GENETIC VARIABILITY OF FRUIT CHARACTERISTICS IN KIYOMI TANGOR PROGENIES OF CITRUS

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LIST OF ABBREVIATIONS AND ACRONYMS

a*	Colour coordinate a* = red to green
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
ARC	Agricultural Research Council
b*	Colour coordinate b* = yellow to blue
CAPS	Cleaved amplified polymorphic sequences
CIE	Commission Internationale de l'Eclairage
DNA	Deoxyribonucleic acid
EMS	Expected mean squares
EST	Expressed sequence tag
F1	First filial
F2	Second filial
ITSC	Institute for Tropical and Subtropical Crops
L*	Colour coordinate L* = lightness; black to white
MAS	Marker assisted selection
NaOH	Sodium hydroxide
PCA	Principal component analysis
RAPD	Randomly amplified polymorphic DNA
QTL	Quantitative trait loci
RFLP	Restriction fragment length polymorphism
SCAR	Sequence characterized amplified region
SSR	Simple sequence repeat
TCA	Tri-carboxylic acid
TSS	Total soluble solids

CHAPTER 1

INTRODUCTION

Citrus is regarded as a universal fruit being produced in over 100 countries and on all six continents. It is the most important tree crop, having a world production far exceeding that of deciduous fruit (Saunt, 2000). Citrus is found between latitude 40°N and 40°S in tropical and subtropical areas where favourable soil and climatic conditions occur (Ray, 2002). The general area of origin of citrus is believed to be South-East Asia, including south China, north-eastern India and Burma, though its introduction into cultivation probably started in China (Saunt, 2000). Today the major producing countries include Brazil, the USA, China, Spain, Mexico, India, Iran, Italy, Egypt, Argentina, Turkey, Japan, Pakistan, South Africa, Greece, Thailand, Morocco, Israel, Indonesia, Korea and Australia (Peña et al., 2007).

Citrus is mainly consumed as fresh fruit or juice, the pulp and rind from the processing can be used as animal feed or compost and the rind oil has many different uses (FABI, 2008). In world trade citrus is the most important fruit crop, its special structure and long shelf life allowing for large scale export as fresh fruit (Spiegel-Roy and Goldschmidt, 1996).

Commercial citrus species and related genera belong to the order Geraniales, family Rutaceae, sub-family Aurantoideae. The group of true citrus fruit trees consists of six genera, three of which are of commercial importance; these are *Poncirus* (trifoliate orange), *Fortunella* (kumquat) and *Citrus*. Within *Citrus* there are eight important commercial species: sweet orange (*C. sinensis*), mandarin (*C. reticulata*), including satsuma (*C. unshiu*) and clementine (*C. clementina*), grapefruit (*C. paradisi*), pummelo (*C. grandis*), lemon (*C. limon*), lime (*C. aurantifolia*), citron (*C. medica*) and sour orange (*C. aurantium*). Some hybrids of commercial importance include citranges (sweet orange x trifoliate orange) and citrumelos (grapefruit x trifoliate orange), used as rootstocks, and tangelos (mandarin x grapefruit), tangors (mandarin x sweet orange) and mandarin hybrids, used as cultivars (Saunt, 2000; Peña et al., 2007).

Many different citrus genotypes are grown in a wide diversity of soil and climatic conditions; therefore trees are subjected to various abiotic and biotic stresses that limit the production and, in some instances, the use of certain rootstocks and cultivars. At the same time that the citrus industry is threatened by important biotic and abiotic stresses, the markets in developed countries demand fruit of increasing quality. In this situation, genetic improvement of citrus is a major priority (Peña et al., 2007).

In South Africa the citrus industry is the second largest agro-industry, after deciduous fruit, and is regarded as one of the largest agricultural industries in the country in terms of export earnings (NDA, 2003). South Africa is the twelfth largest producer of citrus world wide, and even though it produces only 1.8% of the total world production it is the second largest exporting country of fresh citrus fruit (CGA, 2007). South Africa's citrus production is focused on export and is highly competitive; therefore it is extremely important to maintain a high fruit quality and keep abreast of changes in the world market (NDA, 2003). Although South Africa produces the full range of citrus products, there is a constant search for new and improved varieties that will allow the country to remain a competitor on the export market. Therefore it is essential for our country to have a citrus breeding programme in place to breed for these new and improved varieties. The Agricultural Research Council (ARC), Institute for Tropical and Subtropical crops (ITSC), at Nelspruit initiated a citrus scion breeding program in the late 1970's (Miller et al, 1996). With breeding and evaluation sites at Malelane in the north and Addo in the south of the country the program aims at breeding new superior quality citrus varieties for various South African climates (Bijzet and Combrink, 2004).

A citrus breeding programme starts with the selection of suitable parents and the planning of controlled crosses. Information on the breeding value of available parents and the heritability of specific characters is important in a plant breeding programme to aid the breeder in parent selection and the planning of controlled crosses. By quantifying the genetic variability in a population a breeder can study the genetic relationships between hybrids and parents and gain an understanding of how characters are inherited (de Oliveira et al., 2003).

Over the years various authors have stressed the importance of gaining information on the inheritance patterns in citrus. Soost and Cameron (1975) stated that there is a need to gain genetic information on the inheritance of specific characters and the combining ability of available parents. Vardi and Spiegel-Roy (1978) stated that another complicating problem for the breeder is the lack of knowledge on the mode of inheritance of desirable characters, and that few characters are known to be inherited in a simple genetic pattern. Khan and Kender (2007) stated that it is essential that future programmes for citrus cultivar improvement emphasise understanding the inheritance of fundamental qualitative and quantitative traits.

Very little information is available on the genetic variability of mandarin (*Citrus reticulata*) progenies and the inheritance patterns of characteristics in citrus, especially with regard to fruit characteristics. Therefore the aim of this study was to investigate the genetic variability in the progenies of six mandarin families, in the ARC-ITSC's citrus breeding program, with regard to the most important quality traits for citrus fruit in order to provide more information on the inheritance patterns of the traits studied and determine the value of the parents in citrus improvement.

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CHAPTER 2

LITERATURE REVIEW:
THE GENETIC IMPROVEMENT OF CITRUS

INTRODUCTION

Citrus is a tree crop, with a citrus scion cultivar grown on a citrus rootstock. The rootstock influences the performance of the scion by modifying its tree morphology and imparting resistance to biotic and abiotic stresses. Therefore a citrus breeding programme consists of two parts; one part is the breeding of improved citrus scion cultivars and the other part is the breeding of improved citrus rootstock cultivars (Ray, 2002).

Fruit tree breeding, especially using conventional breeding methods, is a difficult task. Researchers can expect the process to take 10 to 15 years from the first step, in which parents with desirable traits are identified and crossed, until the last step, when fruit is produced from trees propagated from the most promising seedlings. Following this comes still another four to five years of extensive field-testing to confirm that a new variety has commercial merit and will continue to thrive and fruit (Soost, 2001). Citrus breeding, based on conventional methods (hybridisation, selection, mutation) needs to be integrated with biotechnological methods (*in vitro* tissue culture, regeneration from protoplasts, somatic hybridisation, *in vitro* mutant selection, genetic transformation and haploid production) in order to obtain larger improvements in a shorter time (Germanà, 2007).

Citrus species have a complex reproductive biology. Some important genotypes have total or partial pollen and/or ovule sterility and cannot be used as parents in breeding programmes. There are also many cases of cross and self-incompatibility and many species are apomictic (poly-embryonic), which means that adventitious embryos initiate directly from maternal nucellar cells precluding the development of zygotic embryos and thus the recovery of sexual progeny populations (Peña et al., 2007). Embryony is a problem with a limited amount of mono-embryonic varieties available for use as female parents. This causes a problem for crosses within or between species such as the orange and grapefruit where few or no mono-embryonic parents are available. This problem has been slightly remedied by the increase in mono-embryonic cultivars produced by breeding and may still improve due to new products of somatic hybridisation by protoplast fusion (Spiegel-Roy and Goldschmidt, 1996).

Citrus has a long juvenile period and most species need at least five years to start flowering in sub-tropical areas, making citrus breeding projects long term and costly. Another difficulty is the expanded trials that need to be performed due to genotype x environment interaction and the copious seed formation in fruit due to cross-pollination in breeding and test plots (Spiegel-Roy and Goldschmidt, 1996).

All these features together with large plant size, high heterozygosity, lack of basic knowledge about how the most important horticultural traits are inherited, and quantitative inheritance of most characters have greatly impeded genetic improvement of citrus through conventional breeding methods (Peña et al., 2007).

In spite of the difficulties mentioned, there has been success in the breeding of new citrus scion and rootstock cultivars and in the mutation breeding by irradiation of budwood. With the recent use of biotechnology such as protoplast fusion and the production of transgenic plants, and their incorporation into breeding programmes, there are new possibilities for the genetic improvement of citrus (Spiegel-Roy and Goldschmidt, 1996).

THE HISTORY OF CITRUS BREEDING

The first organised citrus breeding programme was started in 1893 by W.T. Swingle and H.J. Webber from the United States Department of Agriculture in Florida (Soost and Cameron, 1975). Today many citrus producing countries have their own citrus breeding programmes. These include: Argentina, Australia, Chile, China, France, India, Israel, Italy, Japan, Mexico, Morocco, Pakistan, South Africa, Spain, Turkey and the USA (Turner, 2008).

CONTROLLED POLLINATIONS AND RAISING SEEDLINGS FOR BREEDING PURPOSES

The usual method for breeding citrus is the crossing of two parents to obtain a desired combination of characteristics, and selection in the first filial (F1) generation. This is sometimes followed by the intercrossing of the best F1 selections or crossing the F1 selection with established varieties having desirable traits (Ray, 2002). However the embryony (if the F1 is to be used as a female parent) and the pollen fertility (if the F1 is to be used as a male parent) need to be taken into consideration. The F1 seedlings of two mono-embryonic parents are themselves mono-embryonic, while about half of the seedlings of the F1 progeny of a mono-embryonic x poly-embryonic cross are mono-embryonic (Furr, 1969).

Controlled pollinations in citrus are done by hand and are fairly easy to perform. Flowers to provide the pollen should be picked just before opening, the petals and pistil are removed and the anthers left to dehisce. This generally occurs within 12 to 24 hours. Pollen is then placed in a vial and can be kept in a sealed container at 4℃ or lower for up to five weeks and still remain viable (Soost and Cameron, 1975; Ray, 2002).

Flowers of the female parent that are nearly ready to open are used for pollinations. The terminal bud should be used and the auxiliary buds removed. The flowers are emasculated by opening the petals and removing the stamens, while avoiding contact with the stigma. Pollen is applied with a brush to the stigma of the female flower. Other flowers and fruit are removed from the surrounding area to give the pollinated flower a better chance of setting fruit. Fruit set is reported to be better on leafy inflorescences, therefore leaves should not be removed. Pollination can be done immediately after emasculation or up to several days later. However if the flowers are not pollinated immediately they should be covered with a paper bag to avoid pollination by insects. Following pollination the flowers can again be covered, but this is not necessary since bees seldom visit flowers without petals (Soost and Cameron, 1975; Ray, 2002).

Pollinated flowers should be well marked to later identify the “pollination fruit”. Fruit are harvested at maturity, the seed extracted and germinated (Ray, 2002). Zygotic seedlings may be distinguishable from nucellar seedlings in crosses where the pollen parent has morphological characteristics that differ from the seed parent (Soost and Cameron, 1975).

The seedlings can be tested for resistance to pests and diseases, or environmental factors. Seedlings for evaluation of fruit characteristics need to be planted out to reach maturity and bear fruit for evaluation. Unfortunately there are no correlations between seedling characteristics and mature characteristics that allow the elimination of some seedlings (Soost and Cameron, 1975).

Seedlings can be planted out either on their own roots or grafted to a rootstock. Deciding whether to plant seedlings on their own roots or a rootstock is a difficult decision. A rootstock will protect the hybrid against soil borne diseases; however it may also provide a more uniform basis for tree growth, and therefore a more uniform basis for evaluation (Soost and Cameron, 1975).

EVALUATION OF F1 HYBRIDS

F1 hybrids are usually evaluated visually. Tree ratings are done for tree vigour, tree shape, yield and crop retention. Ratings on fruit size, shape, exterior rind characters, and interior characters, including peel thickness, pulp characteristics, and seediness, are also done visually. A rating of the palatability of the fruit is made organoleptically by judging the level of sugar and acid. The sugar and acid ratings are made several times through the season, if possible, to judge the time of maturity (Soost and Cameron, 1975).

Previously these ratings were defined by a word description; for example a rind colour was described as yellow, yellow-orange, orange or orange-red. To allow the evaluation to be done more rapidly and for statistical handling of data these word descriptions have been replaced by numerical scores. Quantitative measurements and fruit quality tests are usually only done on hybrids that are found to be promising or on populations where specific quality characteristics are of interest (Soost and Cameron, 1975).

BREEDING OBJECTIVES

Almost all commercial citrus is grown as grafted trees, with the scion cultivar budded on a rootstock. A good scion rootstock combination supports the development of trees that yield large quantities of good quality fruit. This combination allows for the best genetic fruit characteristics with the strongest genetic root traits. Creating this combination in a single genotype would be considerably more difficult. There are important breeding goals in citrus for both scions and rootstocks. Many of the goals are longstanding and may be general or related to a particular geographical region (Soost and Cameron, 1975; Khan and Kender, 2007).

The main goals of citrus breeding programmes are to obtain new varieties with a shorter vegetative (non-fruiting) period, an increased yield, a longer ripening season, regular fruit bearing, seedlessness, and improved external and internal quality of the fruit. While another important goal for both scions and rootstocks are to breed for resistance, or tolerance, to biotic and abiotic stresses (Germanà, 2007). So far yield and fruit quality are the traits that have received the most attention in fruit breeding programmes (Ray, 2002).

Breeding for fruit quality

Breeding aims for fruit quality vary with different species and localities and in response to market trends (Spiegel-Roy and Goldschmidt, 1996).

The fruit size is an important characteristic and many hybrids that produce good quality fruit are discarded due to small fruit size. For fresh fruit, an attractive external appearance is important; generally fruit should have smooth rinds and be without stem-end necks and blossom-end nipples. A better standardisation of fruit shape is also important. A deeper orange rind colour is sought for oranges and mandarins but not for lemons and grapefruits (Soost and Cameron, 1975; Spiegel-Roy and Goldschmidt, 1996; Nicotra, 2001; Ray, 2002).

While easy peeling cultivars are desired, fruit with loose rinds are easily damaged. Thick rinds are objectionable; however, very thin rinds do not store well. A good flavour is important, flavour is difficult to define but here the ratio of Brix % to acid plays an important role. Seedlessness is a prime requirement for the fresh fruit market, and seedless fruit or fruit with a very low seed count are desired. Lengthening the time of ripening by breeding varieties that mature earlier and later than the existing varieties is important to extend the season. Other important aspects are adaptability to specific environments, transport and in many cases post-harvest behaviour and storage (Soost and Cameron, 1975; Spiegel-Roy and Goldschmidt, 1996; Nicotra, 2001; Ray, 2002).

Some other aims are the breeding of low acid and possibly less bitter grapefruits and sweet oranges with better external and internal colour that are not due to anthocyanin pigments (Spiegel-Roy and Goldschmidt, 1996).

Breeding for industrial purposes

Breeding fruit specifically for industrial purposes has been performed mainly in Florida. Here a high Brix % as well as a high juice percentage is important. A good juice colour and a lack of bitterness in the sweet orange juice are also important. However, the demand for orange and grapefruit juice as the sole or almost sole component for juice concentrate complicates the breeding of new hybrid varieties (Spiegel-Roy and Goldschmidt, 1996).

Breeding for resistance to pests and diseases

Resistance to pests and diseases depends on the scion in some cases and on the rootstock in other cases, and occasionally on the interactions between the two. Work has been done in breeding for resistance to *Phytophthora*, citrus nematodes and the tristeza virus. Very little has been reported on breeding for resistance to pests such as aphids, mites and scale insects. The work done in this regard has mostly been the breeding of resistant rootstocks. Breeding scion cultivars for disease and pest resistance is an important breeding goal but is difficult to accomplish. The long life cycle of the host plant and the wide variety of pests and diseases drastically reduce the probability of combining resistance with other desirable characteristics (Soost and Cameron, 1975; Ray, 2002).

Breeding for hardy cultivars

Another aspect that should also be considered in citrus breeding is to include hardy cultivars, adapted to particular climatic and soil conditions, to breed for adaptability to problematic environments. In areas that have low winter temperatures, breeding for cold tolerance is important. This was the first goal of the United States Department of Agriculture breeding programme initiated at the turn of the twentieth century. It is also a main objective in Japan and Russia. Another goal is to breed scions and rootstocks that are tolerant to high levels of chlorides in the soil (Soost and Cameron, 1975; Spiegel-Roy and Goldschmidt, 1996; Ray, 2002).

Breeding for improved rootstocks

Objectives specifically for rootstock breeding include better rootstock scion compatibility, reduction in tree size without affecting yield or scion health, resistance to pests and diseases, and hardiness to adverse climatic and soil conditions. In contrast to scions, rootstocks should produce many seeds and be highly nucellar in order to provide uniformity since rootstocks are generally propagated by seed. This method reduces cost and produces more vigorous and uniform nursery stock than by cuttings or tissue culture (Soost and Cameron, 1975; Ray, 2002, Khan and Kender, 2007).

OBSTACLES IN CITRUS BREEDING

There are some obstacles that prevent the plant breeder from fully utilizing the variability in *Citrus*. These are the incompatibility, sterility and poly-embryony which occur in some varieties (Ray, 2002). Heterozygosity and the prolonged juvenile period are also obstacles to the breeder (Spiegel-Roy and Goldschmidt, 1996).

Incompatibility

In citrus, incompatibility is gametophytic and homomorphic; the pollen and ovules are functional but the failure to produce fruit with seed is due to a physiological hindrance during fertilization. Frequently, incomplete pollination occurs where the pollen does not germinate on the stigma, or the pollen tube does not grow from the stigma into the style or from the style in to the ovary. Even though incomplete pollen tube growth may occur as a result of incompatibility, the stimulation may be sufficient in certain cultivars to induce parthenocarpic fruit (Barry, 1995).

Self and to some extent cross-incompatibility occur in citrus (Soost and Cameron, 1975; Spiegel-Roy and Goldschmidt, 1996). Self-incompatible cultivars can set seedless fruit when self-pollinated, but the fruit set and resulting yield may be poor. However, they tend to set seedy fruit when cross-pollinated with compatible pollen. Cross-incompatible cultivars can set seedless fruit when pollinated by incompatible pollen and set seedy fruit when pollinated by compatible pollen (Barry, 1995).

Incompatibility poses a problem for the breeder but at the same time presents the opportunity to produce seedless cultivars provided that there is a prominent parthenocarpic tendency and no cross-pollination. There is little information available on the inheritance of self-incompatibility in citrus, however it has been found that hybrids between self-incompatible cultivars have also been self-incompatible and sometimes cross-incompatible (Soost and Cameron, 1975; Spiegel-Roy and Goldschmidt, 1996).

All cultivars of pummelo, and some cultivars of lemon, sweet orange and mandarin are self-incompatible (Barry, 1995; Ray, 2002). The list of self-incompatible cultivars is on the increase, and with the ancestry of many cultivars unknown, the presence of incompatibility in many progenies cannot be predicted. Using self-incompatible parents in crosses may result in a poor crop in some of the progeny; however self-incompatibility will very often be obscured by a sufficient fruit set due to cross pollination in mixed breeding blocks. Hybrids of interest should therefore be evaluated for fruitfulness in the absence of cross pollination (Spiegel-Roy and Goldschmidt, 1996).

Sterility

Sexual sterility results in the complete inability to reproduce by means of seed (Barry, 1995). Sterility in citrus may be due to different genetic factors such as sterility genes, chromosomal abnormalities and triploidy (Ollitrault et al., 2007a). In citrus, various degrees of sterility occur involving the pollen, the ovule or both of these, embryo abortion is also common and all of these result in seedless fruit (Barry, 1995).

The percentage of functional pollen varies among species and cultivars. Satsuma mandarins and navel oranges are mostly pollen sterile and set parthenocarpic fruit. Marsh grapefruit have very little pollen, lemon and other orange cultivars have low amounts, while mandarin and pummelo produce mostly functional pollen. Cultivars with non-functional pollen very often also show ovule abortion, although Washington navel and satsumas have functional ovules. Pollen degeneration before meiosis is also encountered (Barry, 1995; Spiegel-Roy and Goldschmidt, 1996; Ray, 2002).

Poly-embryony / Nucellar embryony / Apomixis

Most fruit crops have mono-embryonic seeds. Citrus seeds are unusual since they can be mono or poly-embryonic (Bijzet, 2006a). Mono-embryonic refers to a single seed containing one embryo, while poly-embryonic is the development of two or more embryos in one seed (Soost and Cameron, 1975; Spiegel-Roy and Goldschmidt, 1996;).

In poly-embryonic seed the extra embryos develop from tissue (somatic cells) of the nucellus and lie alongside the zygotic embryo. They are called nucellar embryos and are genetically identical to the female or seed parent. The initiation of nucellar embryos requires not only pollination but also the fertilisation of the egg. In poly-embryonic cultivars the zygotic embryo competes for space and nutrients with the nucellar embryos, so the fewer the number of embryos per seed the larger the embryo size and the greater the chance that the zygotic embryo will survive. In most poly-embryonic cultivars the zygotic embryo does not develop and all of the embryos and resulting seedlings are nucellar (Soost and Cameron, 1975). Poly-embryonic seed often contains embryos at different stages of maturation and many embryos fail to germinate and reach the seedling stage. Some cultivars have many embryos in their seed but few seeds produce more than two or three seedlings (Soost and Cameron, 1975; Saunt, 2000).

Poly-embryony complicates the breeding of citrus. Controlled crosses using poly-embryonic female parents produce only nucellar, or a large percentage of nucellar, embryos yielding few or no hybrid progeny. Therefore poly-embryonic cultivars cannot be used successfully as the female parent in crosses. Poly-embryony when accompanied by sterility and inbreeding depression makes it very difficult to create large segregating populations (Spiegel-Roy and Goldschmidt, 1996).

All cultivars of pummelo and citron are mono-embryonic as well as most lemon and lime cultivars. Some cultivars of the mandarin group are mono-embryonic while the grapefruits and oranges have very few mono-embryonic cultivars. The total number of embryos per seed varies greatly within a tree as well as among cultivars and there is very little consistency in poly-embryonic cultivars (Soost and Cameron, 1975; Spiegel-Roy and Goldschmidt, 1996; Saunt, 2000; Bijzet, 2006a). This variation has been suggested as being controlled by minor genes, the pollen source and environmental conditions (Kepiro and Roose, 2007). Additional embryos in a seed are not always nucellar; mono-embryonic cultivars have been reported to produce two or more zygotic embryos per seed, which are zygotic twins or triplets and are genetically identical but genetically different from the mother plant (Soost and Cameron, 1975).

Nucellar seedlings are of no use to the citrus breeder but are very useful in the production of citrus rootstocks since they allow for the propagation from seed of highly heterozygous but genetically uniform rootstock seedlings. These clones are usually free from most of the virus diseases that could be carried by the mother plant (Soost and Cameron, 1975; Ray, 2002). Therefore; for rootstock breeding, parents should be chosen that produce progeny giving poly-embryonic seeds and progeny should be selected that yield a high percentage of nucellar seedlings and few sexual seedlings (Kepiro and Roose, 2007).

Reduction in the number of embryos per seed has been achieved by high temperature treatment, treatment of flower buds with gamma rays, and by the treatment of young fruits with gibberellic acid a month after anthesis. When the plant breeder does obtain seed containing both zygotic and nucellar embryos the nucellar seedlings can be identified and separated from the zygotic ones. Other than using discriminating morphological characteristics, chromatography, browning shoot extracts and isozymes have been used to identify nucellar seedlings, but nowadays these techniques are being replaced with new deoxyribonucleic acid (DNA) marker techniques such as restriction fragment length polymorphisms (RFLPs) (Spiegel-Roy and Goldschmidt, 1996; Ray, 2002).

Heterozygosity

The *Citrus* genus is highly heterozygous, resulting in a high variability among F1 hybrids in breeding populations (Soost and Cameron, 1975; Ray, 2002). This is a problem for the citrus breeder as it makes the production of large segregating populations for selection of a specific trait almost an impossible task (Grosser and Gmitter, 1996).

The high degree of heterozygosity in citrus also makes it impossible to obtain homozygosity by conventional methods, and the absence of pure lines makes genetic studies on citrus rather difficult (Germanà, 2007).

The juvenile period

The juvenile period is the long period of time from the making of the cross until the first fruiting of the progeny; this varies between cultivars but is generally five to ten years. Thorniness is especially prominent in juvenile seedlings and the first years' fruits can also be of inferior quality. Although many horticultural techniques have been used to try and shorten the juvenile period, there has been very limited success. The juvenile period seems to be under multigenic control and varies according to the genotype and parents used; however it is also influenced by environmental conditions (Spiegel-Roy and Goldschmidt, 1996; Ray, 2002). More vigorous cultivars will have a shorter juvenile period and plants in hotter areas will also have a shorter juvenile period than their clones in cooler areas (Bijzet, 2006b).

GENETIC VARIABILITY IN CITRUS

There is a tremendous amount of variability within the genus with which the plant breeder can work and closely related genera provide an even wider array of characteristics (Soost and Cameron, 1975).

The tree and fruit characteristics vary greatly within and between citrus species. Fruit vary in size from very small, such as the kumquats that can be just 3 cm in diameter, to very large, such as the pummelo that can be up to 30 cm in diameter. Fruit rind colour varies from the yellow-green of limes to the red-orange of some mandarins. The fruit shape also shows a full range of forms from oblate to pyriform. While the acid of some varieties is still high at maturity, other varieties have almost no acid (Ray, 2002). This variation is strongly expressed in hybrid progenies and occasionally hybrids will exceed the limits of their parents in some character (Spiegel-Roy and Goldschmidt, 1996).

Many manmade and natural hybrids are now available in breeding programmes as parents and most breeding programmes have increased their collection of gene material over the years. There is however, concern about the maintenance of these collections and the loss of wild resources. Citrus tissue in culture is difficult to handle; however there has been some progress in the *in vitro* conservation of citrus germplasm (Ray, 2002).

HYBRIDISATION IN CITRUS

The citrus species hybridises freely. There is generally compatibility between the species within the genus *Citrus* and more or less fertile F1 hybrids result. The genera *Poncirus*, *Fortunella* and *Microcitrus* are also compatible with *Citrus*; however most F1 hybrids from these crosses are sterile (Barry, 1995; Spiegel-Roy and Goldschmidt, 1996; Ray, 2002). Therefore both interspecific and intergeneric hybrids frequently occur. New hybrids have evolved by controlled breeding or by chance hybridisation (Ray, 2002).

Some citrus species are the result of interspecific crosses. For example the sweet orange is believed to be a natural pummelo x mandarin cross, the grapefruit a pummelo x sweet orange cross and the lemon possibly a combination of the lime, citron and pummelo. Many of today's important commercial citrus varieties are of hybrid origin and many of these have resulted from natural hybridisation events. Many controlled interspecific crosses have also been performed in citrus. The most important of these are the tangelo (mandarin x grapefruit), tangor (mandarin x orange), orangelo (orange x grapefruit) and citrange (*Poncirus* x sweet orange). There is an increase in the scope of crosses between genera, in an attempt to produce novel types of citrus rootstocks and cultivars, and in the future to use tetraploid products of somaclonal fusion (Soost and Cameron, 1975; Spiegel-Roy and Goldschmidt, 1996; Ray, 2002).

On the one hand citrus represents a remarkable degree of variation, with abundant natural crossing giving rise to a wide range of heterozygosity, while on the other hand a free exchange of genes is prevented by wide spread apomixis. The best results of deliberate hybridisation in the citrus species have been obtained by artificial crosses of various mandarin-like species (Vardi and Spiegel-Roy, 1978; Nicotra, 2001).

INHERITANCE OF CHARACTERISTICS

In citrus breeding programmes, groups of specific characters are desired. However there is a high variability among F1 hybrids; this is due to the high heterozygosity that occurs in *Citrus* (Cooper et al., 1962; Soost and Cameron, 1975; Ray, 2002). The F1 hybrids from any two parents show the variability usually expected in the second filial (F2) hybrids between varieties differing in many genes. In any particular character hybrids can be very diverse. They may be similar to one of the parents, fall between the two parents or be outside the parents' range (Cooper et al., 1962). Single gene inheritance is rarely found in citrus; occasionally however there is segregation of a character in citrus progenies which indicates the action of one or a few genes (Soost and Cameron, 1975). The purple anthocyanin colouration of young leaves, found in many lemon cultivars, is reported to be controlled by one dominant gene, while nucellar embryony, which is frequently found in citrus, also appears to be controlled by one or two dominant genes (Ray, 2002).

Research on inheritance in citrus faces many barriers due to the facts that citrus is highly heterozygous and it has a long juvenile phase, nucellar embryo interference, sterility or incompatability, and because most citrus physiological and morphological traits are controlled by quantitative trait loci (QTL's) (Spiegel-Roy and Goldschmidt, 1996).

Inheritance in citrus being mostly quantitative, characters determined by the additive effect of many genes are more difficult to select for. However, the analysis, interpretation and prediction of polygenes can be carried out. This is based mostly on statistical and genetical analysis and has been referred to as 'biometrical genetics'. As the number of genes selected for in a crop increases so does the number of plants needed to be evaluated to obtain the superior genotypes possessing the desired combination of genes. Therefore the citrus breeder needs to work with large numbers of plants, making a citrus breeding programme large and costly (Ray, 2002).

THE ESTIMATION OF HERITABILITY IN FRUIT TREE BREEDING

In fruit tree breeding populations the phenotypic variance can be partitioned in to components corresponding to the grouping of individuals into families (Falconer and Mackay, 1996). The relationship among genetic traits can therefore be investigated between families, within families and within individuals propagated as clones (Labuschagne, 2002a). The heritability of quantitative traits is then based on partitioning the phenotypic variance (σ^2_p) into genetic (σ^2_g) and non-genetic (σ^2_e) components of variance (Falconer and Mackay, 1996);

$$\sigma^2_p = \sigma^2_g + \sigma^2_e$$

In fruit tree breeding populations these variances are easily estimated, σ^2_p is the phenotypic variance among individuals (trees in a family) and σ^2_e is the variance occurring within clones of a common genotype and embraces all variation of a non-genetic origin. The genetic variance between clones (σ^2_g) can then be determined by subtraction (Falconer and Mackay, 1996; Labuschagne, 2002a).

The ratio of the genetic variance to phenotypic variance σ^2_g / σ^2_p expresses the extent to which the phenotypes of the individuals are determined by the genotypes. This provides an estimate of the maximum value of heritability, referred to as heritability in the broad sense. According to this definition of heritability the additive and non-additive components of genetic variation are inseparable (Falconer and Mackay, 1996).

In order to determine the additive component of genetic variance (σ^2_A) an experimental design that allows for the estimation of the covariance between half-sibs or parents and progeny, or realised response to selection with adequate control is required (Labuschagne, 2002a). The additive genetic variance (σ^2_A) allows for the determination heritability in the narrow sense given by the ratio of σ^2_A / $\sigma^2_{p.}$ Heritability in the narrow sense determines the extent to which phenotypes are determined by the genes transmitted from the parents and is the main determinant of the observable genetic properties of the population, therefore being of great importance in breeding programs. However, the most important function of heritability is its role in predicting the reliability of the phenotypic value as a guide to the breeding value (Falconer and Mackay, 1996). Therefore a high broad sense heritability estimate indicates that selection should be effective (Labuschagne, 2002a).

In citrus breeding populations, families are usually planted in single rows without clonal replication or randomization. This experimental design does not allow for the determination of broad sense heritability by the ratio of σ^2_g / σ^2_p (Labuschagne, 2002a). Therefore the repeatability, calculated from multiple measurements on an individual, can be useful in providing an upper limit for the estimate of broad sense heritability (Falconer and Mackay, 1996).

Repeatability

When more than one measurement of a character can be made on each individual, such as trees within families, the phenotypic variance can be partitioned into the variance within individuals (σ^2_w) and variance between individuals (σ^2_b). The ratio of the between individual component to the total phenotypic variance can be determined and is known as the repeatability and is given as (Becker 1992; Falconer and Mackay, 1996);

$\sigma^2_b /(\sigma^2_b + \sigma^2_w)$

The within individual variation (σ^2_w) is entirely environmental in origin and can also be given as σ^2_e, while the between individual component (σ^2_b) is partly environmental and partly genetic in origin and is given by ($\sigma^2_g + \sigma^2_b$). The estimation of repeatability separates the component of variance within an individual (σ^2_w), but it leaves the other component, the between individual variance (σ^2_b) confounded with the genetic variance (σ^2_g). In order to separate the genetic variance (σ^2_g) from the between individual variance (σ^2_b) repeatability needs to be calculated in a genetically uniform group such as the clonal replication of each individual (Falconer and Mackay, 1996).

Repeatability estimates are useful in making predictions of future performance of the phenotype from past records. Repeatability is usually much easier to determine than heritability and can often be estimated where heritability cannot. Since σ^2_b estimates all the genetic variance plus a portion of the environmental variance repeatability is an overestimate of heritability (Falconer and Mackay, 1996) and can be used to set an upper limit for broad sense heritability of the characters analysed (Lima et al., 1981; de Souza and Byrne, 1998). Heritability may therefore be much less than the repeatability however it can never be greater (Becker, 1992; Falconer and Mackay, 1996).

Another application of the estimate of repeatability is to determine the gain in accuracy expected from multiple measurements. An increase in the number of measurements on an individual reduces the amount of variation due to the within individual variance (σ^2_w) that appears in the phenotypic variance, thereby increasing the accuracy. A high repeatability estimate therefore indicates a small gain in accuracy from multiple measurements while a low repeatability indicates that multiple measurements may lead to a worthwhile gain in accuracy (Falconer and Mackay, 1996).

The intraclass correlation coefficient

The repeatability of a character, as discussed above, is the correlation between multiple measurements on the same individual and is also known as the intraclass correlation coefficient. The intraclass correlation coefficient (t) is however, the preferred term when multiple measurements are used to determine the resemblance between related individuals, such as families, or trees within a family and is given as (Falconer and Mackay, 1996);

$$t = \sigma^2_b / (\sigma^2_b + \sigma^2_w)$$

In plant breeding populations the phenotypic variance can be partitioned into the variance between families and the variance within families and can be looked at as either as the variance between individuals in a family or as the variance between individuals in different families. The degree of resemblance is then expressed as the between group component to the total phenotypic variance (Falconer and Mackay, 1996). Therefore multiple measurements taken from the same families in different years may involve genotype x environment interactions at two levels (Labuschagne, 2002b);

1. year x family interaction, $\sigma^2_{\text{family x year}}$

2. year x tree interaction within families, $\sigma^2_{\text{tree x year}}$

The analysis of variance (ANOVA) and expected mean squares (EMS) can therefore be done in two parts, with *y* years of measurement and *N* trees per family (Connor et al., 2002; Labuschagne, 2002b)

1.	Years	$\sigma^2 + N\sigma^2_{year.} + Nfamily\sigma^2_{year}$	
	Families	$\sigma^2 + N\sigma^2_{family\ x\ year.} + Ny\sigma^2_{family}$	(A)
	Y x F interaction	$\sigma^2 + N\sigma^2_{family\ x\ year}$	
	Residual	σ^2	
2.	Trees within families	$(\sigma_e^2 + N\sigma^2_{tree\ x\ year}) + y\sigma^2_{tree}$	(B)
	Y x trees within families	$(\sigma_e^2 + N\sigma^2_{tree\ x\ year})$	(C)

In the second part the environmental variance (within an orchard) and genotype x environment interaction cannot be estimated separately since measurements are taken on only one tree of each genotype (Labuschagne, 2002b).

Intraclass correlation coefficients can therefore be calculated (Falconer and Mackay, 1996; Labuschagne, 2002b);

1. Relevant to selection between families

$t_1 = \sigma^2_B / \sigma^2_B + \sigma^2_W$ or A / total variance

Where σ^2_B is the variance between the families and $(\sigma^2_B + \sigma^2_W)$ is the total phenotypic variance for both between and within the families

2. Relevant to selection between individuals within a family

$t_2 = \sigma^2_b /(\sigma^2_b + \sigma^2_w)$ or B / (B+C)

Where σ^2_b is the variance between the trees within a family and $(\sigma^2_b + \sigma^2_w)$ is the total phenotypic variance within a family including the y x tree interaction (Labuschagne, 2002b).

In a citrus breeding program selection is conducted according to the phenotypic value, therefore knowledge of the reliability of the phenotypic value as the breeding value is of great use to the breeder. Heritability estimates are used to predict the reliability of the phenotypic value as the breeding value (Falconer and Mackay, 1996), however, in citrus breeding populations where a breeding design containing clonal replication of seedlings is not the norm heritability cannot be determined in the usual way by the ratio of σ^2_g / σ^2_p (Labuschagne, 2002a). In this case repeatability and the intraclass correlation coefficient are extremely useful in setting an upper limit to broad sense heritability of the characters studied (Falconer and Mackay, 1996).

SELECTION OF PARENTS

Citrus breeding programmes usually have several objectives, each with a different inheritance pattern. This can complicate the process of parent selection. A breeding programme requires the testing of many generations of plants; therefore a lot of time and patience is required to achieve tangible results. It is therefore advisable to proceed gradually and take a few objectives at a time (Ray, 2002).

