2012). In similar manner, van Hooijdonk (2009) also found reduction in the growth of primary shoots when NPA was applied to the stem junction of 'Royal Gala' scions. However, regardless of vigour

of inter-specific hybrid rootstocks, the primary shoots of 'Hayward' scions from untreated vines continued growing, indicating sufficient supply of IAA or gibberellins (GA) to keep shoot apical meristem (SAM) active. Moreover, reductions in the final length and node number were also observed (Table 4.3), indicating that IAA is needed in regulating SAM and production of node neo-formation of primary shoots of kiwifruit scions (Table 4.3). However, these evidence were observed on particular rootstocks only, such as No.55, No.84, No.87, No.100 and on green-cuttings (self-rooted control) (Figure 4.6). NPA treatment on the particular inter-specific hybrid kiwifruit rootstocks such as No.19, Bruno and No.86 did not cause the primary shoots of scions terminated earlier, suggesting that the reduction in basipetal transport of IAA did not affect the meristematic activity of scions when grafted onto these rootstocks. The results are somewhat surprising; however, we postulate that these rootstocks possibly may have had sufficient amount of IAA in the stem to keep the SAM of scions active. The internode length of scion primary shoots was not affected by the NPA treatment indicating that the inhibition of IAA transport did not affect the level of GA_1 in the root system. According to Ross et al., (2003), IAA is needed to maintain the level of GA_1 and more importantly GA_1 stimulated internode elongation is normally associated with an increase of IAA level. The vigour of 'Hayward' scions as assessed by the total length and node number of proleptic shoots was also affected by the kiwifruit rootstocks and NPA treatment (Table 4.7). In contrast, a previous study found that inhibition of basipetal IAA transport by NPA did not completely reduce the growth of young potted 'Hayward' vines, with only a small reduction of growth on primary shoots was observed and there was no effect on the total shoot length (Vattiprolu, 2012).

In Chapter Five, the bark grafting in an inverted orientation was used as a mean to restrict the basipetal transport of IAA in young potted 'Hort16A' kiwifruit vines (Figure 5.2). As noted, the polarity of auxin (IAA) translocations may be a primary factor in controlling plant growth (review by Teale et al., 2006). In the shoot system, IAA is transported from cell to cell and moves basipetally from the shoot apex (i.e. shoot tip) to the base (Lomax et al., 1995). Since the polarity of cells can be retained regardless of the orientations (Antoszewski et al., 1978; Sheldrake, 1973); therefore theoretically, the transport of IAA from shoots to roots may be reduced by reversing the polarity of cells, and subsequently it may modify the vigour of shoots (Lockhard & Schneider, 1981; Sax, 1954). In our study, inverting a single ring of bark taken from the same vines and

grafted back to the main stem did not significantly affect final mean length, node number, internode length and shoot cross-sectional area of long, medium and short sylleptic axillary shoots (Figure 5.6, Figure 5.7 and Figure 5.8). Therefore, the restriction of basipetal transport of IAA by using a single inverted ring of bark imparted only a small reduction in the growth of 'Hort16A' vines (*GM*1). We suggest that the lack of significant reduction in a single inverted ring of bark is possibly due to the regeneration of newly formed tissues under the bark graft, and presumably a new translocation is resumed, as well as rapid establishment of the new phloem tissues, since only a small piece of bark was used to restrict the IAA transport.

Further assessment of bark grafting was conducted using a ring of bark from other kiwifruit cultivars; G3, G9 and G14 (*GM*2). Three rings of barks were used to increase the dwarfing effects. This also enables us to evaluate the responses of kiwifruit vines to the bark grafting from other kiwifruit cultivars as tested in other fruit trees such as apples (Brase & Way, 1959; Dickson & Samuels, 1956; Lockhard & Schneider, 1981; Sax, 1954; Sax & Dickson, 1956) and peaches (Mosse, 1960). We found that the vines grafted with three rings of bark in an inverted orientation had reduced the mean total length of sylleptic axillary shoots compared to vines that have been grafted with the bark in normal orientation and control (Figure 5.11). Comparing between the insertion of bark from different cultivars, the insertion of barks from G3 and G9 cultivars in an inverted orientation on the main stem of 'Hort16A' kiwifruit had significantly reduced the mean total length of sylleptic axillary shoots (Figure 5.11A and Figure 5.11B), but less noticeable on the insertion of bark from G14 cultivar (Figure 5.11C). Therefore, two major significant findings have been demonstrated in this study. Firstly, the insertion of bark from other cultivars especially in an inverted orientation may have a significant influence on the growth of young kiwifruit vines. Secondly, the dwarfing effect can be increased by increasing the bark length with the use of three rings of barks, since the longer barks length used may result in more dwarfing effect than shorter barks (i.e. one single ring). Both of these findings are in agreement with previous studies with apples (Lockhard & Schneider, 1981; Poniedziałek et al., 2000). Our results also have strengthened the previous hypothesis that the levels of IAA transport in the phloem tissues may be controlled by the genetic of the bark from the original plants since the level of IAA transported in the phloem bark may vary between cultivars or species (Lockhard & Schneider, 1981). It is reasonable to suggest that the bark of genetically

different bark compositions may contain and/or possibly may regulate different amount of IAA to pass through their phloem cells (Lockhard & Schneider, 1981). These results are noteworthy in terms of how vigour of kiwifruit can be manipulated by using the bark grafting technique. Therefore, in the future studies, it is suggested that this technique could be tested in the mature vines by taking a ring of bark from low-vigour vines and grafted bark to the stem of commercial kiwifruit cultivars. Additionally, assessment on the level of endogenous IAA together with IAA metabolism may also provide valuable information on how the hormonal signalling is working, especially IAA transport in the bark grafted vines. Overall, it is suggested that there were strong trends that restriction of basipetal transport of IAA may have reduced the vigour of 'Hayward' scions. Similarly, restriction of IAA by bark grafting in an inverted orientation may have reduced the growth and vigour of young 'Hort16A' vines (Chapter Five). However, it is still unknown whether the level of endogenous IAA was related to the vigour of inter-specific hybrid kiwifruit rootstocks (Chapter Four). Although reductions in the vegetative growth of kiwifruit vines for NPA-treated vines (Chapter Four) and for inverted bark grafting (Chapter Five) were small, they had a pronounced effect on the overall vegetative growth, as well as on the overall architecture of 'Hayward' scions when compared with untreated vines. Although the effects are not as distinct as reported for apple (e.g. Kamboj et al., 1997; van Hooijdonk et al., 2010), these findings have revealed an important role of IAA signalling in regulating shoot architecture of kiwifruit. Although the previous study suggested that restriction of basipetal transport of IAA in stem did not affect the growth of kiwifruit cuttings (Vattiprolu, 2012), we suggest that the discrepancies between our results and previous study are probably due to the differences in the experimental materials used in these studies. The hormonal interactions in the kiwifruit stem might be different between self-rooted (i.e. cuttings) and composite vines, due to self-rooted vines do not have graft-union and stem shank separating shoot from the root system (see Figure 1.4). Even though lack of significant interactions was found between kiwifruit rootstocks and NPA treatment (Chapter Four), trends were evident that endogenous hormonal signalling (especially auxin) may involve in regulating shoot growth and architecture of grafted 'Hayward' scions. Similar indications also appeared in the case of bark grafting/inserts studies (Chapter Five). Therefore, trials with a large number of replicates are needed in the future studies to reveal further interaction effects between auxin (i.e. IAA) and other endogenous hormones in regulating shoot vigour of kiwifruit.

7.3.2 Auxin and leaf growth of kiwifruit

It should be noted that auxin is not only involved in regulating the SAM. As reported in other plants, auxin (i.e. IAA) has been implicated in many activities of vascular tissues development (Aloni, 1995). For example, in the cell division phase of leaf enlargement (Keller et al., 2004; Ljung et al., 2001), in leaf vascular development (Mattsson et al., 2003; Sieburth, 1999), and in the initiation of new leaves (Reinhardt et al., 2000). Therefore, any alteration of basipetal transport of IAA in plants may also affect other aerial parts of the plants such as the leaves. However, until now, the role of IAA in regulating the leaves growth of kiwifruit is poorly understood. Nevertheless, our study in Chapter Four demonstrated that the restriction of basipetal transport of IAA by NPA treatment on the rootstock stem was largely affecting the leaf growth of kiwifruit scions. Leaf characteristics of scions (leaf area, fresh and dry weight) were significantly reduced in NPA-treated vines regardless of rootstock vigour (Table 4.4 and Figure 4.8). NPA treatment also reduced the mean total leaf area of proleptic axillary shoots in the following season (Table 4.7). In Chapter Five, the mean total leaf area of young 'Hort16A' vines may have been reduced by inverting a single ring of bark (Table 5.1), indicating reduction in the transport of IAA. It was also found that the vines with bark grafting in an inverted orientation had 20% reduction in the leaf area compared with control vines (Table 5.1). Remarkably, with the present of bark from other cultivars (i.e. G3, G9 and G14), the mean total leaf area of sylleptic axillary shoots of bark grafted vines was significantly reduced compared to control vines (Table 5.4 and Figure 5.13). The reduction in mean total leaf area was approximately 42% and almost two-fold higher than a reduction found by using a single ring of bark. In a similar manner, reduction in the leaf area of the non-terminated long shoots of 'Hort16A' scions was observed when grafted onto low-vigour kiwifruit rootstocks such as *A. polygama* and *A. kolomikta*, and this effect has contributed to the reduction in total leaf area per scion (Clearwater et al., 2006).

In *Arabidopsis*, Ljung et al., (2001) reported that reduction in leaf growth of NPA-treated in growing medium appeared to coincide with the reduction in IAA content of the leaves. A recent study by Vattiprolu (2012) also found a reduction in the leaf area of primary shoots of 'Hort16A' stem cuttings when treated with NPA. Therefore, it seems

that the restriction basipetal transport of IAA from the shoot to root system may also affect the leaf growth and development of kiwifruit, since any growth manipulations are largely affecting the leaf growth of kiwifruit as demonstrated in our study and previous study by using NPA (Vattiprolu, 2012), as well as low-vigour rootstocks (Clearwater et al., 2006). In summary, our results in Chapter Four and Chapter Five indicate that sufficient amount of IAA in the shoot system is important for the leaf growth and development of kiwifruit. Therefore, we suggest that the reduction in basipetal transport of IAA in low-vigour kiwifruit rootstocks may affect the leaf growth by reducing the leaf size of kiwifruit scions. Besides affecting the SAM, the evidence found in our study (Chapter Four and Chapter Five) was an important phenomenon in what way auxin (i.e. IAA) is controlling the leaf growth of kiwifruit. It is also suggested that the leaf characteristics (e.g. leaf size) could be used as a criteria in selecting the new potential low-vigour rootstocks in kiwifruit.

7.3.3 The role of auxin in bud break, subsequently branching in kiwifruit

As outlined earlier in the Section 7.2.3, we found that the bud break of 'Hayward' scions was significantly affected by the kiwifruit rootstocks. The effect of kiwifruit rootstocks on the bud break can be detected as early as in the initial stage of vines development (Table 2.2), as well as when the kiwifruit vines were transplanted in the field (Table 3.1, Figures 3.2, 3.3 and 3.4). These results are consistent with the previous studies in mature kiwifruit vines (Anon., 2012; Wang et al., 1994b) and also other vine species such as grape (Nikolaou et al., 2000) that found rootstocks may have the ability to influence scions bud break. Another finding that is worthy to take note, there was a strong trend (P=0.06) that the insertion of bark from other kiwifruit cultivars (G3, G9 and G14) either normal or inverted orientation may have an effect on the spring bud break of 'Hort16A' vines (Table 5.5). Therefore, we suggest that the effects of kiwifruit rootstocks or grafting a ring of bark from other kiwifruit cultivars on the bud break could be mediated by the endogenous hormones signalling between shoot (i.e. scion) and root system (i.e. rootstock) or *vice versa*.

