THE INHERITANCE AND GENETIC EXPRESSION OF THE RIPENING INHIBITOR (*rin*) GENE IN TOMATO

By

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CHAPTER 1

INTRODUCTION

The cultivated tomato (*Lycopersicon esculentum* Mill) is relatively new to the world's most important food crops. In the past century, tomatoes became one of the most popular and widely consumed vegetable crops. The annual world production of tomatoes in the early 1990's was already 65 million ton. The main tomato growing countries include the USA, several countries of Europe, China, Turkey, Egypt and Russia.

In South Africa the tomato is the second biggest cultivated vegetable crop with an annual production of about 400 000 tons fresh tomatoes and 200 000 tons processed tomatoes. In 1995 the value of the tomatoes sold on the 15 National Freshproduct Market was R330.9 million. Processed items like paste, puree, soup, juices, ketchup, drinks and whole peeled tomatoes are also a considerable source of income (Laurie, personal communication).

The quality of tomatoes is a very complicated and comprehensive subject as color, shape, keeping quality and flavor are all aspects, which need to be taken into account when determining quality. The best eating quality occurs when tomatoes are slightly underripe and flavor benefits from retaining fruit on the plants for as long as possible (*Richardson and Hobson*, 1987). Most commercial tomatoes are picked at the green-mature or early color stages to ensure sufficient storage life for transport. This problem of harvesting fruit at the early stages for transport and then treated with ethylene for marketing has led to

frequent complaints about the lack of flavor by the consumers. Although the present commercial cultivars normally keep well, their storage life is barely long enough to enable picking at an early color stage for distant markets (Nguyen *et al*, 1991).

One of the main factors influencing the general quality of tomatoes is the keeping quality. One of the abilities of changing the length of the tomato fruit storage period is by using ripening mutants like the 'ripening inhibitor' (*rin*). This ripening mutant (*rin*) delay many ripening processes in the tomato fruit. The mutant has, however, been linked with a reputation for poor flavor (*Richardson and Hobson*, 1987).

The longer storage life of *rin* hybrid fruit should be advantageous to the tomato industry as the fruit can be harvested at a more advanced stage than present. This might allow good quality to be combined with a reasonable product life.

The aim of the study was:

1) To determine the expression and combining ability of the *rin*-gene in some of South African tomato cultivars,

2) To investigate suitable genetic correlations between the *rin*-gene and other yield and quality characteristics,

3) To determine the heritability of shelf life and other yield and quality characteristics,

4) To identify the expression of heterosis of the *rin*-gene containing genotypes.

CHAPTER 2

LITERATURE REVIEW

2.1 Origin and early history

The tomato (*Lycopersicon esculentum* Mill) is one of the most popular members of the *Solanaceae* family and originated in the western parts of South America. Numerous wild and cultivated relatives of the tomato can still be found in the mountainous region of the Andes in Peru, Equador and Bolivia as well as in the Galapagos Islands (Tigchelaar, 1986).

The first written account documenting the arrival of the tomato in Europe was in 1554 in Italy and it probably originated from Mexico. These early introductions were presumably yellow, rather than red in color, since the plant was first known as the golden apple. The tomato was first mentioned in North America in 1710. However, it was not until 1830 that the tomato began to acquire the popularity that has made it the indispensable food commodity it has become today.

The precise date when tomatoes were introduced in South Africa is uncertain, but it was already generally cultivated in 1847 in the Eastern-Cape. Breeding of tomatoes in South Africa started in 1932 by dr. J.J.D. Hofmeyer at the Subtropic Horticultural Research Station at Nelspruit. Breeding of tomatoes thereafter, resulted in the release of many cultivars with improved yield, quality and disease

resistance to the most important tomato diseases in South Africa (Laurie, personal communication).

2.2 The ripening-inhibitor (*rin*) mutant

The *rin* mutation, first reported by Robinson and Tomes (1968), is recessive, maps to chromosome 5 and is closely linked to the macrocalyx locus. The ripening inhibitor (*rin*) is a non-allelic mutant, which inhibit or greatly slow down a wide range of ripening processes in the tomato fruit.

The *rin* fruit fail to attain a normal level of pigmentation as a result of decreased accumulation of carotenoids, particularly lycopene and a decreased rate of chlorophyll loss, thus *rin* fruit remain green when wild-type fruit are fully red (Sink *et al*, 1974). The mutant fruit eventually 'ripen' to a lemon yellow color after several weeks, but fail to achieve normal flavor or aroma (McGlasson *et al*, 1987). Examination of total proteins extracted from wild-type and *rin* fruit reveals differences during ripening, some proteins being more abundant and others reduced in the mutant fruit as compared to the wild-type (Mizrahi *et al*, 1976).

Fruit of the *rin* mutant demonstrate an increased resistance to many common post-harvest pathogens (Robinson and Tomes, 1968) and had been maintained for a year or more without further signs of normal ripening or deterioration. Other aspects of growth and early fruit development appear unaffected by the *rin* mutation.

2.3 Fruit ripening and the *rin* tomato mutant

2.3.1 Biosynthesis and action of ethylene

Ethylene is a plant hormone, which regulates many aspects of growth, development and senescence (Abeles, 1973). Yang (1985a) reported that, as in the case of other hormones, ethylene is thought to bind to a receptor, forming an activated complex, which in turn triggers the primary reaction. The primary reaction then initiates a chain of reactions, including the modification of gene expression, and it leads to a wide variety of physiological responses.

According to Yang (1985b) there are four levels of manipulation that can be used to regulate ethylene responses namely a) to control the level of ethylene in the tissue by addition or removal of ethylene, b) to regulate the level of ethylene in the tissue by stimulating or inhibiting ethylene biosynthesis, c) to modify the binding characteristics of ethylene to the receptor, or amount of receptor, and d) to manipulate the ethylene-dependent gene expression.

2.3.1.1 Regulation of ethylene biosynthesis

Adams and Yang (1979) elucidated the sequence for the pathway of ethylene biosynthesis in ripening apples, and this pathway (Fig 2.1) has since been shown to be operative in all other tested plant tissues. According to the pathway, 1-aminocyclopropane-1-carboxylic acid synthase (ACC-synthase), which converts

S-adenosyl methionine (SAM) to ACC, is the main site of control of ethylene biosynthesis (Yang, 1980).

ACC-synthase seems to be a pyridoxal enzyme, because the enzyme requires pyridoxal phosphate for maximal activity, and is strongly inhibited *in vivo* as well as *in vitro* by N-[2-(2-amino-ethoxy)-ethenyl] glysine (AOA) and (aminooxy) acetic acid (AVG) (Boller *et al*, 1979). AOA and AVG are well known inhibitors of pyridoxal phosphate-dependent enzymes. Cameron *et al* (1979) showed that the conversion of SAM to ACC is the rate-limiting reaction in most plant tissues, because the application of ACC to various plant organs including root, stem, leaf and fruit resulted in a marked increase in ethylene production.