When selecting parents in citrus breeding, several factors need to be considered. The female or seed parents should produce only zygotic seedlings and therefore be mono-embryonic. However, if no suitable mono-embryonic cultivars are available as parents, then poly-embryonic cultivars that produce some zygotic and some nucellar seedlings can be used. Over and above this, some cultivars have a high degree of ovule sterility and these cannot be used as seed parents. Pollen parents need to be chosen from cultivars that are not pollen sterile or have too low a pollen viability to achieve fertilisation (Soost and Cameron, 1975; Ray, 2002).

INBREEDING IN CITRUS

Most citrus cultivars are highly heterozygous; therefore selfing would appear to be a useful technique. However selfing has produced mostly weak and inferior progeny. Narrow crosses also tend to produce many weak offspring while wider crosses tend to produce vigorous offspring (Soost and Cameron, 1975).

Due to the high degree of nucellar embryony, the self-incompatibility in some mono-embryonic types, a high heterozygosity and a prolonged juvenile phase it is almost impossible for the citrus breeder to obtain or use inbred lines (Vardi and Spiegel-Roy, 1978).

POLYPLOIDY IN CITRUS

Plants of the genus *Citrus* are diploid. However polyploidy occurs in many cultivars (Soost and Cameron, 1975; Ray, 2002). The number of chromosomes is 2n=18 (Spiegel-Roy and Goldschmidt, 1996). Citrus chromosomes are small (1.0 to 4.0 µm) and not very favourable for extensive studies (Soost and Cameron, 1975).

Spontaneous tetraploids have been obtained as variant nucellar seedlings in *Citrus* and *Poncirus;* about 2.5% of all nucellar progeny are tetraploid. Tetraploid breeding parents have also been induced using colchicine as well as produced by somatic hybridisation via protoplast fusion. Tetraploids are characterised by their slower growth, compact growth habit, broader, thicker, darker, leaves and fruit with thicker rinds, less juice, and larger oil glands. They also often have a lower fertility than the corresponding diploids (Spiegel-Roy and Goldschmidt, 1996; Ollitrault et al., 2007a). Tetraploids do not have commercial value but are useful in breeding programs for the production of triploids (Soost and Cameron, 1975; Ray, 2002).

Spontaneous triploids occur in about 5% of the seeds obtained from diploid parents. They are found in the small seed, weighing less than 0.1 g (Jaskani et al., 1997; Ollitrault et al., 2007a). Triploids are desired as citrus cultivars since they are sterile and therefore yield seedless fruit, an important aim in citrus breeding. Triploid plants are bred by crossing diploid and tetraploid parents. However the breeding of triploids is very limited in citrus due to the fact that for the 4n x 2n cross there is a lack of mono-embryonic tetraploid parents and the 2n x 4n cross yields many tetraploid individuals (Soost and Cameron, 1975; Ray, 2002). Another problem is that the survival of the triploid embryo is negatively affected by the poor endosperm development and failure of embryo growth (Spiegel-Roy and Goldschmidt, 1996).

Pentaploids in citrus are rare but 2n x 2n crosses may give a few incidental pentaploids (Soost and Cameron, 1975; Ray, 2002). Pentaploids, hexaploids and tetraploids have been obtained from crosses between triploids and diploids, and may have arisen from the functioning of doubly unreduced female gametes. Haploids have also been obtained from crosses with diploid and triploids (Jaskani et al., 1997).

Several types of meiotic irregularities capable of producing aneuploids have been reported. Aneuploids ranging from chromosome number 19 to 41 have been found in citrus, but they are slow growing and weak and therefore do not have any use in breeding (Soost and Cameron, 1975; Jaskani et al., 1997; Ray, 2002).

MUTATIONS IN CITRUS

Mutation refers to any heritable change in the DNA. However the breeder is generally interested only in those mutations that alter a phenotype. At the molecular level, mutations can alter DNA by base substitution, insertion, deletion or sequence rearrangement. Any of these types of changes may cause a phenotypic change (Roose and Williams, 2007). Sectorial, periclinal and mericlinal chimeras are found in citrus and add a further complexity to mutation breeding (Ray, 2002). It is therefore important to distinguish between a permanent change and a temporary change (Roose and Williams, 2007).

Citrus trees produce spontaneous mutations very readily. These can be seen as bud or branch mutations or sectors on fruit and can even be detected amongst nucellar seedlings (Soost and Cameron, 1975). The frequency of observed mutations varies according to cultivar and with the environment, cultural practices (such as pruning) and the type and number of trees being observed (Spiegel-Roy and Goldschmidt, 1996).

Navel oranges and grapefruits tend to produce more natural mutations than other varieties and most of today's important varieties in these two groups resulted from natural mutations. Many lemon varieties are however also a result of natural mutations (Soost and Cameron, 1975). New mutants with valuable characteristics have also been found and exploited in clementine, satsuma and several other Japanese cultivars. The most interesting mutants are those that show seedlessness, pigmented fruit, early and late ripening, and a lower acidity (Spiegel-Roy and Goldschmidt, 1996).

Man-made mutations can be achieved in citrus by exposing budwood or seeds to radiation for short periods (Soost and Cameron, 1975). Ionising radiation from X-rays and gamma rays are the most widely used and effective type of mutagen for citrus. The one trait that can be obtained relatively easily with mutation breeding is seedlessness (Roose and Williams, 2007).

Genetic improvement in citrus by hybridisation has been much hampered by heterogeneity, reproduction by nucellar embryony and juvenility. Because of the taxonomic nature of many of the commercial cultivar groups, such as sweet oranges, grapefruit, lemons and some mandarin types, they are not amendable to breeding strategies based on sexual hybridisation. Improvement in citrus has therefore been largely by the selection of naturally occurring somatic mutants, with many of today's important cultivars having arisen through somatic mutation. Therefore mutation breeding in citrus has been and will always be an important tool (Spiegel-Roy and Goldschmidt, 1996; Gmitter et al., 2007a).

THE USE OF BIOTECHNOLOGY TO ASSIST CITRUS BREEDING

Conventional cross breeding is and will continue to be the foundation of citrus variety improvement. However, conventional breeding cannot be used to develop improved cultivars in many economically important citrus species such as sweet orange, grapefruit and lemon due to barriers of sterility, self and cross incompatibility and the widespread poly-embryony. In addition to this, the heterozygosity makes the breeding for specific traits extremely difficult. Therefore citrus variety improvement programmes have in the past relied on limited sources of genetic variation. In addition to conventional breeding they have included spontaneous mutations, irradiation of seed and budwood and importing germplasm from other locations (Grosser and Gmitter, 1996).

The development of new knowledge, biotechnology and advances in the development of *in vitro* cell and tissue culture methods and plant molecular biology have opened up new opportunities for the creation of improved citrus varieties in the future (Grosser and Gmitter, 1996).

Triploid plants from crosses between diploid and tetraploid parents have been recovered by embryo rescue and *in vitro* culture of the triploid embryo. This is necessary because an unfavourable endosperm balance number in such crosses causes endosperm failure and makes seeds from such crossings incapable of germinating *in vivo* (Grosser and Gmitter, 1996; Ray, 2002). Triploid plants have also been produced from the culture of endosperm (Ray, 2002). Seedless triploids may even be produced using cell level techniques which can expand the parental combinations available for these interploid crosses (Grosser and Gmitter, 1996).

By culturing immature ovules from seedless cultivars such as navel oranges, new nucellar lines have been obtained. Somaclonal variation from ovule culture can be exploited to alter a wide range of characteristics of existing varieties (Grosser and Gmitter, 1996; Ray, 2002). Ovule culture has also been used to recover plants from sectorial chimera mutations on fruit (Grosser and Gmitter, 1996).

Autotetraploid breeding parents can be produced by colchicine treatment of ovules or embryonic tissues followed by *in vitro* plant regeneration via somatic embryogenesis. While somatic hybridisation via protoplast fusion can be used to produce allotetraploid breeding parents that combine complementary elite scion varieties (Grosser and Gmitter, 1996). Plant regeneration via somatic embryogenesis *in vitro* has greatly widened the scope of genetic manipulation and the long term cryoconservation of the germplasm (Ray, 2002).

Plant protoplasts have been isolated from citrus plant tissue and somatic hybrids have been produced through the protoplast fusion of sexually incompatible species. Somatic hybridisation allows for the addition of all dominant traits irrespective of the heterozygosity of the breeding material. Hybrids developed by protoplast fusion are tetraploid and their pollen can be cultured to develop diploid plants (Grosser and Gmitter, 1996; Ray, 2002; Ollitrault et al., 2007b).

Somatic hybridisation has also allowed the direct synthesis of triploids by protoplast fusion of diploid and haploids. The breeding of somatic hybrids at the tetraploid level allows for the mixture of genes from three or four parents simultaneously thereby maximising the genetic diversity of the progeny. Several alloplasts and cybrids have also been obtained by somatic hybridisation (Ollitrault et al., 2007b). Cybrids, where the mitochondrial genome of one species is replaced with that of another, have been obtained by electrofusion of protoplasts of nucellus derived embryogenic callus tissue with protoplasts derived from leaves (Ray, 2002).

Pollen culture of diploids can be used to obtain haploids, totally homogeneous diploids can then be obtained by doubling the chromosome number of the haploids. Haploid plants have also been produced from diploid x triploid crosses (Ray, 2002). Haploids can be used to produce homozygous lines from heterozygous parents in a single step. These haploids and doubled haploids are important in genome mapping and provide excellent material to obtain reliable information on the location of major genes and QTLs (Germanà, 2007).

Tri-haploids have also been formed by the fusion of three haploid protoplasts and diploid somatic hybrids by haploid protoplast fusion. Gametoclonal variation, the variation among cultured gametic cells, is yet another way to use haploids in plant improvement (Germanà, 2007).

Isozyme analysis is useful in the identification of somatic hybrids and studies in phylogeny (Spiegel-Roy and Goldschmidt, 1996) and for the classification of citrus species/cultivars. However isozymes cannot distinguish between closely related cultivars. RFLPs and random amplification of polymorphic DNA (RAPDs) have been used to separate hybrids further into groups (Ray, 2002).

More than 20 isozyme loci have been genetically characterised in citrus, most of these are highly polymorphic. Construction of a genetic map of the citrus genome using isozymes and RFLPs has been initiated and may be useful in locating genes with a specific function(s) (Spiegel-Roy and Goldschmidt, 1996; Ray, 2002).

Linkage maps have been created using isozymes, RFLPs, RAPDs, sequence characterized amplified regions (SCARs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs) and cleaved amplified polymorphic sequences (CAPS) (Peña et al., 2007). Several linkage maps of citrus have been published (Luro et al., 1995; Cristofani et al., 1999; Recupero et al., 2000; Roose et al., 2000; Sancar and Moore, 2001; Ruiz and Asins, 2003) and additional maps are in the process of being developed. Several QTL studies, where a measurable trait in the progeny of a segregating population for which a linkage map has been developed is studied, have been reported in citrus (Garcia et al., 1999; Tozlu et al., 1999a and b). Many different computer packages are available to conduct QTL analyses (Van Ooijen and Maliepaard, 1996; Basten et al., 1998), however many of these are not easy to use for citrus crosses as they require populations derived from homozygous parents. Therefore an alternative approach, linkage disequilibrium mapping, is now being developed, which depends only on natural linkage disequilibrium between traits and markers and does not require a mapping population derived from specific parents (Roose, 2007). Although these studies have served to determine the mode of inheritance of these traits and they could be useful for breeding purposes, map-based cloning of the corresponding genes is still a long way off (Peña et al., 2007).

Marker-assisted breeding and selection can increase the efficiency of a citrus breeding programme and markers for several genes have been identified (Roose, 2007). Markers for dwarfing by the rootstock Flying dragon trifoliate orange (Cheng and Roose, 1995), the citrus tristeza virus resistance gene in trifoliate orange (Gmitter et al, 1996; Mestre et al, 1997; Fang et al., 1998a), the *acitric* gene (Fang et al., 1998b), genes involved in nucellar embryony (Garcia et al., 1999) and citrus nematode tolerance (Ling et al., 2000) and salinity tolerance (Tozlu et al., 1999b) have been located and are useful in marker assisted selection (MAS). However citrus biology limits what can be achieved by MAS, since citrus populations are usually too small to be able to select for a large number of traits in a single generation. A two-generation strategy, selecting for different sets of traits in two populations and then intercrossing selections from these populations, may be a more effective approach for MAS in citrus (Roose, 2007).

Work has been done on genetic transformation of citrus through particle bombardment and the Agrobacterium-mediated technique (Ray, 2002). Research is now underway to incorporate transgenes into citrus species with the aim of obtaining resistance to tristeza virus, higher tolerance to *Phytophthora* and higher tolerance to salinity and shortening the juvenile period (Peña et al., 2007).

The science of genomics driven by the rapidly expanding capability of technology is revolutionising the whole field of biology and genetics. An understanding of the genetic control of agriculturally important traits, together with the ability to manipulate and modify citrus genomes, provides a base for more precise and specific manipulation of tree and fruit characteristics (Gmitter et al., 2007b).

Since the mid 1990's many new technological developments have been seen. These include expressed sequence tag (EST) libraries and even complete genomes, microarray technologies, bioinformatics capabilities that enable processing large volumes of informative data, and high-throughput marker systems for mapping projects that can yield high density maps containing thousands of markers. Despite this, citrus is a plant in which genetic studies are difficult to conduct. The difficulty with which citrus can be transformed is a limitation to making the leap from fundamental genomic information and understanding of a trait to practical deployment of genetically improved citrus plants for the benefit of producers and consumers (Gmitter et al., 2007b).

CONCLUSIONS

The rapid expansion of the world citrus industry over the past few decades has lead to an oversupply of the markets; as a result, premium prices are being paid for high quality fruit. For South Africa, as a citrus producing and exporting country, to stay a competitor on the international markets we need to breed and produce new improved varieties in line with consumer demands (Bijzet, 2002). Therefore, the ARC-ITSC citrus breeding program fulfills an important role by breeding and selecting for new and improved citrus varieties (Bijzet and Combrink, 2004).

A knowledge and understanding of the inheritance of important characteristics in citrus fruit is extremely valuable to the breeder. In the past, conventional citrus breeding involved crosses between commonly known varieties, and often the same crosses were repeated year after year without learning much about the characteristics targeted (Sykes, 1997). However, by understanding the way that key fruit characteristics are inherited, a breeding programme can progress by way of more informed breeding and selection strategies.

It is evident from the literature that citrus is a complex and diverse crop. Advances in breeding and genetics by conventional methods will continue to be slow, because of constraints such as the quantitative inheritance of most characters, sterility, self and cross incompatibility, nucellar embryony and a long juvenile period that can hamper progress in a conventional breeding programme. However, recent advances in molecular genetic techniques and tissue culture-based manipulation of plants have yielded new opportunities for developing advanced citrus cultivars.

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CHAPTER 3

GENOTYPIC VARIATION OF RIND COLOUR IN KIYOMI TANGOR FAMILIES

INTRODUCTION

Rind colour is one of the main cosmetic preferences consumers use when purchasing fruit. Colour has an important psychological effect on people, who generally think 'the nicely coloured one is the better one', and even though rind colour bears no relation to palatability or flavour of the fruit it is still used to gauge the fruit quality (Ladaniya, 2008).

Consumers prefer brightly coloured citrus fruit (Ladaniya, 2008); fruit with a deep orange or orange-red rind are perceived to be more attractive than orange or yellow fruit (Spiegel-Roy, 1988) and consumers are willing to pay a higher price for them. Green coloured fruit are considered unripe and fetch lower prices and consumers are generally reluctant to purchase green fruit except in the case of limes and lemons. Fruit rind colour is therefore one of the most important characteristics when determining a fruit's marketability (Ladaniya, 2008).

The outermost layer of the citrus fruit is called the flavedo. It is a rough, robust and brightly coloured rind which covers the fruit and protects it from damage (Bijzet, 2006a). During the early stages of fruit development the flavedo is dark green and consists of photosynthetically active tissue containing chloroplasts (Spiegel-Roy and Goldschmidt, 1996). The chloroplasts gradually change into carotenoid-rich chromoplasts as the fruit matures, causing the change in rind colour. The citrus rind colour changes from green to yellow, orange or orange-red as per the genetic character of the variety (Ladaniya, 2008).

The development of rind colour in citrus is affected by many factors such as fruit maturity, tree nutrition, rootstock, cultivation practices, water availability, temperature and even the ground cover in the orchard. However, the climate and endogenous growth regulators seem to play the largest role (Ladaniya, 2008). It is during the third stage of the fruit's development, comprising of the last 11 weeks before fruit reach maturity, that the fruit undergo a change in rind colour. To initiate this change in rind colour, night time temperatures of less than 13℃ are needed, from abo ut six to 16 weeks prior to harvest, to break the chlorophyll down and reveal the yellow and red carotenoids in the peel (Bijzet, 2006b). Carotenoids are temperature sensitive and at these lower temperatures even very low concentrations produced by fruit are sufficient to induce colour (Ladaniya, 2008).

Warm temperatures, on the other hand, interfere with the loss of chlorophyll and the build up of carotenoids and cause the fruit to remain pale and greenish and prevent mandarins and oranges from attaining their attractive orange or orange-red rind colour (Spiegel-Roy and Goldschmidt, 1996).

Changes in citrus fruit's rind colour on the tree are mainly due to the climate. However, endogenous growth substances also play an important role and the transformation of chloroplasts to chromoplasts is the major physiological trait affected by growth substances (Spiegel-Roy and Goldschmidt, 1996). Ethylene destroys chlorophyll and causes small changes in the carotenoids, hastening the rind colour development. Plant hormones play a role in the induction of ethylene production, while a low temperature can also provide sufficient stress to produce ethylene (Ladaniya, 2008). Gibberellins and cytokinin, on the other hand, cause a delay in the loss of chlorophyll and delay the change in rind colour. Gibberellins can even enhance the regreening of the citrus rind (Spiegel-Roy and Goldschmidt, 1996; Ladaniya, 2008).

A combination of environmental, nutritional and hormonal signals all play a role in the chloroplast - chromoplast interconversion. Therefore, during summer, when the warm temperatures permit root growth and the root hormones (gibberellins, cytokinins) and other nitrogenous substances can reach the tree canopy, they delay the change in rind colour. When autumn sets in, temperatures drop and this halts root growth and causes a decline in the root hormones, allowing the change in rind colour to begin. Again in spring when the temperatures rise, root growth begins causing a rise in the level of root hormones and this leads to regreening. Regreening occurs in some citrus fruits that are held on the tree past maturity. Chromoplasts revert back to chloroplasts and photosynthetic activities are also partly restored (Ladaniya, 2008).

The rind colour of citrus fruit is not always a reliable indication of the fruit's internal maturity, but it does give an indication of maturity under certain climatic conditions in the field (Ladaniya, 2008). In the warm tropical climates temperatures are high all year round, interfering with fruit colouration, therefore mandarins may only show colour break (the beginning of colour development) when they have already reached maturity (Spiegel-Roy and Goldschmidt, 1996). In the cooler sub-tropics the fruit, depending on their natural colour, can attain the nice orange or orange-red colour found in some mandarin hybrids. However, the most attractive coloured citrus fruit develop in a dry climate with cool nights and warmer days during the fruit's maturation stage (Ladaniya, 2008).

Fruit that reach internal maturity when the rind colour has not fully developed, such as occurs with some early varieties in the sub-tropical areas and many varieties in the warm tropics, are usually degreened to improve the rind colour. This postharvest treatment uses externally applied ethylene to accelerate the break down of chlorophyll and accumulate carotenoids resulting in a change in rind colour, thereby improving the fruit's appearance and making it more marketable (Ladaniya, 2008).

Fruit rind colour is an economically important characteristic concerning citrus fruit; therefore one of the main aims of citrus breeding programmes is to breed fruit with an improved rind colour (Soost and Cameron, 1975; Ray, 2002). In citrus breeding populations, crosses between two parents yield F1 progenies that display a wide quantitative range of character expression for rind colour (Vardi and Spiegel-Roy, 1978), and crosses between mandarins have been found to yield progenies with rind colours ranging from a pale yellow to a deep red-orange (Ray 2002).

Very little information is available on the inheritance of rind colour in fruit breeding populations. A study on broad sense heritability estimates in open pollinated half-sib mango families, gave high heritability estimates for rind colour with L* at 0.82, a* at 0.89 and b* at 0.94 (Brown et al., 2009). However, the only publication relating to this study, 'Studies on the rind colour heredity in Citrus' (Chen et al., 1993), was published in Chinese. Therefore the aim of this study was to investigate the genetic variability of fruit rind colour among the progenies of six mandarin families, to provide more information on the inheritance patterns of citrus rind colour and determine the value of the parents in citrus improvement.

MATERIALS AND METHODS

Experimental site

The study was conducted at the ARC–ITSC experimental farm, the Addo Research Station at Addo in the Eastern Cape, South Africa, 33°34'14 ''S, 25°42'36''E.

Selection of families

Hybrids used for this study were selected from the ARC-ITSC's citrus breeding program at Addo. Six mandarin families, with Kiyomi tangor (*Citrus unshiu x Citrus sinensis*) as female parent and Dancy, Hansen, Rishon, Roma, Shani and Sunburst mandarins (*Citrus reticulata*) as male parents, were selected for the study (Table 3.1).

Table 3.1 Set of citrus parents selected for the study of rind colour

Female parent	Male parents
Kiyomi	Dancy
	Hansen
	Rishon
	Roma
	Shani
	Sunburst

Because of the long juvenile phase of citrus, hybrids chosen for the study were taken from crosses made previously that were already in production. The crosses were made by the ARC-ITSC citrus breeding team in September 1998. Controlled hand pollinations were done by collecting pollen from flowers of the male parents, Dancy, Hansen, Rishon, Roma, Shani and Sunburst, and pollinating flowers of the female parent Kiyomi. The 'pollination fruit' was harvested in July 1999, the seed extracted and germinated. The resulting seedlings were grafted to Troyer citrange rootstocks in the Addo Research Station nursery in September 2000. The trees were planted out in orchards at the Addo Research Station, together with parent cultivars as controls in February 2002. Each progeny tree resulted from a seedling and is of a different genotype; therefore there are no replications. However, the parent cultivars were planted with three replications. Families were planted in rows without randomisation and all trees were grown under the same environmental conditions of soil, irrigation and fertilisation. Table 3.2 shows data on the flowers pollinated, 'pollination fruit' set, seed collected, seedlings germinated, trees budded in the nursery and trees planted in the orchard for the crosses involved in the study.

Kiyomi was used as a female parent since it is mono-embryonic and yields zygotic seedlings (Saunt, 2000). Dancy, Hansen, Rishon, Roma, Shani and Sunburst on the other hand are all poly-embryonic (Hearn, 1981; Patent PP08377, 1993; Miller et al., 1996; Saunt, 2000; Patent PP13634, 2003) and were therefore used as male parents.

The aim of these crosses was to breed new citrus mandarin hybrids with an improved rind colour. The female parent Kiyomi is described as having an attractive orange like appearance; however under South Africa's climate the rind is less well coloured (Saunt, 2000). The rind colour of the male parents, Dancy, Hansen, Rishon, Roma, Shani and Sunburst are all described as ranging from deep orange to a reddish-orange colour (Hearn, 1981; Patent PP08377, 1993; Miller et al., 1996; Saunt, 2000; Patent PP13634, 2003). Images of the parents, showing their characteristic rind colour, are shown in Figure 3.1.

Table 3.2 Data on pollination, fruit set, seed collection, germination, seedlings budded and trees planted for the six crosses studied for rind colour

Cross	Flowers pollinated	Fruit set	Seed collected	Germin-ated	Seedlings budded	Trees planted
Kiyomi x Dancy	16	6	277	216	142	125
Kiyomi x Hansen	16	6	339	304	213	202
Kiyomi x Rishon	16	12	291	216	204	146
Kiyomi x Roma	16	9	284	176	130	116
Kiyomi x Shani	16	12	312	276	242	208
Kiyomi x Sunburst	13	13	383	278	250	234

Determination of sample size

To determine the number of trees to be evaluated within each family the following three published studies were considered;

1. Genetic divergence among hybrids of 'Cravo' mandarin with 'Pêra' sweet orange (de Oliveira et al., 2003). The variability among the hybrids and their relation to the parents were analysed using an F1 generation of 94 hybrids and the two parents.
2. Genotypic variation of prolonged dormancy symptoms in apple families (Labuschagné, 2002a). Eight apple families and 60 seedlings from each family were randomly selected for evaluation.
3. Genetic variation in chilling requirement in apple families (Labuschagné, 2002b). Four apple families, with 60 seedings from two of the families, and 100 seedlings from the other two families were randomly selected for evaluation.

Kiyomi Tangor

Dancy

Hansen

Rishon

Roma

Shani

Sunburst

Figure 3.1 Images of the seven citrus cultivars used as parents for the study of rind colour

Considering these three studies, it was decided to use 100 trees from each of the six Kiyomi families for evaluation. Where 100 trees bearing fruit were not found the maximum number of trees up to 100 was sampled. The trees were selected at random and are expected to be a representative sample of each family. Three trees (three replications) of each parent were included to obtain parent values.

To determine the number of fruit to be sampled per tree, data collected from a pilot study in 2008 (unpublished), from two Kiyomi families, a Kiyomi x Daisy family and a Kiyomi x Fremont family, was used. The families each consisted of 75 trees and 15 fruit were sampled from each tree. Data for rind colour coordinates L*, a* and b* were collected. This data was used to determine the variance component estimates for the two families. The error variance, which is the variance between the 15 fruit sampled per tree, was determined using 15 fruit, 10 fruit and five fruit (Table 3.3).

Table 3.3 Error variance for a sample size of 15, 10 and 5 fruit from a preliminary study of two citrus families for three rind colour coordinates

Number of fruit	L*	a*	b*
15 fruit	22.91	58.12	92.59
10 fruit	22.40	56.86	90.08
5 fruit	23.32	60.32	97.50

From Table 3.3 it can be seen that there is no increase in the error variance when decreasing the sample size to five fruit per tree. Therefore it could be concluded that a sample of five fruit per tree gave a homogenous sample representative of the tree and could be used in this study.

A sample of five fruit was picked from each of the progeny trees, randomly from all areas of the tree. In the same way a sample of five fruit was picked from the parent trees. Data was collected for two years, 2009 and 2010. Samples were picked between the week of the 29 June and the week of 17 August in 2009 and between the week of 28 June and the week of 16 August in 2010. Fruit samples for colour were picked when the fruit rind had reached its orange or orange red colour and there were no traces of green colouration left on the fruit.

Data collection

To allow colour to be expressed as a number, a full quantitative measurement needs to be done. Since colour is associated with light energy reflected off a surface, a controlled light source is projected onto the fruit's surface and the reflected light is measured (Ladaniya, 2008).

Colour meters measure light in terms of a tristimulus colour space that relates to human vision. The 'Commission Internationale de l'Eclairage' (CIE), L*, a*, b* colour space is the closest to the human eye's perception of colour and is therefore this best system to use for measuring fruit colour in citrus (Ladaniya, 2008). The CIE system records colour values in a uniform three dimensional colour space (Figure 3.2). L* represents the light factor and a* and b* are chromaticity coordinates. L* has a minimum value of 0 and a maximum value of 100, L=0 would be black while L=100 indicates a perfect reflecting diffuser. Coordinate a* represents the colours red and green, a positive a* indicates red and a negative a* green. Coordinate b* represents the colours yellow and blue, a positive b* indicates yellow and a negative b* a blue colour (Brown et al., 2009).

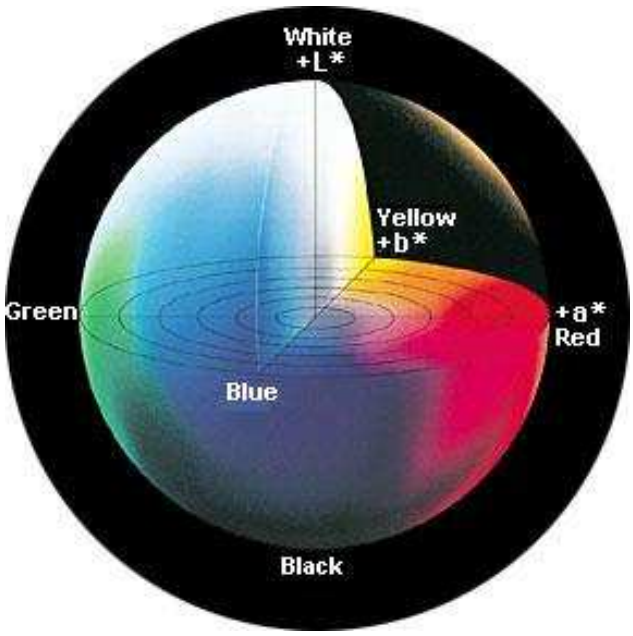

Figure 3.2 The CIE, L*, a*, b* colour space (Konica Minolta, 2007)

A Chroma meter, CR-400 tristimulus colorimeter (Konica Minolta, Osaka, Japan) with Spectramagic software was used for measuring the fruit rind colour. The instrument was set to L*, a*, b* mode and calibrated with a white calibration tile (L*=98.15, a*=-0.13, b*=1.92). The fruit colour, of each fruit, was measured by holding the fruit against the optical opening of the instrument and taking a reading. The fruit was rotated so that three readings could be taken per fruit and the average was recorded automatically.

Data analysis

Data was transferred to Microsoft Excel (2003) spreadsheets for analysis and statistical analyses were done by ARC- Biometry, Stellenbosch using SAS/STAT (1999 and 2008).

ANOVA's were carried out for both the parents and the families and a Student's t LSD test divided them into groups. Separate analyses were carried out for year 1 and year 2 and a joint analysis over the two years was used to test for year and year x family interaction effects.

Variance components for the families were determined for year 1 and year 2 separately; to investigate the variation between the families, within the families and within the trees, while excluding any possible year interaction. The repeatability between trees in a family was calculated using the ratio $\sigma^2_b /(\sigma^2_b + \sigma^2_w)$, where σ^2_w is the within tree variance component and σ^2_b the between tree variance component (Becker 1992; Falconer and Mackay, 1996).

Variance components determined from the joint analysis then allowed for the determination of the year and year x family interactions contribution to the total variation. Intraclass correlation coefficients were calculated and allow for the determination of an upper limit for broad sense heritability of the traits studied (de Souza and Byrne, 1998). Intraclass correlation coefficients were determined at two levels;

1. Relevant to selection between families; $t_1 = \sigma^2_B / \sigma^2_B + \sigma^2_W$

Where σ^2_B is the variance between the families and $(\sigma^2_B + \sigma^2_W)$ is the total phenotypic variance for both between and within the families.

2. Relevant to selection between individuals within a family; $t_2= \sigma^2_b /(\sigma^2_b + \sigma^2_w)$

Where σ^2_b is the variance between the trees within a family and $(\sigma^2_b + \sigma^2_w)$ is the total phenotypic variance within a family including the y x tree interaction (Labuschagne, 2002a).

Distribution curves were drawn for the six families. Parent values were included on the curves and allowed the progeny to be compared to the parents. Pearson correlation coefficients were calculated from the data for the families and determined the correlation between the traits studied.

RESULTS AND DISCUSSION

The ANOVA for the parents (Table 3.4) showed a significant level of variation between the parents for all the colour coordinates. A significant year x parent variation for L* and b* and between the years for b* was seen. L* showed less between parent variation than a* and b*, while b* showed a larger year and year x parent variation than L* and a*.

Table 3.4 ANOVA for rind colour of the seven citrus parents

	Year 1		Year 2		Joint analysis	
	Df	**MS**	**Df**	**MS**	**Df**	**MS**
L*						
Parent	6	35.57**	6	67.15**	6	88.29**
Rep (parent)					14	2.35
Year		-		-	1	3.27
Year x Parent		-		-	6	14.43**
Error	14	2.11	14	1.53	14	1.29
a*						
Parent	6	118.11**	6	143.90**	6	252.49**
Rep (parent)					14	2.18
Year		-		-	1	0.00
Year x Parent		-		-	6	9.52
Error	14	2.31	14	3.30	14	3.42
b*						
Parent	6	99.91**	6	164.42**	6	219.15**
Rep (parent)					14	11.22
Year		-		-	1	68.64**
Year x Parent		-		-	6	45.18**
Error	14	4.85	14	11.14	14	4.76

**** P ≤ 0.01**

The ANOVA for the families (Table 3.5) showed a significant level of between family variation, between year variation and year x family variation for all the colour coordinates. As with the parents, L* for the families showed less between family variation than a* and b* and b* had a larger year and year x family variation than L* and a*.

Table 3.5 ANOVA for rind colour of the six citrus families

	Year 1		Year 2		Joint analysis	
	Df	**MS**	**Df**	**MS**	**Df**	**MS**
L*						
Family	5	37.95**	5	138.62**	5	120.18**
Family (tree)					570	9.72
Year		-		-	1	142.18**
Year x Family		-		-	5	65.97**
Error	530	10.51	560	9.15	520	9.87
a*						
Family	5	434.52**	5	566.44**	5	952.02**
Family (tree)					570	36.74
Year		-		-	1	415.17**
Year x Family		-		-	5	86.45*
Error	530	32.80	560	39.54	520	35.38
b*						
Family	5	390.40**	5	684.72**	5	415.73**
Family (tree)					570	31.70
Year		-		-	1	1026.33**
Year x Family		-		-	5	651.62**
Error	530	35.42	560	27.88	520	31.54

**** P ≤ 0.01, * P ≤ 0.05**

The Student t test for the parents (Table 3.6) showed that for L* female parent Kiyomi had the highest mean and differed significantly from the male parents. Among the male parents, Dancy and Roma had the highest means and differed significantly from Sunburst, Rishon, Hansen and Shani, while Shani had the lowest mean and differed significantly from Dancy, Roma and Sunburst. The ranking and grouping among the male parents for L* varied between year 1 and year 2 as indicated by the significant year x parent variation for L* (Table 3.4).

For a* of the parents (Table 3.6) female parent Kiyomi had the lowest mean and differed significantly from the male parents. There was a significant difference between the male parents except between Roma and Dancy and Hansen and Shani. Male parents Roma and Dancy had the lowest means and Sunburst had the highest mean. The ranking and grouping for a* did vary between year 1 and year 2 for some of the male parents; however this variation was small and the ANOVA did not show a significant year x parent variation for a* (Table 3.4).

For b* of the parents (Table 3.6), female parent Kiyomi had the highest mean but did not differ significantly from male parents Roma and Dancy. Among the male parents, Roma and Dancy had the second highest means and differed significantly from the other male parents. Male parent Shani had the lowest mean and differed significantly from male parents Roma, Dancy and Hansen. The ranking and grouping for b* varied between year 1 and year 2 for the parents as indicated by the ANOVA's significant year x parent variation (Table 3.4). The large mean square value in the ANOVA for year x parent shows a larger variation in the ranking and grouping of the parents for b* than L* and a* (Table 3.4), while the large mean square for year shows a larger year variation in parent means for b* than for L* and a*.

The data revealed that female parent Kiyomi had the highest mean for L* and b* and the lowest mean for a*, indicating a light orange to orange yellow rind colour. Among the male parents Dancy and Roma had higher means for L* and b* and lower means for a*, having a rind colour more similar to the female parent Kiyomi. While male parents Hansen, Rishon, Shani and Sunburst had lower means for L* and b* and higher means for a*, indicating a deeper, more orange-red rind colour.

Table 3.6 Means and standard errors for rind colour of the seven citrus parents

Year 1				Year 2				Joint analysis			
Parent	**Mean**	**Group**	**Error**	**Parent**	**Mean**	**Group**	**Error**	**Parent**	**Mean**	**Group**	**Error**
L*											
Kiyomi	65.54	**A**	± 1.78	Kiyomi	66.72	**A**	± 0.70	Kiyomi	66.13	**A**	± 0.90
Roma	63.17	**AB**	± 0.21	Dancy	64.26	**B**	± 0.58	Dancy	62.48	**B**	± 1.06
Dancy	60.69	**BC**	± 0.14	Roma	61.21	**C**	± 0.57	Roma	62.19	**B**	± 0.51
Sunburst	59.82	**C**	± 0.24	Rishon	58.63	**D**	± 1.06	Sunburst	58.03	**C**	± 0.84
Hansen	58.70	**CD**	± 0.88	Sunburst	56.24	**E**	± 0.54	Rishon	57.19	**CD**	± 0.84
Shani	56.80	**DE**	± 0.73	Shani	55.00	**E**	± 0.63	Hansen	56.60	**CD**	± 1.08
Rishon	55.75	**E**	± 0.57	Hansen	54.50	**E**	± 0.77	Shani	55.90	**D**	± 0.59
LSD (p≤0.05) : 2.54				LSD (p≤0.05) : 2.17				LSD (p≤0.05) : 1.90			
a*											
Sunburst	45.25	**A**	± 0.37	Sunburst	43.66	**A**	± 0.76	Sunburst	44.45	**A**	± 0.52
Shani	40.79	**B**	± 0.24	Hansen	43.40	**A**	± 0.58	Hansen	41.62	**B**	± 1.17
Hansen	39.85	**BC**	± 1.83	Shani	41.00	**AB**	± 0.84	Shani	40.90	**B**	± 0.39
Rishon	37.97	**C**	± 0.91	Rishon	39.46	**B**	± 0.17	Rishon	38.72	**C**	± 0.53
Dancy	34.99	**D**	± 0.87	Roma	34.64	**C**	± 0.80	Roma	34.14	**D**	± 0.46
Roma	33.64	**D**	± 0.40	Dancy	30.55	**D**	± 2.26	Dancy	32.77	**D**	± 1.47
Kiyomi	25.62	**E**	± 0.30	Kiyomi	25.45	**E**	± 0.57	Kiyomi	25.54	**E**	± 0.29
LSD (p≤0.05) : 2.66				LSD (p≤0.05) : 3.18				LSD (p≤0.05) : 1.83			
b*											
Roma	51.62	**A**	± 0.71	Dancy	53.87	**A**	± 2.04	Kiyomi	51.55	**A**	± 1.26
Kiyomi	49.65	**A**	± 1.62	Kiyomi	53.44	**A**	± 1.33	Roma	49.97	**A**	± 0.95
Dancy	42.74	**B**	± 0.28	Roma	48.31	**AB**	± 1.12	Dancy	48.31	**A**	± 2.65
Hansen	38.90	**BC**	± 2.05	Hansen	45.32	**B**	± 3.29	Hansen	42.11	**B**	± 2.25
Shani	38.57	**C**	± 1.79	Rishon	42.53	**BC**	± 1.97	Rishon	40.33	**BC**	± 1.37
Sunburst	38.28	**C**	± 0.36	Sunburst	37.80	**CD**	± 0.77	Sunburst	38.04	**BC**	± 0.40
Rishon	38.12	**C**	± 0.77	Shani	34.51	**D**	± 1.88	Shani	36.54	**C**	± 1.47
LSD (p≤0.05) : 3.86				LSD (p≤0.05) : 5.84				LSD (p≤0.05) : 4.15			

Means with the same letter are not significantly different

A Student t test for the families (Table 3.7) showed that for L* the Kiyomi x Roma and the Kiyomi x Dancy families had the highest means and differed significantly from the Kiyomi x Sunburst, Kiyomi x Rishon and Kiyomi x Hansen families. The Kiyomi x Hansen family had the lowest mean and differed significantly from all the other families. The ranking and grouping among the families for L* varied between year 1 and year 2 as indicated by the ANOVA's significant year x family variation (Table 3.5), while the significant year variation (Table 3.5) showed a variation in family means between the two years for L*.

