PHENOTYPIC AND GENOTYPIC VARIATION IN KIWIFRUIT (*Actinidia deliciosa* (A. CHEV.) C.F. LIANG *et* A.R. FERGUSON) SEEDLING POPULATIONS

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ABSTRACT

Kiwifruit breeding programmes in New Zealand have produced a large number of seedling populations. Effective methods are required to assess seedlings, and knowledge of phenotypic and genotypic variation would facilitate the design of breeding programmes.

1. Multivariate analyses of phenotypic data

Multivariate analyses were used to quantify the characters most powerful in distinguishing between seedlings and between crosses, and to examine their relationships. Seedlings and crosses were placed in order of their overall merit, and the best ones were determined. Bruno was a superior female parent for producing both floriferous male vines and productive female vines with high fruit vitamin C content, and D1-20 was the better male parent in all crosses. Multivariate analysis of variance and discriminant analysis were more suitable to sort cross combinations, while factor analysis was more efficient for screening single seedlings within a population. Factor patterns varied between crosses, and between years for the same cross.

Canonical correlation analysis proved a useful tool to obtain better understanding of the aggregates of useful vine characters and the relationship between them in fruit breeding.

2. Quantitative genetics studies

Relatively high heritability was shown for the beginning date of bloom (0.48) and flowering duration (0.50) in male vines, and total crop weight (0.46), percentage of shoots flowering (0.54), fruit elongation (0.65), and mean fruit weight (0.52) in female vines. For these traits, the selection of superior seedlings as parent could therefore lead to rapid genetic improvement to their subsequent generations. Hairiness of fruit (-0.07) and percentage soluble solids in fruit (-0.19) gave negative heritability value, thus could not be improved efficiently by individual selection. However, their broad sense heritability was reasonably high (0.22 and 0.37 respectively), showing there were some dominant and/or epistatic effects involved. This indicates a possible chance improvement may occur in a large seedling population. Fruit vitamin C content (0.22), fruit symmetry (0.30) and relative core size (0.13) gave moderate or low heritability values, indicating that individual selection may have only moderate success in improving these traits.

Simple recurrent individual selection was shown to be an effective breeding strategy for characters of high heritability. For characters of low heritability, the family selection methods yielded a greater rate of genetic gain. Selection indices were constructed to provide a technique for improving several traits simultaneously.

3. SDS-polyacrylamide gel electrophoresis of leaf proteins

Three regions of the protein profile were found useful for the characterisation of cultivars and seedlings. The banding patterns found in seedlings provided evidence that in the hexaploid kiwifruit the inheritance of these polypeptides occurred in a manner similar to that of a diploid. Hence the kiwifruit may have arisen as a diploidized polyploid and it is proposed the kiwifruit may be considered as allohexaploid.

Progressive changes of some bands with leaf growth and development may be of interest in the study of leaf development. The possible application of leaf protein analysis to kiwifruit breeding was discussed.

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INTRODUCTION

Kiwifruit, the common name for Actinidia deliciosa (A. Chev.) C.F. Liang et A.R. Ferguson, adopted for commercial purposes belongs to the relatively undeveloped (commercially) genus Actinidia. It is a deciduous vine, usually growing on the edges of forests on the hills and mountains of southern and central China. In China, the main region of A. deliciosa distribution is the Chang Jiang (Yangtze River) valley, but it extends from the Tsingling Range and Huai River valley, corresponding to the 750 mm annual rainfall line, to the Southern Coast and Taiwan province. Since it was introduced to New Zealand from China in 1904, kiwifruit plantings in New Zealand have increased very rapidly, and at the present time commands most of the international market with a return of $500 million a year for this fruit. Because of its unique properties and successful promotion, the kiwifruit has gained new respect in the 1970's and early 1980's as a cash crop for human consumption.

The success of the kiwifruit in New Zealand has encouraged a rush of plantings in other countries. But in most cases, these countries have used material raised in New Zealand, mainly Hayward, but also some Bruno. Hayward is essentially the only female cultivar now grown in New Zealand and exported. The New Zealand kiwifruit industry is therefore a monoculture based on one cultivar. Such a situation creates a potential for disease epidemics, as well as the problems of harvesting and marketing large quantities of the fruit over a relatively short period of the year. Therefore breeding programmes with the general aims of improving existing cultivars and creating new cultivars are necessary.

Several kiwifruit breeding programmes have already started in New Zealand, and about 11,000 vines of different ages are involved. It is important to develop methods which can assist in efficiently evaluating seedlings and selecting the best vines for kiwifruit breeding. It is also important to know more about phenotypic and genotypic variation in kiwifruit to facilitate the design of breeding programmes.

For this purpose, three types of analyses were used in this study namely, multivariate analysis, quantitative genetic analysis and electrophoretic analysis.

This project was carried out from 1986-1988 with plants produced and maintained at the Riwaka Research Station, of the Department of Scientific and Industrial Research, New Zealand. The plants used were from a full sib family's breeding trial in a factorial mating design, and a number of attributes of both male and female seedlings were investigated. The objectives and techniques of this work were:

1. To screen the crosses and vines within the crosses by multivariate analysis of phenotypic data.
2. To obtain genetic information for establishing the breeding methods and selection procedures of maximum effectiveness and efficiency.
3. To characterise kiwifruit cultivars and seedling populations with SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and to investigate the profiles for gene markers for genetic studies.

CHAPTER 1

LITERATURE SUMMARY

Unlike apple, peach, grape and orange, kiwifruit cultivation and breeding has only a short history. It is a new fruit crop which has not been subjected to enough study and the literature contains limited information on some aspects of kiwifruit.

1. Kiwifruit and its production

(i) *Kiwifruit taxonomy*

Kiwifruit (A. deliciosa) is a new fruit crop with low calories and high nutritional value. According to the nutritional report of the New Zealand Kiwifruit Authority (Anon., 1988), two average size kiwifruit contain twice the amount of vitamin C of an orange, more potassium than a 15 cm banana, twice the Vitamin E of an avocado, more dietary fibre than half a cup of bran flakes, and only 90 calories. Because of its unique properties, in recent years there has been a world wide interest in cultivating this vine crop.

Kiwifruit belongs to the genus Actinidia, family Actinidiaceae, order Theales (see Ferguson 1984). For many years, it was known as Actinidia chinensis, because it originally came from China. The culture of kiwifruit in China can be traced back about 3000 years - it was mentioned in a collection of ancient Chinese poems, The Book of Songs, which was written in the 10th century B.C.

The genus Actinidia consists of more than 50 species of climbing plants which occur naturally in eastern and southern Asia, and the species A. chinensis was described in 1847 (Planchon 1847).

Botanists later realised that there were at least 3 intraspecies varieties of A. chinensis, with fruit varying in size and hairiness (Li 1952; Liang 1975, 1982) viz.

1. A. chinensis Planch. var. chinensis, which bears soft-haired fruit.
2. A. chinensis Planch. var. hispida Liang, which bears stiff-haired fruit.
3. A. chinensis Planch. var. setosa Li, which bears spiny fruit, and is found only in Taiwan.

The differences between var. chinensis and var. hispida have been cytologically examined by the author, and their different chromosome counts (var. chinensis, 2n = 58; var. hispida, 2n = ca.172) reported (Zhu 1982).

From extensive research, botanists determined to treat the three varieties as three separate species. While initially known as A. chinensis, since 1985 the kiwifruit has been correctly described as A. deliciosa, the name given earlier to var. hispida by the French botanist Auguste Chevalier (Ferguson 1985; Liang & Ferguson 1986). It is A. deliciosa which was introduced to New Zealand in 1904, and became the kiwifruit of today. Only this species is usually thought of as kiwifruit, however, in China, the term kiwifruit has been extended to A. chinensis, and even to the whole genus Actinidia. To avoid such ambiguity, the botanical name should be clear when referring to plant breeding material.

(ii) ***Production***

Since kiwifruit was introduced to New Zealand kiwifruit production has increased dramatically. The small early plantings increased to over 8000 ha in 1982, while in 1985 New Zealand plantings exceeded 15000 ha. The 1984 export crop was 13.3 million trays, representing an increase of 27.7% over 1983 (Lyttelton 1984), while in 1987 46.6 million trays of fruit were exported. The export of 74.8 million tray has been projected for 1990 (Anon. 1987), with f.o.b. earnings of $700m.

The success of the kiwifruit industry has led to a rapid expansion of kiwifruit growing in many parts of the world. In 1985, the world total acreage was over 33000 ha, with 6000 ha in Italy, and USA 3000 ha. France, Japan, Chile and Australia are also significant producers (Alvisi, 1987). In 1985-1986 New Zealand exported almost 75000 tonnes (76% of world total) mainly to Western Europe, Japan and USA (Alvisi, 1987). Export earnings from kiwifruit now exceed those of apples - and are the major horticultural export earner for New Zealand.

Increasing production has given rise to doubts about market outlets. In the next few years, competition is likely to become keener between different countries and even between producers with similar harvesting seasons. Drastic price reductions can be avoided only by increasing and diversifying consumption, and through closer international coordination of marketing.

2. Kiwifruit selection and breeding

(i) ***The Challenge***

Within the past decade the consumption of kiwifruit has increased dramatically, as consumers have become more aware of the health-promoting properties of the fruit. But the increased competition between producing countries means that for New Zealand to continue to be successful it must have the best production and marketing system, and this includes being first to have the best cultivars. Palmer pointed out "Our horticultural produce must lead the market, not be led by it" and suggested that a new fruit cultivar of the status of Hayward, Granny Smith and Red Delicious is needed every 5 years or so if New Zealand is to keep ahead of the field (Palmer 1986).

Unfortunately the kiwifruit industry in New Zealand is based on a narrow range of cultivars selected years ago from the original line of seed imported in 1904, the limitations of which indicates that increased attention to cultivar improvement is required.

(ii) *Selection of existing cultivars*

Much has been written describing the history of kiwifruit selection (Ferguson & Lay-Yee 1984). All the commercial kiwifruit plantings in New Zealand can be traced back to a few plants growing on the Wanganui farm of Alexander Allison, who raised plants from seed sent him from China in 1904. His vines were producing fruit by 1910. Allison's kiwifruit created considerable interest. Within a few years, plants had been distributed to a number of amateur horticulturalists and nurserymen in the Manawatu. By July 1917 the first plants were being sold to the public, and about 1925 the early selection began. The selection of most of the improved cultivars that we grow today took place during the decade 1925-1935. Bruno Just, a nurseryman of Palmerston North, selected several types of kiwifruit which he propagated and sold as grafted vines - the cultivar Bruno was selected from a group of 30 vines. Many of the other named cultivars come from the Avondale nursery of Hayward Wright. The cultivars Hayward and Gracie were both selected by Wright from a row of about 40 seedlings.

For adequate pollination, Matua and Tomuri were selected by H M Mouat and W A Fletcher as male cultivars, and various Matua-type males are now widely used.

Amongst these cultivars, Hayward is the main cultivar in New Zealand and only Hayward fruit is now exported. Therefore the New Zealand kiwifruit industry is based on one cultivar , which may lead to a number of problems. Hayward is a good cultivar, but by no means a perfect one, e.g. it is a late-flowering cultivar, fruit quality could be improved, and the yield is not reliable under conditions of low winter chilling. For New Zealand to retain its place as the world's kiwifruit leader there must be a continued search for better varieties and different types of kiwifruit.

As with other tree crops there is continuous, natural mutation taking place within the Hayward population, and these sports sometimes may represent significant improvements. The major improvements compared with Hayward which are sought are listed as follows (Davison, 1985; Dawes, 1977; Ferguson, 1987; Lawes 1978, 1979; Lay Yee 1980; Seal et al 1986):

- Fruits consistently larger and uniformly-sized
- More symmetrical fruit shape, with fewer 'flats'
- Less hairy, with smoother skin
- Thicker skin to increase resistance to wind rub
- A small internal core

- Earlier maturity to spread the use of harvesting, packing and coolstore facilities and to allow earlier access to markets
- More compact growth and less vegetative vigour, which could reduce orchard management costs

In fact, several interesting bud mutations have been claimed in New Zealand over the past decade. For instance, a superior mutation of Hayward was first noticed in Wilkins' orchard, Te Puke. It was longer than Hayward and had a small core. It was named Wilkins Super and protected by plant patent (Anon., 1987). Another example is an earlier maturing mutation found in Kennedy's Tauranga orchard. The vine was claimed to flower ten to twelve days earlier than others, and its fruit to ripen correspondingly ahead of other vines (Anon.,1984). These selections were some of many from a nationwide competition. While such chance discoveries may lead to a good new cultivar, controlled breeding programmes hold the greatest potential.

(iii) *Creating new* A. deliciosa *cultivars*

Like other tree crops, kiwifruit is a highly heterozygous plant, and the seedlings resulting from gene recombination of parents can provide an enormous diversity of vine types (McNeilage et al 1986). The old cultivars with valuable characteristics could therefore be used as breeding stock, although they have been discarded in kiwifruit cultivation.

Monty, although it has medium-sized fruit, is highly productive, with a good storage quality (Brooks & Olmo 1972; Minnis, 1976) and may be useful to improve productivity of kiwifruit. Bruno has the highest vitamin C content (Minnis 1976), and has good flavour (Brooks & Olmo 1972), and may be useful to increase vitamin C level of new cultivars. Abbott is the earliest maturing (Sozzi et al 1976), so it may be useful material to breed a new earlier maturing cultivar.

In New Zealand, promising male and female parents have been identified and crossed, with a large seedling population now being grown, and it is possible that particular characters will be developed while retaining other good attributes.

First results of those programmes suggested that progress in obtaining hermaphroditic (self-fertile) types may be more rapid than first expected (Seal et al, 1986). Since kiwifruit is functionally dioecious, hermaphroditism could be important in kiwifruit production and breeding, as apart from reducing the need for male vines, the presence of even a few fruit on a fruiting male vine can provide some fruiting information, which enables one to make a more rational choice of the male parent for breeding. In practice, it is difficult to detect flowers with fertile pollen on female vine, but it is easier to find flowers with fertile ovaries on a male vine. This slight deviation from strict dioecism in male plants probably indicates an evolutionary origin from hermaphroditism via a gynodioecious pathway to dioecious (Lloyd, 1980; Webb, 1979). Selective breeding has produced hermaphrodite cultivars in some dioecious or gynodioecious species, such as grapes, strawberries and papaya.

In 1985, one fruiting male vine from seed of an open pollinated fruiting male parent was found with flowers of large ovaries and long, thick styles, and the largest fruit weighed 81 g and contained more than 800 seeds. It was early maturing and had good shape and flavour. The good size and shape of the fruit was confirmed in subsequent seasons, and another vine from the same population has been found with even larger fruit, and the added advantage of producing only bisexual flowers - a stable hermaphrodite (Seal 1987, 1988). By using them as parent, a range of self-fertile selections will emerge, provided that the fruiting male syndrome is both heritable and sufficiently constant.

But until commercial hermaphrodite cultivars are obtained, male cultivars will play an important role in kiwifruit production and breeding. Because of the dioecious property of kiwifruit, two important constraints are imposed on breeding work. First, as two promising female types cannot be crossed directly, a male intermediary must be used and thus an extra generation is needed before desirable fruit characters can be combined. Second the contribution made by a male parent to the fruit characters of its female progeny is unknown. Therefore it is desirable to choose the best male parents. At the present time the progeny of more than 20 male selections, crossed to the same female parent are being evaluated by DSIR, and the new male selections which can pass particular characters onto their offspring will emerge from this work (Seal et al 1986).

Like many other fruit trees, the kiwifruit is often a composite vine consisting of scion and rootstock. In every block, there are some vines with decidedly inferior growth and yield, which may be associated with inherited characters of the individual seedling rootstock. It therefore would be possible to select a clonal rootstock to maintain good growth and reproductive ability of the scion, and perhaps to provide resistance to soil borne pests and diseases. Research on rootstock effect on yielding efficiency may lead to improved rootstocks being selected (Lawes 1986a, 1986b; Lowe, 1987).

In other parts of the world, Actinidia breeding is also proceeding. In China, fruit of 14 improved cultivars is similar to Hayward in weight, flavour and vitamin C content, most of them being bred from A. chinensis and the yield of a three year old planting was as high as 22.5 tonnes/ha. (Gao 1987).

In the United States, two kiwifruit selections "Blake" and "Vincent" have proved adaptable to climates in Southern California where the warm winters preclude the successful cropping of Hayward due to the low winter chilling (Meyer, 1987). The selection "Dexter" in the warmer parts of Australia has a lower requirement for winter chilling, but the fruit are visually very similar to Hayward fruit and store well (Winks, 1986).

In Italy, scientists have released a number of selections and strains including two Hayward selections named HE1 and HE2 whose pollen was about 20% viable. This character could be of value in breeding and further research for hermaphroditism is being carried out (Bellini et al 1987). Another promising selection is a mini-kiwifruit 'Fatma', a chance seedling of Hayward with smaller fruit. Its male progenitor is unknown, probably A. kolomikta. The plant is quite resistant to a dry season and to excess calcium in the soil. Its keeping quality is extremely high, the shelf-life without refrigeration exceeding 9 months (Paglietta 1987).

(iv) ***Interspecific crosses of*** Actinidia

Some breeding objectives for kiwifruit improvement pointed out by horticulturists and breeders (Dawes 1977; Ferguson 1987; Lawes 1978; 1979; Lay Yee 1980; Seal et al 1986,) include:

- for extending the climatic and soil range over which kiwifruit can be cultivated (e.g. tolerant to alkaline soil, high soil moisture levels and drought-tolerant)
- for processing: good flavour, beautiful colour, good texture (firmness) and appearance (cross-section of kiwifruit is a feature), wholeness of a piece (as decoration of food), Bruno type shape, high vitamin C and high sugar content.
- for ensuring pollination: heavy flowering, well timed male cultivar with necessary pollen quality.
- for rootstock: a dwarfing, cold hardy cultivar with graft compatibility
- for resisting pests and diseases (scale, leafrollers, bark canker, storage rot, root rot, nematodes, crown gall).

For reaching the above goals, sometimes it is necessary to introduce characters not found in A. deliciosa such as different flesh colour, different flavour, higher vitamin C and a higher level of winter hardiness. As mentioned above, the genus which the kiwifruit belongs to contains nearly 100 taxa, many of which have specially desirable attributes. There is the possibility of obtaining a completely new kiwifruit through interspecific crosses.

Ivan Michurin, a Russian horticulturist, did much work bringing Actinidia into cultivation as a fruit crop, and he produced young seedlings both from selfing and interspecific crossing and subjected them to the influence of the new environment, and ultimately produced one adapted to it. In this way a number of cultivars capable of being grown for fruit in Russia were produced (Saskin 1937). In western countries, the first successful interspecific hybrid from A. deliciosa was A. fairchildii (Rehder 1939), produced by pollinating A. arguta with pollen from A. deliciosa (Fairchild 1927).

A. chinensis is of great value to Actinidia breeding. It is productive, precocious and of medium vigour with smooth-skinned fruit of different flesh colours (green, yellow and pink). Most of the Chinese cultivars were bred from this species (Chen & Zhu 1981; Ferguson 1984; Gao & Ma 1988;

Zhu 1982).

The other species with edible fruit, which can be used in Actinidia breeding are:

A. arguta is cold resistant, it is of interest for areas too cold for the kiwifruit and with a vitamin C content of 111 mg/100 g, with maxima of 130 mg/100 g (Kolbasina 1970).

A. eriantha contains exceptionally high levels of vit. C. from 195- 720 mg/100 g, while Hayward usually only contains 80-100 mg/100g (Bank & Ferguson, 1986).

A. kolomikta has considerable value as an ornamental plant, Gerasevic (1966) reported that a seedling of unusually large white flowers having up to 15 petals instead of the usual 5-7, was selected from the cultivar Serzant's New. It is also a good breeding stock for its cold resistance and prolific fruiting (Tyunikov 1975). Several selections and cultivars have been obtained and evaluated for this species in the USSR, the most winter hardy ones can give satisfactory yields and good quality in cold areas (Plekhanova 1983). The species has very high vitamin C content, analyses of 120 forms showed the mean content was 890 mg/100 g (Kolbasina 1970), some cultivars were even higher, over 1000 mg/100 g (Samorodova-Bianki et al 1977, Plekhanova 1983). It is of special interest to mention the report of Kopestinskij et al (1966), who found two seedlings that had both male and female flowers on a normally dioecious vine.

As early as the 1930's, there were attempts to pollinate A. arguta with A. kolomikta but they failed because the latter flowers much earlier than A. arguta. In 1937, the early shoots were destroyed by a May frost and flowers from dormant bud came out somewhat later, made it possible to cross the two species, and an exceptionally frost resistant selection was obtained (Saskin 1940). Subsequently, some promising seedlings were selected from a cross of the two species (Tyunikov 1975).

At present, several programmes concerning interspecific hybridization among 10 species and botanical varieties have been carried out in New Zealand, and hybrids between cultivated kiwifruit and A. arguta were obtained. The pentaploid hybrid was intermediate between its parents for most traits, but less vigorous than either. It had poor male but reasonable female fertility (Pringle 1986).

Another example is a red fleshed hybrid (15 g) between A. arguta and A. melanandra. Its fruits mature in March and have a palatable smooth skin, a sweet taste with maximum total soluble solids of 17 to 20% (compared with 16% for Hayward). It is decorative because of its red flesh which appears as the fruit ripens. Further studies are being undertaken to assess its commercial potential (Seal 1987, Seal and McNeilage 1988). While there have been some successes, due to differences in ploidy levels interspecific crosses will not be easy. Special tissue culture techniques are needed to solve this problem. Plantlets derived from immature hybrid embryos, and from endosperm have been transferred to glasshouse conditions for further growth and evaluation. Through embryo rescue, endosperm culture and colchicine doubling of chromosome numbers, one can greatly increase the range of hybrid vines at all possible ploidy levels (Seal et al, 1988).

(v) *Genetic engineering*

Plant genetic engineering is a technique which allows one to incorporate alien genes into crops to create superior or entirely new types of cultivars. Its power lies in its ability to get the desirable genes from any source, and to manipulate them very precisely. Such innovation therefore would reduce many problems facing the horticultural industry. (Austin 1988; Gardner 1986; Moore 1988).

The two steps involved in genetic engineering are DNA recombination and gene transformation. DNA recombination provides methods to isolate a gene and to multiply it in bacteria, to characterize it completely and to modify it at will. Gene transfer usually involves a soil bacterium, Agrobacterium tumefaciens, which takes the isolated gene and introduces it into a plant, to add new traits to a cultivar without altering its intrinsic characters. In New Zealand four strains of Agrobacterium have been tested on kiwifruit, and two gave indications of DNA transfer. With the technique of cell cultivation, research attempting to get genetically engineered cells and shoots is being undertaken. New traits of kiwifruit, such as tolerance to the herbicide Roundup, and insect resistance could be obtained (Gardner 1986).

But biotechnology alone cannot achieve the ultimate desirable results, and genetic engineering must be viewed in the proper perspective. It can be a useful tool in creating genetic diversity, but it will remain for the traditional breeder to mould the germplasm into cultivars usable by the kiwifruit industry.

3. Multivariate analyses in plant breeding:

Multivariate analysis is the branch of statistics concerned with analysing multiple measurements on one or more populations. It is a technique of data summarising and reducing, grouping and analysing, and has proved useful in the biological and social sciences for more than two decades. Its application is being extended to crop science, especially with new statistical packages that facilitate the complex and tedious calculations.

In many horticultural studies measurements are concerned with a number of different attributes. The variates are dependent among themselves, hence one cannot split off one or more from the others and consider it by itself. Multivariate methods therefore should be used.

Although far less than in other crops, a few papers have been published in fruit crop research, where this technique has been applied to deal with kinship of cultivars, numerical taxonomy of germplasm collections (Blazek & Vondracek 1988; Challice & Westwood 1973; Jalikop et al 1984; Handa & Ogaki 1985; Hillig & Iezzoni 1988; Kajiura et al 1979; Kramer et al 1985; Potvin et al 1983), to evaluate commodity quality of fruit products (Forina et al 1986; Genizi and Cohen, 1988), and to examine the relative importance of rootstock and scion (Moore, 1975).

Since the methods below have been employed in this work, their history and application will be briefly reviewed (for detail, see Bose 1977).

(i) ***Manova and discriminant analysis:***

(a) Manova: In 1900, Karl Pearson created the correlation coefficient and obtained the Chi-square distribution, and in 1908, W.S. Gosset, "Student", developed the t distribution. Building on the work of Pearson and "Student", Ronald Fisher in 1923 began to create the analysis of variance (Anova) which proved to be the most widely used and basically useful approach to study differences among several populations or treatments. But Anova only handles one dependent variable, and the dependent variable is assumed to be normally distributed with the same variance in each population, and the research issues concern the "realness" of the differences among the population means for this variable. If one wants to deal with many characters together, Anova cannot do it and the multiple analysis of variance (Manova) is needed to solve this kind of problem. In Manova one is concerned with the multivariate generalisation (vector variable) of analysis of variance, which is the study of group differences with a number of variables combined.

Because Manova deals with the vector variables rather than scalars, it looks at the whole dispersion of the variables, i.e. variance and covariance, it is the more suitable method of handling combined variables. It is also a very important tool for genetic studies because most quantitative genetics information derives from partitioning variance and covariance of phenotypic values, and Manova can generate a dispersion matrix for this purpose.

In fact, complex Anova methods such as factorial designs are multivariate in the independent, or grouping, variable. The distinctive nature of Manova designs is that the dependent variable is a vector variable. This dependent vector variable is assumed to be multivariate, normal in distribution and with the same dispersion, or variance-covariance matrix, for each population. Equality of dispersions is the Manova extension of the assumption of homogeneity of variance in the Anova design. In Manova the research issues concern the "realness" of the differences among the population centroids, or the mean vector.

There are two null hypotheses established for Manova. Hypothesis one is used to test that the populations have a common dispersion; whereas hypothesis two is to identify whether the populations have a common centroid. A test criterion for the null hypothesis H1 of the equality of group dispersion matrices was presented by Box (1949), which extended from a development of

Bartlett (1937). A test of H2 was formulated by Wilks in 1932 in terms of the distribution of a ratio of the determinant of within group dispersion to the determinant of total dispersion - the ratio statistic is usually denoted as Lambda(/\):

/\= | W | / | T |

The general utility of the determinant ratio statistic is based on its transforms. Bartlett gave the Chi-square approximation in 1938; Rao derived an F approximation in 1952 which is superior to the chi-square method in that it gives very close fits to the /\ even for very small numbers of degrees of freedom (Cooley & Lohnes 1971).

In practical research, Manova proved to be a unified approach with all of the power, scope and flexibility of Anova (Cole & Grizzie 1966). Although Manova has been mainly used in social science (Cooley & Lohnes, 1971), several reports have been found in the other research areas. For example, Smith et al (1962) examined the effect of body weight on bio-chemical aspects such as pH, chloride content, in urine of man. Cole and Grizzie (1966) used Manova to investigate the effect of drugs on histamine release in dogs. In agricultural research, Manova was used to analyse sequential observations on grazing animals and perennial plants (Evans & Roberts, 1979). However, no such report was found in fruit crop science.

(b) Discriminant analysis: When two or more populations have been measured for several characters, special interest attaches to certain linear functions called discriminant functions by Fisher (1936), by which the populations are best discriminated. It can therefore be interpreted as a special type of factor analysis that extracts orthogonal factors of the measurement battery, for the specific task of displaying all differences among groups. Since Manova cannot make a comparison of various means, we need to use a discriminant function to get test scores for a means comparison. So it is of great importance in multivariate analysis, and it has been widely used in social science, for example, to group countries on the basis of their employment patterns (Manly, 1986), and to classify the careers of people on the basis of their abilities, interests and motives tests (Cooley & Lohnes, 1971).

(ii) *Factor analysis:*

Like other living things fruit trees are a highly integrated system, all the overt characters of which are interrelated. When using a multivariate approach to solve problems, the intercorrelations among variates must be considered. But the intercorrelations are always the bane of the multivariate researcher's struggle for meaning because of the covariance (Cooley & Lohnes 1971). One must transform the data in order to produce an uncorrelated vector variable. This orthogonal transformation can be completed by factor analysis.

Factor analysis includes principal component (PC) and common factor analysis. PC analysis was first described by Pearson (1901) but a description of practical computing methods came much later from Hotelling (1933). The early development of factor analysis was due to Spearman (1904), who formulated his two-factor theory of mental test. Later this theory was modified to allow for each test result to consist of a part due to several common factors plus a part specific to the test. This gave the general factor analysis model which has a number of potentially useful applications in horticultural research. It can be used to order multivariate data in one or more orthogonal dimensions called factors, which express most of the variance of the original data. Scores on these factors may be used as criteria for plant selection and as a non-measured variable to replace subjective visual attributes in conventional statistical analyses (Broschat 1979). Plotting of multivariate data in 2 dimensional factor space has proved useful for indicating the kinship of a sour cherry germplasm collection (Hillig & Iezzoni, 1988). Compared with Manova and discriminant analysis, factor analysis has been more widely used in agricultural (e.g. Denis & Adams, 1978; Gordon, 1983; Seiler & Stafford, 1985) and horticultural research (e.g. Broschat, 1979; Forina et al, 1986; Hillig & Iezzoni, 1988; Jalikop et al, 1984; Kajiura et al, 1979; Moore, 1975; Verlodt et al, 1985).

It is of interest to note the study made by Godshalk and Timothy (1988) in switch grass selection, in which both PC and common factor analysis were involved. They found that PC analysis had its highest correlation with the Smith-Hazel index (Smith, 1936; Hazel, 1943), and indicated the PC analysis of a simple correlation matrix had some potential for selection purposes.

Another important application of factor analysis is to get the genetic distance between parents in plant breeding using PC analysis of the genetic correlation matrix of parents' effects (Adams & Wiersma, 1978; Kong, 1986), and the superior parent combinations can be selected on the basis of their genetic distance.

(iii) *Canonical correlation:*

The canonical correlation is the maximum correlation between the linear functions of the variables in two data sets. It was developed by Hotelling (1935, 1936). Canonical analysis is the most general of the multivariate techniques, multiple regression and Manova are all special cases in it. It is a descriptive or screening procedure rather than a hypothesis-testing one (Tabachnick & Fedell 1983). Besides the canonical correlation coefficient, interest centres on the interpretation of the canonical factors. It is necessary to know which elements in the vector variables contribute most heavily to the maximally correlated factors. Thus, a factor structure giving the correlations of the canonical factors with the elements of the vector variables on which the factors are defined is desirable.

Since canonical correlation analyses the relationships between two sets of variable, it is suitable for dealing with relationships of yield components and physiological (or morphological etc.) characters in agricultural research (e.g. Ledent, 1982: Sun & Huang, 1987; Zhang et al. 1989). The application of the technique in fruit crops is rare, but Genizi & Cohen (1988) examined the canonical correlation between maturity and sensory properties in tangerines.

4. The practical application of genetic studies

(i) *Application of quantitative genetics to plant breeding*

Plant breeding continued to be mainly based on empirical knowledge until the rediscovery of Gregor Mendel's great 1865 paper on inheritance which gave rise to the science of genetics in the beginning of this century (Moore & Janick, 1975).

In the early days of genetics, it was thought that the segregation law from the single gene differences would revolutionise plant breeding, and so it was for some plants. But when fruit trees were crossed the results did not fit any of the simple segregations and no single gene characters were known for a long time. In fact, most attributes of breeding value, such as fruit weight, shape, colour and cropping are controlled by polygenes (Brown 1975; Lawrence, 1981), which means that when two cultivars are crossed there will be a wide and continuous range of expression of all these traits in the seedlings. The seedlings will not segregate into discrete categories, hence the simple segregation law cannot be applied to analyse these attributes, and a quantitative genetic approach should therefore be used.

For many field crops such as maize, genetic studies of quantitative traits have been widely demonstrated to be useful for developing breeding methods and selection procedures, and for predicting genetic gain of following generations (Hallauer & Miranda, Fo. 1981). This is even more important in fruit tree breeding. The long period of juvenility and the long generation cycle greatly delay the exploitation of genetic recombination, and increase the costs of rearing and evaluating seedlings. In addition, most fruit tree species have been maintained in a highly heterozygous condition, and large seedling populations are required. Therefore the designs which maximise the rate of genetic improvement of breeding material and minimise the cost of obtaining the improvement are more important than for other crops. But in fruit tree breeding, there have been only a few quantitative studies of attributes, and this is even more rare in kiwifruit breeding. Therefore more information of quantitative genetics is needed for designing breeding methods and experiments.

(ii) *A brief description of the history of quantitative genetics*

A detailed review of the history of quantitative genetics can be found in the book of Allard (1960), and Falconer (1981).

Galton and his students first studied quantitative variation by statistical methods in the last part of the 19th century, and their results showed that the quantitative characters were at least partly heritable. Yule then suggested in 1906 that many genes having small and similar effects might give rise to continuous inheritance. About the same time Johannsen showed that seed weight in beans varied continuously as a result of the joint and more or less equal effects of heritable and non-heritable agencies.

Nilsson-Ehle (1909) soon found a naturally occurring model for the type of inheritance suggested by Yule: in wheat and oats there are three genes for red versus white kernels. Any one of these genes gave a ratio of 3:1; two segregating together gave a 15:1 ratio and all three a ratio of 63:1. When more than one gene was segregating, differences in intensity of colour were observed. It was thus established that different genes could have similar and cumulative effects and any single one of those genes was subject to the same Mendelian laws of transmission. Thus quantitative genetics is an extension of Mendelian genetics, resting squarely on Mendelian principles as its foundation.

The further development of the theoretical basis of quantitative genetics was established about 1920 by the work of East (1916), Fisher (1918), Haldane (1932) and Wright (1921). The methods were elaborated by many geneticists and statisticians over the succeeding years. It also became clear that quantitative variation between individuals arises from the joint action of the genotype and environment, therefore the basic idea in the study of quantitative genetics is to partition this variation into components attributable to different causes. The amount of variation is measured and expressed as the variance, and from its partitioning much genetic information can be obtained.

(iii) *Phenotypic and genotypic correlation:*

Sometimes it is necessary to examine correlated characters and to know how the improved character will cause simultaneous change in other characters in plant breeding. For example, fibre quality in cotton is equally as important as yield, and it is necessary to improve quality along with yield. To effect these simultaneous improvements, an understanding of the nature and the magnitude of the association of the two characters is necessary, because the direction of the correlation could enhance or inhibit selection progress (Tyagi 1987).

There are two causes of a relation between characters, genetic and environmental. The former is mainly pleiotrophy, though linkage disequilibrium is a cause of transient correlation particularly in populations derived from crosses between divergent strains. It is the overall effect of all segregated genes that affect both characters. The same is true of correlation resulting from environmental causes, where seedlings reflect the overall effect of all environmental factors, some causing a positive correlation, some a negative one. Phenotypic correlation comes from both causes - shared genetic effects and a common response of independent systems to shared environments. Therefore a separate analysis of genetic correlation is needed.

Correlations have been determined for some fruit trees, and their interpretation has assisted in determining the relative importance and genetic properties of characters included in selection programmes (Hansche et al, 1966; Shaw 1988).

(iv) ***Heritability and its standard error:***

The absolute magnitude of the genetic variance for a character is of critical importance in that it determines how effective different selection and breeding procedures will be, but equally, if the genotypic variance is only a small part of the total variability, then environmental change may be more important. The heritability (h^2) of a trait is often used to describe this proportion.

The heritability can be estimated from the degree of resemblance between relatives, and several methods are used to estimate heritability (Falconer, 1981). The offspring-parent regression is one of the most useful methods, and the sampling errors of the estimates obtained by this method are likely to be less serious than those of estimates based on individuals less closely related. Furthermore the estimates of degree of heritability based on parent and offspring do not include dominance deviations as do the ones based on full sibs (Kempthorne & Tandon, 1953). However when records of parents are not available, sib analysis becomes essential for heritability estimation. Several statistical models and their genetic interpretations have been described for sib analysis, including biparental progeny model and models for three North Carolina Experiment Designs (Baker, 1986; Becker, 1967).

Since the concept of h^2 is associated with the relative importance of heredity and of environment in sib analysis, the estimation of heritability is based on partitioning the variance of phenotypes into various genetic and environmental components. For a balanced design, the estimation of variance components is straightforward. It is usually accomplished by computing the mean squares in the standard analysis of variance, equating these mean squares to their expectations, and solving for the unknown variance components. But most research data is unbalanced, and estimating variance components from such data becomes very complicated. Henderson's (1953) paper has been widely used as a foundation paper dealing with unbalanced data, and based on this paper many methods have been developed. A detailed review of variance component estimation was made by Searle (1971).

To determine the level of reliability of h^2 which can be used as a predictor of the rate of genetic gain when parents are selected on the basis of their own performance, the development of accurate measures of precision for h^2 estimators is needed. Kempthorne and Tandon's method (1953) has been used to estimate the variance of heritability based on regression, and there are two methods for determining the precision of heritability estimators on a progeny mean basis - the standard error method and the exact confidence interval method.

The standard error method traditionally has been used to measure the precision of heritability estimates. Since heritability is a statistic involving the ratio of the genetic variance to the total phenotypic variance, its standard error is large but unknown (Jensen & Barr, 1971). However an approximate variance of the estimated heritability can be obtained by using the approximation to the variance of a ratio (Baker, 1986; Becker, 1967; Gordon, 1972, 1979; Osborne & Paterson, 1952). But the procedure developed for the purpose is limited since it requires equal numbers of observations in the subclasses, and most biological data does not meet that requirement. Searle (1958) derived expressions for calculating variance and covariance of variance components for the two-way classification, and they can be used to approximate the standard error of heritability from an unbalanced design. Because of the number of calculations necessary in Searle's methods, an approximate method similar to Searle's two-way procedure was developed by Jensen & Barr (1971).

Since the distribution of heritability estimates are non-normal, Knapp et al (1985) pointed out that the correct measure of the precision of heritability estimates was the exact confidence interval, not the approximate standard error, and derived the exact confidence intervals for heritability on a progeny mean basis in a one-factor mating design. Knapp (1986) developed the method for a two-factor model, and Knapp et al (1987) further applied the method to estimate the precision of heritability estimates from sorghum populations. However none of these procedures have been derived for unbalanced data.

The values of heritability have been determined for a variety of fruit tree species as a guide in selecting parents for breeding, and have provided quantitative predictors of the rate of genetic improvement in the traits of commercial importance (Barritt, 1982; Hansche, 1983, 1986a, 1986b; Hansche & Boynton, 1986; Hansche et al, 1966, 1972a, 1972b, 1975; Kester et al, 1977). By using the value of h^2, selection response (genetic gain) under mass selection can be estimated. The basic paper of selection response theory in fruit breeding was written by Hansche (1983), and the selection response and correlated response for several importance characters have been calculated by Hansche (1986a, 1986b) and Hansche & Boynton (1986).

(v) ***Selection index:***

The use of a selection index was originally proposed by Smith (1936), who, following a suggestion by Fisher (1936), argued that since it could not be directly evaluated, genetic worth might be estimated by a linear function of observable phenotypic values. The observed value for each trait is weighted by an index coefficient and thus a selection index is developed which provides a guide for plant improvement by identifying the genetically superior ones.

Hazel and Lush (1942) compared the relative efficiencies of index with tandem and independent culling selection, and pointed out the index selection was the most efficient when the traits involved were independent. Young (1961) further evaluated their relative efficiency when the traits were correlated and concluded that the index is never less efficient than independent culling levels, though in some cases it is no more efficient than tandem selection. He further mentioned that the superiority of the index increases with an increasing number of traits under selection, but decreases with increasing differences in relative importance, its superiority being at a maximum when the traits are of equal importance. The theoretical evaluation of the relative efficiency of these methods was confirmed experimentally by Elgin et al (1970) in alfalfa, and Eagles & Frey (1974) in oats.

However there are some potential problems frequently associated with use of the selection index. Lin (1978) pointed out that selection may increase or decrease the genetic variance, depending upon initial gene frequencies. However, selection should decrease the genetic parameters in the long run. The changes of the parameter estimates due to selection may justify the reconstruction of the index at intervals during the breeding programme. Therefore the index should be seen as a dynamic index and not a static one. Brim et al (1959) pointed out that inaccurate estimation of population parameters could bias estimates of the theoretical gains. They suggested an alternative index such that each trait is weighted according to its relative economic value. Williams (1962) called this the base index, and indicated that the base index is superior to the estimated index unless a large amount of data is available for the estimation of the parameter. Heidhues (1961) pointed out the decrease in accuracy of the selection index method depended on the ratio of genotypic to total variance of the selected trait. If estimates of elements of the phenotypic or genotypic covariance matrix are 'unreasonable' i.e., if they exceed theoretically determined limits, they should be modified to increase the accuracy. Experimentally, the base index was found to be as efficient as the estimated index when based on poor estimation of parameters (Elgin et al, 1970). Eagles and Frey (1974) confirmed the estimated index was only slightly more efficient than the base index. However the base index has certain advantages because of its simplicity and its freedom from errors of parameter estimation, and its utility or when population parameter estimates are not available.

5. Protein markers as tools for genetic studies:

Since Peirce & Brewbaker (1973) outlined the applications of electrophoretic analysis of isozymes in horticultural science, many related papers have been published in many areas of fruit science.

Firstly, the banding patterns of isozymes have been used as tools for cultivar and rootstock identification (Bringhurst et al, 1981; Mazzola & Carter, Jr, 1988; Menendez et al. 1986; Weedem & Lamb, 1985). In addition to cultivar identification, the isozyme patterns were used in distinguishing nucellar seedlings from those of zygotic origin in citrus (Torres et al, 1978), abnormal from normal citrus plants resulting from somatic embryogenesis (Navarro et al, 1985), hybrids of peach and plum from their parents (Parfitt et al, 1985), intergeneric hybrids from wingnut and walnut (McGranahan et al, 1986), and in identifying interspecific cell hybrids and fusion products in citrus somatic hybridisation (Ben-Hayyim et al, 1982).

Secondly, different isozymic zymograms of peroxidase have been found in the stalk of apple fruit during the various ripening stages. These zymograms represented excellent markers for study of tissue differentiation (Ao et al, 1983). But Arulsekar et al (1985) examined GPI and AAT isozymes in callus tissues, juvenile leaves and adult leaves of walnuts and found no tissue specific differences in the isozyme patterns.

Thirdly, Arulsekar & Parfitt (1986) refer to many studies of single gene markers in date palm, strawberry and walnut, which indicated that isozymes were often co-dominant and offered the most reliable single gene markers for a breeder. Further studies of inheritance and variability of these gene markers are needed, and the work with apple (Bournival and Korban, 1987) and peach (Arulsekar et al, 1986) have shown some potential for such research.

In kiwifruit, the application of electrophoresis is rare, but work with A. deliciosa showed that the callus tissue, its subcultures and cell suspensions obtained from female plants contained characteristic isoperoxidase fractions, although no difference was found in stem segment cultures between male and female plants (Hirsch & Fortune, 1984).

It is interesting to note that with the exception of two of these papers (Mazzola & Carter, Jr., 1988; Navarro et al, 1985) in which the soluble proteins were analysed, isoenzyme analysis was used.

6. DNA probe technique in plant breeding

In 1986 Alec Jeffries in England found that several regions of human DNA were highly variable from person to person, so much so that everyone had his/her own unique set, therefore the DNA could be used as a unique fingerprint, much like bar codes, of any individual.

This technique is being used in kiwifruit improvement and initially the kinship of A. deliciosa has been examined. The results showed the profiles of A. deliciosa and A. chinensis clearly had some bands in common, and were judged to be closely related; A. eriantha and A. latifolia had no bands in common with A. deliciosa, suggesting that they are unrelated (Crowhurst et al 1988). The further development of DNA probes should be made in order to characterise cultivars and even to distinguish male plants within a seedling population.

CHAPTER 2

MULTIVARIATE ANALYSIS OF PHENOTYPIC VARIATION

SECTION 1 MANOVA & DISCRIMINANT ANALYSIS

1. INTRODUCTION

Several kiwifruit breeding programmes have already been established in New Zealand, and with large numbers of seedlings being evaluated and selected for further breeding, effective methods are urgently needed to assess seedlings.

As discussed in Chapter 1, Manova is the statistical technique concerned with analysing the variance of multiple measurements on several populations, and discriminant analysis is the technique for centroid comparison beween populations. Therefore they are multiple-population techniques.

In plant breeding. the criteria for assessing cross combinations, such as commodity quality and productivity are always determined by a number of attributes combined, hence the Manova and discriminant analysis may be suitable for the evaluation of cross combinations and selection of superior parent vines in kiwifruit breeding.

The objectives of the present study were:

(a) To test the "realness" of differences among the centroid or means vectors of a number of kiwifruit crosses. and of vines nested within these crosses.

(b) To discriminate and ordinate crosses and individual seedlings. on the basis of scores of orthogonal factors or discriminant functions which parsimoniously but effectively describe the measured differences of the groups.

(c) To compare these methods with Anova to examine the differences and the relationship between them.

(d) To examine the possibility of applying discriminant scores as selection criteria.

2. MATERIALS AND STATISTICAL PROCEDURES

(a) Materials

Plant materials used in this work came from a full sib families kiwifruit breeding trial in a factorial mating design. But from the view-point of phenotypic analysis, it is a pooled list design with internal replications, and without external replications. Experimental error resulting from soil heterogeneity, cannot be efficiently controlled in this design, but the large number of internal replications and two years' data set was expected to give rise to a reasonable model for analysis.

In November 1982, 2 male seedling plants were crossed with 3 female cultivars at the Riwaka Research Station, DSIR, New Zealand. The seeds from these crosses were sown in August 1983, after a 6 week cold stratification (3-5^{o} C), and the seedlings were transplanted to the field in September 1984. First flowering took place in 1985/86, when 30% of the seedlings flowered. In the 1986/87 season, the third season in the field, 84% of the seedlings flowered.

Data was collected in the 1986/87 and 1987/88 seasons, with the following number of kiwifruit vines investigated for each cross (Table 2.1.1):

Table 2.1.1 Crosses and the vine number investigated for each cross

Female cultivars	Male Parents	
	D-1-6	D-1-20
BRUNO	61 (cross 1)	87 (cross 2)
GRACIE	16 (cross 3)	159 (cross 4)
HAYWARD	46 (cross 5)	147 (cross 6)

(b) Attributes investigated

A great deal of variation in a range of attributes of horticultural value, or of biological interest was found in the materials investigated. Plates 1 to 5 show quantitative inheritance of petiole pigmentation, flower size, bloom date, fruit storage life and fruit size. Therefore the attributes investigated comprised vegetative, flowering and fruiting characters, and samples were collected in a random manner. For vegetative and flowering characters, two canes in the middle of the canopy of each vine were sampled, and three samples of leaf, flower and flowering shoot were chosen randomly from each cane, i.e., a total 6 samples were used for measurement. For fruiting attributes, five to ten fruits were used for investigation. Only the mean values were analysed in this work. Vegetative and flowering data was collected in November and December, and fruit data in April and May.