According to previous studies, the bud break and successive branch growth are dynamically controlled by the endogenous hormonal signalling, particularly CK produced in roots and IAA produced in shoots (Campoy et al., 2011; Costes & Guédon, 2002; Faust et al., 1997). Auxin (i.e. IAA) from the apical shoot is the primary signal that is directly or indirectly responsible for inhibiting bud outgrowth (Leyser, 2003), while CK was implicated in breaking dormancy or initiation of bud outgrowth (Cline, 1994). In *A. chinensis* kiwifruit, it was found that additional IAA by exogenous application to the buds in early spring did not affect the bud break, but it can be increased by using synthetic CK, 6-benzylaminopurine (BAP) (Vattiprolu, 2012). Studies on other fruit trees also reported that higher spring bud break appeared to coincide with the high level of CK in the shoots, as found in apple (Cook et al., 2001; Cutting et al., 1991; Tworkoski & Miller, 2007) and grape (Nikolaou, 2000). In kiwifruit, Currie (1997) found that the restriction basipetal transport of IAA either by decapitated, girdling and NPA on the own-rooted cuttings resulted in the temporary elevation of CK level in root xylem exudate in a similar manner to girdling. These effects were similar to the hypothesis of the existence of a feedback loop between IAA and CK proposed by Bangerth (1994).

According to Bangerth (1994), in this feedback loop, a decrease in the besipetal transport of IAA would stimulate the synthesis and the export of CK from roots via xylem sap. The increase in CK concentration in the xylem sap would increase the synthesis and translocation of IAA out of apical shoots, thereby reducing the CK levels in the xylem sap (Bangerth, 1994). It was also notable in a recent study by Vattiprolu (2012) that found increased in the formation of sylleptic axillary shoots when kiwifruit vines were treated with NPA, indicating built-up of CK in regulating sylleptic branching. Therefore, theoretically, NPA treatment on the kiwifruit rootstocks stem may increase the CK level during initial bud growth, thereby increasing the proportion of scions bud break. However, our results were contradicted to the previous theories, as we found restriction basipetal transport of IAA by NPA treatment at the earliest possible stage of kiwifruit bud growth did not give any effects on the initiation of lateral axillary buds (bud break) (Table 4.6). Nevertheless, the results of the present study similar with the study in pea that also found the application of NPA to the buds had no effect on the initial bud outgrowth and treated buds grew normally a few days after the first application (Brewer et al., 2009). Unfortunately, lack of significance interactions in our

data to demonstrate that there was an interaction between rootstocks and NPA treatment that may be affecting the initiation of kiwifruit bud outgrowth in the early spring (Table 4.6). Nevertheless, we believe that the bud break of kiwifruit scions may be correlated with the internal carbohydrate status transported from the root (i.e. rootstock), and possibly mediated by the endogenous hormones.

In Chapter Four, we found that the inter-specific hybrid kiwifruit rootstocks had a significant effect on the mean number of of scion primary shoots (i.e. branching) (Table 4.7). In addition, there was a trend that restriction of IAA transport by NPA may have reduced the number of proleptic axillary shoots (Table 4.7). Based on these results, it is reasonable to suggest that reduced basipetal transport of IAA from shoot to root system may influence the transport of CK from the root to shoot, thereby affecting the branching of scions. However, it was also found in our study that the number of proleptic axillary shoots produced from scions was highly variable between the kiwifruit rootstocks (Table 4.7), implying that the level of CK transported from root to shoots may be various between the kiwifruit rootstocks. Besides that, the actual level of CK transported by the kiwifruit rootstocks could not be explained by our data until the endogenous level of CK is fully quantified. In our study, we suggested that the reduction in basipetal transport of IAA by NPA treatment may reduce the CK and GA transported to the scion, thereby may limit the branching as proposed by van Hooijdonk et al., (2010). For those inter-specific hybrid kiwifruit rootstocks that have the low-vigour ability, they were expected to reduce the scion growth and limit the branching compared with high-vigour rootstocks.

We also found that the 'Hayward' scions on rootstocks No.87, No.19 and No.100 (low-vigour group) reduced the mean total length, total node number and total leaf area compared to control vines (GN, self-rooted cuttings) (Table 4.7). Besides that, what was unexpected for us were our results showing that the scions on 'Bruno' rootstock had the least mean total length, total node number, branching, which contributed to the reduction in mean total leaf area (Table 4.10). The reason for these effects is unknown; perhaps this rootstock may have had different capability to transport CK from root to shoot as compared to other kiwifruit rootstocks. Nevertheless, we strongly suggest that the differences in the genetic of the inter-specific hybrid kiwifruit rootstocks may have significant influence on the branching ability of the 'Hayward' scions, because the

branching processes in plants are mainly controlled by the genetic signals (Leyser, 2003; Schmitz & Theres, 1999). This has been demonstrated in a study with new selections of apple rootstocks (Fazio & Robinson, 2008). However, it should be noted that branching is highly plastic and can be influenced by the environmental conditioned, endogenous hormonal signalling and growth manipulation (e.g. pruning) (Evers et al., 2011). It is also questionable whether the mechanism (s) of hormonal signalling and responses in stem cuttings of kiwifruit (e.g. Currie, 1997; Vattiprolu, 2012) may also exhibit the similar responses in those of grafted vines. Therefore, further trials on these aspects may reveal additional information on how branching of kiwifruit can be affected by the use of kiwifruit rootstocks.

Recent discoveries about strigolactones (SLs) as a new group of plant hormones that are involved in the inhibition of shoot branching (Gomez-Roldan et al., 2008) may also open new opportunities in kiwifruit research. The evidences found in this thesis, and also evidences from the previous studies (Clearwater et al., 2004; 2006; Cruz-Castillo et al., 1991; 1997; Wang et al., 1994b), together with the findings in molecular and genetic works in kiwifruit (Honda et al., 2011; Ledger et al., 2010) strongly suggest that 'root-derived signals' from rootstocks such as SLs may be involved in the controlling shoot branching and canopy architecture of kiwifruit scions. Even though in this thesis we did not conduct detail studies on SLs, considerable effort was made to evaluate the initial role of SLs in the different phenotype of kiwifruit seedlings in relation to their branching habit (Appendix 11). Based on our preliminary findings in Appendix 11, we suggest that the SLs may interact with other hormones such as CK and GA in regulating branching of kiwifruit shoots. We also believe there will be a strong interaction between SLs and GA in regulating shoot branching in kiwifruit, as a recent study found that the branching in kiwifruit is very responsive to additional GA application (Manandhar, 2016; Vattiprolu, 2012). Therefore, this study needs to be continued in the future in order to elucidate the branching mechanism (s) that regulate shoot architecture of kiwifruit. The role of GA and branching in kiwifruit is discussed in detail in Section 7.4.2. Overall, our results in this study have demonstrated the importance of auxin (i.e. IAA) signalling in regulating shoot architecture of kiwifruit (Chapter Four) and the importance of cell polarity in the basipetal transport of IAA in kiwifruit stem (Chapter Five). However, we noticed that lacking of data of the significance interactions between main effects is probably due to insufficient replication in our study. Although the early

responses of kiwifruit vines indicating that the basipetal transport of IAA was restricted (Figure 4.5, Figure 5.5, Figure 5.10 and Appendix 7), there was a possibility that the NPA concentration used in this study was too low (10 mg/mL^{-1}), thus only gave minimum or no effect at all on the rootstock and scion growth (Chapter Four). In bark grafting treatment, we suggest that the healing processes in bark inserts/grafting were faster especially in bark grafting in normal orientation, and presumably, a new translocation was resumed (Chapter Five).

7.3.4 Auxin transport and vigour of kiwifruit rootstocks

The IAA has been associated with the vigour of the rootstocks in other fruit tree species such as apple (Kamboj et al., 1997; Soumelidou et al., 1994; van Hooijdonk et al., 2010) and peach (Sorce et al., 2002). However, the information on the relationship between auxin transport and vigour is still lacking in kiwifruit. Even though a recent study was conducted by Vattiprolu (2012) in self-rooted kiwifruit cuttings with an attempt to reveal the hormonal relationship between shoot and root system; however, the actual involvement of auxin (i.e. IAA) still remains unclear. Based on the findings in Experiment One of Chapter Four, we suggest that the meristematic activity of 'Hayward' scions was influenced by the basipetal transport of IAA from the shoot, and this could be controlled by the amount of IAA reached on the rootstock. Therefore, it is worthy for us to study the involvement of polar auxin transport and its contribution to the vigour control in the inter-specific hybrid kiwifruit rootstocks (Experiment Two, Chapter Four). This study was the first attempted to assess the actual IAA transport in kiwifruit stem from different vigour. In this study, we found that the 'agar-donor receiver transport system' used in our trial (Figure 4.4) and developed from previous studies (Kamboj et al., 1997; Soumelidou et al., 1994) works really well with the kiwifruit stem. However, due to a limited number of planting materials, only selected kiwifruit rootstocks such as No.18, No.100, No.87, No.45, No.55, No.86 and No.101 including 'Bounty 71' were subjected to this agar-donor receiver transport system. Nevertheless, most of the kiwifruit rootstocks represented a range of vigour from low to high-vigour.

An experiment conducted in the late autumn season for 24h found that the uptake and transport of radioactivity in apical segments was significantly lower in the rootstock No.18 (low-vigour) compared to apical segments from No.101 (high-vigour) (Figure 4.9). However, the uptake and transport of radioactivity in middle segments did not differ between kiwifruit rootstocks (P=0.13), but there was a trend that the uptake and transport of radioactivity were lower in basal segments of rootstock No.18 (Figure 4.9). Another interesting result was that the total uptake and transport of radioactivity (sum of activity in all the segments and agar) in rootstock No.18 were significantly lower compared to No.101 (Table 4.8). These results indicate that the stem tissues of low-vigour kiwifruit rootstock No.18 may have the ability to reduce basipetal transport of IAA. Besides that, these results may also reflect the influence of IAA transport at the different growing stage and timing of shoot termination, as we observed that the shoots of low-vigour rootstocks tended to terminate early than shoots of vigorous rootstocks, but unfortunately, we did not collect the data on shoot growth to support our results.

Further assessment was conducted during summer 2014 season when a few more rootstocks were included in this trial. In this assessment, the uptake and transport of radioactivity in the stem segments of inter-specific hybrid kiwifruit rootstocks were evaluated at two different hours, 24h and 48h. This enables us to find out whether there are differences in the time period of IAA uptake and activity between inter-specific hybrid kiwifruit rootstocks. Our results found that the rootstock, hour and rootstock x hour interactions had significant effects on the total uptake and transport of radioactivity (Table 4.9). In addition, the transport of radioactivity into agar receptor was also significantly different between inter-specific hybrid kiwifruit rootstocks and hours (Table 4.9). These results indicate that the kiwifruit rootstocks may have different capacity to transport and uptake of IAA in their stem tissues similar to what have been found in apple (Kamboj et al.,1997; Soumelidou et al., 1994). There was a trend that the total uptake and activity of IAA was increased with increasing the vigour of the inter-specific hybrid kiwifruit rootstocks (Figure 4.10). However, the pattern could only be found on low and intermediate-vigour rootstocks (rootstock No.18 until rootstock No.45), but after that, the total uptake and transport of radioactivity in more vigorous kiwifruit rootstocks of No.55, No.86 and No.101 were reduced. These results were unexpected because previous studies in other fruit trees have found that the level of IAA in the stem is increased with increasing the vigour (Jindal et al., 1974; Kamboj et al.,

1997; Martin & Stahly, 1967; Michalczuk, 2002; Noda et al., 2000; Sorce et al., 2002; Soumelidou et al., 1994). We suggest that possibly a few factors may have influenced the level of IAA transport in the kiwifruit rootstocks observed in our study, especially in rootstocks of No.55, No.86 and No.101. Sufficient growth resources in the nursery stage such as nutrients (i.e. fertiliser), water supply (i.e. irrigation), and pest and disease control may have influenced the vigour of the rootstocks. Furthermore, we also strongly believe that the root growth of kiwifruit rootstocks especially rootstocks No.55, No.86 and No.101 were confined to small 5 L polybags, thereby affecting the shoot growth of scions. Similar evidence has been also demonstrated in the recent study with apple rootstocks (Tworkoski & Fazio, 2015).

