Strigolactones and hormonal interaction in control of branching in *Zantedeschia* and other horticultural species

A thesis presented in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy in Plant Science

at Massey University, Palmerston North,
New Zealand

Sarina Manandhar

2016

Abstract

Shoot branching that involves development of lateral buds into shoots is one of the important factors influencing crop productivity. Strigolactones have recently been found to be involved in the control of branching, but the actual bioactive compound/s that inhibits bud outgrowth is still unknown. A germination assay utilizing the seeds of a parasitic weed (*Orobanche minor*), detected strigolactones within the xylem exudates of different horticultural crop species; the strigolactone concentration negatively correlated with branching of cultivars or mutants. In *Zantedeschia* grown *in vivo*, the concentration of strigolactones was independent on the volume of guttation fluid (xylem exudates) suggesting the difference in concentration of strigolactones in high and low branched cultivars was due to the difference in potential of producing strigolactones between these cultivars and not due to differences in volume of guttation fluid. While identifying a bioactive compound using germination and branching assays in combination with liquid chromatography and mass spectrometry, compounds containing 'N' were detected in the low branched wild-type *Petunia*, but not in the highly branched mutant, suggesting the possibility of such compounds being SL-conjugates which may be associated with bud outgrowth inhibition.

In *Zantedeshia* (*in vitro*) and pea stems, strigolactone reduced the axillary shoot number stimulated by the cytokinin suggesting an antagonistic interaction between these two hormones on bud release. However, as cytokinin may stimulate subsequent growth of released buds by increasing the auxin transport out of the bud, strigolactone may have reduced subsequent growth by reducing auxin transport. Since GA_3 enhanced subsequent growth of buds in pea stems, but not the release, an antagonistic interaction between strigolactone and gibberellins on subsequent growth is possible. Interestingly, strigolactone successfully reduced adventitious bud formation in *Zantedeschia* grown *in vitro,* adding a new role for strigolactones in plant development.

Despite correlation between strigolactone and branching inhibition in different horticultural crops such as apple, kiwifruit, *Zantedeschia* and *Acer*, further studies relating to strigolactone and its interaction with other hormones on branching of these crops could be performed using *in vitro* techniques for a clear understanding of strigolactones' role on branching inhibition. More importantly, quantification of strigolactones using the germination assay may have significant implications in horticultural crop breeding for obtaining desired shoot branching.

Extended abstract

Shoot branching, one of the important factors influencing crop productivity, involves development of lateral buds into shoots on an actively growing primary shoot. Recently, a new hormone, which may be a strigolactone, has been found that inhibits bud outgrowth, however, the precise chemical identity of the bioactive compound(s) is unknown. A bioassay based on the germination of a parasitic weed (*Orobanche minor*) was optimized to detect strigolactones. Although there has been controversy in the literature related to whether or not strigolactones are present in xylem exudates, in this thesis strigolactones were found in xylem exudates of a range of horticultural species. The strigolactone concentration correlated with branching of cultivars or mutants mainly at the stage of the growth cycle before the branches were visually evident.

As the germination assay detects all/most strigolactones, not necessarily specifically those associated with branching, a more specific bioassay based on branching was developed. This bioassay was combined with liquid chromatography and mass spectrometry in an attempt to identify a specific branching hormone, whether or not this was a strigolactone. In *Petunia,* four compounds containing 'N' were detected in the xylem sap of the wild-type, low branched, V26, but not in highly branched dad3 mutant, suggesting the possibility of such compounds being SL-conjugates which may be associated with branching inhibition. In *Zantedeschia* grown *in vivo*, since the concentration of strigolactones was independent of the volume of guttation fluid, it was suggested that difference in concentration of strigolactones in high and low branched cultivar was due to the difference in potential of producing strigolactones between these cultivars and not due to differences in volume of guttation fluid.

The synthetic strigolactone GR24 (0.1 or 1 mg L^{-1}) was able to reduce axillary shoot number stimulated by cytokinin in un-decapitated pea stems, and *Zantedeschia* grown *in vitro,* suggesting an antagonistic interaction between these two hormones on bud release, as opposed to subsequent growth. Likewise, strigolactone (1 mg L^{-1}) reduced decapitation-induced bud release, supporting the hypothesis that strigolactone may have interacted with endogenous cytokinin and/or sucrose. Strigolactone was also able to

reduce subsequent growth of the shoot, but the effect was stronger in buds of pea stems orientated horizontally, compared to those orientated vertically. Such disparity was possibly due to the fact that, in addition to vascular stream, the buds of horizontally orientated stems received strigolactones directly. Although exogenously applied cytokinin appeared to enhance subsequent growth of the released bud, the effect of cytokinin on subsequent growth may be via increasing the auxin transport out of the bud. Hence, rather than interacting with cytokinin, strigolactone may have reduced subsequent growth of the buds by reducing auxin transport. As GA_3 enhanced the subsequent growth of buds in pea stems, but not the release, an antagonistic interaction between strigolactone and gibberellins on subsequent growth is considered highly likely. Interestingly, strigolactone successfully reduced cytokinin-stimulated adventitious bud formation in *Zantedeschia* grown *in vitro*. Interaction studies of strigolactone with cytokinin, and probably ethylene, is recommended within highly branched cultivars of *Zantedeschia* spp. or other species, such as gentians and kiwifruit, to further explore the role of strigolactone in adventitious bud formation and development in order to obtain desirable shoots for commercial purposes.

