Phenotypic Variation of Kiwifruit in a Factorial Mating Design

(***Actinidia deliciosa*** (A.CHEV.) C.F. LIANG *et* A.R. FERGUSON **var. deliciosa**)

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Abstract

Systematic evaluation of the New Zealand *A.deliciosa* var. *deliciosa* breeding population was conducted using a two-factor factorial mating design (FMD). Two separate populations were used to study the genetic properties of the New Zealand *A.deliciosa* germplasm. Crosses were made in spring 1991, between seven female and seven male parents, resulting in one population [referred to as *Factorial One*] and another population resulting from six female and seven male parents [referred to as *Factorial Two*]. These ninety-one biparental families each had thirty-six seedlings planted during spring 1992 in a randomised complete block (RCB) design. Trunk diameters on all seedlings (at 20 cm above ground level) were measured annually from 1993 to 1996. Flowering characters were measured from both male and female seedlings in spring 1995. The sexual maturity of these populations had reached 68 %, with a female to male ratio of 1:1.32 by the 1995 flowering season. From these sexually mature seedlings, almost six hundred females were assessed for various fruiting characters of interest during the 1996 fruit season. Significance tests for vegetative and fruiting characters were reported based on their '*female*' (main effect), '*male*' (main effect) and '*female* by *male*' (interaction effect). Results from these statistics showed significant genetic variation exists for many of the vegetative, floral and fruiting characters measured within the two reference populations. Narrow sense (additive) heritabilities of ≥ 0.4 were reported in 11 of the 29 characters from Factorial One and 14 of the 29 characters in Factorial Two. The high additive genetic variation, present in both reference populations, indicates reasonable genetic advances are possible for at least some of these characters. Fruit weight and fruit shape characters in both factorial sets were highly heritable, while fruit quality aspects such as taste, flavour and texture generally had low heritabilities. Genetic and phenotypic correlations were also estimated for some selected vegetative and fruiting characters. These results showed distinct differences (as well as similarities) existed between the two reference populations. Genetic correlations r_a of $\geq |0.7|$ were reported, in 8 and 9 of the 30 (selected) vegetative correlations in Factorials One and Two respectively. From the 34 (selected) fruiting correlations in Factorials One and Two there were 17 and 7 genetic correlations $\geq |0.7|$ respectively. When comparing the same r_a correlations, between both Factorial data sets, it was evident that at least some of correlations could be in opposite directions to each other. General combining abilities (GCA), based on family means, were calculated and significant differences were evident amongst these means for several characters from both female and male classifiers of their half sib arrays. In addition, male (paternal) lines were equally as potent when compared to female (maternal) lines for their effects on their female offspring's fruiting characters. Therefore it is important to identify male parents breeding value (viz. progeny tests) for various characters, in order to improve the response to selection, because only half the genetic gain is possible when selecting one parent (female) as opposed to applying selection pressure to both parents (male and female). The ability to apply selection pressure to both parents, for a single character, could possibly lead to twice the genetic gain.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF EQUATIONS

LIST OF FIGURES

Chapter One

Introduction

I. Introduction

A. *New Zealand kiwifruit*

The kiwifruit is a relatively new commercial crop in comparison to many other fruit tree crops. The fruit is sold on the international fresh fruit market. The prime sensory attributes of kiwifruit include its sweet and acidic flavours (sugar/acid balance), high nutritional value and emerald green flesh (McMath *et al.,* 1991).

The world wide production of kiwifruit is based on the single cultivar, 'Hayward', in the species *Actinidia deliciosa* (A.Chev) C.F. Liang et A.R. Ferguson var. *deliciosa* (*herein referred to as kiwifruit*) (Ferguson and Bollard, 1990). Thus the kiwifruit industry is considered a *monocultural* industry i.e. being based on only one cultivar. The worldwide supply of this cultivar has saturated the international marketplace and eroded the profitability of growing kiwifruit for export in New Zealand. The once lucrative 'Hayward' kiwifruit has now become a marginal fruit crop on the international fruit market (Ferguson *et al.,* 1996).

New Zealand's kiwifruit industry is currently facing much uncertainty, due to the increasing pressure from overseas competitors and the fluctuating market returns. The situation is further exacerbated by the steadily rising cost of production. Therefore it is essential that the kiwifruit industry diversify into new cultivars, allowing New Zealand to reassert its position as the leader in the international kiwifruit market.