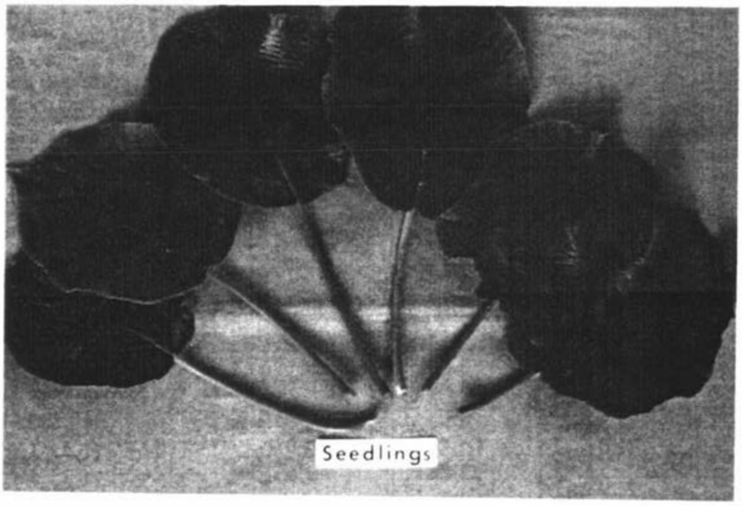


Plate 1 Petiole pigmentation of 6 F1 seedlings, showing a gradient of colour from red to dark red.

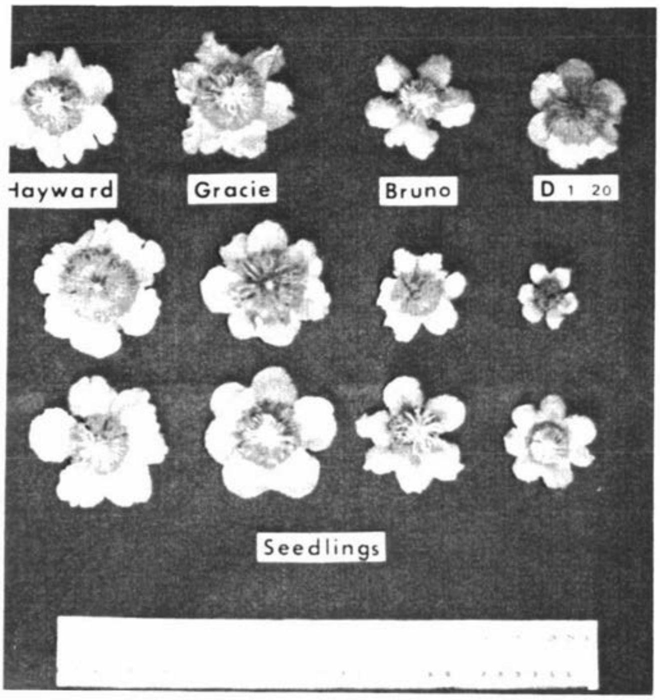


Plate 2 Quantitative inheritance of flower size, showing 4 parents and range of variation in 8 samples of F1 seedlings. Upper seedling row are male flowers, lower row are female flowers.

Plate 3 Early flowering vine versus late flowering vine, showing two adjacent male vines with distinct dates of blossom.

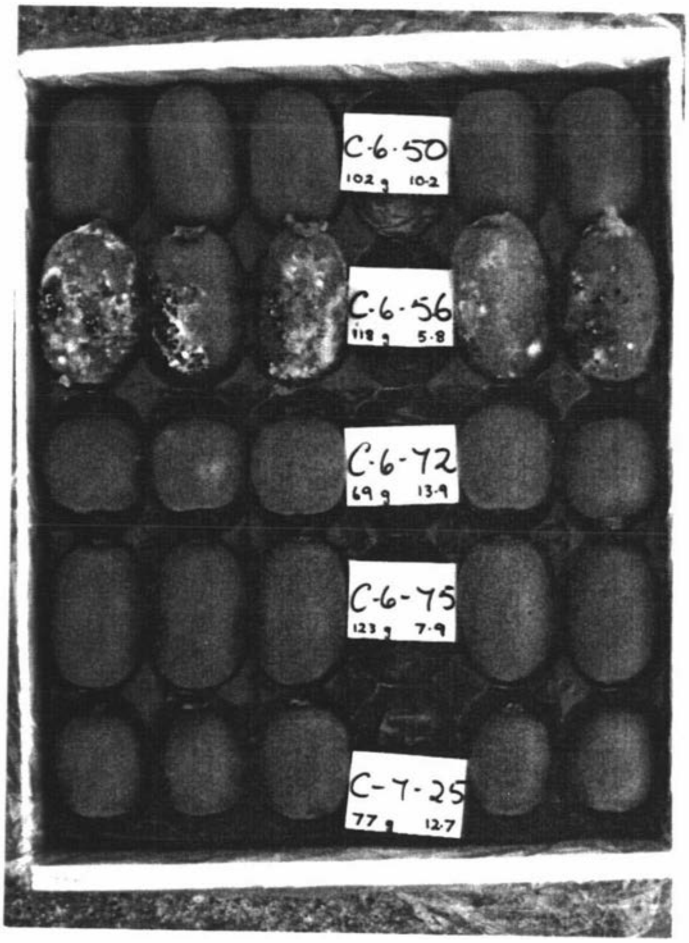


Plate 4 Comparison of fruit storage life of 5 F1 seedlings (Hayward X D-120) after 6 months cool storage. Also showing variation in fruit weight and Brix level among these seedlings. Labels show cross identification, fruit weight and Brix level. (With acknowledgements to Dr R. A. Beatson).

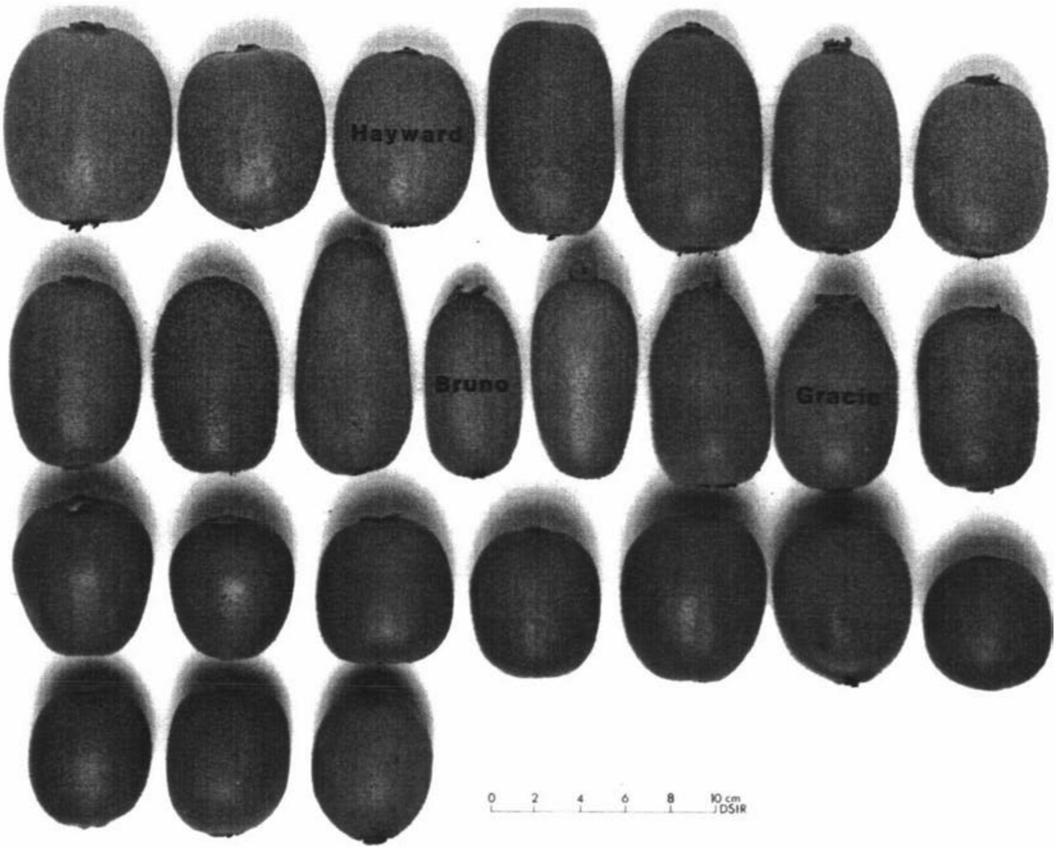


Plate 5 Quantitative inheritance of fruit size, showing 3 parents and range of variation in 23 samples of F1 seedlings. (With acknowledgements to Dr R. A. Beatson).

VEGETATIVE AND FLOWERING ATTRIBUTES:

(i) Leaf shape index (LWRATI):

Because leaf size varies along the shoot, the leaf shape index which is less variable than size was used in this analysis. It was the ratio of leaf length (cm) to width (cm), and its possible application for pre-selection of breeding stock was examined in the work.

(ii) Petiole colour (PETICO):

Five scales were used for this character: light red, medium light red, medium red, medium dark and dark red.

(iii) Flower diameter in cm (FD): diameter at full bloom.

(iv) Pedicel length in cm (PL): length at full bloom.

(v) Percentage floral shoots per vine (FSH%):

The percentage of the current season's shoots that bore flowers was determined and analysed following arcsine transformation. (Asin(sqrt(FSH%))*57.297). (see Steel & Torrie 1981, p236).

(vi) Total shoot (TSH):

Total number of current season shoots per vine.

(vii) Terminal flower number per shoot (TF):

Inflorescences occur along a shoot at each axil. The number of terminal flowers on a flowering shoot was recorded for both male and female plants, and indicates the number of flowering nodes per shoot.

(viii) Lateral flower number per shoot (LF):

Usually only one flower was found in each female inflorescence, thus lateral flower number was recorded only for male seedlings.

(ix) Trunk diameter in spring (DIASP):

Measured (in cm) at 40 cm above ground.

(x) Increment of area of trunk cross section in cm^2 (INCRS):

The trunk was assumed to have a circular cross section, and the formula (3.14*((diameter in autumn/2)^2- (diameter in spring/2)^2)) used to calculate the approximate value for this attribute. Trunk diameter was measured in spring and in the following autumn.

(xi) Beginning of bloom in the season (BEGDA):

This is expressed as the deviation of two dates - the date of first blossom in the season of the vine observed from the date of first blossom in the whole planting.

(xii) Flowering duration (FLPERI):

The period, in days, between the dates of first bloom open and last flower open.

FRUITING CHARACTERS:

(xiii) Total weight (kg) per vine (TW):

A logarithmic transformation, lg (TW*100), was used since the standard deviation was proportional to the mean and the data set had a positive skew.

(xiv) Mean fruit weight (g) (FW):

Determined from the total weight (kg) and total fruit number per vine.

(xv) Fruit number per flowering shoot (FNF):

Determined from the total number of fruit and flowering shoots per vine.

(xvi) Locule number (LOCN):

Counted in sampled fruit at harvest.

(xvii) Hair:

Hair density/coarseness was rated for each fruit at harvest, on a 5-point scale viz. very high, high, medium, low, very low.

(xviii) Elongation (ELONG):

Fruit shape was expressed as the ratio length/diameter, where the diameter was the mean of the maximum and minimum diameter at the fruit equator.

(xix) Relative core size (RELCOR):

The ratio of the sum of the core's maximum and minimum width to the sum of the fruit's maximum and minimum width.

(xx) Symmetry (SYMM):

The ratio of fruit maximum to minimum width.

(xxi) Total soluble solids (BRIX):

The stalk and blossom ends of the fruit were cut 15mm from each end. Two drops of juice were squeezed from each end onto a refractometer and total soluble solids read as % sucrose. Tests were performed immediately after harvest in both seasons.

(xxii) Titratable acidity (AC):

Expressed as the equivalent amount of citric acid (g/100 ml fruit juice), the major acid in kiwifruit (Heatherbell 1975). 2 ml of juice diluted with 20 ml distilled water was titrated against 0.1 N NaOH using phenolphthalein indicator (AOAC 1965). For minimising the variation of AC content the fruit sampled were put into a freezer at -20^{o} immediately after harvest.

(xxiii) L-ascorbic acid (VC):

Vitamin C content varies between species of Actinidia as well as with region, altitude, fruit age, harvesting and storage time (Yin &Li 1982). It is an important character to look at in kiwifruit breeding. The Na-2,6-dichlorophenol-indophenol method (AOAC 1965) was used in this study to determine L-absorbic acid content (mg/100 g) - it does not measure dehydro-ascorbic acid formed on storage. Analyses were performed in the 1986/87 and 1987/88 seasons. For minimising the variation of Vitamin C content, the fruits sampled were put into a freezer at -20^{o}C immediately after harvest.

(a) Extracting solution

To 40% W/V sodium hexametaphosphate 50 ml, add glacial acetic acid 50 ml, then dilute to 500 ml.

(b) Standard Vitamin C

Weigh accurately 100 mg Vitamin C, transfer to 100 ml volumetric flask, make up to volume with extracting solution.

(c) Standard indophenol solution

Dissolve Na-2,6-dichlorophenol-indophenol 0.25 g in distilled water 50 ml, add 0.21 g $NaHCO_3$, dissolve the dye completely, make up to 1 litre with distilled water.

(d) Standardisation

Take standard Vitamin C solution 2 ml, add extracting solution 5 ml, then titrate with indophenol to rose colour, which lasts at least 5 seconds.

(e) Estimation

Pipette 2 ml sample, add 5 ml extracting solution, then titrate until rose colour developed.

(f) Calculation

mgs L-ascorbic acid in 2 ml juice is given by:

2 * (net titre for juice/net titre for standard Vitamin C)

(xxiv) Fruiting efficiency (EFFI):

The ratio of total fruit weight to area of trunk cross section at 40 cm height in spring.

(xxv) Duration of the juvenile period (FY)

The period (in years) from seed to first bloom was recorded. This character determines how early vines come into cropping, and how soon they can be assessed.

(xxvi) Force reading: (kg) (PENE):

Fruit penetrometer tests were made in the 1987-88 season, and used for PC analysis only. Two readings were taken for each fruit, after removing a thin slice of skin, immediately after harvest.

(c) Statistical model

Because 2 years' data was investigated, the year effect can be obtained by partitioning a total sum of squares into components associated with recognised sources of variation. The model used in this work for estimating variance components was:

$$Y_{ijk} = \mu + C_i + V_{j(i)} + Y_k + (CY)_{ik} + (VY)_{j(i)k}$$

where Y_{ijk} stands for the kth year sample on the jth vine of the ith cross. The μ is the grand mean; C_i the random effects contributed by genotype, $V_{j(i)}$ the random effects of vine nested in the crosses; Y_k is the effect of year; $(CY)_{ik}$ is the interaction of cross and year; $(VY)_{j(i)k}$ is residual variances from the interaction of vine and year (equal $E_{j(i)k}$).

(d) Procedures of manova and discriminant analysis

A SAS procedure Manova of GLM (General linear model) which deals with an unbalanced data set, was used in this analysis.

In Anova, for the model depicted above, a pooled set of scalar lists would be compared with different F values. However Manova involves pooling of data vector lists rather than scalar lists since a number of characters are examined in this analysis. Because of intercorrelations among the elements of a vector variable, the total effect, the deviations of all subjects from the grand centroid (means vector) is a matrix (of sum of squares and sum of cross products) rather than scalar. This total effect T was partitioned as matrices C, V, Y, CY, and VY in this work, which correspond to the effect of cross, vine, year, interaction of cross and year, and interaction of vine and year (or error effects) respectively, and each of the partitions divided by its degree of freedom is a matrix of dispersion, i.e. variance and covariance matrix, which is expressed as D_c,

D_v, D_y, D_{cy}, D_{vy} respectively. They are independent estimators of the common population's dispersion, and based on them, the first hypothesis that the groups of seedlings are dispersed equally, was tested.

A test criterion for the null hypothesis of the equality of g group dispersion matrices was presented by Box (1949), which extended from a development of Bartlett's (1937). Box defines the test criterion M for this hypothesis (H1) as:

$$M = (N-g)\text{Log}_e \mid D_w \mid - \Sigma(N_k-1)\text{Log}_e \mid D_k \mid$$

where D_k is a dispersion estimate for the kth group and D_w is the pooled-groups estimate based on within group dispersions, N is the total number of subjects in all groups, N_k is the number of subjects for kth group and g is the number of groups.

Like the homogeneity test in Anova, many research workers prefer to ignore the issue of homogeneity of group dispersions on the grounds that the test of hypothesis two (H2) is probably fairly robust under departures from its assumptions. Also, Box's test is quite powerful, so research on large samples is quite likely to lead to a rejection of H1 with some consequent embarrassment to a Manova theory for the data (Cooley and Lohnes 1971).

A test of H2, which may be viewed as a test of the discriminating power of the measurement battery for the grouping criterion, or as a test of the "realness" of differences among the centroid or means vectors of the treatments represented by the groups, was formulated by Wilks (1932) in terms of the distribution of a ratio of determinants:

$$\Lambda = \mid W \mid / \mid T \mid$$

The general utility of the determinant ratio statistic is based on its transforms. Bartlett gave the Chi-square (x^2) approximation in 1938; Rao's (1952) F approximation gives a very close fit to Λ even for very small degrees of freedom.

Just like a means comparison in Anova, in Manova if H1 cannot be rejected and H2 is significant, one may proceed with the discriminant analysis for centroid comparison between the groups. For the best separation of the groups, the discriminant function, which is a linear transformation of the deviations of group means from the grand centroid, should maximise the ratio of the among-groups sum of squares to the within-groups, so that among-group differences will be large relative to the within-group scatter. Let y_1 be the first desired function and

$$y_1 = \mathbf{v}_1' \mathbf{x}$$

where **x** is a deviation vector of group means from the grand centroid, and **v** is a coefficient vector transforming the deviations into a discriminant function.

The among-groups sum of squares will have the following quadratic form from the matrix of among-groups sum of squares and cross products (matrix A):

$$\mathbf{v}_1'A\,\mathbf{v}_1$$

Similarly the within-groups sum of square will be:

$$\mathbf{v}_1'\,W\,\mathbf{v}_1$$

The task for the calculus of partial differentiation to the unknown weights in **v** is to maximise the ratio of these two quadratic forms subject to $\mathbf{v}_1'\mathbf{v}_1 = 1$.

$$\mathbf{v}_1'(W^{-1}A)\mathbf{v}_1 \mid \text{maximum}$$

Let $\phi_1 = \mathbf{v}_1'(W^{-1}A)\mathbf{v}_1 - \lambda_1(\mathbf{v}_1'\mathbf{v}_1 - 1)$,

and to maximise it:

let $d\phi_1/d\mathbf{v}_1 = 2(W^{-1}A)\mathbf{v}_1 - 2\lambda_1\mathbf{v}_1$

and set equal to 0, we get

$$(W^{-1}A)\mathbf{v}_1 - \lambda_1\mathbf{v}_1 = 0$$

and $(W^{-1}A - \lambda_1 I)\mathbf{v}_1 = 0$

This is recognisable as the problem of the eigenstructure of $(W^{-1}A)$. The maximum value of lambda (λ_1) and the associated vector of weights $\mathbf{v}_1$ are indicated by differential calculus to be the largest eigenvalue and its eigenvector.

The next step is to find the linear combination

$$y_2 = \mathbf{v}_2'\mathbf{x}$$

which, out of all functions uncorrelated with y_1, makes the ratio of two quadratic forms maximum.

But the uncorrelatedness of y_1 and y_2 can be obtained only if

$$\mathbf{v}_2'\mathbf{v}_1 = 0$$

so the second Lagrange multiplier k is introduced to get the orthogonality.

Let $\phi_2 = \mathbf{v}_2'(W^{-1}A)\mathbf{v}_2 - \lambda_2(\mathbf{v}_2'\mathbf{v} - 1) - k\mathbf{v}_2'(W^{-1}A)\mathbf{v}_1$ and to maximise it:

Let $d\phi_2/d\mathbf{v}_2 = 2(W^{-1}A)\mathbf{v}_2 - 2\lambda_2\mathbf{v}_2 - 2k(W^{-1}A)\mathbf{v}_1$

and set equal to 0, and pre-multiplying by $\mathbf{v}_1'$,

$$\mathbf{v}_1'(W^{-1}A)\mathbf{v}_2 - \lambda_2\mathbf{v}_1'\mathbf{v}_2 - k\,\mathbf{v}_1'(W^{-1}A)\mathbf{v}_1 = o$$

The result shows that k must be 0 and the lambda 2 and $\mathbf{v}_2$ must be the second root and its associated vector of eigenstructure. This process may be continued to get p-1 maximal ratios of the two quadratic forms for discriminant functions.

so the second Lagrange multiplier k is introduced to get the orthogonality.

Let $\phi_2 = \mathbf{v}_2' (W^{-1} A)\mathbf{v}_2 - \lambda_2(\mathbf{v}_2'\mathbf{v}-1) - k\mathbf{v}_2'(W^{-1}A)\mathbf{v}_1$ and to maximise it:

Let $d\phi/d\mathbf{v}_2 = 2(W^{-1}A)\mathbf{v}_2 - 2\lambda_2\mathbf{v}_2 - 2k(W^{-1}A)\mathbf{v}_1$

and set equal to 0, and pre-multiplying by $\mathbf{v}_1'$,

$$\mathbf{v}_1'(W^{-1}A)\mathbf{v}_2 - \lambda_2\mathbf{v}_1'\mathbf{v}_2 - k\,\mathbf{v}_1'(W^{-1}A)\mathbf{v}_1 = o$$

The result shows that k must be 0 and the lambda 2 and $\mathbf{v}_2$ must be the second root and its associated vector of eigenstructure. This process may be continued to get p-1 maximal ratios of the two quadratic forms for discriminant functions.

The entire structure will be

$$(W^{-1}A)V = VL$$

where L is a diagonal matrix of λ .

In order to view the discriminant function as a factor of the test battery one derives new coefficients for a standardised function, and the entire structure is

$$F = BX$$

where F is a factor (standardised function) matrix, and B is called raw coefficient matrix, it appears to have limited use as X has not been standardised. If X is standardised,

$$F = CZ$$

where C is called a standardised coefficient matrix. As both x and y are standardised, this structure is scale-free and variance equal. In this study matrix C was used to get net function scores for each group by multiplying the standardised group means (here grand mean = 0) by the standardised coefficients, and the groups were distinguished on the basis of these scores.

The greatest interest in discriminant analysis centres on the correlations between the original variables and the functions. The matrix of such correlations is called the factor structure, and will be

$$S = RC$$

where R is a correlation matrix based on T (Cooley & Lohnes, 1971). The S matrix is very important in discriminant analysis, and it is the primary interpretative device. Based on the magnitude and nature of correlations (factor loadings) between the original variables and functions, the functions can be interpreted and named. In this study, it was called a function structure in order to avoid the confusion with factor structure in factor analysis.

Based on the principle of Manova mentioned above and Fisher's theorem for partitioning the sum of squares in Anova into orthogonal, additive components, it is possible to extend Manova to any experimental design.

According to the model, Wilks' Λ for each of the partitions of the whole effect in this work should be

$$\Lambda_c = |CY| / |CY+C|$$
$$\Lambda_y = |CY| / |CY+Y|$$
$$\Lambda_{cy} = |V| / |V+CY|$$
$$\Lambda_v = |E| / |E+V|$$

Unfortunately, insufficient degrees of freedom prevented valid tests to be made for cross and year, so, the vine effect had to be considered as an error term. Therefore, Wilks' Λ for cross and year became

$$\Lambda_c = |V| / |V+C|$$
$$\Lambda_y = |V| / |V+Y|$$

The corresponding eigen structures for each component are

$$|V^{-1}C - I| = 0$$
$$|V^{-1}Y - I| = 0$$
$$|V^{-1}(CY) - I| = 0$$
$$|E^{-1}V - I| = 0$$

Based on the eigen structures above, the discriminant analysis was carried out.

(e) *Missing data*

The procedure adopted was firstly to enter the integer nearest to the mean of the given variable as the missing score, then pick the one other variable with which the given variable correlated highest, and the raw-score regression of the given variable on the other one computed. This regression equation was used to re-estimate the missing score on the given variable (Cureton & D'Agostino 1983).

3. RESULTS AND DISCUSSION

(a) Male vines

(i) Univariate analysis

The original data set was collected for 12 traits on each of the vines, and was analysed with 12 separate Anova's to determine the effects of various sources on each of the 12 variables measured. Anova statistics are given in tables 2.1.2-2.1.4 with sources vine, cross (CR), year (Y), and cross-year interaction (CR*Y). Significant differences ($P<0.05$) were detected among vines for all traits, and among crosses for most of the traits (Table 2.1.2). No differences were detected for PETICO and TSH among crosses, indicating petiole colour and total shoots per vine were rather consistent characters between genotypes. Year effects were significant for vegetative characters DIASP and INCRS, and also for some flower characters TF, LF, FSH% and FLPERI, indicating these characters changed with growing season. Significant differences were not detected for FD, PL, PETICO, TSH, BEGDA and LWRATI, indicating they were stable across years. Cross-year interactions were not significant for all characters except PETICO, showing petiole colour gave high interaction effects between genotypes and growing seasons.

Table 2.1.2 **F test of 12 dependent variables for male vines**

VARIABLE	PR > F			
	VINE[1]	CROSS[2]	YEAR[2]	CR*Y[3]
FD	0.01	0.04	0.70	0.07
PL	0.01	0.01	0.06	0.06
TF	0.01	0.01	0.01	0.63
LF	0.01	0.01	0.01	0.92
PETICO	0.01	0.38	0.74	0.01
DIASP	0.01	0.01	0.01	0.98
TSH	0.01	0.08	0.15	0.43
FSH%	0.01	0.01	0.04	0.77
BEGDA	0.01	0.02	0.40	0.36
LWRATI	0.01	0.02	0.94	0.19
INCRS	0.01	0.03	0.02	0.16
FLPERI	0.01	0.01	0.03	0.19

[1] Vine, tests of hypotheses using the type III MS for residual variance as an error term
[2] CR and Y, tests of hypotheses using the type III MS for CR*Y as an error term
[3] CR*Y, tests of hypotheses using the type III MS for vine as an error term

Table 2.1.3 **Means of variables, by cross in the male population**

CR	N	FD	PL	TF	LF	PETICO	DIASP	TSH	FSH%	BEGDA	LWRAT	INCRS	FLPE
1	56	6.07	4.37	3.98	4.27	3.28	2.00	39.53	55.38	7.00	1.08	1.09	17.96
2	80	6.16	5.03	3.83	4.66	3.22	2.29	45.68	61.15	5.50	1.03	1.43	19.47
3	6	5.95	3.80	2.53	0.38	2.50	1.54	33.33	28.43	9.16	1.22	0.76	10.66
4	132	6.80	5.68	3.18	3.70	2.93	2.36	37.93	52.40	5.28	1.06	1.80	15.98
5	28	6.08	5.12	3.01	2.16	2.89	2.08	40.14	35.47	8.39	1.10	1.29	13.53
6	126	6.42	5.88	3.44	4.77	3.00	2.43	39.29	62.85	4.91	0.97	1.84	16.67
GM		6.41	5.38	3.46	4.12	3.04	2.29	40.07	56.06	5.68	1.04	1.60	16.86
SE		0.42	0.61	0.73	1.63	0.47	0.11	6.84	14.89	1.95	0.08	0.63	3.12

CR:	Cross
N:	Number
SE:	standard error
GM:	Grand mean

Table 2.1.4 **Means of variables, by CR*Y in male population**

CR	Y	N	FD	PL	TF	LF	PETICO	DIASP	TSH	FSH%	BEGDA	LWRAT	INCRS	FLPER
1	1	28	6.10	3.94	4.24	4.67	3.42	1.78	38.28	57.97	7.32	1.07	0.80	18.92
1	2	28	6.04	4.80	3.72	3.87	3.14	2.23	40.78	52.78	6.67	1.08	1.38	17.00
2	1	40	6.24	4.49	4.21	5.18	3.42	2.00	42.72	62.99	5.40	1.03	1.27	19.75
2	2	40	6.09	5.56	3.46	4.14	3.02	2.57	48.65	59.32	5.60	1.04	1.59	19.20
3	1	3	5.83	3.46	2.66	0.43	2.33	1.38	28.00	31.66	7.33	1.23	0.46	12.66
3	2	3	6.06	4.13	2.40	0.33	2.66	1.69	38.66	25.20	11.00	1.20	1.06	8.66
4	1	66	6.90	5.32	3.66	4.45	2.89	2.08	38.77	58.10	5.54	1.06	1.45	17.42
4	2	66	6.69	6.05	2.70	2.95	2.96	2.64	37.09	46.69	5.03	1.06	2.14	14.54
5	1	14	5.93	4.77	3.59	3.05	2.57	1.83	38.35	39.18	7.64	1.06	0.97	16.21
5	2	14	6.22	5.47	2.44	1.27	3.21	2.32	41.92	31.76	9.14	1.14	1.61	10.85
6	1	63	6.70	5.83	3.80	5.41	2.96	2.15	38.41	65.22	5.34	0.99	1.34	17.53
6	2	63	6.14	5.93	3.09	4.13	3.03	2.70	40.17	60.47	4.47	0.94	2.33	15.80

(ii) Multivariate analysis

Based on this demonstration of significant effects the data was analysed by the Manova procedure. Table 2.1.5 shows the descriptive statistics of the Manova, where Wilks' Lambda was transformed as an F approximation. Strong significant differences were detected among all effects - CR*Y, CR, Y and vine, which suggested the need for discriminant analysis for centroid comparison between groups.

Table 2.1.5 **Manova test criteria for the hypothesis of no overall effect in male population**

```
CR*Y
WILKS' CRITERION  L = DET(E)/DET(H+E) = 0.66074869
                  F(60,926.25) = 1.43     PROB > F = 0.0203
CR
WILKS' CRITERION  L = DET(E)/DET(H+E) = 0.19098034
                  F(60,926.25) = 6.55     PROB > F = 0.0001
Y
WILKS' CRITERION  L = DET(E)/DET(H+E) = 0.62900774
                  F(12,197) =    9.68     PROB > F = 0.0001
VINE
WILKS' CRITERION  L = DET(E)/DET(H+E) = 0.00000009
                  F(2496,2413.12) =  2.79 PROB > F = 0.0001
```

(a) Cross and year interaction

Table 2.1.6 shows a discriminant function structure for CR*Y effects and related statistics including eigenvalue, proportion of total discriminant power accounted for by each function, and cumulative amount of discriminant power of functions. In this structure, each function may be regarded as the dependent variable that is regressed on all original variables, therefore not only can we determine the relationship between function and the original variables, but one can also determine the relationship between the original variables in their own right. Variables which have relatively high positive regression weights on a function are positively intercorrelated as a group. Similarly, variables which have high negative weights are also positively intercorrelated, but they are negatively correlated with those variables having positive weights. The magnitude of the weights shows the relative contribution of the original variables to each function, and is used to interpret and name the function.

The first two functions account for 81% of total discriminant power, i.e., for 47% and 34% of the discriminant power respectively. Since the two account for most of the discriminant power and the rest only account for a small part of the discriminant power due to random effect, and is of no value in this analysis, only these two functions were retained for further analysis.

Function 1 was mainly associated with FD, and negatively with INCRS, hence it was regarded as a flower size and slow vine growth rate function. The second function made up mainly of PETICO, and a negative value of FLPERI, was called a short flowering period and petiole colour function. Examination of the third function suggested it may be called a floriferousness function since it was mainly associated with TF, TSH, FSH% and FLPERI. It accounted for only 9% of the discriminant power, and so it was too weak to use in grouping CR*Y effect.

Table 2.1.6 **Function structure and related statistics for CR*Y effects in male population**

	STRUCTURE				
	FUN1	FUN2	FUN3	FUN4	FUN5
FD	0.35	0.35	-0.20	-0.21	0.25
PL	0.27	-0.16	-0.46	0.16	-0.15
TF	0.15	0.00	0.45	-0.59	0.24
LF	0.10	0.05	0.29	-0.38	0.10
PETICO	-0.34	0.51	0.07	0.23	-0.33
DIASP	-0.16	-0.25	-0.22	0.44	-0.17
TSH	0.18	-0.03	0.57	0.02	0.09
FSH%	0.06	-0.05	0.46	-0.04	0.50
BEGDA	0.21	0.32	0.28	0.00	-0.42
LWRATI	0.28	0.34	-0.30	0.06	0.30
INCRS	-0.53	-0.07	-0.10	0.08	0.04
FLPERI	0.12	-0.33	0.40	-0.12	0.13
EIGENVALUE	0.21	0.15	0.04	0.03	0.01
PROPORTION	0.47	0.34	0.09	0.08	0.02
CUMULATIVE	0.47	0.81	0.90	0.98	1.00

The score for each cross in each year on each function was obtained by multiplying the standardised means of a cross in each year (Table 2.1.4) by standardised coefficients (i.e. C matrix, see 'procedures').

Table 2.1.7 displays net scores for CR*Y effect on the first 2 functions. Thus, 12 original variables were reduced to 2 functions to sort CR*Y effect. Not only was the number of comparisons between CR*Y effect reduced, but the meaningfulness of these comparisons was enhanced, since these 2 functions accounted for most of the total discriminant power.

Table 2.1.7 Net scores for CR*Y effects in male population

	CROSS	YEAR	FUNCTN1	FUNCTN2
1	1	1	9.04	9.54
2	1	2	8.95	8.96
3	2	1	8.79	9.30
4	2	2	9.24	8.31
5	3	1	9.31	9.04
6	3	2	9.42	9.96
7	4	1	9.39	9.35
8	4	2	8.79	8.92
9	5	1	9.46	8.49
10	5	2	9.20	9.58
11	6	1	9.43	9.17
12	6	2	8.17	8.47

Graphically, the mean scores on these two functions can be plotted to show cross differences between years in 2 dimensions, and then the differences can be readily conceptualised. Figure 2.1.1 shows the cross distance between years based on the first two functions. The numbers represent cross identity and the year as follows, and the score distance in mm for the same cross between 2 years was:

Cross 1 Bruno x D1-6	(1-2)	3.8
Cross 2 Bruno x D1-20	(3-4)	6.7
Cross 3 Gracie x D1-6	(5-6)	5.9
Cross 4 Gracie x D1-20	(7-8)	3.8
Cross 5 Hayward x D1-6	(9-10)	7.2
Cross 6 Hayward x D1-20	(11-12)	6.7

The crosses 2, 5 and 6 gave the greatest distance, which means that the male progeny produced from these crosses tended to vary across years in the characters flower size, petiole colour, flowering duration and growth rate, and had high CR*Y effects. In contrast the male plants from the other crosses tended to be stable for these characters, and have lower CR*Y effects.

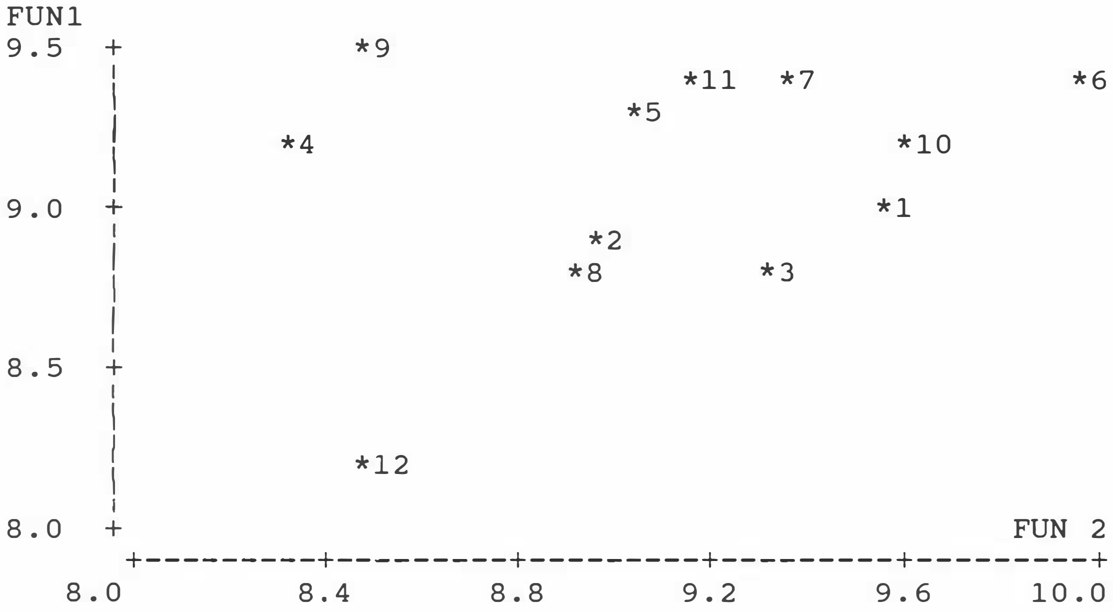


Figure 2.1.1 Plot of net scores of discriminant functions 1 and 2 in male population (6 crosses, 2 years).

(b) Cross effects

Table 2.1.8 shows a function structure and related statistics for cross effect. The first two functions accounted for 84% of the total discriminant power, therefore they were retained for analysis. Function 1 was mainly associated with PL, DIASP and INCR, hence it was regarded as an early vigour function.

The second function can be called a floriferousness function since it was mainly associated with LF, FSH% and FLPERI, and would be a useful criterion for selecting the better crosses. Function 2 with function 1 would improve the effectiveness of selection because both amount of flowering and early vine vigour would then be taken into account. The net scores (Table 2.1.9) of the two functions determined from the standardised coefficients and cross means (Table 2.1.3), were plotted together, so the higher the score, the better the cross (Figure 2.1.2).

The relationship between the original variables which comprise a function may also be examined in the analysis. It is worthwhile noticing that LWRATI was considerably negatively associated with function 2, that is to say, there is a close relationship between broad leaf shape and floriferousness. Further investigation should be carried out in order to test the possibility of using leaf shape as a criterion for selecting for high flowering in male vines.

Table 2.1.8 **Function structure and related statistics for cross effects in male population**

	STRUCTURE				
	FUN1	FUN2	FUN3	FUN4	FUN5
FD	0.22	-0.31	0.62	0.05	0.34
PL	0.69	0.14	-0.08	0.30	0.31
TF	-0.49	0.34	0.37	-0.24	0.34
LF	-0.11	0.48	0.44	-0.22	0.22
PETICO	-0.23	0.30	0.13	0.00	0.38
DIASP	0.53	0.23	-0.07	0.43	0.08
TSH	-0.11	0.24	-0.08	0.53	-0.12
FSH%	0.02	0.62	0.47	-0.17	-0.22
BEGDA	-0.30	-0.30	-0.41	-0.21	0.22
LWRATI	-0.31	-0.59	0.04	0.08	-0.08
INCRS	0.59	0.08	0.04	0.19	0.12
FLPERI	-0.32	0.51	0.52	0.29	0.06
EIGENVALUE	1.34	0.61	0.21	0.10	0.02
PROPORTION	0.58	0.26	0.10	0.04	0.02
CUMULATIVE	0.58	0.84	0.94	0.98	1.00

Table 2.1.9 **Net scores for crosses in male population**

CROSS	FUNCTN1	FUNCTN2
1	7.55	9.04
2	8.28	9.47
3	8.01	7.10
4	9.44	8.50
5	8.69	8.26
6	9.75	9.45

Figure 2.1.2 shows the function scores on the first two functions, the numbers represent cross identity. Hayward cross with D1-20 gave the highest score, which means that the progeny from this cross tended to be both floriferous and vigorous. However, if only function 2 was considered, Bruno crosses with either male parent gave higher function scores than Gracie crosses and Hayward x D1-6, indicating Bruno should be used as the female parent for producing floriferous male seedlings. It is of interest to note the male parent D1-20 crosses surpassed D1-6 crosses constantly, suggesting D1-20 was the better male parent to produce progeny that were both floriferous and had early vigour.

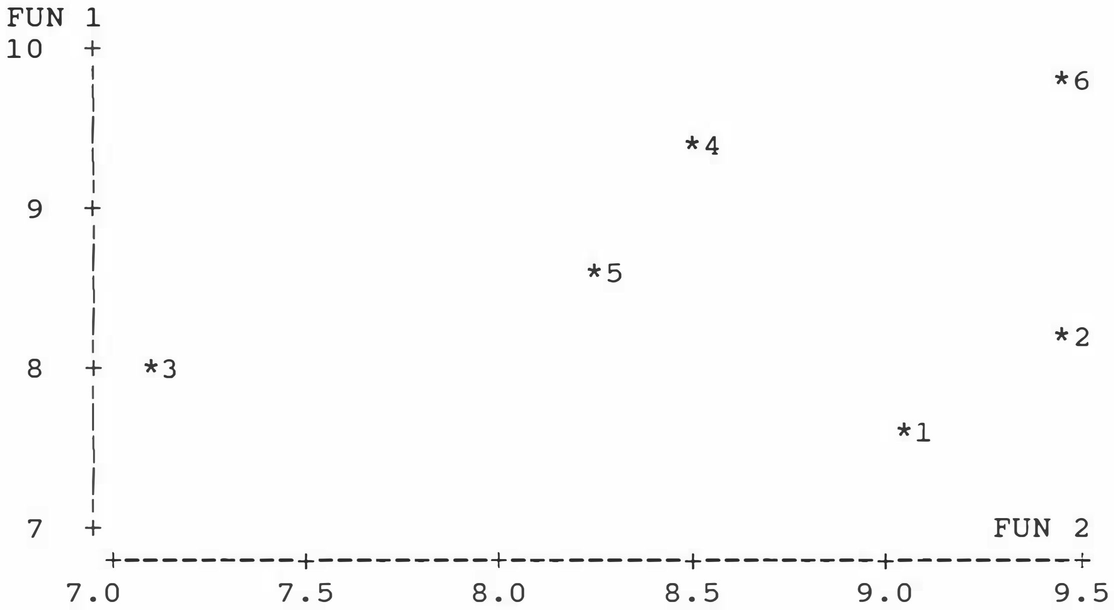


Figure 2.1.2 **Plot of net scores of discriminant functions 1 and 2 for each cross in male population**

Cross 1 Bruno x D1-6	Cross 3 Gracie x D1-6	Cross 5 Hayward x D1-6
Cross 2 Bruno x D1-20	Cross 4 Gracie x D1-20	Cross 6 Hayward x D1-20

(c) Year effects

Table 2.1.10 contains parameters for the year effect, but as only two years data was obtained, only one very simple function was generated. This function was positively associated with vegetative characters, and negatively associated with most of the flower characters, indicating vigorous vines with less flowers tended to have high year effects.

Table 2.1.10 **Function structure and related statistics for year effects in male population**

	STRUCTURE
FD	-0.14
PL	0.50
TF	0.62
LF	-0.37
PETICO	0.04
DIASP	0.63
TSH	0.07
FSH%	-0.22
BEGDA	-0.04
LWRATI	-0.11
INCRS	0.54
FLPERI	-0.44
EIGENVALUE	0.58
PROPORTION	1.00
CUMULATIVE	1.00

(d) Vine effects

Table 2.1.11 shows a function structure and related statistics for vine effect. Function 1 was mainly associated with DIASP and INCRS, thus was regarded as a vegetative function, and held most of the discriminant power (60%).

Table 2.1.11 **Function structure and related statistics for vine effects in male population**

	STRUCTURE				
	FUN1	**FUN2**	**FUN3**	**FUN4**	**FUN5**
FD	0.28	-0.61	-0.37	0.18	-0.01
PL	0.55	-0.50	-0.48	-0.06	0.49
TF	-0.06	0.37	0.12	-0.21	-0.07
LF	0.18	0.25	0.03	-0.39	0.41
PETICO	0.08	0.01	-0.07	-0.05	-0.42
DIASP	0.99	-0.02	-0.17	-0.11	0.09
TSH	0.21	0.65	-0.24	0.44	0.19
FSH%	0.11	0.34	-0.11	-0.28	0.13
BEGDA	-0.22	-0.16	0.68	0.48	-0.24
LWRATI	-0.13	-0.13	-0.08	0.50	-0.12
INCRS	0.64	0.04	-0.27	-0.06	0.00
FLPERI	0.01	0.37	0.11	-0.05	0.36
EIGENVALUE	39.16	4.76	3.85	3.22	2.89
PROPORTION	0.60	0.07	0.06	0.05	0.04
CUMULATIVE	0.60	0.67	0.73	0.78	0.82

The second function was mainly associated with TSH which is also a growth variable, so function 2 was also considered a vegetative function. This means that the vines can only be grouped vegetatively. The other 10 functions only account for 33% of the discriminant power, and are too weak to determine vine effect.

For comparison, the net scores of the first 2 functions were obtained by using standardised coefficients, and plotted in Figure 2.1.3. The vines with the highest score on both functions have the greatest vigour and branching ability. The scatter graph shows that most vines were of moderate value, and skewed towards low value, with very few vines of high value.

```
 FUN 1
150  +
     |
     |
     |                            *
     |                 *      *                *
100  +               *
     |       *  * 2    *   *****  *  2
     |              42 **3*52* 3 2 ***  2*
     | *      *      *32223424522**2232
     |     *  *  * *** 22*233 *22*2 2222    * *     *
 50  +             **  *2* 2 **23**3*2***2
     |          *  * 4 2*2  2 *  2** 2    *2
     |       *2    * ** 2  22 2
     |  *            ** *2 *    ** **
     |        *     * * *                  *
  0  +          *                                   FUN 2
      +---------+---------+---------+---------+---------+
      0         4         8        12        16        20
```

Figure 2.1.3 Plot of net scores of 214 male vines on discriminant functions 1 and 2.