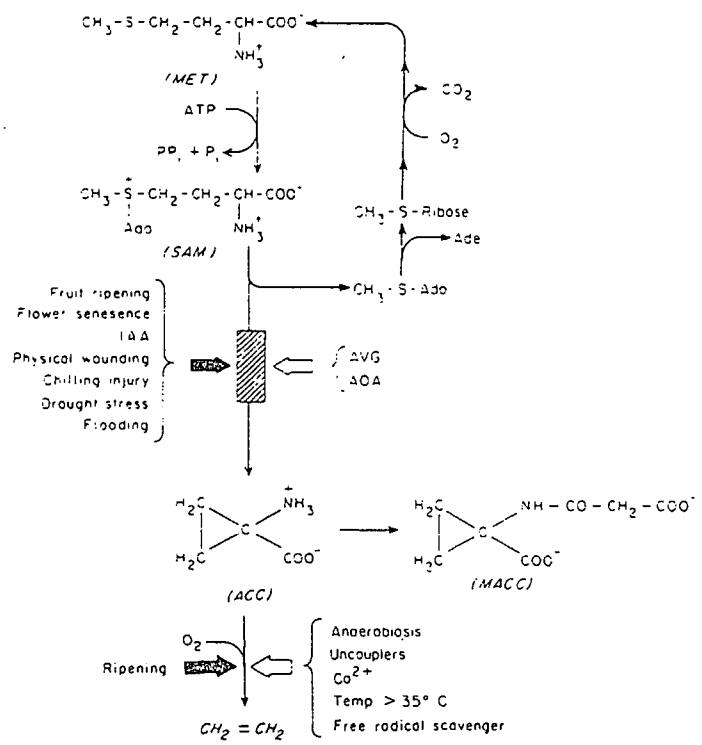

Fig. 2.1 Regulation of ethylene biosynthesis (Yang, 1985a)

This indicates that the enzyme converting ACC to ethylene (EFE) is present in most plant tissues. Lieberman (1979) reported that this enzyme, however, has not yet been identified, but is known to be very labile and is assumed to be membrane-bound. ACC-synthase activity, and therefore ethylene formation, increases dramatically in ripening tomato fruit (Su *et al*, 1984).

2.3.2 Ethylene production and respiration of the *rin* mutant

Fruit ripening is a complex process that includes increased ethylene and carbon dioxide production, softening, and changes in colour and levels of volatiles and soluble sugars (Tong and Gross, 1990).

Biale (1960) has classified fleshy fruits into two general categories, namely climacteric and nonclimacteric, depending upon the changes in respiration, which occur during ripening and the response to exogenous ethylene.

In nonclimacteric fruits, changes in color and composition are not accompanied by a rise in ethylene or CO_2 (Biale *et al*, 1954). Exogenous ethylene causes a rise in respiration when it is applied and after it is removed, the respiration rate returns to normal. In contrast, a large increase in respiration and ethylene production accompanies ripening in climacteric fruit and exogenous ethylene stimulates respiration and ripening of mature unripe fruit (Biale, 1960). Once stimulated by exogenous ethylene or by their own ethylene, climacteric fruits produce ethylene autocatalytically (Burg and Burg, 1965). McMurchie *et al* (1972)

concluded that the biogenesis of ethylene in climacteric fruit is regulated by two systems : System 1 is the low level of ethylene present in fruit before the onset of ripening and System 2, is responsible for the autocatalytic increase in ethylene production, which accompanies ripening. It was further postulated that nonclimacteric fruit have System1, but not System 2 (McMurchie *et al*, 1972). The present evidence is that ethylene envolved in both systems is produced by the ACC-synthase pathway (Yang, 1980).

Normal tomato fruits have been shown to be of the climacteric type, while the *rin* tomato mutant is a nonclimacteric fruit, because of its peculiar respiratory and ethylene production behavior (Herner and Sink, 1973). Evidence presented for classifying the *rin* mutant as a nonclimacteric fruit includes a) its lack of a respiratory climacteric and of a rise in ethylene production, b) the response to exogenous ethylene which resulted in enhanced respiratory activity only while ethylene was presented, c) the repeated stimulation of CO_2 production by repeated ethylene treatments, and d) its response to propylene where CO_2 production was stimulated, but ethylene production was not. All of these responses have been shown to be typical of nonclimacteric fruits (Biale, 1960; McMurchie *et al*, 1972).

According to Herner and Sink (1973), the *rin* tomato mutant lacks the genetic capacity for autocatalytic production of ethylene or, in terms of McMurchie *et al* (1972), lacks the System 2 of ethylene production as do other nonclimacteric fruits. McGlasson (1985) concluded that *rin* fruit lack the ability to produce

ripening-specific ethylene receptor(s) or a specific cellular component, which binds ethylene as evidenced by the failure of added ethylene to induce normal ripening. This specific cellular component is conceived to develop in fruits of normal strains during growth (McGlasson *et al*, 1975).

Ethylene production *in vivo* is regulated by a variety of developmental and environmental factors. Ethylene production is therefore induced during certain stages of development, such as seed germination, fruit ripening, flower and leaf senescence and abscission. It is also induced by various environmental stresses, such as wounding, chilling, drought and by treatments with auxins.

According to Acaster and Kende (1983), the ACC synthase enzyme is readily "turned on" by many stimuli, including wounding. The fact that *rin* fruits produced ethylene in response to wounding by cutting suggest that either a) the stress ethylene was not produced through the same pathway as the ethylene during the climacteric of normal fruit, or b) cutting or wounding stimulated the synthesis of ethylene through the normal pathway, but exogenous ethylene was unable to do so, for undetermined reasons. Abeles and Abeles (1972) have suggested that wound or stress ethylene does come through the same pathway from methionine as that produced during the ripening of normal fruit. They also showed that the efficiency of conversion of labeled methionine to ethylene fell 50 percent after wounding, which might indicate another pathway for at least part of the stress-induced ethylene. There is also the possibility that wounding stimulates the

System 1 production of ethylene, but is incapable of stimulating System 2 in nonclimacteric fruits (McMurchie *et al*, 1972).

Rin mutant fruits were also observed to produce ethylene in response to fungal invasion, but it was not determined if the ethylene was produced by the organism or by the fruits (Herner and Sink, 1973).