The collection of *Actinidia* germplasm and systematic breeding in New Zealand began in the mid-1970's. Since its establishment, in 1992, the Horticulture and Food Research Institute of New Zealand Limited (*herein referred to as HortResearch*) has been charged with responsibility for the New Zealand kiwifruit breeding programme (NZKBP). Consequently the breeding programme itself is very much in its infancy. The main objective of the kiwifruit breeding programme is to increase the probability of selecting a range of superior genotypes for successful commercialisation. The on-going development of new kiwifruit cultivars will depend on the efficacy of the New Zealand kiwifruit breeding programme.

B. Factorial Mating Design (FMD)

The aims of this study were to evaluate the genetic properties of the New Zealand kiwifruit breeding programme *A.deliciosa* germplasm populations as at 1991. This information will increase the scientific knowledge of kiwifruit quantitative genetics. Plant breeding programmes should rely on applied quantitative genetics to develop knowledge about the variation within the plant breeding populations. The information derived by quantitative genetics will allow the plant breeder to make informed decisions about the most efficient selection strategies to employ for achieving the plant breeding objectives. Quantitative genetics provide estimates of various genetical statistics by partitioning the variation into classifiable sources. Several designs such as the diallel, hierarchical and factorial mating designs can be used in order to develop the familial structures necessary for genetical interpretation of the variation. Several issues must be considered when planning the genetic studies of any plant species. These can generally be classified into three broad areas that will help to determine the most appropriate mating design to use, and they are as follows -

- reproductive biology of the plant species
- objectives of the plant breeding programme
- available resources to the programme

The main objective of the New Zealand Kiwifruit Breeding Programmes (NZKBP) was to establish a genetical experiment that would derive information about the significance of utilising controlled crosses (bi-parentals - BiP's) and their effects on several economic fruit traits in kiwifruit. The dioecious nature of kiwifruit makes it difficult to ascertain the breeding value of the male (used as a parent) and its' effects on fruit quality of its female progeny. Consequently, the application of the North Carolina Model II Design i.e. factorial mating design (Comstock and Robinson, 1948), was considered as the most appropriate design, in order to discern these paternal (male) effects. The FMD study is based on a random selection of parents from the New Zealand *A.deliciosa* germplasm.

The inference base population (IBP) is assumed to represent a diverse sampling of the New Zealand *A.deliciosa* population. Selected parents were crossed during November - December 1991 in a factorial mating design (FMD) to produce 91 full sib families. These progenies were planted out in the field, in a randomised complete block design (RCB) in November 1992. Several characters of interest have been measured since. The focus of the factorial mating design (FMD) study for 1996, was the evaluation of fruiting characteristics of 430 female vines across the 91 full sib families. Most of these vines flowered for the first time during November 1995 and the fruit was harvested and assessed during April and May of 1996.

The factorial mating design (FMD) was used in order to estimate genetical statistics about the *A.deliciosa* germplasm currently available in New Zealand. The estimation of these various genetical components and their subsequent use in predicting genetic gain should improve the efficiencies of the New Zealand kiwifruit breeding programme. Future plant breeding decisions concerning the population improvement of *A.deliciosa* germplasm in New Zealand will be enhanced as a result of this FMD study.

1. Vegetative characteristics

Several vegetative characters such as trunk diameter, leaf measurements and flowering attributes (first bloom, mid-bloom and full bloom) have been reported in genetic studies of various perennial fruit crop plants (Hansche *et al.,* 1983; Hansche and Beres 1980; Hayward *et al.,* 1993; Kearsey, 1993). In this study over 3,000 plants were monitored in two RCB factorial designs. Vegetative characters were measured on all individuals in both factorials including trunk diameter, number of prominent (swollen) buds per 500 mm cane, juvenile period, flowering range and median flowering dates.

The objective of both the vegetative and fruiting measurements was to estimate the various genetical statistics pertaining to the 1991 *Actinidia deliciosa* germplasm.

- heritabilities (both narrow and dominance) and their respective standard errors
- general and specific combining ability (GCA/SCA) effects of parental genotypes
- phenotypic and genetic correlations amongst vegetative (and fruiting) characters

The vegetative data could also be used to investigate any significant genetic and/or phenotypic correlations between vegetative and fruiting characters. That is, identifying moderate to high *genetic* correlations between vegetative characters and fruiting characters, that could prove to be very useful as an early (indirect) selection tool for kiwifruit plant breeding. As previously mentioned, the lengthy generation cycle of many perennial fruit tree crops is a major limitation to genetic progress. Therefore, the development of an early selection tool to identify the fruiting potential of seedlings could improve efficiency in perennial fruit breeding programmes. The main reason behind measuring trunk diameters over the four-year period (1993-96) was to investigate if any possible correlation between the early phase (immature) trunk diameters and their floral/fruiting stage i.e. mature phase.