In this study, considerable effort has been made to assess the rooting system of two inter-specific hybrid kiwifruit rootstocks with contrasting vigour, No.18 and No.101 (Appendix 12). Based on our preliminary assessment, the root system of high-vigour kiwifruit rootstock No.101 may have had larger root system compared to the low-vigour kiwifruit rootstock No.18 (Appendix 12C). In order to confirm our visual observation, replicated root samples (n=4) from both rootstocks were analysed by destructive sampling to measure their characteristics of the root system. We found that there were trends that both rootstocks may be differed in their root systems as assessed by fresh and dry weight of roots (P=0.06 and P=0.08, respectively) (Appendix 12D). Therefore, restriction of root system by the polybags may have limited the root growth, subsequently may have an influence on the IAA transport in the stem, and thereby may affect the vigour of rootstocks, especially rootstock No.101 (Appendix 12A and 12B). In the future research, it would be interesting to study the ability of inter-specific hybrid kiwifruit rootstocks to transport endogenous hormones to the grafted scions as revealed in current studies with apple (Li et al., 2012; Tworkoski & Fazio, 2015; van Hooijdonk et al., 2011).

7.4 PART 3: Possible approaches for vigour manipulation of kiwifruit

7.4.1 Utilization of seedling architectural characteristics

Most of the potential architectural traits or characteristics in fruit trees could be identified as early as in the seedling stages. However, there is no information available for kiwifruit on how the architectural traits in the early seedlings stage may influence their future growth and development. Therefore, in Chapter Six, we studied and explored the important of architectural characteristics or growth habits of kiwifruit during the initial stage of seedlings growth. One of the traits that we focussed is the branching habit of kiwifruit seedlings since no one has ever studied the branching that contributes to the architectural form during the initial stage of seedling development. The architectural trait based on the branching pattern has been used to predict the agronomic importance of the fruit trees. In the early stage of tree development, branching is important and considered as an advantage for early establishment of orchards (Robinson, 2004; Van Oosten, 1976). Since branching is mainly developed during the early stage of tree growth (Costes & Guédon, 2002), this trait is expected to be a potential early selection criteria, because it may influence agronomic performance at mature phase of trees or vines (Costes et al., 2006a; Lauri et al., 2008).

Kiwifruit seedlings obtained from the specific crosses were evaluated as early as in the first growing season (Figure 6.1). Detailed architectural characteristics produced from the kiwifruit seedlings were also presented and described (Figure 6.1, Tables 6.1, 6.2 and 6.3). We found there were two different major groups within kiwifruit seedling population; i) the seedlings with branching and ii) the seedling with non-branching form (Figure 6.2). The first group can be further classified as the seedlings that have produced multiple shoots either long or short shoots as their main primary shoots. The second group found from the seedlings population was the seedlings that have produced only one single shoot, either long or short as their primary shoot. Further segregation of these seedlings revealed that the seedlings can be classified into four distinct phenotypes based on their primary shoots which were; i) Long Multiple Stems-LMS (Figure 6.2A), ii) Short Multiple Stems-SMS (Figure 6.2C), iii) Long Single Stem-LS (Figure 6.2B),

and iv) Short Single Stem-SS (Figure 6.2D). The branching group represented the highest proportion in the seedling population with 62.8% compared with the non-branching group with only 37.3% (Figure 6.3). Highly significant differences were found (P<0.0001) in the characteristics of the main primary shoots between the phenotypes. The phenotypes that have long main primary shoots such as LS and LMS may have longer shoot length than other phenotypes, with the values are almost two or three-times longer than the shoots from SS and SMS phenotypes (Table 6.2). These results indicate that there was a huge variability in the initial vigour and growth habit of kiwifruit seedlings similar to the observation in other fruit trees such as apple (De Wit et al., 2004), avocado (Barrientos-Pérez & Sánchez-Colín, 1983) and olive (Hammami et al., 2011; 2012).

Based on the architectural description made by Seleznyova et al. (2002), the primary shoots from LS and LMS phenotypes could be classified as long shoots and for SS and SMB phenotypes, their primary shoots could be possibly classified as medium shoots on the basis of the internode length and node number. This evidence may reflect the important of these two characteristics because internode length and node number distribution within the vines structure of kiwifruit are important variables in modelling their architecture, and may also contribute to the characteristics forming of vines (Seleznyova et al., 2002). Other characteristics such as shoot length, node number and stem size (i.e. diameter or shoot CSA) are often used for estimating seedling vigour. In other fruit trees, the seedling vigour based on height and stem size of primary shoots have been widely used in practice in order to discard those seedling plants with a long juvenile period. Vigorous seedlings are considered as an advantageous trait for obtaining a short juvenility period, as demonstrated in studies with apple (De Wit et al., 2004; Lapins, 1969; Segura et al., 2006), olive (De la Rosa et al., 2006; Pritsa et al., 2003; Santos-Antunes et al., 2005), and plum (Hartmann & Engelhorn, 1990). In contrast with our study, we are interested in the seedlings genotype or phenotype that has low-vigour ability. These types of seedlings may be valuable for developing low-vigour scions or could be used as vigour-controlling rootstocks. However, little attention has been given to these characteristics during the selection of potential kiwifruit seedlings in the early stage of the breeding programme. We believe that these types of seedlings may have long juvenility period based on their initial growth characteristics. Therefore, this might be the reason of why these seedlings were

discarded during initial kiwifruit breeding selections. In this study (Chapter Six), the LMS and SMS phenotypes produced almost three-fold higher in the number of main primary stems compared to LS and SS phenotypes (Table 6.2). However, the number of stem was inversely correlated with the length, node number and stem CSA, and was not correlated with internode length of main primary shoots (Table 6.3). Other studies reported similar evidence that the stem number did not correlate with the characteristics of primary shoots (Barrientos-Pérez & Sánchez-Colín, 1983; De Wit et al., 2002; Segura et al., 2006). These results indicate that the number of stem was not necessarily correlated with the vigour of the main primary shoots, and this evidence is also similar with our findings in Chapter Two. We suggest that highly significant differences in branching and shoot architectural structures among phenotypes may be attributed to the differences in the genetic components, origin or parentage of the kiwifruit seedlings.

In this study, we tried to reveal the most significant characteristics with the higher ability for describing the differences in the architectural structure of the kiwifruit seedlings. We believe that these characteristics will be largely useful in the future kiwifruit breeding programme. Even though some studies mentioned that the characteristics such as length, node number, internode length and CSA of main primary shoots are important criteria and relevance in describing the vigour during the initial stage of seedlings growth, we found that these characteristics were not sufficient for estimating the 'vigour' of the kiwifruit seedlings. Although branching in kiwifruit vines is not correlated with the vigour of the vines, it may have a significant impact on the future physiological architecture and agronomic characteristics of the kiwifruit related to the planting design, canopy light interception, tree size and flowering and fruiting habits. Moreover, in our study, it would be reasonable to suggest that the selection based on the phenotypic architecture of kiwifruit may also segregate the genotypic differences of kiwifruit seedlings. Our study is also limited to the small number of seedling samples (n=102). Therefore, for the future studies, it would be interesting to carry out a similar evaluation with a large number of samples. Additionally, more parameters should be taken and included in the measurements, particularly total dry weight, in order to reveal more information on the relationship between initial architecture or growth habit and their growth potential. Nevertheless, based on our findings, we believe that dwarfing habits of the kiwifruit seedlings may have a potential to be used as low-vigour rootstocks or low-vigour scions. Studies on the endogenous

level of GA and other hormones (e.g. IAA and CK) could also provide an interesting information on how the vigour of the seedlings can be related to the hormones, as it is known that the genetic dwarf plants produce insufficient amount of GA such as found in dwarf apples (Grochowska et al., 1984; Looney & Lane, 1984; Steffens & Hedden, 1992; Yadava & Lockard, 1977). Furthermore, it is really worthy for further studies to investigate if these types of kiwifruit seedlings can be clonally propagated in order to be used as rootstocks or scions.

7.4.2 Manipulation of vines architecture by gibberellin application

Based on the study in Experiment One of Chapter Six, we believe that the four phenotypes (LMS, LS, SMS and SS) from the kiwifruit seedlings population that have been obtained from the specific-crosses may have a different level of endogenous hormones, especially gibberellins (GA) that contributed to the differences in the shoot architecture structures. We would like to test whether application of GA treatment (GA_3 + GA_{4+7}) can modify their architecture and morphology. Theoretically, this approach may allow fulfilment of the desired canopy size by GA application especially low-vigour vines. The application of GA could then be stopped to when the canopy have established to channel a greater carbohydrate allocation to the reproductive instead of the vegetative stage. Besides that, using low-vigour scions will increase the number of rootstocks available regardless of the rootstock vigour, as demonstrated in a study with apple (Bulley et al., 2005).

Application of GA to all phenotypes may have altered the morphology of proleptic shoots. We expected that application of GA may increase the length of long proleptic lateral axillary shoots, but the length was significantly reduced with the GA treatment in all phenotypes, possibly due to the reductions in the node number, internode length and smaller stem CSA (Table 6.4). We also noticed that the long lateral axillary shoots from GA-treated vines developed very thin and very slender shoots compared to the long shoots from untreated vines, regardless of the phenotypes. However, less effects of GA were found on the medium and short shoots except for the internode length (Table 6.4 and Table 6.7). One of the interesting findings in this study was that the internode

length of kiwifruit was not affected by the GA treatment regardless of the phenotypes. Our results demonstrated that the shoots of the same node number have a similar length irrespective of whether GA is applied or not (Figure 6.6A, B, C and D). Even though many studies in other fruit crops (e.g. le Roux & Barry, 2010; Tagliavini & Looney, 1991) including kiwifruit (Vattiprolu, 2012) concluded that typical effect of GA on shoot growth is greater internode elongation (Brian, 1959), and the actual interpretation of the GA effect on mean internode lengths can be completely misleaded by the previous studies. Therefore, any conclusion on the treatment effects on the internode length of kiwifruit shoots should be interpreted with caution.

Application of GA also increased the branching in terms of a total number of lateral axillary shoots (proleptic + sylleptic shoots) per phenotype (Table 6.6). This result has confirmed the previous study suggesting that kiwifruit vine is very responsive to the GA application (Vattiprolu, 2012). Interestingly, the low-vigour phenotypes such as SS and SMS still produced the lowest number of branching compared with the other phenotypes (i.e. LS and LMS) (Table 6.6). This result may reflect the low-vigour ability of these phenotypes as we found less branching was produced, even though after GA application. Increased branching of lateral shoots by GA treatment also had caused significantly greater mean total length and a node number of lateral axillary shoots for all phenotypes (Table 6.6). The proportion of non-terminated shoots was significantly higher in SS phenotype, whereas LS phenotype produced the lowest proportion of non-terminated shoots among the phenotypes (Table 6.5). We suggest that SS phenotype may be having a lower and/or insufficient level of endogenous GA, and additional GA may have active both apical and sub-apical meristem, thereby increasing the proportion of non-terminated shoots. However, additional exogenous GA could not be utilised by the LS phenotype, presumably this phenotype may already had sufficient amount of endogenous GA.