For colour coordinate a* of the families (Table 3.7), the Kiyomi x Dancy family had the lowest mean and differed significantly from the other families except the Kiyomi x Roma family which had the second lowest mean. The Kiyomi x Sunburst family had the highest mean and the Kiyomi x Hansen family the second highest mean and these two families differed significantly from all the other families. The ranking and grouping of the families for a* varied between year 1 and year 2 as indicated by the ANOVA's significant year x family variation (Table 3.5), while the significant year variation (Table 3.5) showed a variation in family means between the two years for a*.

For colour coordinate b* of the families (Table 3.7), the Kiyomi x Roma and Kiyomi x Dancy families had the highest means and differed significantly from all other families, while the Kiyomi x Rishon family had the lowest mean and differed significantly from all the other families. The ranking and grouping among the families for b* varied between year 1 and year 2 as indicated by the significant year x family variation (Table 3.5), while the significant year variation (Table 3.5) showed a variation in family means between the two years. As with the parents, the families had a large mean square for year x family for b* showing a larger variation in the ranking and grouping of the families for b* than L* and a* (Table 3.5), while the large mean square for year showed a larger year variation in family means for b* than for L* and a*.

The Kiyomi x Dancy and the Kiyomi x Roma families had the highest means for L* and b* and the lowest means for a*, indicating a population with a lighter, more yellow-orange rind colour than the other families. The Kiyomi x Hansen family had the lowest mean for L*, the Kiyomi x Sunburst family the highest mean for a* and the Kiyomi x Hansen family the second highest mean, while the Kiyomi x Rishon family had the lowest mean for b*. These families therefore, have one or more colour coordinates that indicates a population with a deeper, more orange-red rind colour than the other families.

Table 3.7 Means and standard errors for rind colour of the six citrus families

Year 1				Year 2				Joint analysis			
Family	**Mean**	**Grp**	**Error**	**Family**	**Mean**	**Grp**	**Error**	**Family**	**Mean**	**Grp**	**Error**
L*											
Kiyomi x Roma	61.90	**A**	± 0.34	Kiyomi x Dancy	63.61	**A**	± 0.31	Kiyomi x Roma	62.45	**A**	± 0.23
Kiyomi x Sunburst	61.75	**AB**	± 0.42	Kiyomi x Roma	62.94	**AB**	± 0.30	Kiyomi x Dancy	62.29	**A**	± 0.24
Kiyomi x Shani	61.26	**AB**	± 0.28	Kiyomi x Shani	62.44	**B**	± 0.31	Kiyomi x Shani	61.85	**AB**	± 0.21
Kiyomi x Rishon	61.19	**AB**	± 0.37	Kiyomi x Sunburst	61.37	**C**	± 0.37	Kiyomi x Sunburst	61.57	**BC**	± 0.28
Kiyomi x Dancy	60.85	**BC**	± 0.31	Kiyomi x Rishon	61.19	**CD**	± 0.29	Kiyomi x Rishon	61.19	**C**	± 0.23
Kiyomi x Hansen	60.14	**C**	± 0.30	Kiyomi x Hansen	60.37	**D**	± 0.29	Kiyomi x Hansen	60.25	**D**	± 0.21
LSD (p≤0,05) : 0.96				LSD (p≤0.05) : 0.87				LSD (p≤0.05) : 0.64			
a*											
Kiyomi x Hansen	32.72	**A**	± 0.48	Kiyomi x Sunburst	32.18	**A**	± 0.68	Kiyomi x Sunburst	32.34	**A**	± 0.46
Kiyomi x Sunburst	32.49	**A**	± 0.64	Kiyomi x Hansen	31.78	**A**	± 0.56	Kiyomi x Hansen	32.27	**A**	± 0.37
Kiyomi x Shani	29.90	**B**	± 0.64	Kiyomi x Shani	28.97	**B**	± 0.64	Kiyomi x Shani	29.44	**B**	± 0.45
Kiyomi x Rishon	29.84	**B**	± 0.65	Kiyomi x Rishon	27.55	**BC**	± 0.62	Kiyomi x Rishon	28.55	**BC**	± 0.46
Kiyomi x Roma	29.73	**B**	± 0.59	Kiyomi x Dancy	27.03	**C**	± 0.63	Kiyomi x Roma	27.95	**CD**	± 0.51
Kiyomi x Dancy	26.72	**C**	± 0.59	Kiyomi x Roma	26.36	**C**	± 0.76	Kiyomi x Dancy	26.88	**D**	± 0.43
LSD (p≤0,05) : 1.70				LSD (p≤0.05) : 1.80				LSD (p≤0.05) : 1.25			
b*											
Kiyomi x Roma	49.09	**A**	± 0.74	Kiyomi x Dancy	50.72	**A**	± 0.49	Kiyomi x Roma	48.47	**A**	± 0.46
Kiyomi x Sunburst	45.57	**B**	± 0.65	Kiyomi x Hansen	49.28	**AB**	± 0.57	Kiyomi x Dancy	47.57	**A**	± 0.44
Kiyomi x Shani	45.41	**B**	± 0.48	Kiyomi x Roma	47.92	**B**	± 0.56	Kiyomi x Hansen	45.91	**B**	± 0.49
Kiyomi x Rishon	45.06	**B**	± 0.75	Kiyomi x Shani	46.18	**C**	± 0.50	Kiyomi x Shani	45.80	**B**	± 0.35
Kiyomi x Dancy	44.12	**BC**	± 0.53	Kiyomi x Sunburst	46.00	**C**	± 0.63	Kiyomi x Sunburst	45.77	**B**	± 0.45
Kiyomi x Hansen	42.55	**C**	± 0.63	Kiyomi x Rishon	43.23	**D**	± 0.50	Kiyomi x Rishon	44.03	**C**	± 0.44
LSD (p≤0,05) : 1.76				LSD (p≤0.05) : 1.51				LSD (p≤0.05) : 1.16			

Means with the same letter are not significantly different

Variance components determined, for year 1 and year 2 separately, for rind colour followed the same trend over the two years (Table 3.8). The variation within the families was greater than between the families for all colour coordinates, indicating a high level of genetic variation within the families. The between family variation, expressed as a percentage of the total variation, was 2% and 11% for L*, 10% and 14% for a* and 9% and 13% for b*, for year 1 and year 2 respectively. While the within family variation was 81% and 72% for L*, 72% and 66% for a* and 73% and 71% for b*. The within tree variation (error variance) was lower than the within family variation for all the colour coordinates and showed that a sample of five fruit per tree was sufficient. Expressed as a percentage of the total variation the within tree variation was 16% for both year 1 and year 2 for L*, and 18% and 19% for a* and 18% and 16% for b*, for year 1 and year 2 respectively. Colour coordinate L* had a lower between family, within family and within tree variation showing less variation than a* and b*.

The repeatability, calculated between trees within a family (Table 3.8), was high for all families and all colour coordinates. Repeatability estimates ranged from 0.76 to 0.88 for L*, 0.76 to 0.86 for a* and 0.60 to 0.87 for b*. The repeatability estimate in this case was used to determine the gain in accuracy expected from multiple measurements within a tree. The high repeatability estimate, therefore, indicates that only a small gain in accuracy would be attained by increasing the number of fruit sampled per tree (Falconer and Mackay, 1996).

Variance components determined from the joint analysis (Table 3.9) allowed for the year and year x family contribution to the total variance to be calculated.

The between family variation was again found to be lower than the within family variation for all colour coordinates. Expressed as a percentage of the total variation, the between family variation was 1% for L*, 9% for a* and 0% for b*, while the within family variation was 36% for L*, 35% for a* and 32% for b*. The variation between the years and the year x family variation were both found to be low and were less than within family variation for all the colour coordinates. Expressed as a percentage of the total variation, the year variation was 1% for L* and a* and 0% for b*. The year x family variation was slightly higher than the year variation at 6% for L*, 2% for a* and 13% for b*.

Table 3.8 Variance components and repeatability for rind colour of the six citrus families

	Source of variation								Repeatability (between trees in a family)	
	Between families		Within families (between trees)		Within trees		Total			
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
L*	0.30	1.38	10.11	8.75	2.01	2.00	12.42	12.13		
Kiyomi x Dancy			7.69	7.98	1.80	2.16	9.49	10.14	0.81	0.79
Kiyomi x Hansen			8.88	7.91	1.76	1.91	10.64	9.82	0.83	0.80
Kiyomi x Rishon			10.33	8.00	2.17	2.23	12.50	10.23	0.83	0.78
Kiyomi x Roma			8.41	7.06	1.58	2.17	9.99	9.23	0.84	0.76
Kiyomi x Shani			7.58	9.42	2.43	1.95	10.01	11.37	0.76	0.83
Kiyomi x Sunburst			16.94	12.11	2.19	1.57	19.13	13.68	0.88	0.88
a*	4.48	5.71	31.22	26.34	7.92	7.62	43.62	39.67		
Kiyomi x Dancy			27.05	34.54	8.20	8.14	35.25	42.68	0.77	0.81
Kiyomi x Hansen			22.23	30.15	6.46	8.68	28.69	38.83	0.77	0.78
Kiyomi x Rishon			32.28	37.38	7.71	10.81	39.99	48.19	0.81	0.78
Kiyomi x Roma			24.86	46.93	7.83	9.67	32.69	56.60	0.76	0.83
Kiyomi x Shani			39.42	38.83	10.18	8.37	49.60	47.20	0.79	0.82
Kiyomi x Sunburst			39.31	40.05	7.09	6.53	46.40	46.58	0.85	0.86
b*	4.24	6.79	33.74	37.74	8.32	8.71	46.30	53.24		
Kiyomi x Dancy			21.60	19.35	8.74	9.37	30.34	28.72	0.71	0.67
Kiyomi x Hansen			37.94	31.36	6.99	8.03	44.93	39.39	0.84	0.80
Kiyomi x Rishon			43.49	23.83	6.65	7.32	50.14	31.15	0.87	0.76
Kiyomi x Roma			39.53	25.06	6.52	6.63	46.05	31.69	0.86	0.79
Kiyomi x Shani			20.02	24.00	13.53	7.71	33.55	31.71	0.60	0.76
Kiyomi x Sunburst			41.19	34.41	6.65	6.56	47.84	40.97	0.86	0.84

Table 3.9 Variance components and intraclass correlation coefficients for rind colour of the six citrus families

	Source of variation						Intraclass correlation coefficients	
	Between families	**Within families**	**Year**	**Year x Family**	**Error**	**Total**	**t_1**	**t_2**
L*	0.18	4.55	0.17	0.73	7.03	12.67	0.01	0.39
a*	4.32	16.84	0.67	0.93	26.05	48.81	0.09	0.39
b*	0.00	14.36	0.75	5.79	23.86	44.76	0.00	0.38

Intraclass correlation coefficients were determined; relevant to selection between families (t_1) and relevant to selection between trees within a family (t_2) (Table 4.9). Intraclass correlation coefficient t_1 was very low at 0.01 for L*, 0.09 for a* and 0 for b* indicating very little variation between the families. Intraclass correlation coefficient t_2 was fairly low at 0.39 for L* and a* and 0.38 for b*. The intraclass correlation coefficient sets an upper limit to broad sense heritability for the traits studied (de Souza and Byrne, 1998). Therefore, the low value for t_2 indicates that the variation found within the families was only partly genetic and non-genetic factors (environment) also played a role.

Rind colour coordinate L* gives an indication of the light component. As previously mentioned, L* can have a minimum value of 0, indicating a completely dark / black colour, and a maximum value of 100, indicating a total reflection / white colour (Brown et al, 2009). Since a deeper rind colour is desired, progeny with a lower L* value are sought.

Distribution curves were drawn for the six families for rind colour coordinates L*, a* and b*. Parent values were included on each curve to allow the progeny to be compared to the parents (Figure 3.3 – 3.5).

All families showed a continuous distribution for L* indicating a quantitative expression of the trait, with all the curves following a normal distribution, having a p-value > 0.05 (Figure 3.3). The Kiyomi x Sunburst curves had a slightly wider distribution than those of the other families, showing more within family variation, as seen in the variance component analysis (Table 3.8) where Kiyomi x Sunburst had a larger between tree variance than the other families.

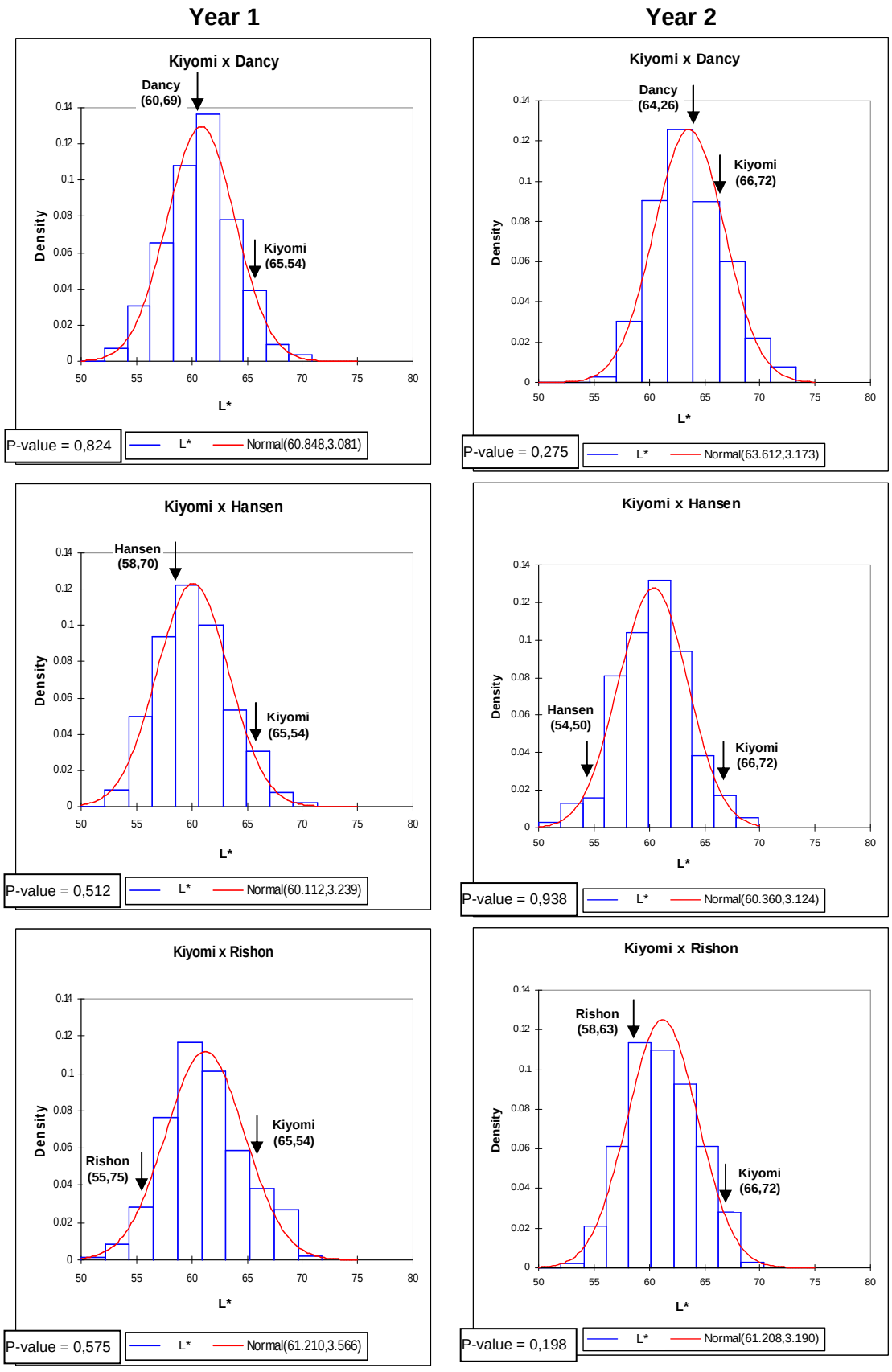

Figure 3.3 Distribution curves for the six citrus families for rind colour factor L*

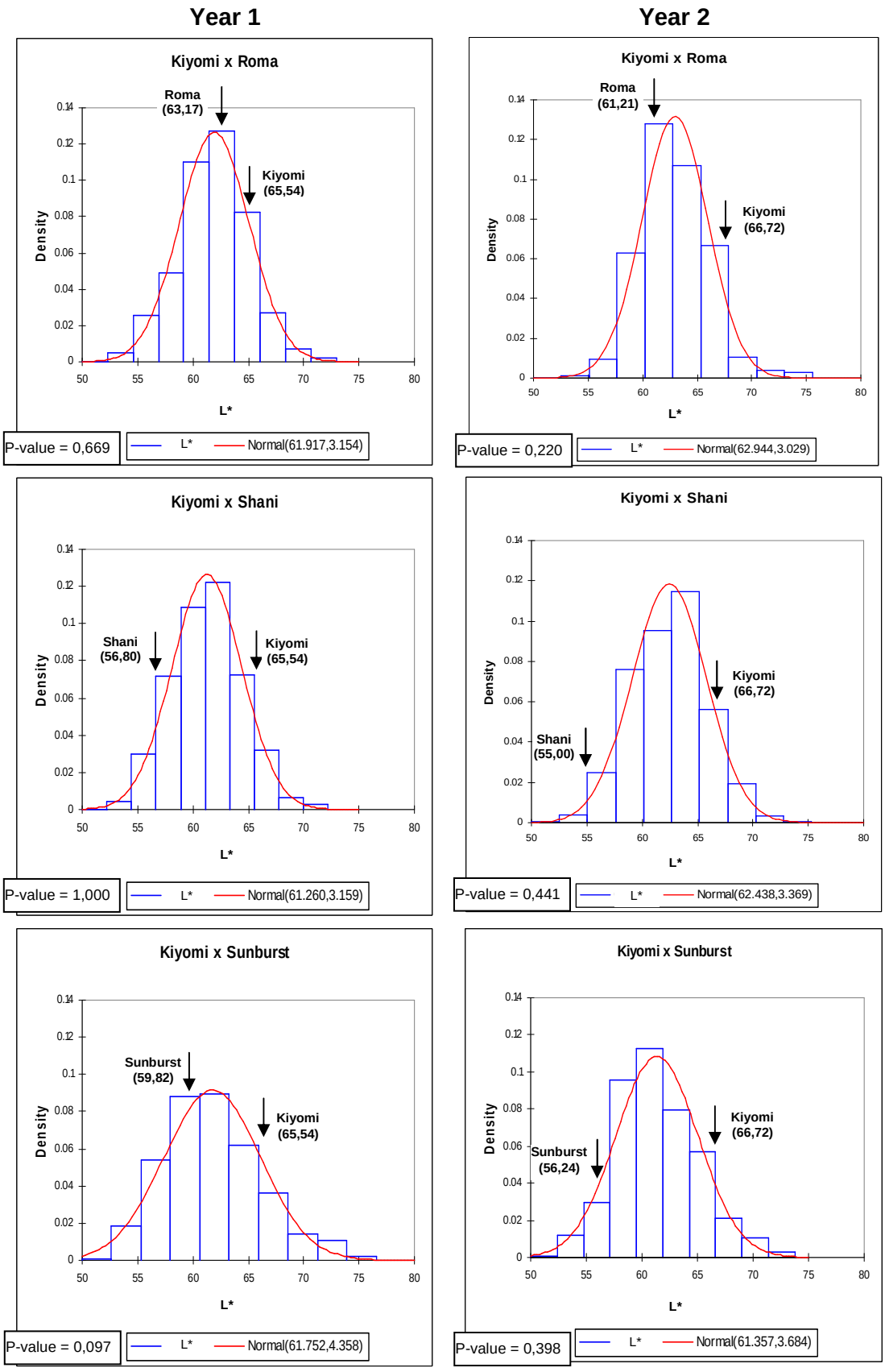

Figure 3.3 (cont.) Distribution curves for the six citrus families for rind colour factor L*

For L* the Kiyomi x Hansen, Kiyomi x Rishon, Kiyomi x Shani and Kiyomi x Sunburst families had means between the two parents and the Kiyomi x Dancy and Kiyomi x Roma families had means close or equal to that of the male parents (Figure 3.3). Therefore all the families showed an improvement in the progeny for L* over female parent Kiyomi, while in the Kiyomi x Dancy and Kiyomi x Roma families the male parent was more dominant. All families however showed heterosis and contained some individuals with lower L* values than both of the parents. The Kiyomi x Hansen family had the lowest mean for L* and was significantly lower than all the other families (Table 3.7).

When comparing the year 1 and year 2 distribution curves for L* (Figure 3.3), it could be seen that the families generally showed similar trends over the two years. However the differences between the years can be attributed to the significant year and year x family variation for the families (Table 3.5) as well as the significant year x parent variation for the parents (Table 3.4).

Rind colour coordinate a* represents the colours red and green, with a positive a* indicating red and a negative a* indicating green (Brown et al., 2009). Since a deeper orange to orange red rind colour is desired, progeny with a high positive a* value are sought.

All families showed a continuous distribution for a* indicating a quantitative trait (Figure 3.4). The curves for the Kiyomi x Dancy, Kiyomi x Hansen and Kiyomi x Rishon families for both years and the Kiyomi x Roma family for year 1 had a p-value > than 0.05 and followed a normal distribution. The curves for the Kiyomi x Roma family for year 2 and the Kiyomi x Shani and Kiyomi x Sunburst families for both years did not follow a normal distribution with all of the curves being skewed towards higher a* values. The Kiyomi x Dancy, Kiyomi x Hansen and Kiyomi x Roma curves had a slightly narrower distribution in year 1 than year 2, showing less within family variation in year 1, as seen in the variance component analysis (Table 3.8) where the Kiyomi x Dancy, Kiyomi x Hansen and Kiyomi x Roma families had a lower within family variation for year 1 than year 2.

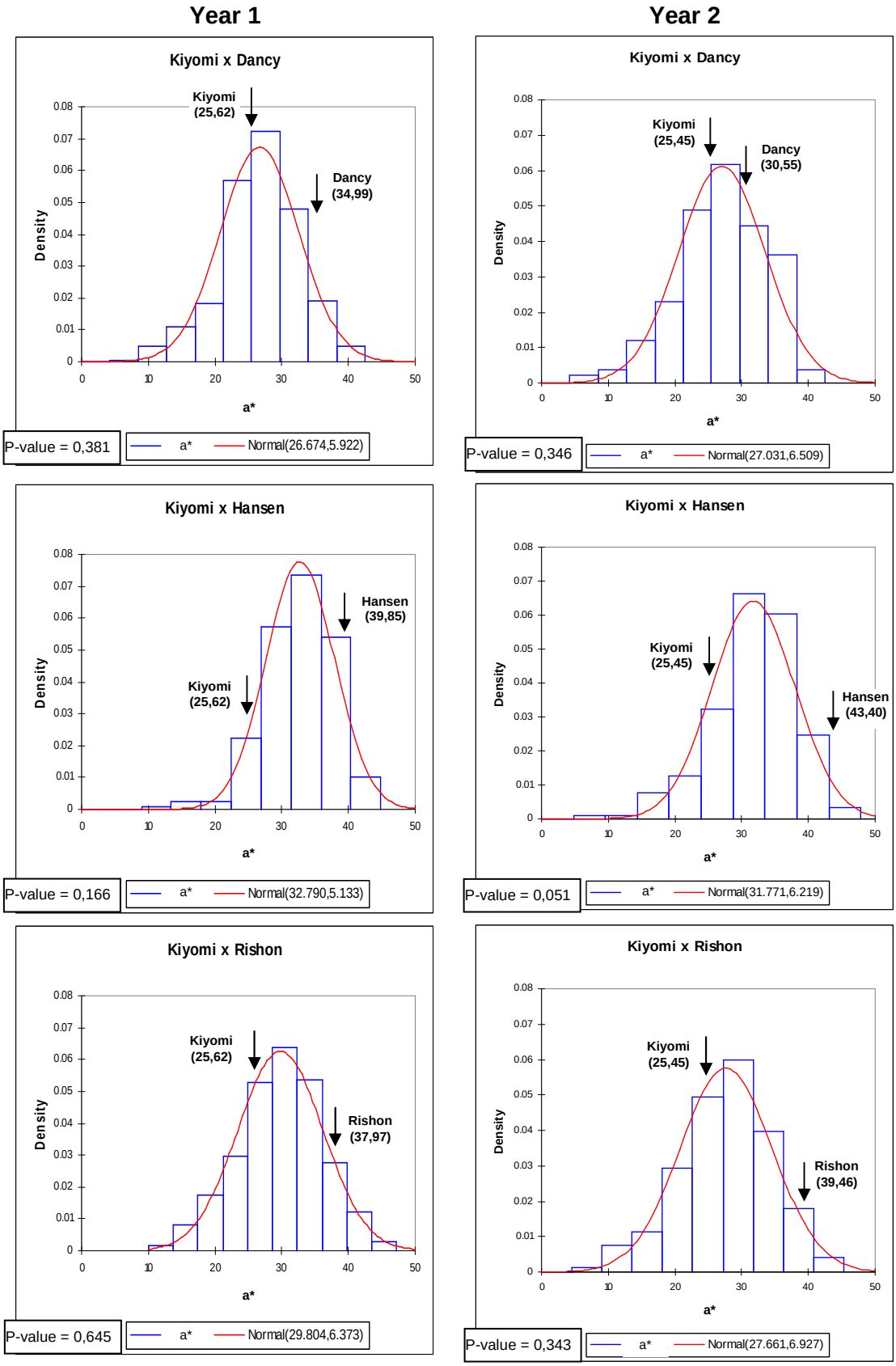

Figure 3.4 Distribution curves for the six citrus families for rind colour factor a*

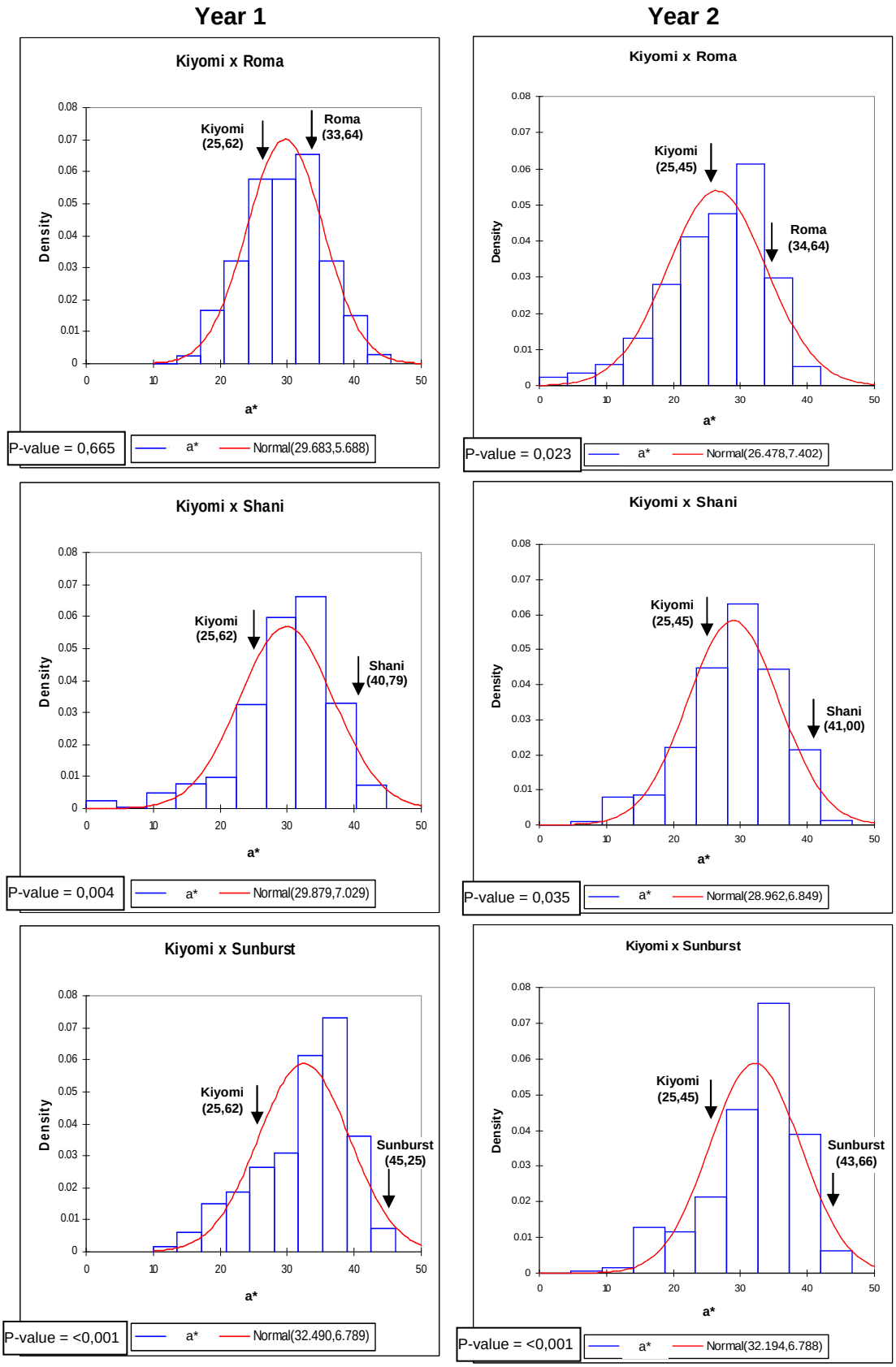

Figure 3.4 (cont.) Distribution curves for the six citrus families for rind colour factor a*

For a* the Kiyomi x Hansen, Kiyomi x Shani and Kiyomi x Sunburst families had means between the two parents and the Kiyomi x Dancy family had means close to the female parent. The Kiyomi x Rishon and Kiyomi x Roma families had means between the two parents in year 1 but had means close to the female parent in year 2 (Figure 3.4). Therefore all families except the Kiyomi x Dancy family showed some improvement in the progeny for a* over the female parent Kiyomi, while the Kiyomi x Dancy, Kiyomi x Rishon and Kiyomi x Roma families showed the female parent Kiyomi to be more dominant. All families however showed heterosis and contained some individuals with higher a* values than both of the parents. The Kiyomi x Sunburst and Kiyomi x Hansen families had the highest means for a* and differed significantly from the other families (Table 3.7).

When comparing the year 1 and year 2 distribution curves for a* (Figure 3.4) it could be seen that the families generally showed similar trends over the two years, except for the Kiyomi x Rishon and Kiyomi x Roma families. The differences between the years can be attributed to the significant year and year x family interaction for the families (Table 3.5).

Rind colour coordinate b* represents the colours blue and yellow, with a positive b* indicating yellow and a negative b* indicating blue (Brown et al., 2009). Since an orange or orange-red rind colour is preferred over a yellow colour, progeny with a low positive b* value are sought.

All families showed a continuous distribution for b*, indicating a quantitative trait (Figure 3.5). All of the distribution curves had a p-value > than 0.05 and followed a normal distribution, except for the Kiyomi x Sunburst family in year 1 which was slightly skewed towards lower b* values. The Kiyomi x Dancy and Kiyomi x Shani curves for both years and the Kiyomi x Rishon and Kiyomi x Roma curves for year 2 had a narrower distribution, as seen in the variance component analysis (Table 3.8) where the Kiyomi x Dancy and Kiyomi x Shani families for both years and the Kiyomi x Rishon and Kiyomi x Roma families for year 2 had a lower within family variation for b*.

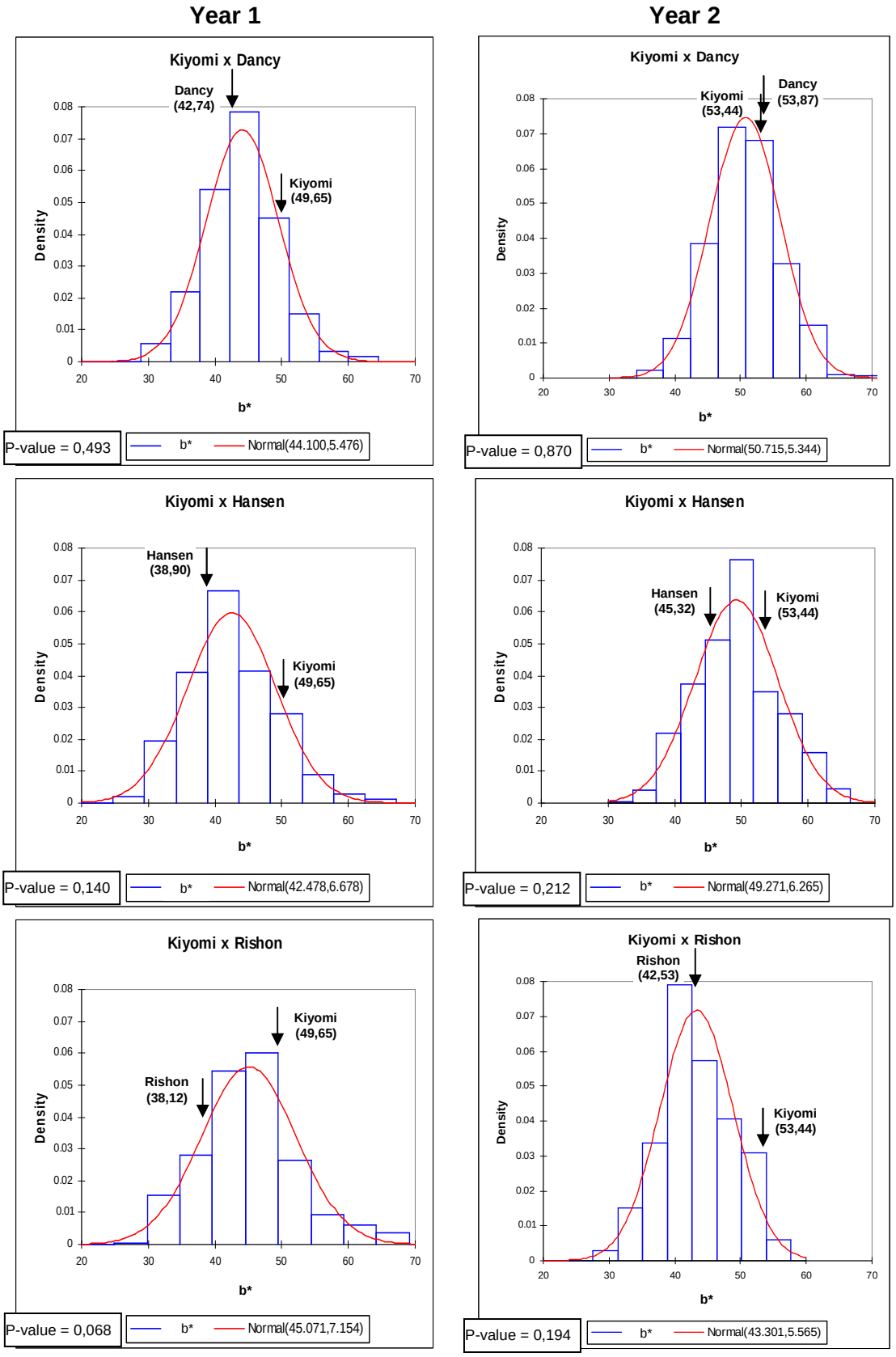

Figure 3.5 Distribution curves for the six citrus families for rind colour factor b*

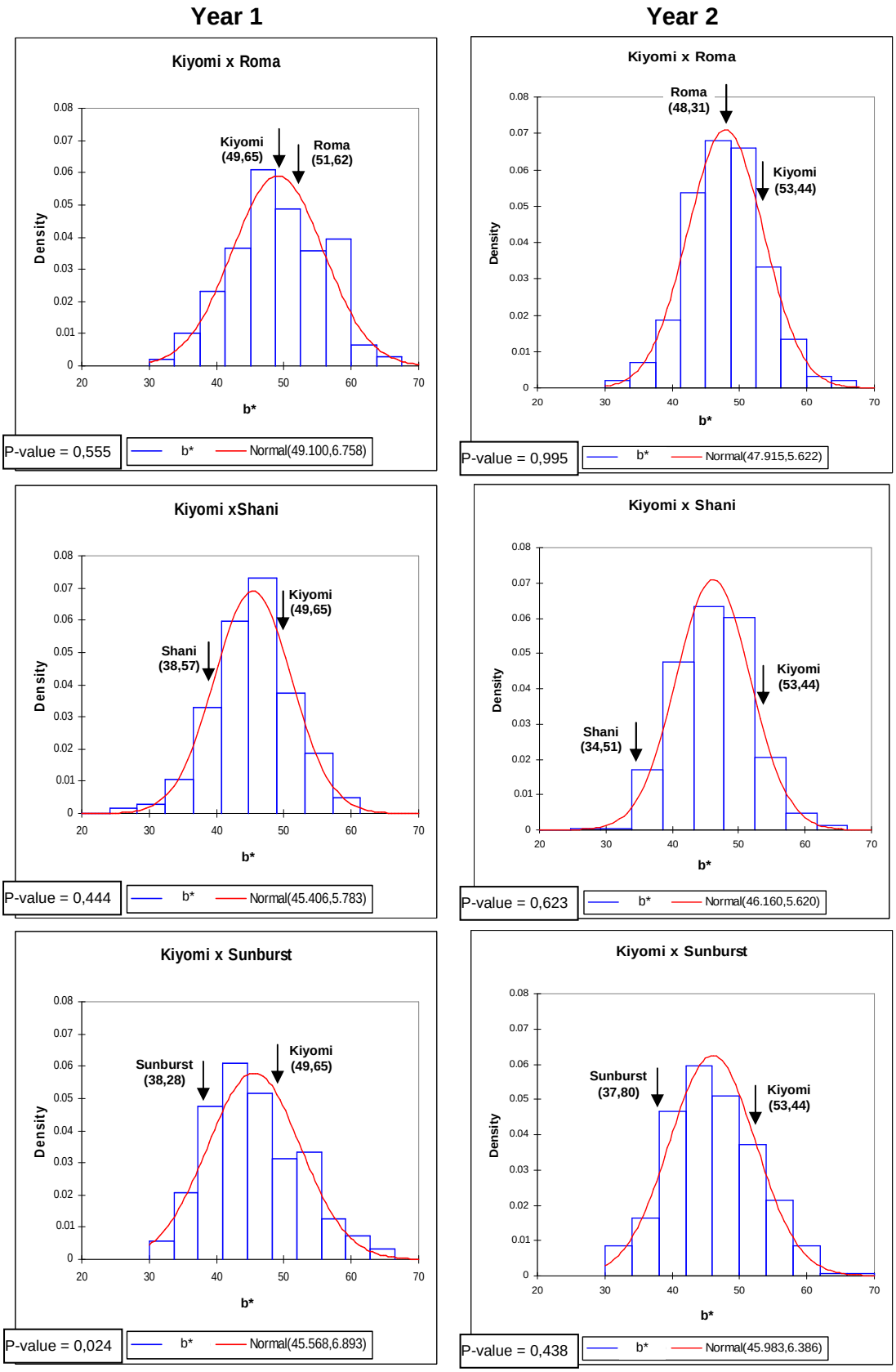

Figure 3.5 (cont.) Distribution curves for the six citrus families for rind colour factor b*

For b* the Kiyomi x Hansen, Kiyomi x Shani and Kiyiomi x Sunburst families had means between the two parents, while the Kiyomi x Rishon family had a mean between the two parents in year 1 but had a mean closer to the male parent in year 2. The Kiyomi x Dancy family had a mean closer to the male parent Dancy in year 1 and close to both parents in year 2, while the Kiyomi x Roma family had a mean closer to the female parent Kiyomi in year 1 and to the male parent Roma in year 2 (Figure 3.5); however parent values for Dancy and Roma did vary between the two years (Table 3.6). Therefore all of the families showed some improvement in the progeny for b* over the female parent Kiyomi. The Kiyomi x Dancy family in year 1 and the Kiyomi x Rishon and Kiyomi x Roma families in year 2 showed the male parent to be more dominant, while the Kiyomi x Roma family in year 1 showed the female parent to be more dominant. All families however showed heterosis and contained some individuals with lower b* values than both the parents. The Kiyomi x Rishon family had the lowest mean for b* and differed significantly from the other families (Table 3.7).