(b) Female vines

(i) Univariate analysis

As for the male vines, the original data set was analysed with 23 separate anova's to determine the effects of various sources on each of the 23 variables measured. Anova statistics are given in Tables 2.1.12-2.1.14. Like the male vines significant differences ($P<0.05$) were detected among vines for all traits except TSH and FLPERI, and among crosses for most of the traits (Table 2.1.12). No differences were detected for PETICO, HAIR, TSH, FSH%, BRIX, FNF and EFFI, suggesting these characters changed very little between genotypes. Year effects were significant for PL, AC, DIASP, BEGDA, FLPERI, BRIX, LWRATI, INCRS, ELONG, RELCOR, FW and EFFI, demonstrating these characters were easily changeable with growing season. The rest of the characters including a number of economically important ones such as VC, TW, SYMM and FNF, did not change much across years, indicating they were immune to seasonal changes. Cross-year interactions were not significant for all characters except for PETICO, HAIR and TSH.

Table 2.1.12 F test of 23 dependent variables for female vines

VARIABLE	PR > F			
	VINE[1]	CROSS[2]	YEAR[2]	CR*Y[3]
FD	0.01	0.01	0.41	0.88
PL	0.01	0.01	0.01	0.95
TF	0.01	0.01	0.06	0.78
PETICO	0.01	0.23	0.65	0.04
HAIR	0.01	0.66	0.08	0.01
VC	0.01	0.01	0.16	0.93
TW	0.01	0.01	0.41	0.66
AC	0.01	0.01	0.02	0.50
DIASP	0.01	0.01	0.01	0.96
TSH	0.14	0.77	0.28	0.01
FSH%	0.03	0.06	0.23	0.10
BEGDA	0.01	0.01	0.02	0.98
FLPERI	0.11	0.02	0.01	0.79
BRIX	0.01	0.26	0.02	0.42
LOCN	0.01	0.03	0.71	0.32
LWRATI	0.01	0.01	0.01	0.42
INCRS	0.01	0.01	0.01	0.73
ELOGN	0.01	0.01	0.01	0.77
SYMM	0.01	0.01	0.79	0.77
RELCOR	0.01	0.01	0.01	0.62
FNF	0.05	0.09	0.96	0.09
FW	0.01	0.01	0.01	0.99
EFFI	0.01	0.07	0.01	0.30

[1] Vine, tests of hypotheses using the type III MS for residual variance as an error term.
[2] CR & Y, tests of hypotheses using the type III MS for CR*Y as an error term.
[3] CR*Y, tests of hypotheses using the type III MS for vine as an error term.

Table 2.1.13(1) Cross means by year in female population

CR	Y	N	FD	PL	TF	PETICO	HAIR	VC	TW	AC	DIASP	TSH	FSH%	BEGDA
1	1	25	6.56	3.96	3.01	3.28	1.84	105.84	2.13	1.32	1.84	40.36	35.68	8.56
1	2	25	6.62	4.29	2.58	3.52	2.64	108.60	2.10	1.27	2.40	43.24	27.56	9.28
2	1	30	6.90	4.91	3.22	3.60	2.06	126.03	2.67	1.56	2.02	45.46	54.73	7.33
2	2	30	6.81	5.12	2.82	3.10	2.60	115.60	2.40	1.39	2.60	40.70	39.23	8.56
3	1	3	6.86	4.46	2.33	2.33	2.00	103.33	1.85	1.37	1.77	41.66	21.66	8.00
3	2	3	7.00	5.33	1.93	2.66	2.33	96.66	1.80	1.22	2.29	36.00	19.33	7.00
4	1	55	7.82	5.66	2.57	2.98	1.80	76.30	2.27	1.40	2.13	44.56	39.09	6.50
4	2	55	7.56	6.09	2.42	3.03	2.41	74.25	2.19	1.16	2.84	33.20	35.69	7.54
5	1	11	6.81	4.74	2.28	3.18	2.00	85.72	1.93	1.12	1.92	41.90	27.63	7.36
5	2	11	6.76	5.12	2.07	3.54	2.63	79.90	1.83	1.02	2.52	43.09	21.90	9.00
6	1	63	7.25	5.60	2.59	3.19	2.00	91.04	2.35	1.33	2.18	43.90	40.06	6.95
6	2	63	6.99	5.80	2.55	3.20	2.01	85.12	2.43	1.18	2.85	33.12	41.15	7.90

Table 2.1.13(2) Cross means by year in female population

CR	Y		FLPERI	BRIX	LOCN	LWRAT	INCR	ELOG	SYMM	RELCO	FNF	FW	EFFI
1	1	25	14.28	7.87	32.98	1.08	1.11	1.42	1.07	0.24	2.55	53.28	0.59
1	2	25	9.68	8.95	31.72	1.07	2.29	1.70	1.06	0.26	3.01	64.75	0.32
2	1	30	14.60	7.59	36.10	1.03	1.56	1.54	1.11	0.25	2.96	64.66	0.61
2	2	30	10.60	7.94	33.50	0.96	2.55	1.79	1.10	0.29	2.45	81.61	0.31
3	1	3	9.33	8.23	38.86	1.20	0.93	1.51	1.09	0.27	1.91	78.06	0.54
3	2	3	8.00	9.26	39.00	1.14	2.13	1.71	1.12	0.31	2.47	96.96	0.28
4	1	55	13.34	7.65	36.01	1.07	1.89	1.48	1.12	0.28	2.18	82.95	0.43
4	2	55	10.09	9.21	37.67	1.02	2.86	1.68	1.12	0.31	2.00	99.38	0.26
5	1	11	9.90	7.34	38.25	1.13	1.49	1.20	1.11	0.27	1.93	77.58	0.44
5	2	11	8.00	7.98	38.54	1.00	2.51	1.35	1.13	0.32	1.53	93.24	0.25
6	1	63	12.66	7.68	37.68	0.99	1.82	1.25	1.14	0.28	2.34	84.78	0.45
6	2	63	9.03	8.85	36.85	0.92	3.22	1.41	1.12	0.30	2.49	99.83	0.27

Table 2.1.14(1) Cross means in female population

CR	N	FD	PL	TF	PETICO	HAIR	VC	TW	AC	DIASP	TSH	FSH%	BEGDA
1	50	6.59	4.13	2.79	3.40	2.24	107.22	2.11	1.30	2.12	41.80	31.62	8.92
2	60	6.85	5.01	3.02	3.35	2.33	120.81	2.54	1.48	2.31	43.08	46.98	7.95
3	6	6.93	4.90	2.13	2.50	2.16	100.00	1.82	1.29	2.03	38.83	20.50	7.50
4	110	7.69	5.88	2.49	3.00	2.10	75.28	2.23	1.28	2.49	38.88	37.39	7.02
5	22	6.79	4.93	2.17	3.36	2.31	82.81	1.88	1.07	2.22	42.50	24.77	8.18
6	126	7.12	5.70	2.57	3.19	2.00	88.08	2.39	1.25	2.51	38.51	40.61	7.42
GM		7.15	5.37	2.62	3.19	2.14	92.01	2.29	1.29	2.39	40.03	38.22	7.93
SE		0.40	0.62	0.73	0.44	0.51	9.91	0.49	0.10	0.14	10.96	15.67	2.27

Table 2.1.14(2) Cross means in female population

CR	Y	FLPERI	BRIX	LOCN	LWRAT	INCR	ELOG	SYMM	RELCO	FNF	FW	EFFI
1	50	11.98	8.41	32.35	1.07	1.70	1.56	1.07	0.25	2.78	59.02	0.45
2	60	12.60	7.76	34.80	1.00	2.05	1.66	1.10	0.27	2.70	73.14	0.46
3	6	8.66	8.75	38.93	1.17	1.53	1.61	1.10	0.29	2.19	87.51	0.41
4	110	11.71	8.43	36.84	1.05	2.38	1.58	1.12	0.30	2.09	91.17	0.35
5	22	8.95	7.66	38.40	1.06	2.00	1.27	1.12	0.30	1.73	85.41	0.34
6	126	10.84	8.26	37.27	0.95	2.52	1.33	1.13	0.29	2.42	92.30	0.36
GM		11.39	8.22	36.18	1.01	2.25	1.49	1.11	0.28	2.37	83.96	0.38
SE		3.34	0.99	3.74	0.08	0.70	0.07	0.04	0.02	0.87	12.30	0.10

(ii) Multivariate analysis

Based on the anova results, the manova result was obtained and listed in Table 2.1.15. Wilks' Lambda and Hotelling - Lawley Trace were transformed as an F approximation, and strong significant differences were detected among all effects - CR*Y, CR, Y, and vine. Such an analysis shows whether differences exist between the vines and crosses, but in no way indicates how each effect compared with respect to the combined variables. Discriminant functions were therefore calculated as they permit one to order the vines and crosses according to their net scores, and based on the scores the effects are grouped and sorted.

Table 2.1.15 **Manova test criteria for the hypothesis of no overall effect for female vines**

```
------------------------------------------------------------
CR*Y
WILKS' CRITERION  L = DET(E)/DET(H+E) =    0.40602506
                  F(115,785.11) = 1.38  PROB > F = 0.0085
CR
WILKS' CRITERION  L = DET(E)/DET(H+E) =    0.05686273
                  F(115,785.11) = 5.42  PROB > F = 0.0001
Y
WILKS' CRITERION  L = DET(E)/DET(H+E) =    0.41831882
                  F(23,159) = 9.61      PROB > F = 0.0001
VINE
WILKS' CRITERION  L = DET(E)/DET(H+E) =    0.00000000
                  F(4163,3840.83) =      . PROB > F =   .

HOTELLING-LAWLEY TRACE=TR(E**-1*H)=138.25864297
                  F(4163,3613) = 5.22   PROB > F = 0.0001
 -----------------------------------------------------------
```

(a) Cross year interaction:

Table 2.1.16 shows a function structure and related statistics. Because the first three functions account for 89% of total discriminant power, they were retained for further analysis, while the other 3 were dropped from the test. Function 1 was mainly positively associated with DIASP, and negatively with TSH, and LWRATI and so may be regarded as a function for vigorous vines with broad leaves and less shoots. The second function was made up mainly of FSH%, TW, FNF and so was called a productivity function. The second function seems to imply that the prolific crosses have a high year effect possibly reflecting biennial bearing behaviour.

The third function may be described as a hairiness function since it mainly associated with PETICO and HAIR.

Table 2.1.16 **Function structure and related statistics for CR*Y effects in female population**

	FUN1	FUN2	FUN3	FUN4	FUN5
LWRATI	-0.35	0.22	0.18	0.17	0.12
PETICO	0.06	0.27	0.44	-0.31	0.14
FD	-0.13	0.05	-0.08	-0.10	-0.19
PL	0.15	-0.02	-0.05	0.07	-0.32
TSH	-0.58	0.20	0.17	-0.46	0.21
FSH%	0.18	0.35	-0.02	-0.28	-0.02
TF	0.08	0.10	-0.00	-0.17	0.11
DIASP	0.37	-0.09	-0.03	0.33	0.00
INCRS	0.23	0.10	-0.25	0.12	0.02
BEGDA	0.04	-0.11	0.02	0.06	0.32
FLPERI	-0.10	0.03	0.11	-0.44	-0.26
TW	0.04	0.33	-0.11	-0.18	0.06
FW	0.24	-0.19	-0.13	0.20	-0.22
FNF	-0.08	0.58	0.00	-0.02	-0.04
LOCN	0.18	-0.05	0.21	0.06	-0.11
HAIR	-0.15	-0.23	0.50	0.29	0.22
ELOGN	-0.07	0.02	0.12	0.34	0.12
RELCOR	0.12	-0.19	0.09	0.12	-0.21
SYMM	0.06	-0.11	0.07	-0.14	-0.28
BRIX	0.23	0.01	0.15	0.33	0.05
AC	-0.24	0.17	0.01	-0.32	0.17
VC	-0.09	0.13	0.20	-0.02	0.26
EFFI	0.00	0.13	0.18	-0.42	0.16
EIGENVALUE	0.53	0.23	0.16	0.08	0.02
PROPORTION	0.50	0.23	0.16	0.08	0.03
CUMULATIVE	0.50	0.73	0.89	0.97	1.00

Thus, twenty three original variables were reduced to 3 functions to sort CR*Y effects, and therefore the analysis of CR*Y effects was simplified. Moreover, from the function structure the relative contribution of the characters to CR*Y effects and their relationship can be easily examined. The first function is a vigour function and accounted for 50% of total discriminant power, therefore it was the most important function contributing to CR*Y effects. In this function TSH was the most important character. The second function which accounted for 23% of total discriminant power was the second important function, and FNF was the most important character for this function. The third function was made up mainly of HAIR and PETICO, and accounted for 16% of total discriminant power. To sum up, in the kiwifruit populations investigated, TSH, FNF, HAIR and PETICO were the most important characters contributing to CR*Y effects, and the finding agrees with the results from Anova analysis (see Table 2.1.12). However Anova only gives separated analysis of variables, it does not examine the relationship between variables. Table 2.1.17 displays the function scores of 6 crosses between 2 years. Based on these function scores the CR*Y effects can be easily detected for each cross.

Table 2.1.17 **Net scores for CR*Y effects in female population**

			FUNCTION		
	CR	**Y**	**1**	**2**	**3**
1	1	1	2.26	7.80	7.13
2	1	2	3.22	8.16	8.96
3	2	1	3.31	8.64	8.83
4	2	2	5.12	6.46	9.11
5	3	1	0.27	8.16	7.38
6	3	2	2.50	8.58	9.05
7	4	1	2.26	7.75	6.93
8	4	2	8.13	6.05	9.35
9	5	1	1.60	7.70	6.38
10	5	2	5.15	5.46	8.62
11	6	1	3.88	6.83	7.13
12	6	2	9.25	6.11	7.80

Since the second function represents the yield characters, the CR*Y effects on this function is of great interest for kiwifruit selection. The score deviation between 2 years for each cross on this function was calculated, and the results are:

Cross 1	Bruno x D1-6	(1-2)	0.36
Cross 2	Bruno x D1-20	(3-4)	-2.18
Cross 3	Gracie x D1-6	(5-6)	0.42
Cross 4	Gracie x D1-20	(7-8)	-1.70
Cross 5	Hayward x D1-6	(9-10)	-2.24
Cross 6	Hayward x D1-20	(11-12)	-0.72

In kiwifruit cultivation, one always hopes the yield does not vary very much with the year, so crosses 1, 3 and 6 should be better selections.

The CR*Y effects can be also examined on both functions 1 and 2. Figure 2.1.4 shows the cross distance between years based on these two functions, for which the score distance of each cross between 2 years was as follows:

Cross 1	Bruno x D1-6	(1-2)	1.4
Cross 2	Bruno x D1-20	(3-4)	6.3
Cross 3	Gracie x D1-6	(5-6)	2.9
Cross 4	Gracie x D1-20	(7-8)	8.0
Cross 5	Hayward x D1-6	(9-10)	7.9
Cross 6	Hayward x D1-20	(11-12)	6.3

Once again the crosses 1 and 3 have less CR*Y effects on these two functions, indicting that the female progeny produced from the two crosses tend to vary very little across years for both vine vigour and vine cropping. Therefore they are better cross combinations in kiwifruit breeding.

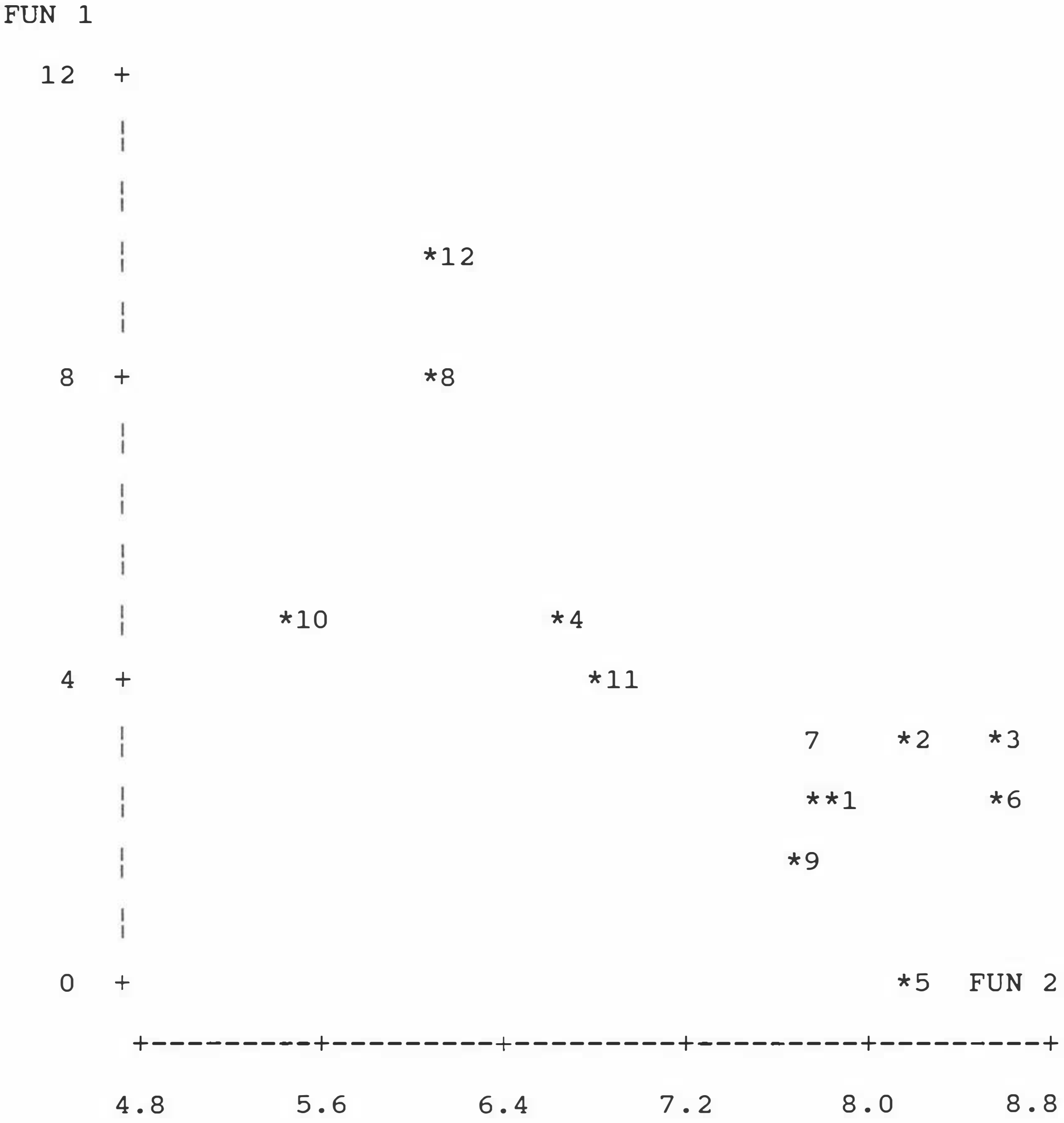


Figure 2.1.4 **Plot of net scores of discriminant function 1 and 2 in female population (6 crosses, 2 years)**

(b) <u>The effect of cross:</u>

Table 2.1.18 shows a function structure and related statistics for cross effect. The first three functions account for 90% of the total discriminant power, and were therefore retained for analysis. Function 1 was mainly associated with LWRATI, VC and EFFI, and negatively with PL, DIASP, INCRS, FW, RELCOR and SYMM, - it was therefore regarded as a function for weak vines with longer leaves and small fruit with high Vitamin C content. With regards to EFFI, caution should be taken interpreting the function. EFFI was the ratio of TW to DIASP and the relatively high loading of EFFI in this function resulted from high negative loading of DIASP rather than from loading of TW. Therefore function 1 actually was a less productive function rather than an efficient one.

As the second function was mainly associated with FD, PL, LWRATI and ELOGN, it may be called a big flower and longer fruit function. The third function was made up mainly of FSH% TF, TW, VC and AC, and so may be viewed as a productivity function with high fruit acidity.

Table 2.1.18 **Function structure and related statistics for cross effects in female population**

	FUN1	FUN2	FUN3	FUN4	FUN5
LWRATI	0.47	0.36	-0.53	0.13	0.04
PETICO	0.16	-0.31	0.04	0.02	-0.39
FD	-0.21	0.57	-0.06	-0.11	-0.22
PL	-0.56	0.33	0.20	0.04	-0.01
TSH	0.34	0.05	-0.11	0.27	-0.23
FSH%	-0.02	0.06	0.58	-0.02	-0.21
TF	0.21	-0.06	0.33	-0.07	-0.15
DIASP	-0.43	0.00	0.28	-0.11	0.13
INCRS	-0.44	-0.02	0.25	-0.09	0.02
BEGDA	0.09	-0.24	-0.02	-0.02	0.00
FLPERI	0.27	0.21	0.15	-0.19	-0.38
TW	-0.02	0.01	0.51	-0.06	-0.08
FW	-0.63	0.12	0.03	0.13	0.23
FNF	0.21	-0.16	0.29	-0.32	0.20
LOCN	-0.31	0.09	-0.03	0.36	0.19
HAIR	0.13	-0.07	0.07	0.21	-0.01
ELOGN	0.33	0.36	0.31	-0.03	0.13
RELCOR	-0.39	0.18	0.05	0.22	0.09
SYMM	-0.37	0.00	0.11	0.17	0.01
BRIX	-0.15	0.02	-0.03	-0.31	0.26
AC	0.34	0.14	0.35	0.03	-0.05
VC	0.45	-0.31	0.37	0.09	0.13
EFFI	0.40	0.01	0.10	-0.04	-0.20
EIGENVALUE	2.11	1.22	0.70	0.31	0.13
PROPORTION	0.47	0.27	0.15	0.06	0.02
CUMULATIVE	0.47	0.74	0.90	0.97	1.00

Thus, twenty-three variables were reduced to 3 important functions to classify the crosses. Table 2.1.19 displays the scores of 6 crosses on 3 functions.

Table 2.1.19 Net scores for crosses in female population

CROSS	FUNCTION		
	1	2	3
1	8.98	6.91	8.49
2	8.48	8.41	11.51
3	10.72	7.57	6.93
4	5.34	10.24	8.85
5	4.27	5.17	5.80
6	2.84	6.13	8.71

Since a grower and breeder is always seeking a vine that is high yielding, function 3 is important in kiwifruit selection. Bruno x D1-20 gave the highest score on this function, suggesting it was the best cross combination for improving cropping. In contrast Hayward x D1-6 gave the lowest score, indicating it was not an appropriate combination for this purpose. Just like in the male vine population where D1-20 was the better male parent to produce floriferous progeny, D1-20 proved the better parent to produce female vines with high early yields, elongate fruit and large flowers, since D1-20 crosses were always better than D1-6 crosses on the functions 2 and 3.

Although function 1 was a weak vine function, it was reasonably associated with VC. Therefore functions 1 and 3 may be used together to indicate productive crosses with high fruit vitamin C content. Figure 2.1.5 shows the mean scores on the first and third functions. Bruno crosses gave relatively high scores on both functions, which means the progeny in these crosses tended to be weak and productive, and to produce longer fruit with high Vitamin C content.

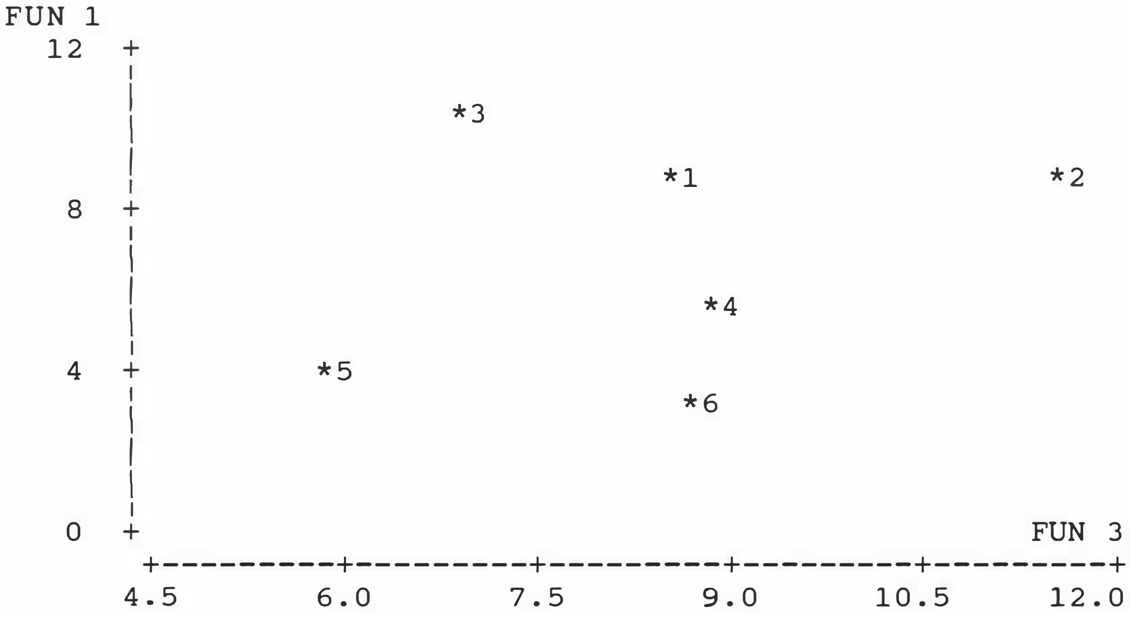


Figure 2.1.5 **Plot of net scores of functions 1 and 3 for each cross in female population**

Cross 1 Bruno x D1-6
Cross 2 Bruno x D1-20
Cross 3 Gracie x D1-6
Cross 4 Gracie x D1-20
Cross 5 Hayward x D1-6
Cross 6 Hayward x D1-20

(c) The year effect:

Table 2.1.20 shows that only one function was obtained for year effects. This function was positively associated with vine vigour, and negatively associated with total shoots, floriferousness and yield, indicating vigorous vines with less flowers and less fruits tended to have high year effects. The results demonstrate that the female seedlings varied in their phenotypic characteristics from one year to the next, and that they varied primarily with respect to their degree of vegetative growth and flowering level. The vine is then inherently inclined to biennial cropping when conditions allow low cropping and high vigour in any year.

Table 2.1.20 **Function structure and related statistics for year effects in female population**

	STRUCTURE
LWRATI	-0.38
PETICO	-0.01
FD	-0.14
PL	0.19
TSH	-0.46
FSH%	-0.18
TF	-0.18
DIASP	0.64
INCRS	0.56
BEGDA	0.15
FLPERI	-0.51
TW	-0.11
FW	0.44
FNF	-0.05
LOCN	0.01
HAIR	0.31
ELOGN	0.33
RELCOR	0.42
SYMM	0.00
BRIX	0.40
AC	-0.37
VC	-0.10
EFFI	-0.63
EIGENVALUE	1.39
PROPORTION	1.00
CUMULATIVE	1.00

(d) The vine effect:

Table 2.1.21 shows the function structure and related statistics for vine effects. The first three functions accounted for 62% of the total discriminant power, and the other 20 only 38%. As in the male vine, function 1 was strongly associated with DIASP and INCRS, i.e. it is a vegetative growth function. Because of this strong growth, the fruiting efficiency was very poor, and the function therefore was strongly negatively associated with EFFI. Since function 1 represented 37% of the discriminant power, it was the most important one, this means that as in male vines, the female vines could most effectively be grouped by vegetative characters. Plants with a high score on this function tend to have relatively low fruit yield in relation to trunk size, i.e., they are not very efficient canopies with respect to fruiting in relation to vine growth. Therefore, from a plant breeding viewpoint, this function could be used to select out inferior vines.

Function 2 was a long fruit function since it strongly associated with ELOGN. Function 3 was a Vitamin C function, and function 4 was for fruit acidity. Function 5 was of horticultural value because it was associated with flower size and average fruit weight, but unfortunately it represented only 5% of the discriminant power.

Table 2.1.21 Function structure and related statistics for vine effects in female population

	FUN1	FUN2	FUN3	FUN4	FUN5
LWRATI	-0.27	0.04	-0.02	0.01	-0.12
PETICO	0.12	-0.01	-0.00	0.21	-0.16
FD	0.29	0.00	-0.28	-0.00	0.69
PL	0.49	-0.05	-0.17	-0.03	0.57
TSH	0.00	-0.05	0.15	0.06	-0.04
FSH%	0.04	-0.03	0.01	0.09	0.03
TF	0.02	0.06	0.11	0.16	-0.06
DIASP	0.97	0.13	0.04	-0.15	0.00
INCRS	0.74	0.06	0.01	-0.02	-0.05
BEGDA	0.00	0.09	0.29	-0.25	0.10
TW	0.17	-0.01	0.04	0.09	0.07
FW	0.43	-0.03	-0.28	-0.11	0.62
FNF	0.03	-0.00	0.17	0.12	-0.09
LOCN	0.09	-0.39	-0.12	-0.17	0.36
HAIR	0.25	0.21	0.15	-0.10	-0.10
ELOGN	0.29	0.93	0.30	0.16	0.07
RELCOR	0.24	-0.11	-0.03	-0.25	0.29
SYMM	0.08	-0.17	-0.00	-0.04	0.31
BRIX	0.21	0.17	-0.00	-0.04	-0.29
AC	-0.25	-0.10	-0.08	0.89	-0.00
VC	-0.29	-0.00	0.84	0.48	-0.20
EFFI	-0.80	-0.13	0.02	0.16	0.01
EIGENVALUE	50.84	20.06	14.06	9.15	7.26
PROPORTION	0.37	0.15	0.10	0.07	0.05
CUMULATIVE	0.37	0.52	0.62	0.69	0.74

For comparison, the net scores of the first 3 functions were obtained by using standard coefficients, and plotted in Figures 2.1.6 and 2.1.7. The vines with the high scores in Figures 2.1.6 tend to be vigorous and to bear longer fruit, and the vines with the high scores in Figures 2.1.7 tend to be vigorous and to bear fruit with high vitamin C content.

```
FUN 1
120  +
     |
     |
     |
     |        *        ***
 80  +        *     * 22*     *   **
     |   *  2    23*  ***  **   2** **    *        *
     |      *2**  332  233 *  2 * *** *   ** *
     | *  *       *3* *  *   * 2** 2  *  2  *
     |     2 3  * 2*  2*2  4  * ***  *3 **2 22
 40  +    *   *   **32**3  2* 2  **  * *
     |    **   * 2 *2**   222* 2     2*   *
     |       *   *  2  *******  2          *
     |                             *
     |           *          *    *        *
  0  +                                               FUN2
      +---------+---------+---------+---------+---------+
      0        15        30        45        60        75
```

Figure 2.1.6 Plot of net scores of 187 female vines on discriminant functions 1 and 2

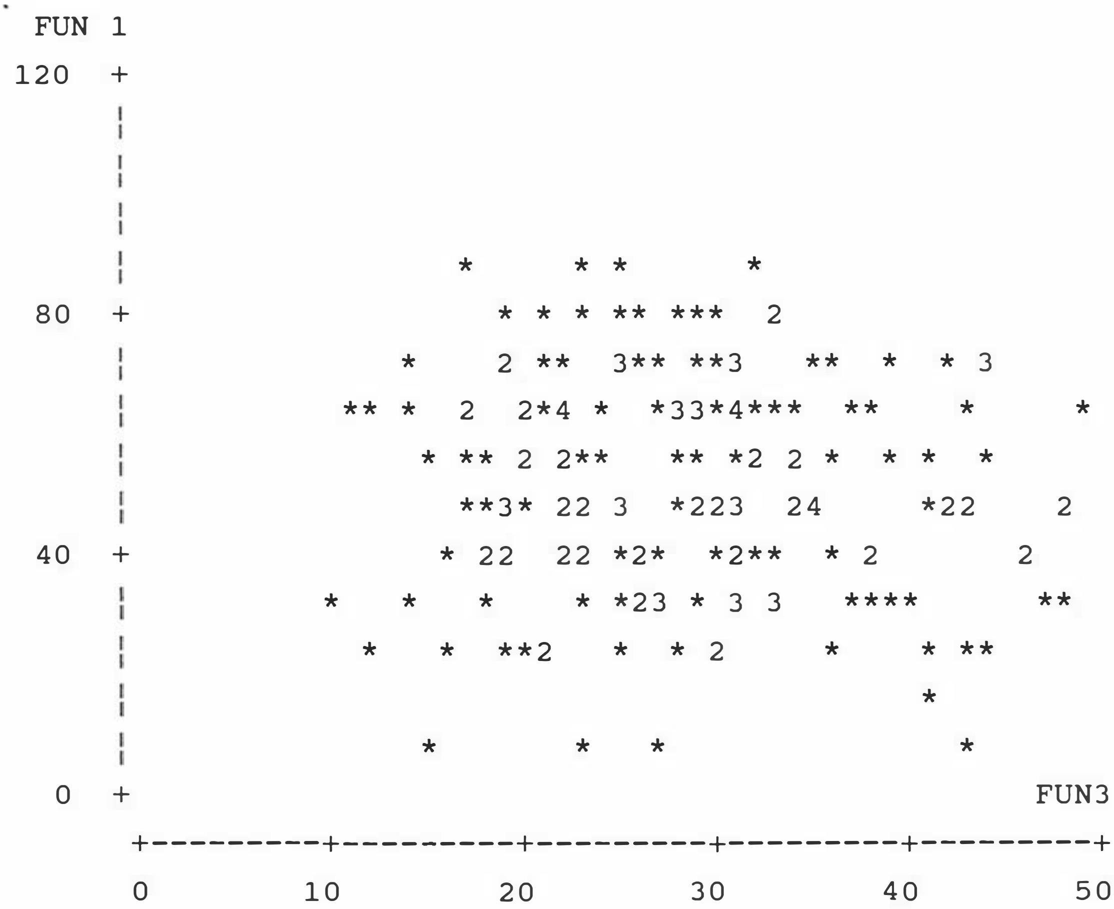


Figure 2.1.7 **Plot of net scores of 187 female vines on discriminant functions 1 and 3**

To sum up, the results based on Anova and Manova indicated that Manova was indeed a useful analytical tool with all of the power and scope of Anova. Moreover, as it considers dependent variables (DVs) in combination, sometimes it was more powerful and simplified than separate Anova. With the use of discriminant analysis following Manova, the complex interrelationship among DVs can not only be revealed, but also can be taken into account in statistical inference, while Anova ignores and fails to exploit the interrelationships. The main purpose of discriminant analysis is to find a linear combination of the variables that maximises differences among the pre-existing populations, and to sort the objects into their appropriate group with minimal error (see Bryant & Atchley 1971, Introduction). Plant taxonomy is concerned with the classification of plant material based on relationships inferred from the characters of whole plants. This requires that the statistical technique used takes into consideration simultaneously the covariance of as many characters of the plant as possible. Thus this technique has been used in plant taxonomy research (Rohlf 1971).

In plant breeding, the exploitation and utilisation of germplasm of plant material is of great importance, and it is necessary to characterise germplasm before using it. This is actually a taxonomic problem, hence manova and discriminant analysis should be suitable for dealing with this sort of problem.

The results obtained in this study with both male and female plants demonstrated the technique of discriminant analysis was useful for grouping and sorting plant material.

In male vines, the DVs flower diameter, pedicel length, increment of stem, petiole colour and flower period were detected as the most important DVs for distinguishing between interaction effects of cross and year, because these DVs were strongly associated with the first two functions which accounted for 81% of the discriminant power. Based on these two functions, the deviation between 2 years' mean scores for each cross was obtained. Hayward crosses gave the highest value of deviation, indicating Hayward progeny tended to have a high cross and year interaction, while the crosses of Gracie were relatively weaker in this effect. Since most of the DVs mentioned were of no breeding value, the functions based on them did not make much sense in a selection programme. Thus, they could be good discriminant functions for grouping crosses on CR*Y effects, but of limited value for kiwifruit selection.

However for cross effect, it was a quite different story. The first two functions which accounted for 85% of the discriminant power were mainly associated with the characters vigour and floriferousness, and may therefore be useful as selection criteria. The mean scores based on these two functions indicated that Hayward x D1-20 was the best cross because it produced vigorous and floriferous vines; while Gracie x D1-6 was the worst cross because it gave weak vines sparse in flowering.

In female seedling populations, two functions for cross and year interaction, which accounted for 73% discriminant power, were mainly concerned with vine vigour and productivity, so they may be used as selection criteria. But these functions were used for grouping the interaction effect of cross and year, where what was wanted was the deviation between mean scores in different years in each cross, not the scores themselves. For example, Bruno x D1-6 gave the lowest value of the deviation between two years' scores based on the first two functions, indicating there was not much variation in vine vigour and productivity between years in this cross. Therefore it was shown to be a particularly good cross for producing vines with consistency of vigour and yield.

For cross effects in female vine, function 3 was of horticultural interest since it was related to the productivity of kiwifruit. Bruno x D1-20 gave the highest score on this function, suggesting it was the best cross combination for improving vine productivity. However, compared with the first two functions, function 3 accounted for only 15% of the total discriminant power, it was weaker than functions 1 (47% power) and 2 (27% power) for classifying crosses. Although the first two functions were more powerful than function 3, they only classified crosses in terms of characters of less importance. Once again, discriminant analysis proved a useful technique for taxonomic grouping, but it was found to have limited value in plant selection. The reason is that the objectives of taxonomy differ in an important way from those of plant selection. In plant selection one is usually interested in a number of particular variables, whereas in taxonomy often there is no special interest in the particular character used. In discriminant analysis, characters are weighted according to how well they discriminate between groups. This may or may not be related to the relative importance of characters as perceived by the plant breeder. Therefore this analytical technique has limited uses for selecting the best plants unless the most important functions happen to be very good horticultural criteria for choosing vines and crosses.

SECTION 2 FACTOR ANALYSIS

1. Introduction

Factor analysis has become the generic term for principal component analysis and common factor analysis. The two procedures have a similar aim, the difference between the two is that the former is not based on any particular statistical model, while the latter is based on a rather special model (Manly, 1986). That is, in the former analysis data are fitted to a model, whereas in factor analysis a model is fitted to the data (Timm, 1975). The factor sometimes refers to a hypothetical, unobservable variable and this analysis must be distinguished from component analysis since a component is an observable linear combination of variables (SAS Institute Inc. 1985).

Principal component analysis was first described by Karl Pearson (1901) and subsequently developed by Hotelling (1933, 1936). The early development of factor analysis was due to Charles Spearman (1904). Later modification gave the general factor analysis model which consists of several unobserved factors plus a random error vector (Timm, 1975).

In contrast to multivariate analysis of variance, factor analysis is essentially a screening technique rather than a technique of analysing variance. It is used to group individuals within one population, while discriminant analysis is used to discriminate between two or more populations. Factor analysis does not require an experimental design, hence is useful in surveys. Since it is a technique for grouping, synthesis and reduction of information, it has a number of potentially useful applications in fruit tree science. It can be used to order multivariate data in reduced orthogonal dimensions called factors, which express most of the variance of the original data. Scores on these factors can be used as criteria for grouping plants within the germplasm collection, and as a non-measured variable to replace subjective visual attributes in conventional statistical analyses (Broschat, 1979). Plotting of multivariate data in 2 or 3 dimensional factor space can be useful for indicating the morphological variants within a selection programme. In this study factor analysis was performed with phenotype data as previously described (Section 1), to evaluate its suitability for identifying distinctive and superior kiwifruit vines in a breeding programme.

2. Methods

The factor analysis was executed by using the FACTOR procedure of the SAS statistical package (SAS Institute Inc. 1985).

Suppose there are p correlated x variates which are so standardised to z variates as to have zero means and unit standard deviations, then a linear function

$$y = V'z$$

will be found to be independently distributed with unit variance and such that **y** is the vector of components whose contribution to the variance of **z** is as great as possible. V is a p x n coefficients matrix which carries the p-element vector variable z into the derived n-element vector variable **y**. Thus the elements of **y** will be principal components. It can be used for data reduction to describe a set of p variables in terms of a smaller number of indices or components.

For the best separation of individuals in a population, V is sought so as to make **y** have the above properties. To obtain the V matrix the eigenstructure employed was:

$$RV = VL$$

where R is the matrix of correlation between the original variables, V is a coefficients matrix, and L is a diagonal matrix containing the successive eigenvalues (Cooley and Lohnes, 1971).

Since factors are standardised principal components, they may be obtained as

$$f = L^{-(1/2)}y$$

where f is a vector variable of a factor, and L and **y** are as previously defined (Cooley and Lohnes, 1971).

The possible rank n of the factor structure matrix depends on the number of individuals (i) and the number of original variables (p). If the quantity i-1 is less than p, then i-1 is the maximum possible rank, if i-1 is equal or greater than p, then p is the maximum possible rank for the matrix.

The first factor is generated such that it accounts for a large portion of the total variance of the data. The second factor is extracted such that it is completely uncorrelated with the first factor, and accounts for the maximum possible remaining variance of the set of original variables. This procedure is repeated until all of the variance of the data is accounted for by p factors. Usually only factors having eigenvalues greater than one are retained for further consideration, although the relative value of the variance explained by the factor and the meaningfulness of the component should be considered as well.

The matrix of regression coefficients for the multiple regression of each element of the observation vector variable on each factor is the factor pattern. It is the primary interpretative device in factor analysis, as it shows the relative contribution of the individual variables to each factor, and is used to name and interpret the factors. The factor score for each plant was obtained by multiplying the original data set by the factor score coefficients.

A critical value of 0.5 and above for the MSA (a measure of sampling adequacy) was used to determine whether the matrix was suitable for factor analysis (Kaiser & Rice, 1974).

3. RESULTS AND DISCUSSION

(1) *Factor patterns and factor comparison*

Adequate samples is a pre-requisite for factor analysis, and as only crosses 2, 4 and 6 reached an appropriate level of the measure of sampling adequacy (MSA > 0.5), the analysis for only these crosses is reported. Factors were calculated for each cross separately, and the manner in which the factors differed was identified. Factors having eigenvalues greater than one were retained for full analysis.

A. Male vine populations

Tables 2.2.1-2.2.3 display the factor patterns, the eigenvalue and the proportion of total variance explained by each factor in male seedlings for each of two seasons. The first factor always has the largest eigenvalue and explains the largest proportion of total variance. Maximum variance does not necessarily mean maximum information, but in the absence of contrary evidence they are regarded as equivalent (Moore, 1975). Therefore factor 1 is the most important factor.

Table 2.2.1 Factor pattern of first 4 PCs for male seedlings in cross 2 (Bruno x D120)

	1987				1988			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
FD	0.57	0.63	0.15	-0.04	-0.14	0.58	-0.52	0.24
PL	0.46	0.56	0.21	-0.42	-0.37	0.65	-0.17	-0.36
TF	0.66	-0.29	-0.46	-0.02	0.73	0.01	0.34	0.02
LF	0.77	-0.24	-0.37	-0.23	0.64	0.36	0.35	-0.22
PETICO	0.38	0.33	0.52	0.02	-0.37	0.12	0.30	0.70
DIASP	0.83	0.26	-0.13	-0.06	-0.07	0.70	0.53	0.11
TSH	0.27	-0.01	0.14	0.80	0.74	0.05	-0.22	0.08
FSH%	0.77	-0.16	-0.19	0.19	0.89	0.07	0.05	0.01
BEGDA	-0.32	0.58	-0.52	0.29	-0.52	0.03	0.60	-0.25
LWRATI	-0.09	-0.14	0.48	0.09	-0.20	0.39	-0.15	-0.49
INCRS	0.62	0.10	0.12	0.40	0.20	0.66	-0.12	0.32
FLPERI	0.55	-0.61	0.40	-0.09	0.83	0.04	-0.03	-0.09
EIGENVALUE	3.92	1.83	1.47	1.21	3.66	2.04	1.38	1.19
PROPORTION	0.32	0.15	0.12	0.10	0.30	0.17	0.11	0.09
CUMULATIVE	0.32	0.47	0.60	0.70	0.30	0.47	0.59	0.69

Table 2.2.2 Factor pattern of first 4 PCs for male seedlings in cross 4 (Gracie x D120)

	1987				1988			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
FD	0.61	0.42	-0.48	-0.14	0.43	0.62	-0.37	-0.06
PL	0.65	0.49	-0.33	-0.13	0.43	0.59	-0.16	-0.29
TF	0.77	-0.35	0.15	0.21	0.78	-0.22	0.09	-0.08
LF	0.82	-0.27	0.13	0.09	0.88	-0.16	0.19	-0.13
PETICO	-0.00	0.09	0.29	-0.79	-0.20	0.47	0.75	-0.17
DIASP	0.79	0.38	0.10	0.10	0.10	0.75	0.20	-0.00
TSH	0.51	0.17	0.61	0.25	0.69	-0.08	0.22	0.27
FSH%	0.60	-0.40	-0.36	-0.05	0.87	-0.25	-0.12	0.07
BEGDA	-0.69	0.32	0.10	0.21	-0.64	-0.08	0.20	0.35
LWRATI	-0.16	0.51	-0.17	0.41	-0.58	0.09	-0.41	-0.21
INCRS	0.49	0.47	0.38	-0.16	0.22	0.46	-0.16	0.75
FLPERI	0.78	-0.20	-0.05	0.06	0.78	-0.00	-0.00	-0.01
EIGENVALUE	4.71	1.61	1.19	1.05	4.50	1.91	1.13	0.96
PROPORTION	0.39	0.13	0.09	0.08	0.37	0.15	0.09	0.08
CUMULATIVE	0.39	0.52	0.62	0.71	0.37	0.53	0.62	0.70

Table 2.2.3 Factor pattern of first 4 PCs for male seedlings in cross 6 (Hayward x D120)

	1987				1988			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
FD	0.45	0.52	-0.49	-0.06	0.23	0.56	-0.54	-0.12
PL	0.58	0.43	-0.24	-0.27	0.27	0.49	-0.52	-0.17
TF	0.70	0.15	0.27	-0.18	0.80	-0.09	-0.16	0.02
LF	0.80	-0.12	0.24	-0.05	0.89	-0.05	-0.14	-0.13
PETICO	0.07	0.53	-0.15	0.09	-0.13	0.62	0.04	0.35
DIASP	0.61	0.28	0.04	0.18	0.24	0.68	0.32	0.00
TSH	0.31	0.12	0.75	-0.02	0.65	-0.13	0.30	-0.05
FSH%	0.73	-0.40	0.10	-0.02	0.89	-0.22	0.05	0.02
BEGDA	-0.47	0.38	0.45	-0.24	-0.44	-0.08	-0.28	0.65
LWRATI	-0.17	0.51	0.30	0.35	-0.39	0.25	0.39	-0.41
INCRS	0.23	-0.02	0.00	0.86	0.31	0.54	0.44	0.22
FLPERI	0.64	-0.29	-0.13	0.00	0.67	-0.08	0.16	0.43
EIGENVALUE	3.43	1.55	1.37	1.09	3.80	1.87	1.26	1.04
PROPORTION	0.28	0.12	0.11	0.09	0.31	0.15	0.10	0.08
CUMULATIVE	0.28	0.41	0.52	0.62	0.31	0.47	0.57	0.66

One of the major aspects of factor analysis is the biological interpretation of the important factors. In the data set of cross 2 in 1987 (Table 2.2.1), factor 1 was strongly associated with LF, DIASP, and FSH% and thus was regarded as a vigour and floriferous factor. The second factor was made up mainly of FD, PL, BEGDA and negatively associated with FLPERI, and was described as a large flower, late flowering and short bloom duration factor. The third factor, mainly associated with PETICO and negatively associated with BEGDA, was viewed as an early blossom and dark petiole colour factor. Factor 4 may be called a branching factor since it was mainly associated with TSH. Factors represent the patterns of relationship of the original variables, and one may examine the differences in these patterns between populations and between years. Cureton and D'Agostino (1983) developed the coefficient of congruence (CC) for factor comparison to show how closely the pairs of corresponding factors resemble each other, and suggested that if corresponding factors from two studies are really the same factor, the coefficient of congruence should be at least 0.90. The formula is

$$CC=\sum^{n} L_1 L_2 / \sqrt{(\sum^{n} L_1^2 \sum^{n} L_2^2)}$$

where L_1 and L_2 are the loadings on corresponding factors from two studies, and n is the number of variables investigated.