Although strigolactone correlated with branching inhibition in different horticultural crops such as apple, kiwifruit, *Zantedeschia* and *Acer*, further studies for answering the direct role of strigolactone on bud outgrowth in these crops as well as its interaction with other hormones can be performed using *in vitro* techniques. More importantly, quantification of strigolactones using the germination assay may have significant implications in horticultural crop breeding for obtaining desired shoot branching. Since guttation fluid from *Zantedeschia* was found to give a true estimate of the concentration of strigolactones present in the xylem of the shoot system, future experiments may benefit through the use of guttation fluid for hormonal analysis and/or interaction studies *in vivo*. Consideration of the stages of shoot branching during such studies would be valuable for a clear understanding of the shoot branching mechanism and help modify the branching of commercially important crops.

Acknowledgments

Foremost, I would like to express my sincere gratitude to my chief supervisor **Dr David Woolley** for the continuous support of my PhD study and research; for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me during the research and writing of this thesis. His unending encouragement, constructive criticism throughout the course of my PhD as well as his kindness to understand me is highly appreciated.

Thanks to my co-supervisor Dr Keith Funnell for helping me learn technical things right from the beginning of my research until end of my thesis write-up. Your remarkable quality of planning, organizing and implementing things is really appreciated. Your constructive feedback and suggestions helped me drive towards achieving perfection.

I would like to extend my thanks to staff of the IAE, as well as at the Plant Growth Unit; especially Chris, Kay, James and Lesley for their technical help and moral support. Kay, also thank you for your advice when dealing with the health of my son.

I wish to acknowledge the staff of Plant & Food Research, especially Andrew Mullan for helping me prepare media during tissue culture experiments, Maree Debenham for teaching me skills associated with tissue culture, and Duncan Hedderley for statistical help while analyzing data. I am also grateful to Revel Drummond for the supply of petunia seed and helping me learn sample extraction in petunia plants. My sincere thanks go to Dr Tony McGhie and Dr Daryl Rowan for assisting me in learning LC/MS and also thanks to Dr. Janney Cooney and her team for analyzing cytokinins and strigolactones through LC/MS.

I would particularly like to acknowledge the financial support from a Massey University Doctoral Scholarship, without which I my PhD would not have run as smoothly. I am also grateful for the financial support covering the research costs provided by The New Zealand Institute for Plant & Food Research Limited Core funding: 1198 – "Fashionable Plants for the Ornamentals Industry", 1972 – "Breeding Technology Development", as well as both the Helen E. Akers PhD scholarship and Johannes Anderson PhD scholarship.

Special thanks to Marzieh, Fadhil and my senior colleagues for their help, sharing ideas and creating a friendly environment during the period of study. This thesis would be incomplete if I do not mention the help and support of dear friends Rachael, Mahesh, Bhawana, Rajiv, Srijan and my entire circle of friends in Palmerston North. Thank you so much my dear sisters Jamuna and Saraswati for looking after my son during such a busy time.

Most importantly, I would like to express my deep gratitude to my husband Bishad for your many sacrifices, constant support and encouragement. My son Aaron, his innocent words and funny activities were a tonic to recover from having a frustrated mind. My heart-felt thanks to both of you boys. At last, my mother, brothers, sisters, and in-laws, who are far from me; thank you so much for your constant support.

Table of contents

List of Figures

List of Tables

List of Abbreviations

ABA	Absisic acid
ANOVA	Analysis of variance
BAP	Benzylaminopurine
BPC	Base peak chromatogram
BRC1	Branched1
CCD	Carotenoid cleavage dioxygenases
CK	Cytokinin
CRD	Completely randomised design
D27	Dwarf27
DAD	Decreased apical dominance
DMRT	Duncan's multiple range test
DW	Dry weight
FW	Fresh weight
GA	Gibberellin
GA_3	Gibberellic acid
GLM	General linear model
GR24	a synthetic strigolactone (3aR*,8bS*,E)-3-(((R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
IAA	Indole-3-acetic acid
IPT	Isopentenyl transferase
LC/MS	Liquid chromatography/ Mass spectrometry
LSD	Least significant difference

M.9	Malling 9
MAX	More axillary shoot
MRM	Multiple reaction monitoring
NAA	Naphthalene 1-acetic acid
NMR	Nuclear magnetic resonance
NPA	1-N-Naphthylphthalamic acid
NZ	New Zealand
PAT	Polar auxin transport
PIN	Pin-Formed
PPFD	Photosynthetic photon flux density
RO	Reverse osmosis
RMS	Ramosus
RWC	Relative water content
SAM	Shoot apical meristem
SE	Standard error
SL	Strigolactone
TW	Turgid weight
V26	Velm 26
X-CK	Xylem cytokinin