Another interesting result found in our study was that GA treatment had stimulated the buds located at the shoot apex and base to break and form the sylleptic axillary stem, even though the shoot apex of proleptic axillary shoots was still actively growing (Figure 6.5). These results are consistent with the recent studies that also found similar evidence on the branching of kiwifruit (Vattiprolu, 2012) and *Jatropha curcas* seedlings when treated with GA (Ni et al., 2015). Overall, we found that both branching (i.e. LMS

and SMS) and non-branching (i.e. LS and SS) phenotypes of kiwifruit seedlings are so responsive to the exogenous application of GA, particularly the seedlings from low-vigour phenotypes. Our findings are in agreement with the previous studies that also found seedlings to be very responsive to GA application, as demonstrated in apple (Lee & Looney, 1977), cherry (Oliveira & Browning, 1993) and current study in *Jatropha curcas* (Ni et al., 2015).

In this study, we have demonstrated that the genetic dwarf in kiwifruit can be manipulated by GA application, by promoting the meristematic activity of both apical and sub-apical meristems. It was also observed in our study that GA treatment not only affecting the apical and sub-apical meristems of kiwifruit, but also altering the leaf growth of the vines. We observed that the leaves of kiwifruit vines treated with GA were smaller than the leaves from untreated vines regardless of phenotypes. Unfortunately, in this study, we did not measure the size and area of leaves between GA-treated and untreated vines. Therefore, it is likely that the application of GA has altered the transport and allocation of photoassimilates, and possibly carbohydrate accumulation in kiwifruit vines. We believe that the GA has stimulated the transport of photoassimilates and carbohydrate accumulation by diverting them into the growing tip of the shoots (i.e. apical and sub-apical meristems, as well as elongating internode) instead of the leaves. Therefore, GA may have altered the source-sink relationship in the kiwifruit vines. As currently reviewed by Iqbal et al. (2011), GA is believed to mediate assimilate translocation through an increase in the extracellular invertase, which is responsible for phloem unloading into the sink, thereby increasing the sink activity and also sink strength (Iqbal et al., 2011). We suggest that the developing organs such as new shoots outgrowth after the bud break that actively elongating may become a potential site for GA activity. All these suggestions may open new possibilities for future studies.

7.5 Directions for future research in kiwifruit

The research covered in this thesis aims to investigate the mechanism (s) of vigour control in kiwifruit, in relation to the hormonal physiology as well as the opportunity to manipulate the vigour of kiwifruit. In kiwifruit, as highlighted before, the vigour control of vegetative growth is important in order to achieve optimal yield with high-quality fruits. The findings of this thesis have revealed a few mechanisms of vigour control in kiwifruit vines. Nevertheless, our study may have also opened other research opportunities or questions that need to be answered in the future. A number of possible research questions or approaches are highlighted below:

1. Our findings have demonstrated that the architectural modifications of 'Hayward' scions imposed by the inter-specific hybrid kiwifruit rootstocks can be identified during the first and the second year following grafting (Chapter Two). Therefore, further experimental works could be directed to study the effects of inter-specific hybrid kiwifruit rootstocks using different scion cultivars such as G3, G9 and G14. Besides that, the rootstocks could be tested under different environmental conditions and climatic regions to assess whether modification (s) of scions by the inter-specific hybrid kiwifruit rootstocks is similar or different across the environment and climatic region. Rootstocks expression could be changed under different climatic regions as demonstrated in previous studies with apple rootstocks (Foster et al., 2016; Seleznyova et al., 2008; van Hooijdonk, 2009).

2. Still potential dwarfing or vigour-controlling rootstocks in kiwifruit is could not be identified based on a single trial and need a few years for evaluation. As demonstrated in this thesis, the inter-specific hybrid kiwifruit rootstocks have potential to affect the scions vigour, architectural pattern and possibly flowering and fruiting (Chapter Three). Therefore, further evaluation of effect of the inter-specific hybrid kiwifruit rootstock on flowering and fruiting is warranted with a large number of replicates. Furthermore, it would be also interesting to study whether the inter-specific hybrid kiwifruit rootstocks have potential to confer resistant, tolerant or susceptible reaction to diseases such as *Pseudomonas syringae* (PSA) (Vanneste et al., 2011). In this study, there was an indication that a particular kiwifruit rootstock such as rootstock No.45 was susceptible to the disease (data not shown).

3. It has been proposed that dwarfing rootstocks may reduce the basipetal transport of IAA to the root (Li et al., 2012; Lockhard & Schneider, 1981; van Hooijdonk et al., 2010), subsequently decreasing the amount of CK and GA in the root system transported to the scion (Tworkoski & Fazio, 2015; van Hooijdonk et al., 2010, 2011). In this study we have identified that IAA is involved in regulating the shoot architecture in the grafted kiwifruit vines and possibly that amount transported from shoot to root may be differed according to the vigour of kiwifruit rootstocks (Experiment One of Chapter Four). Besides that, the uptake and transport of IAA were significantly different between various inter-specific hybrid kiwifruit rootstocks and may be influenced by the growing season (Experiment Two of Chapter Four). Therefore, endogenous hormonal signalling between IAA, CK and GA in grafted kiwifruit vines need to be quantified periodically throughout the growing season.

4. Recent studies have identified the genes, *Dw1* and *Dw2* that are responsible for rootstock-induced dwarfing in apple (Fazio et al., 2014; Foster et al., 2015; Pilcher et al., 2008) and pear (Knäbel et al., 2015). However, little is known about the genetic basis of kiwifruit rootstocks. Therefore, studies on the genetic background of kiwifruit rootstocks are an important step in unravelling the mechanism behind the dwarfing ability of kiwifruit rootstocks.

5. There were strong trends that the transport of IAA in the stem of kiwifruit rootstocks may be related to the size of the root system (Appendix 12) because of the reduced amount of IAA reaching the roots from shoots and that limits the root growth (Lockhard & Schneider, 1981). Therefore, it is interesting to identify whether there is a correlation between the endogenous transport of IAA and possibly other hormones (i.e. CK and GA), and the root growth of the kiwifruit rootstocks. Moreover, it is also suggested that further investigation could be focused on the metabolic fate of IAA on the stem of different vigour kiwifruit rootstocks, as we found that part of stem shank (and stem bark) could be responsible for dwarfing effects (Lockhard & Schneider, 1981) (Chapter Five).

6. In our study (Experiment Two of Chapter Six) and in a previous study (Vattiprolu, 2012), it has been demonstrated that GA alone can regulate shoot branching in kiwifruit. Therefore, fine scale phenotyping through scanning electron microscopy studies is needed to evaluate the actual mechanism (s) of GA regulating branching in kiwifruit. Trials using and application of GA in a lanolin paste on the stem of young kiwifruit could also be tested to assess whether endogenous application of GA also may regulate the branching of kiwifruit, similarly to the foliar application. Furthermore, it would be also interesting to study the role of GA in stimulating transport of photoassimilates and carbohydrate accumulation in kiwifruit vines, as well as changes in organ allometry in relation to the vigour.

7. Our study had identified that the differences in the vigour, branching and shoot architectural structures among LMS, LS, SMS and SS phenotypes of seedlings may be attributed to the differences in the genetic components and/or origin of the kiwifruit seedlings (Experiment One of Chapter Six). Therefore, further studies are needed to evaluate the use of low-vigour seedlings phenotypes as rootstocks or low-vigour scions, whether the low-vigour ability of these can be transferred to the grafted scion or can be retained if use as a scion regardless of rootstock vigour, as well as whether the potential kiwifruit seedlings can be easily propagated clonally.

8. Anatomical characteristics of rootstocks have been shown to correlate well with the vigour of the scion. However, in kiwifruit, this aspect is still lacking enough evidence and need to be further investigated. It is relatively unknown whether anatomical characteristics of kiwifruit rootstocks such as xylem to phloem ratio, vessel diameter etc. may have an influence on the vigour of grafted scions. Besides that, experiments looking at mucilage output of various kiwifruit rootstocks in relation to the precocity and flowering are worthwhile to investigate. Until now, only one study was conducted on anatomical studies in kiwifruit rootstocks, however, not on the vigour aspect but on flowering ability (Wang et al., 1994a). Therefore, studies on the anatomical characteristics in kiwifruit rootstocks would also provide additional information on how the anatomy of rootstocks could be related to the vigour-control ability in kiwifruit vines.

7.6 Conclusion and final comments

The first important results of this thesis revealed the mechanisms of kiwifruit rootstocks in controlling the scion vigour. Our results in this thesis have strengthened the previous findings that kiwifruit rootstocks may have affected the scions vigour by altering the different proportion of shoots, leaf size and bud break of 'Hayward' scions. Besides that, the new findings also proposed that kiwifruit rootstocks may have the ability to affect the shoot growth rate, precocity and stem development of grafted scions. Our findings reveal that kiwifruit rootstocks have the ability to modify the scion growth as early as during the first and the second year following grafting. We have identified that auxin (i.e. IAA) is an important signal in regulating scions growth and this effect could be controlled by the kiwifruit rootstocks because the stem of different kiwifruit rootstocks have different ability to uptake and transport of IAA. There are lots of potential in the different phenotypes of kiwifruit seedlings obtained from inter specific-crosses, and these phenotypes should be utilised by the kiwifruit breeders whether as low-vigour rootstocks or low-vigour scions. We also have identified that GA had a direct role in regulating branching in kiwifruit, and the architectural structures of kiwifruit can be modified by using GA application.

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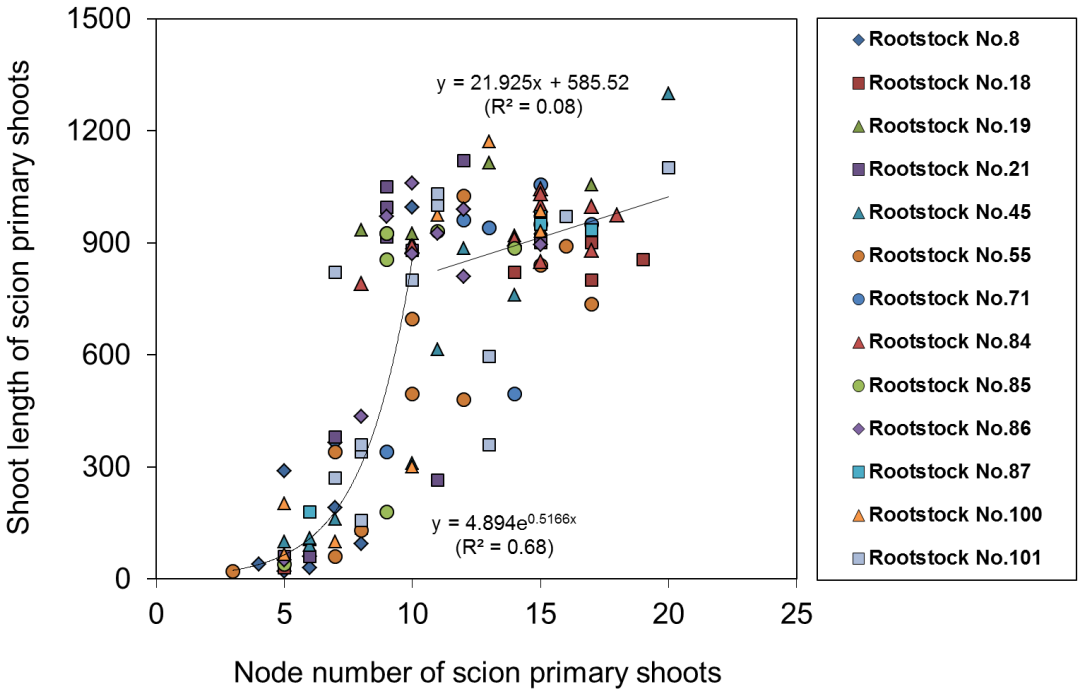
APPENDICES

<u>APPENDIX 1</u>. The relationship between shoot length (mm) and node number of scion primary shoots.