Table 2.2.4 shows the CCs of the 4 pairs of PCs between the 3 crosses in 1987 and 1988, and between two years in the same cross. In 1987, there was no difference between the first factor of all crosses since all the CC values were above 0.90. In 1988, the CC values between all crosses were close to 0.90, suggesting consistency in the patterns of association of the characters which mainly contribute to factor 1. However, between crosses, different factor patterns were found for all subsequent factors in 1987, and in factors 3 and 4 in 1988. This experimentally demonstrates that factor analysis is a single population technique, and the factor patterns of investigated characters can be obtained for each cross by separated analyses. Thus the crosses can be grouped on the basis of the consistency of their factor patterns.

Table 2.2.4 The coefficients of congruence for factor comparison in male seedling populations

	1987			1988			1987 VS 1988		
	CR2&4	CR2&6	CR4&6	CR2&4	CR2&6	CR4&6	CR2	CR4	CR6
PC1	0.94	0.95	0.98	0.84	0.88	0.98	0.58	0.89	0.92
PC2	0.70	0.71	0.75	0.81	0.83	0.97	0.45	0.76	0.71
PC3	-0.06	-0.35	0.61	0.65	0.06	0.09	-0.55	0.63	0.40
PC4	0.20	0.35	-0.09	0.26	0.33	0.48	0.25	0.08	-0.01

It is of interest to note that the CC values between crosses 4 and 6 were always higher than for the other cross combinations on factors 1 and 2 in both growing seasons, which suggests a similarity of the two crosses. In crosses 4 and 6, factor 1 represented the same plant variable relationships in each of the two successive years, but in all other instances there was a clear change in the factor pattern between years.

Moore (1975) examined the changes in factor patterns with year, and indicated that the annual records can best be summarised by the trend of the change. This enlarged the application of factor analysis. Denis and Adams (1978) made separate factor analyses of plant variables related to yield in dry beans in two locations, and found that factor patterns were almost identical. This may indicate the year effect is more important than location in affecting the factor pattern. Floriferousness is a desirable property in a male kiwifruit and as factor 1 was a floriferous factor, the seasonal effects were studied more closely.

In cross 2, factor one was strongly associated with LF, DIASP and FSH% (loading magnitudes 0.77, 0.83, and 0.77 respectively), and reasonably associated with FD, TF, INCRS and FLPERI (0.57, 0.66, 0.62 and 0.55 respectively). This factor represented a vine's degree of floriferousness (expressed by FSH%. TF and LF) and growth (expressed by DIASP and INCRS) in 1987. In 1988, this factor remained as a floriferous factor since it was strongly associated with TF, FSH% (0.73 and 0.89 respectively), but its relationship with DIASP and INCRS dramatically changed (-0.07 and 0.20 respectively). Hence the nature of factor 1 changed markedly from one year to the next.

In contrast, in crosses 4 and 6, the factor 1 pattern indicated a lower level of vigour in 1987, and a more similar vigour level in the following season. The significant congruence of factor 1 between years may therefore reflect a balanced pattern of growth in these seedlings - whereas in cross 2 annual growth relationships were more variable.

B. Female vine populations

Tables 2.2.5 - 2.2.7 display the factor patterns, the eigenvalues and the proportion of total variance explained by each factor in female seedlings in two seasons. Table 2.2.8 shows the CCs of the 4 pairs of PCs between 3 crosses in 1987 and 1988 and between the two years in the same cross.

Table 2.2.5 Factor pattern of first 5 PCs for female seedlings in cross 2 (Bruno x D120)

	1987				1988			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
FD	0.73	0.32	-0.10	-0.29	0.44	-0.55	-0.14	-0.01
PL	0.56	0.52	0.03	-0.13	0.62	-0.34	0.04	0.33
TF	0.68	-0.34	0.38	-0.02	0.76	0.43	0.06	0.20
PETICO	0.14	-0.51	-0.00	0.18	0.56	0.03	0.15	0.15
HAIR	0.21	-0.01	0.28	-0.36	0.33	-0.01	-0.51	0.11
VC	-0.09	-0.54	0.28	0.29	-0.08	0.40	0.17	-0.33
TW	0.91	-0.16	0.28	-0.03	0.87	0.34	0.08	0.16
AC	-0.01	-0.10	0.07	0.34	-0.19	0.17	0.42	-0.46
DIASP	0.84	0.05	-0.26	0.19	0.80	-0.40	0.07	-0.14
TSH	0.67	-0.05	-0.03	0.49	0.29	0.38	0.23	-0.41
FSH%	0.38	-0.40	0.66	-0.05	0.69	0.37	0.05	0.14
BEGDA	-0.34	0.27	0.38	-0.17	-0.40	-0.48	-0.03	0.31
FLPERI	0.20	-0.47	-0.48	0.33	0.56	0.58	-0.06	0.13
BRIX	-0.43	0.36	-0.11	0.30	-0.18	0.46	0.22	0.18
LOCN	-0.26	0.54	0.14	0.19	-0.01	-0.45	0.60	0.52
LWRATI	-0.07	-0.24	0.07	-0.53	-0.38	0.30	-0.15	0.16
INCRS	0.36	0.67	-0.04	0.27	0.57	-0.24	0.12	-0.54
ELOG	0.50	-0.26	-0.46	-0.34	0.48	-0.17	-0.68	-0.22
SYMM	0.18	0.49	0.50	0.05	0.24	-0.28	0.68	0.12
RELCOR	-0.07	0.69	0.42	0.06	0.15	-0.24	0.54	-0.28
FNF	0.67	-0.00	0.50	0.15	0.66	0.51	0.00	0.11
FW	0.34	0.25	-0.37	-0.65	0.33	-0.79	-0.08	0.24
EFFI	-0.54	-0.45	0.56	-0.27	-0.35	0.65	0.08	0.50
EIGENVALUE	5.29	3.60	2.71	2.06	5.63	4.03	2.30	1.98
PROPORTION	0.23	0.15	0.11	0.08	0.24	0.17	0.10	0.08
CUMULATIVE	0.23	0.38	0.50	0.59	0.24	0.42	0.52	0.60

Table 2.2.6 Factor pattern of first 5 PCs for female seedlings in cross 4 (Gracie x D120)

	1987				1988			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
FD	0.47	-0.34	0.52	-0.03	0.07	0.29	0.36	-0.05
PL	0.44	-0.30	0.40	-0.04	-0.02	0.30	0.55	0.28
TF	0.76	0.22	-0.24	0.10	0.71	-0.21	0.26	-0.28
PETICO	-0.34	0.19	0.02	0.37	0.31	0.26	0.46	0.17
HAIR	0.38	0.05	0.31	0.05	-0.26	-0.07	-0.20	0.16
VC	-0.21	0.03	-0.25	0.16	0.28	-0.40	0.43	0.23
TW	0.92	0.18	-0.02	0.02	0.90	0.20	0.14	-0.07
AC	-0.50	0.06	-0.12	0.34	0.28	-0.54	-0.02	0.17
DIASP	0.72	-0.53	0.05	-0.03	-0.42	-0.14	0.53	-0.50
TSH	0.74	-0.20	-0.11	0.14	0.20	-0.20	0.23	0.70
FSH%	0.86	0.33	-0.09	-0.01	0.87	0.15	-0.00	-0.13
BEGDA	-0.24	-0.04	-0.11	-0.42	-0.54	-0.31	0.07	0.22
FLPERI	0.75	0.09	-0.11	0.35	0.55	0.06	-0.31	-0.04
BRIX	-0.39	0.02	0.16	0.45	-0.35	-0.05	-0.15	-0.04
LOCN	0.18	0.30	0.69	0.27	-0.12	0.42	-0.10	-0.05
LWRATI	0.17	-0.30	0.11	-0.28	-0.25	-0.24	-0.32	0.05
INCRS	0.01	-0.53	0.03	0.60	-0.32	-0.02	0.50	-0.14
ELOG	0.08	-0.47	-0.21	-0.49	-0.07	-0.02	0.41	0.42
SYMM	0.25	0.44	0.43	-0.05	-0.10	0.66	-0.19	0.18
RELCOR	-0.07	0.28	0.44	-0.36	-0.31	0.76	-0.08	0.12
FNF	0.82	-0.01	-0.23	-0.00	0.85	0.01	0.12	-0.20
FW	-0.37	-0.10	0.55	-0.11	-0.12	-0.53	0.32	-0.01
EFFI	0.17	0.87	-0.13	-0.19	0.76	0.12	-0.40	0.34
EIGENVALUE	6.03	2.54	2.06	1.79	5.01	2.58	2.32	1.56
PROPORTION	0.26	0.11	0.08	0.07	0.21	0.11	0.10	0.06
CUMULATIVE	0.26	0.37	0.46	0.54	0.21	0.33	0.43	0.49

Table 2.2.7 Factor pattern of first 5 PCs for female seedlings in cross 6 (Hayward x D120)

	1987				1988			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
FD	0.53	0.47	0.15	-0.18	0.03	0.54	0.24	0.33
PL	0.45	0.48	-0.03	-0.01	0.12	0.61	0.21	0.36
TF	0.78	-0.29	-0.07	0.03	0.80	0.16	-0.17	0.16
PETICO	0.07	0.51	0.23	-0.22	0.08	0.59	-0.03	-0.10
HAIR	0.44	0.23	0.03	0.06	-0.08	0.09	0.18	0.00
VC	-0.08	-0.62	-0.15	0.18	-0.13	-0.34	0.09	0.37
TW	0.91	-0.27	0.01	-0.01	0.90	0.14	-0.12	0.17
AC	-0.10	-0.07	-0.20	-0.17	0.03	0.24	-0.45	-0.02
DIASP	0.63	0.56	-0.01	0.18	-0.01	0.86	0.02	-0.23
TSH	0.72	0.06	0.09	0.33	0.37	0.37	-0.00	0.06
FSH%	0.79	-0.44	-0.08	0.08	0.87	0.05	-0.15	0.09
BEGDA	-0.57	0.12	-0.07	0.60	-0.60	-0.01	0.16	0.49
FLPERI	0.70	-0.28	0.03	-0.30	0.75	0.03	-0.20	-0.28
BRIX	-0.38	0.09	-0.13	0.13	-0.32	0.15	-0.26	-0.46
LOCN	0.16	-0.05	0.83	0.08	0.26	-0.06	0.72	-0.27
LWRATI	-0.16	0.05	0.12	-0.38	-0.27	-0.24	-0.27	-0.33
INCRS	0.19	0.68	0.17	0.19	0.05	0.57	0.12	-0.29
ELOG	0.33	0.32	-0.38	0.11	-0.20	0.40	-0.19	0.23
SYMM	-0.11	-0.10	0.67	0.41	0.45	-0.19	0.67	-0.16
RELCOR	-0.21	-0.33	0.69	-0.18	0.34	-0.23	0.61	-0.28
FNF	0.78	-0.27	0.00	0.21	0.77	-0.11	-0.23	0.05
FW	0.03	0.57	0.08	-0.53	-0.03	0.26	0.36	0.37
EFFI	0.06	-0.88	0.00	-0.27	0.50	-0.69	-0.15	0.32
EIGENVALUE	5.60	3.84	2.03	1.62	4.84	3.38	2.28	1.75
PROPORTION	0.24	0.16	0.08	0.07	0.21	0.14	0.09	0.07
CUMULATIVE	0.24	0.41	0.49	0.56	0.21	0.35	0.45	0.53

Table 2.2.8 The coefficient of congruence for factor comparison in female seedling populations

	1987			1988			1987 VS 1988		
	CR2&4	CR2&6	CR4&6	CR2&4	CR2&6	CR4&6	CR2	CR4	CR6
PC1	0.75	0.86	0.91	0.44	0.66	0.82	0.90	0.56	0.77
PC2	-0.23	0.44	-0.75	-0.41	-0.38	0.01	-0.66	0.30	0.80
PC3	0.00	0.31	0.71	-0.06	0.43	0.10	0.41	0.04	0.80
PC4	0.66	0.39	-0.00	-0.10	0.17	0.33	-0.42	-0.18	0.06

With only one exception, in two years the four factors were found to differ between crosses. Thus the degree of consistency of the factor patterns was less for the female progeny where a larger number of variables were recorded. Each population therefore, produced its own peculiar group of distinguishing characteristics.

As for male vines the factor 1 CC values between crosses 4 and 6 were always higher than for the other cross combinations.

Factor 1 was a yield factor since it was strongly associated with TW and FNF in all crosses in both seasons (Tables 2.2.5-2.2.7). Inspection of the factor pattern indicates that, as for the male vines, it was primarily differences in the vigour variables that effected the changes between years. Female seedlings from cross 2 were the most consistent over two years in being characterisable by their yield/vigour features.

Because the factor pattern changed between years, superior vines were identified by factor analysis of the mean of the two years' phenotype data.

(2) *Selection of distinctive and superior vines*

Since cross 6 reached the required level of Kaiser and Rice's measure of sampling adequacy (namely 0.69 for male vines, 0.62 for female vines) and had the highest value among the crosses, only the analyses for those seedlings are described. Also variables FY (duration of the juvenile period) and PENE (force readings from penetrometer tests) were included in this analysis of female vines.

A. Male Vines

Table 2.2.9 displays the factor patterns and the statistics of the first 4 factors (of a total of 12 factors) for 63 male seedlings. The first principal factor had an eigenvalue of 3.85, and explained 32% of the total variance, hence was the most important factor in this analysis. As is usual, the first few factors accounted for most of the variance of the original variables, and in this analysis, only factors with eigenvalues greater than one were retained. The first 4 factors together explained 66% of the total variance of the data set and were retained for further analysis.

Table 2.2.9 Factor pattern of first 4 PCs for male vines (mean of 2 years)

	PC1	PC2	PC3	PC4
FD	0.34	0.62	-0.44	-0.31
PL	0.43	0.54	-0.29	-0.09
TF	0.77	-0.10	-0.12	-0.19
LF	0.89	-0.17	-0.11	-0.10
PETICO	-0.02	0.62	-0.17	0.47
DIASP	0.49	0.54	0.30	0.18
TSH	0.55	-0.17	0.34	0.14
FSH%	0.84	-0.32	-0.01	0.05
BEGDA	-0.43	-0.00	-0.23	0.55
LWRATI	-0.28	0.38	0.47	-0.30
INCRS	0.40	0.31	0.64	0.14
FLPERI	0.67	-0.16	-0.11	0.42
EIGENVALUE	3.85	1.82	1.26	1.04
PROPORTION	0.32	0.15	0.10	0.08
CUMULATIVE	0.32	0.47	0.57	0.66

In the factor pattern matrix each factor may be regarded as the dependent variable that is regressed on all the original variables, therefore one can determine the relationship between a factor function and the original variables, and also the relationship between the original variables.

Factor 1 was strongly associated with TF, LF, TSH, FSH% and FLPERI, hence may be regarded as a prolific factor. The second factor was made up mainly of FD, PL, PETICO and DIASP, suggesting it is a robustness factor. The third factor may be called a vigour factor (INCRS) and Factor 4 (BEGDA) a late blossom factor.

The first factor was also important because it represents a number of desirable properties in a male kiwifruit selection. Numerous flowers and an extended flowering period are necessary for good pollination, and the factor scores of each vine on this factor could therefore be used as an index of prolificness to order the male vines. If the best 10% of the male plants were selected, the 6 vines which had the highest scores on this factor would be vines 76, 86, 100, 103, 114, 128, with scores 1.1, 1.5, 1.5, 1.2, 1.6 and 1.4 respectively.

Factor 2 is of less obvious interest, being associated with large flowers and also a thick stem which reflects good early vigour. Both factors 1 and 2 may be considered together for selecting male vines. By plotting the factor scores of each vine on these two factors, differences between vines can be conceptualised. The vines may be grouped according to their distances, and the best vines selected. The scatter graph (Figure 2.2.1) shows that most vines had moderate scores on these two factors, and skewed towards the higher scores. Very few vines had a low score on both factors.

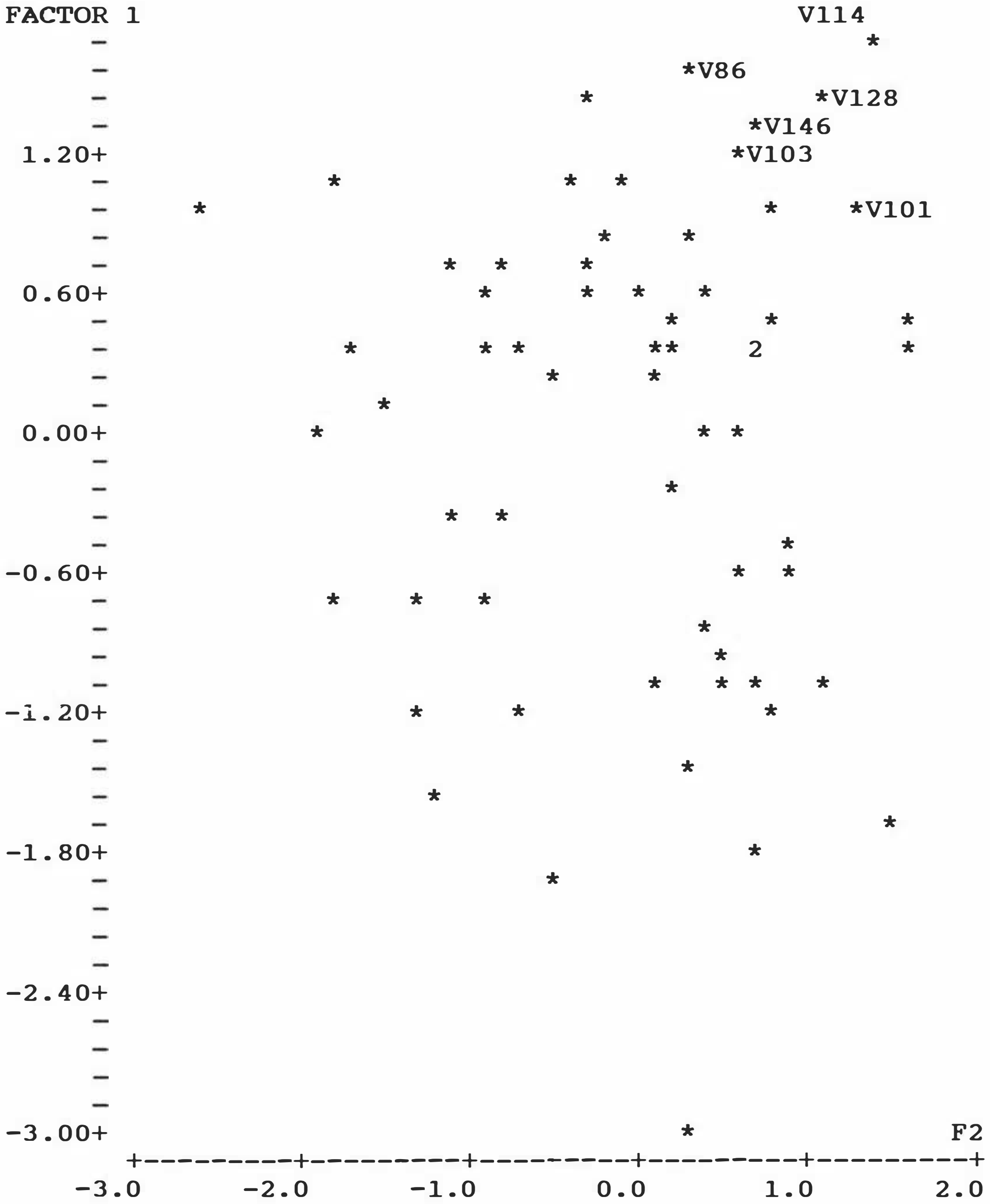


Figure 2.2.1 Plot of the factor scores of 63 male vines on first 2 factors.
V86 (1.5,0.3), V101(0.9,1.3), V103(1.2,0.6)
V114(1.6,1.4), V128(1.4,1.1), V146(1.3,0.7)

If the best 10% of the male plants were selected, the 6 vines which had the highest scores on both factors were vines 86, 101, 103, 114, 128, and 146. Thus by factor analysis, the 12 original variables were transformed into a small number of factors, reducing the size of the data set but also enhancing the meaningfulness of the data. This then gave an opportunity for distinguishing between plants, and identifying phenotypically superior ones. Similarly factor 1 and factor 3 could be plotted together to express floriferous vines with a fast growth rate of the main stem, and factor 1 and factor 4 could be plotted together to identify prolific vines with a late flowering season.

It is interesting to note that the male vines that bloom early, with a short flowering period are desirable for collecting pollen for artificial spray pollination in commercial kiwifruit cultivation. Table 2.2.9 indicates vines with a high score on function 1 are early flowering but with a long bloom period. Although function 2 was negatively associated with the bloom period, the magnitude of the loading for this character was small. This indicated that factor analysis was not satisfactory for identifying superior males for artificial pollination, that were both early flowering and of short flowering duration.

Factor 1 was strongly associated with TF, LF, TSH, FSH% and FLPERI, therefore a close relationship between these characters was found. Not surprisingly TF and LF were closely associated with FLPERI, with more terminal and lateral flowers extending the flowering duration. The close relationship between TSH and FSH% implies that vines with more shoots tend to be floriferous. Therefore TSH may be used as a criterion for male vine selection in the juvenile period of kiwifruit.

B. Female vines

Twenty-five attributes were recorded for the 63 female seedlings from cross number 6. Table 2.2.10 displays the factor patterns and the statistics of the first 8 factors. In this analysis, only the first 8 factors which together explain 74% of the total variance were retained for further analysis.

Factor 1 was mainly associated with FD, TF, DIASP, TSH, FSH%, FLPERI, and FNF, and especially strongly associated with TW, hence it was regarded as a prolific factor - a desirable property in a female kiwifruit selection. The factor scores of each vine on this factor give an index of prolificness to order the female vines. On this basis, the best 10% of the female plants were vines numbered 1, 88, 109, 124, 134, 142, with scores 1.6, 1.5, 1.4, 1.7, 1.6 and 1.7 respectively.

Table 2.2.10 Factor pattern of first 8 PCs for female vines (mean of 2years)

	FACTOR							
	1	2	3	4	5	6	7	8
FD	0.57	-0.37	0.23	-0.24	-0.17	0.38	-0.00	0.11
PL	0.47	-0.48	0.16	-0.30	-0.16	0.15	-0.10	-0.20
TF	0.70	0.39	-0.11	-0.06	-0.11	-0.21	0.18	-0.03
PETICO	0.31	-0.52	0.13	0.14	-0.26	-0.10	-0.29	-0.17
HAIR	0.42	-0.05	-0.01	0.17	0.17	0.38	-0.30	0.53
VC	-0.25	0.36	-0.11	-0.19	0.36	0.12	0.00	0.21
TW	0.83	0.42	-0.11	-0.00	-0.06	0.06	0.09	-0.11
AC	-0.04	-0.15	-0.18	-0.09	-0.39	-0.52	-0.27	0.13
DIASP	0.70	-0.59	-0.01	0.09	0.13	-0.17	-0.03	0.04
TS	0.69	-0.04	-0.03	0.06	0.42	-0.05	0.18	-0.06
FSH%	0.69	0.52	-0.19	-0.07	0.06	0.04	-0.09	-0.15
BEGDA	-0.39	-0.26	0.06	-0.64	0.35	-0.06	-0.01	-0.14
FLPERI	0.56	0.43	-0.15	0.49	-0.09	0.06	-0.09	-0.05
BRIX	-0.21	-0.25	-0.34	0.57	0.27	0.03	-0.16	-0.32
LOCN	0.07	0.07	0.75	0.31	0.05	-0.02	0.04	-0.08
LWRATI	-0.28	-0.00	-0.17	0.56	-0.21	0.08	0.01	0.40
INCRS	0.34	-0.49	0.21	0.29	0.27	-0.38	0.17	0.07
ELOGN	0.30	-0.31	-0.36	-0.05	0.34	0.26	0.31	0.13
SYMM	0.05	0.29	0.69	0.19	0.29	0.05	0.04	-0.15
RELCOR	-0.18	0.33	0.75	0.14	0.01	0.05	-0.14	-0.04
FNF	0.58	0.49	-0.08	-0.11	-0.15	-0.32	0.15	0.01
FW	0.23	-0.37	0.25	-0.05	-0.53	0.40	0.32	-0.02
EFFI	-0.33	0.81	-0.08	-0.07	-0.17	0.20	0.02	-0.10
FY	-0.48	-0.16	0.01	0.24	-0.17	-0.13	0.66	0.04
PENE	0.21	0.23	0.45	-0.27	0.05	-0.32	0.00	0.50
EIGENVALUE	5.20	3.76	2.47	1.99	1.57	1.37	1.12	1.10
PROPORTION	0.20	0.15	0.09	0.08	0.06	0.05	0.04	0.04
CUMULATIVE	0.20	0.35	0.45	0.53	0.60	0.65	0.70	0.74

The second factor was associated with PETICO, FSH%, and negatively associated with DIASP, and strongly associated with EFFI and may be described as a cropping efficiency factor. Factor 3 made up mainly of LOCN, SYMM and RELCOR, was called a fruit quality factor. The fourth factor may be called maturity date factor since it mainly associated with BRIX and LWRATI, and negatively associated with BEGDA. The fifth factor may be called a small fruit factor (negatively associated with FW), Factor 6 low acidity factor (negatively associated with AC), Factor 7 long juvenility factor (associated with FY), and Factor 8, a hairy factor, was mainly associated with HAIR.

The first factor represented productivity of the kiwifruit vine and together with factor 2 which represented fruiting efficiency, offers a useful technique for selecting superior female vines.

Figure 2.2.2 shows most vines had moderately high factor scores on the first two factors, and skewed towards the higher scores, with very few vines with low scores on both factors.

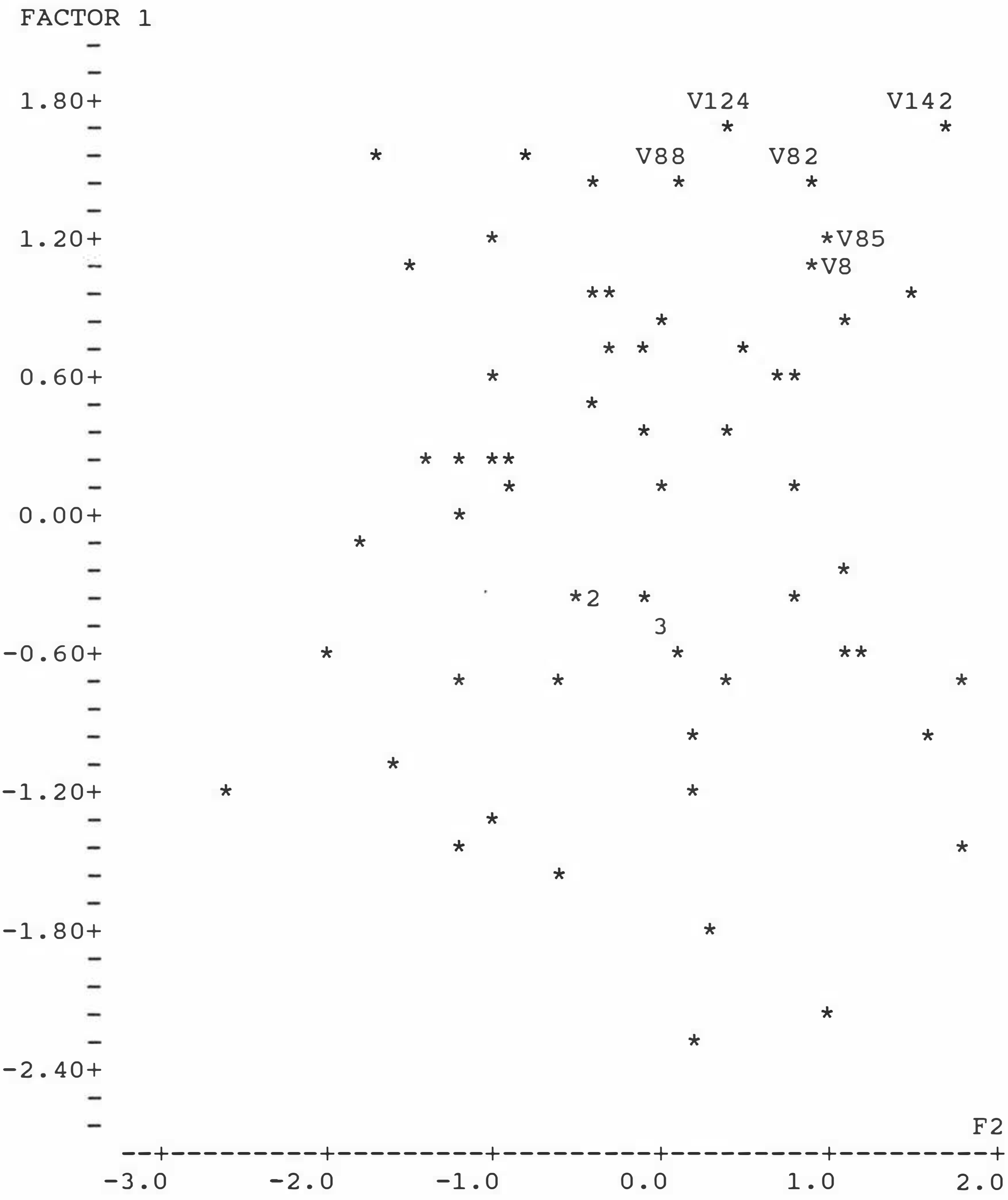


Figure 2.2.2 Plot of the factor scores of 63 female vines on first 2 factors.
V8 (1.1,0.9), V82 (1.4,0.9), V85 (1.2,1.0). V88 (1.5,0.1), V124(1.7,0.4), V142(1.7,1.7)

The best 10% of the female plants having the highest scores on both factors were vines 8, 82, 85, 88, 124 and vine 142. Thus these vines can be readily visualised by this analysis. Similarly factor 1 and factor 3 could be plotted together to help identify prolific vines with good quality fruit, and factor 1 and factor 4 could be plotted together to represent prolific vines with early fruit maturity.

A 3-dimension plot may be used to allow consideration of 3 factors together, but with a large number of points, the plot becomes cluttered and difficult to interpret. Figure 2.2.3 shows the factor scores of 63 female vines on factors 1, 2 and 4 which together rate vines for prolificness, efficiency, and early fruit maturity. The scatter graph shows that most vines had moderate scores on these three factors, and only a few vines with a high score on each of the factors 1, 2 and 4 are located in the upper left hand corner.

In addition, the relationship between the original variables which together comprise a factor may also be examined in factor analysis. Factor 1 was strongly associated with TW, and also had a close relationship with FD, TF, DIASP, TSH, FSH%, FLPERI, and FNF. Since variables which have relatively high loadings on a factor are intercorrelated as a group (Broschat 1979), the close relationship between these characters is obvious. The fruiting character FNF (fruit number per fruiting shoot) could be considered as the component of the yield (TW), and the others as the basis of high yielding.

TSH was strongly associated with floriferous male vines, but also with FSH% and TW in female vines, hence it could be an indicator for the selection of floriferous males and productive female kiwifruit.

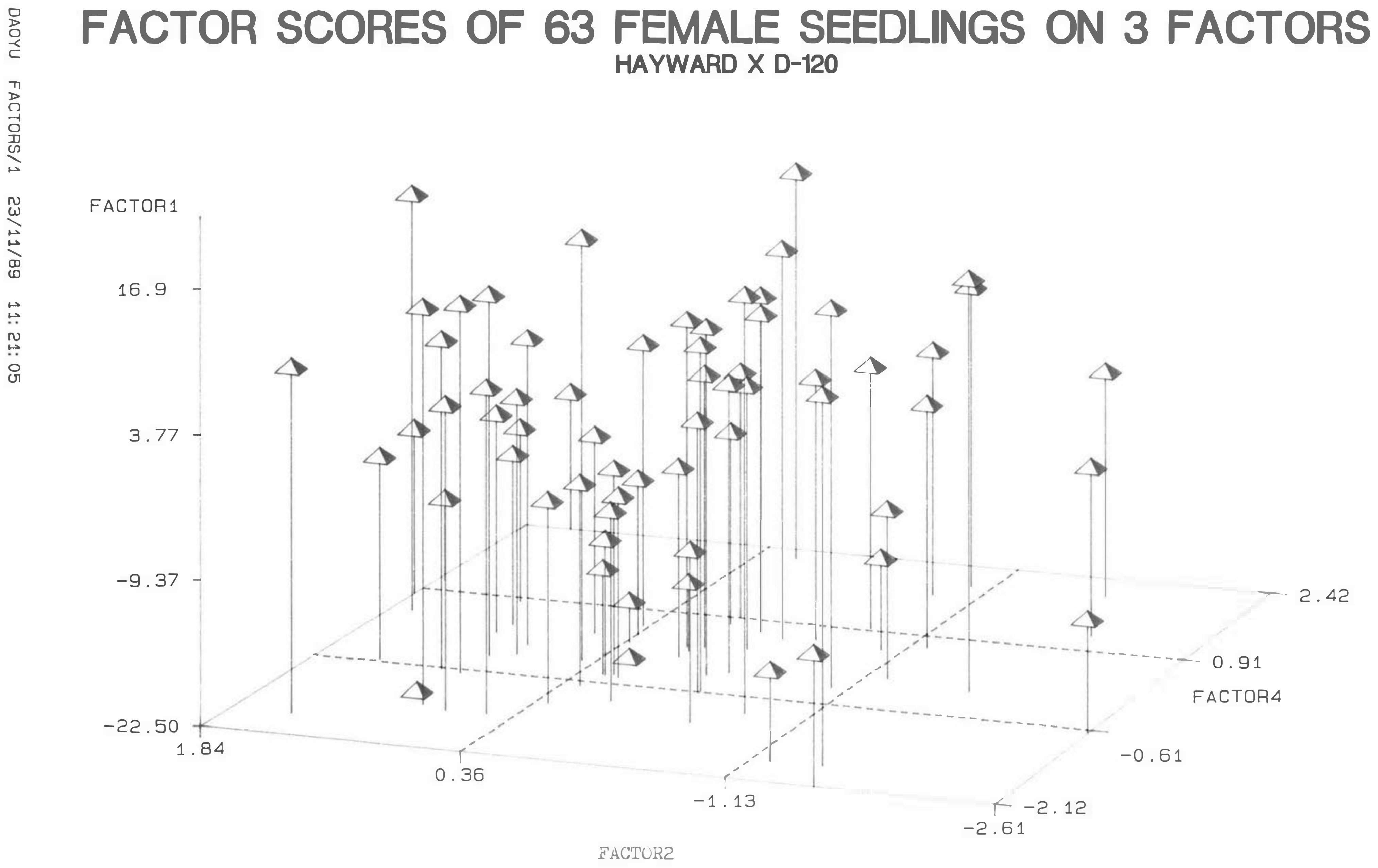


Figure 2.2.3 Factor scores of 63 female kiwifruit seedling

To sum up, factor analysis is a data reduction and screening technique. The main purpose of factor analysis is to find linear combinations of the measured variables, which may be used to group individuals within a single population. In discriminant analysis the linear combinations of the original variables, which maximise differences among pre-existing populations, are used to order and to group a number of different populations. Therefore factor analysis is a one-population technique, and is a suitable screening technique in single plant selection and in a germplasm collection. Discriminant analysis is appropriate for determining the best cross combinations following hybridisation.

In this work, 12 variables for male vines and 25 variables for female vines were reduced to a 4 factor model and 8 factor model respectively according to the eigenvalue of each factor. Based on these retained factors, the vines were scored so that they could be compared on several characters in combination. Not only the number of comparisons were reduced, but the meaningfulness of the comparisons was enhanced, and based on these comparisons, the best vines could be selected for different breeding purposes.

While factor analysis proved to be a useful tool for screening kiwifruit plants, it was based on phenotypic data. Genetic studies are necessary to provide information on the inheritance of these plant characters.

(3) ***Comparison between principal component and principal factor analysis***

Since principal component analysis and factor analysis are both invoked to analyse the structure of variance-covariance and correlation matrices, they are easily confused. Common factor analysis is covariance oriented, whereas principal component analysis is variance oriented. Factor analysis, although similar to principal component analysis, is considerably more complex. However, principal factor analysis is the simplest method of the factor analysis, and is generated the same way as principal component analysis except that it sets the squared multiple correlations (SMC) of each variable with all the other variables as the prior communality estimates, whereas principal component analysis sets all prior communalities to one (SAS Institute Inc. 1988). The two methods were compared using the coefficient of congruence of two factors.

Tables 2.2.11 and 2.2.12 are principal factor patterns for male vines and female vines respectively. Compared with Tables 2.2.9 and 2.2.10 which are principal component patterns, a similar magnitude of loadings was found. The CC values between the two methods are 0.99, 0.99, 0.96, 0.90 respectively for 4 factors of male vines, and 0.99 0.99, 0.99, 0.99, 0.93 and 0.91 respectively for 6 factors of female vines. This indicates there was no difference between the two methods in identifying factors for characterising and grouping plants.

Table 2.2.11 Factor pattern of first 4 factors for male vines (mean of 2 years)

	FACTOR			
	1	2	3	4
FD	0.30	0.55	-0.35	-0.10
PL	0.37	0.46	-0.19	0.01
TF	0.75	-0.06	-0.17	-0.24
LF	0.90	-0.13	-0.12	-0.08
PETICO	-0.03	0.44	-0.04	0.28
DIASP	0.43	0.47	0.28	0.08
TSH	0.49	-0.12	0.24	-0.01
FSH%	0.83	-0.30	0.03	0.09
BEGDA	-0.37	-0.01	-0.12	0.23
LWRATI	-0.24	0.26	0.21	-0.26
INCRS	0.34	0.26	0.46	-0.01
FLPERI	0.61	-0.12	0.00	0.34
EIGENVALUE	3.44	1.23	0.64	0.41
PROPORTION	0.63	0.22	0.11	0.07
CUMULATIVE	0.63	0.86	0.98	1.06

Table 2.2.12 Factor pattern of first 6 factors for female vines (mean of 2 years)

	FACTOR					
	1	2	3	4	5	6
FD	0.53	-0.36	0.22	-0.26	-0.24	0.25
PL	0.44	-0.46	0.14	-0.29	-0.17	0.10
TF	0.69	0.37	-0.09	-0.06	-0.04	-0.25
PETICO	0.28	-0.46	0.10	0.11	-0.17	-0.09
HAIR	0.38	-0.06	0.00	0.13	0.05	0.29
VC	-0.22	0.32	-0.09	-0.15	0.23	0.19
TW	0.85	0.41	-0.09	-0.02	-0.08	0.07
AC	-0.04	-0.12	-0.15	-0.05	-0.14	-0.36
DIASP	0.70	-0.62	-0.02	0.11	0.18	-0.12
TSH	0.67	-0.05	-0.01	0.06	0.36	0.09
FSH%	0.71	0.52	-0.17	-0.08	0.07	0.11
BEGDA	-0.38	-0.24	0.02	-0.59	0.34	0.03
FLPERI	0.55	0.41	-0.10	0.48	-0.11	0.07
BRIX	-0.19	-0.21	-0.29	0.51	0.16	0.16
LOCN	0.07	0.05	0.70	0.24	0.04	-0.03
LWRATI	-0.25	0.00	-0.12	0.44	-0.17	-0.02
INCRS	0.31	-0.46	0.18	0.27	0.26	-0.19
ELOG	0.27	-0.28	-0.32	-0.02	0.16	0.32
SYMM	0.04	0.26	0.67	0.15	0.24	0.16
RELCOR	-0.17	0.31	0.73	0.10	0.02	0.05
FNF	0.58	0.46	-0.06	-0.12	-0.03	-0.38
FW	0.22	-0.36	0.25	-0.09	-0.64	0.18
EFFI	-0.32	0.84	-0.06	-0.09	-0.23	0.17
FY	-0.45	-0.13	0.00	0.21	-0.15	-0.17
PENE1	0.19	0.19	0.37	-0.23	0.12	-0.22
EIGENVALUE	4.98	3.54	2.11	1.59	1.23	0.95
PROPORTION	0.30	0.21	0.12	0.09	0.07	0.05
CUMULATIVE	0.30	0.51	0.64	0.74	0.82	0.87

SECTION 3 CANONICAL ANALYSES

1. Introduction

In some sets of multivariate data the variables can be divided naturally into two domains. Although they are investigated concurrently on the subjects, conceptually they are different. For example domain 1 may be physiological characters, and domain 2 may be morphological ones;or domain 1 may be characters of productivity, and domain 2 may be quality characters; or domain 1 may be environment attributes, and domain 2 may be plant characters. Research to determine the interrelatedness of the two domains may be of interest. The numerous interrelationships may make this unsuited to a bivariate correlation method, but canonical correlation analysis can then be used to examine the relationships between the two groups.

The objective of this section was to examine the relationship between plant vegetative and flowering characters, and between flowering characters and fruiting properties, in order to understand the intercorrelatedness of various aggregates of these characters for screening plants in kiwifruit breeding.

2. Methods

The canonical correlation analysis was performed by the CANCORR procedure of the SAS package with data averaged over 2 years for 126 seedlings in cross 6, using 12 characters for male vines and 25 characters for female vines. Cooley and Lohnes (1971) provide a good understanding of the canonical correlation model. First the model derives a pair of linear functions x_1 and y_1 from each original vector variable such that the covariance between the functions is maximised. Next, the canonical model derives another pair of functions x_2 and y_2 that are maximally correlated,subject to the constraint that they must be uncorrelated with the first pair of functions from each domain. Then the third pair of functions is generated, and so on up to the pth pair of functions, where p equals the function number in the smaller data set.

The conditions are

$$x = \mathbf{c}'\mathbf{z}_1 \text{ and } y = \mathbf{d}'\mathbf{z}_2$$

and $$R_c = (1/n)\Sigma x_i y_i \mid \text{maximum}$$

where x and y are linear functions for the original data set of $\mathbf{z}_1$ and $\mathbf{z}_2$, and both $\mathbf{z}_1$ and $\mathbf{z}_2$ have zero means and unit variances; **c** and **d** are coefficient vectors which carry the original vector variables into the derived variables x and y. R_c is the canonical correlation between two functions.

To maximise R_c, **c** and **d** are sought to transfer the original data sets into appropriate components x and y. Once again a complicated eigenstructure, which can be expressed in terms of the partitions of the correlation matrix for $\mathbf{z}_1$ and $\mathbf{z}_2$ together was used to obtain the **c** or **d** vector:

$$(R_{22}^{-1} R_{21} R_{11}^{-1} R_{12} - \lambda_j I)\mathbf{d}_j = o$$

with the restriction equation

$$\mathbf{d}_j' R22\, \mathbf{d}_j = 1$$

where R22 is the matrix of correlation between the elements of $\mathbf{z}_2$, R11 is the matrix of correlation between the elements of $\mathbf{z}_1$; R21 and R12 contains the cross-correlations between elements of $\mathbf{z}_1$ and $\mathbf{z}_2$ (Cooley and Lohnes 1971).

When **d** has been computed for canonical functions of $\mathbf{z}_2$, the corresponding weights **c** for $\mathbf{z}_1$ can be obtained from the equation

$$\mathbf{c} = (R11^{-1}\, R12\, \mathbf{d})/Rc$$

Finally in this formulation of the canonical correlation, the eigenvalue is the square of the canonical correlation coefficients.

Bartlett (1941, 1947) derived a procedure for testing the significance of the canonical correlation. The null hypothesis that $\mathbf{z}_1$ is unrelated to $\mathbf{z}_2$ is tested by a function of "Lambda" that is distributed approximately as chi-square with $p_1 p_2$ degree of freedom, where p_1 is the function number in $\mathbf{z}_1$, p_2 is the function number in $\mathbf{z}_2$ (see Cooley and Lohnes, 1971).

Besides the canonical correlation coefficient, interpretation of the canonical functions is important, and a canonical structure giving the correlations of the canonical functions with the original variables was examined for this purpose.

3. Results and discussion

A. Male vines

The 12 variables investigated for male vines can be divided naturally into two groups, i.e. vegetative characters and flowering characters. The vegetative group included PETICO, DIASP, TSH, LWRATI and INCRS called "VAR" variables in this analysis, and the flowering group included FD, PL, TF, LF, FSH%, BEGDA and FLPERI which were called "WITH" variables. A canonical correlation analysis was used to examine the relationships between the two groups.

Table 2.3.1 displays the statistics of the 5 canonical correlations for 5 pairs of canonical variates of the two groups. The first canonical correlation was the most important and explained 61% of the covariance between the vegetative and flowering characters.

Table 2.3.1 Eigenvalue and the proportion of total covariance explained by each canonical correlation in male seedlings

	Canonical Correlation	Approx Standard Error	Eigenvalue	Proportion	Cumulative
1	0.66	0.07	0.795	0.612	0.61
2	0.49	0.09	0.320	0.246	0.85
3	0.34	0.11	0.135	0.104	0.96
4	0.19	0.12	0.039	0.030	0.99
5	0.09	0.12	0.008	0.006	1.00

The null hypothesis that group 1 was unrelated to group 2 was tested for each canonical correlation (Table 2.3.2) and as only correlation 1 reached a significant level, only this correlation was investigated.