2.4 Polygalacturonase and fruit ripening

2.4.1 Differential expression of isozymes of polygalacturonase

Tomato fruit polygalacturonase [poly (1.4-α-D-galacturonide) glycanohydrolase, EC3.2.1.15 is a cellwall hydrolases catalyzing pectin solubilization and degradation during ripening (Zheng *et al*, 1994). Polygalacturonase (PG) is synthesized *de novo* during ripening and accumulates in the tomato in several forms (Tucker *et al*, 1980; Ali and Brady, 1982; DellaPenna and Bennett, 1988). Polygalacturonase isoform 1 (PG 1) accumulates early during ripening with a *mol wt* of 110 kDa as determined by column chromatography (Pressey, 1986b). As fruit development continues, two smaller isoforms, polygalacturonase isoform 2A (PG 2A) and polygalacturonase 2B (PG 2B) of approximately 42 and 46 kDa, respectively, accumulate (Brady *et al*, 1982). According to Ali and Brady (1982), all three isozymes are glycoproteins and antibodies to PG 2A. PG 2A and PG 2B have identical isoelectric points, and are composed of single catalytic PG polypeptides, but only differ in the level of glycosylation (DellaPenna and

Bennett, 1988). Digestion of PG 1 and PG 2A with trypsin and chymotrypsin yield nearly identical peptide patterns (Tucker *et al*, 1980).

PG 1 is a complex composed of at least one catalytic PG 2 polypeptide tightly associated with a 38 kDa noncatalytic glycoprotein, known as the converter or β subunit protein (Tucker *et al*, 1980; Pogson *et al*, 1991). The β subunit of PG 1 is a heat-stable glycoprotein found in high levels in fruit cell tissues and at lower levels in leaf tissue (Pressey, 1986a). The amount of immunologically detectable β subunit protein increases in developing tomato fruit well before the appearance of catalytic PG 2 protein.

The role of the β subunit in regulating PG activity *in vivo* remains unresolved, but line evidence from molecular and biochemical studies suggest that PG 1 is the physiologically active complex *in vivo* and have implicated the β subunit as playing an important role in immobilizing or regulating the catalytic PG 2 protein *in vivo* (Giovannoni *et al*, 1989; DellaPenna *et al*, 1990).

During ripening, the first detectable dissolution of the middle lamella occurs very early in ripening, when the fruit contains mainly PG 1. This suggests that *in vivo* PG 1 is responsible for initiating wall disruption, by attacking the middle lamella. According to Crookes and Grierson (1983) later stages of disorganization, in which the primary wall is attacked, may be due to the action of PG 2.

The relative amounts of polygalacturonase isoenzymes in different cultivars vary widely and differ in fruit sampling, extraction and assay techniques (Pressey, 1986b).

2.4.2 Polygalacturonase and fruit softening

The tomato fruit cell wall (Fig 2.2) is similar to other plant cell walls consisting of cellulose microfibrils embedded in a matrix of crosslinking molecules (Huber, 1983).

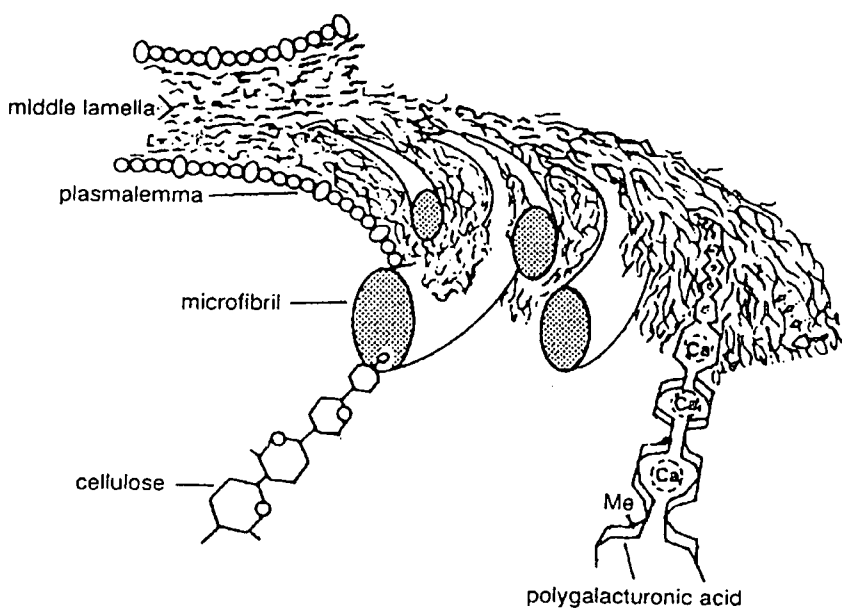

Fig. 2.2 The primary cell wall at the interface of two fruit cells (Kramer *et al*, 1989).

These matrix components include glycoproteins, hemicelluloses and polyuronides. Polyuronides or pectins are the middle lamella region, joining adjacent plant cell walls and provide adhesion to juxtaposed cell walls, thereby imparting firmness to plant organs, including unripe fruit (Crookes and Grierson, 1983).

One of the most characteristic changes in the cell wall associated with fruit softening is the solubilization of pectin (Fig 2.3), which is accompanied by dissolution of the middle lamella and eventual disruption of the primary cell wall (Crookes and Grierson, 1983). Enzymes involved in the metabolism of pectin, include pectinmethylesterase (PE) and polygalacturonase (PG) (Huber, 1983; Giovannoni *et al*, 1989). PE and PG are both physically associated with the cell wall fraction. PG has been implicated as an important enzyme in fruit softening because a) its appearance during ripening corresponds to the increase in fruit softening; b) there is a correlation between levels of PG activity and the extent of fruit softening; c) it degrades isolated fruit cell walls *in vitro* in a manner similar to that observed during ripening and d) several ripening-mutants that have been described with delayed or decreased softening are deficient in PG activity.

Hobson (1964) and Tucker *et al* (1980) found that PG activity is absent from mature green tomato fruits and that the activity develops rapidly during ripening. There is, however, disagreement as to just when the increase in polygalacturonase activity commences. Poovaiah and Nukaya (1979) reported an increase in polygalacturonase activity before the onset of the respiratory

climacteric. Tigchelaar *et al* (1978) have suggested that the appearance of PG activity may be the initial trigger of fruit ripening and that ethylene synthesis and other events occur as a consequence of PG activity. The results of Brady *et al* (1982) and Tucker *et al* (1982) clearly indicate that ethylene synthesis began before any polygalacturonase activity was detected, and that the hypothesis of Tighelaar *et al* (1978) should be rejected.