APPENDIX 2. The relationship between shoot length and node number for individual treatment (± NPA).

Rootstock No.	Treatment (±NPA)	R^2	*P*-value	Equations
19	+NPA	0.35	$P=0.40^{ns}$	y = 90.882x-650.88
	-NPA	0.90	$P=0.04^{*}$	y = 29.874x + 961.3
55	+NPA	0.91	$P=0.04^{*}$	y = 100.81x - 939.97
	-NPA	0.86	$P=0.23^{ns}$	y = 81.00x - 532.00
84	+NPA	- [y]	- [y]	- [y]
	-NPA	0.93	$P=0.008^{**}$	y = 76.889x - 339.62
86	+NPA	0.89	$P=0.21^{ns}$	y = 95.385x - 597.69
	-NPA	0.19	$P=0.55^{ns}$	y = 48.372x + 564.42
87	+NPA	0.56	$P=0.08^{ns}$	y = 78.686x - 405.4
	-NPA	0.95	$P=0.02^{*}$	y = 63.673x + 60.204
100	+NPA	0.93	$P=0.007^{**}$	y = 74.564x - 376.02
	-NPA	0.95	$P=0.02^{*}$	y = 101.75x - 756.41
GN	+NPA	0.98	$P=0.008^{**}$	y = 105.07x - 905.64
	-NPA	0.68	$P=0.08^{ns}$	y = 31.559x + 1706.7
Bruno	+NPA	- [x]	- [x]	- [x]
	-NPA	- [x]	- [x]	- [x]

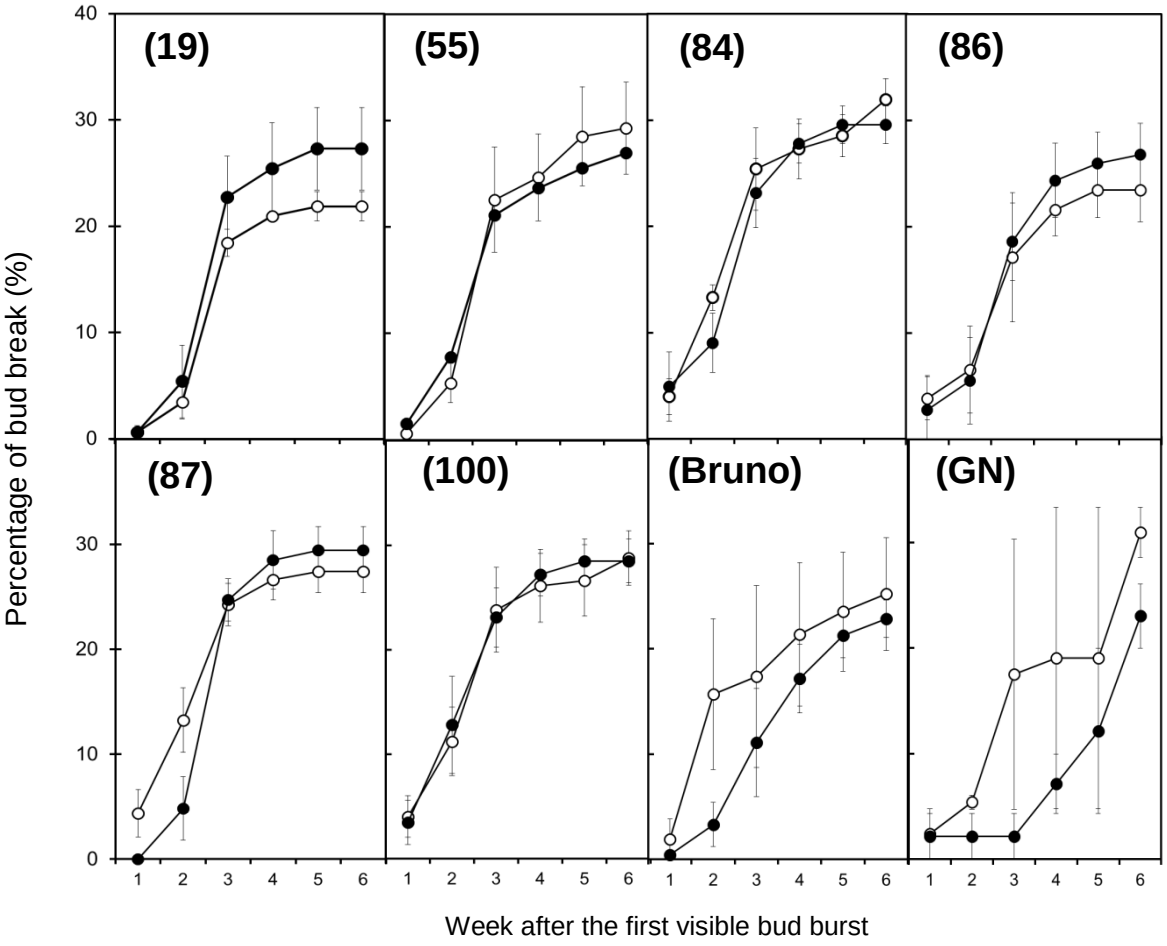
[x]Only two replicates available.

APPENDIX 3. Effect of rootstocks and auxin transport inhibitor (± NPA) on the the mean proportions of bud break of 'Hayward' scions in the early spring growing season (October 2013).

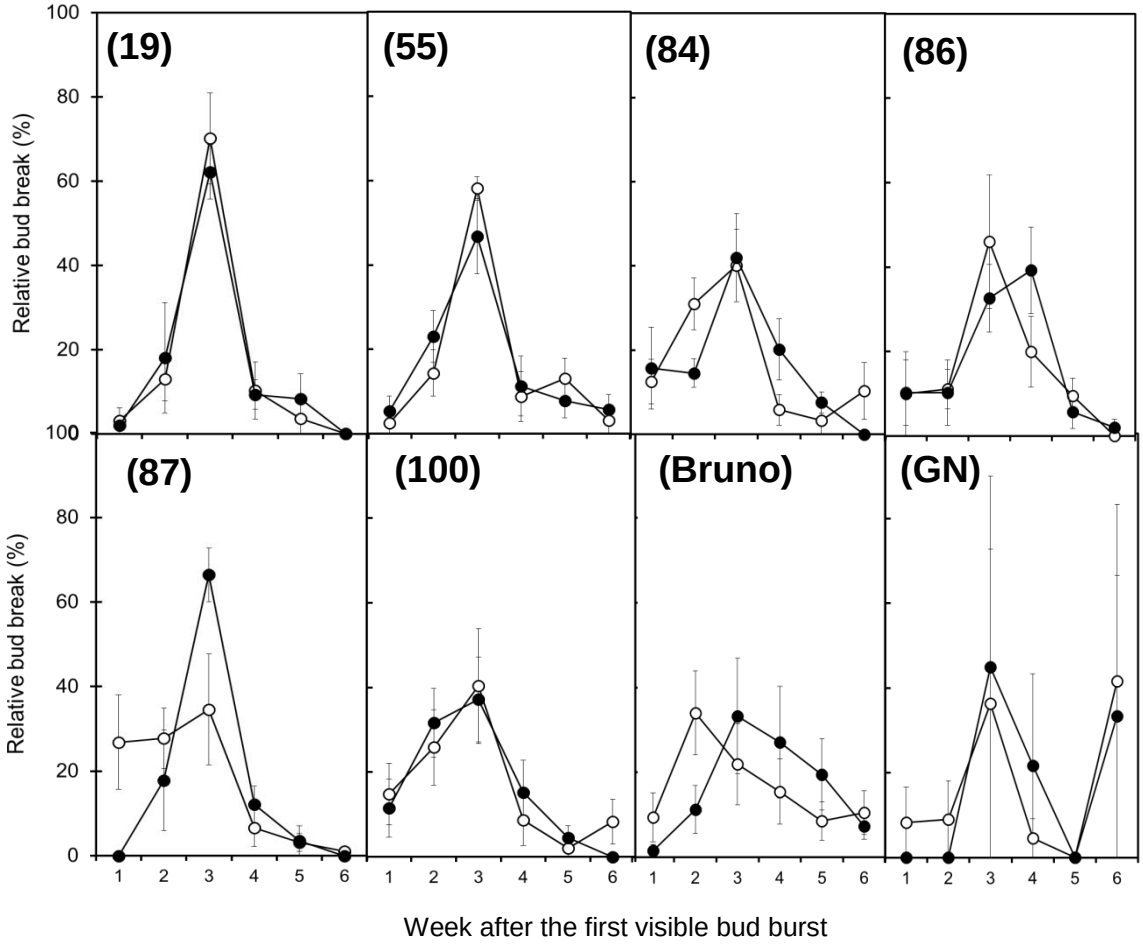
Main effects		Mean proportion of budbreak (%)
Rootstock	**Treatment (± NPA)**	
19	+ NPA	22.0 (± 2.8)
	- NPA	27.6 (± 2.8)
	***P*-value**	**(*P*=0.15) ^{ns}**
55	+ NPA	29.1 (± 2.8)
	- NPA	27.0 (± 2.5)
	***P*-value**	**(*P*=0.16) ^{ns}**
84	+ NPA^{x}	31.9 (± 2.5)
	- NPA	29.6 (± 2.5)
	***P*-value**	**(*P*=0.56) ^{ns}**
86	+ NPA	23.5 (± 2.5)
	- NPA	26.4 (± 2.8)
	***P*-value**	**(*P*=0.42) ^{ns}**
87	+ NPA	26.8 (± 2.5)
	- NPA	29.6 (± 2.8)
	***P*-value**	**(*P*=0.46) ^{ns}**
100	+ NPA	28.7 (± 2.5)
	- NPA	28.5 (± 2.5)
	***P*-value**	**(*P*=0.94) ^{ns}**
Bruno	+ NPA^{x}	31.9 (± 3.9)
	- NPA^{x}	24.0 (± 3.9)
	***P*-value**	**(*P*=0.16) ^{ns}**
Green cuttings (Own root)	+ NPA	24.7 (± 3.2)
	- NPA	22.8 (± 2.5)
	***P*-value**	**(*P*=0.64) ^{ns}**

^{x}Only two replicates available.