2. Fruiting characteristics

Fruiting characters such as fruit weight, fruit yield (per vine) and post harvest fruit quality characters (sensory, shape descriptors and storage life) are the basis of plant selection in the NZKBP. Fruiting characters were measured on all pistillate (female) vines in Factorial One and Factorial Two, during March to August of 1996, and these data will be referred to, herein, as being from the '1996 fruiting data'. The objective was to determine various genetical statistics relating to these fruiting characteristics accordingly the data were used to estimate the same genetic estimates as for the vegetative characters (listed above). These include heritabilities (both additive and dominance) and their respective standard errors, GCA/SCA effects (general/specific combining abilities) of parental genotypes and phenotypic and genotypic correlations amongst fruiting character.

The main goal of selection in the kiwifruit breeding programme is the development of an improved pistillate selection for commercial release. In order to achieve this, a number of fruiting traits must be monitored, the data analysed appropriately and then individuals selected based on these results. If the primary objective of selecting new commercial cultivars cannot be achieved from the current cycle of F_1 plants, the fruiting data can be analysed for genetic parameters and used to determine the best selection strategy to employ in order to achieve the objective in subsequent cycles (recurrent selection).

C. *Biology of A.deliciosa - kiwifruit*

All vines in the genus Actinidia have a perennial climbing or scrambling habit. Most species in the genus are deciduous, although there are some evergreen species found in subtropical regions. The genus is mainly confined to China, particularly forested riparian hillsides in central and southern China. Currently, there are some 60 species, most of which have been subdivided so that more than 100 taxa are currently recognised. Of these only two species, *A. deliciosa* and *A. chinensis* are considered commercially important. In general, *A. chinensis* is distributed mainly in the warmer eastern regions of China, while *A. deliciosa* is predominantly found in more western regions (Ferguson *et al.*, 1996). For the purpose of the FMD study, only the *A. deliciosa* will be considered.

The Actinidia genus is assumed to be functionally dioecious, although a genus-wide systematic survey to confirm this has never been conducted. The dioecious condition means male and female flowers are borne on separate vines. These male and female vines are functionally staminate and functionally pistillate vines respectively (McNeilage, 1991). Staminate vines (pollen bearing) have flowers with a rudimentary or undeveloped ovary and have no ovules, however their stamens contain viable pollen. The flowers on the pistillate (egg-bearing) vines have a fully functional ovary containing ovules, but their stamens contain non-viable pollen. These two types of vines are assumed to result from a genetic sex-determining mechanism that is, as yet, poorly understood. There are, at times, deviations from strict dioecism. Fruiting or inconstant males are staminate vines with some flowers capable of fruit set i.e. some flowers with functional ovaries. Conversely, hermaphrodites are morphologically indistinguishable from pistillate vines, but have stamens with viable pollen as well as functional ovaries. Both the fruiting males and hermaphrodite vines are capable of self pollination and fruit set (McNeilage, 1991). These two types of vines occur (naturally) in very low frequencies in the New Zealand *A. deliciosa* germplasm. Therefore female vines from dioecious kiwifruit populations (whose sex is unknown at planting) have an unusual situation with regard to the statistical design and analyses of these fruiting data. The primary concern involves the random distribution of the females (and therefore males) throughout the physical layout of the design leading to possible imbalance in these data (see Chapter Three).

Kiwifruit *A. deliciosa* is a hexaploid with 2n = 6x = 174 and is considered amphidiploid i.e. behaves in a diploid manner with respect to segregating populations (Ferguson *et al.,* 1996; Zhu, 1990; McNeilage and Considine, 1989). This assumption, of amphidipliod behaviour, is crucial for the successful application of genetic theory and variance component analysis for the understanding of polygenic gene action (Hayward and Breese, 1993; Nyquist, 1991; Mayo, 1980; Mettler and Gregg, 1969). There are various polyploid levels across the 60 species within the *Actinidia* genus itself, ranging from diploid through to octaploid types (McNeilage and Considine, 1989). Subsequently, a great deal of natural diversity exists amongst the species within the *Actinidia* genus and high phenotypic variation for many characters has been reported (Beatson, 1991; Zhu, 1990; McNeilage and Considine, 1989).