Table 2.3.2 Statistics for significance tests of each canonical correlation in male seedlings

Canonical Correlation	Likelihood Ratio	Approx F	Number DF	PR > F
1	0.35	1.73	35	0.009**
2	0.63	1.05	24	0.401
3	0.84	0.63	15	0.840
4	0.95	0.32	8	0.956
5	0.99	0.15	3	0.926

Table 2.3.3 shows the canonical structure matrix, where each canonical variable may be regarded as the dependent variable that is regressed on all original variables, and it can be named according to the relationship between the canonical variable and original variables which together comprise the canonical variable.

The first canonical variable (V1) was called a total shoot and leaf shape function, since it was associated mainly with TSH, and negatively associated with LWRATI, hence the vines with high scores on this canonical variable tended to have many shoots and relatively round leaves.

Table 2.3.3 Canonical structure: correlations between the vegetative variables and their canonical variables (males)

	Canonical variables				
	V1	V2	V3	V4	V5
PETICO	-0.35	0.76	-0.25	0.40	0.24
DIASP	0.20	0.67	0.70	-0.04	-0.09
TSH	0.77	0.05	0.16	0.13	0.58
LWRATI	-0.50	-0.30	0.50	0.45	0.43
INCRS	0.36	0.15	0.44	0.67	-0.43

The significant first canonical variable for flowering characters (W1) was primarily associated with TF, LF, FLPERI, and strongly associated with FSH% (Table 2.3.4) and therefore may be called a floriferous canonical variable. The canonical correlation of 0.66 between the first two canonical variables of Table 2.3.1 implies that vines with many shoots and relatively round leaves tend to be prolific flowering. It also suggests that in the selection of male kiwifruit, the leaf shape index may be used as a selection criterion for heavy flowering. It also suggests large shoot number was associated with many lateral flowers, or that floriferousness was related to the presence of many lateral flowers (rather than increased flower nodes/shoot).

The results from the other crosses confirm the above conclusion. For example, in cross 4, the first vegetative canonical variable was mainly associated with TSH (0.74), and negatively associated with LWRATI (-0.72); the floral one was mainly associated with FSH% (0.92) and FLPERI (0.71). The canonical correlation between the two was 0.80, implying the above conclusion was true in Gracie x D-120 progenies.

Table 2.3.4 Canonical structure: correlations between the flowering variables and the canonical variables (males)

	Canonical variables				
	W1	W2	W3	W4	W5
FD	-0.19	0.74	0.33	-0.08	0.29
PL	0.11	0.60	0.54	0.04	-0.10
TF	0.62	0.08	0.42	-0.06	0.63
LF	0.75	0.29	0.33	-0.38	0.14
FSH%	0.92	0.26	-0.03	-0.02	-0.02
BEGDA	-0.26	-0.03	-0.57	-0.14	0.43
FLPERI	0.57	0.49	-0.13	0.28	0.15

B. Female vines

The variables investigated for female vines can be divided naturally into three groups, i.e. vegetative characters, flowering characters, and fruiting characters. Three canonical correlation analyses were examined:

1. *Canonical correlation between vegetative and flowering attributes*

The vegetative group ('var' variables) included PETICO, DIASP, TSH, INCRS, and LWRATI, and the flowering group ('with' variables) included FD, PL, TF, FSH%, BEGDA, FLPERI and FY.

Table 2.3.5 displays the statistics of 5 canonical correlations for 5 pairs of canonical variates of the two groups. The largest proportion (0.62) of the total covariance between the vegetative and flowering groups of variables was given by the first canonical correlation. Canonical correlations 2 and 3 were of relatively minor importance.

Table 2.3.5 Eigenvalue and the proportion of total covariance explained by each canonical correlation between vegetative and flowering characters (female seedlings)

	Canonical Correlation	Approx Standard Error	Eigenvalue	Proportion	Cumulative
1	0.71	0.06	1.014	0.623	0.62
2	0.53	0.09	0.382	0.235	0.85
3	0.40	0.10	0.189	0.116	0.97
4	0.18	0.12	0.034	0.020	0.99
5	0.08	0.12	0.006	0.004	1.00

On testing the null hypothesis that group 1 was unrelated to group 2 for each canonical correlation, only correlation 1 was found to be significant (Table 2.3.6) and analysed further.

Table 2.3.6 Statistics for testing the significance of each canonical correlation between vegetative and flowering characters

Canonical Correlation	Likelihood Ratio	Approx F	Number df	PR > F
1	0.28	2.12	35	0.0006 **
2	0.58	1.26	24	0.1922
3	0.80	0.78	15	0.6914
4	0.96	0.27	8	0.9733
5	0.99	0.12	3	0.9465

Table 2.3.7 shows the canonical structure matrix for the vegetative characters, where each canonical variable may be regarded as the dependent variable that was regressed on all the original variables. The first canonical variable, being mainly associated with DIASP and LWRATI was called main stem and leaf shape canonical variable.

Table 2.3.7 Canonical structure: correlations between the vegetative variables and their canonical variables

	V1	V2	V3	V4	V5
PETICO	0.43	-0.53	0.31	-0.46	0.45
DIASP	0.79	-0.16	0.53	0.23	-0.05
TSH	0.47	0.61	0.53	0.11	0.30
LWRATI	0.60	-0.26	0.62	-0.12	-0.40
INCRS	0.22	-0.35	0.41	0.66	0.46

Of the canonical variables for flowering characters (Table 2.3.8), only the significant first canonical variable (W1) was examined. It was mainly associated with FD and PL, and negatively associated with FY, and may be called a flower size and precocity canonical variable. From Table 2.3.5, the canonical correlation between the first two canonical variables was 0.71, implying that the vines with a thick stem (strong early vine growth) and long leaves tended to produce big flowers and to be precocious.

Since nearly the same characters were investigated for canonical correlation between vegetative and floral characters in both male and female vines, it is possible to compare the extent and nature of the canonical correlation between the two types of kiwifruit. The first floral canonical variable no longer was a floriferous function in female vines, but a flower size function; and the first vegetative canonical variable changed from a total shoot (male) to a main stem function. The leaf shape index was a main contributor to the first canonical functions, but the direction was changed. A further study is suggested to understand the mechanism of these changes.

Table 2.3.8 Canonical structure: correlations between the flowering variables and their canonical variables

	Canonical variables				
	W1	W2	W3	W4	W5
FD	0.70	-0.13	0.37	0.18	-0.44
PL	0.89	-0.38	-0.02	0.03	0.18
TF	0.37	0.60	0.37	0.23	0.16
FSH%	0.41	0.80	0.20	-0.14	0.12
BEGDA	0.09	-0.03	-0.83	0.17	-0.11
FLPERI	0.06	0.36	0.72	-0.23	0.52
FY	-0.50	-0.34	-0.06	0.70	0.14

2. *Canonical correlation between vegetative and fruiting attributes*

Table 2.3.9 displays the statistics of 5 canonical correlations for 5 pairs of canonical variates of the two groups. The largest proportion (0.95) of the total covariance between the flowering and fruiting groups of variables was given by the first canonical correlation, which was shown to be a significant relationship (Table 2.3.10).

Table 2.3.9 Eigenvalue and the proportion of total covariance explained by each canonical correlation between vegetative and fruiting characters

	Canonical Correlation	Approx Standard Error	Eigenvalue	Proportion	Cumulative
1	0.98	0.004	27.89	0.950	0.95
2	0.64	0.073	0.71	0.024	0.97
3	0.51	0.093	0.35	0.012	0.98
4	0.47	0.098	0.28	0.009	0.99
5	0.29	0.115	0.09	0.003	1.00

Table 2.3.10 Statistics for testing the significance of each canonical correlation between vegetative and fruiting characters

Canonical Correlation	Likelihood Ratio	Approx F	Number df	PR > F
1	0.01	5.40	65	0.0001 **
2	0.30	1.35	48	0.0823
3	0.52	1.03	33	0.4244
4	0.70	0.90	20	0.5805
5	0.91	0.53	9	0.8433

The first canonical variable (**V1**) being mainly associated with DIASP, TSH and INCRS (Table 2.3.11) was called a vigour canonical variable.

Table 2.3.11 Canonical structure: correlations between the vegetative variables and their canonical variables

	V1	V2	V3	V4	V5
PETICO	0.42	-0.44	0.27	0.71	-0.19
DIASP	0.99	-0.07	-0.02	0.02	-0.01
TSH	0.62	0.71	0.26	0.17	0.03
LWRATI	-0.12	-0.28	-0.07	0.09	0.94
INCRS	0.59	-0.25	0.32	-0.32	0.03

Of the canonical variables for fruiting characters (Table 2.3.12), the significant first canonical variable being strongly negatively correlated with EFFI, was called an efficiency canonical variable. Table 2.3.9 indicated that the canonical correlation between the first two canonical variables was 0.98. This implies that the vigorous vines tended to be less efficient in cropping.

Table 2.3.12 Canonical structure: correlations between the fruiting variables and their canonical variables

	Canonical Variables				
	W1	W2	W3	W4	W5
HAIR	0.33	0.16	-0.16	0.18	0.36
VC	-0.32	0.42	-0.11	-0.28	0.20
TW	0.37	0.70	-0.05	0.34	-0.15
AC	0.05	-0.47	-0.15	0.10	-0.22
BRIX	0.05	-0.21	0.21	-0.01	0.62
LOCN	0.05	-0.00	0.60	0.16	0.14
ELOGN	0.35	0.15	0.12	-0.12	0.05
SYMM	-0.10	0.26	0.49	-0.15	-0.25
RELCOR	-0.31	-0.08	0.23	-0.19	-0.09
FNF	0.19	0.52	-0.16	-0.05	-0.17
FW	0.21	-0.24	-0.09	0.39	-0.29
EFFI	-0.86	0.37	-0.00	0.19	-0.05
PENE	0.03	0.12	0.33	-0.25	-0.52

3. *Canonical correlation between flowering and fruiting attributes*

The flowering group included FD, PL, TF, FSH%, BEGDA, FLPERI and FY , and the fruiting group included HAIR, VC, TW, AC, BRIX, LOCN, ELOGN, SYMM, RELCOR, FNF, FW, EFFI, and PENE .

Table 2.3.13 displays the statistics of 7 canonical correlations for 7 pairs of canonical variates of the two groups. The largest proportion (0.81) of the total covariance between the flowering and fruiting groups of variables was given by the first canonical correlation although the first two correlations were significant (Table 2.3.14).

Table 2.3.13 Eigenvalue and the proportion of total covariance explained by each canonical correlation between flowering and fruiting characters

	Canonical Correlation	Approx Standard Error	Eigenvalue	Proportion	Cumulative
1	0.97	0.006	17.90	0.814	0.81
2	0.85	0.034	2.64	0.120	0.93
3	0.64	0.073	0.72	0.033	0.96
4	0.48	0.097	0.29	0.013	0.98
5	0.40	0.105	0.20	0.009	0.99
6	0.32	0.113	0.11	0.005	0.99
7	0.27	0.117	0.08	0.003	1.00

Table 2.3.14 Statistics for testing the significance of each canonical correlation between flowering and fruiting characters

Canonical Correlation	Likelihood Ratio	Approx F	Number df	PR > F
1	0.004	4.20	91	0.0001 **
2	0.084	1.96	72	0.0001 **
3	0.307	1.11	55	0.2841
4	0.531	0.79	40	0.7956
5	0.690	0.69	27	0.8687
6	0.829	0.58	16	0.8858
7	0.924	0.57	7	0.7736

Table 2.3.15 shows the canonical structure matrix for flowering characters, where each canonical variable may be regarded as the dependent variable that was regressed on all the original variables. The first canonical variable, as for male vines, being mainly associated with TF, FLPERI, and strongly correlated with FSH%, was called a floriferous canonical variable. The second canonical variable was mainly associated with FD and PL, and may be described as a big flower canonical variable.

Table 2.3.15 Canonical structure: correlations between the flowering variables and their canonical variables

	V1	V2	V3	V4	V5	V6	V7
FD	0.15	0.855	-0.15	-0.09	-0.25	-0.28	0.25
PL	0.01	0.724	-0.07	-0.59	0.10	0.12	-0.30
TF	0.74	0.239	0.45	0.04	-0.19	0.35	-0.10
FSH%	0.97	-0.003	-0.08	-0.09	0.06	-0.17	-0.07
BEGDA	-0.38	-0.209	0.38	-0.62	-0.08	-0.42	0.26
FLPERI	0.69	0.015	-0.35	0.14	0.12	0.56	0.19
FY	-0.46	0.023	0.36	0.44	0.67	-0.01	0.08

Of the canonical variables for fruiting characters (Table 2.3.16), only the significant first two were examined. The first canonical variable was mainly associated with TW and FNF, and called a yield canonical variable. The second canonical variable was mainly associated with FW and negatively associated with EFFI, and may be called a big fruit canonical variable.The results imply that the vines which were floriferous tended to be high yielding, and vines which produce big flowers tend to bear large fruit.

Table 2.3.16 Canonical structure: correlations between the fruiting variables and their canonical variables

	Canonical Variables						
	W1	W2	W3	W4	W5	W6	W7
HAIR	0.22	0.30	-0.493	0.20	-0.41	-0.111	0.12
VC	0.03	-0.44	0.051	-0.26	0.25	-0.320	-0.01
TW	0.95	0.24	0.004	-0.01	0.08	-0.002	0.03
AC	-0.08	0.05	0.079	0.01	0.09	0.102	-0.65
BRIX	-0.18	-0.25	0.375	0.11	0.49	0.421	-0.02
LOCN	-0.03	0.07	-0.081	0.21	-0.06	0.169	0.38
ELOGN	0.11	0.31	0.124	-0.17	0.17	-0.136	0.46
SYMM	0.07	0.04	-0.042	-0.01	0.09	0.366	0.29
RELCOR	-0.04	-0.08	-0.206	0.22	-0.03	-0.182	0.01
FNF	0.71	0.13	-0.497	0.27	-0.10	0.055	-0.21
FW	-0.12	0.76	0.127	0.14	0.29	-0.119	0.02
EFFI	0.26	-0.55	-0.046	0.46	0.17	-0.189	0.08
PENE	0.15	0.05	0.212	0.05	-0.59	0.068	-0.09

The above results showed that the canonical correlation provided interesting information concerning the relationship between three domains of kiwifruit properties. This analytical method becomes important where there are many characters in each domain, and it would otherwise be difficult to generalise the extent and nature of the variable interrelationships.

In the male seedlings, a relatively high canonical correlation (0.66) was detected between aggregates of vegetative characters (explained mainly by total shoot number per vine and leaf shape index) and flowering characters (explained mainly by flower number per shoot and percentage flowering shoots per vine). This correlation reflects an association between the number of shoots developing in spring, and the vine's flowering efficiency. This is expressed in the field when comparing vine behaviour following mild and cool winters. For instance, following a cool winter there is a high level of bud burst (hence numerous shoots), and many flowers develop on each shoot. Also, warm spring temperatures from the start of bud break result in high bud burst and more flowers are produced. Therefore this analysis supports experimental observation by Warrington and Stanley (1986), and indicates the vine is an integrated system with vegetative development interacting with flower development - and one expects this relationship to have a genetic basis.

The characters TSH and LWRATI appear to be potentially useful indicators to help breeders select a floriferous male vine - possibly even in the kiwifruit's juvenile period. It is interesting to notice that TSH was associated with floriferous characters in both the principal factor analysis and the canonical correlation. However, association of LWRATI with floral characters was identified only in the canonical correlation. This may be due to the differences between the two methods. Although canonical correlation uses the same analytical technique as factor analysis to reduce the dimensionality to a few linear functions, the canonical model selects linear functions that have maximum covariance between domains, while the factor model selects linear functions of original variables that have maximum variance within a domain. In correctly identifying correlations. the canonical model would be the more powerful.

In the analysis of female vines, some associations between flowering and the important characters of yield and fruit size were identified. The canonical correlations showed flower numbers (FSH%, TF and LF) were a good indicator of yield (TW and FNF). While this analysis did not provide any new insights into vine productivity, it serves to illustrate the analytical technique can provide realistic statements about vine performance.

The correlation coefficient between flower size variables (FD and PL) and fruit size was high in the canonical correlation 2, so that a large flower and long pedicel always correlated with large fruit size - therefore FD and PL could be used as selection criteria to select vines with large fruit. However, not all fruit characters of commercial value were found to be associated with any recorded flower characters, for instance, BRIX, SYMM, PENE, ELONG and RELCOR. This suggests more flowering characters, especially physiological characters of the flowers need to be recorded for canonical analysis. Zhang et al (1989) working with wheat found that the canonical correlation of physiological characters with qualitative grain characters was significant, and higher than that with yield-component characters. This indicates the importance of physiological characters in qualitative improvement, and their usefulness in plant selection.

CHAPTER 3

GENETIC ANALYSIS

The relatively long juvenile period of fruit trees, and the high cost of rearing and evaluating seedlings provides a strong impetus for establishing effective breeding methods and also for procedures which permit accurate predictions of population behaviour under the influence of selection. The development of such methods and procedures depends on the exploitation of genetic variability.

A limited number of papers on quantitative genetics of tree fruits have been published. (Barritt, 1981, 1982; Hansche, 1983, 1986a, 1986b; Hansche and Beres 1966; Hansche and Boynton, 1986; Hansche et al 1966, 1972a, 1972b, 1975; Kester et al 1977), but none for kiwifruit. Therefore the objectives of the present study were:

(a) to establish a statistical model of variance component analysis for a factorial mating design.
(b) to estimate heritability and its standard error
(c) to estimate genetic advance for different selection strategies.
(d) to estimate genetic correlations, and to estimate the correlated response amongst a range of characters.
(e) to construct a selection index and to compare index selection with multivariate analysis.
(f) to evaluate the application of these analyses to kiwifruit breeding.

SECTION 1 : GENETIC VARIANCE AND HERITABILITY

1. INTRODUCTION

Variance component analysis, which partitions the phenotypic variance into its genetic and environmental components, is used to estimate the types and extent of gene action. From the results of variance component analysis, heritability values can be generated, and genetic advance can then easily be estimated using the heritability values.

The procedure used in variance component analysis can also be used in the analysis of components of covariance between two metric attributes. Based on the dispersion of genetic effects, genetic correlations and a selection index can be established. This provides further information on the co-inheritance of the observed characters, and indicates effective breeding practices.

The first report of variance component analysis was by Fisher (1918), and others contributed to advancements in this field, but mainly for animal breeding. An interest in the application of quantitative genetics to plant improvement developed in the 1940's, following publications by Comstock and Robinson (1948), and Comstock et al (1949). Genetic studies of quantitative traits have subsequently proved useful in developing effective and efficient breeding methods for many field crops.

2. MATERIALS AND STATISTICAL MODEL

The materials and attributes used for the phenotype analyses (Chapter 2) were used in this study. However a different model was used in the analyses to incorporate the effects of male and female plants and their interaction. The following model was employed:

$$y_{ijkv} = \mu + Y_k + F_i + M_j + (MF)_{ij} + (FY)_{ik} + (MY_{jk}) + (FMY)_{ijk} + V_v + E_{ijkv}$$

Where y_{ijkv} stands for the kth year sample on the vth vine (full sib progeny) from a mating of the ith paternal and jth maternal plant. The μ is a grand mean: Y_k is the effect of year; F_i is the random effect contributed by the maternal parent, (the general combining ability of the genes in the crosses), and M_j by the paternal parent; $(MF)_{ij}$ is the interaction for paternal and maternal parents (specific combining ability of the genes in specific biparental combinations); $(FY)_{ik}$ is the interaction of paternal parent and year, $(MY)_{jk}$ is the interaction of maternal parent and year; $(FMY)_{ijk}$ is the interaction of biparental cross and year; V_v is full-sib individual vine effects; and E_{ijkv} is the residual variance. This model is a years-pooling of a factorial mating design with vines nested within crosses. and without externally blocked replications.

Like most fruit tree crops, the kiwifruit is outbred, with levels of heterozygosity approaching panmictic expectations , and very variable in the F1 hybrid population of any cross. Therefore, genetic parameters were estimated using the F1 populations. Since in plant breeding most estimates of genetic parameters rest on the assumption that tested individuals are a random sample from a large panmictic population, the complication of different procedures for the so-called 'fixed', 'mixed' and 'random' models is in general ignored, random models being preferred (Mayo 1980). Similarly in this study all variance components were treated as random effects.

Based on this model, the dispersion due to the various effects mentioned above were determined by multiple analysis of variance (MANOVA) performed using SAS procedure GLM (SAS Institute Inc. 1985). When samples have unequal subsample number, the basic analysis is that the square of any total is divided by the number of observations in the total, and the computation of the coefficients for the components of variance is therefore far less obvious than when equal numbers prevail (Steel & Torrie, 1981, p 161-162).

Components of variance for the above sources were estimated using the method of Gaylor et al

(1970) which is a single unified procedure for obtaining the expected mean squares from a balanced or unbalanced design. Tables 3.1-3.2 show the expected mean squares for male and female populations. Based on these expected mean squares, the variance components can be obtained (see Tables 3.4 & 3.6).

Table 3.1 The expected mean squares for male vines (following Gaylor et al, for this internally unbalanced design).

SOURCE	DF	EXPECTED MEAN SQUARE
F	2	VAR(ERROR) + 1.3595 VAR(V) + 50.7053 VAR(F*M*Y) + 4.7426 VAR(M*Y) + 71.2196 VAR(F*Y) + 101.4106 VAR(F*M) + 9.4853 VAR(M) + 142.4392 VAR(F)
M	1	VAR(ERROR) + 1.7988 VAR(V) + 26.6748 VAR(F*M*Y) + 61.5893 VAR(M*Y) + 53.3496 VAR(F*M) + 123.1787 VAR(M)
F*M	2	VAR(ERROR) + 1.6476 VAR(V) + 17.4572 VAR(F*M*Y) + 34.9145 VAR(F*M)
Y	1	VAR(ERROR) + 51 VAR(F*M*Y) + 142.9252 VAR(M*Y) + 71.5607 VAR(F*Y) + 214 VAR(Y)
F*Y	2	VAR(ERROR) + 50.7053 VAR(F*M*Y) + 4.7426 VAR(M*Y) + 71.2196 VAR(F*Y)
M*Y	1	VAR(ERROR) + 26.6748 VAR(F*M*Y) + 61.5893 VAR(M*Y)
F*M*Y	2	VAR(ERROR) + 17.4572 VAR(F*M*Y)
V	208	VAR(ERROR) + 3.2755 VAR(V)
ERROR	208	VAR(ERROR)

Table 3.2 The expected mean squares for female vines (following Gaylor et al, for this internally unbalanced design)

SOURCE	DF	EXPECTED MEAN SQUARE
F	2	VAR(ERROR) + 1.6218 VAR(V) + 44.5282 VAR(F*M*Y) + 5.0202 VAR(M*Y) + 61.7754 VAR(F*Y) + 89.0565 VAR(F*M) + 10.0405 VAR(M) + 123.5508 VAR(F)
M	1	VAR(ERROR) + 1.6583 VAR(V) + 21.8017 VAR(F*M*Y) + 51.6921 VAR(M*Y) + 43.6034 VAR(F*M) + 103.3842 VAR(M)
F*M	2	VAR(ERROR) + 1.6292 VAR(V) + 14.9451 VAR(F*M*Y) + 29.8903 VAR(F*M)
Y	1	VAR(ERROR) + 46.2513 VAR(F*M*Y) + 125.2673 VAR(M*Y) + 63.4491 VAR(F*Y) + 187 VAR(Y)
F*Y	2	VAR(ERROR) + 44.5282 VAR(F*M*Y) + 5.0202 VAR(M*Y) + 61.7754 VAR(F*Y)
M*Y	1	VAR(ERROR) + 21.8017 VAR(F*M*Y) + 51.6921 VAR(M*Y)
F*M*Y	2	VAR(ERROR) + 14.9451 VAR(F*M*Y)
V	181	VAR(ERROR) + 3.0166 VAR(V)
ERROR	181	VAR(ERROR)

The experimental design was similar to the North Carolina Design II described and evaluated by Comstock and Robinson (1952). Males were crossed in all combinations with the females to produce full-sib families. Individuals may be classified as full sib (FS) within each biparent cross, or as half-sib (HS) sharing a common male parent, or as HS sharing a common female parent. Because both male and female plant effects were built into this design, the model was especially suitable for dioecious species like kiwifruit.

Based on the fundamental equations $Va = 2pq\ [a + d(q-p)]^2$ and $Vd = (2pqd)^2$ in a population at Hardy-Weinberg equilibrium (Falconer 1981), the following genetic interpretation of resemblance (expressed as covariance) between relatives was derived for this factorial design (Baker 1986, Becker 1967):

Table 3.3 The genetic interpretation of variance components

variance components	Relatives	Va	Vd	Vaa	Vad	Vdd
F	covHS	1/4	0	1/16	0	0
M	covHS	1/4	0	1/16	0	0
MF	covFS-(covHS(F) +covHS(M))	0	1/4	1/8	1/8	1/16
V	Total-covFS	1/2	3/4	3/4	7/8	15/16

F is the variance of half sib families with female parent in common, M is the variance of half sib families with male parent in common, MF is the variance of full sib families. V is variance among vines in FS families; Va = additive genetic variance, Vd = dominance genetic variance, Vaa = variance due to additive x additive epistatic interaction, Vad = variance due to additive x dominance epistatic interaction, etc. (Baker 1986).

Three types of heritability were estimated in this study, and they were all obtained from the sib analysis outlined above. They were:

1. h^2 (narrow), the heritability in the narrow sense. It is defined as the ratio of additive genetic variance to phenotypic variance;
2. h^2 (broad), the heritability in the broad sense. It is defined as the ratio of total genetic variance to phenotypic variance; and
3. h^2 (Sca), the heritability of specific combining ability. It is defined as the ratio of non-additive genetic variance to phenotypic variance.

In the following text, any reference to 'heritability' indicates narrow sense heritability unless otherwise specified.

Since both male and female components have the same interpretation, it is best to use the average of the two to estimate genetic variance (Baker, 1986; Becker 1967), therefore heritabilities can be estimated as:

$$h^2\text{ (narrow)} = 2(V_f + V_m)/V\text{ total}$$
$$h^2\text{ (sca)} = (4V_{mf})/V\text{ total}$$
$$h^2\text{ (broad)} = (V_f + V_m + V_{mf} + V_v)/V\text{ total}$$

where Vtotal = $Vf + Vm + Vmf + Vv + Vy + V_{fy} + V_{my} + V_{fmy} + Ve$.

Using these equations the heritability values (Tables 3.5, 3.7) were calculated.

The precision of an estimate of heritability is indicated by its standard error. For estimating its standard error, the above definitions of heritability can be reconstructed as:

$$h^2\text{ (narrow)} = x1/y$$
$$h^2\text{ (SCA)} = x2/y$$
$$h^3\text{ (broad)} = x3/y$$

Where $x1 = 2(V_f + Vm)$, $X2 = 4Vmf$, $X3 = V_f + Vm + Vmf + Vv$, and $y = Vtotal$.

The variance of h^2 may be obtained approximately from:

$$V_{\text{heritability}} = [(U_y{}^2 * V_x{}^2) + (U_x{}^2 * V_y{}^2) - 2U_x U_y * COV(x,y)]/U_y{}^4$$

where $U_y = E(y)$, $U_x = E(x)$ (Kempthorne, 1957; Osborne & Paterson, 1952). In practice, the parameters are replaced by the corresponding estimates.

Sample variances of estimated variance components for a balanced design may be obtained by

$$Vi = 1/n^2\Sigma[2(MS_i)^2/(f_i + 2)]$$

Where n = the divisor appropriate in the estimator of a specific variance component. MS_i = the ith mean square in estimator, and f_i = the degree of freedom of the ith mean square (Crump 1946, 1951). According to Kempthorne (1957), when calculating the variance of an estimated variance and covariance, one should replace df by (df + 2) to remove bias.

For unbalanced data, the procedure for variance estimation of variance components is very complex (see Searle, 1971), therefore the approximation method of Jensen and Barr (1971) was used in this study. The variance-covariance matrix of the components, V(cc'), can be calculated as:

$$V(cc') = X^{-1}\, V(RR')X^{-1'}$$

where X is a matrix of the coefficient of components, and V(RR') is a diagonal matrix, each diagonal element being the variance of the appropriate mean square approximated by the well-known expression $2(MS_i)^2/(f_i+2)$, MS_i is the ith mean square and f_i is the associated degree of freedom.

3. RESULTS AND DISCUSSION

Male Vines:

Table 3.4 shows the values of variance components of 12 characters for male vines. It is obvious that these components vary with the character investigated.

Table 3.4 Variance component estimation for male vines

	F	M	F*M	Y	M*Y	M*Y	F*M*Y	V	ERROR
FD	- 0.0035	0.0326	0.0100	-0.0047	-0.0353	0.0078	0.0464	0.1243	0.181
PL	0.0434	0.3269	0.0636	0.0893	-0.0035	-0.0225	0.0429	0.2726	0.375
TF	0.0913	0.0026	0.0496	0.1053	-0.0198	-0.0183	0.0319	0.1437	0.536
LF	- 0.4109	0.8010	1.1574	0.2074	0.0173	-0.0126	-0.0212	1.2052	2.679
PETICO	0.0028	-0.0033	0.0049	-0.0215	0.0390	0.0075	0.0078	0.0742	0.229
DIASP	- 0.0099	0.0607	0.0155	0.0416	-0.0004	0.0026	0.0005	0.1054	0.012
TSH	1.6107	0.5165	2.9681	3.7630	-5.1464	-1.8191	6.8598	40.5676	46.722
FSH%	-41.4032	68.8683	94.0976	6.6337	5.2337	1.0389	-10.7214	59.8930	221.630
BEGDA	- 0.2131	2.3079	0.0761	0.0712	-0.8439	-0.0847	1.2875	2.405	3.812
LWRATI	- 0.0001	0.0031	0.0008	0.0004	-0.0011	-0.0006	0.0015	0.0028	0.008
INCRS	- 0.0066	0.1152	0.0141	0.0764	-0.0012	-0.0111	0.0124	0.0945	0.402
FLPERI	2.5652	2.5858	0.8361	0.8464	0.4044	0.4560	-0.0153	3.2070	9.721

As mentioned above, the different components had different genetic interpretations (Table 3.3), based on these variance components, the heritability values were calculated and listed in Table 3.5.

Table 3.5 Heritability estimates for male vines

TRAITS	h^2(narrow)	h^2(sca)	h^2(broad)
FD	0.16 (0.09)*	0.11	0.46
PL	0.62 (0.10)	0.21	0.60
TF	0.20 (0.08)	0.22	0.31
LF	0.14 (0.07)	0.82	0.49
PETICO	0.00 (0.10)	0.06	0.23
DIASP	0.44 (0.16)	0.27	0.75
TSH	0.04 (0.10)	0.12	0.48
FSH%	0.14 (0.08)	0.93	0.45
BEGDA	0.48 (0.09)	0.03	0.52
LWRATI	0.41 (0.36)	0.23	0.45
INCRS	0.31 (0.08)	0.08	0.31
FLPERI	0.50 (0.08)	0.16	0.45

*Standard error

According to Falconer (1981), h^2 (narrow) determines the degree of average resemblance between relatives, and is therefore of the greatest importance in breeding programmes.

The standard of Hansche et al (1966) was used in this study to examine the heritability level - namely, very high (above 0.8), moderate to high (0.3-0.6) and low (below 0.2).

Study of Table 3.5 reveals that for male vines, PL, DIASP, BEGDA, LWRATI and FLPERI have high heritability, indicating that for these traits, the selection of superior seedlings for use as parents in successive crosses could result in rapid genetic improvement to subsequent generations. Some of these characters are of interest for kiwifruit breeding. Selection for FLPERI, for example, could be used to produce a superior male of either long or short flowering period to cover the flowering duration of present and future female cultivars, or to facilitate harvesting flowers in a male orchard.

PETICO and TSH had very low narrow-sense heritability in this study. Three possible explanations for low values for narrow sense heritability are:

(1) genetic homogeneity of the sample population investigated.

(2) low levels of average gene effects relative to dominance/epistatic effects in the expression of these traits, or

(3) large relative effect of non-genetic (environmental) variability (Hansche, 1972). Whatever the case, in breeding practice such characters cannot be improved efficiently by individual selection, which operates through improving the average genetic worth of successive generations.

The rest of the traits in male vines gave low or moderate heritability (narrow), indicating individual selection may be an effective and efficient method to improve these traits, but the rate of genetic gain would be lower than that of traits with high heritability.

It is of interest to notice that LF and FSH% gave very high value for h^2(sca) (see Table 3.5). This means that great differences can be expected among different segregation combinations for these two characters.

Female Vines:

Table 3.6 show the values of variance component of 23 characters for female vines. Some characters have relatively large values of genetic components, while some have not. Therefore different heritability estimates were obtained on the basis of these components, and were listed in Table 3.7.

Table 3.6 Variance component estimation for female vines

Traits	F	M	F*M	Y	F*Y	M*Y	F*M*Y	V	ERROR
FD	0.019	0.0569	0.0086	-0.0064	0.0068	0.0111	-0.0139	0.178	0.240
PL	0.105	0.2585	-0.0147	0.0200	0.0143	0.0115	-0.0223	0.320	0.393
TF	0.056	0.0343	-0.0018	0.0068	0.0219	0.0067	-0.0329	0.115	0.537
PETICO	0.012	-0.0235	0.0192	-0.0198	0.0017	0.0276	0.0051	0.092	0.196
HAIR	-0.009	-0.0057	0.0089	0.0423	-0.0037	-0.0045	0.0144	0.095	0.266
VC	105.005	-41.2351	85.2941	8.2813	-12.0045	-7.1306	15.9743	338.573	97.948
TW	0.010	0.0727	-0.0093	0.0034	-0.0020	-0.0046	0.0003	0.047	0.242
AC	0.007	0.0031	0.0016	0.0029	0.0003	0.0013	-0.0004	0.024	0.012
DIASP	0.000	0.0305	0.0002	0.0658	0.0001	0.0010	-0.0002	0.104	0.021
TSH	-2.134	-7.0365	3.6506	-4.1849	4.0034	11.5308	-5.7057	6.826	119.669
FSH%	8.157	91.6582	-10.4611	6.2784	3.8892	-5.5871	2.3828	26.125	244.589
BEGDA	0.278	0.2327	-0.3212	0.0980	0.0052	-0.0188	-0.0412	3.604	5.164
FLPERI	0.738	0.9391	0.1890	1.6476	0.2039	0.0114	-0.1374	2.135	11.128
BRIX	-0.101	-0.0785	0.1687	0.2029	-0.0728	-0.0607	0.1055	0.696	0.994
LOCN	1.704	-1.1938	2.5993	-0.1071	0.6380	0.0002	-0.6020	7.006	13.935
LWRATI	0.001	0.0035	-0.0004	0.0009	-0.0003	-0.0005	0.0008	0.002	0.007
INCRS	0.020	0.1084	-0.0066	0.2317	-0.0048	-0.0088	-0.0014	0.216	0.495
ELONG	0.017	0.0002	0.0003	0.0074	0.0012	0.0000	-0.0002	0.023	0.005
SYMM	0.000	0.0002	-0.0001	0.0000	-0.0001	-0.0000	0.0001	0.001	0.002
RELCOR	0.000	-0.0000	0.0001	0.0002	-0.0001	-0.0000	0.0001	0.000	0.001
FNF	0.084	-0.0042	0.0027	0.0376	-0.1263	-0.0622	0.1720	0.070	0.767
FW	102.402	19.8385	4.0174	44.7006	1.9416	-0.5341	-5.3802	148.072	150.789
EFFI	0.002	-0.0000	-0.0002	0.0092	0.0008	-0.0001	-0.0002	0.008	0.010

For female vines, PL, TW, FSH%, LWRATI, ELONG, and FW were shown to have a moderate to high heritability indicating that the selection of superior lines for these traits would result in rapid genetic progress among successive offspring. Moreover, as most of these characters are of interest in kiwifruit breeding, the heritability estimates for them is particularly relevant to a programme aimed at improving productivity and quality of kiwifruit.

Table 3.7 Heritability estimates for female vines

TRAITS	h^2(narrow)	h^2(sca)	h^2(broad)
FD	0.30 (0.08)*	0.07	0.53
PL	0.67 (0.07)	-0.05	0.62
TF	0.24 (0.09)	-0.01	0.27
PETICO	-0.08 (0.12)	0.25	0.32
HAIR	-0.07 (0.12)	0.09	0.22
VC	0.22 (0.08)	0.58	0.83
TW	0.46 (0.11)	-0.10	0.34
AC	0.39 (0.40)	0.13	0.70
DIASP	0.28 (0.10)	0.00	0.61
TSH	-0.14 (0.15)	0.12	0.01
FSH%	0.54 (0.03)	-0.11	0.31
BEGDA	0.11 (0.10)	-0.14	0.42
FLPERI	0.20 (0.09)	0.04	0.24
BRIX	-0.19 (0.12)	0.36	0.37
LOCN	0.04 (0.10)	0.43	0.42
LWRATI	0.61 (0.14)	0.11	0.42
INCRS	0.24 (0.00)	-0.03	0.32
ELONG	0.65 (0.12)	0.02	0.75
SYMM	0.30 (0.13)	-0.09	0.41
RELCOR	0.13 (0.10)	0.19	0.38
FNF	0.17 (0.11)	0.01	0.16
FW	0.52 (0.08)	0.03	0.59
EFFI	0.12 (0.07)	-0.03	0.33

*Standard error

PETICO and TSH had very low heritability - just as they did in male vines. HAIR, BRIX, and LOCN also had low heritability. These characters cannot be improved efficiently by individual selection or related strategies. However, some of them are considered important characters for the ideotype and further studies are needed to discover if there is genetic variability for them in other gene pools. Although the narrow sense heritability value is low for these characters, the broad sense heritability is reasonably high for some of them. for example BRIX (0.37) and hairiness (0.22), indicating that there are some dominant and/or epistatic effects involved. Because kiwifruit is clonally propagated, all of the genetic variances - additive, dominant and epistatic can be utilised for improving these characters by circumventing the usual sexual generation progression. However, total shoots per vine (TSH) had very low value in all three kinds of heritability estimates. There is apparently no large genetic variance of any kind in this population for this trait, and with this level of heritability it is not possible for selection to be effective in improving this character as far as judged from this population.

The rest of the traits, including such important characters as TF, VC, AC, SYMM, RELCOR and FNF in female vines gave moderate or low heritability, indicating individual selection may have moderate success in improving these traits, but the rate of genetic gain would be lower than that of traits with high heritability. Therefore more sophisticated methods which employ family selection based on dispersion would be highly beneficial.

The standard errors for h^2 (narrow) displayed in Table 3.5,3.7 showed that most estimates have reasonably low value, indicating that the heritabilities for these characters were estimated with considerable precision. This may be resulted from either the appropriate and accurate measurements of these characters or sufficient sample size. To some fruit characters, a particular problem was the variability within a vine. To account for this two canes in the middle of the canopy of each vine were sampled, and measurements for the characters investigated were carried out on these two canes. Thus the sampling error of experimental units was reduced, and the precision of heritability estimates was enhanced.

It is very important that the limitations of estimates of heritability are realised. While a mean is a first degree statistic, variance is a second degree statistic which is inherently less precisely estimated. Heritability, being the ratio of variances, not only shares all biological restrictions for estimating genetic variances, but also shares the higher imprecision of second-degree statistics (Ewing, 1987).

The more common assumptions for estimating genetic variances are:

1. normal diploid behaviour at meiosis
2. no maternal or cytoplasmic effects
3. no selection, and no epistasis in the estimation population (Sprague 1966).

In the current study, only three female cultivars and two male cultivars of kiwifruit were involved, all belonging to one species, A. deliciosa. This is a restricted population of inference, although it does represent a much wider gene pool than that commonly studied in New Zealand.

Based on chromosome numbers, this species appears to be a hexaploid, which would introduce bias from the failure of assumption (1). Kempthorne (1955, 1957) examined the complexities of interpreting genetic relationships for auto-tetraploids. He showed that, because dominance effects are involved in the covariance between half-sibs, the additive effects estimated according to a diploid model will be positively skewed. The same could be true of a hexaploid so that the magnitude of narrow sense h^2 (reflecting additive gene effects) will be larger than it should be. However, it appears that we do not need to be concerned about this source of bias here, because Zhu (1981) found that like A. chinenesis, A. deliciosa also has a regular chromosomal behaviour in the whole process of meiosis for the pollen mother cell. This indicates that kiwifruit is a diploidised polyploid, and that the use of diploid genetic analysis in this work is valid.

An estimate of h^2 strictly applies to a particular metrical character, in a particular population, at a particular moment and in a particular environment. Thus even for a single character, h^2 is not constant, so caution is necessary in interpreting estimates of h^2 (Ewing et al. 1987). However, some inference can still be obtained by comparison of h^2 for the same character in different species. In this study, a zero estimate of h^2 for BRIX was obtained (small negative estimate),and a similar result (0.01) was reported for the peach tree (Hansche et al. 1972). Other estimates are 0.17 for dwarf peach, and 0.35 for dwarf nectarine (Hansche, 1986). While the values differ, in general the h^2 of BRIX is relatively low. But for fruit size, weight and length, Hansche (1983) pointed out that the traits in different species had similarly high h^2. The results of this work agree with this conclusion, the h^2 for mean fruit weight was 0.52, and 0.65 for fruit elongation. This implies that considerable genetic potential for increasing fruit size exists in fruit tree breeding, even by simple individual selection.

Apart from the problems discussed above, due to sampling error genuinely low values for some variation components may be obtained. In the estimation of h^2, negative estimates are frequently equated to zero, but this obviously, leads to bias in the results. In this work, such bias was not introduced and PETICO, TSH, HAIR, and BRIX have been reported as showing low negative h^2 values.

In this work some standard errors of heritability estimates were found to be uncomfortably large. This may be caused by inherently large non-genetic variability in the data, the use of crude subjective scores, or insufficient sample size. Hansche (1972) pointed out that there was considerable evidence that the variability associated with subjective scores, such as crop rating were large and may be a major cause of low heritability estimates. Increasing sample size can increase precision, thus smaller differences between cross means become detectable at a given probability level (Hansche and Beres, 1966a). But if the sample size is larger than required, more work is done than necessary. Therefore it is suggested the optimum sample size should be estimated before measurement starts.

SECTION 2 : GENETIC CORRELATION

1. PROCEDURES

Genetic correlation is the ratio of the additive genetic covariance to the geometric mean of the components of additive genetic variance. It implies a relationship between the genes that influence the two traits.

The estimation of genetic correlations is based on the resemblance between relatives and the method is analogous to the estimation of h^2. Instead of computing the variance components of one character, the components of covariance of the two characters are computed, and rather than partitioning sums of squares, the sum of cross-products of the two characters are partitioned according to the sources of variation. This gives estimates of the observational components of covariance, and the interpretation of these estimates is exactly the same as that of the variance components shown in Table 3.1. For example, in an analysis of HS families, the component of covariance between males and that between females estimates one-quarter of the covariance of the breeding values of the two characters. Therefore the additive genetic correlation (r_A) is:

$$r_A = cov_{xy} / \sqrt{(var_x \, var_y)}$$

where var and cov refer to the components of variance and covariance respectively.

According to Van Vleck and Henderson (1961), the genetic correlation will not be accurately determined if at least one of the 2 traits has a low h^2 estimate. Therefore genetic correlation may not be estimated where the compontents of genetic variance are negative or of very low value (Godshalk et al, 1988, Melchinger 1988). On this basis, excluded from correlation analysis for female vines were PETICO, HAIR, TSH, BRIX (negative h^2), and LOCN (low h^2 of 0.04 and very low value of component of genetic variance). For the male population, PETICO and TSH were excluded because of their low h^2 (0.00 and 0.04) and very low value of component of genetic variance.

2. RESULTS

The genetic correlations are presented in Tables 3.8 and 3.9. The environmental effects have been partitioned out, so these values are purely a genetic correlations. These relationships may contrast with phenotypic observation, due to environmental influences that modify gene expression.

Table 3.8 Additive genetic correlations (above diagonal) and phenotypic correlation (below diagonal) for male seedlings

	FD	PL	TF	LF	DIAS	FSH%	BEGD	LWRA	INCR	FLPE
FD	1	1.00	-1.00	-0.78	0.85	0.09	-0.26	0.17	0.28	-0.14
PL	0.53	1	-0.55	0.61	0.90	1.00	-0.62	-0.69	0.82	0.03
TF	0.14	0.01	1	0.51	0.11	0.44	-0.25	-0.22	-0.05	1.00
LF	0.28	0.30	0.72	1	1.00	1.00	-0.89	-0.80	0.82	1.00
DIASP	0.39	0.59	0.05	0.33	1	1.00	-0.90	-0.92	0.88	0.51
FSH%	0.32	0.30	0.62	0.77	0.30	1	-0.88	-0.69	0.97	1.00
BEGDA	-0.31	-0.33	-0.37	-0.54	-0.33	-0.58	1	0.73	-0.98	-0.65
LWRATI	-0.16	-0.27	-0.23	-0.36	-0.29	-0.42	0.36	1	-0.99	-0.61
INCRS	0.27	0.42	0.01	0.21	0.54	0.23	-0.24	-0.21	1	0.39
FLPERI	0.20	0.14	0.62	0.68	0.18	0.67	-0.58	-0.34	0.13	1

The significant values of phenotypic correlation coefficient based on error df = 208 are 0.14 for P = 0.05 and 0.18 for P = 0.01.