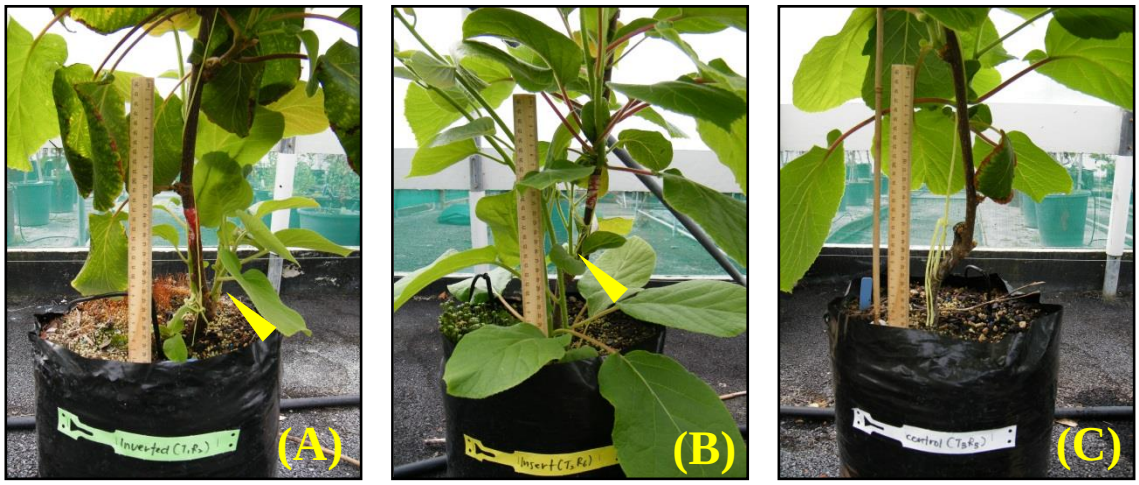
<u>APPENDIX 4.</u> Effect of rootstock and auxin transport inhibitor (± NPA) on the bud break pattern of 'Hayward' scions (%). Symbols indicate; NPA treated (○) and non-treated (●) vines.



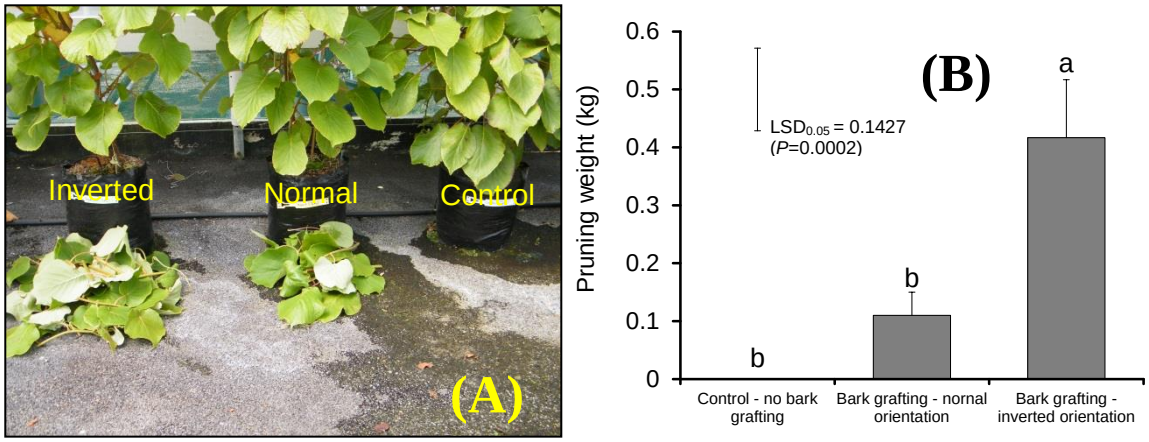
APPENDIX 5. Effect of rootstock and auxin transport inhibitor (± NPA) on the mean relative bud break of 'Hayward' scions. Symbols indicate; NPA treated (○) and non-treated (●) vines.



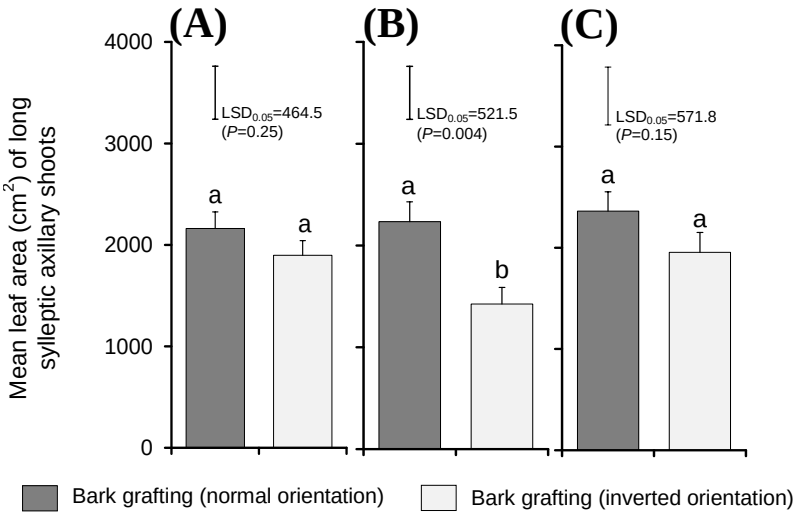
APPENDIX 6. Arrows indicate the axillary bud outgrowth below the graft-union area from the bark grafting vines; (A) in an inverted and (B) normal orientation but no axillary outgrowth from the control vines (C).



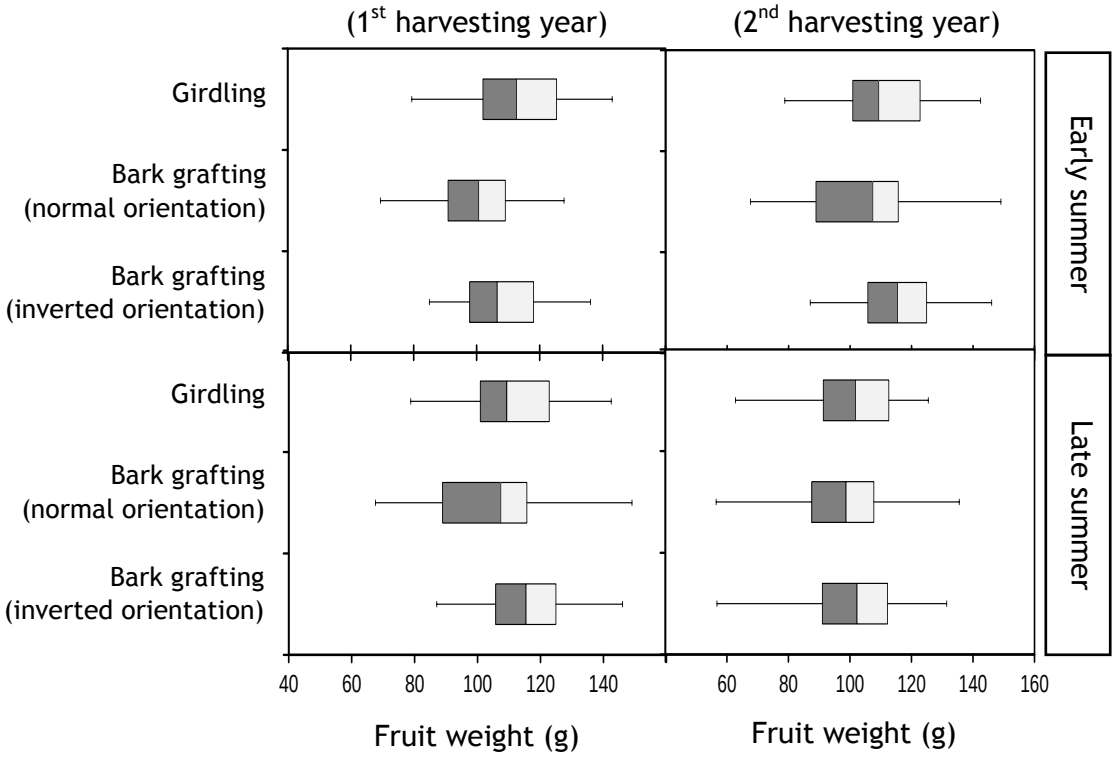
APPENDIX 7. (A) The amount of pruning of axillary outgrowth and suckers below the bark graft-union and; (B) the mean pruning weight (kg) of axillary outgrowth and suckers produced below the bark graft-union. Means sharing same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. Bars denote the LSD at *P*=0.05.



APPENDIX 8. Mean total leaf area of long sylleptic axillary shoots of young 'Hort16A' vines (cm^2) for the first 15 nodes at the end of growing season, means value (± standard error). (A) Bark grafting from G3 cultivar, (B) bark grafting from G9 cultivar, and (C) bark grafting from G14 cultivar. Means sharing same letters are not significantly different at *P*=0.05 according to $LSD_{0.05}$ test. Bars denote the LSD at *P*=0.05.



<u>APPENDIX 9</u>. The horizontal box-plots showing the fruit weight distributions (range) for bark grafting (normal and inverted) and girdling in the first and second harvesting year.



These box-plots displayed the range of distribution of fruit weight (g) and the data were also arranged according to the values from lowest to highest weight of fruits. Briefly, from the box-plots, the distribution of fruit weight (g) can be estimated whether the mean is less than or greater than the median. In the first harvesting season, the vines from the girdling and bark grafting in an inverted orientation conducted in early summer had the fruit weight distribution (g) approximately skewed to the right (positive skewed). However, the vines from bark grafting in normal orientation had the distribution of fruit weight (g) approximately symmetric (mean equal to median). For the vines treated in late summer, the fruit weight distribution (g) was approximately skewed to the right for the vines treated with girdling, skewed to the left for vines treated with bark grafting in normal orientation and symmetric for the vines treated with bark grafting in an inverted orientation. In addition, the distribution of vines with bark grafting in both orientations showed a wider spread of fruit weight than the girdling treatment. In the second harvesting season, for the vines treated in early summer (early December), the range of fruit weight (g) produced was nearly similar for all treatments. The fruit weight distributions of vines treated with girdling and bark grafting in inverted orientation were negatively skewed. However, a positive skew was observed for bark grafting in normal orientation. For the vines treated in late summer (early March), the distributions of fruit weight was negatively skewed for all treatments.

APPENDIX 10: Non-destructive leaf area estimation in green kiwifruit (*Actinidia deliciosa*) by using simple linear regression

Introduction

Models for the non-destructive measurement of leaf area in kiwifruit area are useful tools for scientists in horticultural experiments. Therefore, in order to avoid destruction and damaged to the canopy of the kiwifruit vines, simple and non-destructive models determining leaf area are required and these models also can be used for others experimental purposes. Simple linear mathematical models for estimation leaf area could be developed by using the measurements of length and width of leaves. The main objective of this study was to develop a simple model for estimating leaf area of green kiwifruit, and this model can be used for estimating the leaf size of green kiwifruit (*A.deliciosa*) scions that have been grafted onto inter-specific hybrid rootstocks.

Materials and Methods

The assessment was carried out at kiwifruit orchard, Fruit Crops Unit, Massey University, Palmerton North. A total of 105 green kiwifruit (*A. deliciosa*) leaves with various sizes were harvested. The leaves were kept in brown envelop and immediately transported back to the laboratory for further assessment. By using ruler, the length of leaves (cm) was measured from the tip to the petiole base, and the width (cm) was measured from end-to-end between the widest lobes of the lamina (Figure 1). Values of length and width were recorded to the nearest 0.1 cm. Besides that, the area of each leaf (LA) was measured using leaf area meter (LI-3100, LI-COR, Nebraska, USA). The relationship between LA as a dependent variable and length (L*l*), width (L*w*), length x width (L*l* x L*w*), Ll^2 and Lw^2 as independent variables was conducted using simple linear regression. These data were fitted together to a linear regression equation $y = a + bx$, where y represents the leaf area and *x* either independent variables, L*l*, L*w*, L*l* x L*w*, Ll^2 or Lw^2 (Table 1). The final model was selected based on the combination of the highest coefficient of determination (R^2) and the lowest root mean square root error (RMSE). To validate the developed model, the best model from the estimated experiment was compared with the actual leaf area that has been measured using leaf area meter. Regression analyses were performed using the SAS software (SAS Version 9.2, SAS Institute, NC, USA).