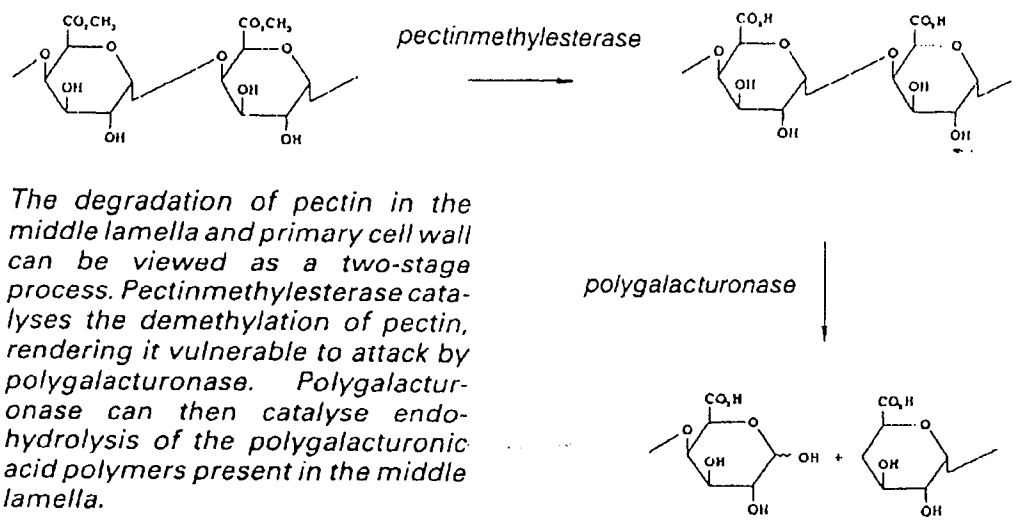

Fig. 2.3 Degradation of pectin (Kramer *et al*, 1989).

In addition to the proposed role of polygalacturonase in fruit softening, it has been speculated that endo-polygalacturonase dependent polyuronide hydrolysis may generate oligosacharide molecules capable of influencing other aspects of the ripening process (Baldwin and Pressey, 1988). Brecht and Huber (1986) demonstrated that cell wall fragments are capable of stimulating ethylene

biosynthesis when applied exogenously to tomato pericarp tissue. Baldwin and Pressey (1988) reported that infiltration of purified polygalacturonase protein into mature green tomato fruit stimulates ethylene production.

2.4.3 Polygalacturonase levels and the *rin* mutant

Polygalacturonase has an important role in tomato softening associated with ripening (Crookes and Grierson, 1983). The correlation of low PG enzyme levels in the *rin* mutant supported the hypothesized role of PG involvement in fruit ripening.

Rin genotypes, which soften the least, display only trace amounts of PG activity and protein at a time corresponding to normal fruit ripening (Buescher and Tigchelaar, 1975; Biggs and Handa, 1989). DellaPenna *et al* (1987) reveals that barely detectable levels of PG 1 isoform accumulate in *rin* fruit at a time corresponding to several weeks after the onset of normal ripening. Themmen *et al* (1982) and Tucker and Grierson (1982) showed that PG 2 isoform degrades tomato-cell-wall preparations *in vitro* and to be the main component absent from the cell-wall-associated proteins of the *rin* mutant.

McGlasson *et al* (1975) and Giovannoni *et al* (1989) reported that the treatment of detached *rin* fruits with ethylene or propylene hastens the normal yellowing of the fruit without noticeable effects on lycopene accumulation, softening, autocatalytic ethylene biosynthesis, or polygalacturonase activity. Mizrahi *et al*

(1975) and Buescher (1977) on the other hand report that treatment of *rin* fruit still attached to the vine with ethylene or 2-chloroethyly phosphonic acid (ethephon) results in 10 to 20 percent of normal lycopene accumulation and increased softness of the fruit.

Tucker and Grierson (1982) suggest that the lack of PG in *rin* arise from a partial or complete failure in the synthesis of PG. They also report that the *rin* mutation may be in a structural gene for PG, which results in an unstable or altered PG protein without enzyme activity and with no affinity for PG antibody. However, since the *rin* mutation has several different phenotypic effects it seems more likely that the mutation is in a regulatory gene, which affects a number of ripening-related changes, including PG synthesis.

2.4.4 Polygalacturonase gene expression and the *rin* mutant

Fruit ripening is a complex, developmentally regulated process resulting from the coordination of numerous biochemical and physiological changes within the fruit tissue. The availability of polygalacturonase cDNA clones made it possible to study the regulation of gene expression during tomato fruit development (Slater *et al*, 1985).

According to DellaPenna *et al* (1989) no PG gene transcription was detected in immature fruit and it first became detectable at the MG3 stage (when a rise in ethylene production occurred). Maunders *et al* (1987) and Biggs and Handa

(1989) have demonstrated that PG gene transcription and mRNA accumulation increases dramatically at the onset of ripening and continues to retain a high level of abundance throughout the remainder of fruit development. Hybridization to radiolabelled cDNA probes has demonstrated increased PG mRNA accumulation in breaker tomato fruit followed by a continual increase through the fully red stage. Biggs and Handa (1989) demonstrated that PG mRNA accumulation does not occur in roots, leaves or stems of the tomato plant. Grierson and Tucker (1983) suggested a correlation between the climacteric increase in the rate of ethylene production and PG levels. Maunders *et al* (1987) also report that ethylene stimulates the accumulation of PG mRNA in tomato fruits. Tieman and Handa (1989) report that PG accumulation is differentially regulated in different sections of tomato fruit. DellaPenna *et al* (1989) found that the changes in PG mRNA accumulation during ripening parallel the change in transcriptional activity of the PG gene, indicating that transcriptional control plays an important role in both the initiation and maintenance of PG expression during ripening in wild-type fruit.

Analysis of *rin* fruit development revealed low levels of PG mRNA and no induction of PG mRNA accumulation was observed when *rin* fruit were treated with exogenous ethylene. In correlation with the patterns of mRNA accumulation, the *rin* mutation showed reduced or barely detectable transcription of PG throughout fruit development. DellaPenna *et al* (1987) suggested that the lower PG mRNA accumulation in *rin* is because the mutation affects a regulatory step prior to translation.

2.5 Ultrastructure and pigment content of normal and *rin* tomatoes

The *rin* tomato mutant produces fruits that develop normally, but do not undergo several of the physiological changes associated with ripening in normal strains (Robinson and Tomes, 1968).

Simpson *et al* (1976) report that no detectable ultrastuctural differences existed between the organelles of the pericarp cells of normal fruits and those of the mutant. All of the cytoplasmic structures observed in normal fruits were also noted in the mutant fruits.

The major ultrastructural difference between normal and mutant fruits was in the behavior of the plastids during the chloroplast-chromoplast transformation. Although the chloroplast of *rin* fruits transform into chromoplast, the time taken is much longer than in normal tomato fruit.

According to Simpson *et al* (1976) there are features peculiar to mutants. The presence of many small vacuoles in the cytoplasm of immature *rin* fruit cells is a distinctive but not a unique feature of this mutant. As the fruits matured, Golgi bodies became less common and were rarely observed in 95-day *rin* fruits, but vesicles containing a fibrillar matrix and found near or in contact with the cell wall were seen more frequently. *Rin* fruits picked 95 days after anthesis could be distinguished from normal fruit of the same age by the presence of spiral tubular membranes in the cells of the epidermis and the layer immediately beneath it.