When comparing the year 1 and year 2 distribution curves for b* the Kiyomi x Hansen, Kiyomi x Shani and Kiyomi x Sunburst families generally showed similar trends over the two years, while the Kiyomi x Dancy, Kiyomi x Rishon and Kiyomi x Roma families differed between the two years. The differences between the years can be attributed to the significant year and year x family variation for the families (Table 3.5) as well as the variation in the parent values for Dancy and Roma between the two years (Table 3.6).

Correlations for year 1 and year 2 showed the same trend, with a positive correlation between L* and b* (Table 3.10), indicating that as the yellow colour increases so does the light factor, while an increase in the blue colour causes a darker colour. However the colour coordinates L*, a* and b* are measured simultaneously; therefore in this case a correlation is of no use and it was determined simply as a point of interest.

Table 3.10 Pearson correlation coefficients for rind colour of the six citrus families

	Year 1			Year 2		
	L*	**a***	**b***	**L***	**a***	**b***
L*		-0.52**	0.78**		-0.61**	0.76**
a*	-0.52**		-0.33**	-0.61**		-0.38**
b*	0.78**	-0.33**		0.76**	-0.38**	

****P ≤ 0.01**

CONCLUSIONS

Rind colour is the most important characteristic contributing to a fruit's appearance (Ladaniya, 2008) and therefore the improvement of rind colour has been a longstanding aim of citrus breeding programmes (Ray, 2002). However, due to the lack of information on the inheritance of rind colour in citrus, the breeder faces a difficult task when planning crosses for breeding new and improved cultivars. In this study six mandarin families, where the female parent Kiyomi tangor was crossed with various male parents in order to produce hybrids with an improved rind colour, were studied. The fruit rind colour data collected were used to quantify the genetic variability within and between the families over two seasons as well as to study the genetic relationships between the hybrids and the parents.

The study revealed a significant level of between parent and between family variation for all the colour coordinates. The variation in fruit rind colour in citrus is, therefore, measurable and can be further explored for breeding purposes. The variance component analysis showed a greater variation within the families than between the families, indicating a high level of genetic variation within the families. Citrus cultivars are highly heterozygous (Ray, 2002) and the high genetic variation found within the families can be ascribed directly to the variation generated by crossing two heterozygous parents. The within tree variation was lower than the within family variation and showed that a sample size as small as five fruit per tree can be used as a homogenous sample. The high repeatability between trees within a family indicated that only a small gain in accuracy would be attained from increasing the number of fruit sampled per tree and again confirmed that a sample of five fruit per tree was sufficient.

The parents had a significant year variation for b* and a significant year x parent variation for L* and b*, while the families had a significant year and year x family variation for all colour coordinates. However, the variance component analysis showed that the year and the year x family variation contributed little to the total variation. The climate, especially temperature, is known to influence the development of rind colour in citrus and possibly played a part in the variation found between the years. The intraclass correlation coefficient t_2 was found to be fairly low and indicated that the variation found within the families was only partly genetic and that the environment (non-genetic factors) contributed to the variation in the phenotype. Therefore, it can be concluded that only two years of testing will not be reliable and a mean performance over multiple years data should be used for effective selection.

In citrus, rind colour is a quantitative genetic trait and all the colour coordinates showed a continuous distribution in rind colour. From the L*, a* and b* values it can be seen that all the families contained progeny with rind colours ranging from a light yellow to a deep red-orange as was noted by Vardi and Spiegel-Roy (1978). The distribution curves showed that all the families had an improvement in rind colour over the female parent Kiyomi for L* and b*, while all the families except for the Kiyomi x Dancy family showed an improvement for a*. Heterosis does occur in citrus and all families contained some individuals with a rind colour superior to both parents.

The Kiyomi x Dancy and Kiyomi x Roma families were found to have a population with a lighter, more yellow-orange rind colour, while the Kiyomi x Hansen, Kiyomi x Rishon, Kiyomi x Shani and Kiyomi x Sunburst families had a population with a deeper, more orange-red rind colour. From the parent values it can be seen that male parents Dancy and Roma had a lighter, more yellow-orange rind colour more similar to the female parent Kiyomi, while other male parents Hansen, Rishon, Shani and Sunburst had a deeper more orange-red rind colour. Therefore, it can be seen that the families with male parents having a deeper, more orange-red, rind colour showed a greater improvement in the population when crossed with female parent Kiyomi.

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CHAPTER 4

GENOTYPIC VARIATION IN FRUIT SIZE AND SHAPE IN KIYOMI FAMILIES

INTRODUCTION

Fruit of the genus Citrus vary greatly in size and range from about 30 mm in diameter for kumquats (*Fortunella* spp.) to more than 300 mm in diameter for pummelos (*C. grandis*) (Ray, 2002). Fruit size in mandarins can vary from small to large but most fruit have a size of between 50 mm and 80 mm in diameter. Fruit size is one of the main factors used to determine consumer acceptance and higher premiums are paid for larger fruit, making fruit size an important factor when determining market returns (Ladaniya, 2008). In addition to this, many grocery stores have strict quality standards regarding citrus fruit and premium mandarins are required to be orange, unblemished and large (Campbell et al., 2006).

Fruit size for citrus is determined by the maximum diameter at the equatorial section of the fruit and used to sort the fruit into grade standards (Ladaniya, 2008). Mandarins graded at sizes above 78 mm are given a code of 1-XXX, a size of 72-78 mm a 1-XX, 68-72 mm a 1-X, 64-68 mm a 1, 59-64 mm a 2, 55-59 mm a 3, 51-55 mm a 4 and 48-55 mm a 5 (SRCC, 2008). There is a minimum requirement regarding fruit size in mandarins and some mandarins including satsuma mandarins less than 45 mm, clementine mandarins less than 35 mm and Kinnow mandarins less than 50 mm are not accepted (Ladaniya, 2008).

The development of a citrus flowers' ovary into a fruit takes about six to 18 months, and varies with the cultivar and the climate (Ladaniya, 2008). This growth and development can be broken up into three main stages. The first stage consists of intense cell division but slow growth over about nine weeks, the second stage is a rapid growth due to cell enlargement over about 30 weeks resulting in a rapid increase in fruit size, while during the third and final stage the fruit reaches horticultural maturity over 11 weeks with virtually no growth (Bijzet, 2006a).

The climate is the most important factor influencing fruit growth in citrus and in a warm tropical climate, where the heat unit requirement for maturation is reached much quicker, the fruit grow and develop faster, allowing them to become very large. In the cooler subtropical climates with lower temperatures and less light intensity, the fruit grow slower and the mature fruit are considerably smaller (Spiegel-Roy and Goldschmidt, 1996).

A large amount of photosynthate is required for fruit enlargement (Spiegel-Roy and Goldschmidt, 1996). Larger fruit are therefore found on leafy, compared to leafless, branches due to the nearby leaves being potent suppliers of photosynthate (Ladaniya, 2008). Mechanical practices such as girdling and fruit thinning can be used to increase the amount of photosynthate available to the fruit and therefore increase fruit size. Girdling involves removing a ring of bark from the trunk or scaffold branches interfering with the downward phloem transport and preventing the escape of the photosynthate from the girdled area to other parts of the tree. Girdling during the fruit-enlargement stage can increase the fruit size by up to 30%. Fruit thinning, where some of the fruit are removed, increases the leafy area per fruit making more photosynthate available to each individual fruit and thereby increasing the fruit size. Intrinsic fruit size is probably a genetic trait while the fruit number reflects the tree's bearing limits. Even though the yield varies considerably between citrus cultivars, all cultivars have shown the fruit size to be inversely proportional to the number of fruit on the tree (Spiegel-Roy and Goldschmidt, 1996), with heavy bearing trees producing smaller fruit (Ladaniya, 2008).

Plant growth regulators can also be used to manipulate fruit size and auxins are used extensively for this purpose. Auxins can increase fruit size in three ways: firstly by thinning the crop and increasing the growth of the remaining fruitlets, secondly when applied at the end of the first stage and beginning of the second stage of fruit development they directly enhance fruit growth, and thirdly when applied to the stylar end of young fruit they enhance the growth of some fruitlets and increase late fruitlet abscission (Ladaniya, 2008).

Mineral nutrition is another factor influencing fruit growth and the macronutrient potassium plays an important role in fruit size. A potassium deficiency in the tree results in smaller fruit, and potassium sprays are used commercially to increase the fruit size (Spiegel-Roy and Goldschmidt, 1996; Ladaniya, 2008).

The regulation of a citrus tree's production at tree level involves a large array of subtle nutritional and hormonal signals. An imbalance in these systems results in productivity disorders such as alternate bearing (Spiegel-Roy and Goldschmidt, 1996), which is common among mandarins and their hybrids (Saunt, 2000). Alternate bearing is a tendency of fruit trees to bear a heavy crop of small fruit one year followed by a light crop of large fruit the next year (Verreynne and Lovatt, 2009) causing a variation in fruit size between years. Several horticultural practices such as girdling the stems, spraying with plant growth regulators and hand thinning of fruit are used to reduce the effect of this irregular bearing behaviour (Saunt, 2000).

Seed content also plays a role in fruit size. Seedless fruit are generally small and an increase in the seed content can increase the fruit size (Chao, 2005). Since fruit development is linked to the development of the ovule, it is possible that a hormonal stimulus from the seeds regulates fruit growth. Pollen has been found to play a role in fruit size and generally larger fruit with more seeds are produced if more pollen is applied to the stigma of the flower (Ladaniya, 2008).

For the citrus breeder fruit size is an important characteristic and many newly bred hybrids that produce good quality fruit have to be discarded because of a small fruit size (Soost and Cameron, 1975; Ray, 2002). No information could be found on the inheritance of fruit size in mandarin breeding populations; however some work has been done on the genetic variability of fruit size among mandarin clones. Repeatability in time, over years, and space, between clones, was determined for fruit height and width measurements in mandarin clones (Lima et al., 1981 and 1992). This was found to be fairly low with repeatability in time at 0.33 for height and 0.29 for width and repeatability in space at 0.35 for height and 0.31 for width (Lima et al., 1992), showing variation between years as well as between clones. Broad sense heritability estimates for acid lime (*C. aurantifolia* Swing) clones were also found to be fairly low at 0.55 for fruit height and 0.37 for width (Prasad and Rao, 1989). Literature was, however, found on heritability estimates in fruit tree breeding populations of peach (*Prunus persica* (L.) Batsch) and mango (*Mangifera indica* L.) (de Sousa and Bryne, 1998; Brown et al., 2009). Fairly low estimates were found for narrow sense heritability in peach at 0.47 for fruit height and 0.38 for fruit width (de Sousa and Bryne, 1998). Open pollinated half-sib mango families; on the other hand, gave high estimates for broad sense heritability at 0.95 for fruit height and 0.94 for fruit width (Brown et al., 2009).

Fruit shape is another important characteristic of citrus fruit and serves as an indication of quality (Ladaniya, 2008). Mandarin fruit have a globose to oblate shape (Bijzet, 2006b) with some cultivars having a low to high collar with a deeply depressed apex. Oblong and pyriform fruit are also sometimes found among mandarins, however these are removed before packing as they are not true to type and can damage the impression of the fruit (Ladaniya, 2008). In citrus round fruit are preferred and are perceived to be more attractive than flat fruit, which are commonly found in mandarin cultivars and hybrids (Spiegel-Roy and Goldschmidt, 1996).

Fruit shape is therefore another characteristic that needs to be considered by the citrus breeder when evaluating breeding populations. Broad sense heritability estimates for acid lime clones were found to be fairly low for fruit shape at 0.58 (Prasad and Rao, 1989), while peach breeding populations also had a low narrow sense heritability estimate for the fruit shape index at 0.43 (de Sousa and Bryne, 1998).

Fruit size and fruit shape are important characteristics pertaining to citrus fruit and need to taken into consideration when planning controlled crosses for breeding purposes. Therefore the aim of this study was to investigate the genetic variability in fruit size and fruit shape among the progenies of six mandarin families, thereby providing more information on the inheritance patterns of these characters and determining the value of the parents in citrus improvement.

MATERIALS AND METHODS

Unless otherwise stated the materials and methods used in this chapter for the determination of fruit size and fruit shape are the same as used in Chapter 3 for the determination of fruit rind colour.

Selection of families

The six mandarin families, with Kiyomi tangor (*Citrus unshiu* x *Citrus sinensis*) as female parent and Dancy, Hansen, Rishon, Roma, Shani and Sunburst mandarins (*Citrus reticulata*) as male parents, used in Chapter 3 for the determination of fruit rind colour, were used for the determination of fruit size and fruit shape.

The aim of these crosses was to breed new citrus mandarin hybrids with a larger fruit size and a more round fruit shape, in combination with other improved fruit characteristics. Fruit of the female parent Kiyomi are described as being medium to large in size (Saunt, 2000), while male parent Dancy is said to have a tendency to alternate bearing and often produces a heavy crop of small fruit (Saunt, 2000), Rishon and Hansen produce small to medium sized fruit (Patent PP08377, 1993; Maritz and Combrink, 2007), Shani and Sunburst produce medium sized fruit (Patent PP13634, 2003; Futch and Jackson, 2009) and Roma produces medium to large sized fruit (Miller et al., 1996).

With regard to the fruit shape, female parent Kiyomi is described as having fruit with a round to flattish, orange-like shape, while the male parents Dancy, Hansen, Rishon, Roma, Shani and Sunburst are all described as having flattened or oblate fruit (Patent PP08377, 1993; Miller et al., 1996; Saunt, 2000; Patent PP13634, 2003; Maritz and Combrink, 2007, Futch and Jackson, 2009).

Sampling of trees and fruit for evaluation

The trees selected and fruit sampled, for the parents and the families, for evaluation of fruit rind colour in Chapter 3, were used for the collection of fruit size and fruit shape data.

To confirm that a sample size of five fruit per tree, as used in Chapter 3, could be used to determine fruit size, data collected from a pilot study in 2008 (unpublished), from two Kiyomi families, a Kiyomi x Daisy family and a Kiyomi x Fremont family was used. The families each consisted of 75 trees and 15 fruit was sampled from each tree. Data for fruit height and fruit width was collected. This data was used to determine the variance component estimates for the two families. The error variance, which is the variance between the 15 fruit sampled per tree, was determined using 15 fruit, 10 fruit and five fruit (Table 4.1).

Table 4.1 Error variance for a sample size of 15, 10 and 5 fruit from a preliminary study of two citrus families for fruit size

Number of fruit	Fruit height	Fruit width
15	138.43	128.72
10	139.89	128.24
5	134.27	127.20

From Table 4.1 it can be seen that there is no increase in the error variance when decreasing the sample size to five fruit per tree. Therefore a sample of five fruit per tree gave a homogenous sample representative of the tree and could be used for the measurement of fruit size.

Data collection

Fruit sizing in mandarins should be done using the fruit's dimensions rather than the fruit weight, since many mandarins have a problem of puffiness and this can lead to erroneous sizing (Ladaniya, 2008). Fruit size was therefore determined by measuring the fruit height, stem end to stylar end, and fruit width, the equatorial diameter, of each fruit. Measurements were taken with a Mitutoyo, Digimatic CD-6, digital calliper measuring in millimetres up to two decimal places.

A fruit shape index can be determined using fruit height and fruit width measurements. This ratio is calculated by dividing the fruit width by the fruit height (Lima et al., 1992). A ratio of 1.0 indicates a perfectly round fruit, while a ratio of < 1.0 indicates an oval fruit and a ratio of > 1.0 an oblate fruit. Data collected for fruit height and fruit width was used to calculate the fruit shape index of each fruit.

Data analysis

Data was transferred to Microsoft Excel (2003) spreadsheets for analysis and statistical analyses were done by ARC- Biometry, Stellenbosch using SAS/STAT (1999 and 2008), as in Chapter 3 for the determination of fruit rind colour.

RESULTS AND DISCUSSION

ANOVA for the parents (Table 4.2) showed a significant level of variation between the parents and between the years for fruit height, fruit width and fruit shape, and a significant year x parent variation for fruit height and fruit width. The parents showed a larger between parent and between year variation for fruit height than for fruit width, while the year x parent variation was larger for fruit width than for fruit height. The fruit shape showed a considerably smaller between parent, between year and year x parent variation than both fruit height and fruit width.

Table 4.2 ANOVA for fruit size and shape of the seven citrus parents

	Year 1		Year 2		Joint analysis	
	Df	**MS**	**Df**	**MS**	**Df**	**MS**
Fruit height						
Parent	6	264.91**	6	357.44**	6	588.96**
Rep (parent)					14	10.05
Year		-		-	1	289.04**
Year x Parent		-		-	6	33.39*
Error	14	14.98	14	4.66	14	9.59
Fruit width						
Parent	6	189.46**	6	327.40**	6	458.91**
Rep (parent)					14	8.54
Year		-		-	1	131.65**
Year x Parent		-		-	6	57.95**
Error	14	11.48	14	7.47	14	10.41
Fruit shape						
Parent	6	0.02**	6	0.01**	6	0.03**
Rep (parent)					14	<0.01
Year		-		-	1	0.03**
Year x Parent		-		-	6	<0.01
Error	14	<0.01	14	<0.01	14	<0.01

**** P ≤ 0.01, * P ≤ 0.05**

The ANOVA for the families (Table 4.3) showed a significant level of between family and between year variation for fruit height, fruit width and fruit shape. As with the parents, the fruit height for the families showed a slightly larger between family and between year variation than fruit width, while fruit shape showed a considerably smaller between family and between year variation than both fruit height and fruit width. The year x family variation was not significant for fruit height, fruit width or fruit shape.

Table 4.3 ANOVA for fruit size and shape of the six citrus families

	Year 1		Year 2		Joint analysis	
	Df	**MS**	**Df**	**MS**	**Df**	**MS**
Fruit height						
Family	5	1968.00**	5	1212.87**	5	2900.24**
Family (tree)					571	114.98
Year		-		-	1	2966.59**
Year x Family		-		-	5	149.78
Error	531	114.60	560	99.48	520	98.82
Fruit width						
Family	5	1256.02**	5	881.62**	5	1979.88**
Family (tree)					571	114.41
Year		-		-	1	2504.72**
Year x Family		-		-	5	157.03
Error	531	109.60	560	103.81	520	97.79
Fruit shape						
Family	5	0.13**	5	0.08**	5	0.18**
Family (tree)					571	0.01
Year		-		-	1	0.05*
Year x Family		-		-	5	0.02
Error	531	0.01	560	0.01	520	0.01

**** P ≤ 0.01, * P ≤ 0.05**

The Student t test for the parents (Table 4.4) showed that for fruit height, female parent Kiyomi had the highest mean and differed significantly from the male parents. Among the male parents, Rishon had the highest mean for fruit height and differed significantly from Hansen, Shani and Dancy, while Dancy had the lowest mean and differed significantly from Rishon, Roma and Sunburst. The ranking and grouping among the male parents for fruit height varied between year 1 and year 2 as indicated by the significant year x parent variation for fruit height (Table 4.2).

Table 4.4 Means and standard errors for fruit size and shape of the seven citrus parents

Year 1				Year 2				Joint analysis			
Parent	**Mean**	**Group**	**Error**	**Parent**	**Mean**	**Group**	**Error**	**Parent**	**Mean**	**Group**	**Error**
Fruit height											
Kiyomi	76.65	**A**	± 3.68	Kiyomi	74.98	**A**	± 1.62	Kiyomi	75.81	**A**	± 1.84
Roma	57.11	**B**	± 1.65	Rishon	53.76	**B**	± 1.67	Rishon	55.02	**B**	± 1.28
Rishon	56.28	**B**	± 1.94	Sunburst	49.30	**C**	± 0.28	Roma	53.07	**BC**	± 2.02
Sunburst	55.84	**B**	± 0.53	Roma	49.04	**C**	± 1.16	Sunburst	52.56	**BC**	± 1.48
Shani	55.12	**B**	± 1.71	Hansen	47.71	**C**	± 0.88	Hansen	49.55	**CD**	± 1.37
Hansen	51.39	**BC**	± 2.29	Dancy	46.39	**C**	± 1.61	Shani	48.12	**D**	± 3.24
Dancy	46.66	**C**	± 2.55	Shani	41.12	**D**	± 0.84	Dancy	46.52	**D**	± 1.35
LSD (p≤0.05) : 6.78				LSD (p≤0.05) : 3.78				LSD (p≤0.05) : 3.93			
Fruit width											
Kiyomi	84.13	**A**	± 3.10	Kiyomi	86.12	**A**	± 1.65	Kiyomi	85.13	**A**	± 1.63
Sunburst	69.62	**B**	± 0.72	Rishon	63.95	**B**	± 1.98	Sunburst	66.47	**B**	± 1.64
Shani	67.82	**B**	± 1.54	Sunburst	63.31	**B**	± 1.72	Roma	64.37	**BC**	± 1.05
Roma	65.91	**BC**	± 0.70	Roma	62.83	**B**	± 1.64	Rishon	62.52	**CD**	± 1.32
Hansen	64.59	**BC**	± 2.41	Hansen	60.36	**B**	± 1.27	Hansen	62.47	**CD**	± 1.54
Rishon	61.08	**C**	± 1.63	Dancy	60.21	**B**	± 1.63	Dancy	60.47	**D**	± 1.27
Dancy	60.74	**C**	± 2.30	Shani	52.32	**C**	± 0.93	Shani	60.07	**D**	± 3.56
LSD (p≤0.05) : 5.93				LSD (p≤0.05) : 4.77				LSD (p≤0.05) : 3.61			
Fruit shape											
Dancy	1.30	**A**	± 0.02	Dancy	1.30	**A**	± 0.01	Dancy	1.31	**A**	± 0.01
Hansen	1.27	**AB**	± 0.06	Sunburst	1.29	**A**	± 0.03	Hansen	1.27	**AB**	± 0.03
Sunburst	1.25	**AB**	± 0.02	Roma	1.29	**A**	± 0.02	Sunburst	1.27	**AB**	± 0.02
Shani	1.23	**AB**	± 0.02	Shani	1.27	**A**	± 0.01	Shani	1.25	**AB**	± 0.01
Roma	1.16	**BC**	± 0.05	Hansen	1.26	**A**	± 0.02	Roma	1.22	**B**	± 0.04
Kiyomi	1.10	**C**	± 0.02	Rishon	1.19	**B**	± 0.02	Rishon	1.14	**C**	± 0.03
Rishon	1.09	**C**	± 0.04	Kiyomi	1.15	**B**	± 0.01	Kiyomi	1.13	**C**	± 0.01
LSD (p≤0.05) : 0.11				LSD (p≤0.05) : 0.06				LSD (p≤0.05) : 0.06			

Means with the same letter are not significantly different

For the fruit width of the parents (Table 4.4) female parent Kiyomi had the highest mean for fruit width and differed significantly from the male parents. Among the male parents; Sunburst had the highest mean for fruit width and differed significantly from Rishon, Hansen, Dancy and Shani, while Shani had the lowest mean and differed significantly from Sunburst and Roma The ranking and grouping among the male parents for fruit width varied between year 1 and year 2 as indicated by the significant year x parent variation for fruit width (Table 4.2).

For the fruit shape of the parents (Table 4.4) female parent Kiyomi had the lowest mean for fruit shape but did not differ significantly from male parent Rishon. Among the male parents, Dancy had the highest mean for fruit shape and differed significantly from Roma and Rishon, while Rishon had the lowest mean and was significantly different from all of the other male parents. The ranking and grouping for fruit shape did vary between year 1 and year 2 for most of the parents, however the ANOVA did not show a significant year x parent variation for fruit shape (Table 4.2).

The data revealed that female parent Kiyomi had the highest mean for fruit height and fruit width, and the lowest mean for fruit shape. Kiyomi therefore had larger fruit with a more round fruit shape than the male parents. Among the male parents Rishon had the highest mean for fruit height and the lowest mean for fruit shape, while Sunburst had the highest mean for fruit width.

A Student t test for the families (Table 4.5) showed that for fruit height the Kiyomi x Rishon family had the highest mean and differed significantly from all the other families. The Kiyomi x Hansen family had the lowest mean and differed significantly from the Kiyomi x Rishon, Kiyomi x Shani and Kiyomi x Sunburst families. The ranking and grouping among the families for fruit height showed little variation between year 1 and year 2; only the Kiyomi x Roma, Kiyomi x Dancy and Kiyomi x Hansen families' had a change in ranking and the ANOVA's year x family variation for fruit height (Table 4.2) was not significant.

For the fruit width of the families (Table 4.5) the Kiyomi x Rishon family had the highest mean and differed significantly from the other families, while the Kiyomi x Hansen family had the lowest mean and differed significantly from the other families. The ranking and grouping among the families for fruit width showed little variation between year 1 and year 2; only the Kiyomi x Shani, Kiyomi x Sunburst, Kiyomi x Roma and Kiyomi x Dancy families had a change in ranking and the ANOVA's year x family variation (Table 4.2) for fruit width was not significant.

Table 4.5 Means and standard errors for fruit size and shape of the six citrus families

Year 1				Year 2				Joint analysis			
Family	**Mean**	**Grp**	**Error**	**Family**	**Mean**	**Grp**	**Error**	**Family**	**Mean**	**Grp**	**Error**
Fruit height											
Kiyomi x Rishon	68.00	**A**	± 1.53	Kiyomi x Rishon	62.30	**A**	± 1.16	Kiyomi x Rishon	64.80	**A**	± 0.96
Kiyomi x Shani	60.01	**B**	± 1.05	Kiyomi x Shani	56.07	**B**	± 0.97	Kiyomi x Shani	58.05	**B**	± 0.73
Kiyomi x Sunburst	57.97	**B**	± 0.85	Kiyomi x Sunburst	54.67	**BC**	± 0.92	Kiyomi x Sunburst	56.41	**BC**	± 0.64
Kiyomi x Roma	57.82	**B**	± 1.06	Kiyomi x Dancy	54.11	**BC**	± 1.02	Kiyomi x Dancy	55.43	**CD**	± 0.81
Kiyomi x Dancy	56.89	**BC**	± 1.26	Kiyomi x Hansen	53.57	**BC**	± 1.08	Kiyomi x Roma	55.00	**CD**	± 0.72
Kiyomi x Hansen	53.73	**C**	± 1.07	Kiyomi x Roma	52.49	**C**	± 0.91	Kiyomi x Hansen	53.65	**D**	± 0.76
LSD (p≤0.05) : 3.17				LSD (p≤0.05) : 2.86				LSD (p≤0.05) : 2.20			
Fruit width											
Kiyomi x Rishon	73.60	**A**	± 1.25	Kiyomi x Rishon	70.95	**A**	± 0.98	Kiyomi x Rishon	72.18	**A**	± 0.78
Kiyomi x Shani	70.81	**AB**	± 1.07	Kiyomi x Sunburst	66.66	**B**	± 0.97	Kiyomi x Shani	68.35	**B**	± 0.74
Kiyomi x Sunburst	69.86	**B**	± 0.95	Kiyomi x Shani	65.86	**B**	± 0.96	Kiyomi x Sunburst	68.34	**B**	± 0.69
Kiyomi x Roma	69.15	**BC**	± 1.01	Kiyomi x Dancy	64.19	**BC**	± 1.16	Kiyomi x Roma	66.48	**BC**	± 0.74
Kiyomi x Dancy	66.10	**CD**	± 1.26	Kiyomi x Roma	64.11	**BC**	± 1.02	Kiyomi x Dancy	65.10	**C**	± 0.85
Kiyomi x Hansen	63.01	**D**	± 1.09	Kiyomi x Hansen	62.36	**C**	± 1.18	Kiyomi x Hansen	62.68	**D**	± 0.80
LSD (p≤0.05) : 3.10				LSD (p≤0.05) : 2.92				LSD (p≤0.05) : 2.20			
Fruit shape											
Kiyomi x Sunburst	1.22	**A**	± 0.01	Kiyomi x Sunburst	1.23	**A**	± 0.01	Kiyomi x Sunburst	1.22	**A**	± 0.01
Kiyomi x Roma	1.21	**AB**	± 0.01	Kiyomi x Roma	1.23	**A**	± 0.01	Kiyomi x Roma	1.22	**A**	± 0.01
Kiyomi x Shani	1.19	**AB**	± 0.01	Kiyomi x Dancy	1.19	**B**	± 0.01	Kiyomi x Shani	1.19	**B**	± 0.01
Kiyomi x Hansen	1.18	**AB**	± 0.01	Kiyomi x Shani	1.19	**BC**	± 0.01	Kiyomi x Dancy	1.18	**B**	± 0.01
Kiyomi x Dancy	1.17	**B**	± 0.02	Kiyomi x Hansen	1.18	**BC**	± 0.01	Kiyomi x Hansen	1.18	**B**	± 0.01
Kiyomi x Rishon	1.10	**C**	± 0.01	Kiyomi x Rishon	1.16	**C**	± 0.01	Kiyomi x Rishon	1.14	**C**	± 0.01
LSD (p≤0.05) : 0.03				LSD (p≤0.05) : 0.03				LSD (p≤0.05) : 0.02			

Means with the same letter are not significantly different

For fruit shape of the families (Table 4.5) the Kiyomi x Sunburst family had the highest mean and differed significantly from the Kiyomi x Shani, Kiyomi x Dancy, Kiyomi x Hansen and Kiyomi x Rishon families. The Kiyomi x Rishon family had the lowest mean and differed significantly from all the other families. The ranking and grouping among the families for fruit shape showed little variation between year 1 and year 2; only the Kiyomi x Shani, Kiyomi x Hansen and Kiyomi x Dancy families had a change in ranking and the ANOVA's year x family variation for fruit shape (Table 4.2) was not significant

The data revealed that the Kiyomi x Rishon family had the highest mean for fruit height and fruit width and the lowest mean for fruit shape, indicating a population with a larger fruit size and a rounder fruit shape than the other families.

Variance components determined, for year 1 and year 2 separately, for fruit height, fruit width and fruit shape followed the same trend over the two years (Table 4.6). The variation within the families was greater than between the families for fruit height, fruit width and fruit shape, indicating a high level of genetic variation within the families. The between family variation, expressed as a percentage of the total variation, was 14% and 9% for height and 9% and 6% for width, for year 1 and year 2 respectively, and 5% for shape for both years, while the within family variation was 69% and 71% for height, 72% and 74% for width and 55% and 55% for shape. The within tree variation (error variance) was lower than the within family variation for fruit height and fruit width and showed that a sample of five fruit per tree was sufficient. Expressed as a percentage of the total variation the within tree variation was 18% and 21% for height and 19% and 20% for width, for year 1 and year 2 respectively. Fruit shape however, did not show a large difference between the within tree and within family variation; with a within tree variation of 37% and 35% of the total variation, for year 1 and year 2 respectively. Therefore, for fruit shape a larger within tree sample should be used to decrease the error variation.

The between family, within family and within tree variation was very low for fruit shape and much lower than for fruit height and fruit width (Table 4.6), this can be seen in the low mean squares for fruit shape in the ANOVA (Table 4.3). The between family variation for fruit height and fruit width was larger in year 1 than year 2, at 14% for year 1 and 9% for year 2 for fruit height and 9% for year 1 and 6% for year 2 for fruit width, expressed as a percentage of the total variation. This can be seen in the ANOVA's larger mean square for family in year 1 compared to year 2 for fruit height and width (Table 4.3). The Kiyomi x Roma and the Kiyomi x Sunburst families had a lower total variation for fruit height and fruit width showing less variation than the other families, while the Kiyomi x Rishon family had a higher total variation for fruit height and fruit shape showing more variation (Table 4.6).

Table 4.6 Variance components and repeatability for fruit size and shape of the six citrus families

	Source of variation								Repeatability (between trees in a family)	
	Between families		Within families (between trees)		Within trees		Total			
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Fruit height	21.89	11.40	108.92	93.90	28.11	27.29	158.93	132.60		
Kiyomi x Dancy			123.90	88.91	30.48	26.61	154.38	115.52	0.80	0.77
Kiyomi x Hansen			110.26	112.86	18.90	20.04	129.16	132.90	0.85	0.85
Kiyomi x Rishon			175.69	127.68	47.75	43.84	223.44	171.52	0.79	0.74
Kiyomi x Roma			80.49	65.22	20.66	24.20	101.14	89.43	0.80	0.73
Kiyomi x Shani			106.43	89.01	26.96	25.27	133.40	114.28	0.80	0.78
Kiyomi x Sunburst			66.59	72.19	27.27	23.24	93.86	95.43	0.71	0.76
Fruit width	12.63	7.88	103.87	98.25	28.01	27.17	144.50	133.30		
Kiyomi x Dancy			124.86	115.30	29.09	30.52	153.96	145.82	0.81	0.79
Kiyomi x Hansen			115.52	134.31	19.54	18.39	135.07	152.70	0.88	0.88
Kiyomi x Rishon			113.76	89.86	44.51	34.41	158.27	124.28	0.72	0.72
Kiyomi x Roma			71.52	81.69	23.45	25.83	94.97	107.52	0.75	0.76
Kiyomi x Shani			110.12	86.04	26.42	27.65	136.55	113.69	0.81	0.76
Kiyomi x Sunburst			85.11	79.23	28.08	26.39	113.19	105.63	0.75	0.75
Fruit shape	0.001	0.001	0.010	0.011	0.007	0.007	0.019	0.020		
Kiyomi x Dancy			0.009	0.009	0.010	0.007	0.019	0.015	0.47	0.57
Kiyomi x Hansen			0.009	0.010	0.006	0.007	0.016	0.017	0.59	0.58
Kiyomi x Rishon			0.013	0.019	0.009	0.009	0.022	0.028	0.59	0.67
Kiyomi x Roma			0.006	0.009	0.007	0.007	0.013	0.015	0.48	0.56
Kiyomi x Shani			0.010	0.011	0.006	0.006	0.016	0.017	0.70	0.63
Kiyomi x Sunburst			0.011	0.012	0.008	0.007	0.019	0.020	0.59	0.62

The repeatability, calculated between trees within a family (Table 4.6), was high for all families for fruit height and fruit width. Repeatability estimates ranged from 0.71 to 0.85 for height and 0.72 to 0.88 for width. The repeatability estimate in this case was used to determine the gain in accuracy expected from multiple measurements within a tree. The high repeatability estimate, therefore, indicates that for fruit height and fruit width only a small gain in accuracy would be attained by increasing the number of fruit sampled per tree (Falconer and Mackay, 1996). The repeatability for fruit shape was however lower ranging from 0.47 to 0.70 and therefore a worthwhile gain in accuracy could be attained by increasing the number of measurements per tree.