Results, discussion and conclusion

Based on the regression analyses, there were strong relationships between LA (cm^2) and L*l*, L*w*, L*l* x L*w*, Ll^2 and Lw^2 (Table 1). All models produced a coefficient of determination (R^2) greater than 0.90. However, the model No.3 (Table 1 and Figure 2) with the independent variable of L*l* x L*w* was found to be the suitable one. This model was chosen based on the highest R^2 (R^2=0.9903) and lowest RMSE (4.83 cm^2). Comparison between measured and calculated leaf area using model No.3 (Figure 3) showed a high degree of correlation (R^2=0.9903). This has shown high-accuracy prediction of individual leaf area observed in this study and the model No.3 (Table 1 and Figure 2) can be used for further evaluation or experiment that related to rootstocks effect on the leaf area of *A. deliciosa* scions.

Table 1. Model tested including regression coefficient (*a*) and regression intercept (*b*) of five different models to estimate the green kiwifruit leaf area (*A.deliciosa*) from width (L*w*) and length (L*l*) measurements (*n*=105 leaves).

Model No.	Model tested	Regression coefficient (*a*)	Regression intercept (*b*)	Coefficient of determination (R^2)	Mean Square Errors (cm^2)	Full model
1.	LA = *a* + *b* (L*l*)	- 82.456	16.957	0.9073	14.93	y = 16.957*x* - 82.456
2.	LA = *a* + *b* (L*w*)	- 59.348	14.075	0.9489	11.08	y = 14.075*x* - 59.348
3.	**LA = *a* + *b* (L*l* x L*w*)**	**- 1.5349**	**0.7879**	**0.9903**	**4.83**	**y = 0.7879*x* - 1.5349**
4.	LA = *a* + *b* (Ll^2)	- 5.0217	0.8577	0.9349	12.51	y = 0.8577*x* - 5.0217
5.	LA = *a* + *b* (Lw^2)	+ 6.3527	0.6753	0.9706	8.41	y = 0.6753*x* + 6.3527

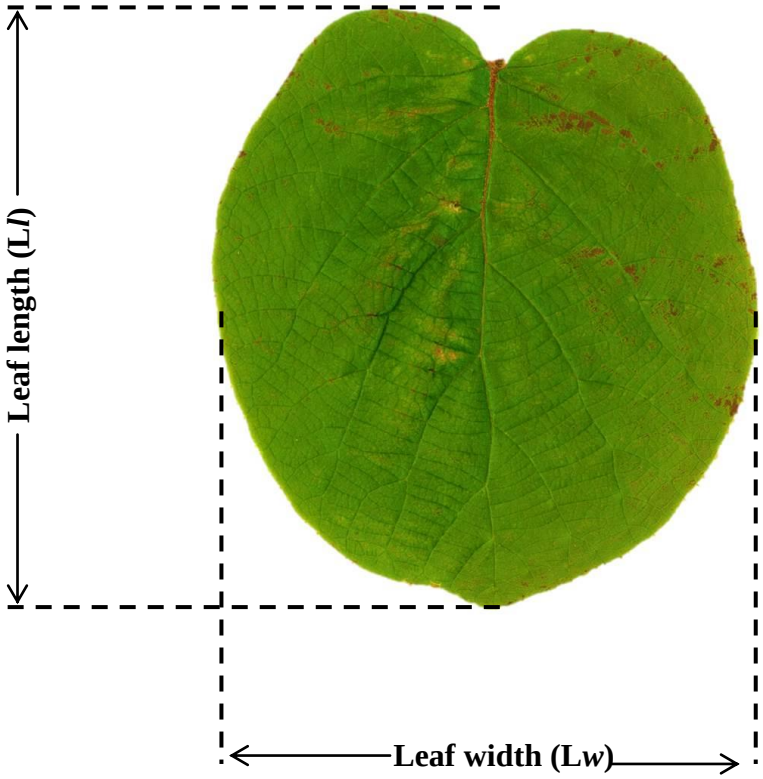


Figure 1. Diagram of green kiwifruit (*Actinidia deliciosa* cv. 'Hayward') leaf showing positions of length (L*l*) and width (L*w*) measurements.

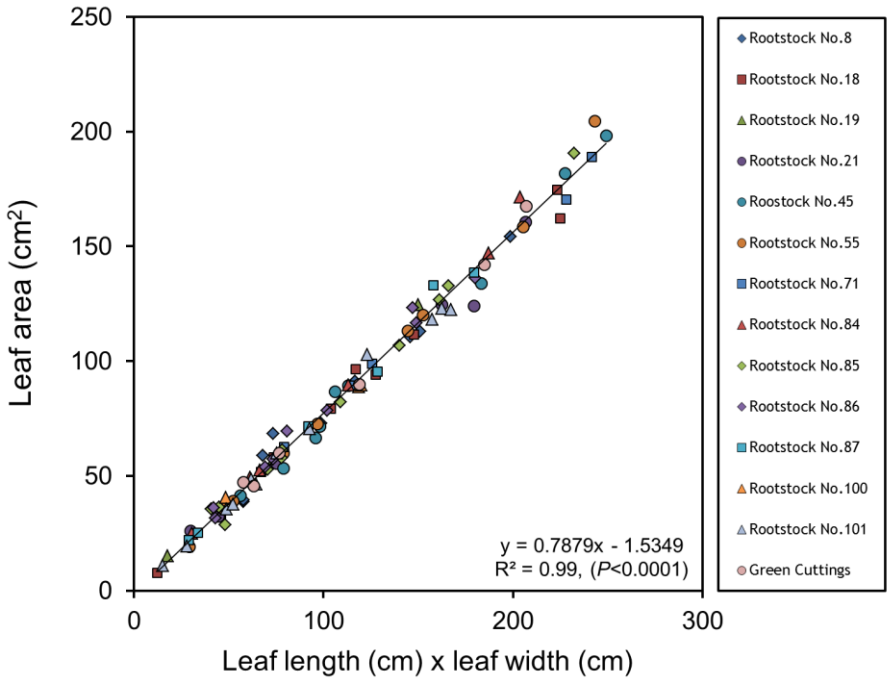


Figure 2. Relationship between leaf area (LA) and leaf length (L*l*) x leaf width (L*w*) of single leaves of *A.deliciosa*. The equation for the regression is LA = 0.7879 (L*l* x L*w*) – 1.5349 (refer Table 1).

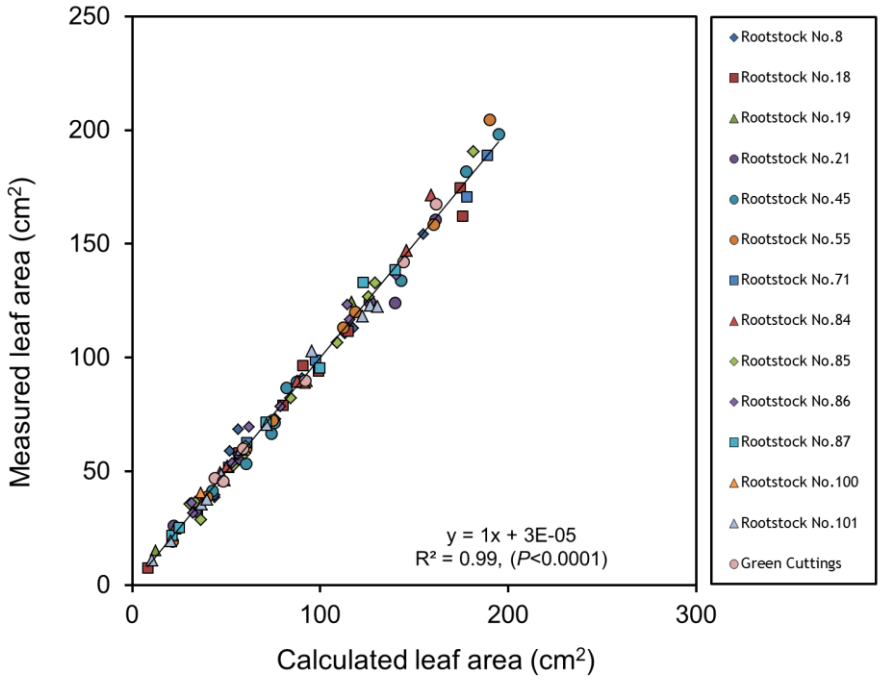


Figure 3.The relationship between measured and calculated of single leaves area of *A.deliciosa* using the equation model LA = 0.7879 (L*l* x L*w*) – 1.5349.

APPENDIX 11: Bio-assay of *Orobanche* seedlings treated with xylem sap and root exudates from different kiwifruit phenotypes for *strigolactones* identification.

Table 1. Germination of *Orobanche* seedlings treated with xylem sap and root exudates of different vigour/phenotype of kiwifruit (± standard error of means).

Treatments/ phenotypes	Germination of *Orobanche* seedlings from xylem sap (%)	Germination of *Orobanche* seedlings from root exudates (%)
Control (Water)	00.0 ^{c} (± 0.0)	00.7 ^{d} (± 0.7)
GR24	70.2 ^{a} (± 1.5)	74.3 ^{a} (± 3.6)
LMS	44.3 ^{b} (± 5.8)	32.5 ^{b} (± 2.8)
SMS	37.7 ^{b} (± 13.1)	20.8 ^{bc} (± 7.3)
LS	34.6 ^{b} (± 5.7)	30.5 ^{b} (± 6.0)
SS	22.1 ^{bc} (± 9.4)	15.0 ^{dc} (± 5.8)
$LSD_{0.05}$	22.9	15.1
P-value	*P*=0.0006	*P*<0.0001

*Means sharing with the same columns are not significantly different according to $LSD_{0.05}$ test.

Materials and method

- To investigate the role of strigolactones (SL) in the shoot architecture and branching of kiwifruit, *Orobanche* seeds were treated with the xylem sap and root exudates from four different phenotypes; Long Multiple Stem (LMS), Short Multiple Stem (SMS), Long Single Stem (LS) and Short Single Stem (SS).
- Kiwifruit xylem sap and root exudates, either diluted (10x) or non-diluted were applied to *Orobanche* seeds, and germination was scored after 7 days according to method developed by Manandhar (2011).
- Only results from non-diluted samples are reported here. Results from diluted (10x) are not reported due to highly variable.

Results and discussion

- In xylem sap, no significant difference was found on the germination of *Orobanche* seeds between branching and non-branching phenotypes (Table 1).
- The xylem sap of highly branching phenotypes (LMS and SMS) induced highest germination rates with 44% and 38%, respectively (Table 1).
- Similarly, germination of *Orobanche* seeds treated with the xylem sap from LS (non-branching phenotype) also induced higher germination rate with almost 35%, whereas treatment with xylem sap from SS only induced 22% germination of *Orobanche* seeds, which is almost 15-20 times lower than branching phenotypes (Table 1).
- Even though the germination is not statistically significant (Table 1), there was an opposite trend (Figure 1A) that amount of SL in xylem sap was low in non-branching phenotypes (LS and SS phenotypes) compared to branching phenotypes (LMS and SMS phenotypes).

- In root exudates, there was no significant difference in germination of *Orobanche* seeds between LMS, SMS and LS phenotypes and only germination of *Orobanche* seeds treated with root exudates of SS phenotype was found significantly lower compared to the other phenotypes (Table 1).
- Again, there was an opposite trend that non-branching phenotypes (LS and SS) have less SL than highly branching phenotypes (LMS and SMS) (Table 1). The germination rates of LS and SS (non-branching phenotype) were almost 15 to 20 times lower than highly branching phenotypes (Table 1).
- Similar germination trend of *Orobanche* seeds was also found in root exudates of different kiwifruit phenotypes (Figure 1B), the germination was reduced from highly branching phenotypes to non-branching phenotypes.
- However, in our study, we found opposite pattern in the present of SL in the xylem sap and root exudates from kiwifruit of different vigour or branching habit.