The chlorophyll content of the normal and *rin* mutant decreased as the fruits matured and in 32-day fruits, the *rin* mutant contained more chlorophyll and colored carotenoids. In contrast to the normal fruits, *rin* fruits slowly lost chlorophyll up to 95 days after anthesis and lost chlorophyll a at a faster rate than chlorophyll b, while the level of colored carotenoids in *rin* fruits increased slightly during this period. The loss of chlorophyll as the fruits matured paralled the observed decrease in the number of phytosynthetic lamellae in the plastids, and the presence of chlorophyll in 95-day fruits of *rin* was confirmed by the existence of grana in these fruits. Although chlorophyll persist in *rin* fruit much longer than in normal fruit, this characteristic does not appear to be the primary effect of the *rin* gene on ripening, but rather one of the modifications of the ripening process that is significantly changed.

According to Simpson *et al* (1976) the colored cartenoid level in mutant fruit began to increase after 50 days from anthesis, and corresponded to the accumulation of plastoglobules and, to a lesser degree, lycopene crystalloids in the plastids. Pigmentation in ripening fruit is generally agreed to be genetically controlled, with various kinds and quantities of carotenoids accumulating depending on the genetic control of each step in the biosynthesis pathway. Phytoene, β-carotene and λ-carotene were all present in lower quantities in *rin* than in normal fruit. Tomato carotenoids generally increase in quantity as normal fruits ripen. This appears to be the situation with phytoene and β-carotene in *rin*, whereas this pattern was not observed for the remaining carotenes. Therefore, the light yellow color, which gradually develops in *rin*, is β-carotene.

Simpson *et al* (1976) report that the delay in starch hydrolysis, granal lysis and carotenoid accumulation observed in the plastids of *rin* fruits is a pleiotropic effect rather than a specific effect. The presence of lycopene in untreated *rin* and the rapid induction of lycopene accumulation in fruits of *rin* by 2-(4-chlorophenylthio) ethyldiethyammonium chloride (CPTA) suggests that the enzyme necessary for *de novo* carotenogenesis are indeed present in the plastids of mature mutant fruit (Sink *et al*, 1974). Therefore, the low concentration of lycopene in *rin* may be due to a delay in lycopene synthesis, or its suppression.

Simpson *et al* (1976) concluded that some of the apparent deficiencies in the mutant fruit, including the protracted transformation of chloroplast to chromoplast, are due to the suppression of nuclear action. The major deficiency in the mutants is the lack of capacity to produce certain cellular components, illustrated particularly in *rin* by the absence of changes in ethylene evolution during coloring.

2.6 Effect of *rin* on the flavour and aroma of tomato fruit

The ripening mutant gene in tomato inhibits, or greatly slows down, a wide range of processes, leading to a markedly extended shelf life and inferior flavor (Kopeliovitch *et al*, 1979; Kopeliovitch *et al*, 1980). McGlasson *et al* (1987) found that a complex mixture of volatile compounds (aroma) interacts with sugars and acids to give characteristic tomato flavor. Since flavor change accompanies

ripening, it might also be affected by the mutant genes in the heterozygous condition (Kopeliovitch *et al*, 1982).

McGlasson *et al* (1987) found that the *rin* mutant fruits envolved numerous volatiles compounds, but generally in smaller amounts than in normal tomato fruit. Sixty-nine odorous compounds were found in volatilize of ripe normal fruit, of which 46 were common to fruit of *rin* and normal tomato fruit. Many of these compounds found in normal fruits were deficient in the mutant *rin* fruits, as shown in Table 2.1.

McGlasson *et al* (1987) identified a number of compounds that were common in both normal and *rin* tomatoes, but relatively few that was lacking or present in different amounts in the mutant. Notable among the compounds lacking in the *rin* fruit were the two strongly odorous sulfur-containing compounds, 2-isobutylthiazole and 2-methylthioethanol. These two compounds have been identified as an important flavor component in tomatoes. The authors also concluded that the compounds which cause intense aromas in normal fruit, but are deficient in the mutants, play a key role in determining the acceptability of different cultivars, whereas the compounds found to be common to both normal and mutants comprise the "normal background aroma" in fresh tomatoes.

Table 2.1 Identified odors intense in normal tomato fruit but deficient in *rin* mutants (McGlasson *et al*, 1987).

1. Hexanal
2. 2-Methylbut-2-enal
3. 3-Methylbutanol
4. 4-Methylpentanol
5. 3-Methylbut-2-enol
6. 3-Methylpentanol
7. 2-Methylpentanol
8. *Trans*-6-methylhept-5-en-2-one
9. Hexanol
10. Dimethyltrisulphide
11. 2-Isobutylthiazole
12. 2-Methylthioethanol
13. *p*-Cymen-8-ol
14. Geranyl acetone
15. *a* Cresol

De Bruyn *et al* (1971) and Stevens *et al* (1979) have suggested a good correlation between high sugar and acid levels in tomato fruit and good taste. Kopeliovitch *et al* (1982) found that the sugar content and acid content of tomatoes increase with ripening. However, its increase starts at an early developmental stage before the initiation of the ripening process, reaching a peak at the orange stage.

Stevens *et al* (1979) reported that while all genotypes with *rin* genes were inferior in flavor to the fruit of the normal cultivars, their reducing sugars and acidity levels were within the range of normal cultivars. It is, therefore, possible to conclude that *rin* do not affect fruit flavor *via* the modification of these

parameters. This conclusion is further supported by the fact that both sugar content and total acidity start to increase already before the onset of ripening and therefore may not be an integral part of ripening. The inferiority of *rin* genotypes to flavor is the lack of some volatile compounds or the increased levels of undesirable volatile compounds.

2.7 Storage life and the *rin* mutant

Tomatoes are one of the most favorable and important vegetables around the world. In order to meet the need of fruit around the year all over, the affecting factors of storability and transportability of tomatoes are very important (Yan, 1999). A major difficulty in the handling of fresh market tomatoes is that they easily get soft and then perish the ripe fruits. The spoilage amount of tomato fruit after harvest is about 20 percent of total yield every year (Chungui *et al*, 1995).

One of the abilities of changing the length of tomato fruit storage period is the growing of hybrids with genes *rin* (ripening inhibitor), *nor* (non ripening) and *nr* (never ripe) (Ignatova *et al*, 1999). Fruits from plants homozygous for these genes, demonstrate an absence of ripening or a very low speed of this process. They express very long shelf life, but are associate with decreased fruit quality, lack of red pigmentation and are not commercially acceptable (Chungui *et al*, 1995).