Table 3.9 Additive genetic correlations (above diagonal) and phenotypic correlation (below diagonal) for female seedlings

	FD	PL	TF	VC	TW	AC	DIAS	FSH%	BEGD	FLPE	LWRA	INCR	ELOG	SYMM	RELC	FNF	FW	EFFI
FD	1	1.00	0.08	-0.31	0.58	0.48	0.86	-0.57	0.58	-0.93	-0.59	0.74	0.11	1.00	1.00	-0.29	1.00	-0.41
PL	0.53	1	0.05	-1.00	0.52	0.06	1.00	-0.47	0.51	-0.74	-0.75	0.95	-0.28	0.88	1.00	-0.25	0.90	-0.74
TF	0.08	0.13	1	1.00	0.84	1.00	0.25	0.84	-0.07	0.95	-0.37	0.16	0.89	-0.19	-0.61	1.00	-0.46	0.75
VC	-0.21	-0.30	0.22	1	0.59	0.65	0.06	0.66	1.00	1.00	-0.03	-0.06	0.91	-1.00	-1.00	1.00	-1.00	1.00
TW	0.28	0.30	0.74	0.13	1	0.86	0.80	1.00	-0.46	0.79	-0.63	0.76	0.44	0.14	-0.23	0.89	0.04	0.22
AC	0.03	-0.06	0.22	0.39	0.17	1	0.58	0.94	0.04	1.00	-0.44	0.43	0.88	-0.55	-1.00	1.00	-0.68	0.69
DIASP	0.31	0.50	0.13	-0.21	0.27	-0.13	1	0.79	-0.82	0.19	-0.78	0.92	0.01	1.00	1.00	-0.08	0.89	-0.46
FSH%	0.21	0.23	0.70	0.16	0.91	0.23	0.20	1	-0.47	0.77	-0.65	0.76	0.47	0.12	-0.35	1.00	0.03	0.22
BEGDA	-0.11	-0.11	-0.29	0.10	-0.39	-0.06	-0.04	-0.34	1	-0.12	0.38	-0.96	0.24	-0.37	-0.31	-0.22	-0.63	0.67
FLPERI	0.08	0.01	0.56	0.19	0.63	0.22	-0.04	0.61	-0.45	1	-0.57	-0.04	1.00	0.25	0.10	0.25	-0.15	0.75
LWRATI	-0.17	-0.37	-0.18	0.00	-0.26	-0.03	-0.35	-0.28	0.03	-0.11	1	-0.63	0.02	-0.96	-1.00	0.48	-0.56	0.35
INCRS	0.20	0.37	-0.03	-0.15	0.11	-0.07	0.58	0.03	-0.03	-0.13	-0.33	1	-0.12	0.86	0.94	0.20	0.86	-0.52
ELOGN	0.11	0.05	0.14	0.24	0.12	0.09	0.30	0.06	0.10	0.05	-0.38	0.19	1	-0.75	-1.00	1.00	-0.79	0.92
SYMM	0.14	0.28	0.05	-0.18	0.22	-0.15	0.12	0.20	-0.10	0.07	-0.20	0.17	-0.20	1	1.00	-0.84	0.87	-1.00
RELCOR	0.09	0.23	-0.17	-0.27	-0.02	-0.26	0.24	-0.03	-0.08	-0.20	-0.21	0.25	-0.13	0.50	1	-0.83	1.00	-1.00
FNF	0.10	0.06	0.68	0.24	0.72	0.14	0.11	0.63	-0.24	0.43	-0.08	0.02	0.16	0.03	-0.11	1	-0.69	1.00
FW	0.36	0.46	-0.29	-0.42	-0.08	-0.29	0.35	-0.22	-0.06	-0.27	-0.21	0.33	0.01	0.22	0.33	-0.30	1	-1.00
EFFI	-0.17	-0.30	0.38	0.32	0.36	0.27	-0.61	0.44	-0.14	0.45	0.14	-0.62	-0.19	-0.01	-0.21	0.29	-0.44	1

The significant values of **phenotypic** correlation coefficient based on error df = 181 are 0.15 for P = 0.05 and 0.20 for P = 0.01.

Male population

Flower diameter: FD was strongly positively correlated with PL, DIASP, and negatively correlated with TF, indicating (1) the larger the flower, the longer the pedicel; (2) a vigorous vine (expressed as diameter and increment of main stem) is associated with large flowers; (3) increasing terminal flower number per shoot is associated with decreasing flower size.

Pedicel length: PL was strongly correlated with DIASP, FSH% and INCRS and negatively correlated with LWRATI, indicating high vigour vine was associated with long pedicels and broad leaves.

Terminal flower number per shoot: TF had a strong correlations with FLPERI. This suggests that the two characters can be improved together in the selection process.

Lateral flower number per shoot: LF had strong correlations with DIASP, FSH%, INCRS, FLPERI, and was negatively correlated with BEGDA, LWRATI, indicating vigorous vines blossomed early in the season, and tended to be interrelated with more lateral and terminal flowers and broad leaves. A high incidence of lateral flowers in male kiwifruit was associated with vines being floriferous with a long flowering duration.

Diameter of main stem in spring: DIASP was strongly correlated with FSH%, INCRS, and negatively correlated with BEGDA, and LWRATI, indicating that the vine with a thick main stem tended to be floriferous, to blossom early and to produce broad leaves genetically.

Flowering shoot % : as expected, FSH% was strongly correlated with FLPERI, because more flowers result in a longer flowering period. FSH% also was negatively correlated with BEGDA and LWRATI, suggesting a vine with many flowering shoots tended to blossom early in the season and to produce broad leaves. Also FSH% was strongly associated with INCRS.

Beginning date of blossom in the season: There was a relatively high positive correlation with LWRATI, and a negative one with INCRS, this means weak vines tended to blossom late in the season and to have elongate leaves.

Leaf shape index: LWRATI was negatively associated with INCRS, suggesting a vigorous vine tended to produce broader leaves.

A study of the phenotypic correlations (Table 3.8) showed they were similar to the genetic correlations in direction, but different in magnitude. It was obvious that genetic correlation in general were higher than corresponding phenotypic correlation. This was in agreement with the results obtained by Godshalk et al (1988), Hansche et al (1966), Tyagi (1987) and several others.

According to Shaw (1988), genetic correlation implies a relationship beween the genes that condition two traits, whereas phenotypic correlation results from both shared genetic effects and a common response of independent genetic systems to shared environments. If the two types of corelation are similar to each other, the selection on phenotype will also be a fairly "accurate" selection of genotype.

In male kiwifruit floriferousness (expressed as percentage floral shoots and lateral flower number) is considered an important character. The results show that it is strongly associated with diameter of the main stem and leaf shape index. This suggests that diameter of main stem and leaf shape index may be used as guides to select superior male vine for heavy flowering, although this relationship may not hold for other populations. In breeding practice, a search is always being made for characters which can be assessed in the juvenile period and are correlated with fruit characters in the mature tree, in order to discard undesirable plants at an early stage. The correlation should be of fairly high order and the character in the juvenile plant must be reasonably easy to recognise. The two characters mentioned may be suitable for such a purpose. These correlations were established on young mature vines (3-4 years) and require confirmation, since there is no proof on even younger plants.

It is of interest to note that the flowering components TF and LF had differing relationships with the vigour indicators INCRS and DIASP. LF was strongly correlated with INCRS and DIASP in genetic correlation (0.82 and 1.00), while TF was not (-0.05 and 0.11). The same was true in phenotypic correlation (Table 3.8). The correlation coefficients of LF with INCRS (0.21) and DIASP (0.33) both were significant at P = 0.01, while the coefficients of TF with these two characters were not (0.01 and 0.05). This suggests that terminal flowers and lateral flowers have different response mechanisms to vine vigour.

In general, the floriferousness which is expressed by FSH% (many floral shoots) and LF (many flowers) was always associated with vine vigour characters (INCRS and DIASP) within the male population in this study, therefore selection for floriferous genotypes with vigour should produce rapid genetic gain among the offspring. On the other hand, where floriferousness and less vigour is the selection goal, genetic progress may be considerably more difficult to obtain, due to the unfavourable genetic correlations between these characters. Under these circumstances, a selection index should be used to make a balance between these characters in order to select parents efficiently.

Female population:

Flower diameter: FD was strongly correlated with PL, DIASP, INCRS, SYMM, RELCOR and FW. This implies a strong relationship between the genes that conditioned FD and the genes that conditioned these other traits, and that one could improve the FD character and these characters simultaneously. In contrast FLPERI had a strong negative correlation with FD, indicating that the vines with short flowering duration were associated with big flowers.

Pedicel length: Like FD, PL had strong correlations with DIASP, INCRS, SYMM, RELCOR and FW. PL had a strong negative correlation with VC, indicating that pedical length could be a useful indicator for high Vitamin C kiwifruit selection.

Terminal flower number per shoot: TF had strong correlations with VC, TW, AC, FSH% FLPERI, ELOGN and FNF. While it could be predicted that TF would have a strong relationship with fruit productivity characters, its relationship with VC and AC was unexpected and indicates these characters can be improved together in the selection process.

Vitamin C: This important character is negatively related to SYMM, RELCOR and FW, indicating that big fruit tend to have a lower vitamin C content. In plant breeding, improvement in both fruit size and vitamin C are required but one cannot improve the two characters simultaneously due to the negative direction of the correlation - selection for one character will inhibit the selection progress of the other. Therefore a selection index is needed. The results also showed that vitamin C was strongly associated with BEGDA, FLPERI, FNF, EFFI and ELOGN, indicating these characters can be improved together.

Total fruit weight: TW had a strong relationship with FNF, but its relatively strong relationships with DIASP (0.80) and INCR (0.76) are of interest. Just like in the male population, in females a vigorous vine tends to be floriferous and tends to be productive.

Acidity: AC was strongly associated with FSH%, FLPERI, ELOGN, FNF, and negatively associated with RELCOR. This indicated that the longer fruits with a smaller core on prolific vines tended to have high acidity. Since AC was associated with a number of characters of horticultural value. it was considered an important character. But increasing AC is undesirable unless BRIX also increases. This is because it is the balance between the two that primarily determine acceptability of the fruit. For example, the majority of successful dessert apples are in the group of medium acid and medium sugar, medium acid and high sugar, and low acid and medium sugar (Brown, 1975).

Diameter of main stem in spring: DIASP was strongly correlated with INCRS, SYMM, RELCOR, FW, and negatively correlated with BEGDA, and LWRATI, suggesting vigorous vines blossomed early, bore big fruit and produced broader leaves.

Flowering shoot %: FSH% was strongly correlated with FNF, indicating vines with many flowering shoots tended to produce floral shoots with many fruits.

Beginning date of blossom in the season: BEGDA showed a strong negative value of correlation with INCRS,this means weak vines tended to be associated with late blossom in the season.

Flowering period: FLPERI had strong relationships with ELOGN, indicating the vines with longer fruit tended to be associated with a long blossom period.

Leaf shape index: LWRATI was negatively associated with RELCOR and SYMM, suggesting the vine with broader leaves tended to be associated with asymetrical fruit with large core size.

Increment of main stem: INCRS strongly associated with SYMM, RELCO, and FW, suggesting a vigorous vine produced big asymmetrical fruits, with large core size.

Elongation of fruit: ELONG was strongly correlated with FNF and EFFI, and negatively associated with SYMM, RELCO, and FW, and it could be predicted that some of these relationships would occur in a seedling population if parent vines were selected according to these characters.

Symmetry of fruit: SYMM was strongly correlated with RELCOR, FW, but negatively associated with FNF and EFFI, indicating the more fruit that set on a fruiting shoot, the smaller the fruit size, and the more regular the fruit's shape.

Relative core size of fruit: RELCOR was strongly correlated with FW, but negatively associated with FNF and EFFI, indicating that the more the fruit set on a fruiting shoot, the smaller the core size of the fruit.

Fruit number per fruit shoot: FNF was strongly associated with EFFI, indicating it is possible to select for improvement in both characters at the same time.

Average fruit weight: FW was negatively associated with EFFI, indicating an efficient vine tended to produce small fruit.

The results showed that as in the male population most phenotypic correlations were similar to the corresponding genetic correlation in direction, but different in magnitude (Table 3.9).

In this work, vine vigour (expressed as DIASP and INCRS) was shown to be an important character because it was strongly correlated with FW and TW and significantly negatively correlated with the BEGDA. This indicates that a vigorous vine tended to be productive, and to produce large fruit and to blossom early in the season. Such a vine would be of commercial interest.

Watkins and Spangelo (1970) in a diallel analysis of apple seedling vigour (expressed as plant height) found that different sets of parents had different additive genetic variability, varying from 50% to 100%. That is, plant vigour is heritable, and can be improved by selection. In the current study, the h^2 of DIASP and INCRS are 0.28 and 0.24 for female plants, 0.44 and 0.31 for male vines, and the results confirm the above finding.

The strong correlation between leaf shape index and vine vigour indicates the leaf shape index may be useful as a guide for pre-selection.

It is important to realise some limitations in the determination of a genetic correlation. Firstly, estimates of genetic correlations from variance and covariance components are usually subject to rather large sampling error and are therefore seldom very precise. Furthermore, since the distribution of such statistics is not known, their variances cannot be calculated (Griffing, 1956). Secondly, genetic correlations are strongly influenced by gene frequencies, so they may differ markedly in different populations (Falconer 1981). For these reasons, the results in this work must be regarded as estimates for this population and are not necessarily valid for other populations. Thirdly, the genetic correlation will be larger in absolute magnitude than the phenotypic correlation. This mainly results from the very low value of genetic variance component (Baker, 1986). Many large values of genetic correlation have occurred in this analysis, and the results are in agreement with this conclusion.

SECTION 3 : SELECTION RESPONSE

1. SELECTION RESPONSE FOR INDIVIDUAL SELECTION

The change in the population mean produced by selection is called the response to selection, which is symbolised by R in individual selection , in which individuals are selected only in accordance with their own phenotypic values.

$R = h^2*S$

where S is the average superiority of the selected parents, which is called the selection differential, and h^2 is the heritability of the average allele effects for the character involved. The chief use of R is for predicting the genetic advance from selection and for monitoring the success of practical selection response.

The R may be generalised if S is expressed in terms of the phenotypic standard deviation, $\sigma(p)$, and this standardised S is called the intensity of selection, symbolised by i. Thus

$S = i\sigma(p)$, and

$R = ih^2*\sigma(p)$

In fruit tree breeding, the common breeding system is recurrent mass selection. This method involves selecting a source population (parents) which contains all the traits desired for cultivar improvement. The parents are intermated to form a heterozygous base population. The best seedlings are then selected to form the second source population after intermating selected plants and bulking the seed. The cycle can be repeated many times, and each cycle should produce progressive population improvement.

In this study, four important characters were examined for genetic advance (Table 3.10). Assuming the best 10% of the female parent population will be selected, the value of i will be 1.755. Since only the female parent population is selected, the genetic advance will be half of the advance which results from the R equation mentioned above (Hallauer & Miranda, Fo. 1981).

Table 3.10 Genetic advance for 6 selection cycles

TRAITS	MEANS	CYCL1	CYCL2	CYCL3	CYCL4	CYCL5	CYCL6	NEW MEANS
VC	92.01	4.61	9.24	13.90	18.60	23.32	28.06	120.07
TW	2.29	0.24	0.46	0.68	0.89	1.08	1.27	3.56
FSH%	38.22	9.14	17.44	25.12	32.33	39.16	45.68	83.90
FW	83.96	9.94	19.21	27.92	36.16	43.99	51.47	135.43

Table 3.10 shows that the expected cumulative selection advance in VC is 4.61, 9.24, 13.90, 18.60, 23.32, 28.06 respectively in 6 selection cycles. After 6 cycles of selection, the mean value would increase from 92.01 mg/100g to 120.07 mg/100g.

Thus for TW, the mean value will increase from 2.29 units to 3.56 units, FSH% from 38.22 to 83.90 units, and FW from 83.96 g to 135.43 g.

The results indicate that much potential exists for kiwifruit improvement in both productivity and fruit quality. These results show only the predicted value, and it is necessary to test the reality in breeding practice. Generally, the predicted and actual responses do not agree very well (Caldwell et al, 1966; Eagles & Frey, 1974). One cause for the lack of agreement between the two could be insufficient sampling. A second could be a change in the mean gene frequency between the time when a population was initially sampled and when it was evaluated some years later (Hallauer, 1981). However, as the estimates of phenotypic variability and heritability are unbiased, the predicted and actual advance will be in close agreement.

Information about actual selection advance in horticultural crops is limited, but Shikata (1982) conducted one of the best controlled divergent selection programmes in sweet potato, selecting the best 10% for high yield for 3 cycles and 10% for low yield for 1 cycle only. Mean yield increased 74% over 3 selection cycles, while negative selection reduced mean yield 52% (cited in Jones 1986). Similar reports have not been found for fruit trees, and further studies need to be carried out.

It is of interest to note that the genetic advance is not consistent in each selection cycle, because h^2 will change with the selection process (Lin, 1978). Li (1975) reported a reduction in h^2 for root weight from 0.57 in generation 4 and 5 to 0.44 in generation 6 and 7 in sweet potato breeding (cited in Jones 1986). This may imply the predicted genetic advance from the first selection cycles is the more believable one and the latter possibly too optimistic.

2. SELECTION RESPONSE FOR DIFFERENT STRATEGIES

To evaluate the different selection strategies, the corresponding R may be estimated from the R equation for individual selection (Falconer 1981):

For line selection, in which whole lines are selected or rejected as units, according to the mean phenotypic value of the line.

$$R(L) = i\sigma(p)h^2[1+(n-1)r]/\sqrt{\{n[1+(n-1)t]\}}$$

For within-line selection, in which the individuals are selected according to the deviation of each individual from the mean value of the line to which it belongs

$R(WL) = i\sigma(p)h^2(1-r)/\sqrt{[(n-1)/n(1-t)]}$

For combined selection, in which both lines and individuals are selected

$R(comb) = i\sigma(p)h^2\sqrt{\{1+[(r-t)^2/(1-t)][(n-1)/(1+(n-1)t)]\}}$

where $\sigma(p)$ is the phenotypic standard deviation of an individual; h^2 is heritability of individual values; r is the coefficient of the additive variance; t is the intraclass correlation between member of lines, and calculated as the between-group component divided by the total variance ($t = \sigma_B/\sigma_T$); n is the number of individuals in the lines.

The between-line component can be expressed as $\sigma_B = t\sigma_T$, and the within-line component as $\sigma_W = (1-t)\sigma_T$.

Since the value of r varies with mating system (with full-sib families, $r = ½$; with half-sib families, $r = ¼$), the selection response not only varies with different selection strategies, but also varies with mating systems and pollen management. Therefore different line structures which result from different ways can be compared for choosing the best breeding methods.

By using Gordon's programme (Gordon 1989, personal communication), the different breeding methods and selection strategies were compared, and the results are displayed in Table 3.11 and Figures 3.1 - 3.4. These results were obtained for the following conditions:

(1) $i = 1.755$ and truncation $z = 1.282$ ($P = 0.1$);
(2) 25 individuals per line;
(3) only female parents were selected;
(4) no wild pollen was involved.
(5) 1 cycle pre-line selection.

Table 3.11 shows the genetic advance for 4 characters after five selection cycles by using different selection strategies in different mating systems in a line selection programme. According to the genetic gains, full sib mating was the most effective method of pollen control for all selection strategies except for within line selection. As to within line selection, random mating would be the most effective method, and full sib mating the least effective.

Table 3.11 Genetic advance in random mating, half sib mating, full sib mating

		RANDOM MATING				HALF SIBS				FULL SIBS			
	CYC	IDVI	WL	AL	COM	IDVI	WL	AL	COMB	INDVI	WL	AL	COMB
VC	1	9.24	8.98	6.26	9.28	9.70	8.44	9.40	10.75	10.28	7.54	12.55	13.08
	2	13.90	13.23	8.55	14.11	15.16	11.86	15.91	18.10	16.52	10.19	21.57	22.48
	3	18.60	17.35	11.40	19.12	20.97	14.88	23.81	26.56	23.38	12.23	32.22	33.32
	4	23.32	21.35	14.76	24.35	27.09	17.56	32.82	35.96	30.79	13.92	43.80	45.03
	5	28.06	25.23	18.60	29.80	33.47	19.93	42.80	46.22	38.34	15.27	56.22	57.51
TW	1	0.46	0.45	0.31	0.46	0.48	0.43	0.42	0.50	0.50	0.38	0.53	0.56
	2	0.68	0.65	0.40	0.68	0.73	0.59	0.65	0.78	0.77	0.50	0.83	0.89
	3	0.89	0.83	0.51	0.89	0.97	0.72	0.90	1.06	1.03	0.59	1.16	1.23
	4	1.08	1.00	0.62	1.10	1.21	0.83	1.17	1.35	1.30	0.66	1.50	1.57
	5	1.27	1.16	0.74	1.30	1.44	0.93	1.45	1.65	1.55	0.77	1.84	1.92
	1	17.44	17.03	11.80	17.47	18.05	16.12	15.46	18.56	18.73	14.47	19.05	20.39
FSH%	2	25.12	24.11	14.95	25.22	26.68	21.87	23.00	28.04	28.10	18.85	29.15	31.40
	3	32.33	30.56	18.45	32.55	35.10	26.62	31.32	37.62	37.34	21.93	40.14	42.82
	4	39.16	36.48	22.19	39.56	43.33	30.60	40.10	47.26	46.36	24.30	51.31	54.23
	5	45.68	41.98	26.12	46.31	51.37	33.93	49.19	56.94	55.13	26.06	62.57	65.63
FW	1	19.21	18.74	12.94	19.24	20.01	17.81	17.17	20.63	20.93	16.05	21.27	22.81
	2	27.92	26.76	16.59	28.05	29.95	24.41	25.95	31.61	31.81	21.15	32.94	35.55
	3	36.16	34.10	20.72	36.48	39.71	29.89	35.74	42.83	42.59	24.77	45.65	48.77
	4	43.99	40.86	25.22	44.59	49.25	34.46	46.10	54.16	53.05	27.53	58.48	61.89
	5	51.47	47.11	30.00	52.47	58.53	38.28	56.80	65.52	63.13	29.57	71.28	74.86

IDVI, Individual Selection
WL, Within Line Selection
AL, Among Line Selection
COM, Combined Selection

Figure 3.1 shows the cumulative genetic gains of vitamin C in 5 selection cycles: In random mating, combined and individual selection strategies would result in a greater genetic advance than within line selection, and much better than among-line selection. But in 2 other mating systems, among line selection became more effective than individual and within line selections as the number increasing of selection cycles increased. The similar trend is found in the analysis of the 3 other characters, TW, FSH% and FW, the results for which are depicted in Figures 3.2-3.4.

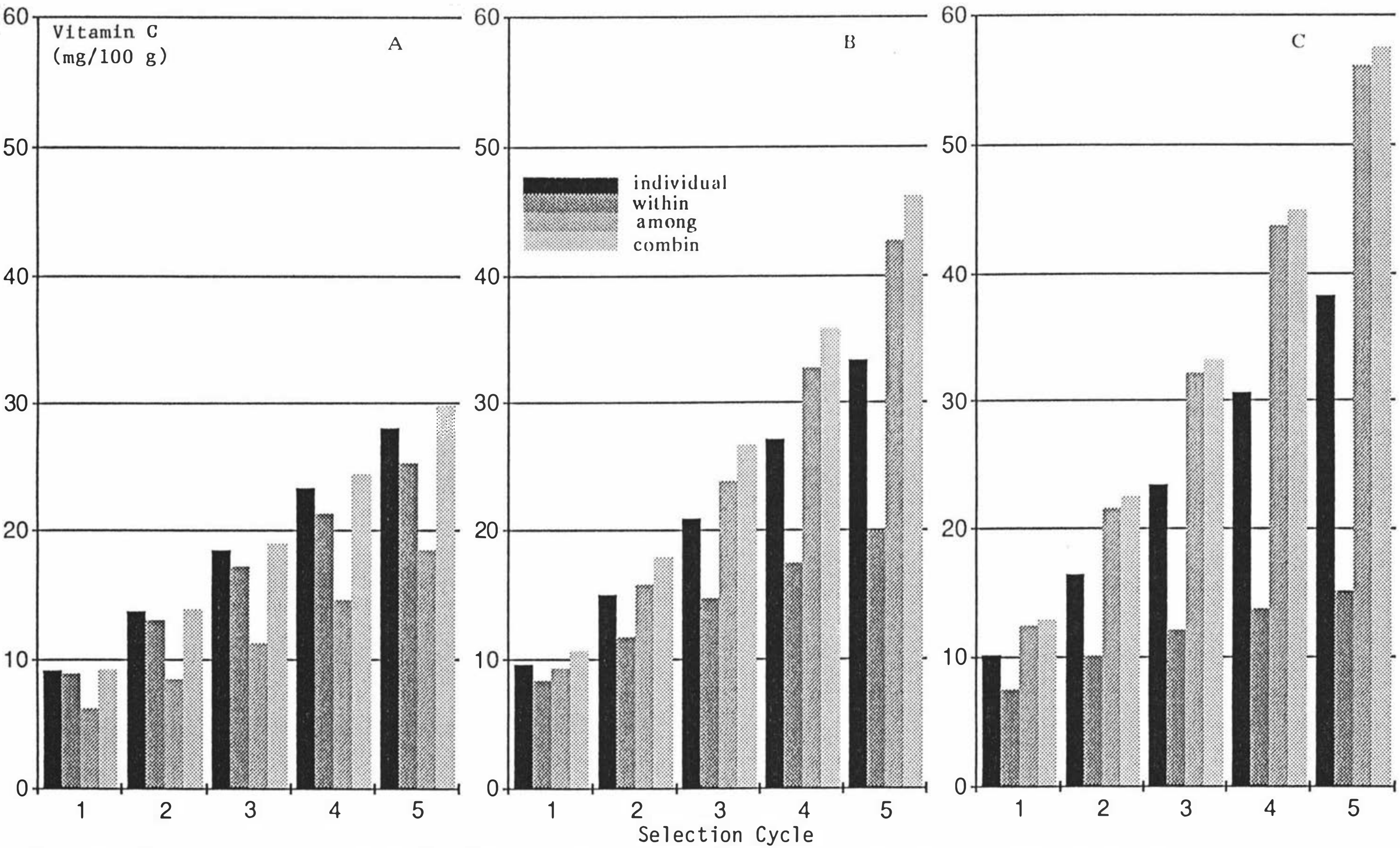


Figure 3.1 Cumulative genetic gains of fruit vitamin C content by using different selection strategies in different mating systems. A. Random mating. B. Half sib mating. C. Full sib mating.

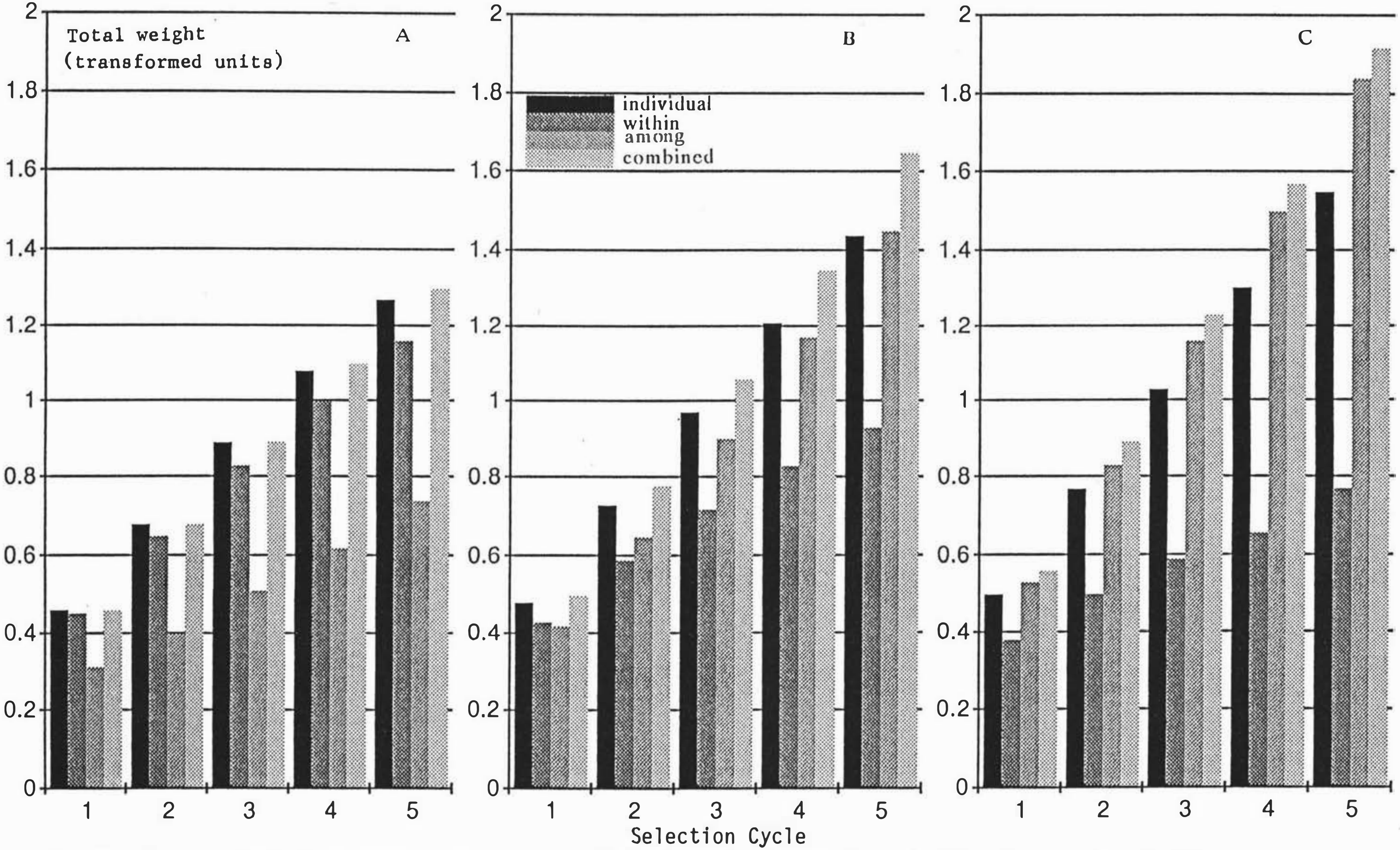


Figure 3.2 Cumulative genetic gains of total fruit weight per vine by using different selection strategies in different mating systems. A. Random mating. B. Half sib mating. C. Full sib mating.

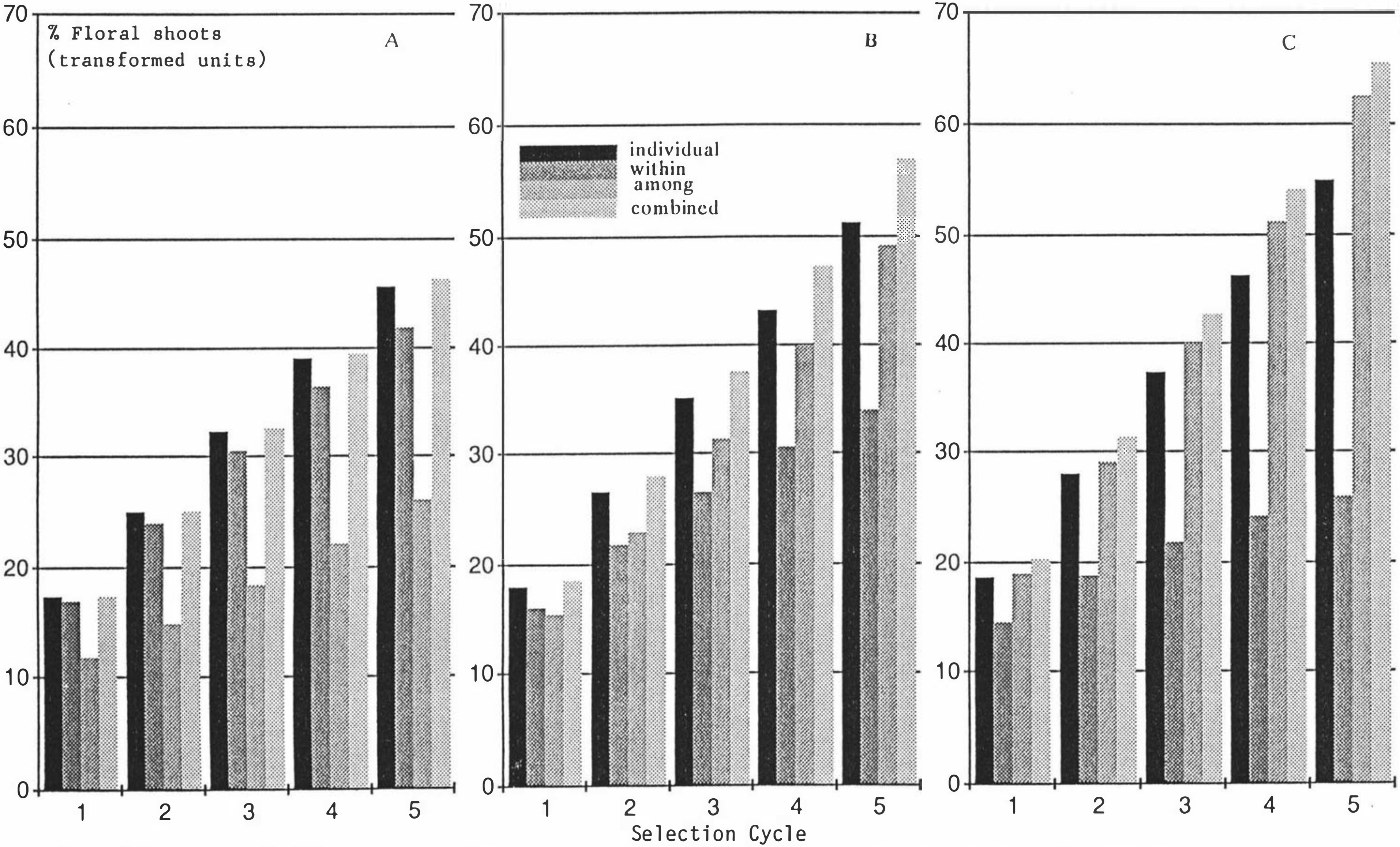


Figure 3.3 Cumulative genetic gains of percentage floral shoots per vine by using different selection strategies in different mating systems. A. Random mating. B. Half sib mating. C. Full sib mating.

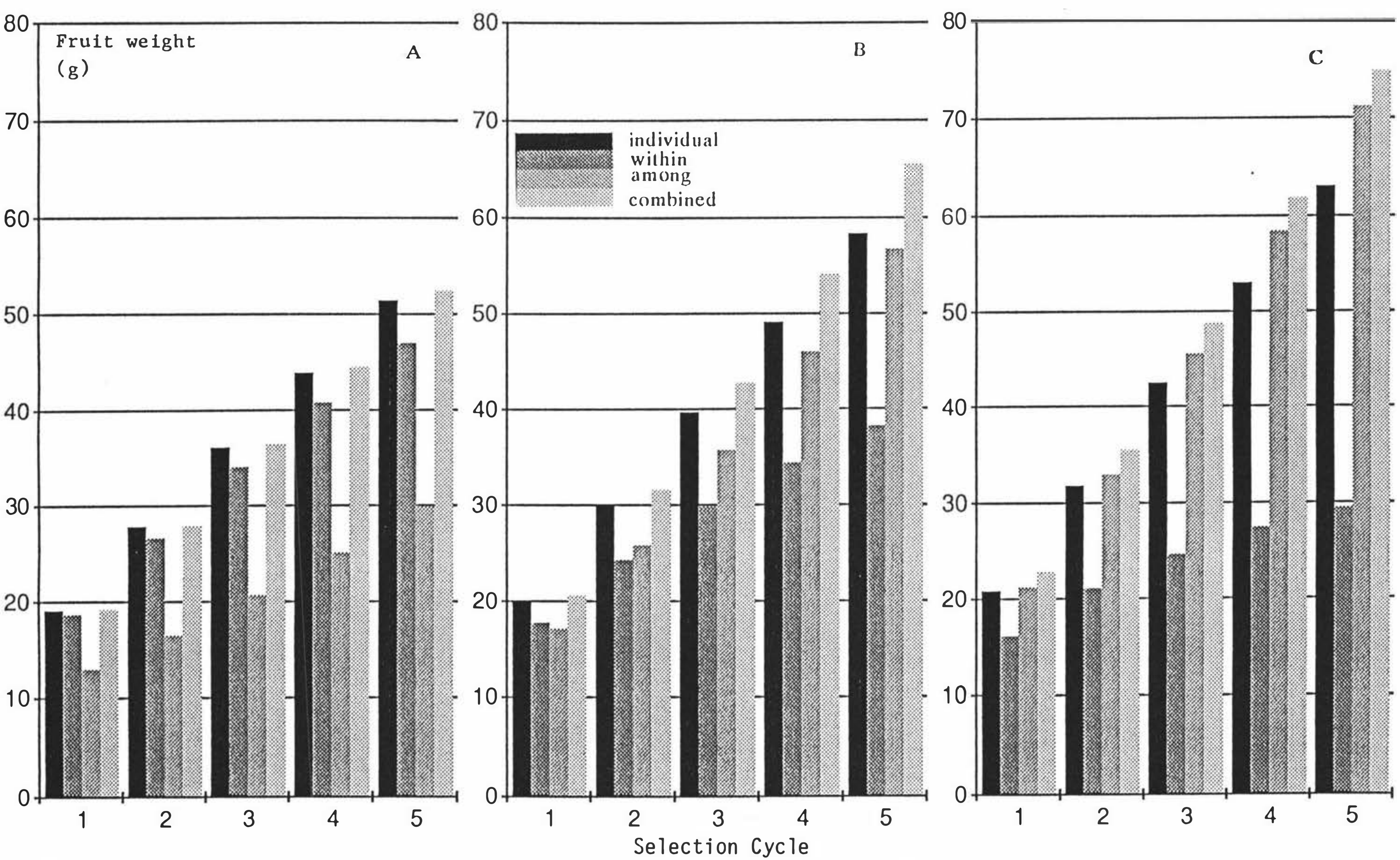


Figure 3.4 Cumulative genetic gains of mean fruit weight by using different selection strategies in different mating systems. A. Random mating. B. Half sib mating. C. Full sib mating.

It is of interest to note that the relative effectiveness of individual selection increases with the heritability values of the characters. In half sib mating, for instance, individual selection for vitamin C (h^2 0.22) becomes less effective than among line selection after 1 selection cycle, for TW (0.46) individual selection becomes less effective only after 5 cycles, but for FSH% (0.54) and FW (0.52) individual selection was always more effective than among line selection within 5 cycles. This indicates that individual selection is more appropriate for the characters with high heritability, but that dispersion-assisted strategies will always be better.

Unlike annual field crops, the kiwifruit has a long juvenile period, (3-4 years each generation) and 5 selection cycles seem to be maximum for any likely breeding programme. Within 5 cycles, the individual selection would be more effective than within line and among line selections in random mating and half sib mating systems if the heritability of the selected character was high. Although combined selection was shown to be the most efficient method in all mating systems, it is more complex than individual selection. In addition, kiwifruit is dioecious and therefore selection of a male vine for fruit characters is very subjective, hence a random mating system without pollen control may be the appropriate selection method for population improvements. But if heritability of the selected character is low, the other methods can yield a greater genetic gain than individual selection in random mating.

3. CORRELATED RESPONSE OF SELECTION

The selection response for one trait may cause simultaneous change in another trait. This is called correlated response to selection. Sometimes it might be possible to achieve more rapid progress under selection for a correlated response than from selection for the desired character itself. This is referred to as indirect selection. In many cases a correlated response results from genetic correlation between the two traits involved. If the change in breeding value of trait y is considered, when selection is on x, then the regression of breeding value of Ay on Ax will give the correlated response of y as (Falconer 1981, Mayo 1980,):

$CRy = bRx$

and $b = COV_A/\sigma^2_{AX} = r_A\,\sigma_{AY}/\sigma_{AX}$

$R_x = i\,h_x\sigma_{AX}$

so that $CRy = i\,h_x r_A \sigma_{AY}$

here CRy is the correlated response of y, b is the regression coefficient of Ay on Ax, **Rx** is the response of x, σ_{Ay} is the standard deviation of breeding value of y.

Thus, the ratio of correlated response (CRy) to the response of direct selection (Ry) can be derived from the above equation:

$$CRy/Ry = h_x\, r_A/h_y$$

According to the above equation, the ratios of indirect selection to direct selection for the 4 characters mentioned above were calculated.

Since VC has a much lower h^2 (0.22) than TW (0.46) and FSH% (0.54), and has a moderate genetic correlation with the two characters (0.59 and 0.66 respectively), selecting VC for TW only gave the ratio of 0.53, and selecting VC for FSH% only gave the ratio of 0.52, indicating indirect selections are far less effective than direct ones.

However, selecting TW for VC gave the ratio of 1.11, and selecting FSH% for VC gave the ratio of 1.27, indicating indirect selections in reversed manner would be more effective than direct ones.

The results show that the indirect selection will be more effective than the direct one, only if both $h^2{}_x$ and r_A are high relative to $h^2{}_y$. In kiwifruit breeding, VC is one of the important characters to be improved, but the vitamin C test is complex. Therefore the indirect selection method is particularly useful in this case. Not only can procedure of the vitamin C test be omitted, but also the selection response increased by using this method.

Since the coefficient of genetic correlation between TW and FSH% was 1, the ratio of indirect to direct selection simply becomes the ratio of two heritabilities. Thus, selecting TW for FSH% gave the ratio of 0.92, and selecting FSH% for TW gave the ratio of 1.08, indicating if the genetic correlation between two characters is very high, the magnitude of correlated selection response is mainly dependent on the deviation between two heritability values.

Since FW had the very low genetic correlation with TW and FSH% (0.04 and 0.03 respectively), selecting TW for FW only gave the ratio of 0.2, and selecting FSH% for FW only gave the ratio of 0.17. Similar results are obtained in reversed way, selecting FW for FSH% gave the ratio of 0.17, and selecting FW for TW gave the ratio of 0.22. This means that indirect selection does not make sense if the genetic correlation between the characters is very low.

It will be of great interest in fruit tree breeding if selection is based on the correlation between traits expressed in the juvenile period and the traits of mature trees. Thus, the genetic gain of fruiting characters could be predicted even in the juvenile period, by using vegetative characters in indirect selection.

SECTION 4 : SELECTION INDEX

1. INDEX THEORY AND WORKING PROCEDURES

A selection index (I) is a linear function of phenotypic values of different traits, and the observed value for each trait is weighted by an index coefficient.

$$I = p'b$$

where p' is a row vector of known phenotypic values, b is a column vector of the weights assigned to each phenotypic value.

Because the purpose of using a selection index in plant breeding is to attempt to select for improved "genotypic worth", the selection index must be based on it. Two approaches for constructing an index have been used in this work.

SINGLE TRAIT: Selection aims at improving just one character, and secondary characters are used as aids to the improvement of the one desired character. The equation for the solution of this index (Baker 1986, Falconer 1981) is

$$Pb = g(i)$$

where P is the matrix of phenotypic variance and covariance of all characters involved, g(i) is the column vector of the additive genetic variance and covariance of the focal character i, and b as defined above. Thus vector b can be obtained by multiplying the inverse of P matrix by vector g(i):

$$b = P^{-1}\, g(i)$$

MULTIPLE TRAITS: Selection aims at improving several characters simultaneously. The objective is to improve the aggregated breeding value, or net merit, which is a particular combination of all the characters to be improved. A linear function is used to define genotypic worth, in which the genotypic value of each trait is weighted by a relative economic value.

$$W = x'a$$

where x' is a row vector of unknown genetic values, a is a vector of known relative economic values for each character.

Selection for W cannot be done directly, as it is the phenotype not the genotype which is measured. Therefore as in the single trait method, a linear function (I) is used as the selection criterion. The following equation was used to describe the relationship between I and W. It minimizes the sum of squared deviations of index values from the linear regression of I on W, and the resulting values of the b's are then the partial regression coefficients of the "genotypic worth" on each phenotypic character (Falconer, 1981). The equation is:

$$Pb = Ga$$

where P is a matrix of phenotypic dispersion, G a matrix of additive genotypic dispersion, and a and b are defined as before.

If G and a are known, then

$$b = P^{-1}(Ga)$$

and yielding the optimal selection index described above.

When selection is based on I, the expected response to selection in the aggregate genotype (W) is:

$Rw = i\sigma(I)$ (Lin 1978).

where i is the selection intensity, $\sigma(I)$ is the standard deviation of I.

And $\sigma(I) = b'Pb$ (Baker 1986, Lin 1978).

The standard deviation of the index provides a simple way of comparing the relative efficiency of different indices for improving merit because the selection response is simply proportional to $\sigma(I)$.

The correlated response for each component character incorporated in the index will be:

$$CR(i) = g(i)'b(i/\sigma(I))$$

where $g(i)'$ is a row vector of genetic variance and covariances between ith character and other characters in the index.

2. ECONOMIC WEIGHT

The early work on indices used actual economic weights in the vector a. In fact, any relevant importance weighting could be used instead. In this study, economic weights were used and estimated as the contributions of the different traits to the efficiency of production, price, etc., as listed in Table 3.12.

Vitamin C

The benefit of an increase in fruit Vitamin C level would primarily be through sales at a higher price following the promotion of this fact. It was assumed that an increase in vitamin C concentration of 22 mg/100g fresh weight from the mean value of 90 mg/100g, could increase returns by 60 cents per tray. At an average price for Hayward of 6 dollars per tray, if vitamin C in a new cultivar was 25% higher than Hayward, the price will be assumed to be 10% higher than for Hayward.

Total soluble solids (BRIX)

An increase in fruit soluble solids level at approximately the first of May might indicate early maturation and better tasting (sweeter) fruit. If soluble solids concentration in a new cultivar was 25% higher, the price may increase 5% - or, if the new cultivar gained 1.5% soluble solids over the original value of 6.2, it would increase returns by 30 cents per tray. This character was excluded in the analysis due to its negative heritability value.

Mean fruit weight (FW)

An increase of 10% in mean fruit size increases the pack size by 1 count (say from 36 to 33 fruit per tray), and so would attract a higher price. Based on data of size distribution and dollar returns in the 1987/88 season, the grower would receive an extra return of 65 cents for each tray with increased fruit size. That is to say, if the weight of a fruit increased 9 g from an original value of 90 g, the return would be increased by 65 cents per tray.

Total weight per vine (TW)

Assuming all fruits are acceptable, an increase of 1 kg (2 logarithmic transformed units) in total yield would increase return by 200 cents a vine.

Assuming mean production is 10 trays per vine, the degree of change for an increased return of $3.00 can be computed, and the relative economic value therefore can be obtained (Table 3.12).

Table 3.12 Relative economic value for 4 traits of breeding importance

Traits	Increment equiv. to $3 gain/vine	Relative economic value
VC	11.00 mg	0.27
BRIX	1.50 %	2.00
FW	4.10 g	0.72
TW	2.18 *	1.38

* Transformed unit

3.CONSTRUCTING SELECTION INDICES

A matrix algebra method was used in this work. From the Manova, phenotypic and genetic dispersion was obtained for female vines (Tables 3.13). BRIX was excluded due to its demonstrating no genetic variation.