Variance components determined from the joint analysis (Table 4.7) allowed for the year and year x family contribution to the total variance to be calculated.

The between family variation was again found to be lower than the within family variation for fruit height, fruit width and fruit shape. Expressed as a percentage of the total variation, the between family variation was 9% for height, 6% for width and 4% for shape, while the within family variation was 35% for height, 37% for width and 29% for shape. The variation between the years and the year x family variation were both found to be low and were less than within family variation for fruit height, fruit width and fruit shape. Expressed as a percentage of the total variation, the year variation was 4% for height, 3% for width and 1% for shape, while the year x family variation was 1% for height, width and shape.

Intraclass correlation coefficients were determined; relevant to selection between families (t_1) and relevant to selection between trees within a family (t_2) (Table 4.7). Intraclass correlation coefficient t_1 was very low at 0.08 for height, 0.06 for width and 0.04 for shape indicating very little variation between the families. Intraclass correlation coefficient t_2 was fairly low at 0.41 for height and width and 0.30 for shape. The intraclass correlation coefficient sets an upper limit to broad sense heritability for the traits studied (de Souza and Byrne, 1998). Therefore, the low value for t_2 indicates that the variation found within the families was only partly genetic and non-genetic factors (environment) also played a role.

Table 4.7 Variance components and intraclass correlation coefficients for fruit size and shape of the six citrus families

	Source of variation						Intraclass correlation coefficients	
	Between families	**Within families**	**Year**	**Year x Family**	**Error**	**Total**	**t1**	**t2**
Fruit height	12.86	52.68	5.73	1.45	76.83	149.56	0.08	0.41
Fruit width	8.49	52.52	4.59	1.42	75.45	142.48	0.06	0.41
Fruit shape	0.0007	0.0056	0.0001	0.0001	0.0128	0.0194	0.04	0.30

Distribution curves were drawn up for the six families for fruit height, fruit width and fruit shape. The parent values were included on each curve to allow the progeny to be compared to the parents (Figure 4.1 – 4.3).

All families showed a continuous distribution for fruit height indicating a quantitative expression of the trait (Figure 4.1). The curves followed a normal distribution, with a p-value > 0.05, except for the Kiyomi x Hansen curves, the Kiyomi x Rishon and Kiyomi x Roma curves in year 1 and the Kiyomi x Sunburst curve in year 2, which were skewed towards lower fruit height values. The Kiyomi x Roma and Kiyomi x Sunburst families had a narrower distribution showing less within family variation, while the Kiyomi x Rishon family in year 1 had a wider distribution showing more within family variation. This can be seen in the variance component analysis (Table 4.6) where Kiyomi x Roma and Kiyomi x Sunburst had a lower between tree variance and the Kiyomi x Rishon family for year 1 a larger between tree variance than the other families for fruit height.

For fruit height the Kiyomi x Dancy and Kiyomi x Rishon families and the Kiyomi x Shani family in year 2 had means between the two parents and the Kiyomi x Hansen, Kiyomi x Roma and Kiyomi x Sunburst families and the Kiyomi x Shani family in year 1 had means close or equal to the male parent (Figure 4.1). Therefore the families with male parents Dancy, Rishon and Shani showed an increase in fruit height over the male parent, while the Kiyomi x Hansen, Kiyomi x Roma and Kiyomi x Sunburst families and the Kiyomi x Shani family in year 1 showed the male parent to be more dominant. However, all families did show heterosis and contained some individuals with larger fruit height values than both of the parents. The Kiyomi x Rishon family had the highest mean for fruit height (Table 4.5) and was significantly higher than all the other families.

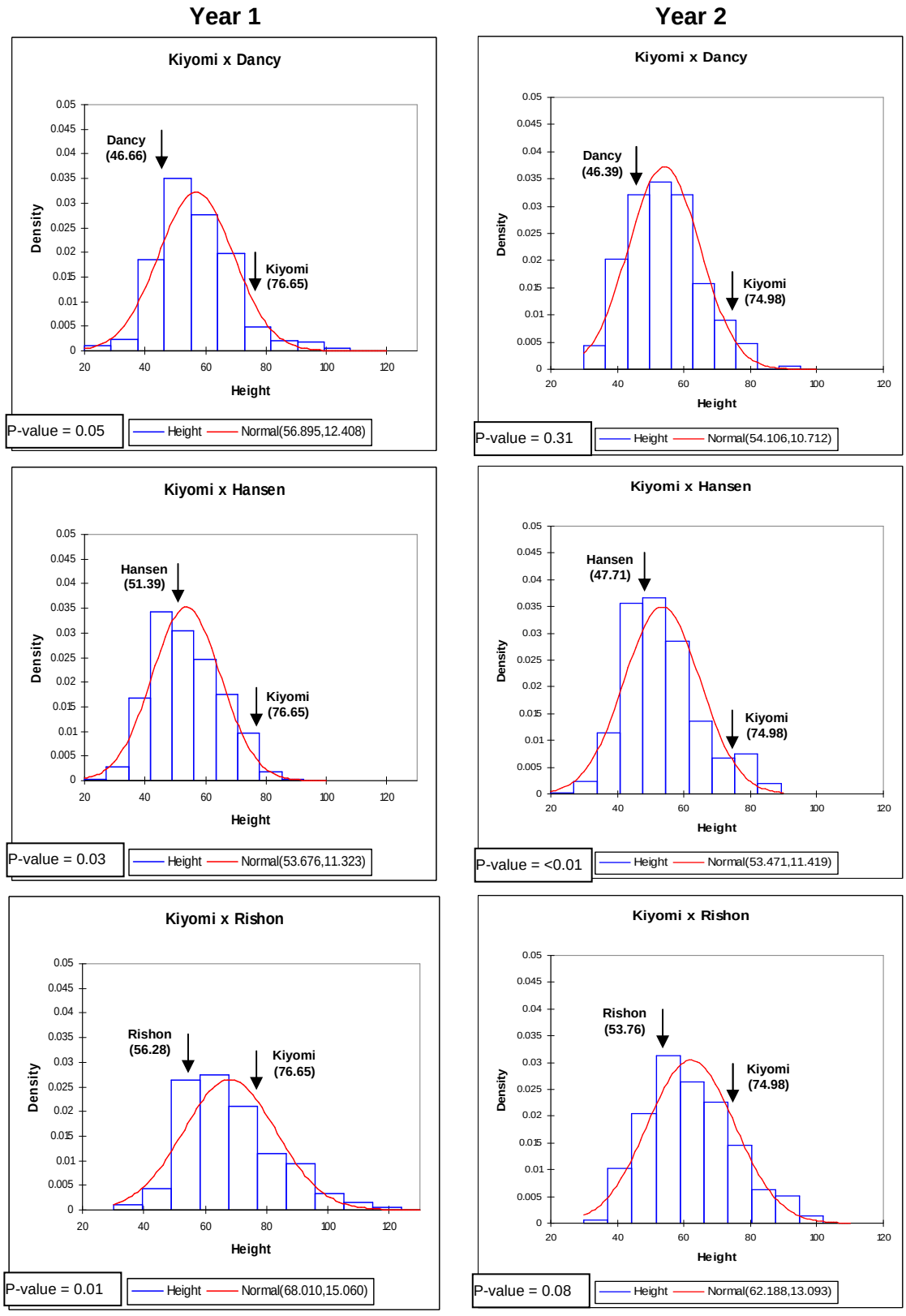

Figure 4.1 Distribution curves for the six citrus families for fruit height

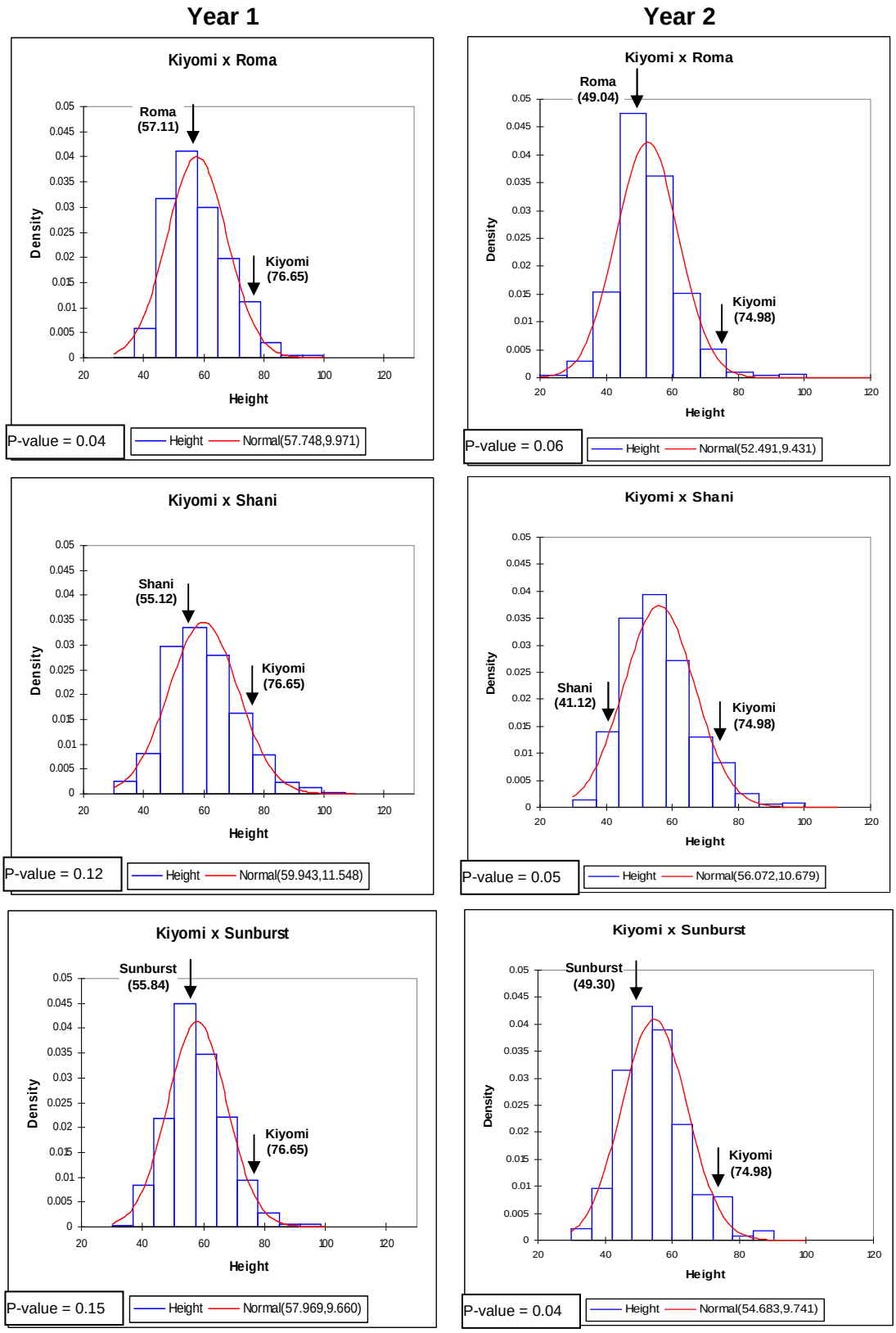

Figure 4.1 (cont.). Distribution curves for the six citrus families for fruit height

When comparing the year 1 and year 2 distribution curves for fruit height (Figure 4.1) the families showed similar trends over the two years except for the Kiyomi x Shani family; however the parent value for Shani differed considerably between the years (Table 4.4). The differences between the years can be attributed to the significant year variation for the families (Table 4.3) as well as the significant year and year x parent variation for the parents (Table 4.2).

All families showed a continuous distribution for fruit width indicating a quantitative expression of the trait (Figure 4.2). The curves followed a normal distribution, with a p-value > 0.05, except for the Kiyomi x Hansen curve in year 2 and the Kiyomi x Roma curve in year 1, which were skewed towards lower fruit width values. The Kiyomi x Roma and Kiyomi x Sunburst families had a narrower distribution showing less within family variation, as seen in the variance component analysis (Table 4.6) where Kiyomi x Roma and Kiyomi x Sunburst had a lower between tree variation than the other families.

For fruit width the Kiyomi x Dancy, Kiyomi x Hansen, Kiyomi x Roma, Kiyomi x Sunburst families and the Kiyomi x Shani family for year 1, had means close or equal to the male parent. The Kiyomi x Rishon family and the Kiyomi x Shani family for year 2 had means between the two parents (Figure 4.2). Therefore only the Kiyomi x Rishon family and the Kyomi x Shani family for year 2 showed an increase in fruit width over the male parent, while the Kiyomi x Dancy, Kiyomi x Hansen, Kiyomi x Roma, Kiyomi x Sunburst families and the Kiyomi x Shani family for year 1 showed the male parent to be more dominant. However all families did show heterosis and contained some individuals with larger fruit width values than both of the parents. The Kiyomi x Rishon family had the highest mean for fruit width (Table 4.5) and differed significantly from the other families.

When comparing the year 1 and year 2 distribution curves for fruit width (Figure 4.2) it could be seen that the families generally showed similar trends over the two years except for the Kiyomi x Shani family, however the parent value for Shani differed considerably between the years (Table 4.4). The differences between the years can be attributed to the significant year variation for the families (Table 4.3) as well as the significant year and year x parent variation for the parents (Table 4.2).

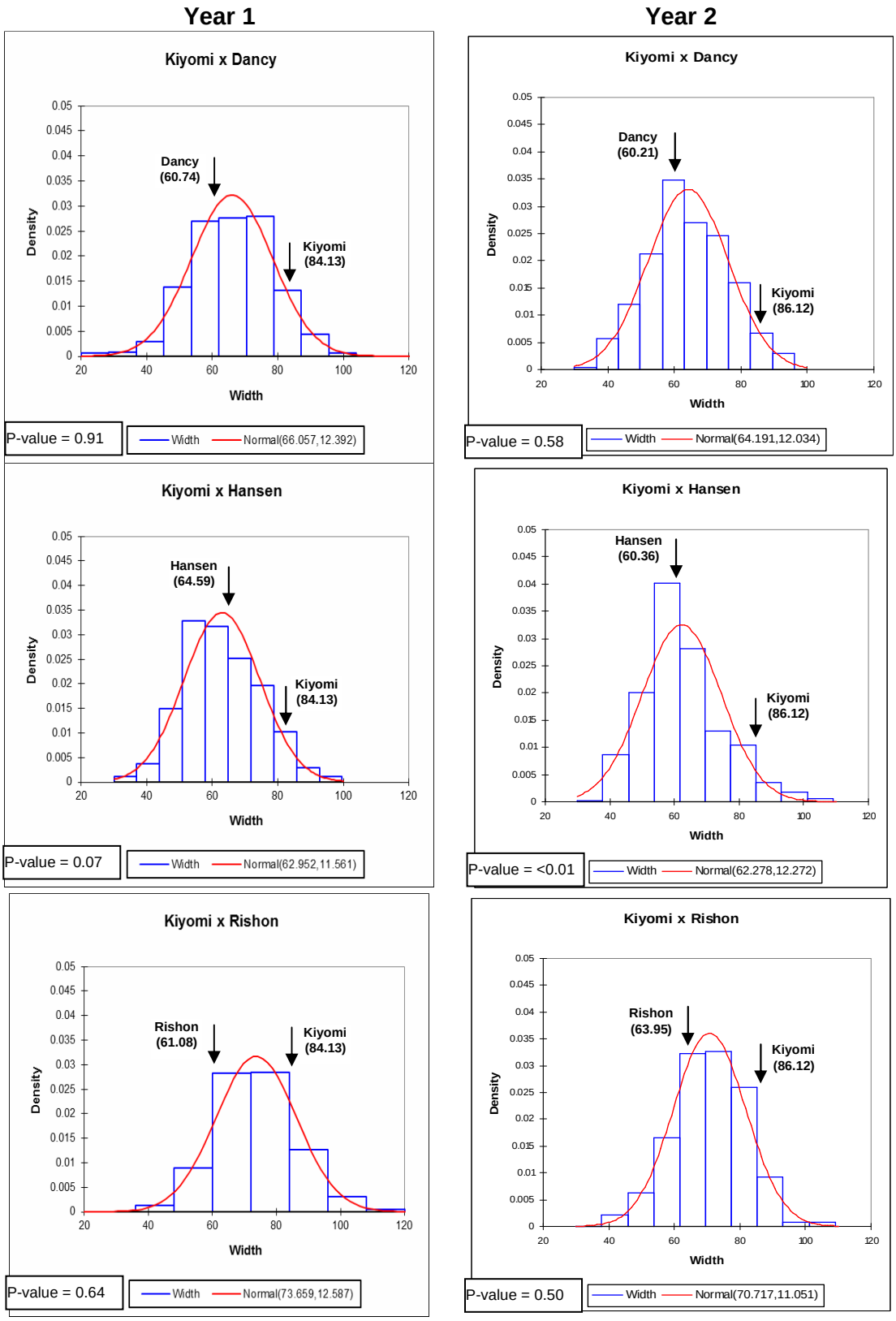

Figure 4.2 Distribution curves for the six citrus families for fruit width

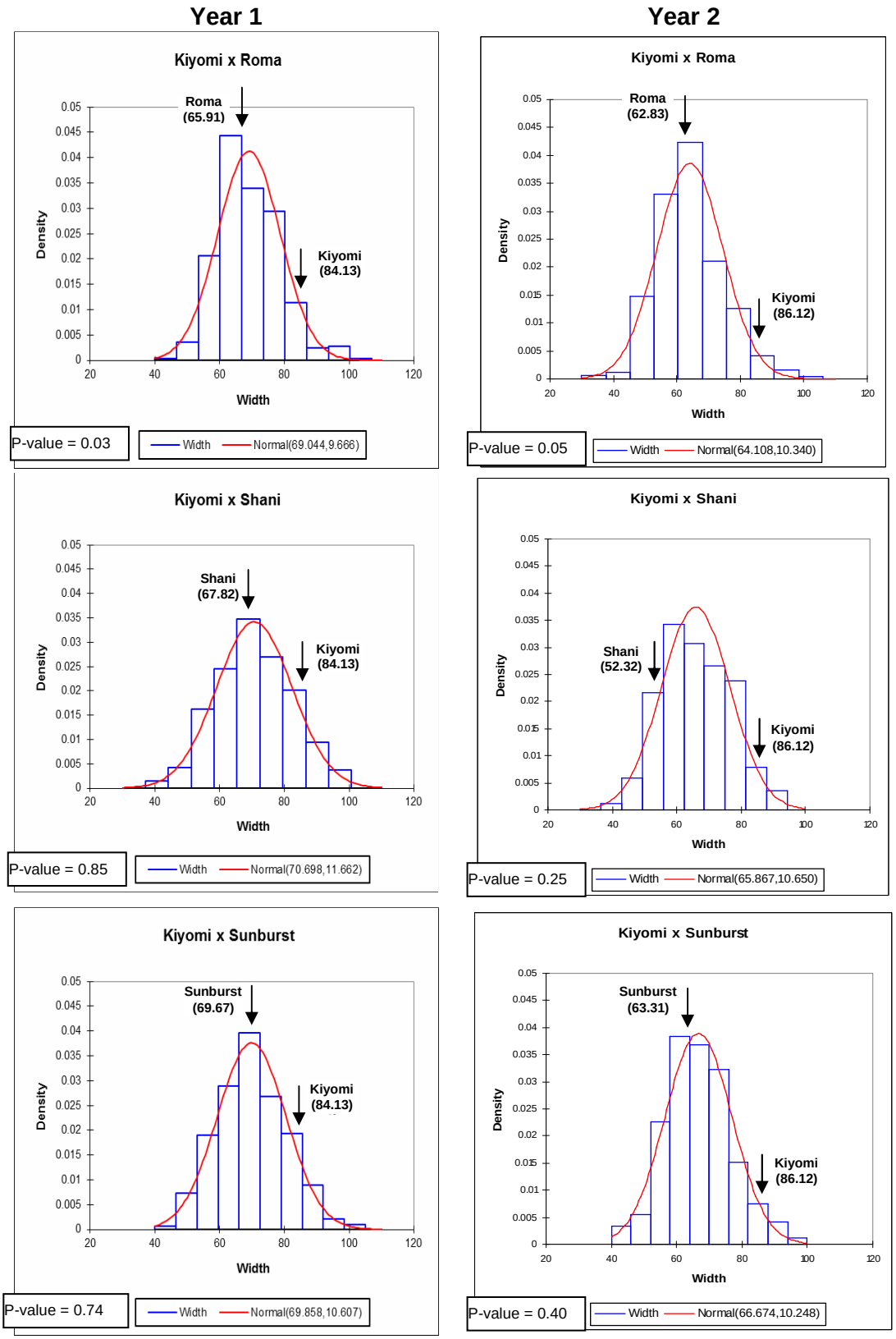

Figure 4.2 (cont.). Distribution curves for the six citrus families for fruit width

All families showed a continuous distribution for fruit shape, indicating a quantitative expression of the trait (Figure 4.3). The curves followed a normal distribution, with a p-value > 0.05, except for the Kiyomi x Sunburst curve in year 1, which was skewed towards lower fruit shape values. The Kiyomi x Rishon family had a wider distribution showing more within family variation, while the Kiyomi x Roma family in year 1 had a narrower distribution showing less within family variation. This could be seen in the variance component analysis (Table 4.6) where the Kiyomi x Rishon family had a larger between tree variance and the Kiyomi x Roma family, in year 1, had a lower between tree variance than the other families.

For fruit shape the Kiyomi x Dancy, Kiyomi x Hansen, Kiyomi x Shani and Kiyomi x Sunburst families and the Kiyomi x Roma family for year 2, had means between the two parents while the Kiyomi x Roma family in year 1 had a mean larger then both parents. The Kiyomi x Rishon family had a mean equal to both the parents in year 1, where both parent means and the family mean were equal, and a mean close to both parents in year 2, where the two parent means and the family mean were very close (Figure 4.3). Therefore all the families, except for the Kiyomi x Roma family in year 1, showed an improvement in the fruit shape index over the male parent. All families however showed heterosis and contained some individuals with a fruit shape index closer to 1.0 than both the parents. The Kiyomi x Rishon family had the lowest mean for fruit shape (Table 4.5) and was significantly lower than the other families.

When comparing the year 1 and year 2 distribution curves for fruit shape (Figure 4.3) it could be seen that the families generally showed similar trends over the two years, except for the Kiyomi x Rishon and Kiyomi x Roma families. The differences between the years can be attributed to the significant year variation for the families (Table 4.3) as well as the significant year variation for the parents (Table 4.2).

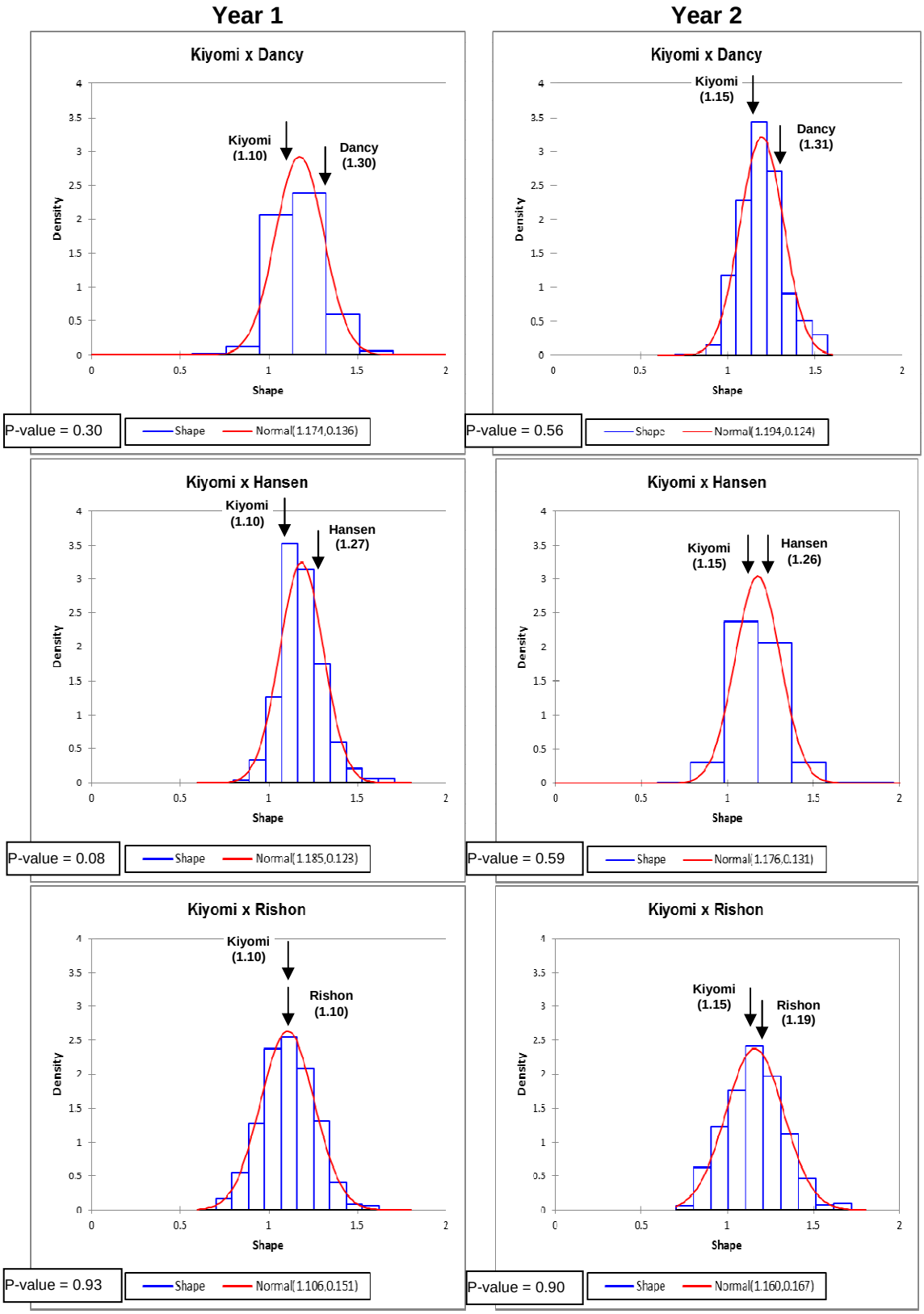

Figure 4.3 Distribution curves for the six citrus families for fruit shape

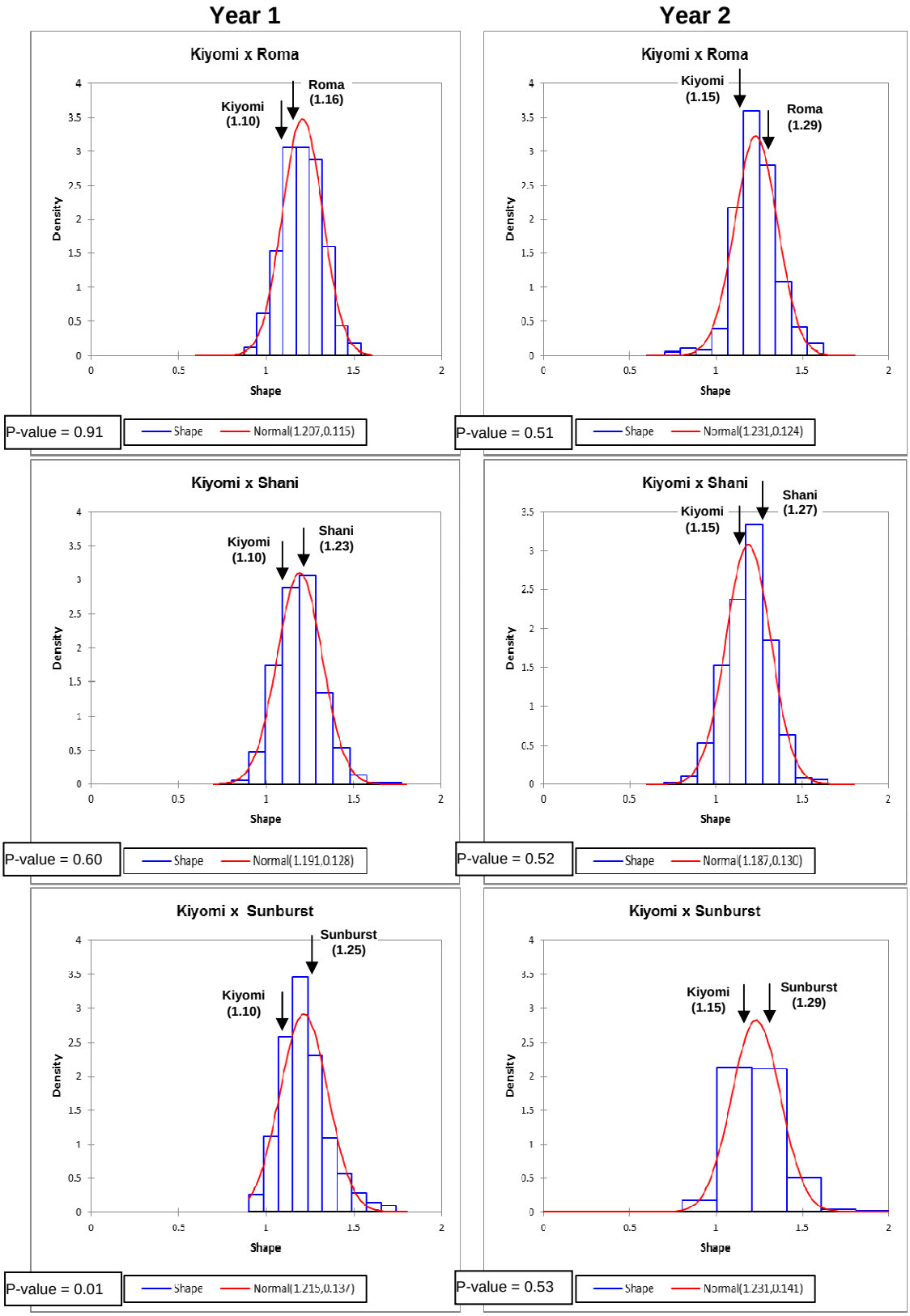

Figure 4.3 (cont.). Distribution curves for the six citrus families for fruit shape

Correlations for year 1 and year 2 showed the same trend, with a positive correlation between fruit height and fruit width (Table 4.8), indicating that as fruit height increases so does the fruit width. Therefore a constant fruit shape index is maintained within a variety and for fruit size measurements only one of these characteristics need be measured.

Table 4.8 Pearson correlation coefficients for fruit height, fruit width and fruit shape of the six citrus families

	Year 1			Year 2		
	Fruit height	**Fruit width**	**Fruit shape**	**Fruit height**	**Fruit width**	**Fruit shape**
Fruit height		0.85**	-0.53**		0.83**	-0.48**
Fruit width	0.85**		-0.02	0.83**		0.08
Fruit shape	-0.53**	-0.02		-0.48**	0.08	

****P ≤ 0.01**

CONCLUSIONS

Fruit size and fruit shape are important characteristics to be considered when evaluating citrus breeding populations as they contribute to the fruit's appearance and influence the fruit quality and marketability (Ladaniya, 2008). There is, however, very little information available on the inheritance of fruit size and fruit shape in citrus to aid the breeder in breeding parent selection and the planning of controlled crosses. In this study six mandarin families, where the female parent Kiyomi tangor was crossed with various male parents, were studied. Fruit size and fruit shape data were collected and used to quantify the genetic variability within and between the families over two years as well as study the genetic relationships between the hybrids and the parents.

The study revealed a significant level of between parent and between family variation for fruit height, fruit width and fruit shape. The variation in fruit size and fruit shape in citrus is, therefore, measurable and can be further explored for breeding purposes. Overall fruit shape showed a considerably lower variation than fruit size; however fruit shape is an index and therefore cannot be compared as such to the fruit size measurements.

The variance component analysis showed a greater variation within the families than between the families, indicating a high level of genetic variation within the families. Citrus cultivars are highly heterozygous and crosses produce progeny displaying a large variability in fruit characteristics (Ray, 2002). The within tree variation was lower than the within family variation for fruit height and width and showed that a sample size as small as five fruit per tree can be used as a homogenous sample for these measurements. The fruit shape, however did not show a large difference between the within tree and within family variation and a larger within tree sample should therefore be used for measuring fruit shape. A high repeatability was found for fruit height and fruit width indicating that only a small gain in accuracy would be attained by increasing the number of fruit sampled per tree, while a lower repeatability was found for fruit shape and indicated that a worthwhile gain in accuracy could be attained by increasing the number of measurements per tree.

Both the parents and the families had a significant year variation for fruit height, fruit width and fruit shape, while the parents had a significant year x parent variation for fruit height and fruit width. However, the variance component analysis showed that the year and the year x family variation contributed little to the total variation. Variation in fruit size between years occurs in mandarins and has been found for fruit height and fruit width measurements of mandarin clones (Lima et al. 1981 and 1992).

The intraclass correlation coefficient t_2 was found to be fairly low for fruit height, fruit width and fruit shape and indicated that the variation found within the families was only partly genetic and that non-genetic factors also contributed to the variation in the phenotype. Fairly low broad sense heritability estimates were also found for fruit height and fruit width measurements in acid lime clones (Prasad and Rao, 1989), while similarly low narrow sense heritability estimates were found for fruit height and fruit width in breeding populations of peach (de Sousa and Bryne, 1998). Therefore, the fairly low value found for intraclass correlation coefficient t_2 combined with the alternate bearing tendency of many mandarins (Saunt, 2000; Verreynne and Lovatt, 2009), as was seen for the male parent Shani, indicates that only two years of testing will not be reliable and a mean performance over multiple years data should be used for effective selection.

The correlation analysis showed a positive correlation between fruit height and fruit width, therefore for fruit size measurements only one of these characteristics need be measured. This is already practised in many packhouses where flatter shaped mandarin fruit are sized and graded using only the fruit's equatorial diameter (width) measurement (Ladaniya, 2008).

Fruit height, fruit width and fruit shape are quantitative genetic traits and gave curves with a continuous distribution. The Kiyomi x Dancy, Kiyomi x Rishon and Kiyomi x Shani families had an increase in fruit height over the male parent, while the Kiyomi x Rishon and Kiyomi x Shani families had an increase in fruit width over the male parent, and all of the families showed an improvement in the fruit shape index over the male parent. Heterosis does occur in citrus and all families contained some individuals with a fruit height, fruit width and fruit shape index superior to both parents.

The Kiyomi x Rishon family had the highest mean for fruit height and fruit width and therefore a larger fruit size than the other families, while male parent Rishon had the highest mean among the male parents for fruit height. The Kiyomi x Rishon family had the lowest mean for fruit shape and therefore more round fruit than the other families, while male parent Rishon had the lowest mean among the male parents for fruit shape. Therefore male parent Rishon showed a greater improvement in the population, giving fruit with a larger size and rounder shape, when crossed with female parent Kiyomi.

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CHAPTER 5

GENOTYPIC VARIATION OF THE INTERNAL FRUIT QUALITY IN KIYOMI FAMILIES

INTRODUCTION

Fresh mandarin fruit have a pleasant desirable flavour which makes them popular among consumers (Ladaniya, 2008). The main flavour attributes in mandarins are sweetness and sourness, the sweetness is due to the presence of sugars and the sourness is due to the presence of acids in the juice (Tietel et. al., 2011). Mature fruit have a fine balance between the sugars and the acids and this creates the mandarins' appealing flavour (Ladaniya, 2008). A fruit's flavour can be associated with its internal quality, which is depicted by the total soluble solids (TSS) (sugars) and acid content as well as the ratio between the TSS and the acid (Van Rensburg, 1985).

The development and maturity of a citrus fruit takes about six to 18 months and as previously mentioned can be divided into three main stages (Ladaniya, 2008). The first stage consists of intense cell division over about nine weeks. However it is during the second stage, which is cell enlargement over about 30 weeks, that there is a rapid growth in the pulp, the juice sacks fill with juice and the sugar and acid levels begin to rise (Spiegel-Roy and Goldschmidt, 1996; Bijzet, 2006). The third and final stage is when the fruit reach horticultural maturity over 11 weeks (Bijzet, 2006) and while the sugars continue to increase the acid levels begin to decrease (Ladaniya, 2008). The ratio between the TSS and the acid is extremely sensitive and is therefore used as a maturity index and can give an indication of the time of harvest (Spiegel-Roy and Goldschmidt, 1996). The time of harvest is critical to the mandarins' flavour and fruit harvested too early will have excessive acid while fruit harvested too late will lack acid and be bland and tasteless (Tietel et. al., 2011). The change in acid therefore sets a limit to the delay of harvest (Spiegel-Roy and Goldschmidt, 1996), as most mandarins lose their internal quality if they are not picked as soon as they reach internal maturity (Ladaniya, 2008).