Preliminary conclusion

- Therefore, different possibilities open for discussion here:
  1. Possibility of SL may interact with other hormones (i.e. ABA, CK and GA) in stem or root system. Higher germination in branching group (indicating high level of SL) may be due to the present of cytokinins (CK) in xylem sap of highly branching groups?
     - During germination of parasitic seeds (in our study *Orobanche* seeds), SL may reduce the ABA levels and increasing GA levels (review by Cheng et al., 2013).
     - It would be reasonable to assume that highly branching groups (LMS and SMS phenotypes) may contain high GA compared to non-branching groups? As we know that GA is involved in regulating axillary branching of kiwifruit (Vattiprolu, 2012) and high germination rates of *Orobanche* seeds show the present of SL in xylem sap or root exudates.
     - There was also evidence that CK can promote germination of *Orobanche* seeds in the present of SL (review by Cheng et al., 2013).
  2. Different mechanisms of SL and GA or other endogenous hormones in stem and root system of kiwifruit?
     - Because recent study has shown that SL action independently from GA (Luisi et al., 2011)
  3. The present of other SL inhibitors in both xylem sap and root exudates of kiwifruit (Sarina Manandhar, *pers. comm.*)

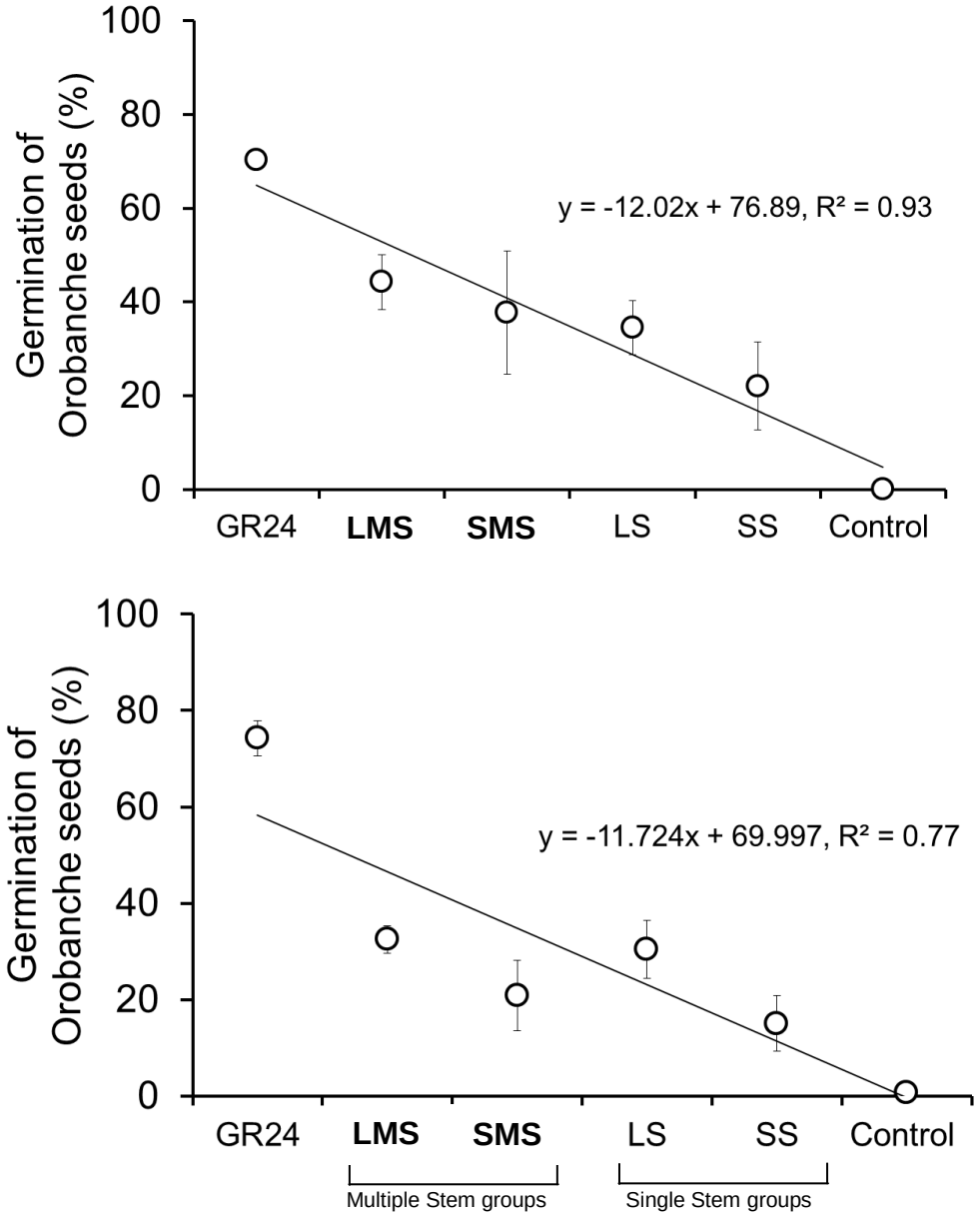


Figure 1. Germination of *Orobanche* seeds treated with; (A) xylem sap and (B) root exudates of different phenotypes/vigour of kiwifruit.

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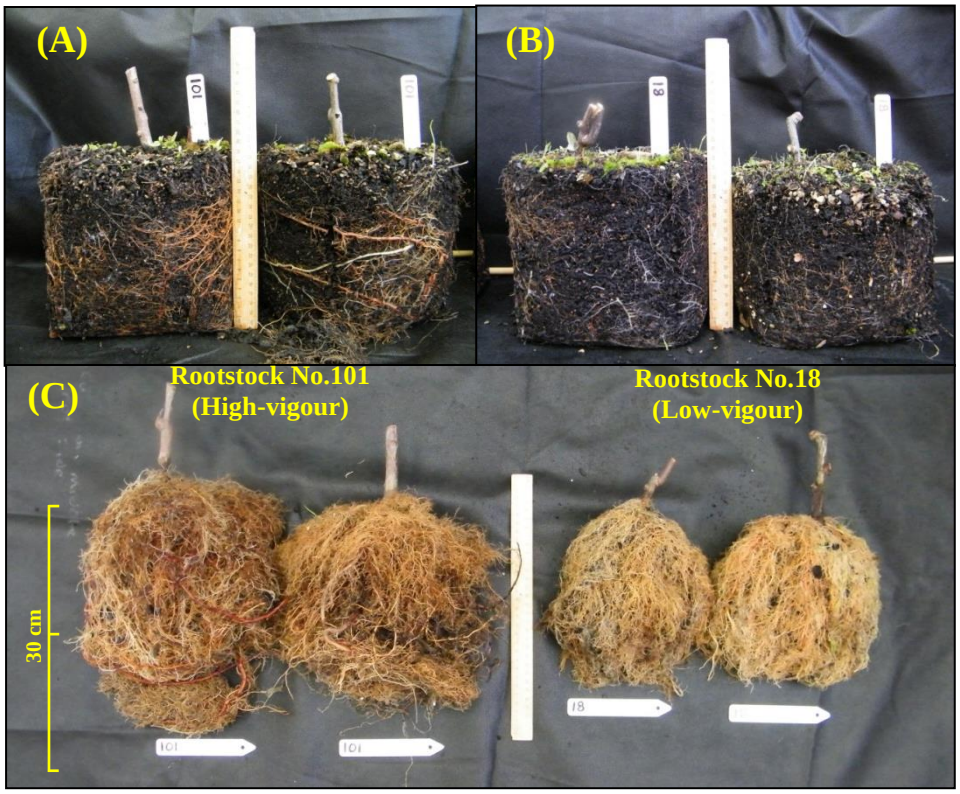
Cheng, X., Ruyter-Spira, C., & Bouwmeester, H. (2013). Interactions between strigolactone and other hormones in plant development. *Frontiers in Plant Science*, *4*, 1-16.

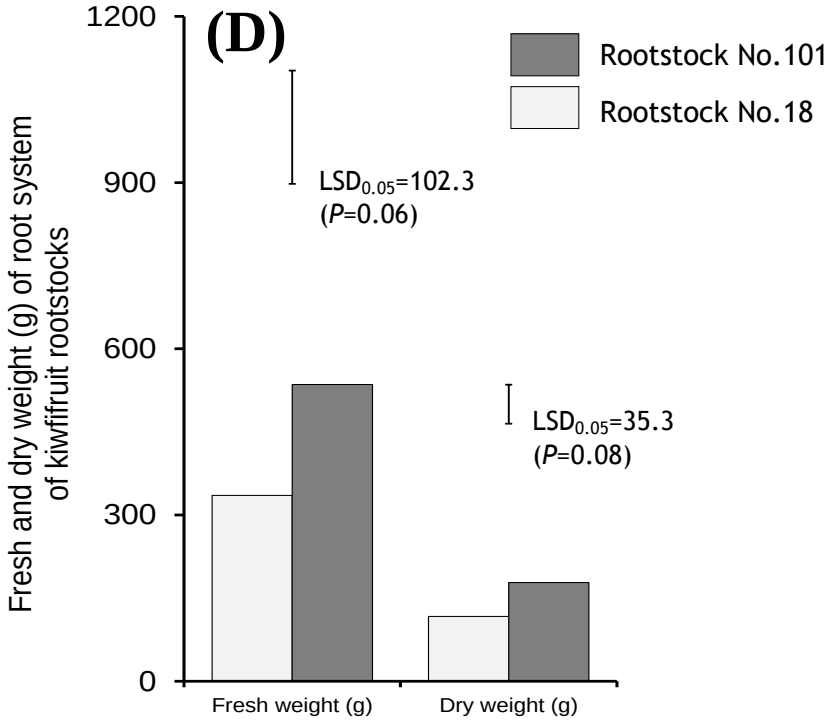
Luisi, A., Lorenzi, R., & Sorce, C. (2011). Strigolactone may interact with gibberellin to control apical dominance in pea (*Pisum sativum*). *Plant Growth Regulation, 65*(2), 415-419.

Manandhar, S. (2011). *Crop Architecture: Investigations into a 'new' branching hormone.* Unpublished PhD Confirmation report, Massey University, Palmerston North, New Zealand.

Vattiprolu, N. M. R. (2012). *Vigour control in apple (Malus domestica) and kiwifruit (Actinidia deliciosa and Actinidia chinensis).* Doctor of Philosophy. Unpublished PhD Thesis, Massey University, New Zealand, Palmerston North.

APPENDIX 12: The morphology of root system of unworked kiwifruit rootstocks





(A) High-vigour kiwifruit rootstock - No.101, (B) Low-vigour kiwifruit rootstock – No.18, and (C) Visual comparison of root morphology of both kiwifruit rootstocks. Noted here, (A) root-confined was clearly observed on the root system of high-vigour than low-vigour rootstock (think arrows), and (C) rootstock No.101 had slightly larger root system than rootstock No.18. (D) Statistical analysis of root system of kiwifruit rootstocks between No.101 (high-vigour) and No.18 (low-vigour) (n=4 for each rootstock).

FULL PUBLICATIONS

Abdullah, F. and Woolley, D. J. (2012). Effects of bark inversion on fruit weight, size and dry matter concentration of green kiwifruit (*Actinidia deliciosa* cv. 'Hayward'). *Acta Horticulturae, 1012,* 213-218.

Abdullah, F., Woolley, D.J., B.M. van Hooijdonk and A.P. Friend (2015). Interspecific hybrid kiwifruit rootstocks have potential to modify scion architecture and vigour of young 'Hayward' vines. *Acta Horticulturae, 1096,* 241-246. (Part of Chapter Two)