Table 3.13 Phenotypic (upper part) and genetic (lower part) dispersion for 3 traits of breeding importance

Traits	VC	TW	FW
VC	590.705	1.837	-221.235
TW	1.837	0.360	-0.992
FW	-221.235	-0.991	465.846
VC	105.004	1.467	-161.645
TW	1.467	0.010	-1.149
FW	-161.645	-1.149	102.401

The index aimed at improving one character in particular was calculated, and the selection coefficients for improving fruit vitamin C concentration, total fruit weight (vine yield) and mean fruit weight are given in Table 3.14.

Table 3.14 Type 1 indices for improving different characters

Traits	Index Coefficients		
	VC Index	TW Index	FW Index
VC	0.05	0.002	-0.23
TW	2.95	0.014	-1.73
FW	-0.32	-0.002	0.11

The results indicate that the type 1 index was effective for TW and FW, but less effective for VC. Although the coefficient for VC was relatively high (0.05) in the VC improving index, the coefficient for TW gave the highest value (2.95) in the index. Therefore this index still emphasized TW selection. However, both TW and FW improving indices did mainly lead to the improvement of the focal characters.

Table 3.15 shows the character-set index where several characters were to be improved at the same time.

Table 3.15 Type 2 indices for multiple character improvement

Traits	Index Coefficients	
	Economic Weights Index	Equal Weights Index
VC	-0.15	-0.18
TW	-0.43	1.23
FW	-0.01	-0.21

It was obvious that two different weight types gave distinct indices. The economic weight index enhanced FW selection, while the equal weight index enhanced TW selection.

Since negative values occurred in the calculation, it was impossible to compare the relative efficiencies of the different indices for improving merit, and to compare the correlated responses of the different characters incorporated in the index. The negative values may result from huge differences between characters in genetic variance and covariance estimates (see Table 3.10), which may swamp the analysis.

Similarly, the selection index for male vines could be calculated. However it would be difficult to accurately estimate the economic value of the important male characters, and the determination of the selection index for male vines was not attempted in this study.

Index scores can be obtained for each vine by multiplying the phenotypic values of the component characters by index coefficients, and therefore superior vines could be selected based on their index scores, as if the index were a single character. The best 10% of female seedlings (6 vines) selected in cross 6 were used to demonstrate the application of selection index to kiwifruit breeding, and the vines selected from the different indices and from the factor analysis (see chapter 2) were compared (Table 3.16).

Table 3.16 The 6 female vines selected from the diffcrent indices and from the factor analysis in cross 6.

Cross	Economic Weights Index		Equal Weights Index		Factor 1	
	Vine	Score	Vine	Score	Vine	Score
6	120	-7.2	142	-18.1	142	1.7
6	142	-10.4	134	-22.3	124	1.7
6	90	-10.6	90	-22.7	134	1.6
6	118	-10.7	35	-24.8	1	1.6
6	95	-10.7	9	-26.3	88	1.5
6	77	-10.8	27	-26.6	109	1.4

The results show the vines selected varied with different selection methods. Only one vine in common (vine 142) was found between two index selections, and two vines in common (vines 142 and 134) between equal weights index and factor 1 selections.

In reviewing some of the recent literature, one finds little evidence that selection indices are being used routinely for crop improvement. These techniques apparently are not an important part of present day crop improvement programmes. However, when several characters are to be improved simultaneously, the selection could be made more effective by adopting some objective method of weighting traits (Baker 1986). In fruit breeding, selection is always applied to several traits simultaneously, since none of these characters alone can adequately express the overall quality of a commodity and vine productiveness. A selection index is suitable for this purpose.

Hazel and Lush (1942) indicated that the index selection was the most efficient one among three methods - tandem, independent culling, and index selection. In index selection, each character is weighted according to its relative economic importance, and its genetic variance, therefore both genetic and phenotypic variations are taken into consideration. In fact, the discriminant functions and principal factors in multivariate analysis (see Chapter 2) can also be treated as indices, but since no genetic information is involved, they are less effective.

CHAPTER FOUR

ELECTROPHORETIC ANALYSIS

1. INTRODUCTION

The electrophoretic analysis of total proteins and isozymes has been widely used in many fruit tree crops, but rarely applied to kiwifruit (Hirsch and Fortune, 1984). The technique may aid study of the following aspects in kiwifruit breeding.

(1) Cultivar identification and taxonomic classification

With the development of the kiwifruit industry, new cultivars will be developed. It will then become important to identify cultivars to prevent fraudulent representation, to assist in designing breeding programmes and for application for plant variety rights. The genus to which kiwifruit belongs contains about 100 taxa, many of which have desirable attributes not found in present kiwifruit cultivars but which may be transferable by interspecific hybridisation to create completely new kiwifruit types. Before using this germplasm for breeding, the origin and relationship of the taxa should be examined to assist in successful hybridisation.

Since proteins are a consequence of the nucleotide sequence at the DNA level, morphological similarity between cultivars and between taxa should have a biochemical basis and be reflected as similarity in protein and isozyme structure. Hence the electrophoretic banding pattern of total proteins and isozymes can be used as a tool for cultivar identification and taxonomic classification of fruit tree crops (Bringhurst et al, 1981, Weeden and Lamb 1985, Menendez et al 1986, Mazzola and Carter, Jr. 1988).

(2) Study of plant adaptation

In plants grown under conditions of temperature, pH, nutritional or moisture stress, changes occur in tissue proteins (Ougham, 1987). Therefore studies on induction and repression of proteins under stress could provide an early indication of potential adaptation of breeding material, if the changes were shown to be linked to undesirable environmental conditions.

(3) Pest and disease resistance

Resistance or susceptibility of fruit trees to pests and diseases often appears to be based on regulation of protein synthesis, therefore changes in the protein pattern in an infected fruit tree could be used as biochemical markers to study host-pest and host-pathogen interaction. In 1974, Rick and Forbes found a tight linkage of the isozyme gene Aps-1 and the nematode resistance gene Mi in tomato, and this linkage has now been used to screen nematode resistant plants in tomato breeding (Tanksley and Rick 1980).

(4) Genetic analysis

Because of their long period of juvenility and multiple gene controlled traits, genetic research on fruit trees is limited. However electrophoretic analysis of total proteins and isozymes could assist in such research.

Many studies of single gene markers in date palm. strawberry and walnut were mentioned in the report of Arulsekar and Parfitt (1986). Studies of inheritance and variability of these gene markers in apple (Chyi and Weeden 1984, Bournival and Korban 1987), peach (Arulsekar et al 1986), grape (Parfitt and Arulsekar 1989) and in strawberry (Arulsekar et al 1981) have shown some potential for such research.

Another important aspect of genetic studies is to construct gene linkage maps. Theoretically. tagging genes which control some characters of commercial value with isozyme alleles and protein bands is possible, especially if the character concerned is controlled by a single gene. In kiwifruit it would be highly desirable to find a strong linkage between genes coding for certain isozymes or proteins and the sex determination factor, thus male vines could be removed from a seedling population early, to allow the most efficient use of time and resources. The callus tissue, callus subcultures and cell suspensions obtained from female plants of A. deliciosa contained characteristic isoperoxidase fractions. but there was no difference in stem segment cultures between male and female plants (Hirsch and Fortune 1984).

2. EXPERIMENTAL

A preliminary study of electrophoretic analysis of isozymes showed no difference in peroxidases between the kiwifruit cultivars investigated. Some cultivar differences in esterase staining occurred, but with poor resolution. Therefore it was decided to examine buffer-soluble leaf proteins, using a stain for total proteins following electrophoresis in the presence of SDS.

Since no information concerning SDS-Polyacrylamide electrophoresis of kiwifruit leaf protein was available, it was necessary first to determine suitable electrophoretic procedures, and then to assess their suitability as a tool in kiwifruit breeding. The objectives of this research were:

(i) to develop procedures for electrophoretic analysis of kiwifruit leaf proteins.
(ii) to characterise kiwifruit cultivars and seedling populations electrophoretically.
(iii) to study the variability and inheritance of polypeptides.

3. MATERIALS

Usually leaves were harvested from kiwifruit cultivars and seedlings growing in Palmerston North and Riwaka aged 3-8 years, between September and March during the 1986/87 and 1987/88 seasons. For some experiments in cultivar identification, hardwood cuttings were used in order to get fresh leaves in July and August.

4. ELECTROPHORESIS PROCEDURES

Discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed, using a system based on that of Laemmli (1970):

(a) General procedures

(i) *Gel preparation*

Initially a comparison was made between running gels of a range of concentrations from 7.5% to 20% polyacrylamide (Table 4.1) in a Biorad vertical slab gel system. Immediately after preparation, the non-polymerised solution was poured between the glass plates, and overlayed with distilled water to form a flat upper gel surface and to allow complete polymerisation. Polymerisation usually took at least one hour. The water was then decanted off and the stacking gel solution (Table 4.1) poured onto the running gel. A comb was then used to form 14 6mm wide wells for sample loading and the comb was removed after 20 minutes when polymerisation was complete. The final gel size was 150x200x1 mm.

Table 4.1 Recipes for making different strength polyacrylamide gels.

Reagents	Running gel					Stacking gel
(ml)	7.5%	10%	15%	20%		4.5%
Lower gel stock[1]	10.0	10.0	10.0	10.0		-
Upper gel stock[2]	-	-	-	-		2.5
Acrylamide stock[3]	10.0	13.4	20.0	26.0		-
Acrylamide stock[4]		-	-	-	-	1.5
Distilled Water	20.0	16.6	-	-		6.0
40% Glycerol	-	-	10.0	3.3		-
10% Ammonium Persulfate	0.2	0.2	0.2	0.2		0.05
TEMED	0.02	0.02	0.02	0.02		0.01

[1] 1.5M Tris base, 0.4% SDS, pH 8.8.
[2] 0.5M Tris base, 0.4% SDS, pH 6.8.
[3] Acrylamide 30g and Bis 0.5g, made up to 100 ml with distilled water.
[4] Acrylamide 30g and Bis 1.6g, made up to 100 ml with distilled water.

(ii) ***Sample loading***

Protein samples were loaded in the wells, volume depending on the results of a protein assay - but usually 5-30 μl, - to give a loading of 50 μg protein.

(iii ***Running conditions***

Upper (negative electrode) and lower (positive electrode) reservoir buffer contained 0.025 M Tris base, 0.19 M glycine, and 0.1% SDS.

Electrophoresis was at constant current of 30 mA for 4-5 hours. To minimise diffusion of protein at high temperature, the equipment was pre-cooled to 4°C, the electrophoresis was performed in a refrigerator, and tap water was circulated through the central core of the apparatus.

(iv) ***Staining and destaining***

After electrophoresis the gel was placed in a staining solution (250 ml isopropanol, 100 ml glacial acetic acid and 0.4g Coomassie Brilliant Blue R-250 made up to 1000 ml with distilled water) for 12 hours. The gel was then destained by immersing in 10% acetic acid which was changed every 2 hours until the gel background was clear. The destained wet gel was then photographed. Sometimes gels were dried using a Biorad gel drier.

(v) *Drying gel*

A Bio-Rad slab dryer was used as follows to dry the gel without cracking:

(a) Filter paper soaked with water placed on the porous gel support and the gel positioned on the filter paper. The gel covered with very thin plastic film.

(b) Laid the transparent sealing gasket over the gel and sealed it with water. The water vacuum turned on and the lid closed.

(c) Dried at 80°C for 2.5 hours.

(d) The lid was then opened to cool the gel. When the gasket was cool to the touch, the gel was removed, and then the vacuum turned off.

(b) Method development

A comparison was made of a number of extraction, running and sampling methods.

(i) *Evaluation of leaf protein extraction buffers*

Two extraction buffers were compared:

(a) Tris buffer system:

Tris buffer: 0.05 M buffer solution at pH 8.5 was used in this study.

SDS sample buffer: 0.125 M Tris base, 10 ml 2-mercaptoethanol, 6.1% SDS.

Bromophenol Blue tracking dye: 0.1% Bromophenol Blue in 80% glycerol.

1 g of fresh leaves were ground with a mortar and pestle in Tris buffer at a ratio of 1 to 1, (w/v) with a little sand added. The extraction was carried out for 4 hours at room temperature, then the slurry was centrifuged at 15000 g at 4°C for 30 minutes. SDS sample buffer was added to the supernatant at a ratio of 1:4, and tracking dye added at 1:10. The mixture was then heated for 3 minutes in a boiling water bath, and the sample used immediately or stored in a freezer at -17°C.

(b) Smith and Payne extraction solution:

Smith and Payne's (1984) stock solution consisting of 12.5 ml of 1 M Tris buffer, pH 6.8; 24 ml water; 20 ml glycerol; 4 g sodium dodecyl-sulphate and 20 mg of pyronin y was prepared. Immediately before use the stock solution was mixed with 2-mercaptoethanol, dimethylformamide and water in the ratio of 17:6:10:17.

1 g of fresh leaves was ground in the extraction solution (1:1 w/v). The mixture was centrifuged and the supernatant heated for 3 minutes at 100°C in a water bath.

The results for the two extraction buffers are shown in Figure 4.1. Tracks 1-6 show banding patterns of proteins extracted by Tris buffer from mature leaves of cultivars Hayward, Bruno, Matua and Tomuri, and from young leaves on shoot tips of Tomuri and Matua respectively. Mature leaves of Bruno and Matua gave an extra band at Rf 0.80 compared with Hayward and Tomuri, and young Tomuri leaves gave rise to an extra band at Rf 0.45 compared with Matua.

Tracks 7-13 show banding patterns of proteins extracted by Smith and Payne's buffer from young tip leaves of Tomuri and Matua, and mature leaves of Hayward. Bruno, Matua and Tomuri. Young Tomuri leaves gave a band at Rf 0.45 that was absent from Matua. Proteins extracted from mature Hayward leaves gave a banding difference at Rf 0.53 compared with Bruno, Matua and Tomuri.

Although the use of Smith and Payne's extraction buffer which contains a number of additives gave rise to protein bands of different relative intensities than resulted from using Tris buffer alone, the latter gave rise to more distinct bands showing differences between cultivars, so it was used routinely.

(ii) ***Estimation of sample loading***

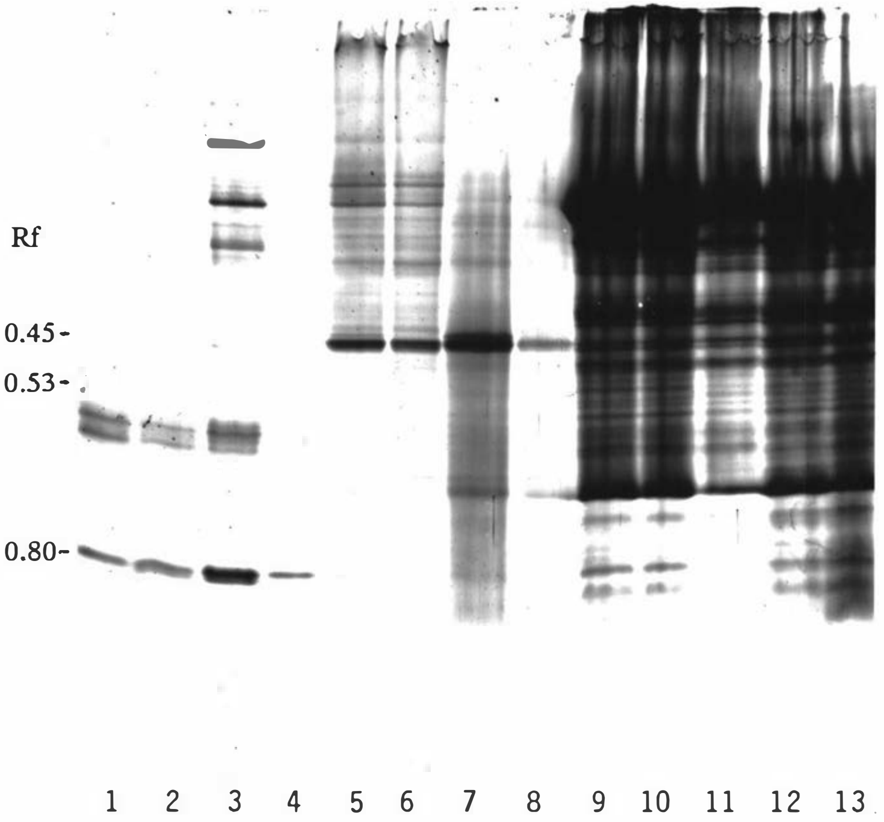
To ensure that a consistent amount of sample protein was applied to the gel a protein assay of Ionas' (1983) based on that of Bradford (1976) was used.

(a) Preparation of Coomassie-Blue Reagent

Coomassie-Blue G250	100 mg
95% ethanol	50 ml
85% (w/v) phosphoric acid	100 ml

Made up to 1000 ml with distilled water

Figure 4.1 Banding pattern of kiwifruit leaf proteins extracted by 2 extraction media and analysed on a 15% polyacrylamide gel



Tracks 1-6 Tris Buffer
Tracks 7-13 Smith and Payne buffer

1 Hayward
2 Bruno
3 Matua
4 Tomuri
5 Tip leaf of Tomuri
6 Tip leaf of Matua
7 Tip of leaf of Tomuri
8 Tip leaf of Matua
9 Hayward
10 Hayward
11 Bruno
12 Matua
13 Tomuri

Figure 4.2 Banding pattern of kiwifruit leaf proteins extracted by 2 different extraction media analysed on 7.5% polyacrylamide gel.

Tracks 1-3 Tris buffer system
Tracks 4-6 Ethanol extractant

(b) Standard curves for protein solution

A range of Bovine Serum Albumin solutions from 0 to 1 mg/ml were prepared in 0.2 M NaOH and heated to 100°C for 3 minutes to simulate the treatment of the leaf protein samples. The solution was cooled and to a 0.1 ml aliquot of each dilution 5 ml of reagent was added. The solutions were mixed, and the absorbance at 595 nm measured and a standard curve was plotted.

(iii) *Estimation of protein content of sample*

180 μl 0.2M NaOH was added to 20 μl sample of extract and 5 ml Coomassie-Blue reagent added. The protein content was derived from the absorbance at 595 nm and the standard curve. A wide range of extractable protein was found in kiwifruit leaves, the levels becoming lower as the leaf aged. Typical values ranged from 1 μg/μl sample of extract in fully expanded leaves to 10 μg/μl in young leaves.

Suitable gel loadings for Tris buffer extracts were 5-10 μl for young leaves, and 30-40 μl for the older leaves, giving the equivalent of 50 μg protein per track.

(iv) *Effect of polyacrylamide concentration on resolution of leaf proteins*

Polyacrylamide gel concentrations ranging from 7.5 to 20% (Table 4.1) were compared. With a 7.5% gel only 6 poorly resolved bands were obtained and a large amount of protein accumulated at the bottom of the gel (Figure 4.2, p126). Both a 15% gel and 10% gel (Figure 4.5 and Figure 4.6, p133) gave good separation of both the high and low molecular weight proteins. The presence of glycerol in the 15% gel made it easier to handle. The 20% gel had no advantage over the 15% gels, so 15% gel were routinely used in this work.

(v) *Determination of protein molecular weight*

In this work, the SIGMA MW-SDS-70L kit which contains seven standard proteins was used to obtain the molecular weight of the kiwifruit leaf proteins.

Albumin, Bovine	66.0 kilodalton
Albumin, Egg	45.0 "
Glyceraldehyde-3-Phosphate Dehydrogenase	36.0 "
Carbonic Anhydrase	29.0 "
Trypsinogen	24.0 "
Trypsin Inhibitor, Soybean	21.1 "
α-Lactalbumin	14.2 "

The protein standards were run on a 15% polyacrylamide gel, and the relative mobility (Rf) values determined. A plot of log MW against Rf was described by a linear function (Figure 4.3) which was used as a calibration curve.

```
Log(MW)
2.0 +
    |
    |           *
    |
    |                  *
1.6 +
    |                     *
    |                           *
    |                               *       *
    |
1.2 +
    |                                                *
    |
    |
    |
0.8 +
     +---------+---------+---------+---------+-----------
    0.0       0.2       0.4       0.6       0.8       1.0
```

Relative Mobility (Rf)

Figure 4.3 Calibration Curve of Protein MW Standards on 15% Polyacrylamide Gel

	log MW		log MW
Albumin, Bovine	1.820	Carbonic Anhydrase	1.462
Albumin, Egg	1.653	Trypsinogen	1.380
Glyceraldehyde-3-Phosphate		Trypsin Inhibitor	1.324
Dehydrogenase	1.556	α-Lactalbumin	1.152

(vi) *Effect of leaf age on protein banding pattern*

For identifying cultivars or seedlings the leaf material used should give a consistent protein banding pattern. Therefore the uniformity and stability of the banding patterns was studied.

An actively growing shoot about 80 cm long was harvested from a vine at Massey University in September, 1987. Four leaves were chosen from different positions along the shoot and analysed separately. While the leaf age effect on the protein pattern was obvious with different protein patterns being found for leaves at different positions on the same shoot (see below, 5(i)), some bands showed little change.

(vii) *Method for leaf sampling*

Because of the leaf age effect, the samples used for cultivar verification should be identical in age, but this is difficult in practice. Therefore several leaves, were combined to provide an average picture of the protein pattern. The sample used consisted of one leaf obtained from the tip, 2 leaves from the middle, and one leaf from the base of the shoot, and this proved to be reliable for giving reproducible protein profiles.

5. RESULTS AND DISCUSSION

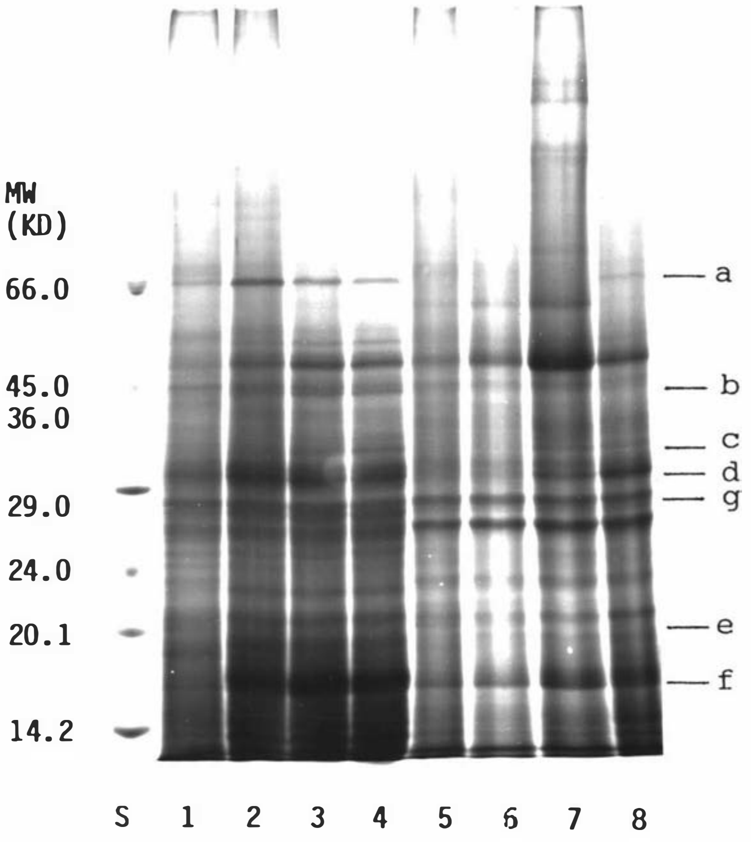
(i) Progressive changes in soluble polypeptide complement during leaf development

The effect of leaf age on the protein banding pattern may be of interest for studying gene expression during leaf development - although this was not an original objective.

According to Ougham et al (1987), growth and differentiation in plants necessitates changes in gene expression which will in the majority of cases be represented by differential synthesis of polypeptide products. The leaf age effect observed on kiwifruit leaf proteins (Figure 4.4) confirms this conclusion.

In spring (late September in NZ), kiwifruit buds swell and after 5-6 days begin to open. It takes 10-15 days for leaves to unfold, after which they start to grow rapidly and the shoot begins to elongate. The shoot constitutes a linear gradient of leaf age, with the young leaves on the tip, mature leaves in the middle, and older leaves on the base, and when analysed both qualitative and quantitative changes in the soluble polypeptide complement were found (Figure 4.4). A loading of 50 μg protein for each sample track permitted comparison of band intensities.

Figure 4.4 Leaf age effect on electrophoretic protein banding pattern from female cultivar Hayward and male cultivar Matua



S	Protein standards
1	Hayward young leaf on the tip of shoot
2	Hayward leaf in the middle of shoot
3	Hayward leaf in the middle of shoot
4	Hayward leaf on the base of shoot
5	Matua young leaf on the tip of shoot
6	Matua leaf in the middle of shoot
7	Matua leaf in the middle of shoot
8	Matua leaf on the base of shoot

- show bands which change with leaf age (see text)

(a) A band "a" at molecular weight 66 KD was very faint in young Hayward leaves (track 1), but was stronger in mature leaves (tracks 2-4). In Matua, this band occurred only in extracts from the basal leaf.

(b) A band "b" corresponding to about 45 KD molecular weight, which was not present in a young Hayward leaf occurred in mature leaves. No change in this band was found in the protein profiles of Matua.

(c) A band "c" between 36 and 29 KD molecular weight was not present in young leaves of Hayward or Matua, but did occur in extracts of older leaves of both cultivars.

(d) A major band "d" of about 30 KD MW was faint in young Hayward and Matua leaves, but became more intense in more mature leaves.

(e) A band "e" at 20.1 KD MW was present only in the younger Hayward and Matua leaves (tracks 1, 5 and 6).

(f) A band "f" at about 17 KD MW became more intense in mature leaves of both Hayward and Matua.

With growth and development of the leaves, both qualitative and quantitative changes in proteins occurred. Monitoring such changes may enhance our understanding of plant growth and development, and these bands may be useful as markers of the stage of a plant's development (Ao et al 1983).

Expression of photosynthesis-related genes alters with distance from the base to the tip of the leaf in wheat, maize, barley and Lolium temulentum, and major photosynthesis-related polypeptides and their mRNAs increase in quantity with the age of leaf tissue (Ougham et al 1987). Therefore in this experiment, most of those polypeptides that increased with leaf age may be located in the chloroplast and concerned with the photosynthetic function.

Some metabolically important isoenzymes exhibit gradients of activity with leaf age. Studies by Ougham et al (1987), Ao et al (1983), Parfitt and Arulsekar (1989), Croxdale and Vanderveer (1986)and Tyson et al (1985) support the contention that leaf tissue should not be treated as enzymically homogeneous for the purpose of classification. Where leaf proteins are to be used as genetic markers by a plant breeder, it is clear that consistency in sampling of leaf material is essential, since if leaves are taken from widely differing positions on a shoot, developmental variations in protein pattern will be superimposed on those due to underlying genetic differences between cultivars. Many papers on cultivar identification by electrophoresis do not comment on the leaf age effect, hence the reality of the genetic differences is not always clear.

However, other isozymes have been found to be consistently present in tissues of different ages, (Arulsekar et al 1985). In this study a band (g) of about 28 KD MW (Figure 4.4) was found to be constant across leaf age in both Hayward and Matua. Such a band could be used as a gene marker for cultivar verification.

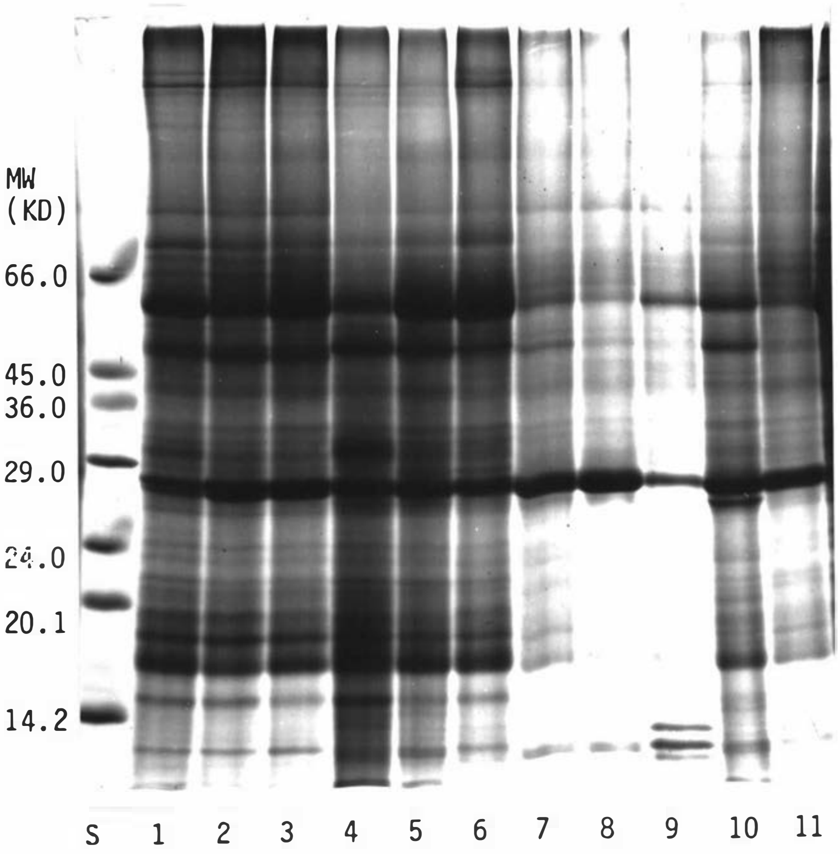
(ii) **Cultivar identification**

Seed proteins as gene markers for cultivar identification of some crops have proved reliable, consistent and essentially unaffected by environmental changes (Ellis and Beminster 1977; Gardiner et al 1986; Marchylo and LaBerge 1980; Shewry et al 1979 Wrigley et al 1982,). Few papers describing cultivar identification of fruit crops by leaf proteins have been published. (Navarro et al 1985, Carter Jr. and Brock 1980, Stegemann et al 1987, Mazzola and Carter Jr.,1988). No such work has been reported for kiwifruit. Initially cultivar identification by SDS-PAGE of proteins extracted from mature leaves was attempted, but protein content was low and the banding patterns were not clear. Similarly when mature leaves were harvested from seedling plants, results were inconclusive. In order to enable comparison between cultivars, results from young leaves harvested from regrowths of hardwood cuttings are presented as these gave the clearest and most consistent protein banding patterns.

Dormant woody cuttings of three Hayward selections and eight other cultivars were collected in July, 1987. After cold storage bud break was induced, and after 4 weeks, the leaf sample which comprised leaves of different ages as described in 4 (vii) were harvested from the new growth and extracted proteins analysed by SDS-PAGE (Figure 4.5, p133).

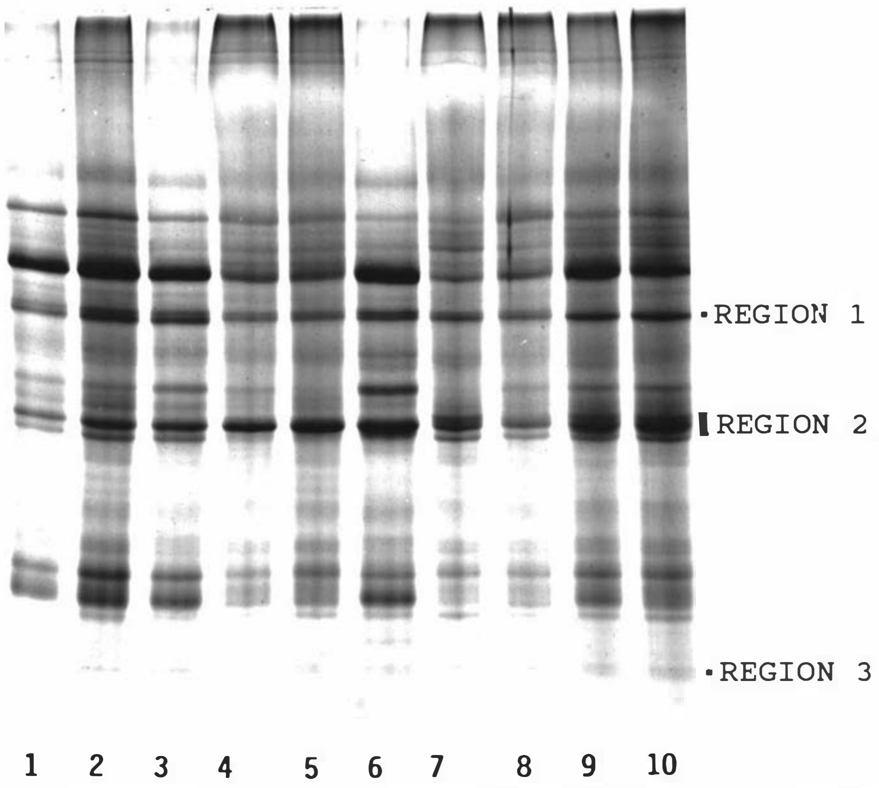
Three regions of the protein banding patterns were useful for cultivar identification. Region 1 with MW between 66 and 45 KD, region 2 with MW about 28 KD and region 3 with MW < 14.2 KD. At least two major bands occurred in each region. In numerous comparisons of protein banding patterns, regions 2 and 3 proved to be consistent and identical in leaves from cuttings and from vines in the field. Therefore it is acceptable to make comparisons between the two sample types in these regions. However, region 1 seemed to occur clearly only in the protein profile of kiwifruit cuttings, so its usage is limited although it is distinct between cultivars.

Figure 4.5 15% Polyacrylamide gel showing leaf protein banding pattern of 9 cultivars (winter cuttings)



S Protein standards
1 Hayward - HRC (Female)
2 Hayward - DES (Female)
3 Hayward - DSIR (Female)
4 Elmwood (Female)
5 Monty (FM)
6 Abbott (FM)
7 Burts' Fav. (Male)
8 McEwan (Male)
9 Berryman (Male)
10 M118 (Hermaphrodite)
11 Baldwin (Hermaphrodite)

Figure 4.6 10% Polyacrylamide gel comparison of protein banding pattern of the 4 parent cultivars (winter cuttings)



1-3 Hayward (Female)
4-6 Bruno (Female); Track 6 from wilted shoot
7-8 Gracie (Female)
9-10 D-120 (Male)

Figure 4.5 shows no differences in leaf protein banding patterns between Hayward from three different sources indicating they are homogeneous genetically. In region 1, Hayward and Baldwin gave 2 bands (Figure 4.5), while only 1 band was present at that position for Burt's Favourite, M118, McEwan D14, Abbott, Monty and Elmwood. The band number for Berryman 13/8 was unclear.

In region 2, Hayward once again gave 2 bands but the 8 other cultivars gave one band only. M118 was distinguished from the other cultivars by a distinct dark band that ran just ahead of this region.

Hayward. Abbott and Berryman each gave one band in region 3. Berryman also gave 2 other distinct bands running just behind and just ahead of this region.

Therefore by reference to the leaf banding patterns in these 3 regions it was possible to distinguish beween each of the cultivars Hayward, Baldwin. M118, Abbott, and Berryman 13/8. The cultivars Monty, Elmwood, McEwan D14 and Burt's Favourite all had the same banding pattern in these regions - however Monty and Elmwood differed in also having a band equivalent to MW of 29 KD.

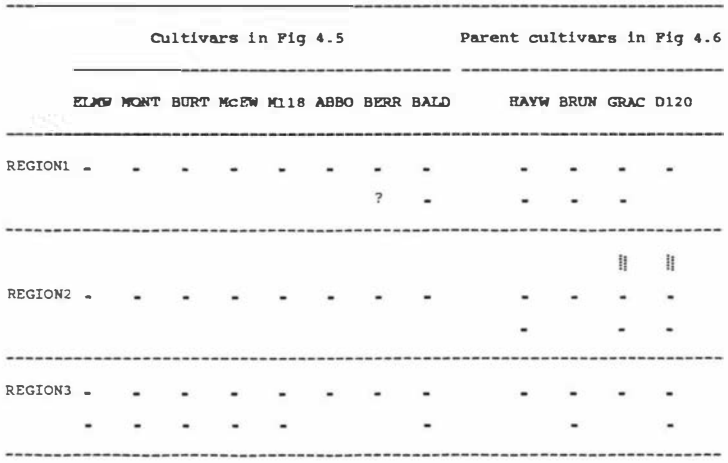
Region 2 was a complex of several polypeptides, which could be separated further by using a lower concentration polyacrylamide gel. Thus a faint trailing band behind the two more prominent bands was found in Region 2 for Gracie and D-120 (Figure 4.6, p133) but only two bands occurred on 15% gels (data not shown).

Figure 4.6 also shows the results are reproducible between vines. Protein profiles from leaves of three Hayward vines, two Gracie vines and two D-120 vines were identical to each other. The profile of proteins extracted from leaves of a wilted Bruno shoot differed from unstressed Bruno samples, indicating protein changes in the stressed condition. Ougham (1987) pointed out that higher plants have a greater need than most other living organisms to protect themselves against transient stresses such as heat, pH changes and salinity. A common response to such stresses is the rapid and large-scale synthesis of a group of proteins known collectively as heat-shock proteins, concomitant with a reduction or cessation of the normal pattern of protein synthesis for the tissue concerned.

Banding patterns for 12 cultivars shown in Figures 4.5 and 4.6 are summarised in

Figure 4.7. The four parent cultivars (Hayward, Bruno, Gracie and D1-20) can be differentiated from one another by reference to Regions 1-3.

Figure 4.7 Diagrammatic drawing of protein banding pattern of 12 cultivars



⁞⁞ faint trailing band.

(iii) Inheritance and variability of gene markers

Studies of inheritance and variability of gene markers of isozymes in fruit trees are limited, but include apple (Chyi and Weeden 1984, Bournival and Korban 1987), peach (Arulsekar et al 1986), grape (Chaparro et al 1989, Parfitt and Arulsekar 1989) and strawberry (Arulsekar et al 1981). No work has been reported for tissue proteins in fruit crops. Hence the possibility of using the banding pattern of polypeptides to ascertain the origin of a genotype was investigated.

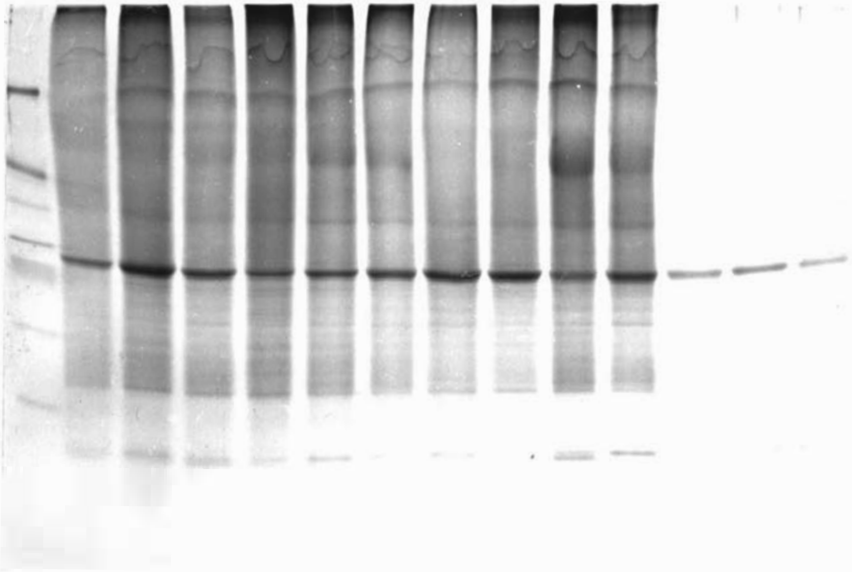
Leaf samples of F1 seedlings of 6 kiwifruit crosses were collected from the Riwaka Research Station in January and February of 1988, and electrophoretic analysis of protein showed some variability in the protein markers within these populations.

Figure 4.8 shows the banding profiles of leaf proteins for 27 seedlings of the cross Bruno x D-16 (Cross 1). Bands in region 1 were not clear in most of the seedling profiles. All seedlings gave identical patterns in region 2, but either one or two bands in region 3.

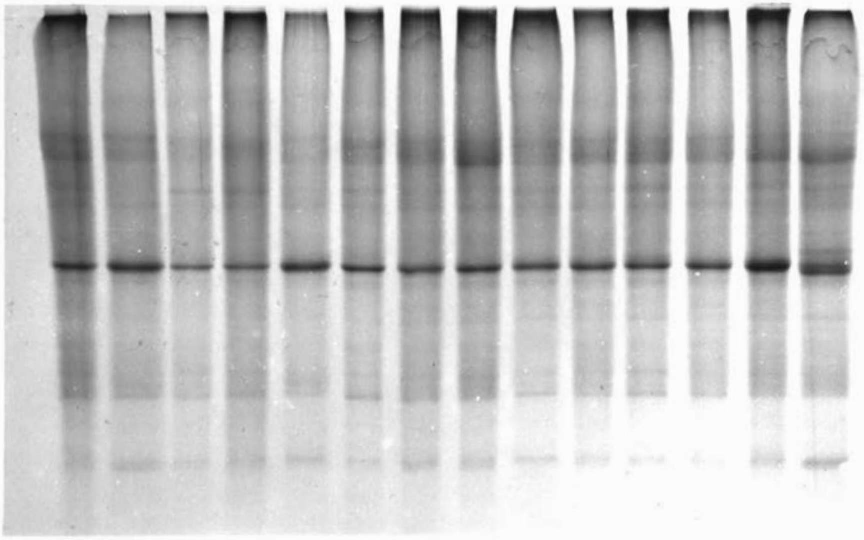
Figure 4.9 shows leaf protein profiles within the cross Bruno x D-120 (Cross 2). Variations in the banding patterns were found in both regions 2 and 3, with either one or two bands respectively in each region. On this basis the 28 seedlings could be classified into 4 groups: two bands in both regions; one band in both regions; two bands in region 1, and one band in region 2; one band in region 1, and two bands in region 2.

Figure 4.8 Protein banding patterns of F_1 seedlings of Cross 1 (Bruno x D-16)

A. Showing individual seedlings 1-14 (left to right)



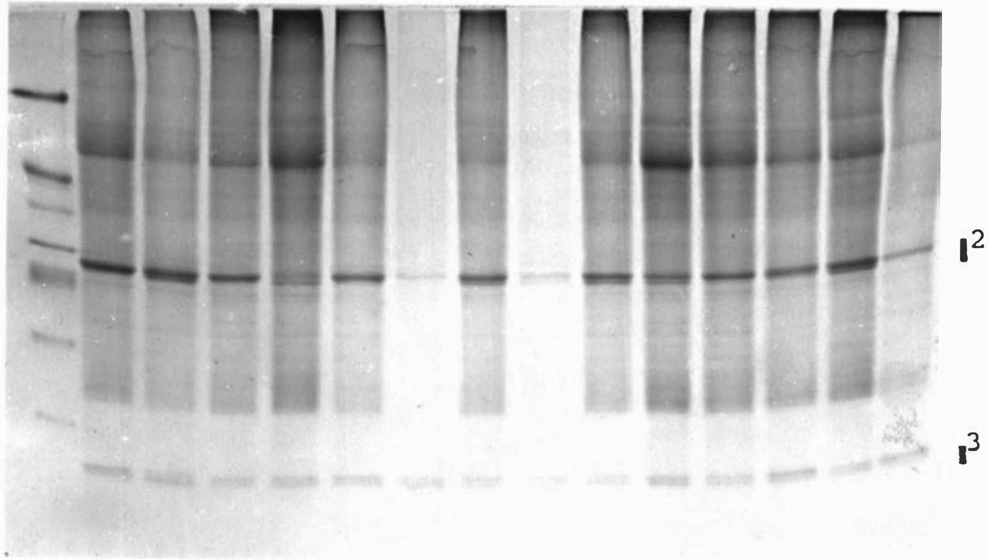
B. Showing individual seedlings 15-28 (left to right)



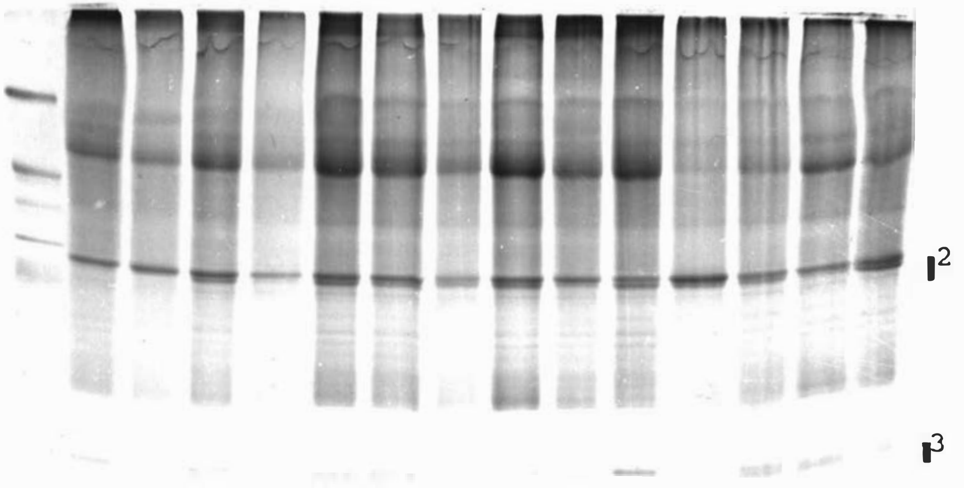
*Seedling in track 28 of Figure 4.8.B belongs to Cross 4.

Figure 4.9 Protein banding patterns of F_1 seedlings of Cross 2 (Bruno x D-120)

A. Showing individual seedlings 1-14 (left to right)



B. Showing individual seedlings 15-28 (left to right)



▌2-3 Regions 2 and 3

The protein banding pattern differed between seedlings of crosses 1 and 2 especially in region 2. As there was a common female parent, the difference must be attributable to the male parent's contribution to the genotypes of the F1 progeny.

Because the male vine D-16 was missing, its protein profile was not determined. However both parents were available for the seedlings in cross 2 which permitted inheritance analysis.

Since Beckman et al (1964) reported that both parental enzyme variants could be present in F1 hybrids of maize, many research papers have confirmed that the isozymes coded by both alleles at a given locus are codominant. For protein analysis, Payne and Lawrence (1983), and Slack et al (1989), identified a number of allelic proteins derived from each of the glutenin genes in wheat, and indicated that many of the alleles occurred as pairs of proteins. Gardiner et al (1986) analysed seed protein extracts from hybrid ryegrass and found that like enzymes the proteins of hybrids possess a combination of patterns inherited from their parents. They showed that like isoenzymes, some polypeptides coded by both alleles at a given locus are codominant.