Herner and Sink (1973) report that fruits from F1 plants (reciprocal crosses between normal and *rin*) produced much less ethylene than normal fruits and the F1 fruits were delayed in ripening compared to normal fruit as measured by ethylene and CO_2 production and color change. Fruits from the F1 crosses were stimulated to ripen by exogenous ethylene, but did not respond as rapidly as normal fruits. McGlasson (1985) report that the ACC synthase system in F1 *rin* is partly suppressed, because the rate of ethylene production during ripening is half normal. Softening and carotenogenesis in heterozygote *rin* fruit proceed at a rate intermediate between the normal and the mutant parents and eventually attain acceptable flavor and color (Kopeliovitch *et al*, 1979).

According to Kopeliovitch *et al* (1979) the *nor* mutant is the most efficient one in improving the storage life of F1 hybrid fruit, but developed a pink color instead of the red pigmentation of normal varieties. They also found F1 with *rin* picked at the breaker stage can be stored about four times longer than normal fruit (cv. Kewalo). Polderdijk (1989) suggested a positive correlation between fruit firmness and keeping quality. Kopeliovitch *et al* (1979) suggested that ripening-inhibitor genes, capable of prolonging storage life, do not necessarily improve its firmness.

Tomatoes taste better when fruit ripen on the plant. Nguyen *et al* (1991) noted that fresh market tomatoes lack flavor when picked at the green-mature to early color stages to ensure sufficient storage life for transport and retailing during the winter. *Rin* hybrid fruits ripen and soften more slowly than present commercial

cultivars, so it may be possible to harvest the fruits at a more advanced color stage without loss of quality and risk of fruit rapidly becoming too soft.

2.8 Sugar and sucrose-degradation in tomato

Stommel (1992) reported that sugars are important component of tomato fruit quality. Sugars (glucose and fructose) in *Lycopersicon esculentum* make up approximately 55 to 65 percent of the fruit soluble solids fraction and contribute significantly to overall tomato fruit flavor and higher amounts of fructose, in comparison to glucose, are typical in ripe fruits (Berry and Uddin, 1991; Stommel, 1992). Davies (1966) determined that the green-fruited tomato species (subspecies L. *eulycopersicon*) accumulate high levels of sucrose in contrast with the red-fruited species (subspecies L. *eriopersicon*) that store predominantly reducing sugars. Fruit of the cultivated tomato, *Lycopersicon esculentum* Mill. are among the latter group and sucrose accumulation is present in very small quantities, generally less than 0.1 percent of the fresh weight (Stommel and Haynes, 1993). In contrast to L. *esculentum,* L. *chmielewskii*, as well as L. *peruvianum* and L. *hirsutum* fruit, accumulate primarily sucrose, rather than glucose and fructose (Yelle *et al*, 1988).

During rapid growth, sucrose is used for respiration and as structural material for cell growth and the remainder is stored as hexose and starch in equal amounts. According to Ho (1999) starch is later degraded to increase the content of

hexose and thus the regulation of the degradation of sucrose in the cytosol and the vacuole is important for the total sugar content of the ripe tomato fruit.

Stommel (1992) reported that L.*esculentum* fruits are characterized by increased levels of invertase activity and declining sucrose synthase activity throughout fruit development. Different enzymatic determinants appear to contribute to sucrose accumulation in the green-fruited species. Yelle *et al* (1988) noted low levels of invertase and nondetectable levels of sucrose synthase in the sucrose-accumulating *Lycopersicon chmielewskii*. Stommel (1992) suggested that sucrose accumulation in wild type tomato is facilitated by a lack of enzymatic degradation of imported sucrose.

According to Dali *et al* (1992) and Ruan and Patrick (1995), fruit insoluble invertase may play a role in the apoplastic unloading of sucrose in mature tomato fruit. Sucrose only accumulates in tomato fruit when soluble acid invertase activity is low, suggesting a role for invertase in the regulation of the composition of the sugar stored (Miron and Schaffer, 1991). High activities of soluble invertase are found in the vacuolar compartment of tomato fruit and any sucrose transported into the vacuole would therefore be immediately hydrolyzed to glucose and fructose and released much slower into the cytosol than sucrose (Husain *et al*, 1999). Sucrose is then resynthesized in the symplast from reducing sugars by sucrose phosphate synthase (Dali *et al*, 1992).

In tomato fruit, the change of sucrose synthase activity in the fruit corresponds with the rate of dry matter accumulating and correlate to the quantity of starch accumulated (Yelle *et al*, 1988) and to the rate of fruit growth (Wang *et al*, 1993). Sun *et al* (1992) observed a very strong correlation between sucrose synthase activity and sucrose unloading. Husain *et al* (1999) suggested that sucrose synthase cleaves sucrose to UDP glucose and fructose. Sun *et al* (1992) reported that sucrose synthase activity was not detectable at any time during fruit development in the wild tomato (L. *chmielewskii*) in contrast to fruits of L. *esculentum*, which reaches a peak about three weeks after anthesis and then decreases to undetectable levels at ripening. They also reported that sucrose synthase is a biochemical determinant of sink strength in growing tomato fruits.

Storage of sucrose in storage organs, such as the cultivated tomato, which typically accumulate hexose sugars offers great potential for increasing total soluble sugar content and percentage of soluble solids (Stommel, 1992).

2.9 Heterosis in tomato fruit

The founder of the heterosis concept defines it as the superiority of the hybrids over their parents in vegetation, adaptiveness and productivity (Hayes, 1952). According to Yordanov (1983) heterosis is confirmed more and more as a basic, highly effective breeding method applied in an ever-growing number of agricultural crops for developing early, high-yielding, uniform cultivars, which combine additionally a number of other valuable economic characters.

Rick and Butler (1956) highlighted the theoretical and practical importance of heterosis in the tomato. According to Yordanov (1983), the ability to adapt better to varying and often unfavourable environmental conditions is one of the most valuable properties of hybrid cultivars. An investigation of heterosis effect in tomatoes proved that in glasshouses the performance of heterosis is higher than in the field. Heterosis manifests itself most strongly in the F1's and decreases progressively in the next segregating generations (Georgiev, 1991).

Yordanov (1983) observed that the direction of crossing has an influence on the heterosis effect in the F1 in respect to earliness, total yield, fruit size and fruit shape and leaf size. He also reported that heterosis have a number of advantages. The heterosis method makes a given breeding task possible in the shortest, most precise way, by combining the valuable dominant characters of both parents.

Georgiev (1991) explains that male parents are chosen which complement those characteristics that are not transferred through the female parent. He also points out that the selection of parents based on the various characteristics to develop a hybrid may differ from place to place, depending upon production problems and consumer demands.