The TSS contributes to the pleasant flavour of citrus fruit and is one of the most important aspects when determining the fruit's eating quality (Van Rensburg, 1985). The TSS constitutes mainly sugars while the rest is made up of citric and other acids and their salts, nitrogenous compounds and other minor soluble substances such as water-soluble vitamins (Ladaniya, 2008). The sugar content varies widely between different varieties and mandarin cultivars generally contain 80 to 85% sugar (Spiegel-Roy and Goldschmidt, 1996). The sugars consist of sucrose, glucose and fructose and in mandarins they are distributed in a ratio of 2:1:1 (Ladaniya, 2008).

Leaves are the main source of nutrition for the growing fruit and sugars are transferred from the leaves to the juice cells of the fruit (Ladaniya, 2008). Sugars are transferred in the form of glucose and fructose and are converted to sucrose in the fruit (Spiegel-Roy and Goldschmidt, 1996). The juice sacks are also capable of synthesising sucrose (Ladaniya, 2008). Sucrose levels increase markedly towards maturation and some mandarins can reach sugar levels of 15 to 18% of the fresh weight (Spiegel-Roy and Goldschmidt, 1996).

The acid content is another important characteristic influencing the eating quality of citrus fruit and a consumer will identify a high acid content before any other characteristic when tasting a fruit (Van Rensburg, 1985). However while many consumers dislike fruit with high acid, a lack of acid can cause the fruit to have a flat taste (Ladaniya, 2008). Citrus fruits are classified as acid fruits since their soluble solids are composed mainly of organic acids and sugars and, even though organic acids are minor components, they make an important contribution to the fruit's flavour. The main acids in citrus juice are citric and malic acids; however, traces of tartaric, benzoic, oxalic and succinic acids have also been reported (Karadeniz, 2004). Citric acid is the predominant acid and accounts for 80 to 95% of the total acids in most citrus fruit (Ladaniya, 2008). Citrus species, however, vary greatly in their acid content and even cultivars of the same species can show differences (Spiegel-Roy and Goldschmidt, 1996).

Organic acids are found dissolved in the cell sap moving from the roots of a tree to the fruit. Acids are also produced in the leaves and the juice vesicles in the fruit can also synthesise acids (Ladaniya, 2008). The acid content of citrus fruit peaks at the middle of the growing period and then slowly decreases towards maturity (Spiegel-Roy and Goldschmidt, 1996). Organic acids are an important source of energy in plant cells and some of the acid is used by the TCA (tri-carboxylic acid) cycle to provide energy for fruit growth and maturation (Ladaniya, 2008). As the fruit mature the sugars and water continue to accumulate in the juice cells and this also dilutes the acid, contributing to the decline (Kimball, 1999).

The TSS to acid ratio is an important determination of the eating quality of citrus fruit and is used to determine the fruit's maturity (Van Rensburg, 1985). Immature fruit have low TSS content and a high acid, as the fruit mature the TSS increase and the acid level drops, bringing about an increase in the ratio (Van Rensburg, 1985). There is, however, a limit to this ratio and fruit with ratios higher than 19 to 20 have a flavour that is sweet but flat due to the low acid content. Therefore the TSS:acid ratio only shows a clear picture when presented together with the TSS % (Ladaniya, 2008). A TSS:acid ratio of at least 14 is considered necessary for good eating quality in mandarins; however this varies between consumers and certain markets do prefer slightly sour fruit (Ladaniya, 2008). In South Africa mandarins are accepted for packing at a TSS:acid ratio of 8.0 or higher, as long as the TSS is above 9.5% and the acid content is between 0.7 and 1.5% (SRCC, 2008).

The internal quality of citrus fruit is affected by climatic conditions, the soil type, water availability, cultural practices and the nutrient supply (Ladaniya, 2008). Water availability has an effect on the acid content and large amounts of water, from either irrigation or rain, can cause acid levels to drop (Bijzet, 2006). Mineral nutrition also affects the fruit's internal quality and excess potassium increases the fruit's acid and reduces the TSS:acid ratio (Ladaniya, 2008). Rootstocks have the ability to improve the fruit quality of the scion and the choice of rootstock can influence both the TSS and acid content of the fruit (Koekemoer, 2006).

The climate, however, has the largest influence on the quality of citrus fruit. In hot tropical climates fruit grow and mature faster due to the high heat units (Ladaniya, 2008). The fruit have a high TSS but the acids decline very quickly, which can result in a poor eating quality and the fruit also remain marketable for only a short period of time where after they deteriorate very rapidly (Spiegel-Roy and Goldschmidt, 1996). In cooler subtropical areas the lower heat units result in a slower growth and the fruit not only mature later but also over a longer period of time (Bijzet, 2006). The fruit have a lower TSS and a higher acid than those produced in tropical climates. The climate most suitable for growing the best quality citrus fruit is a Mediterranean type climate, with low rainfall, hot summers and cool, wet winters at fruit maturity (Ladaniya, 2008).

The market demand is for high quality mandarin fruit with a good flavour. The main factor affecting a mandarin's flavour is its genetic background (Tietel et. al., 2011); therefore in a citrus breeding programme it is essential to select and breed using parents with a superior flavour that can be transmitted to the progeny. Unfortunately there is very little information on inheritance of fruit characteristics in citrus and no specific information could be found on the inheritance of fruit quality in mandarin breeding populations.

Some work, has, however been done on the genetic variability of fruit internal quality among mandarin clones. The repeatability in time, over years, and space, between clones, was determined for the TSS, acid percentage and the TSS:acid ratio (Lima et al., 1981 and 1992). The repeatability in time was very low for TSS at 0.12 and slightly higher for acid percentage at 0.31, while the repeatability for the TSS:acid ratio was fairly high at 0.69. The repeatability in space was slightly higher for TSS at 0.38, while the acid percentage was 0.29 and the repeatability for the TSS:acid ratio was again high at 0.73 (Lima et al., 1992). Both the TSS and the acid percentage showed a variation between years and between clones, while the variation in the TSS:acid ratio was fairly small. Broad sense heritability estimates for acid lime (*C. aurantifolia* Swing) clones were, however, found to be fairly high for TSS and acid percentage at 0.85 and 0.80 respectively, while the broad sense heritability for the TSS:acid ratio was lower at 0.51 (Prasad and Rao, 1989). Literature on heritability estimates were found for fruit tree breeding populations of peach (*Prunus persica* (L.) Batsch) and revealed fairly low estimates of narrow sense heritability for TSS and titratable acid at 0.33 and 0.31 respectively (de Sousa and Bryne, 1998).

Therefore the aim of this study was to investigate the genetic variability in the fruit's internal quality, with respect to Brix %, acid percentage and the Brix:acid ratio, among the progenies of six mandarin families. This will provide more information on the inheritance patterns of this characteristic and determining the value of the parents used as breeding parents in citrus improvement.

MATERIALS AND METHODS

Unless otherwise stated, the materials and methods used in this chapter for the determination of the fruit's internal quality are the same as used in Chapter 3 for the determination of fruit rind colour.

Selection of families

The six mandarin families, with Kiyomi tangor (*Citrus unshiu x Citrus sinensis*) as female parent and Dancy, Hansen, Rishon, Roma, Shani and Sunburst mandarins (*Citrus reticulata*) as male parents, used in Chapter 3 for the determination of fruit rind colour, were used to determine the fruits internal quality.

The aim of these crosses was to breed new citrus mandarin hybrids with an improved flavour, in combination with other improved fruit characteristics. Fruit of the female parent Kiyomi are described as having a sweet navel like flavour and maintaining a high acid until late in the season. Male parent Dancy is described as being sweet with enough acid to give a well balanced flavour (Saunt, 2000), while Hansen, Rishon, Roma, Shani and Sunburst are all described as having pleasant or appealing flavours (Patent PP08377, 1993; Miller et al., 1996; Patent PP13634, 2003; Maritz and Combrink, 2007; Futch and Jackson, 2009).

Sampling of trees and fruit for evaluation

The trees selected for the parents and the families, for evaluation of fruit rind colour in Chapter 3, were used to sample fruit for internal quality tests.

As mandarin fruit mature on the tree, there is a constant change in the TSS and acid levels; therefore in order to compare the families with regard to the fruit's internal quality all the families together with the parents had to be sampled and tested at the same time. Fruit samples for the internal quality tests in the first year were picked when the common parent, female parent Kiyomi's acid level had dropped to approximately 1.00 and was considered to be internally mature (Ladaniya, 2008). Samples in the second year were then taken at as close as possible a date to year one. Samples were picked the week of 3 September in 2009 and the week of 6 September in 2010. A sample of five fruit was picked from each of the progeny trees, randomly from all areas of the tree. In the same way a sample of five fruit was picked from the parent trees.

Due to the time required for the fruit's internal quality tests and the fact that a large amount of samples had to be processed within a limited time frame, the internal quality of each fruit could not be determined separately. Therefore the sample of five fruit from each tree was split into two samples of three and two fruit respectively, allowing for two measurements per tree.

Data collection

The fruit were cut open and the juice extracted, the juice was then used to determine the TSS (Brix) and titratable acid as well as to calculate the Brix:acid ratio.

The TSS in fruit juice is measured by determining the juice's refractive index using a refractometer (Ladaniya, 2008). Refractometers are calibrated to give TSS % or °Brix readings directly. °Brix gives the amount of sugar in a solution and since 1 degree Brix is equivalent to 1 g sugar in 100 g solution Brix can be expressed as a percentage sugar by weight (Ladaniya, 2008). The Brix scale is based on sucrose and water; however most samples also contain other soluble substances and therefore the Brix % represents the total concentration of all soluble solids in the sample. The density of fruit juice, and therefore its refractive index, varies with temperature, making it necessary to correct Brix readings for temperature (Van Rensburg, 1985). However, currently most digital hand held refractometers have a built in mechanism that automatically corrects for temperature (Ladaniya, 2008). The pocket refractometer Pal-1, Atago, Tokyo, with an automatic temperature compensation feature, was used to measure the TSS of the fruit juice samples. A sample of juice was placed on the glass lens and the reading was given automatically as a Brix %.

The titratable acid in citrus juice includes all acids that react with the hydroxide ions from sodium hydroxide (NaOH). Citric acid is the main acid in citrus juice and sometimes the acid percentage is given as percentage citric acid (Van Rensburg, 1985). The acid content of the juice samples was determined by an acid base titration using NaOH. A 10 ml sample of the fruit juice was transferred to a conical flask using a pipette. Three drops of phenolphthalein indicator solution were added and the juice was titrated with a 0.1562 N NaOH solution from a burette, until the solution turned a pink colour (Van Rensburg, 1985). The amount of NaOH used was recorded and the acid percentage was calculated using the following equation (Ladaniya, 2008);

$$\text{Acid (\%)} = \frac{\text{titre (burette) x normality of NaOH x equivalent weight of citric acid (64) x 100}}{\text{Volume of fruit juice x 1000}}$$

The Brix:acid ratio was then determined for each sample by dividing the Brix % by the acid percentage.

Data analysis

Data was transferred to Microsoft Excel (2003) spreadsheets for analysis and statistical analyses were done by ARC- Biometry, Stellenbosch using SAS/STAT (1999 and 2008), as in Chapter 3 for the determination of fruit rind colour.

RESULTS AND DISCUSSION

The ANOVA for the parents (Table 5.1) showed a significant level of between parent, between year and year x parent variation for Brix %, acid percentage and the Brix:acid ratio. The Brix:acid ratio had a larger between parent, between year and year x parent variation than both Brix % and acid percentage, while acid percentage had a smaller between parent, between year and year x parent variation than both Brix % and the Brix:acid ratio.

Table 5.1 ANOVA for Brix %, acid percentage and the Brix:acid ratio of the seven citrus parents

	Year 1		Year 2		Joint analysis	
	Df	**MS**	**Df**	**MS**	**Df**	**MS**
Brix %						
Parent	6	21.16**	6	29.22**	6	44.14**
Rep (parent)					14	0.72
Year		-		-	1	30.52**
Year x Parent		-		-	6	5.88**
Error	14	0.69	14	0.65	14	0.64
Acid %						
Parent	6	0.29**	6	0.88**	6	0.96**
Rep (parent)					14	0.01
Year		-		-	1	0.51**
Year x Parent		-		-	6	0.21**
Error	14	0.03	14	0.02	14	0.03
Brix:acid ratio						
Parent	6	32.26**	6	74.60**	6	79.44**
Rep (parent)					14	1.78
Year		-		-	1	108.26**
Year x Parent		-		-	6	27.49**
Error	14	4.42	14	1.74	14	4.39

**** P ≤ 0.01, * P ≤ 0.05**

The ANOVA for the families (Table 5.2) showed a significant level of between family variation for Brix %, acid percentage and the Brix:acid ratio. As with the parents the Brix:acid ratio for the families had a higher between family variation than the Brix % and the acid percentage, while the acid percentage had a lower between family variation than the Brix % and Brix:acid ratio. The year variation was significant for Brix % and the Brix:acid ratio, while only Brix % showed a significant year x family variation.

Table 5.2 ANOVA for Brix %, acid percentage and the Brix:acid ratio of the six citrus families

	Year 1		Year 2		Joint analysis	
	Df	**MS**	**Df**	**MS**	**Df**	**MS**
Brix %						
Family	5	8.96*	5	24.83**	5	26.65**
Family (tree)					526	4.29
Year		-		-	1	1018.61**
Year x Family		-		-	5	10.28*
Error	490	3.92	511	3.98	475	3.62
Acid %						
Family	5	5.71**	5	8.41**	5	13.90**
Family (tree)					526	0.32
Year		-		-	1	0.45
Year x Family		-		-	5	0.23
Error	490	0.31	511	0.36	475	0.35
Brix:acid ratio						
Family	5	439.48**	5	482.53**	5	903.25**
Family (tree)					526	16.79
Year		-		-	1	959.62**
Year x Family		-		-	5	7.56
Error	490	18.60	511	15.43	475	16.93

**** P ≤ 0.01, * P ≤ 0.05**

The Student t test for the parents (Table 5.3) showed that for Brix %, male parent Roma had the highest mean and differed significantly from all the other parents. Male parent Rishon had the lowest mean and differed significantly from male parents Roma, Shani, Sunburst and Hansen. The ranking and grouping among the parents for Brix % varied between year 1 and year 2 as indicated by the significant year x parent variation for Brix % (Table 5.1).

Table 5.3 Means and standard errors for Brix %, acid percentage and the Brix:acid ratio of the seven citrus parents

Year 1				Year 2				Joint analysis			
Parent	**Mean**	**Group**	**Error**	**Parent**	**Mean**	**Group**	**Error**	**Parent**	**Mean**	**Group**	**Error**
Brix %											
Shani	15.50	**A**	± 0.36	Roma	15.33	**A**	± 0.07	Roma	14.72	**A**	± 0.51
Roma	14.05	**AB**	± 0.46	Sunburst	12.23	**B**	± 0.30	Shani	13.63	**B**	± 1.21
Sunburst	12.92	**BC**	± 0.22	Shani	11.75	**B**	± 0.25	Sunburst	12.60	**B**	± 0.32
Hansen	12.05	**C**	± 1.01	Hansen	7.92	**C**	± 0.12	Hansen	10.02	**C**	± 1.44
Kiyomi	9.97	**D**	± 0.17	Rishon	7.90	**C**	± 0.95	Kiyomi	8.92	**D**	± 0.67
Dancy	9.68	**D**	± 0.18	Kiyomi	7.83	**C**	± 0.04	Dancy	8.42	**D**	± 0.92
Rishon	8.00	**E**	± 0.38	Dancy	7.08	**C**	± 0.67	Rishon	7.97	**D**	± 0.65
LSD (p≤0.05) : 1.46				LSD (p≤0.05) : 1.42				LSD (p≤0.05) : 1.05			
Acid %											
Shani	1.31	**A**	± 0.14	Hansen	1.98	**A**	± 0.14	Hansen	1.58	**A**	± 0.30
Hansen	1.16	**A**	± 0.18	Shani	1.81	**A**	± 0.03	Shani	1.56	**A**	± 0.18
Kiyomi	1.12	**A**	± 0.05	Sunburst	1.18	**B**	± 0.09	Kiyomi	1.06	**B**	± 0.06
Roma	1.10	**A**	± 0.06	Kiyomi	1.00	**BC**	± 0.05	Sunburst	0.98	**B**	± 0.14
Sunburst	0.79	**B**	± 0.04	Roma	0.85	**C**	± 0.05	Roma	0.98	**B**	± 0.09
Dancy	0.69	**BC**	± 0.09	Dancy	0.79	**C**	± 0.05	Dancy	0.74	**C**	± 0.07
Rishon	0.44	**C**	± 0.04	Rishon	0.52	**D**	± 0.04	Rishon	0.48	**D**	± 0.04
LSD (p≤0.05) : 0.30				LSD (p≤0.05) : 0.22				LSD (p≤0.05) : 0.14			
Brix:acid ratio											
Rishon	18.24	**A**	± 0.77	Roma	18.28	**A**	± 1.02	Rishon	16.73	**A**	± 1.30
Sunburst	16.52	**AB**	± 0.66	Rishon	15.22	**B**	± 1.16	Roma	15.57	**A**	± 1.89
Dancy	14.68	**ABC**	± 2.53	Sunburst	10.57	**C**	± 0.52	Sunburst	13.54	**B**	± 1.96
Roma	12.85	**BCD**	± 0.70	Dancy	9.07	**CD**	± 1.08	Dancy	11.87	**C**	± 2.50
Shani	12.12	**CDE**	± 1.20	Kiyomi	7.87	**DE**	± 0.39	Shani	9.30	**D**	± 1.94
Hansen	10.62	**DE**	± 0.92	Shani	6.48	**E**	± 0.06	Kiyomi	8.39	**DE**	± 0.46
Kiyomi	8.9	**E**	± 0.29	Hansen	4.03	**F**	± 0.29	Hansen	7.33	**E**	± 2.17
LSD (p≤0.05) : 3.68				LSD (p≤0.05) : 2.31				LSD (p≤0.05) : 1.65			
Means with the same letter are not significantly different											

For the acid percentage of the parents (Table 5.3) male parent Hansen had the highest mean and differed significantly from female parent Kiyomi and male parents Sunburst, Roma, Dancy and Rishon. Male parent Rishon had the lowest mean for acid percentage and differed significantly from all of the other parents. The ranking and grouping among the parents for acid percentage varied between year 1 and year 2 as indicated by the significant year x parent variation for acid percentage (Table 5.1).

For the Brix:acid ratio of the parents (Table 5.3), male parent Rishon had the highest mean and differed significantly from male parents Sunburst, Dancy, Shani and Hansen, and female parent Kiyomi. Male parent Hansen had the lowest mean and differed significantly from all the other male parents but was not significantly different from female parent Kiyomi. The ranking and grouping among the parents for the Brix:acid ratio varied between year 1 and year 2 as indicated by the significant year x parent variation (Table 5.1).

The data revealed that Roma had the highest mean for Brix % and therefore had sweeter fruit. Hansen and Shani had the highest means for acid percentage and are therefore later maturing than the other parents, while Rishon had the lowest mean for acid percentage and is therefore earlier maturing. Rishon and Roma had the highest means for the Brix:acid ratio; however the Brix % needs to be taken into consideration when looking at the Brix:acid ratio and even though Rishon had the highest Brix:acid ratio it had the lowest mean for Brix % and the high mean for the ratio is only as a result of the low acid percentage. Roma had the second largest mean for the Brix:acid ratio and had the highest mean for Brix %; therefore at the time of sampling Roma had an internal quality superior to the other parents.

A Student t test for the families (Table 5.4) showed that for Brix % the Kiyomi x Hansen family had the highest mean and differed significantly from the Kiyomi x Sunburst, Kiyomi x Roma, Kiyomi x Dancy and Kiyomi x Rishon families. The Kiyomi x Rishon family had the lowest mean and differed significantly from the Kiyomi x Hansen, Kiyomi x Shani and the Kiyomi x Sunburst families. The ranking and grouping among the families for Brix % varied between year 1 and year 2 as indicated by the significant year x family variation for Brix % (Table 5.2).

Table 5.4 Means and standard errors for Brix %, acid percentage and the Brix:acid ratio of the six citrus families

Year 1				Year 2				Joint analysis			
Family	**Mean**	**Grp**	**Error**	**Family**	**Mean**	**Grp**	**Error**	**Family**	**Mean**	**Grp**	**Error**
Brix %											
Kiyomi x Hansen	13.71	**A**	± 0.20	Kiyomi x Hansen	11.78	**A**	± 0.21	Kiyomi x Hansen	12.70	**A**	± 0.16
Kiyomi x Dancy	13.34	**AB**	± 0.27	Kiyomi x Shani	11.48	**A**	± 0.24	Kiyomi x Shani	12.35	**AB**	± 0.17
Kiyomi x Shani	13.20	**AB**	± 0.22	Kiyomi x Sunburst	11.40	**AB**	± 0.18	Kiyomi x Sunburst	12.16	**B**	± 0.14
Kiyomi x Roma	13.14	**AB**	± 0.21	Kiyomi x Roma	10.86	**BC**	± 0.22	Kiyomi x Roma	11.96	**BC**	± 0.18
Kiyomi x Rishon	12.86	**B**	± 0.24	Kiyomi x Dancy	10.76	**C**	± 0.23	Kiyomi x Dancy	11.95	**BC**	± 0.20
Kiyomi x Sunburst	12.83	**B**	± 0.18	Kiyomi x Rishon	10.36	**C**	± 0.20	Kiyomi x Rishon	11.52	**C**	± 0.19
LSD (p≤0.05) : 0.61				LSD (p≤0.05) : 0.60				LSD (p≤0.05) : 0.44			
Acid %											
Kiyomi x Hansen	1.56	**A**	± 0.08	Kiyomi x Shani	1.57	**A**	± 0.06	Kiyomi x Hansen	1.54	**A**	± 0.06
Kiyomi x Shani	1.50	**A**	± 0.06	Kiyomi x Hansen	1.53	**A**	± 0.09	Kiyomi x Shani	1.54	**A**	± 0.04
Kiyomi x Dancy	1.43	**A**	± 0.09	Kiyomi x Dancy	1.51	**A**	± 0.09	Kiyomi x Dancy	1.48	**A**	± 0.06
Kiyomi x Sunburst	1.22	**B**	± 0.04	Kiyomi x Sunburst	1.31	**B**	± 0.05	Kiyomi x Sunburst	1.26	**B**	± 0.03
Kiyomi x Roma	1.07	**B**	± 0.04	Kiyomi x Roma	1.06	**C**	± 0.05	Kiyomi x Roma	1.07	**C**	± 0.03
Kiyomi x Rishon	0.87	**C**	± 0.04	Kiyomi x Rishon	0.78	**D**	± 0.03	Kiyomi x Rishon	0.82	**D**	± 0.03
LSD (p≤0.05) : 0.17				LSD (p≤0.05) : 0.18				LSD (p≤0.05) : 0.12			
Brix:acid ratio											
Kiyomi x Rishon	16.42	**A**	± 0.57	Kiyomi x Rishon	14.69	**A**	± 0.53	Kiyomi x Rishon	15.50	**A**	± 0.39
Kiyomi x Roma	13.60	**B**	± 0.51	Kiyomi x Roma	11.68	**B**	± 0.48	Kiyomi x Roma	12.61	**B**	± 0.36
Kiyomi x Sunburst	11.68	**C**	± 0.40	Kiyomi x Sunburst	9.78	**C**	± 0.40	Kiyomi x Sunburst	10.80	**C**	± 0.29
Kiyomi x Dancy	11.25	**CD**	± 0.53	Kiyomi x Hansen	9.21	**CD**	± 0.37	Kiyomi x Dancy	9.89	**D**	± 0.34
Kiyomi x Hansen	10.56	**CD**	± 0.49	Kiyomi x Dancy	8.73	**CD**	± 0.41	Kiyomi x Hansen	9.85	**D**	± 0.31
Kiyomi x Shani	10.02	**D**	± 0.40	Kiyomi x Shani	8.39	**D**	± 0.37	Kiyomi x Shani	9.22	**D**	± 0.28
LSD (p≤0.05) : 1.33				LSD (p≤0.05) : 1.18				LSD (p≤0.05) : 0.88			

Means with the same letter are not significantly different

For the acid percentage of the families (Table 5.4), the Kiyomi x Hansen family had the highest mean and differed significantly from the Kiyomi x Sunburst, Kiyomi x Roma and Kiyomi x Rishon families. The Kiyomi x Rishon family had the lowest mean and differed significantly from all the other families. The ranking and grouping of the families for acid percentage showed little variation between year 1 and year 2, only the Kiyomi x Hansen and Kiyomi x Shani families had a change in ranking and the year x family variation for acid percentage was not significant (Table 5.2).

For the Brix:acid ratio of the families (Table 5.4), the Kiyomi x Rishon family had the highest mean and differed significantly from all the other families. The Kiyomi x Shani family had the lowest mean and differed significantly from the Kiyomi x Rishon, Kiyomi x Roma and Kiyomi x Sunburst families. The ranking and grouping among the families for the Brix:acid ratio showed little variation between year 1 and year 2, only the Kiyomi x Dancy and Kiyomi x Hansen families had a change in ranking and the year x family variation for the Brix:acid ratio was not significant (Table 5.2).

The data revealed that the Kiyomi x Hansen family had the highest mean for Brix % and therefore had sweeter fruit, however it was not significantly higher than the Kiyomi x Shani family. The Kiyomi x Hansen family had the highest mean for acid percentage and is therefore later maturing, but was not significantly different from the Kiyomi x Shani and Kiyomi x Dancy families, while the Kiyomi x Rishon family had the lowest mean for acid percentage and is therefore earlier maturing than the other families. The Kiyomi x Rishon family had the highest mean for the Brix:acid ratio; however this family had the lowest mean for Brix % and the high mean for the ratio is as a result of the low acid percentage. The Kiyomi x Roma family had the second largest mean for the Brix:acid ratio and, even though this family did not have the highest mean for Brix %, the ratio between the Brix % and the acid percentage gave an internal quality superior to the other families at the time of sampling.

Variance components determined, for year 1 and year 2 separately, for Brix %, acid percentage and the Brix:acid ratio followed the same trend over the two years (Table 5.5). The variation within the families was greater than between the families for Brix %, acid percentage and the Brix:acid ratio, indicating a high level of genetic variation within the families. The between family variation, expressed as a percentage of the total variation, was 2% and 6% for Brix %, 18% and 20% for acid percentage and 22% and 26% for the Brix:acid ratio, for year 1 and year 2 respectively. While the within family variation was 98% and 88% for Brix %, 82% and 78% for acid percentage and 77% and 71% for the Brix:acid ratio.

Table 5.5 Variance components and repeatability for Brix %, acid percentage and the Brix:acid ratio of the six citrus families

	Source of variation								Repeatability (between trees in a family)	
	Between families		Within families (between trees)		Within trees		Total			
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Brix %	0.06	0.24	3.90	3.84	0.02	0.26	3.98	4.34		
Kiyomi x Dancy			5.16	4.07	0.01	0.44	5.17	4.51	0.99	0.90
Kiyomi x Hansen			3.29	4.16	0.03	0.25	3.32	4.41	0.99	0.94
Kiyomi x Rishon			4.18	3.13	0.02	0.48	4.20	3.61	0.99	0.87
Kiyomi x Roma			3.31	3.52	0.04	0.10	3.35	3.62	0.99	0.97
Kiyomi x Shani			4.62	5.09	0.02	0.20	4.64	5.29	0.99	0.96
Kiyomi x Sunburst			3.03	2.79	0.02	0.12	3.05	2.91	0.99	0.96
Acid %	0.07	0.09	0.31	0.35	0.00	0.01	0.38	0.45		
Kiyomi x Dancy			0.54	0.64	0.00	0.01	0.54	0.65	0.99	0.98
Kiyomi x Hansen			0.52	0.59	0.00	0.04	0.52	0.63	0.99	0.94
Kiyomi x Rishon			0.13	0.09	0.00	0.00	0.13	0.09	0.99	0.95
Kiyomi x Roma			0.14	0.19	0.00	0.00	0.14	0.19	0.99	0.98
Kiyomi x Shani			0.34	0.34	0.00	0.02	0.34	0.36	0.99	0.96
Kiyomi x Sunburst			0.17	0.21	0.00	0.00	0.17	0.21	0.99	0.97
Brix:acid ratio	5.42	5.54	18.50	15.10	0.19	0.61	24.11	21.25		
Kiyomi x Dancy			20.45	13.89	0.08	0.33	20.53	14.22	0.99	0.98
Kiyomi x Hansen			20.33	12.89	0.07	0.72	20.40	13.61	0.99	0.95
Kiyomi x Rishon			23.16	22.33	0.47	1.65	23.63	23.98	0.98	0.93
Kiyomi x Roma			18.19	17.65	0.36	0.50	18.55	18.15	0.98	0.97
Kiyomi x Shani			15.40	12.49	0.16	0.18	15.56	12.67	0.99	0.98
Kiyomi x Sunburst			15.28	12.25	0.09	0.48	15.37	12.73	0.99	0.96

The within tree variation (error variance) was lower than the between family and within family variation for Brix %, acid percentage and the Brix:acid ratio (Table 5.5), showing very little variation between the two fruit samples tested per tree. Expressed as a percentage of the total variation the within tree variation was 1% and 6% for Brix %, 0% and 2% for acid percentage and 1% and 3% for the Brix:acid ratio, for year 1 and year 2 respectively.

Acid percentage had a lower variation than Brix % and the Brix:acid ratio (Table 5.5), as seen in the ANOVA's low mean squares for acid percentage (Table 5.2). The between family variation for Brix % was larger in year 2 than year 1, at 6% for year 2 and 2% for year 1, expressed as a percentage of the total variation (Table 5.5), as seen in the ANOVA's larger mean square for family in year 2 (Table 5.2). The within tree variation was larger in year 2 than year 1 for Brix %, acid percentage and the Brix:acid ratio (Table 5.5). Expressed as a percentage of the total variation Brix % was 1% and 6%, acid percentage 0% and 2% and the Brix:acid ratio 1% and 3% for year 1 and year 2 respectively, showing more variation between the two fruit samples tested per tree in year 2.

The variance components determined per family (Table 5.5) showed that for acid percentage the Kiyomi x Rishon, Kiyomi x Roma and Kiyomi x Sunburst families had a lower within family variation than the other families. In year 2 the Kiyomi x Dancy and Kiyomi x Rishon families had a larger within tree variation for Brix %, while the Kiyomi x Rishon family had a larger within tree variation for the Brix:acid ratio than the other families.

The repeatability, calculated between trees within a family (Table 5.5), was high for all families for Brix %, acid percentage and the Brix:acid ratio. Repeatability estimates ranged from 0.87 to 0.99 for Brix %, 0.94 to 0.99 for acid percentage and 0.93 to 0.99 for the Brix:acid ratio. The repeatability estimate in this case was used to determine the gain in accuracy expected from multiple measurements within a tree. The high repeatability estimates therefore indicate that a small gain in accuracy would be attained by increasing the number of samples per tree (Falconer and Mackay, 1996).

Variance components determined from the joint analysis (Table 5.6) allowed for the year and year x family contribution to the total variance to be calculated.

Table 5.6 Variance components and intraclass correlation coefficients for Brix %, acid percentage and the Brix:acid ratio of the six citrus families

	Source of variation						Intraclass correlation coefficients	
	Between families	**Within families**	**Year**	**Year x Family**	**Error**	**Total**	**t1**	**t2**
Brix %	0.07	1.61	2.12	0.10	2.38	6.28	0.01	0.40
Acid %	0.08	0.11	0.00	0.00	0.23	0.42	0.19	0.32
Brix:acid	5.63	6.06	1.82	0.01	11.16	24.68	0.23	0.35

The between family variation was again found to be lower than the within family variation for Brix % and acid percentage (Table 5.6). Expressed as a percentage of the total variation, the between family variation was 1% for Brix % and 19% for acid percentage, while the within family variation was 26% for both Brix % and acid percentage. For the Brix:acid ratio the between family variation and the within family variation were fairly similar with the between family variation at 23% and the within family variation at 25% of the total variation. The year variation contributed largely to the total variation for Brix % at 34%, while the year x family had a fairly small contribution at 10% of the total variation. For acid percentage neither the year nor year x family variation contributed to the total variation, while for the Brix:acid ratio the year variation contributed 7% of the total variation, while the year x family variation did not contribute.

Intraclass correlation coefficients were determined; relevant to selection between families (t_1) and relevant to selection between trees within a family (t_2) (Table 5.6). Intraclass correlation coefficient t_1 was very low for the Brix % at 0.01 indicating very little variation between the families. For acid percentage and the Brix:acid ratio t_1 was slightly higher at 0.19 for acid percentage and 0.23 for the Brix:acid ratio but still indicated a small variation between the families. Intraclass correlation coefficient t_2 was fairly low at 0.40 for Brix %, 0.32 for acid percentage and 0.35 for the Brix:acid ratio. The intraclass correlation coefficient sets an upper limit to broad sense heritability for the traits studied (de Souza and Byrne, 1998). Therefore, the low value for t_2 indicates that the variation found within the families was only partly genetic and non-genetic factors (environment) also played a role.

Distribution curves were drawn up for the six families for Brix %, acid percentage and the Brix:acid ratio. The parent values were included on each curve to allow the progeny to be compared to the parents (Figures 5.1 – 5.3).

All families showed a continuous distribution for Brix % indicating a quantitative expression of the trait (Figure 5.1). The curves followed a normal distribution, with a p-value > 0.05, except for the Kiyomi x Dancy, Kiyomi x Rishon and Kiyomi x Sunburst curves in year 1. The Kiyomi x Dancy and Kiyomi x Rishon curves were skewed towards lower Brix % values while the Kiyomi x Sunburst curve was skewed towards higher Brix % values. The Kiyomi x Sunburst family had a narrower distribution showing less within family variation, while the Kiyomi x Shani family and the Kiyomi x Dancy family in year 1 had a wider distribution showing more within family variation. This can be seen in the variance component analysis (Table 5.5) where the Kiyomi x Sunburst family had a lower between tree variance and the Kiyomi x Shani family and the Kiyomi x Dancy family in year 1 had a larger between tree variance than the other families.

For Brix % the Kiyomi x Dancy, Kiyomi x Hansen and Kiyomi x Rishon families had means greater than both parents and showed an overdominance for the trait. The Kiyomi x Roma family and the Kiyomi x Shani family in year 1 had means between the two parents. While the Kiyomi x Sunburst family and the Kiyomi x Shani family in year 2 had means close to the male parent showing the male parent to be more dominant (Figure 5.1). Therefore all of the families showed an improvement in the progeny over the female parent Kiyomi for Brix %. The Kiyomi x Dancy, Kiyomi x Hansen and Kiyomi x Rishon families showed heterosis with means greater than both parents. All families, however, showed some heterosis and contained individuals with a higher Brix % than both of the parents. The Kiyomi x Hansen family had the highest mean for Brix % (Table 5.4); however it was not significantly higher than the Kiyomi x Shani family.