Bruno gave rise to only one band in region 2, and 2 bands in region 3 whereas D-120 gave 2 bands in both regions 2 and 3 (Figure 4.6, p133). Assuming the slow band was coded by allele "A", and the fast band by allele"a", and assuming the polypeptides coded by the same alleles had identical migration rates (discussed later) either one or two bands would occur in F1 seedlings as follows.

The protein patterns of F1 in region 2 would be:

Bruno D-120
AA x Aa
F1
50% AA, 50% Aa,

and the protein patterns of F1 in region 3 would be:

Bruno D-120
Bb x Bb
F1
25% BB, 50% Bb, 25% bb

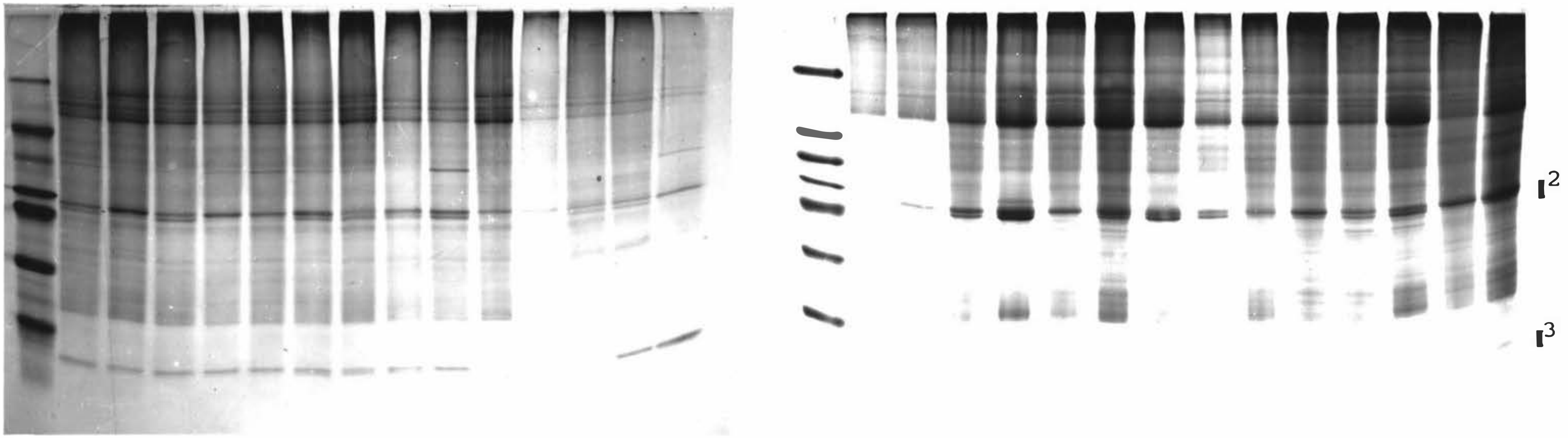
When seedlings of cross 2 were examined, the two expected banding patterns were found in region 2. Some seedlings gave both a slow moving band and a fast band in this position, others gave only a slow band. The fast band, which came from the male parent D-120 was absent from protein profiles of nearly half of its progeny, indicating that the allele coding for the fast band was separated from the allele coding for the slow band in a manner that followed the Mendelian law of independent assortment of alleles. The ratio of the two patterns in region 2 was 1.3:1 (28 seedlings), which was close to the theoretical ratio of 1:1 for a diploid species, suggesting a similar inheritance behaviour in kiwifruit. The Chi-square test for simple 1:1 inheritance of region 2 ($X^2 = 0.32$, where $X^2_{0.05} = 3.84$) supported this conclusion.

However, chromosome numbers confirm kiwifruit is hexaploid, therefore the assumption of Arulsekar et al (1981) may assist in the interpretation of the banding patterns obtained. The assumption was that functional enzymes coded by the same alleles at different 'loci' (assumed to be due to polyploidy) have identical migration rates, and therefore in the polyploid kiwifruit there must be several polypeptides which migrate together to give a single band. Thus, one may hypothesize that the hexaploid kiwifruit, which in some respects behaves as a diploid, is a diploidized polyploid derived from unknown ancestors.

Since both Bruno and D-120 gave 2 bands in region 3, according to the Mendelian laws of segregation and independent assortment of genes, the progeny should show the 3 banding patterns shown above, corresponding to BB, Bb and bb. The inheritance ratio of these protein patterns should be 1:2:1. However within cross 3 only 3 were found to belong to pattern 1 among 28 seedlings, and no seedlings were found to give the third pattern. As this differed substantially from the theoretical ratio, it suggests that strong selection had occurred against homozygous genotypes. Parfitt and Arulsekar (1989) discussed a similar phenomenon in a study of inheritance of isozymes among grape cultivars, and pointed out that lethal recessive alleles may explain the absence of the represented genotype. In fact, similar cases were found in region 2 in following analyses, (see Figure 4.10) suggesting that the different lethal alleles had become associated with different gene loci.

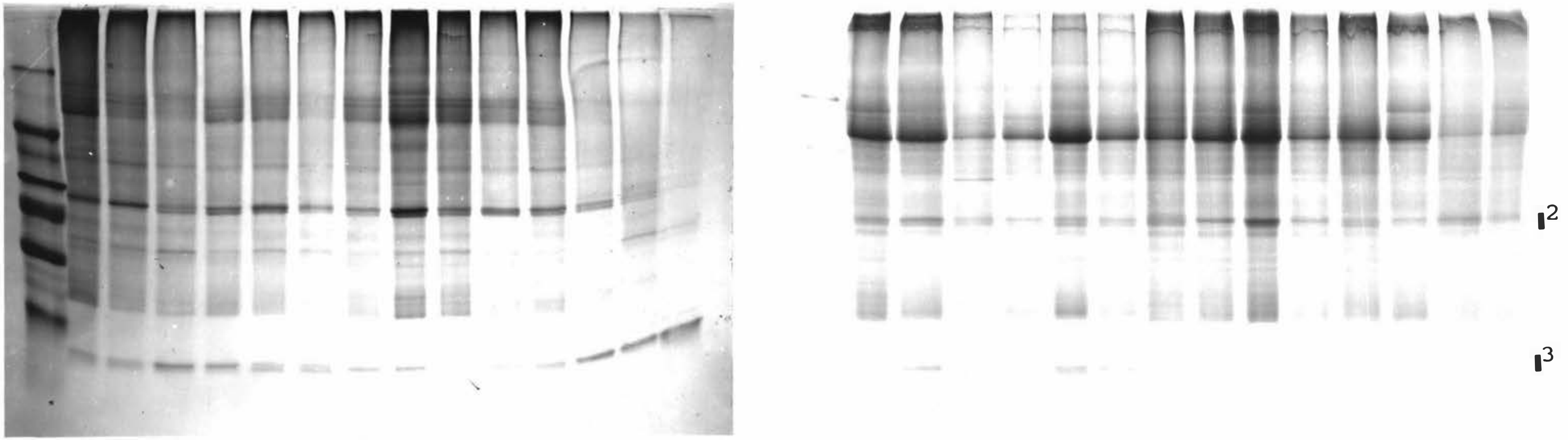
No protein profiles were available in Cross 3 due to a very small sample size, thus only protein patterns from cross 4 (Gracie x D-120) are presented (Figure 4.10). Since both Gracie and D-120 gave 2 bands in region 2 (Figure 4.6, p133) three patterns (corresponding to AA, Aa, aa) with the ratio of 1:2:1 could be predicted in their F1 population. But the protein profiles of only 12 among 56 seedlings were found to belong to pattern 1 and no seedlings were found to have the third pattern. Again the assumption of lethal recessive alleleles (Parfitt and Arulsekar 1989) may explain its absence.

Figure 4.10 Protein banding pattern of F_1 seedling of Cross 4 (Gracie x D-120)



A. Showing individual seedlings 1-14 (left to right)

B: Showing individual seedlings 15-28 (left to right)



C. Showing individual seedlings 29-42 (left to right)

D: Showing individual seedlings 43-56 (left to right)

▌2-3 Regions 2 and 3

The parents D-120 and Gracie gave 2 and 1 band respectively in region 3. Therefore 2 patterns in equal proportions could be expected in their F1 seedling population. The observed ratio of the two patterns in region 3 was 1.08:1, close to the theoretical ratio of 1:1 for diploid species. The Chi-square test for simple 1:1 inheritance of region 3 ($X^2 = 0.41$, where $X^2_{0.05} = 3.84$) supported the conclusion.

Because vine D-16 was missing, inheritance analysis for cross 5 (Figure 4.11) was not possible. However, in region 2, 2 protein patterns were found. One pattern was with only one fast band, the other had 2 bands. The ratio 1.2:1 of these 2 patterns was close to the theoretical ratio of 1:1 for gamete segregation of an Aa genotype. It was shown that Gracie gave 2 bands in this region, hence D-16, whose protein banding pattern could not be determined, must give only one band in region two.

This conclusion was supported by the results (Figure 4.8, p136) for the cross Bruno x D-16. Bruno and all the seedling progeny gave only one band in this region, therefore D-16 must be considered as giving only one band in region 2, being the slow band of the pair.

Both Hayward and D-120 gave 2 bands in region 2, thus the progeny of cross 6 (Figure 4.12) could be expected to give 3 banding patterns here, in the ratio of 1:2:1, as previously discussed. In fact, among 24 seedlings, 7 gave pattern 1 and none gave the third pattern. An observed ratio of 1:2.42:0 was close to the theoretical ratio of 1:2:1 with pattern 3 absent probably due to the effect of lethal recessive alleles (Parfitt and Arulsekar 1989).

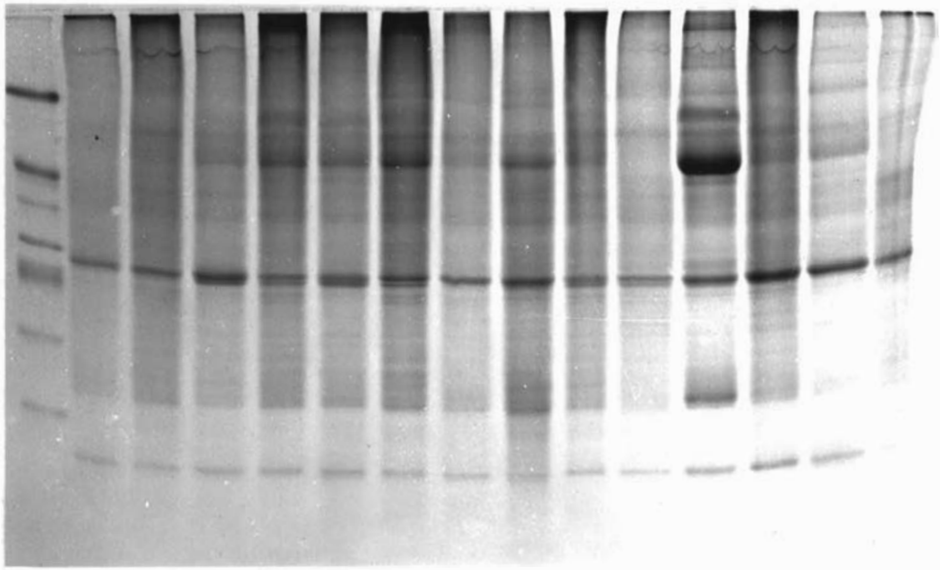
In region 3, D-120 had 2 bands and Hayward had only one, hence 2 patterns in equal proportions could be expected in their seedlings. However, only 3 were found to belong to pattern 2 among 24 seedlings, suggesting that strong selection had occurred against seedlings of the second pattern of protein banding.

To sum up, the following findings were obtained in these experiments:

(i) Three regions of the protein profiles were found useful for cultivar identification. Some of the named cultivars investigated could be distinguished from one another by using these three regions. No difference was found between the selections of Hayward, indicating they were homogeneous genetically in these regions. For the cultivars which could not be distinguished in this study, alternative techniques or tissues may be more satisfactory.

Figure 4.11 Protein banding pattern of F_1 seedling of Cross 5 (Hayward x D-16)

A. Showing individual seedlings 1-14 (left to right)



B. Showing individual seedlings 15-28 (left to right)

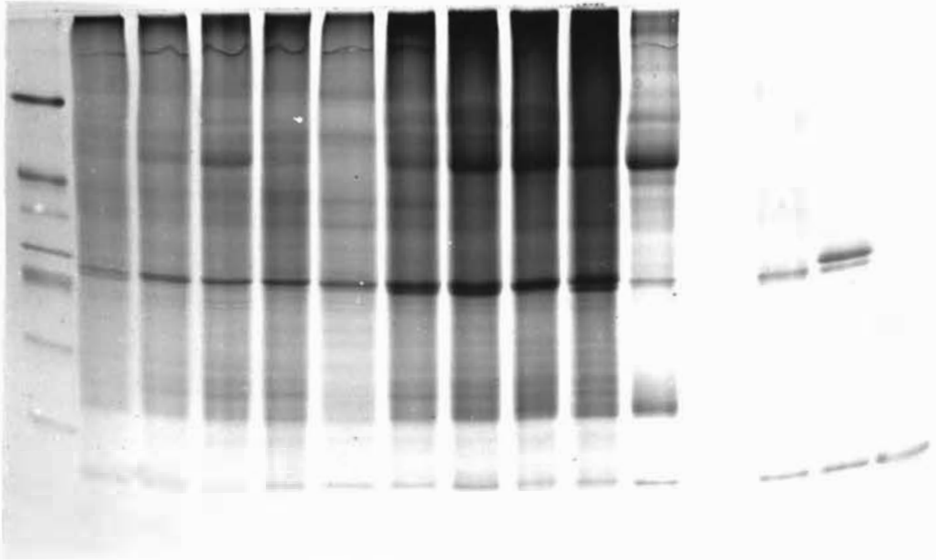
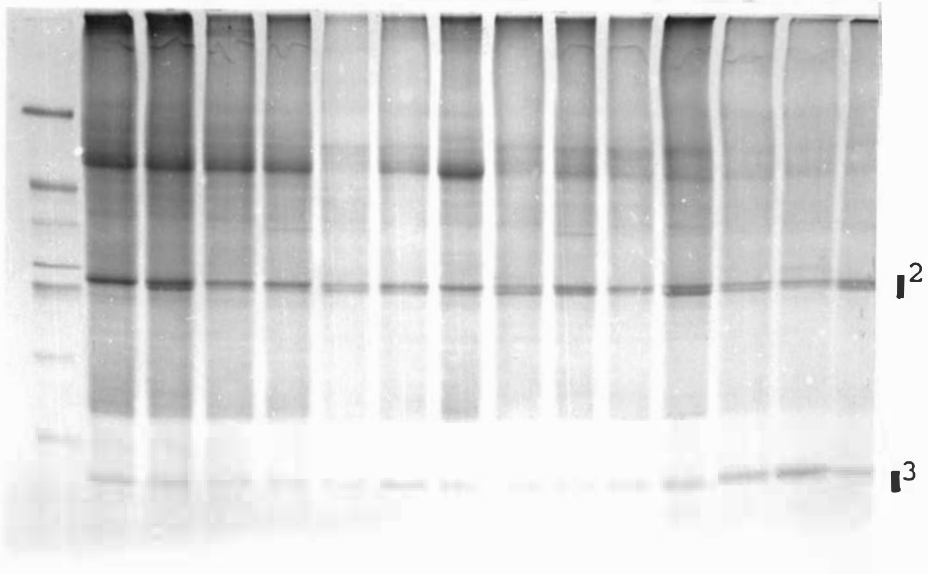
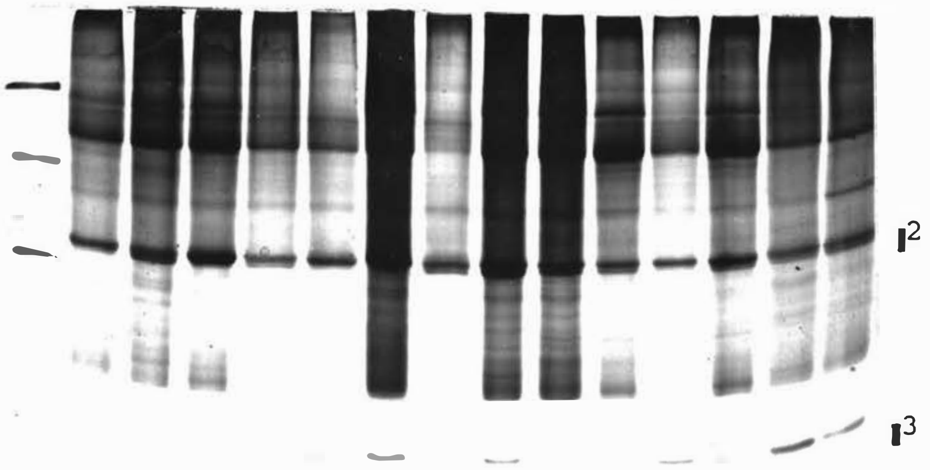


Figure 4.12 Protein banding pattern of F_1 seedling of Cross 6 (Hayward x D-120)

A. Showing individual seedlings 1-14 (left to right)



B. Showing individual seedlings 15-28 (left to right)



▌$^{2-3}$ Regions 2 and 3

While the three identified regions proved useful in cultivar identification, they were not adequate to characterise individual F1 progeny. This was because both parental polypeptide variants could be present in F1 hybrids, and gene recombination makes the protein patterns of F1 more complex than their parents. Regions were characteristic of some crosses, for example, in cross 1 (Bruno x D-16) all seedlings analysed had only 1 band in region 2. This region therefore distinguished cross 1 from the other crosses, but on a group basis rather than for individual plants, i.e. there were not enough proteins which could be demonstrated to show variability in banding patterns in the vines studied.

(ii) There was some variation in the protein markers in the F1 populations investigated, implying the progeny varied genetically. In plant selection, it would be useful to link some agronomic character to variants in protein banding pattern and band intensity and such a linkage has been reported by Tanksley and Rick (1980) in tomato and by Kosmolak et al (1980) in wheat. The multivariate analysis technique especially canonical correlation is suggested for this purpose. With quality and productivity characters on one domain, and data derived from protein profiles on the other domain, the canonical correlation coefficient between the two domains can be obtained. For doing so, a scanning-densitometric approach could be used to capture the protein profile and to quantify band patterns and band intensity. Then the digital representation of SDS-PAGE results could be analysed statistically and related to plant characteristics.

(iii) The banding pattern of some polypeptides could be used to indicate the mode of origin of a genotype. This work showed that kiwifruit had a similar inheritance behaviour to diploids. However, kiwifruit is a hexapoloid, suggesting that the ancestors of modern kiwifruit cultivars were diploidized polyploids. Because allopolyploids tend to behave as a functional diploid in inheritance, the kiwifruit may be an allohexaploid. It possibly arose from hybridisation of diploid and tetraploid species to give a sterile triploid hybrid, followed by chromosome doubling and the restoration of fertility. However, to our knowledge, this is the first time leaf polypeptides have been used in genetic analysis, and the assumptions made in this work need to be confirmed.

(iv) Apart from differences between banding patterns, different banding intensities were also found within the seedling populations. Such changes may be due to the effect of gene dosage. Chyi and Weeden (1984) analysed two triploid apple cultivars and their diploid parents electrophoretically, and a skewed intensity pattern in the hybrids indicated the female parent was the source of the 2n gamete in both triploid cultivars. This example implied that it is possible to use the effect of gene dosage to explain different banding intensities.

(v) There was some evidence in late March (autumn), differences in protein band intensity were associated with vine sex, but no qualitative band differences were clearly evident. It would be highly desirable to distinguish between male and female seedlings of kiwifruit in a selection nursery in order to shorten breeding programmes. Hence further work is desirable, investigating other extraction mediums for proteins in different plant tissues, and also DNA determination. Because of the possible influence of environmental conditions on relative banding intensities, it would be preferable to identify qualitative differences in banding patterns.

CHAPTER 5

GENERAL DISCUSSION

1. KIWIFRUIT BREEDING

Kiwifruit is one of the most recently domesticated fruit crops, first developed as a major cash crop in New Zealand. Because of its unique properties and successful commercial cultivation in New Zealand, kiwifruit plantings throughout the world have increased dramatically in the last 10 to 20 years as consumers have been quick to accept this new fruit. Kiwifruit has become an important horticultural crop in a number of different countries. In the early stage of kiwifruit cultivation, work concentrated on how to grow and market the fruit, but more recently there has been increased study on vine physiology and cultivar improvement and selection.

Unlike other fruit crops, such as apple and orange, kiwifruit cultivation has only a very short history, and initially only a small number of cultivars were selected from a very narrow gene base. In most cases, the kiwifruit growing countries have used material either raised or originating in New Zealand, mainly the female cultivar Hayward and the male cultivars Matua and Tomuri. Such a situation has its problems, and means for example a potential for high losses from disease epidemics, and provides a very narrow product range for marketing. Furthermore, Hayward is a good cultivar, but by no means perfect. The following traits need to be improved further:

- it is slow to come into bearing as a young plant.
- it responds poorly to spur pruning methods, the options for training and pruning are therefore more limited.
- it often has a relatively low percentage bud break and it is less floriferous under conditions of low winter chilling.
- the 'Hayward' mark on the fruit is a common fault of this cultivar and leads to downgrading of the fruit.
- the fruit tend to have too sharp or acid a flavour for some consumers.

(Ferguson et al, 1987)

In addition to the problems of current cultivars, marketing competition also requires cultivar improvement. Within the past decade kiwifruit production has increased dramatically. This situation must bring drastic competition between the kiwifruit growing countries, and between growers within these countries. This competition in part can be met by making available cultivars that are cheaper to produce, more productive or with different and superior fruit characters. Therefore improving current cultivars and creating new cultivars is necessary.

At present in New Zealand some 15 ha of land of the Department and Scientific and Industrial Research (DSIR) are dedicated to kiwifruit breeding and many inter- and intra- species crosses have been made and about 11,000 vines of different ages are involved (Feguson et al. 1988). Seedlings from a number of crosses within Actinidia deliciosa in this programme were used in this work, and the results of this study indicated a great deal of variation in vegetative, floral and fruit characters among the seedlings investigated, and many of these seedlings are of horticultural interest. Some progenies surpassed their parents in certain characters. In male populations, for example, the earliest date of blossom of seedlings was 3 days earlier than the male parent D-120, and the latest flowering plant blossomed 14 days later than its male parent. This gives a great chance to select an appropriate polleniser to match the flowering date of female cultivars, such as Hayward which is relatively late blooming. In female populations, the vitamin C content of seedling fruit varied considerable from 25 mg/100 g fresh weight to 196 mg/100 g, while in Bruno the cultivar with the highest vitamin C it was 133 mg/100 g on average and in Hayward, only 83.5 mg/100 g. It was also found the average fruit weight from female seedlings varied from 31 to 153 g, while the average weight of Hayward was 84 g. All these examples indicate that there is a great potential to improve kiwifruit by breeding.

However, while some promising material has emerged in these F1 populations, no efficient methods have yet been reported to assess these vines. There was also very little quantitative genetic information in this species. Therefore three analyses were proceeded in this work to assess the potential to uncover, identify and capitalise on the gene pool present in New Zealand and which is initially likely to be the basis of breeding programmes in the country.

2. MULTIVARIATE ANALYSIS OF PHENOTYPIC DATA

Multivariate analysis is the branch of statistics concerned with analysing multiple measurements on one or more populations. For an overall evaluation of seedlings and selecting superior vines in kiwifruit breeding, the assessment should not be based on only one or two horticultural traits, but on several traits together. This can only be done with multivariate analysis which was the basis of this study.

Three important multivariate techniques, which included multivariate analysis of variance (Manova) and discriminant analysis, factor analysis, and canonical correlation analysis, were applied in this work, and the results indicated they were useful tools to sort crosses and vines and to examine the relationships between the variables investigated.

(1) Discriminant analysis for cross sorting

In plant breeding, it is important to select superior parents and this can be done with cross evaluating. Manova was used to handle multiple characters, and the results showed strong significant differences between crosses in both the male and female seedling populations on the combination of characters observed. Discriminant analysis then revealed the superior parents for kiwifruit breeding. In male vine selection, the Hayward and D1-20 cross produced heavy flowering progeny with early vigour, which was shown as best cross combination; Bruno crosses with either of two male parents produced floriferous vines, hence it was the superior female parent cultivar for breeding floriferous male vines. Compared with D1-6, D1-20 was a better male parent to give rise to progeny with early vigour and floriferousness. In the production of female vines, Bruno proved the best parent for producing vines with high yield and high fruit vitamin C content.

One may think that evaluating crosses can also be done just by mean comparisons of Anova results. Often in horticultural research, results are analysed for only individual responses separately, but the relationship between the character is unknown, much less for which is the most important character. Furthermore, the criteria for assessing crosses, such as commodity quality are always determined by a number of attributes combined, and if there are more than 3, there is no way to plot them together to get an overall conclusion. To solve this problem, discriminant analysis can be used to find linear combinations of the variables that maximise differences among pre-existing populations. Since the first few linear functions are usually the most important, the original variables which mainly contribute to these functions therefore are important variables. And the relationship of these variables in the structures of these functions can also be examined. The function scores of each cross on the functions can be plotted together to show the distance between these crosses. To sum up, discriminant analysis can be used to quantify those characters most powerful in distinguishing between groups, whereas Anova fail to compare the relative importance of the characters.

However, in the current study the most important discriminant functions did not sort vines on the basis of characters with horticultural value, and both male and female vines were only grouped by vegetative characters by these functions. Therefore factor analysis was further used to solve this problem.

(2) Factor analysis for selecting superior vines

Factor analysis has been the generic term for principal component analysis and various common factor analyses. But only principal component and principal factor analysis were used in the study, and the two procedures gave similar factor patterns for interpreting and naming each factor. Compared with Manova, factor analysis is essentially a screening technique rather than a technique of analysing variance. It is used to group individuals in one population, therefore it is desirable to use it to group vines that are nested within a cross. Different factor patterns were found between crosses, and between years for the same cross. These findings confirmed experimentally that factor analysis is a single population technique, and one should make separate analyses if many populations are involved.

The results in this study showed that the best male vines for early vigour and high floriferousness, and the female vines with high yield and vine efficiency could be easily determined by factor analysis by plotting the factor scores of the first two most important functions. Sometimes it may be desirable for 3 factors to be considered together in selection, and a 3-dimension plot may be utilised for this purpose. But with a large number of points, the plot becomes too cluttered to be useful (see Figure 2.2.3).

Another virtue of factor analysis is that the relationship between the original variables which together comprise a factor can be examined, and the magnitude and nature of these relationships can be guides for the improvement of a certain character. For example, factor 1 in female vines was strongly associated with TW, and also associated with FD, TF, DIASP, TSH, FLORI, FLPERI, and FNF. Therefore the fruit characters FNF could be considered as the components of the yield, and the others as the basis of high yielding. These relationships also indicate the necessity of good early vine vigour and bud break (DIASP and TSH) in order to give high yield.

As mentioned above, the most powerful discriminant functions only grouped vines vegetatively, but the most important factors sorted vines according to their economical attributes. It is of interest to compare the factor analysis with the discriminant analysis as the two methods gave different solutions. this may be because discriminant functions are so established that the ratio of between groups-variance to within group-variance is a maximum, while the factors represent the largest amount of variance among individuals within one population. Discriminant analysis may be able to group vines if two or more years' data is used, where the records of the same vine in different years are treated as one group. The variance of a cross includes both variance between years and variance between vines nested in the cross. Therefore discriminant analysis is more suitable to select the best cross combinations following hybridisation, while factor analysis is suitable to screen single plant in a selection programme.

Because factors represent the patterns of relationships within the original variables, it is possible to examine the constancy difference of these patterns between populations and between years. By using the coefficient of congruence (CC) of Cureton and D'Agostino (1983), the difference between the factor patterns of different crosses, and that between years in the same cross were examined. The former may be mainly from genotypical difference, while the latter may be from environmental variance or in response to the vine maturing/ageing. By examining the factor patterns, one can investigate which characters mainly contribute to genotypical difference and which characters mainly contribute to environmental variation, and also the relationship between the various characters. In addition, the different growth types may be detected by factor pattern comparison. For example, factor 1 was a floriferous factor in male vines for all crosses investigated, but its relationship with vine vigour characters was different between crosses. There was a strong positive relationship between vigour and floriferousness in a Bruno cross and a relatively weak one in both Hayward and Gracie crosses. The pattern of factor 1 in Bruno seedlings changed markedly from one year to the next, but in contrast, the Hayward and Gracie crosses gave a more similar pattern in the following season. This may therefore reflect a balanced pattern of growth and development in Hayward and Gracie seedlings at an age of 3 to 4 years whereas in Bruno seedlings annual growth relationships were more changeable. This indicates that some aspects of vine development were susceptible to increased yearly variation in some genotypes. The identification of such relationships may enhance our understanding of the mechanism of balance between growth and development in various types, and assist in selection of the best plant types.

Since no genetic information is involved in factor analysis, it is less efficient than the selection index for vine selection. However, Godshalk and Timothy (1988) reported that principal component plant scores had a high correlation with those of selection index and indicated the PC analysis of simple correlation matrices had the potential to be used for selection purposes, since there were several limitations of index selection that reduced its effectiveness (see Chapter 1). Although further research is needed to confirm the results of Godshalk and Timothy(1988), factor analysis can be used as a selection criterion in some circumstances such as where no genetic information is available or there is no experimental design.

(3) Canonical correlation between aggregated characters:

The canonical correlation is the maximum correlation between a linear function of the two vector variables. The results showed that the canonical correlation provided interesting information concerning the relationship between three domains of kiwifruit properties. In the male seedlings, a relatively high canonical correlation (0.66) was detected between aggregates of vegetative characters (explained mainly by total shoot number per vine and leaf shape index) and flowering characters (explained mainly by flower number per shoot and percentage of flower shoots per vine). This correlation revealed an association between the number of shoots developing in spring, and the flowering efficiency of the vine, and indicated the vine was an integrated system with vegetative development interacting with flower development - and possibly this relationship had a genetic basis. In addition, the characters TSH and LWRATI appear to be potentially useful indicators to help breeders select a floriferous male vine - possibly even in the kiwifruit's juvenile period, although that remains to be confirmed.

In the analysis of female vines, a high canonical correlation between flower size variables (FD and PL) and fruit size was found, suggesting FD and PL could be used as selection criteria to select vines with large fruit. However, not all fruit characters of commercial value were found to be associated with vegetative or flowering characters, indicating more characters, including physiological characters need to be recorded for canonical analysis.

3. GENETIC ANALYSIS

In fruit tree breeding, the long period of juvenility and the long generation cycle greatly slows the exploitation of genetic recombination and increase the costs of rearing and evaluating seedlings, therefore the designs which maximise the rate of genetic improvement of breeding material and minimise the cost of obtaining the improvement are important. But it is difficult to predict the success of different breeding strategies without information on variation and inheritance of the characters of horticultural value. In this study quantitative genetics methods were employed to provide such information.

It has been demonstrated that genetic studies of quantitative traits are useful for developing breeding methods and selection procedures in fruit trees, and for predicting genetic gain of following generations (Barritt, 1982; Hansche, 1986a, 1986b; Hansche and Boynton, 1986; Hansche et al, 1966, 1972a, 1972b, 1975, Shaw, 1988). But there is no similar report for kiwifruit, therefore the estimation of genetic parameters is necessary to facilitate kiwifruit breeding.

(1) Genotypic correlation

Sometimes it is necessary to examine correlated characters and to know if the improvement of one character in plant breeding will also result in a simultaneous change in the other characters. To effect a simultaneous improvement in two characters, an understanding of the nature and the magnitude of the association of the two characters is necessary, because the direction of the correlation could enhance or inhibit selection progress (Tyagi, 1987). Such correlations have been determined for some fruit crops, and the interpretation of these results has assisted in determining the relative importance and genetic properties of characters included in selection programmes (Hansche et al, 1966, 1972; Shaw, 1988).

The genetic correlations between 10 characters in male vines and 18 characters in female vines were examined in this work, and the phenotypic correlations between these characters were also calculated for comparison. The results showed that the genetic and phenotypic correlation between the same characters were similar in direction but different in magnitude. This is because that genetic correlations imply a relationship between the genes that condition the traits, whereas phenotypic correlation result from both shared genetic effects andshared environments (Shaw, 1988).

In male vines high genetic correlations were found between floriferous characters (expressed as percentage of flowering shoots and lateral flower number) and vine vigour characters (expressed as diameter of main stem and increment of main stem). Therefore selection for floriferous male genotypes with high vigour will/can be expected to produce greater genetic gain among their offspring than selection for floriferous vines with less vigour. Similarly in female vines, the vine vigour characters were strongly associated with total cropyield and mean fruit weight, indicating these characters can be improved together in the selection process. It also means that in selecting for high vine vigour, an improvement in the FW will also tend to follow.

It is interesting to note the fruit vitamin C content was strongly negatively associated with mean fruit weight. Where big fruit and high vitamin C content are the selection goal, genetic progress may be considerably difficult to obtain due to the unfavourable genetic correlation between the two characters. Thus a selection index is needed to make a compromise between the characters.

In breeding pratice, a search is always being made for characters which can be assessed in the juvenile period and are correlated with fruit characters in the mature tree, in order to discard undesirable plants at an early stage. Leaf shape index was examined for this purpose in this work. It was positively associated with bloom date in the male population, and strongly negatively associated with fruit symmetry and core size in female plants. Therefore it may be a useful character in the juvenile period to predict the male vines that will have either an early or late bloom date, and the female vines with symmetrical fruit with a small core size. But these genetic correlations were established on young mature vines. There is no proof that the same correlation will hold when juvenile materials are used, therefore an overall study from young seedlings to mature vines is needed.

(2) Heritability estimation

For a number of important species of fruiting plants, heritability estimates (h^2) have been made for a range of plant and fruit characters. They provide a quantitative predictor of the effectiveness of parent selection in influencing progeny quality, and can be used to estimate the rate of genetic improvement in the traits of commercial importance (Barritt, 1982; Hansche, 1983, 1986a, 1986b; Hansche and Boynton, 1986, Hansche et al, 1966, 1972a, 1972b, 1975; Kester et al, 1977).

The values for the heritability of the earliness of bloom (0.48) and flowering duration (0.50) in male vines, and total weight (0.46), the percentage of flowering shoot (0.54), fruit elongation (0.65) and mean weight (0.52) in female vines were high in this study, indicating that individual selection of superior plants with these traits could give rise to rapid genetic progress among offspring.

Terminal flower number per shoot in both male and female vines, and fruit vitamin C concentration, earliness of bloom, and flowering duration in female vines had moderate or low values for h^2. This indicates that for these traits, the individual selection of superior seedlings as parent would contribute to genetic improvement in their subsequent generations, but the rate of genetic gain would be lower than that of the traits with higher heritability. Therefore family selection based on dispersion should be used.

Hairiness of fruit, total shoots per vine and percent soluble solids of fruit in female vines gave very low h^2 (negative values), indicating that these characters cannot be improved efficiently by individual selection. However, although the narrow sense h^2 value was low for these characters, the broad sense h^2 was reasonably high for some of them. For example, percent soluble solids of fruit gave a negative value for h^2 (-0.19), but 0.37 for broad sense h^2,indicating that there are some dominant and epistatic effects involved. Because kiwifruit is clonally propagated, all of the genetic variances - additive, dominant and epistatic can be utilised for improving these characters.

In conclusion, since the date at which flowering began and flowering duration in male vines gave high heritability values, one could expect to fairly quickly develop males that were either early or late flowering, and with short or long bloom periods to meet the requirement of current female cultivars or pollination practices, by using simple individual selection. Similarly for female vines, big genetic gains are feasible in improving the characters such as vine yield, fruit size and fruit shape, and fruit acidity due to their high heritability values.

It is important to note that the heritability is a property not only of a character but also of the population and of the environmental circumstance to which the individuals are subjected (Falconer, 1981). The value of the heritability estimate depends on the magnitude of all the components of variance, and a change in any one of these will affect it. All the genetic components are influenced by gene frequencies and may therefore differ from one population to another, according to the past history of the population. So, whenever a value is stated for the heritability of a given character it must be understood to refer to a particular metrical character, in a particular population, at a particular moment and in a particular environment (Ewing et al, 1987).

In the current study, only 3 female cultivars and 2 male cultivars of kiwifruit were involved, and they all belong to A. deliciosa. Therefore the estimates of h^2 are relevant only to this species and to the relatively narrow range of germ plasm used. However, as this material represents the main part of the gene pool of the species available in New Zealand, the inferences from this study are of relevance to most of the current New Zealand kiwifruit breeding work.

There is some similarity to the h^2 values published for a number of different species of fruit crops. Table 5.1 shows that fruit length and fruit weight had a similarly high heritability for most species investigated, indicating great genetic potential for increasing fruit size exists in these breeding stocks. Date of bloom also gave high heritability for most species, although the value was low for female kiwifruit. The heritability of fruit soluble solids and yield varied considerably between species, and some species had very low values. Compared with soluble solids, fruit acidity is relatively consistent between peach and kiwifruit, indicating it is easier to improve fruit acidity than to improve soluble solids by individual selection.

Hansche et al (1972a, 1972b) pointed out that crude subjective measures may be a major cause of a low heritability value, since the crude subjective scale used to measure the character would, by itself, preclude the discrimination of any but gross genetic differences, and thus also preclude the possibility of determining a reasonable rate of genetic gain. The total yield per vine was accurately measured in kiwifruit, while crude scores were used for the other species in Table 5.1. This may be the reason the heritability of yield for kiwifruit was much higher than reported for other species. This indicates that accurate measurement of plant characters is necessary for heritability estimation.

Table 5.1 **The heritabilities (h^2) of some characters of horticultural value in various fruit crop species**

	Fruit Length	Fruit Weight	Bloom Date	Soluble Solids	Acidity	Yield
Almond	0.77					
Apricot	0.61		1.02			
Peach	0.31		0.39	0.01	0.19*	0.08
Plum	1.00	0.97	0.86	0.49		-0.17
Peach (Dw)		0.50		0.17		
Nectarine (Dw)		0.20		0.35		
Sweet Cherry			1.00			0.03
Walnut	0.82	0.86	0.91**			0.03
Kiwifruit (M)			0.48			
Kiwifruit (FM)	0.65	0.52	0.11	-0.19	0.39	0.46

* By tasting

** Fruit elongation

(Table 5.1 is based on the data of Hansche, 1983, 1986a; Hansche et al, 1966, 1972a, 1972b, 1975; Kester et al, 1977).

Heritability cannot be estimated with any great precision if small samples are used. Nevertheless, despite the lack of precision, it is very clear that heritability differ greatly according to the character. In terms of the relative value of h^2, a comparison between traits is valid.

(3) Selection response

The results of this study showed that some characters concerned with fruit productivity and fruit quality had relatively high heritability, and therefore they can be improved by simple recurrent mass selection. The calculation of selection response showed that great potential exists for kiwifruit improvement in those characters. For example, the expected cumulative selection advance in fruit Vitamin C is 4.21, 9.24, 13.9, 18.6, 23.32, 28.06 mg/100 g respectively in 6 selection cycles. After 6 cycles selection, the mean value would increase from 92.01 mg/100 g to 120.07 mg/100 g. Another example is the projected increase of fruit weight, and after 6 selection cycles, mean fruit weight would increase from 83.96 to 135.43 g. But as these results are only predicted values, it is necessary to test the validity of these estimates of expected genetic gains. Caldwell et al, (1966) compared selection methods for soybeans, and indicated that the expected gains were more extreme than the actual ones, but both ranked the various selection methods similarly. Eagles and Frey (1974) computed actual gains of grain and straw yield for oats, and found that estimated genetic gains invariably overestimated the actual ones for all traits and for all methods of selection. No information on this aspect is available for fruit tree crops, therefore further research is needed to facilitate the evaluation of a theoretical value.

The genetic gains for 4 important characters of commercial value after five selection cycles were examined in this work by using different selection strategies in different mating systems in a line breeding programme. Based on the calculated genetic gains, full sib mating was the most effective method followed by half sib mating and random mating for individual, among line and combined selection strategies, but random mating was the most effective method for within line selection.

Within 5 cycles, individual selection would be more effective than within line and among line selections in a random mating design/system. Although combined selection was the most efficient method in all mating systems, it is more complex than individual selection. Moreover kiwifruit is dioecious, and the selection of a male vine as parent is subjective, thus recurrent selection without pollen control may be the appropriate selection method for improving the characters of high heritability, because it combines the advantages of reasonably high genetic gain and simplicity of application. In addition to the mating problem, the estimates of genetic and environmental variances and heritability for many characters of economic value suggest recurrent individual selection should facilitate a greater rate of improvement in kiwifruit breeding stock than the more sophisticated alternatives that exploit nonadditive genetic variance but double the minimum length of the selection cycles, and therefore demand more time and space. But since the relative effectiveness of individual selection decreases with the decreasing of the heritability values of the characters, if h^2 of selected character is low - more than half of genetic variance present in a given breeding stock is due to non-additive gene effects, other methods (those involving inbreeding, back cross and the development of hybrids) can yield a greater rate of response than recurrent individual selection (Hansche, 1983).

By using information of both heritability and genetic correlation, the correlated responses of selection were compared with direct selection responses for several characters of economical value. The results showed the indirect selection could be interesting to some related characters. For example, vitamin C has a much lower h^2 (0.22) than TW (0.46) and TSH% (0.54), and has a moderately high genetic correlation with the two characters (0.59 and 0.66 respectively). Thus selecting by VC for improvements in TW and FSH% are far less effective than selecting for them directly. However, selecting TW for VC and selecting FSH% for VC are more effective than direct ones. In kiwifruit breeding vitamin C is an important character to improve, but the vitamin C test is a little involved. As an effective indirect selection method appears available, not only the procedure of a vitamin C test can be omitted, but also the selection response increased.

(4) Selection index

When selection is used to improve the commodity quality and productivity of tree crops, it is always applied to several traits simultaneously, since no one of these characters can adequately express the overall quality of a commodity and its productiveness. A selection index is suitable for quantifying these properties.

A selection index is a linear function of phenotypic values of different traits, and the observed value for each trait is weighted by an index coefficient. Three methods which are recognised as appropriate for simultaneous improvement of two or more traits in a breeding programme are independent culling, tandem and index selection. Hazel and Lush (1942) investigated the relative efficiencies of these 3 methods and pointed out the selection index is the most efficient one when the traits involved are independent. Young (1961) evaluated relative efficiency when the traits are correlated, and concluded that in most cases the index was a more effective method than independent culling levels and tandem selection.

A selection index for the selection of 3 important fruit characters was constructed in this work, the component characters, vitamin C concentration, total fruit weight per vine, and average fruit weight being combined together into a score. Superior vines could be selected using this index as if it were a single character. In fact, the discriminant functions and principal factors in multivariate analysis (see Chapter 2) can also be treated as indices of plant superiority or inferiority, but since no genetic information is involved, they are less effective than selection index.

In another application of the selection index Falconer (1981) constructed an index for male individual selection according to the additive covariance of the individual with its mother and with its half sister, viz.

$$I = 1/2\ p2 + 1/4\ p3$$

where p2 is the value of character investigated for the mother, and P3 for the half sister. But this index selects male vines only on a group basis rather than for individual selection. In kiwifruit selection, it is a problem to select male vine for a female character since the information about male vine cannot itself be measured. Therefore the above male selection index may be used to get some information for male vine selection.

4. SDS-PAGE TO CHARACTERISE KIWIFRUIT CULTIVARS AND SEEDLING POPULATIONS

Because of their long period of juvenility and important traits that are controlled by multiple genes, it is very difficult to make genetic studies on fruit trees. However electrophoretic techniques may help plant breeders resolve this problem. The profiles of specific isoenzymes and proteins may be used as gene markers to study variability and inheritance of these markers, so as to understand the linkage between parents and progeny. These techniques also assist the breeder to characterise cultivars and seedlings to examine their origin and kinship.

In this study SDS-PAGE of kiwifruit leaf extracts was used to characterise cultivars and seedlings. Three regions of the protein profile were found to be useful for cultivar identification, and the four parent cultivars could be differentiated easily by the unique banding patterns in these identity regions. The protein patterns of seedlings were examined and based on the protein patterns of their parents, the inheritance of these polypeptides was studied. The banding patterns found in seedlings within different crosses provided evidence that in kiwifruit the inheritance of these polypeptides occurred in a manner similar to that of a diploid. Therefore it is considered the kiwifruit may have arisen as a diploidized polyploid. Because an allopolyploid tends to behave as a functional diploid in inheritance, it is proposed the kiwifruit may be considered as allohexaploid. This therefore validates the results obtained in the genetic analysis which was based on the genetic model of diploid plant.

Another important aspect of genetic studies is to construct gene linkage maps. Theoretically tagging genes which control some characters of commercial value with isozyme alleles and protein bands is possible, especially if the character concerned is controlled by a single gene. Rick and Fobes (1974) found a tight linkage of the isozyme gene Aps-1 and the nematode resistance gene Mi in tomato, and this linkage has now been used to screen nematode resistance plants in tomato breeding (Tanksley and Rick, 1980). In kiwifruit it would be highly desirable to find a strong linkage between, for instance, genes coding for certain isozymes or polypeptides and fruit characters. Then superior vines could be selected from a seedling population early, to allow the most efficient use of time and resources and to shorten a breeding programme. This could be done by using a scanning densitometric approach to capture the protein profile and to quantify band patterns and band intensity, then the digital representation of the banding pattern could be analysed by multivariate analysis methods and related to plant characters.

Although no difference was found between male and female vines in any region of a protein track in this work, there was some evidence in late March (autumn), differences in protein band intensity were associated with vine sex. Hence further work is desirable, investigating other extraction mediums for proteins in different plant tissues, and also DNA determination.

In conclusion, for possibly the first time with kiwifruit, the multivariate analysis has been used to quantify these characters most powerful in distinguishing between vines and between crosses, and to examine their relationships. Thus, vines and crosses have been placed in order of their merit in meeting a breeder's requirements - based not on the individual characters, but rather on an overall linear combination of characters. This has been followed by the determination of genetic parameters, including variance components, heritabilities and genetic correlations. Based on these parameters, the success of different breeding strategies for different characters has been predicted, therefore the strategies with maximum efficiency and effectiveness can be adopted for kiwifruit improvements. Also a selection index has been constructed to facilitate improving several characters simultaneously. In addition, the electrophoretic analysis of kiwifruit leaf proteins has proved useful technique to characterise cultivars and seedlings, and to determine the linkage between genes coding for certain polypeptides and fruit characters to shorten a breeding programme.

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