CHAPTER 3

MATERIAL AND METHODS

3.1 Experimental material

Six tomato genotypes, *Lycopersicon esculentum* Mill. (2n = 2x = 24) and three tester lines (*rin* 1, *rin* 2 and *rin* 3) were used in a Line x Tester analysis. A code was assigned to each of the tomato genotypes. Table 3.1 gives a summary of the codes, type of variety and the origin of each of the genotypes and tester lines used.

Table 3.1 Experimental material used in this study

CODE	TYPE	ORIGIN
RDP 1	Advance breeding line	Roodeplaat
RDP 2	Advance breeding line	Roodeplaat
R 1	Pure breeding line	Roodeplaat
KOM 1	Cultivar	Stark Ayres
KOM 2	Cultivar	Stark Ayres
F1B	F1- Hybrid	Stark Ayres
RIN 1	Tester line	Mayfords
RIN 2	Tester line	Mayfords
RIN 3	Tester line	Mayfords

3.2 Production of F1 hybrids

Seeds of the six parents and three testers were planted on 8 March 2000 in seedling trays filled with a commercial seedling mixture and placed in a heated glasshouse at the University of the Free State (UFS). The seedling trays were watered twice daily and were fertilized once a week with Chemicult at the recommended concentration, once the seedlings germinated.

The seedlings were transplanted after five weeks into 2 litre plastic pots with two seedlings per pot. The pots were filled with a pre-sterilised potting medium to reduce the possibility of soilborne diseases. After two weeks one seedling was removed from each pot so that the strongest one remained. A total of 5 plants from each parental line and tester line with two replications were used. The crossing block is given in Table 3.2. The plants were watered as required and fertilized once a week with Chemicult at the recommended concentration.

Table 3.2 Crossing block used in this study

	RIN 1	**RIN 2**	**RIN 3**
RDP 1	RDP 1 x RIN 1	RDP 1 x RIN 2	RDP 1 x RIN 3
RDP 2	RDP 2 x RIN 1	RDP 2 x RIN 2	RDP 2 x RIN 3
R 1	R 1 x RIN 1	R 1 x RIN 2	R 1 x RIN 3
KOM 1	KOM 1 x RIN 1	KOM 1 x RIN 2	KOM 1 x RIN 3
KOM 2	KOM 2 x RIN 1	KOM 2 x RIN 2	KOM 2 x RIN 3
F1B	F1B x RIN 1	F1B x RIN 2	F1B x RIN 3

Emasculation was done one day prior to anthesis as recommended by Tigchelaar and Edward (1986) to avoid accidental self-pollination. Pollen was transferred two days after emasculation from the donor plant (*rin*1, *rin* 2 or *rin* 3) to the female by removing the anther of the donor and rubbing it over the stigma of the female. The female flower was then covered for six days. The fully matured (red ripe stage) fruit resulting from the pollination were then harvested for seed extraction.

Seed extraction was done as recommended by Opena and Chen (1993). The fruits of each of the different F1 crosses were harvested and put in pre-marked plastic bags. The fruit of each bag was crushed and pectolytic enzyme was added. Pectolytic enzyme helps in breaking down the cell walls of the fruit. Natural fermentation continued for 24 hours so that the seed mucilage could be broken down and the seed be separated from their gelatinous coating. After the fermentation was complete, water was added and stirred so that the seeds and refuse could be separated. The refuse was sieved and the seeds were cleaned. The seeds were then placed in paper bags and put in a dryer for two to three days at 28°C. The dry seed was then placed in pre-marked envelopes.

3.3 Experimental method

The 18 F1 hybrid combinations and their 9 parental genotypes were planted in seedling trays on 9 October 2000 and placed in a heated glasshouse. The

seedling trays were filled with a commercial sterilized seedling mixture. The seedlings were watered and fertilized as described previously.

After four to five weeks the seedlings were transplanted in 5 litre plastic pots filled with pre-sterilized soil and placed in a heated glasshouse at the UFS. Five seedlings of each F1-hydrid and parental genotype were transplanted in a randomized complete block design with three replications. Seedlings that died were replaced until one week after the original planting date to ensure that there were 15 plants per replication.

The plants were watered daily as required and fertilized with Chemicult once a week. Three weeks after the transplanted date the plants were lined-up to ensure that the plants grew upwards and to prevent them from tipping over. High temperatures in the glasshouse, during the fruit-set stage, led to stress conditions of the plants. Insecticides like Telstar and Metasystox were sprayed in succession to kill red spider mites and plant-aphids. The fruits were harvested at the red ripe stage and not in the breaker stage as normal. The harvested fruits were stored in a dark room at room temperature and then used to measure the yield, quality and shelf life characteristics.

3.4 Measurements

3.4.1 Yield characteristics

The following yield characteristics were measured :

Total yield : Total yield is the total mass in kilogram (kg) of marketable and unmarketable fruit harvested from a cross.

Marketable yield : Marketable yield is the mass of the fruit with no physiological or other defects.

Unmarketable yield : Unmarketable yield is the mass of fruit with physiological and other defects. Physiological defects include growth cracks, catface, sunscald, puffiness and blossom-end rot. Other defects refer to fruit that has been damaged by insects, birds or other. Marketable and unmarketable tomatoes were picked and weighed separately.

Average fruit mass : The mass of each tomato of the different crosses were measured separately and the average fruit mass per plant was determined.

Average fruit size : Each tomato were measured separately with a Precision Vernier caliper (± 0.1mm) and the average fruit size per plant was determined.

3.4.2 Shelf life

The fruits of each genotype were harvested at the red ripe stage and stored in a dark room at room temperature. The results are expressed in days from harvest to deterioration (fruit that were to soft to handle). The deteriorated fruit was removed to prevent the possible spread of pathogens.

3.4.3 Sugar-content

The tomatoes of each genotype were sliced and blended separately in a commercial food blender. A single drop of the tomato juice was used to determine the sugar-content using an ATAGO refractometer. The results are expressed in percentage. The ATAGO refractometer was calibrated with distilled water. The sugar-content was measured at day 1 (day of harvest), 4, 8, 12, 16, and day 20 with six replications.

3.4.4 Fruit acidity (pH)

A portion of the separately blended sample was used to measure the fruit pH. The pH of each tomato juice sample was measured with a Crison pH-meter. The fruit pH was measured at day 1, 4, 8, 12, 16 and day 20 with six replications.

3.4.5 Fruit color

Tomato fruit color is determined by the color of the skin and the flesh. The skin is usually colorless or yellow, depending on the content of an unidentified alkali-soluble pigment. The color of the flesh is determined mainly by the content of the carotenoid pigments. The fruit color was determined by the human eye and classified as red, red-orange, red-yellow, orange-yellow, yellow-orange-red, yellow and green.