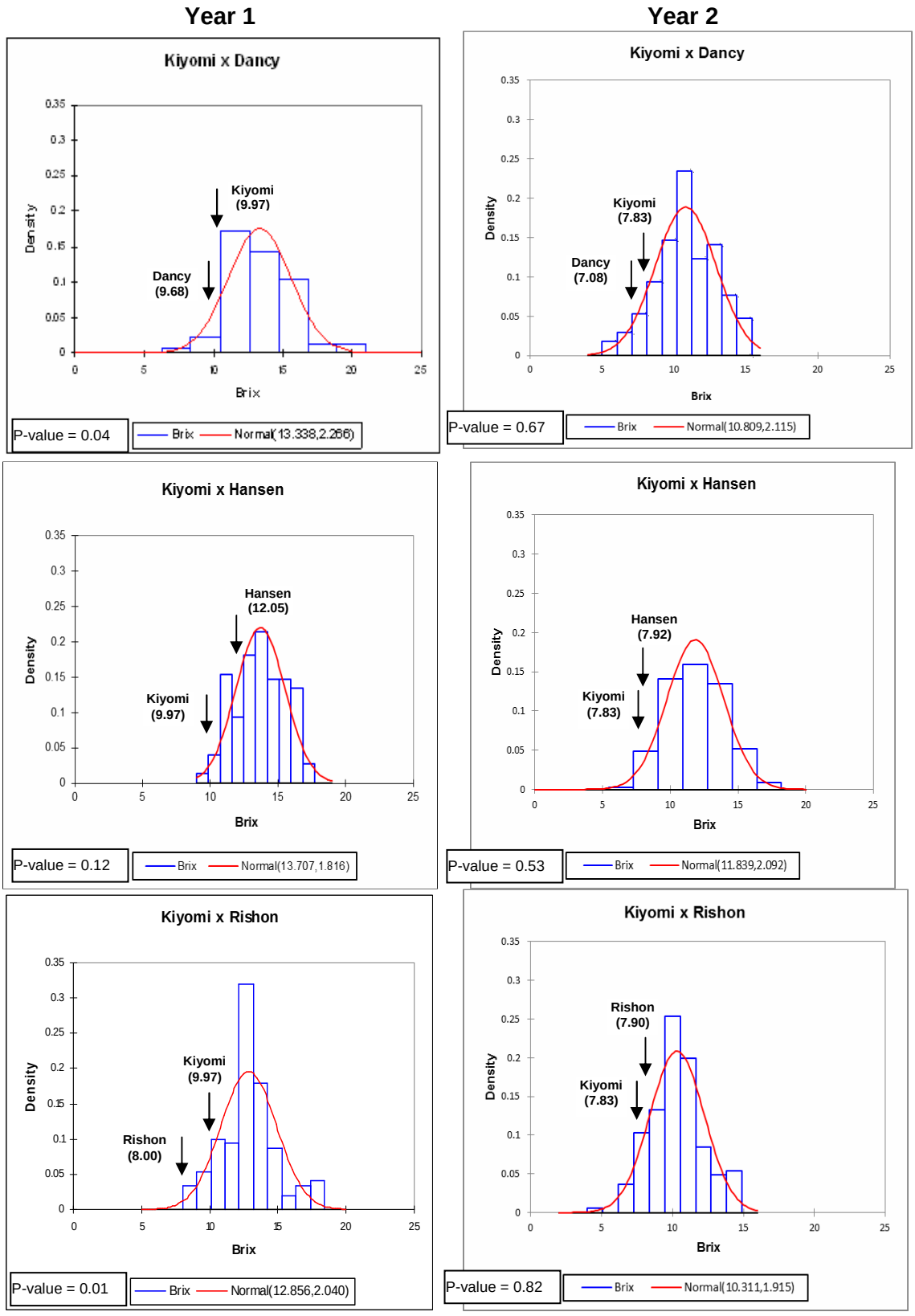

Figure 5.1 Distribution curves for the six citrus families for Brix %

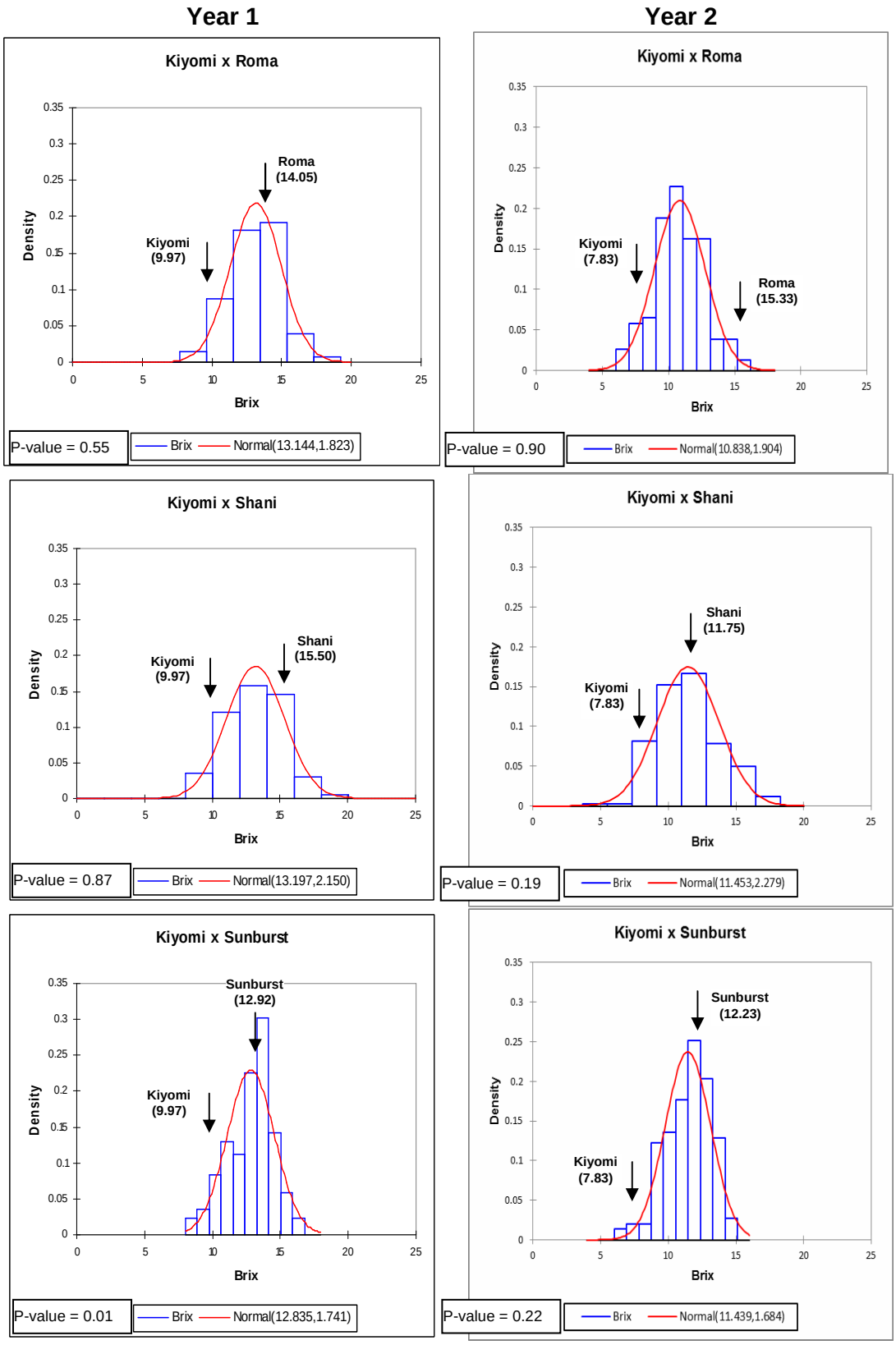

Figure 5.1 (cont.). Distribution curves for the six citrus families for Brix %

When comparing the year 1 and year 2 distribution curves for Brix % it could be seen that the families showed similar trends over the two years except for the Kiyomi x Shani family; however the parent value for Shani as well as the family means differed between the years. Parent values for Kiyomi, Dancy and Hansen as well as family means for all of the families differed between the years (Table 5.3 and 5.4). This can be seen in the significant year and year x parent variation for the parents (Table 5.1) and the significant year and year x family variation for the families (Table 5.2).

All families showed a continuous distribution for acid percentage indicating a quantitative expression of the trait (Figure 5.2). The curves followed a normal distribution, with a p-value > 0.05, except for the Kiyomi x Dancy and the Kiyomi x Hansen curves as well as the Kiyomi x Rishon curve in year 1 and the Kiyomi x Roma curve in year 2, which were skewed towards lower acid percentage values. The Kiyomi x Rishon, Kiyomi x Roma and Kiyomi x Sunburst families had a narrower distribution showing less within family variation, as seen in the variance component analysis (Table 5.5) where these families had a lower between tree variance than the other families. The Kiyomi x Dancy and Kiyomi x Hansen curves had a wider distribution showing more within family variation, as seen in the variance component analysis (Table 5.5) where these families had a larger between tree variance than the other families.

For acid percentage the Kiyomi x Dancy and Kiyomi x Sunburst families, and the Kiyomi x Hansen and Kiyomi x Shani families for year 1, had means greater than both parents showing an overdominance for the trait. The Kiyomi x Rishon family and the Kiyomi x Hansen and Kiyomi x Shani families for year 2 had means between the two parents. The Kiyomi x Roma family had a mean close to both parents in year 1 and a mean close to the female parent Kiyomi but larger than the male parent Roma in year 2 (Figure 5.2). Therefore all of the families, except the Kiyomi x Roma family in year 1, had an increase in acid percentage over one of the parents, with the Kiyomi x Dancy and Kiyomi x Sunburst families, and the Kiyomi x Hansen and Kiyomi x Shani families for year 1 showing heterosis with means greater than both parents. All families, however, showed some heterosis and contained individuals with a higher acid percentage than both of the parents. The Kiyomi x Hansen family had the highest mean for acid percentage (Table 5.4); however it was not significantly different to the Kiyomi x Shani and Kiyomi x Dancy families.

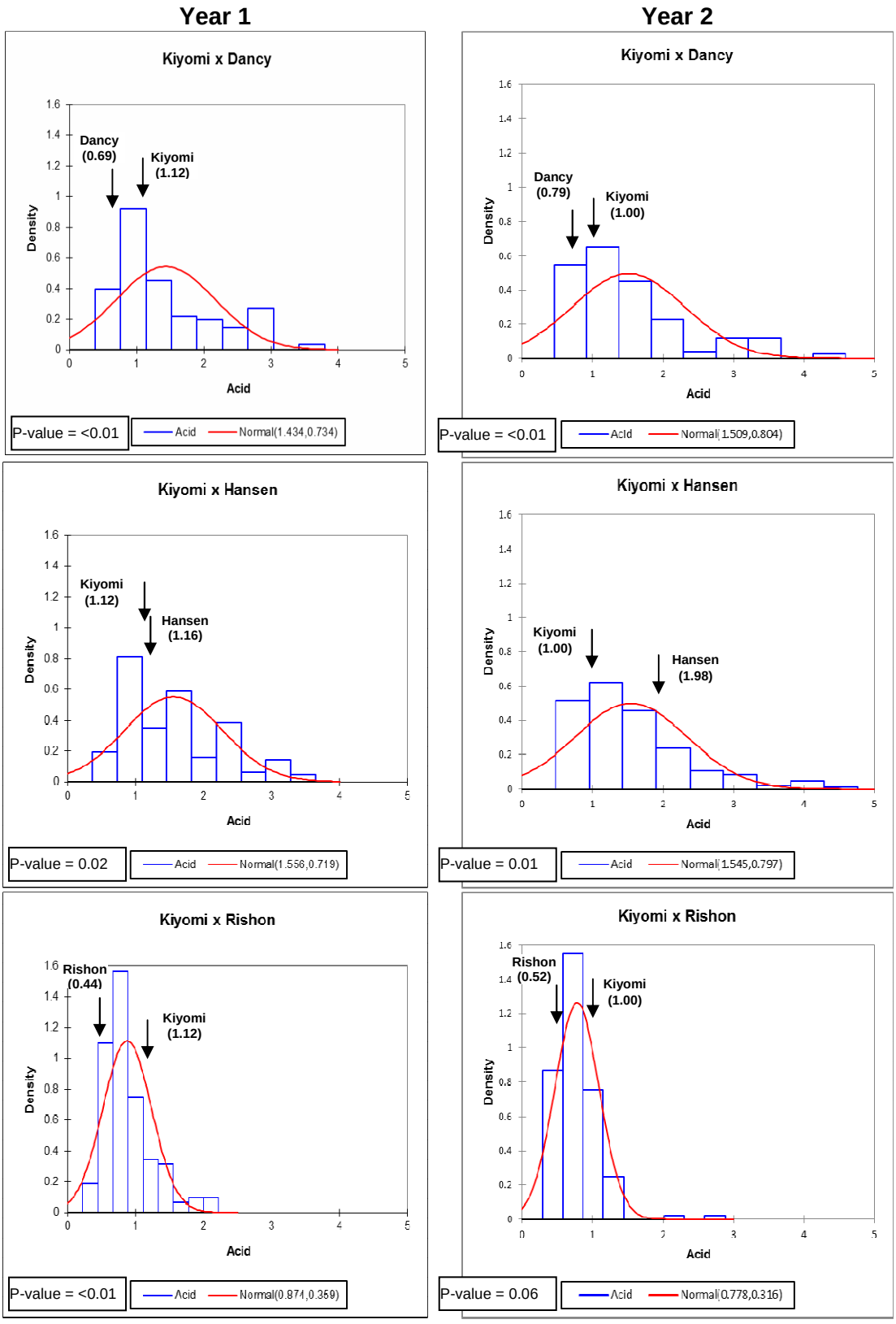

Figure 5.2 Distribution curves for the six citrus families for acid percentage

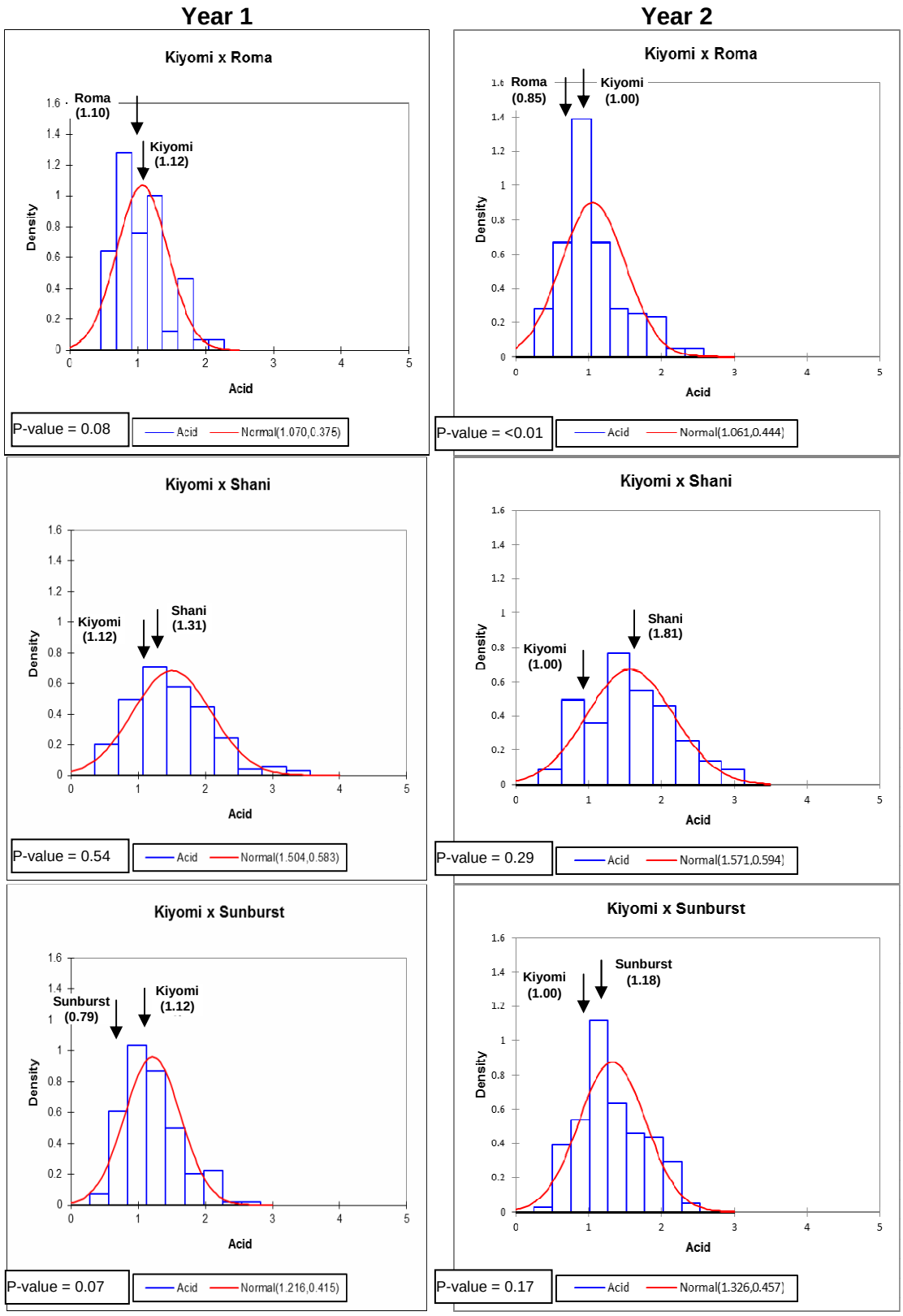

Figure 5.2 (cont.). Distribution curves for the six citrus families for acid percentage

When comparing the year 1 and year 2 distribution curves for acid percentage, it could be seen that the families generally showed similar trends over the two years except for the Kiyomi x Hansen and Kiyomi x Shani families; however the parent values for both Hansen and Shani differed considerably between the years, while the other male parents also showed some variation over the years (Table 5.3). This can be seen in the significant year and year x parent variation for the parents (Table 5.1). The families did not have a significant year or year x family variation for acid percentage (Table 5.2) and therefore the difference between the years in the parent progeny relation of these families can be attributed to the difference in the parent values between the years.

All families showed a continuous distribution for the Brix:acid ratio indicating a quantitative expression of the trait (Figure 5.3). The curves followed a normal distribution, with a p-value > 0.05, except for the Kiyomi x Shani curves and the Kiyomi x Rishon curve in year 2, which were skewed towards lower Brix:acid ratio values. The Kiyomi x Rishon family had a wider distribution showing more within family variation, while the Kiyomi x Dancy, Kiyomi x Hansen, Kiyomi x Shani and Kiyomi x Sunburst families in year 2 had a narrower distribution showing less within family variation. This could be seen in the variance component analysis (Table 5.5) where Kiyomi x Rishon family had a larger between tree variance and the Kiyomi x Dancy, Kiyomi x Hansen, Kiyomi x Shani and Kiyomi x Sunburst families in year 2, had a lower between tree variance than the other families.

For the Brix:acid ratio the Kiyomi x Hansen and Kiyomi x Shani families for year 2 had means larger than both parents showing an overdominance of the trait. The Kiyomi x Sunburst family and the Kiyomi x Dancy, Kiyomi x Rishon, and Kiyomi x Shani families for year 1 and the Kiyomi x Roma family for year 2 had means between the two parents. While the Kiyomi x Hansen and Kiyomi x Roma families for year 1 and the Kiyomi x Dancy and Kiyomi x Rishon families for year 2 had means close to the male parent showing the male parent to be more dominant (Figure 5.3). Therefore all the families showed an improvement in the Brix:acid ratio over the female parent Kiyomi, with the Kiyomi x Hansen and Kiyomi x Shani families in year 2 showing heterosis having means greater than both parents. All families however showed some heterosis and contained individuals with a Brix:acid ratio larger than both the parents. The Kiyomi x Rishon family had the highest mean for the Brix:acid ratio (Table 5.4) and was significantly higher than all the other families.

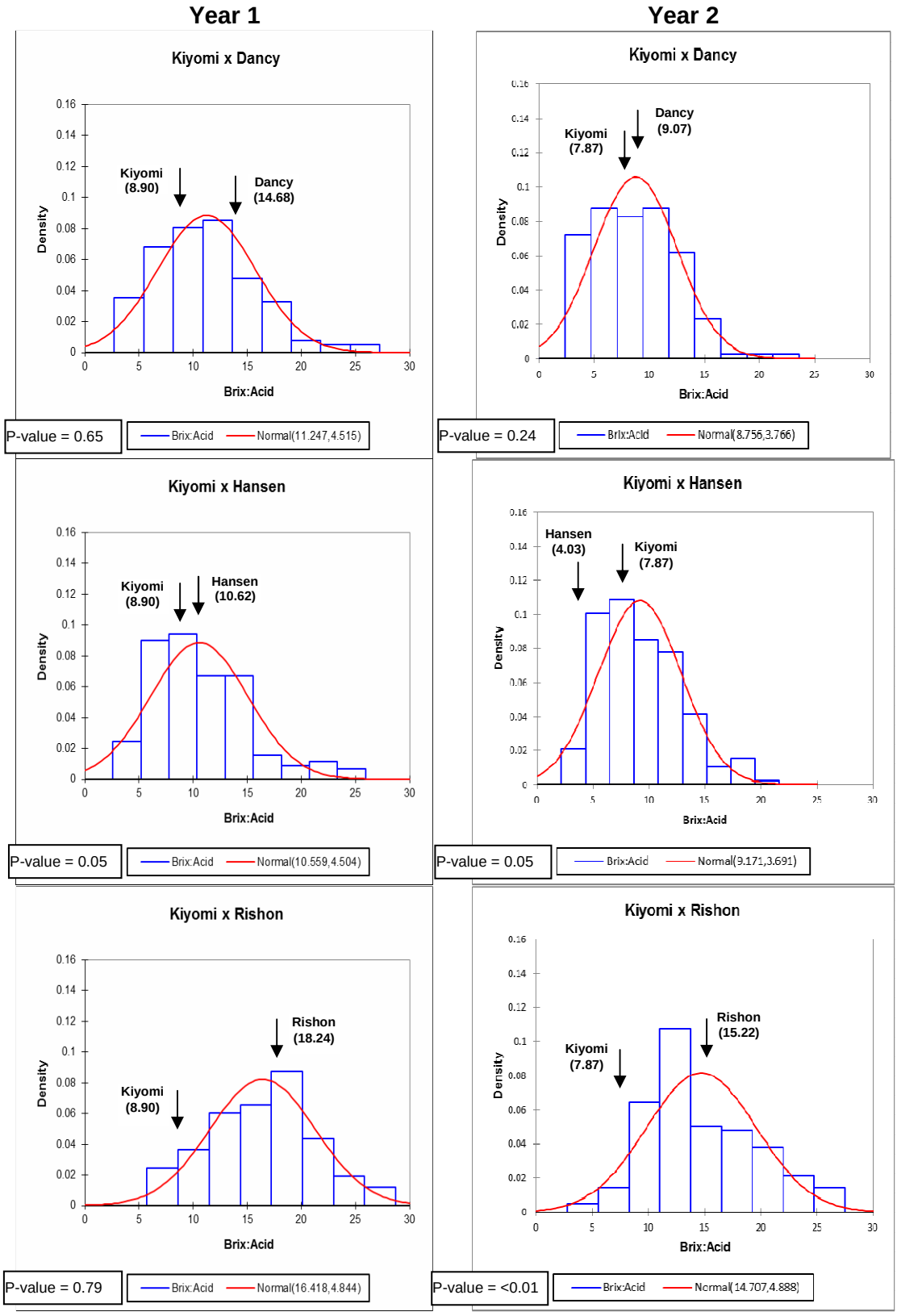

Figure 5.3 Distribution curves for the six citrus families for the Brix:acid ratio

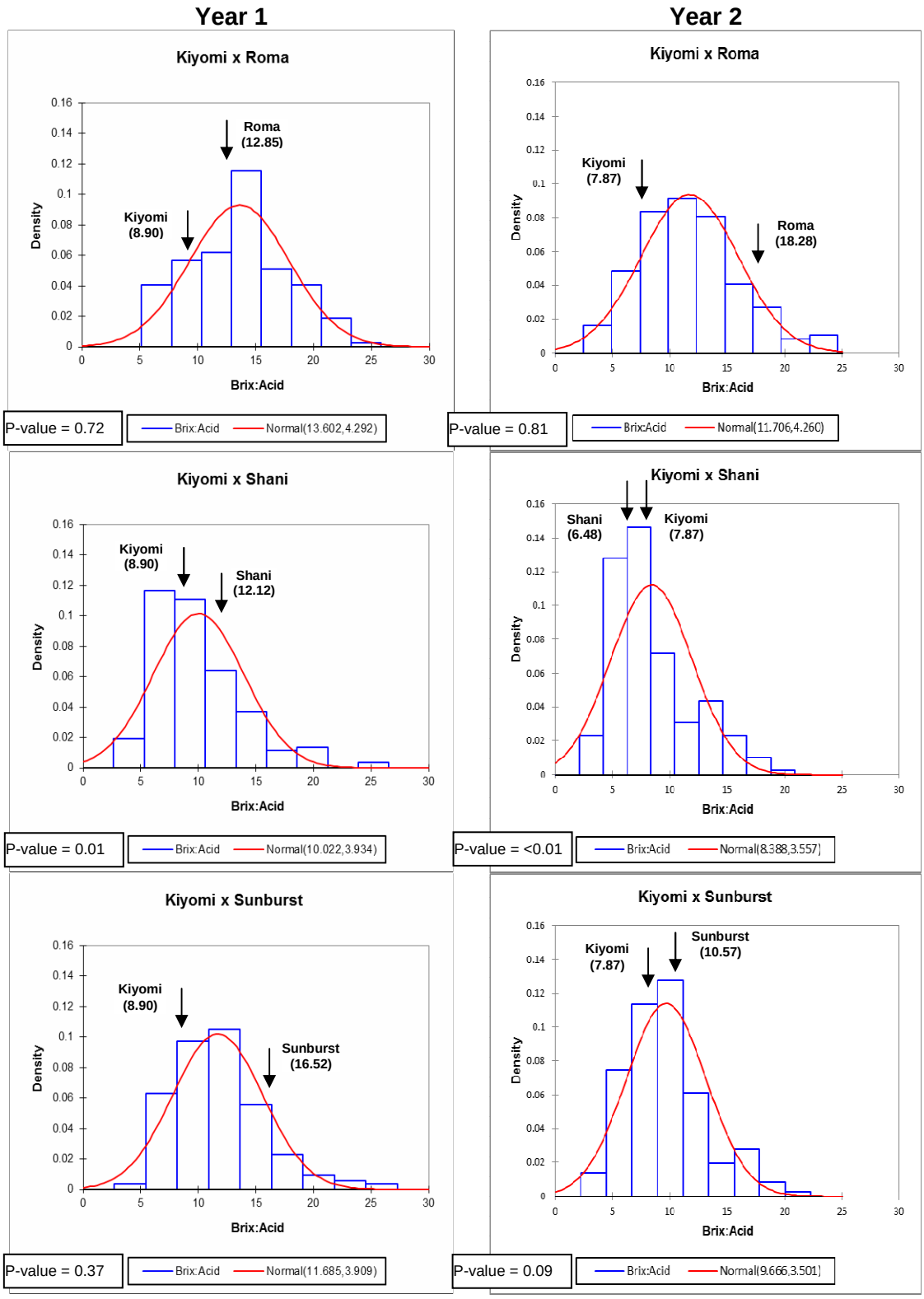

Figure 5.3 (cont.) Distribution curves for the six citrus families for the Brix:acid ratio

When comparing the year 1 and year 2 distribution curves for the Brix:acid ratio, it could be seen that the families differed between the years with only the Kiyomi x Sunburst family showing similar trends over the two years. Parent and family means differed between the years and attributed to the differences in the parent progeny relation seen in the distribution curves. The difference between the years in the parent and family means can be seen in the significant year and year x parent variation for the parents (Table 5.1) and the significant year variation for the families (Table 5.2).

Correlations for year 1 and year 2 showed the same trend, with a negative correlation between acid percentage and the Brix:acid ratio (Table 5.7), indicating that as the acid percentage decreased so the Brix:acid ratio increased. The Brix:acid ratio is calculated using both Brix % and acid percentage readings however the increase in the ratio is mainly due to the decline in the acid percentage and even though the Brix % increases it does not have as great effect on the ratio as the acid percentage (Van Rensburg, 1985).

Table 5.7 Pearson correlation coefficients for Brix %, acid percentage and the Brix:acid ratio of the six citrus families

	Year 1			Year 2		
	Brix %	**Acid %**	**Brix:acid ratio**	**Brix %**	**Acid %**	**Brix:acid ratio**
Brix %		0.39**	-0.08		0.36**	-0.01
Acid %	0.39**		-0.84**	0.36**		-0.80**
Brix:acid ratio	-0.08	-0.84**		-0.01	-0.80**	

****P ≤ 0.01**

CONCLUSIONS

The improvement of fruit quality is one of the main goals of citrus breeding programmes worldwide, and while the fruit's external appearance with regard to factors such as colour and size are important, the fruit's internal quality plays an essential role in producing high-quality fruit (Miyazaki et al., 2011). A citrus fruit's internal quality is determined by the Brix %, acid percentage and the Brix:acid ratio and information on the inheritance of these characteristics can aid the breeder in the selection of parents and planning of crosses for breeding new and improved citrus cultivars. In this study six mandarin families, where the female parent Kiyomi tangor was crossed with various male parents were studied. Data for Brix %, acid percentage and the Brix:acid ratio were collected and used to quantify the genetic variability within and between the families over two seasons as well as study the genetic relationships between the hybrids and the parents.

The study revealed a significant level of between parent and between family variation for Brix %, acid percentage and the Brix:acid ratio. Therefore the variation in internal fruit quality in citrus is measurable and can be further explored for breeding purposes. Overall the acid percentage showed a lower variation than the Brix % and the Brix:acid ratio.

The variance component analysis, for year 1 and year 2 separately, showed a greater variation within the families than between the families for Brix %, acid percentage and the Brix:acid ratio, indicating a high level of genetic variation within the families. Citrus cultivars are highly heterozygous (Ray, 2002) and the high genetic variation found within the families can be directly ascribed to the variation generated by crossing two heterozygous parents. The within tree variation was lower than the within family variation showing little variation between the two fruit samples tested per tree. The variance components from the joint analysis again showed a greater variation within the families than between the families for Brix % and acid percentage, however for the Brix:acid ratio the within family variation and between family variation were very similar. A high repeatability was found for Brix %, acid percentage and the Brix:acid ratio and indicated that for these measurements only a small gain in accuracy would be attained by increasing the number of samples per tree.

The parents had a significant year and year x parent variation for Brix %, acid percentage and the Brix:acid ratio, while the families had a significant year and year x family variation for Brix % and a significant year variation for the Brix:acid ratio. The variance component analysis for the families showed that for Brix % the year variation contributed largely to the total variation, while the year x family variation for Brix % and the Brix:acid ratio also had a small contribution. The internal quality in mandarins does vary over years and a significant year and year x cultivar variation has been found for both TSS and acid percentage for mandarin cultivars (Russo and Fanizza, 1992), while a fairly large variation between years was found for TSS measurements of mandarin clones (Lima et al. 1981 and 1992). The climate, especially temperature, is known to influence the Brix % and most probably played a part in the variation found between the years.

The intraclass correlation coefficient t_2 was found to be fairly low for Brix %, acid percentage and the Brix:acid ratio and indicated that the variation found within the families was only partly genetic and that non-genetic factors contributed to the variation in the phenotype. Estimates of repeatability in time for mandarin clones (Lima et al., 1992) were found to be very low for TSS measurements, while the repeatability for acid percentage was slightly higher and similar to the value found for intraclass correlation coefficient t_2 in this study. However while the repeatability for the Brix:acid ratio was found to be fairly high. Fairly low narrow sense heritability estimates similar to those for t_2 in this study were also found for Brix % and acid percentage in breeding populations of peach (de Sousa and Bryne, 1998). Therefore it can be seen that for the fruit internal quality only two years of testing will not be reliable and a mean performance over multiple years data should be used for effective selection.

The correlation analysis showed a positive correlation between acid percentage and the Brix:acid ratio, therefore a lower acid percentage should indicate a higher Brix:acid ratio. However the Brix:acid ratio only gives a clear picture of the fruits internal quality when presented with the Brix % (Ladaniya, 2008), therefore it is not possible to use only the acid percentage to predict the Brix:acid ratio.

In citrus, Brix %, acid percentage and the Brix:acid ratio are quantitative genetic traits and showed a continuous distribution. All of the families showed an increase in the Brix % and the Brix:acid ratio over the female parent Kiyomi, while all the families except the Kiyomi x Roma family in year 1 showed an increase in the acid percentage over one of the parents. The Kiyomi x Dancy, Kiyomi x Hansen and Kiyomi x Rishon families showed heterosis for Brix %, while the Kiyomi x Dancy, Kiyomi x Dancy, Kiyomi x Shani and Kiyomi x Sunburst showed heterosis acid percentage and the Kiyomi x Hansen and Kiyomi x Shani families showed heterosis for the Brix:acid ratio having means greater than both parents. All of the families however, did contain some individuals with a Brix %, acid percentage and Brix:acid ratio superior to both parents.

The Kiyomi x Hansen family had the highest mean for Brix % and acid percentage and the second lowest mean for the Brix:acid ratio, while male parent Hansen had the highest mean for acid percentage and the lowest mean for the Brix:acid ratio. The Kiyomi x Rishon family had the highest mean for the Brix:acid ratio and the lowest mean for Brix % and acid percentage, while male parent Rishon had the highest mean for the Brix:acid ratio and the lowest mean for Brix % and acid percentage. Male parents with higher means resulted in families with higher means while male parents with lower means resulted in families with lower means. Therefore it can be seen that the Brix %, acid percentage and the Brix:acid ratio of the male parent had a large influence on the progeny.

The Kiyomi x Hansen and Kiyomi x Shani families had the highest means for Brix % and acid percentage; however they had the lowest means for the Brix:acid ratio. The low mean for the ratio is as a result of the high acid percentage indicating that these families were not internally mature at the time of sampling. However, later in the season when the acid percentage drops and the Brix:acid ratio rises these families have the potential to yield high quality late maturing varieties.

Therefore the results of this study are only relevant to the specific time of the season when the fruit was sampled. Male parent Roma had an internal quality superior to the other parents, while the Kiyomi x Roma family had an internal quality superior to the other families. Therefore crosses using male parent Roma resulted in progeny with a superior fruit quality at the time of sampling and testing, when crossed with female parent Kiyomi.

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CHAPTER 6

INTERRELATIONSHIPS AMONG FRUIT CHARACTERISTICS IN KIYOMI FAMILIES

INTRODUCTION

Mandarins are the most popular group of fresh citrus fruit due to their attractive appearance, pleasant taste, seedlessness and easy peeling characteristics (Ladaniya, 2008). Mandarins are a very diverse group consisting of many different varieties (Saunt, 2000), with the different varieties varying considerably with regard to the fruits' appearance, flavour and time of maturity (Ladaniya, 2008). Even with the wide variety of mandarins available on the fresh fruit market there is still an ongoing search for new varieties with improved fruit quality (de Oliveira et al., 2003).

Fruit quality can be defined as the combination of fruit characteristics that have significance in determining the degree of consumer acceptance. High quality fruit are more readily accepted by the consumer and can therefore attain a higher price (Ladaniya, 2008). The fruit's external quality is determined by external physical characteristics such as rind colour, fruit size, shape and any visible defects. The fruit's internal quality is governed by the taste, aroma, mouth-feel (as a result of the viscosity and presence of water-insoluble solids) and appearance. However the internal quality or maturity index is mainly based on the juice content, the TSS content and the TSS to acid ratio (Spiegel-Roy and Goldschmidt, 1996; Ladaniya, 2008).

Many factors need to be taken into account when cultivating citrus; however the climate and especially temperature has a large influence on a citrus fruit's growth and quality (Bijzet, 2006). The climatic effect is more predominant than any other factor, including soil, cultural practices and even genetic factors (Ladaniya, 2008). Mandarins have a wide range of climatic adaptability and can be grown under desert, semi-tropical and sub-tropical Mediterranean climates; however different mandarin varieties are very specific in their climatic requirements for good production and fruit quality (Saunt, 2000).

Hot tropical climates with their prevailing warm temperatures interfere with and delay fruit colouration, preventing many mandarin varieties from attaining their attractive orange rind colour at maturity (Spiegel-Roy and Goldschmidt, 1996). The heat unit requirement for maturation is also reached much faster, allowing the fruit to grow and develop quite rapidly and become very large (Ladaniya, 2008). The fruit have a high TSS but the acids decline very quickly, which can result in a poor eating quality. The fruit also remain marketable for only a short period of time where after they deteriorate quite rapidly (Spiegel-Roy and Goldschmidt, 1996). Under the cooler subtropical climates with their lower temperatures, mandarin fruit can attain the nice orange or orange-red colour found in some varieties (Ladaniya, 2008). The lower heat units under these cooler conditions result in a slower growth and the fruit not only mature later but also over a longer period of time (Bijzet, 2006) and the mature fruit are considerably smaller than those grown in warmer climates. The fruit have a lower TSS and higher acid content but do tend to have a better keeping quality (Spiegel-Roy and Goldschmidt, 1996). Optimal climatic conditions that produce the highest quality mandarin fruit is a Mediterranean type climate, where the low rainfall, hot summers and cool, wet winters at fruit maturity result in attractive fruit with a deep orange rind colour and a superior eating quality (Ladaniya, 2008).

Citrus breeding through hybridisation has been practised for almost 80 years, with the aim of developing high quality new cultivars (Ray, 2002a). Many important breeding goals exist in citrus, most of these are long standing and are similar to other tree fruits (Soost and Cameron, 1975). Breeding goals for mandarins may be general or related to a specific geographical area and tend to vary in response to market trends (Soost and Cameron, 1975; Spiegel-Roy and Goldschmidt, 1996). Breeding goals for the improvement of fruit quality in mandarins are to breed fruit with an attractive appearance having a deep orange or orange red rind colour, good fruit size and attractive fruit shape (Spiegel-Roy and Goldschmidt, 1996; Nicotra, 2001). A good flavour, easy peeling ability, seedless or very low seed count and extension of the ripening season (earlier and later ripening cultivars) are also important goals for mandarins. While adaptability to different environments, low tendency to alternate bearing, post harvest quality and storage and resistance to the most damaging pests and diseases also need to be taken into consideration (Soost and Cameron, 1975; Nicotra, 2001).

Mandarin breeding programmes generally aim at improving several different characteristics, each with a different inheritance pattern, and this can complicate the process of parent selection and the planning of controlled crosses. In addition to this, these characteristics are generally distributed over many cultivars and some desirable characters may be associated with undesirable ones (Ray, 2002b). Information on the interrelationships among fruit characteristics is important in a mandarin breeding programme as the probability of breeding a new superior quality cultivar depends largely on these relationships (Russo and Fanizza, 1992). An understanding of how these fruit characteristics are inherited can aid the breeder in the selection of parents and planning of controlled crosses. In this study the parents and progenies of six mandarin families were investigated with regard to some important fruit quality characteristics, in order to gain information on the interrelationships among the fruit characteristics studied as well as how they are inherited.

MATERIALS AND METHODS

Unless otherwise stated, the materials and methods used in this chapter for the determination of fruit characteristics are the same as used in Chapter 3 for the determination of rind colour.

Selection of families

Six mandarin families, with Kiyomi tangor (*Citrus unshiu x Citrus sinensis*) as female parent and Dancy, Hansen, Rishon, Roma, Shani and Sunburst mandarins (*Citrus reticulata*) as male parents, were selected for the study. The aim of these crosses was to breed new citrus mandarin hybrids with improved fruit characteristics, the main focus being on an improved rind colour, larger fruit size, more round fruit shape and a superior flavour.

Sampling of trees and fruit for evaluation

The materials and methods for the sampling of trees and fruit are given in Chapter 3 for rind colour, Chapter 4 for fruit size and shape and Chapter 5 for the fruit's internal quality.

Data collection

Data were collected for fruit rind colour as chroma coordinates L*, a* and b*, for fruit size as fruit height and fruit width, for fruit shape and for fruit internal quality as Brix %, acid percentage and the Brix:acid ratio. Data was taken for two years, 2009 and 2010. The materials and methods for the data collection are given in Chapter 3 for rind colour, Chapter 4 for fruit size and shape and in Chapter 5 for fruit internal quality.