D. *Production of kiwifruit*

The first known introduction of kiwifruit into New Zealand, was seed from the Hubei Province, China, in 1904 (*hereafter known as the '1904' introduction*) (Beatson, 1991; Blanchet and Chartier, 1991). Subsequently, the kiwifruit cultivar 'Hayward' was selected from a small seedling population in the mid-1920's by Mr. Hayward Wright; this population is assumed to be one or two generations on from the '1904' introduction. Several other selections were made around the same time i.e. 'Bruno', 'Monty', 'Gracie' and 'Abbott'. However, by the mid-1960's, 'Hayward' had established itself as the preferred commercial cultivar and went on to form the basis of today's kiwifruit industry (Ferguson, 1990). The rapid rise in the popularity of kiwifruit on the international market, saw a dramatic increase in New Zealand kiwifruit planting's between 1970 and 1990. Total planting's in 1994 were estimated at 10,161 ha producing about 70 million trays (225,000 tonnes) of export fruit. More recently, however, the New Zealand production has declined and fluctuates around 50 - 55 million export trays annually (New Zealand Fruit Council, 1995). The other major producers of kiwifruit are Italy, France, Greece, USA, Australia and Chile. These countries collectively produce significant volumes of kiwifruit, with Italy being the largest individual producer. The New Zealand Kiwifruit Marketing Board *(hereafter known as NZKMB)* now has to compete vigorously in the international kiwifruit market.

The oversupply of kiwifruit to the international marketplace has meant diminishing returns for both the local and international growers' alike (Ferguson *et al.,* 1996). The reduced economic viability of many New Zealand orchards has meant some orchardists have been forced out of business. The New Zealand Kiwifruit Marketing Board Limited was established in 1988 to control the export of New Zealand kiwifruit. The NZKMB has sole rights to export kiwifruit to all markets other than Australia (New Zealand Fruit Council, 1995).

Until recently, the common name *kiwifruit* was used by the NZKMB for the international marketing of the fruit, but this has since been supplanted by the generic brand name of '*Zespri*'. The brand name change is an attempt to differentiate the New Zealand kiwifruit, the "world's finest", from the rest of the world kiwifruit production (Kiwifruit Marketing Board, 1997). The increasing pressure from overseas producers has encouraged the NZKMB to look towards new kiwifruit cultivars.

Accordingly, there has been an increase in funding resources given to the HortResearch breeding programme. The relationship between NZKMB and HortResearch is vital to the development of new kiwifruit cultivars to supplement and perhaps even replace the existing single cultivar 'Hayward'. The 'Hayward' cultivar, being a pistillate vine, requires a polleniser (staminate vine) with coincident flowering period in order to achieve fruit set. Currently, a minimum planting ratio of 1:8 male:female is recommended (Sale and Lyford, 1990).

In New Zealand, *A. deliciosa* vines begin flowering in November of each year and their fruit mature in April-May of the following year i.e. some six months after flowering. The 'Hayward' fruit must reach a threshold brix level of 6.2 % soluble solids concentration (%SSC) before being harvested, as measured by refractometer (Beever and Hopkirk, 1990). The average storage life of 'Hayward' fruit can vary from between 6 - 8 months when stored at 0°C and high humidity conditions. The long storage life is one of the prime postharvest attributes of 'Hayward', allowing the fruit to be shipped to international marketplaces. Generally, the fruit arrives in excellent condition for retail distribution and also maintains a good shelf life, which is another important postharvest attribute (McDonald, 1990).

The current planting density for individual kiwifruit vines is 4.8 - 5.0 m within row and 5.5 - 6.0 m between row. This plant density can produce average yields of 25 tonnes/ha and higher. Kiwifruit can be sexually propagated by seed, although these seedlings are not true to type. There are several methods of asexual propagation, which include grafting, softwood or hardwood cuttings, root cuttings and micropropagation (tissue culture). Grafting is the most practicable method of reproducing clones of a cultivar or genotype, for commercial purposes (Lawes, 1990).

Grafting of kiwifruit requires that dormant wood of the cultivar is taken during winter. Scion wood is usually cut into '*two bud scion*' sections. Grafting is best done in early spring, to a suitable seedling rootstock either in a nursery situation or *in-situ* in the field. The most commonly used rootstock for kiwifruit are 'Bruno' seedlings. The relative ease with which kiwifruit can be replicated in this manner facilitates the quick establishment of new cultivars for commercial scale planting's (Lawes, 1990).

1. New cultivars in kiwifruit breeding

Systematic kiwifruit breeding has only been established during the last 10 - 15 years. A major constraint being the long generation cycle of kiwifruit (*3 - 7 years*) from seed raising to the final evaluation cycle (Ferguson *et al.,* 1996). As a consequence, a number of new cultivars are just beginning to be developed. Recent selections from the New Zealand kiwifruit breeding programme (NZKBP) include 'Tomua', a new early maturing cultivar that resulted from *individual selection* from an F_1 population between two accessions of *A. deliciosa* (HortResearch Kiwifruit Breeding, 1996). The cultivar 'Tomua' is very similar to 'Hayward' in many respects but the fruit matures 4-6 weeks earlier than 'Hayward' and has a reduced storage life of 2 - 3 months in comparison to 6 - 8 months for 'Hayward' (Figure I-1). Therefore, 'Tomua' is seen by the Kiwifruit Marketing Board as an 'early niche market' fruit with a possible annual production of 1-2 million trays (Lamb, 1995). Incremental improvements, such as those offered by new cultivar 'Tomua', will not be of medium to long term benefit to the New Zealand industry. Instead, there must be a significant improvement in a suite of characters (several traits) focusing on key attributes considered desirable from both the 'consumer' (taste convenience) and the grower (productivity).

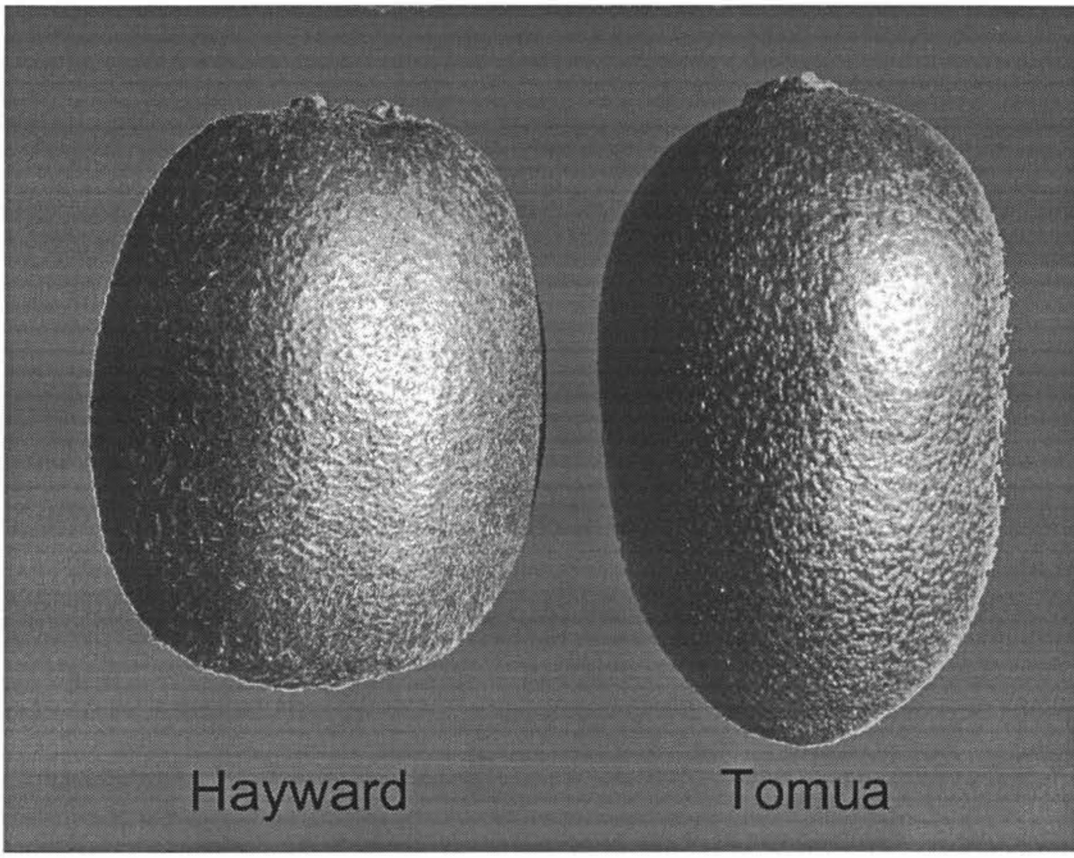

Figure I-1 : New *A.deliciosa* cultivars 'Tomua' and 'Hayward'

Other examples of recently released cultivars include

- 'MontCap' - early maturing F_2 seedling from 'Hayward' with limited storage life and developed in France (Blanchet, 1995)
- 'Xuxiang' – large fruited seedling with higher %SSC soluble solids content than 'Hayward' originating from a 'wild' population in China (Wei, 1993)
- 'Miliang 1' – large fruited seedling with higher %SSC soluble solids content than 'Hayward' and originates from another 'wild' population in China (Wang, 1995)

In China many of the selections of *A. deliciosa* and *A. chinensis* have being selected straight from the wild and most of these are not readily available outside of China. However in order to challenge the status of 'Hayward' these new selections must have substantial improvements in composite (generic) characters such as fruit quality and yield, guaranteeing both a market premium and grower enthusiasm, before they can revolutionise the commercial status of *A. deliciosa*.