Data analysis

Data was transferred to Microsoft Excel (2003) spreadsheets and statistical analyses were done by ARC- Biometry, Stellenbosch using SAS/STAT (1999 and 2008) and XLSTAT (2008).

ANOVA's were done combining the parents and families data and a Student's t LSD test divided them into groups. A Principal Component Analysis (PCA) based on Pearson's correlations was used to investigate the relationships between the parents and the families with regard to the fruit characteristics studied. The PCA is a multivariate statistical technique which is useful in investigating the genetic relationships between the parents and families. By looking at correlations in a group of variables the dimensions of the group of data can be reduced. A smaller number of abstract variables are created and this maximises the variance of the linear combinations of the variables (de Oliveira et al, 2003).

RESULTS AND DISCUSSION

Parent and family means for fruit characteristics; rind colour coordinates L*, a* and b*, fruit height, fruit width, fruit shape, Brix %, acid percentage and the Brix:acid ratio are given in Table 6.1 for 2009 and Table 6.2 for 2010.

Table 6.1 Means for fruit characteristics of the seven citrus parents and six citrus families for 2009

	L*	a*	b*	Height	Width	Shape	Brix %	Acid %	Brix:acid
Kiyomi	65.54 a	25.62 g	49.65 ab	76.65 a	84.13 a	1.10 c	9.97 cd	1.12 abcd	8.92 d
Dancy	60.69 bc	34.99 bcde	42.74 bcde	46.66 d	60.74 c	1.30 a	9.68 d	0.69 de	14.68 abc
Hansen	58.70 cde	39.85 abc	38.90 de	51.39 cd	64.59 bc	1.27 ab	12.05 bc	1.16 abcd	10.62 cd
Rishon	55.75 e	37.97 bcd	38.12 e	56.28 bcd	61.08 c	1.09 c	8.00 d	0.44 e	18.24 a
Roma	63.17 ab	33.64 cde	51.62 a	57.11 bcd	65.91 bc	1.16 bc	14.05 ab	1.10 abcd	12.85 bcd
Shani	56.80 de	40.79 ab	38.57 de	55.12 cd	67.82 bc	1.23 ab	15.50 a	1.31 abcd	12.12 bcd
Sunburst	59.82 bcd	45.25 a	38.28 e	55.82 bcd	69.62 bc	1.25 ab	12.92 b	0.79 cde	16.52 ab
Kiyomi x Dancy	60.85 bc	26.72 fg	44.12 bcde	56.89 bcd	66.10 bc	1.18 bc	13.34 ab	1.43 abc	11.25 cd
Kiyomi x Hansen	60.14 bcd	32.72 def	42.55 cde	53.73 cd	63.01 bc	1.18 abc	13.71 ab	1.56 a	10.56 cd
Kiyomi x Rishon	61.19 bc	29.84 efg	45.06 abcde	68.00 ab	73.60 ab	1.10 c	12.86 b	0.87 bcde	16.42 ab
Kiyomi x Roma	61.90 abc	29.73 efg	49.09 abc	57.82 bcd	69.15 bc	1.21 abc	13.14 b	1.07 abcde	13.60 abcd
Kiyomi x Shani	61.26 bc	29.90 efg	45.41 abcd	60.01 bc	70.81 bc	1.19 abc	13.20 ab	1.50 ab	10.02 cd
Kiyomi x Sunburst	61.75 abc	32.49 def	45.57 abcd	57.97 bcd	69.86 bc	1.21 abc	12.84 b	1.22 abcd	11.68 bcd

Means with the same letter are not significantly different

Table 6.2 Means for fruit characteristics of the seven citrus parents and six citrus families for 2010

	L*	a*	b*	Height	Width	Shape	Brix %	Acid %	Brix:acid
Kiyomi	66.72 a	25.45 e	53.44 a	74.98 a	86.12 a	1.15 c	7.83 c	1.00 cdef	7.87 ef
Dancy	64.26 ab	30.55 de	53.87 a	46.39 cd	60.21 bc	1.31 a	7.08 c	0.79 ef	9.07 de
Hansen	54.50 f	43.40 a	45.32 bcd	47.71 cd	60.36 bc	1.26 abc	7.92 c	1.98 a	4.03 f
Rishon	58.63 ed	39.46 abc	42.53 de	53.76 bc	63.95 bc	1.19 abc	7.90 c	0.52 f	15.22 ab
Roma	61.21 bcd	34.64 bcd	48.31 abcd	49.04 cd	62.83 bc	1.29 abc	15.33 a	0.84 def	18.28 a
Shani	55.00 f	41.00 ab	34.51 f	41.12 d	52.32 c	1.27 abc	11.75 b	1.81 ab	6.48 ef
Sunburst	56.24 ef	43.66 a	37.80 ef	49.30 cd	63.31 bc	1.29 ab	12.23 b	1.18 bcdef	10.57 cde
Kiyomi x Dancy	63.61 abc	27.03 e	50.72 ab	54.11 bc	64.19 bc	1.19 abc	10.76 b	1.51 abcd	8.73 de
Kiyomi x Hansen	60.37 cd	31.78 de	49.28 abc	53.57 bc	62.36 bc	1.18 abc	11.78 b	1.53 abcd	9.21 de
Kiyomi x Rishon	58.63 de	27.55 de	43.23 cde	62.30 b	70.95 b	1.16 bc	10.36 b	0.78 ef	14.69 abc
Kiyomi x Roma	62.64 bc	26.36 e	47.92 abcd	52.49 bcd	64.11 bc	1.23 abc	10.86 b	1.06 cdef	11.68 bcd
Kiyomi x Shani	62.44 bc	28.97 de	46.18 bcd	56.07 bc	65.86 b	1.19 abc	11.48 b	1.57 abc	8.39 def
Kiyomi x Sunburst	61.37 bcd	32.18 cde	46.00 bcd	54.67 bc	66.66 b	1.23 abc	11.40 b	1.31 abcde	9.78 de

Means with the same letter are not significantly different

From the nine principal components obtained, the first principal component accounted for 44.23 % of the total variance (Table 6.3) and included L*, a* and b*, height, width and shape (Table 6.4). These variables made the highest contribution to the first factor and were inter-correlated as a group. The second principal component accounted for 21.50 % of the total variance (Table 6.3) and included acid percentage and the Brix:acid ratio (Table 6.4). These variables made the highest contribution to the second factor and are correlated. The third and fourth principal components accounted for 13.63 % and 11.35 % of the total variance respectively (Table 6.3) and consisted of the Brix % (Table 6.4).

Tabel 6.3 Principal components for the fruit characteristics of the seven citrus parents and the six citrus families for both years combined

Principal component	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eigenvalue	3.98	1.94	1.23	1.02	0.53	0.22	0.07	0.02	0.00
Variability (%)	44.23	21.50	13.63	11.35	5.88	2.38	0.75	0.26	0.02
Cumulative %	44.23	65.73	79.36	90.71	96.59	98.98	99.72	99.98	100.00

Table 6.4 Squared cosines of the fruit characteristics of the seven citrus parents and the six citrus families for both years combined

	F1	F2	F3	F4
L*	0.75	0.03	0.06	0.11
a*	0.69	0.06	0.01	0.05
b*	0.51	0.15	0.14	0.07
Height	0.78	0.03	0.11	0.06
Width	0.72	0.01	0.07	0.02
Shape	0.45	0.06	0.15	0.12
Brix %	0.02	0.01	0.46	0.51
Acid %	0.06	0.70	0.23	0.00
Brix:acid ratio	0.00	0.88	0.01	0.09

The first two principal components accumulated 65.73 % of the total variance (Table 6.3). This is sufficient to represent a reliable dispersion and was used to plot the parents and the families on a dispersion graph (Figure 6.1).

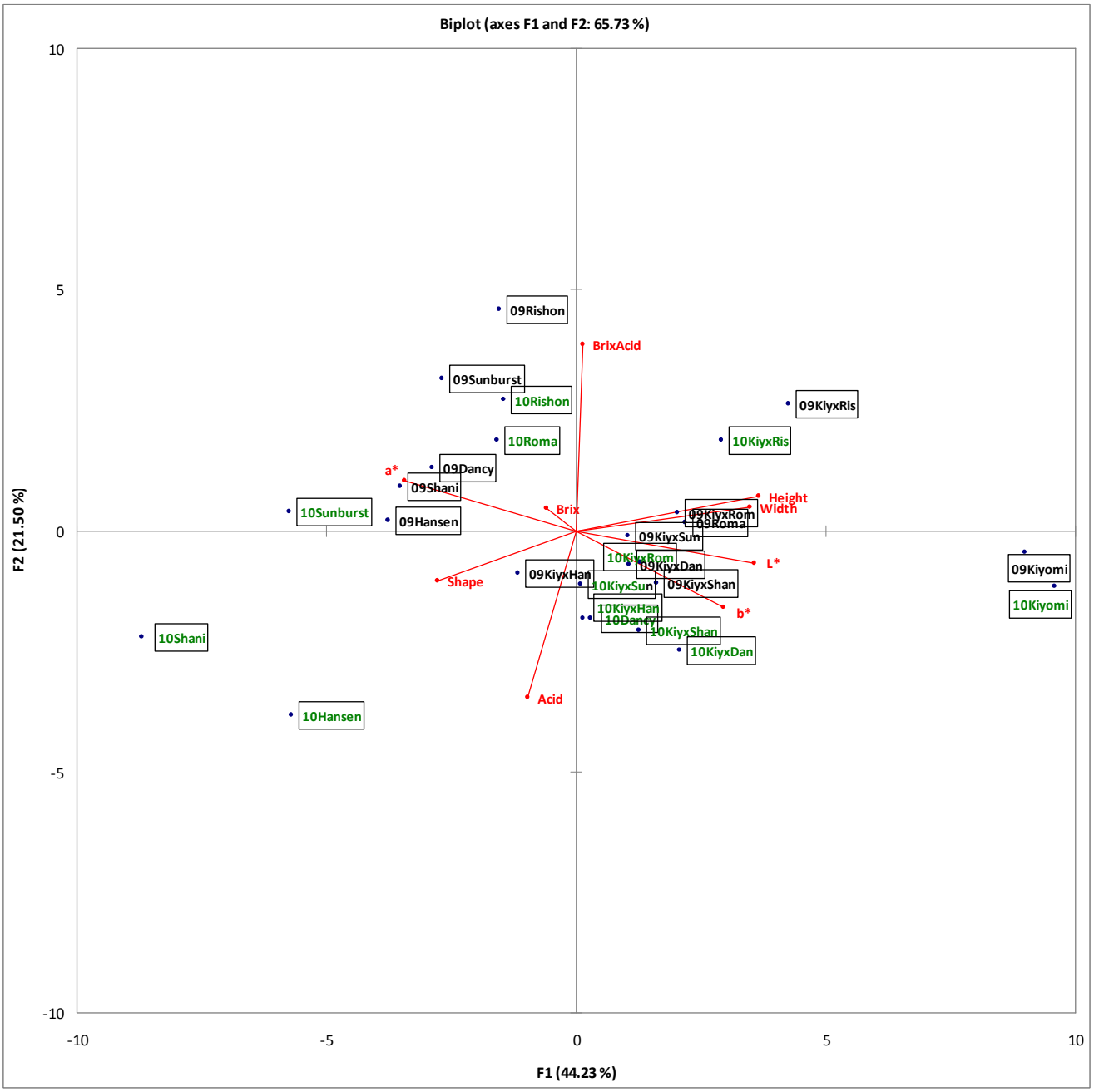

Figure 6.1 PCA using fruit characteristics of the seven citrus parents and the six citrus families for years 2009 and 2010

The fruit's external quality characteristics, L*, a*, b*, height, width and shape, contributed to the variation in the direction of the x axis, F1 (Figure 6.1).

Height, width, L* and b*, were located on the right hand side of the y axis in the second (top right) and third (bottom right) quadrants (Figure 6.1), and were positively correlated. Height and width were located close to each other and had a very strong positive correlation (r=0.95, Table 6.5), while L* and b* were located close to each other and had a strong positive correlation (r=0.82, Table 6.5). L* was located closer to height and width than b* and was slightly stronger positively correlated with height (r=0.59, Table 6.5) and width (r=0.63, Table 6.5) than b* with height (r=0.38, Table 6.5) and width (r=0.40, Table 6.5).

Shape and a* were located on the left hand side of the y axis in the first (top left) and fourth (bottom left) quadrants (Figure 6.1) and were positively correlated. Shape and a* were located some distance apart and the correlation was fairly weak (r=0.48, Table 6.5).

Shape and a* were located on the opposite side of the y axis to height, width, L* and b* (Figure 6.1). Shape and a* were therefore negatively correlated with height, width, L* and b*. a* had a fairly strong negative correlation with L* (r=-0.82, Table 6.5) and b* (r=-0.72, Table 6.5) and a slightly weaker negative correlation with height (r=-0.56, Table 6.5) and width (r=-0.50, Table 6.5). Shape had a fairly strong negative correlation with height (r=-0.78) and a slightly weaker negative correlation with width (r=-0.56). Shape had a weak negative correlation with L* (r=-0.29, Table 6.5).and b* (r=-0.20, Table 6.5).

The fruit's external quality characteristics of L*, a* and b*, height width and shape did not show any correlations with the fruit internal quality characteristics of Brix %, acid percentage and the Brix:acid ratio.

The fruit's internal quality characteristics, acid percentage and the Brix:acid ratio, contributed to the variation in the direction of the y axis, F2 (Figure 6.1).

Acid percentage was located in the fourth quadrant while the Brix:acid ratio was located in on the opposite side of the x axis in the second quadrant (Figure 6.1) and had a strong negative correlation (r=-0.80, Table 6.5). Acid percentage and the Brix:acid ratio showed no correlation with Brix % or any of the fruit's external quality characteristics of L*, a* and b*, height, width and shape.

The Brix % accounted for the variation in the third and fourth principal components (Table 6.4) and did not contribute to the variation in either the x or y axis. Brix % also showed no correlation with any of the other fruit characteristics (Table 6.5).

Table 6.5 Pearson correlation coefficients for fruit characteristics of the seven citrus parents and the six citrus families

	L*	a*	b*	Height	Width	Shape	Brix %	Acid %
a*	-0.82**							
b*	0.82**	-0.72**						
Height	0.59**	-0.56**	0.38					
Width	0.63**	-0.50**	0.40**	0.95**				
Shape	-0.29	0.48**	-0.20	-0.78**	-0.56**			
Brix %	-0.07	0.08	-0.20	-0.05	-0.02	0.07		
Acid %	-0.20	0.01	-0.01	-0.20	-0.20	0.14	0.25	
Brix:acid	-0.04	0.11	-0.21	0.08	0.03	-0.18	0.25	-0.80**

****P ≤ 0.05**

Female parent Kiyomi was located far right of the y axis in the third quadrant while the male parents were located on the left side of the y axis in the first and fourth quadrants (Figure 6.1). Female parent Kiyomi was therefore clearly different from the male parents.

Kiyomi was associated with fruit characteristics L*, b*, height and width (Figure 6.1). Kiyomi 2009 and Kiyomi 2010 had the highest means for L*, b*, height and width (Table 6.1 and Table 6.2) and in the case of height and width were significantly different from the male parents and the families. Kiyomi 2009 and Kiyomi 2010 were located close to each other, showing little variation between the years.

The male parents were mostly distributed over the first and fourth quadrants, while Roma 2009 was located in the second quadrant and Dancy 2010 in the third quadrant.

Rishon 2009, Sunburst 2009, Rishon 2010 and Roma 2010 were located in the first quadrant close to the y axis and were associated with each other and the Brix:acid ratio (Figure 6.1). Rishon 2009 had the largest mean and Sunburst 2009 the second largest mean for the Brix:acid ratio (Table 6.1), while Roma 2010 had the largest mean and Rishon 2010 the second largest mean for the Brix:acid ratio (Table 6.2).

Dancy 2009, Shani 2009, Hansen 2009 and Sunburst 2010 were located in the first quadrant close to the x axis. They were associated with each other and with a* and shape (Figure 6.1). Dancy 2009 had the largest mean for shape and a high mean for a*, Shani 2009 had the second largest mean for a* and a high mean for shape, Hansen 2009 had the second largest mean for shape and a high mean for a* (Table 6.1) and Sunburst 2010 had the largest mean for a* and the second largest mean for shape (Table 6.2).

Shani 2010 and Hansen 2010 were located in the fourth quadrant, some distance apart. They were not associated with each other or any of the other male parents (Figure 6.1). Shani 2010 was associated with a*, shape and acid percentage and had high means for a* and shape and the second highest mean for acid percentage. Hansen 2010 was associated with shape and acid percentage and had a high mean for shape and the highest mean for acid percentage (Table 6.2).

Roma 2009 was located in the second quadrant close to the x axis and was not associated with any of the other male parents. Roma 2009 was associated with the families and with height, width, L* and b* (Figure 6.1). Roma 2009 had the second highest mean for L* and the highest mean for b* but means for height and width were fairly low (Table 6.1).

Dancy 2010 was located in third quadrant close to the y axis and was not associated with any of the other male parents. Dancy 2010 was associated with the families and with b*, acid percentage and shape (Figure 6.1). Dancy 2010 had the highest mean for b* and shape but a low mean for acid percentage (Table 6.2).

The male parents were found to be widely distributed in all four the quadrants showing a large variation between the male parents as well as between the years.

The families were located fairly close together, mostly in the third quadrant, and were not associated with either the female or the male parents. The families were distributed intermediately to the two parents, along the x axis, for the external fruit characteristics L*, a*, b*, height, width, and shape. The families generally had means between the female and male parents for the external fruit characteristics L*, a*, b*, height, width, and shape (Table 6.1 and 6.2).

For the internal fruit characteristics of acid percentage and the Brix:acid ratio, the families, except for the Kiyomi x Rishon family, were located on the negative side of the y axis and were more associated with the acid percentage (Figure 6.1). The families, except the Kiyomi x Rishon family, had high means for the acid percentage (Table 6.1 and 6.2). The Kiyomi x Rishon family for 2009 and 2010 were located in the second quadrant and were not associated with the other families (Figure 6.1). The Kiyomi x Rishon family was more associated with the Brix:acid ratio than the other families and had the largest mean among the families for the Brix:acid ratio for both 2009 and 2010 (Table 6.1 and 6.2).

The families were located fairly close together, except for the Kiyomi x Rishon family, showing a small amount of variation between the families. The different families for 2009 and 2010 were, however, not located close to each other, indicating a variation in the families between the years.

CONCLUSIONS

One of the main goals of mandarin breeding programmes is to breed new varieties with improved fruit quality (Miyazaki et al., 2011). Important characteristics contributing towards a mandarin fruit's quality are the external fruit characteristics of rind colour, fruit size and fruit shape and the fruits internal quality characteristics of Brix %, acid percentage and the Brix to acid ratio (Ladaniya, 2008). Information on the interrelationships among these fruit characteristics as well as understanding how they are inherited is important to the citrus breeder when selecting parents and planning controlled crosses for breeding purposes. In this study, data was collected on some specific fruit characteristics contributing to fruit quality from the parents and progeny of six mandarin families. A principal component analysis was used to examine the interrelationships among these characteristics as well as to determine how they are inherited.

The fruit's external quality characteristics, L*, a*, b*, height, width and shape were found to be inter-correlated. Height, width, L* and b* were positively inter-correlated, while shape and a* were positively correlated. Shape and a* were then negatively correlated to height, width, L* and b*. The fruit's internal quality characteristics, acid percentage and the Brix:acid ratio were negatively correlated. The internal quality characteristic of Brix % showed no correlation with any of the other fruit characteristics.

Strong correlations were found among rind colour coordinates L*, a* and b*, fruit size characteristics height and width and the fruit's internal quality characteristics acid percentage and the Brix:acid ratio. Correlations between fruit size and fruit shape were fairly strong, while correlations between fruit size and rind colour were slightly weaker and correlations between fruit shape and rind colour were found to be fairly weak.

This study revealed that the fruit's external quality characteristics were inter-correlated as a group while the internal fruit characteristics of acid percentage and the Brix:acid ratio were correlated. No correlations were found between the fruit's external quality characteristics and the fruit's internal quality characteristics, while Brix % was found to be independent and showed no correlation with any of the other fruit characteristics studied. Strong correlations were found among fruit characteristics contributing to the same trait such a rind colour, fruit size and fruit internal quality, while fruit characteristics contributing to different traits were found to be mostly independent from each other. It is therefore possible to improve traits such as rind colour, fruit size, fruit shape and the fruits internal quality through breeding in mandarins.

Female parent Kiyomi and the male parents were very different from each other and while Kiyomi was associated with fruit characteristics L*, b*, height and width the male parents varied considerably with regard to the fruit characteristics studied. The male parents Rishon 2009, Rishon 2010, Roma 2010 and Sunburst 2009 were associated with each other and the Brix:acid ratio. Dancy 2009, Hansen 2009, Shani 2009 and Sunburst 2010 were associated with each other and with a* and shape. Male parents Hansen 2010 and Shani 2010 were not associated with any of the other male parents. Shani 2010 was associated with a*, shape and acid percentage and Hansen 2010 was associated with shape and acid percentage. Dancy 2010 and Roma 2009 were not associated with the other parents but were associated with the families. Dancy 2010 was associated with b*, acid percentage and shape and Roma 2009 was associated with height, width, L* and b*.

The families were located fairly close together, except for the Kiyomi x Rishon family, showing a small amount of variation between the families. The families were not associated with either the female or the male parents. They were distributed intermediately to the two parents for the external fruit characteristics L*, a*, b*, height, width, and shape and for the internal fruit characteristics the majority of the families were associated with the acid percentage, while the Kiyomi x Rishon family was associated with the Brix:acid ratio.

All of the families showed an improvement in the population over one of the parents for the external fruit characteristics L*, a*, b*, height, width, and shape. For the internal fruit characteristics the Kiyomi x Rishon family was found to be superior to the other families for the Brix:acid ratio, while the rest of the families were found to have inherited a high acid percentage. It is therefore possible to improve the fruit characteristics studied through breeding and selection.

Data over the two years revealed little variation between the years for female parent Kiyomi, while the male parents were widely distributed showing large variation between the years. The families had little variation between them; however, the different families did show a variation between the years. In citrus the climate, especially temperature, is known to influence fruit characteristics such as the development of rind colour, fruit size and the internal fruit quality and most probably contributed to the variation found between the years among the male parents and the families. Therefore it is recommended that for effective selection a mean performance over multiple years should be used, as only two years of testing will not be reliable.

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CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

On the fresh fruit market the fruit quality determines consumer acceptance and ultimately the price, making fruit quality equally important as yield (Van Rensburg, 1985). Therefore most of the research conducted on fruit crops has been to improve the fruit quality via breeding rather than increasing the yield of existing varieties (Possinghan, 1998). Fruit quality is determined by a combination of external and internal fruit characteristics. The external characteristics contribute to the fruit's appearance and marketability (Ladaniya, 2008) while the fruit's internal quality characteristics determine the flavour and therefore the eating quality (Van Rensburg, 1985). In citrus the most important external characteristics are rind colour, fruit size and fruit shape while the internal quality characteristics of Brix %, acid percentage and the Brix:acid ratio play the greatest role in the fruits flavour (Spiegel-Roy and Goldschmidt, 1996; Ladaniya, 2008). Therefore the main breeding goals for the improvement of fruit quality in mandarins is to breed fruit with an attractive appearance having a deep orange or orange red rind colour, good fruit size and attractive fruit shape together with a good internal quality (Spiegel-Roy and Goldschmidt, 1996; Nicotra, 2001).

A citrus breeding programme starts with the selection of suitable parents and the planning of controlled crosses (de Oliveira et al., 2003). Parents are chosen from the best available cultivars and unrelated plants are crossed to develop a highly heterozygous population from which superior genotypes can be selected. However, the breeder will not know whether the crosses were effective until the population is evaluated. Therefore information on the breeding value of available parents and the heritability of specific characters can be a great aid to the citrus breeder in predicting which parent combinations will produce superior progeny (Ray, 2002).

By quantifying the genetic variability in a population, a breeder can study the genetic relationships between hybrids and parents and gain an understanding of how characters are inherited (de Oliveira et al., 2003). However, very little information is available on the genetic variability of mandarin (*Citrus reticulata*) progenies and the inheritance patterns of fruit characteristics in citrus.

A study was therefore conducted to determine the genetic variability in the progeny of six mandarin families where female parent Kiyomi tangor (*Citrus unshiu x Citrus sinensis*) was crossed with male parents Dancy, Hansen, Rishon, Roma, Shani and Sunburst mandarins (*Citrus reticulata*). Using data collected on the fruit quality traits of rind colour, fruit size, fruit shape and fruit internal quality the genetic variability within and between the families was determined in order to gain information on the inheritance patterns of the traits studied and to determine the value of the parents used as breeding parents in citrus improvement.

The parents had a significant level of between parent variation for all the fruit characteristics studied. A principal component analysis (PCA) showed female parent Kiyomi to be very different from the male parents, while the male parents differed considerably from each other. The PCA associated Kiyomi with colour coordinates L* and b* and height and width, while the male parents were associated with the colour coordinate a*, shape, Brix %, acid percentage and the Brix:acid ratio.

Female parent Kiyomi had fruit with a light orange to orange yellow rind colour, while male parents Dancy and Roma had a rind colour more similar to the female parent Kiyomi. The male parents Hansen, Rishon, Shani and Sunburst had a deeper, more orange-red rind colour. Kiyomi had a larger fruit size and a more round fruit shape than the male parents, while among the male parents Rishon and Sunburst had the largest fruit size and Rishon had a more round fruit shape. For the fruit internal quality Roma had the highest mean for Brix %, Hansen and Shani had the highest means for acid percentage and Rishon and Roma had the highest means for the Brix:acid ratio. However, when considering the Brix % together with the Brix:acid ratio it could be seen that, at the time of sampling, Roma had an internal quality superior to the other parents.

There was a significant level of between family variation for all the fruit characteristics studied. A PCA showed a lot less variation between the families than between the male parents, except for the Kiyomi x Rishon family which was found to be different from the other families. The families were not associated with either the female or the male parents. They were distributed intermediately to the two parents for the external fruit characteristics L*, a*, b*, height, width and shape. For the internal fruit characteristics the majority of the families were associated with the acid percentage, while the Kiyomi x Rishon family was associated with the Brix:acid ratio.

All of the families showed some improvement in the population's rind colour over the female parent Kiyomi. The Kiyomi x Dancy and Kiyomi x Roma families had a population with a lighter, more yellow-orange rind colour while the Kiyomi x Hansen, Kiyomi x Rishon, Kiyomi x Shani and Kiyomi x Sunburst families had a population with a deeper, more orange-red rind colour. Parent values showed that male parents Dancy and Roma had rind colour more similar to the female parent Kiyomi, while other male parents Hansen, Rishon, Shani and Sunburst had a more orange-red rind colour. Therefore it can be seen that the families with male parents having a superior rind colour showed a greater improvement in the population when crossed with female parent Kiyomi.

The Kiyomi x Dancy, Kiyomi x Rishon and Kiyomi x Shani families showed an improvement in fruit size over the male parent, while all of the families showed an improvement in the fruit shape over the male parent. The Kiyomi x Rishon family had a larger fruit size and a more round fruit shape than the other families, while male parent Rishon had the largest fruit height and more round fruit than the other male parents. Therefore crosses using male parent Rishon with female parent Kiyomi showed a greater improvement in the population, giving fruit with a larger size and rounder shape.

All of the families had an increase in the Brix %, acid percentage and the Brix:acid ratio over one of the parents. Many of the families showed heterosis, having family means greater than both parents for Brix % and the Brix:acid ratio, while all the families except the Kiyomi x Rishon family showed heterosis for acid percentage. The Kiyomi x Hansen family had the highest Brix % and acid percentage but a low Brix:acid ratio, while male parent Hansen had the highest acid percentage and the lowest Brix:acid ratio. The Kiyomi x Rishon family, on the other hand, had the highest Brix:acid ratio and the lowest Brix % and acid percentage, while male parent Rishon had the highest Brix:acid ratio and the lowest Brix % and acid percentage. Therefore it can be seen that male parents with higher means resulted in families with higher means while male parents with lower means resulted in families with lower means. In this study the Kiyomi x Roma family had an internal quality superior to the other families, while male parent Roma had an internal quality superior to the other parents. Therefore crosses using male parent Roma with female parent Kiyomi resulted in progeny with a superior fruit quality. However there is constant change in a citrus fruits' internal quality through out the season, therefore this data is only relevant to the time of sampling and testing.

The study revealed that all of the families showed an improvement in the population for rind colour, fruit shape and the fruits internal quality while half of the families showed an improvement for fruit size. All of the families did, however, show heterosis for all of the fruit characteristics, containing some individuals superior to both parents. Male parents with a deeper more orange-red rind colour, larger fruit size, rounder fruit shape and superior internal quality resulted in families with a greater improvement in the population. Therefore the male parent has a large influence on the progeny and only male parents of the highest quality should be used in breeding programmes to increase the chance of obtaining a superior new cultivar.

A variance component analysis for the families revealed a greater variation within the families than between the families for all the fruit characteristics studied. This high genetic variation found within the families is due to the high heterozygosity that occurs in citrus and it is therefore essential to use the largest possible within family sample. The within tree variation was lower than the within family variation for all the fruit characteristics studied. This indicated that the sample of five fruit per tree used for the rind colour, fruit size and fruit shape data gave a homogeneous sample of the tree, while the two fruit samples tested per tree for the fruit's internal quality showed little variation between the samples and can be used to obtain a reliable result. A high repeatability, between trees within a family, was found for the rind colour, fruit size and fruit internal quality measurements and therefore only a small gain in accuracy would be attained from increasing the number of measurements per tree. A lower repeatability was found for fruit shape and an increase in the number of measurements per tree could lead to a worthwhile gain in accuracy for this characteristic.

The consistency in performance was determined for the parents and the families over the two years of the study. The parents had a significant year x parent variation for all the fruit characteristics studied except a* and shape and a significant year variation for all the fruit characteristics except L* and a*. The PCA showed very little variation for female parent Kiyomi between the years, however the male parents were widely distributed and accounted for the large variation found for the parents between the years.

The families had a significant year x family variation for all of the rind colour coordinates and Brix % and a significant year variation for all the fruit characteristics except acid percentage, while the PCA also showed some variation between the years for the families. The variance component analysis for the families showed that the year and the year x family variation contributed little to the total variation for the rind colour, fruit size and fruit shape measurements, however for Brix % the year variation contributed largely to the total variation, while the year x family variation for Brix % and the Brix:acid ratio also had a small contribution. The intraclass correlation coefficient, relevant to selection within the families, was found to be fairly low for all the fruit characteristics studied and indicated that the variation found within the families was only partly genetic and non-genetic factors contributed to the variation in the phenotype. Therefore, it can be concluded that only two years of testing will not be reliable and a mean performance over multiple years data should be used for effective selection.

Interrelationships among the fruit characteristics studied were determined using a PCA based on Pearson's correlations. The fruit's external quality characteristics L*, a*, b*, height, width and shape were found to be inter-correlated as a group while the internal fruit characteristics of acid percentage and the Brix:acid ratio were correlated. No correlations were found between the fruit's external quality characteristics and the fruit's internal quality characteristics, while Brix % was found to be independent and showed no correlation with any of the other fruit characteristics studied. Strong correlations were found among rind colour coordinates L*, a* and b*, fruit size characteristics height and width and the fruit's internal quality characteristics of acid percentage and the Brix:acid ratio. Therefore it can be seen that strong correlations exist among fruit characteristics contributing to the same traits such a rind colour, fruit size and the fruit's internal quality, while fruit characteristics contributing to different traits are mostly independent from each other. It is therefore possible to improve traits such as rind colour, fruit size, fruit shape and the fruit internal quality through breeding methods in mandarins.

Conventional cross breeding is and will always be the foundation of citrus variety improvement. However due to obstacles, such as sterility, self and cross incompatibility and poly-embryony that occur in many important citrus varieties the development of improved citrus cultivars through conventional breeding methods is very limited (Grosser and Gmitter, 1996). New developments in biotechnology, *in vitro* cell and tissue culture methods and plant molecular biology and their incorporation into breeding programmes have now opened up new opportunities for the genetic improvement of citrus (Grosser and Gmitter, 1996; Spiegel-Roy and Goldschmidt, 1996).

Genetic maps of the citrus genome can be useful in locating genes with a specific function(s) (Spiegel-Roy and Goldschmidt, 1996) and marker-assisted breeding and selection can greatly increase the efficiency of a citrus breeding programme (Roose, 2007). While linkage maps can be used for QTL studies (Garcia et al., 1999) and allow for the determination of the mode of inheritance of traits and are therefore very useful in to the citrus breeder (Peña et al., 2007). An understanding of the genetic control of agriculturally important traits, together with the ability to manipulate and modify citrus genomes, can therefore provide a base for more precise and specific manipulation of tree and fruit characteristics in the future (Gmitter et al., 2007).

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SUMMARY

In a citrus breeding programme, information on the breeding value of available parents and the heritability of specific characters can be an important aid to the breeder when planning controlled crosses for cultivar improvement. By quantifying the genetic variation in a population the breeder can study the relationships between the hybrids and parents and gain an understanding of how certain characteristics are inherited. Therefore a study was undertaken to investigate the genetic variation in the progenies of six mandarin families, where female parent Kiyomi tangor was crossed with male parents Dancy, Hansen, Rishon, Roma, Shani and Sunburst mandarins. Data were collected over a two year period for the fruit quality characteristics of rind colour, fruit size, fruit shape and fruit internal quality.

A significant level of variation was found between the families for all the fruit characteristics studied. The within family variation was larger than the between family variation and indicated a high level of genetic variation within the families, while the within tree variation was found to be lower than the within family variation. Data collected over a two year period showed significant year and year x family variation for many of the fruit characteristics. The intraclass correlation coefficient, relevant to selection within the families, was found to be fairly low for all the fruit characteristics studied and indicated that the variation found within the families was only partly genetic and non-genetic factors contributed to the variation in the phenotype. Therefore only two years of testing will not be reliable and a mean performance over multiple years data is recommended for effective selection.

All the families showed an improvement in the population for rind colour, fruit shape and the fruit internal quality while half of the families showed an improvement for fruit size. All the families showed heterosis with some individuals being superior to both parents. Families with male parents Hansen, Rishon, Shani and Sunburst had a rind colour superior to the other families, while the Kiyomi x Rishon family had larger fruit with a more round fruit shape and the Kiyomi x Roma family had a superior internal quality. This study revealed the male parent to have a large influence on the progeny and male parents with a deeper orange rind colour, larger fruit size, better fruit shape and superior internal quality resulted in families with a greater improvement in the population. Therefore only male parents of the highest quality should be used as parents in breeding programmes in order to increase the chance of obtaining a superior new cultivar.

Key words: *Citrus reticulata*, fruit tree breeding, breeding parents, genetic variation, inheritance, fruit quality, rind colour, fruit size, fruit shape, internal quality.

OPSOMMING

In ‘n sitrus teelprogram is die teelwaarde van die beskikbare teelouers sowel as die erflikheid van spesifieke eienskappe van onskatbare waarde tydens die beplanning van gerigte kruisings vir spesifieke teeldoelwitte. Deur die genetiese variasie in ‘n teelpopulasie te kwantifiseer kan die teler die verwantskap tussen kruisingsnageslag en ouers bestudeer en sodoende meer leer oor hoe sekere eienskappe oorgeërf word. Dit is dus met dié doel dat die genetiese variasie in die nageslag van ses mandaryn families ondersoek is waar Kiyomi tangor as vroulike ouer gekruis is met Dancy, Hansen, Rishon, Roma, Shani en Sunburst mandaryne as manlike ouers. Data t.o.v. die vrugkwaliteitseienskappe van skilkleur, vruggrootte, vrugvorm en interne kwaliteit is oor ‘n tydperk van twee jaar versamel.

Daar is betekenisvolle variasie tussen die families t.o.v. alle vrugkwaliteitseienskappe gevind. Die variasie t.o.v. vrugkwaliteitseienskappe binne families was groter as die variasie tussen families en dui op ‘n hoë genetiese variasie binne families, terwyl die variasie binne ‘n boom kleiner was as in ‘n familie. Data ingesamel oor ‘n tydperk van twee jaar wys ‘n beduidende jaar x familie variasie t.o.v die meeste vrugeienskappe. Die intraklas korrelasiekoeffisiënt wat gebruik word vir seleksie binne families was redelik laag vir al die vrugeienskappe wat bestudeer is. Dit dui daarop dat die variasie van ’n fenotipe sowel geneties as nie-geneties van aard is. Twee jaar van evaluering is nie voldende nie en evaluering oor 'n aantal jare sal meer betroubaar wees vir doeltreffende seleksie.

Al die families het ‘n verbetering in skilkleur, vrugvorm en interne kwaliteit getoon terwyl die helfte van die families ‘n verbetering in vruggroote gehad het. Heterose het in al die families voorgekom en sommige individue van die nageslag het beter vertoon as beide die ouers. Families met Hansen, Rishon, Shani en Sunburst as manlike ouers se skilkleur was beter as die ander families, terwyl die Kiyomi x Rishon familie groter en ronder vrugte gehad het en die Kiyomi x Roma familie ‘n beter interne vrugkwaliteit. Hierdie studie het getoon dat manlike ouers ‘n beduidende invloed op vrugeienskappe van die nageslag het en dat manlike ouers met ‘n dieper rooi skilkleur, groter vrugte, beter vrugvorm en beter interne kwaliteit aanleiding gegee het tot families waarvan die nageslag ‘n groter verbetering getoon het. Dit is dus belangrik dat slegs manlike ouers van uitstaande kwaliteit as teelouers gebruik moet word in ‘n teelprogram ten einde die kanse te verhoog om ‘n uitstaande nuwe kultivar te verkry.