3.4.6 Blossom-end rot

Blossom-end rot is a physiological disorder caused by a local calcium (Ca) deficiency in the distal fruit tissue. The susceptibility to blossom-end rot in tomato varies among tomato types and cultivars. There is a wide range of susceptibility among round tomato cultivars and they are related to both the plant growth and fruit growth characteristics. The occurrence of blossom-end rot is often related to the growing conditions and by optimizing the growing conditions for both fruit growth and Ca uptake and transport, it can be largely prevented. Blossom-end rot was visible in many genotypes and hybrids and was measured as none, very little, medium and heavily affected.

3.4.7 Fruit cracks

Fruit cracks are a physiological disorder common in tomatoes. Cracks on the fruit may develop during ripening when the elasticity of the fruit wall decrease and the transport of water and sugars increase. Cracks of the fruit are more common in some cultivars than in others. Cracks can also develop on green fruit that are caused by environmental factors like irregular irrigation, high temperatures, high light-intensity and large fluctuation between day and night temperatures. Fruit cracks were measured and divided into none, little and heavily affected.

Correlations were done between fruit color, blossom-end rot and fruit cracks, using AGROBASE 98, sub-menu Statistic corr. command.

3.5 Statistical analysis

3.5.1 Analysis of variance (ANOVA)

Analysis of variance is an arithmetric technique by which total variation presents in a set of data is partitioned into different components. The ordinary factorial analysis of variance for data was analyzed with Agrobase 98 for each yield and quality parameters as a randomized block design with 27 treatments and three replications. Differences among significant means were separated using least significant differences (LSD) at $P \leq 0.05$.

3.5.2 Genetic analysis

Genetic parameters were calculated using the Line x Tester analysis. The components of variance of the ANOVA were interpreted genetically by translating them into covariance of relatives (Table 3.2) based on the factorial model (Wricke and Weber, 1986). The statistical model for the ANOVA was :

$$Yhijk - \mu + \alpha i + \beta j + (\alpha\beta)ij + Rh + \Sigma hijk$$

Where : Yhijk = the observation of the k-th full sib progeny in a plot in h-th replication of the I-th parental plant and the j-th maternal plant. Mμ (μ) is common to all observations, αi is the effect of the I-th parental plant, βj is the effect of the j-th maternal plant, ($\alpha\beta$)ij is the interaction of the parental and maternal plants;

Rh is the effect of the h-th replication, and Σhijk is the environmental effect and reminder of the genetic effect between full sibs on the sample plot.

Table 3.2. Analysis of variance and expected mean squares (EMS) from a factorial design (Wricke and Weber, 1986).

Source	**Df**	**MS**	**EMS**	**Variance components**
Lines(l)	l-1	M1	$\sigma^2e + r\sigma^2lt + rt\ \sigma^2l$	$\sigma^2l = C(HSl)$
Testers(t)	t-1	M2	$\sigma^2e + r\sigma^2lt + rl\ \sigma^2t$	$\sigma^2t = C(HSt)$
L x T	(l-1)(t-1)	M3	$\sigma^2e + r\sigma^2lt$	$\sigma^2lt = C(FS)- C(HSl) – C(HSt)$
Blocks	r-1	M5		
Error	(lt-1)(r-1)	M4	σ^2e	σ^2e □ σ^2

Translation of model variance components to casual components as applied for non-inbred parents follows Wricke and Weber (1986) :

$\sigma^2A = 4\ \sigma^2l = 4.$ M1 – M3/rt and $\sigma^2A = 4\ \sigma^2f = 4.$M2 – M3/rt

$\sigma^2D = 4\ \sigma^2lt = 4.$ M3 – M4/rt and $\sigma^2 =$ M4

$\sigma^2e = (\sigma^2G – C(FS) + \sigma^2)/n$

Where : σ^2A and σ^2D are the variances due to additive and dominance genetic effects respectively. The analysis of variance consisted of two variance components, which estimate the covariance between half-sibs, one from the sample of lines, and one from the samples of testers. These estimates might differ due to maternal effects (Wricke and Weber, 1986).

3.5.2.1 General and specific combining ability (GCA and SCA) effects.

3.5.2.1.1 GCA effects

Combining ability is the ability of a parent to produce inferior or superior combinations in one or a series of crosses (Chaudhary, 1982). Poehlman (1962) defined general combining ability as the average performance of a line in a hybrid combination, and as such, general combining ability is recognized as primarly a measure of additive gene action. Falconer and Mackay (1996) defined general combining ability as the mean performance of the line in all crosses, when expressed as a deviation from the mean of all crosses. A line with good or high combining ability values for traits of economic importance can be selected to improve these traits. The general combining ability of lines and testers was computed from a Line x Tester analysis using the AGROBASE 98 computer program. The GCA estimates for lines and testers for all characters were calculated to select the best line and tester for each characteristic.

a) Lines : (gi)

$$gi = xi.../tr - x../ltr$$

Where : l = no. of lines

t = no. of testers

r = no. of replications

Standard error (SE) for gi effects

S.E. (gca for lines) = (Me/r x t)

Where : Me = error mean square

b) Testers : (gt)

gt = x.j./lr – x.../ltr

Standard error for gt effects

S.E. (gca for tester) = (Me/r x l)

The LSD between GCA was calculated as :

LSD = qα; t,f. S^2□E/r (t = 0.5)

qα; t,f = α value at t treatment's degree of freedom and error's degree of freedom.

3.5.2.1.2 SCA effects

Poehlman (1962) defined specific combining ability (SCA) as the performance of specific combinations of genetic strains in crosses in relation to the average performance of all combinations and as an estimate of the effects of non-additive gene actions. Falconer and Mackay (1996) described specific combining ability as the deviation to a greater or lesser extent from the expected value of any particular cross, which is the sum of the general combining abilities of its two parental lines. The specific combining ability estimates for crosses was also performed. This also shows the minimum and maximum genetic gain of hybrids

from certain lines by certain testers. The SCA effects estimation (Sij) for crosses was calculated as follow :

SCA effects (Sij) :

$$Sij = xij/r - xj../tr - x.j./lr + x.../ltr$$

Standard error for Sij effects :

$$S.E.\ (sca\ effects) = (Me/r)$$

The LSD between SCA effects was calculated as :

$$LSD = q\alpha;\ t,f..S^2\square E/r \qquad (t = 0.5)$$

$q\alpha$; t,f = α value at t treatment's degree of freedom and error's degree of freedom.

3.5.2.2 GCA : SCA ratio

The GCA : SCA ratio was calculated to study the performance of the effects and to assess the relative importance of additive gene or non-additive gene effects. The ratio indicates whether a character is mainly controlled by additive or non-additive gene action. The GCA : SCA ratio was computed from the estimates of genetic components of the Line x Tester analysis of variance, as the ratio of sum of additive genetic variances to the dominance genetic variance (σ^2A ; σ^2D). A high ratio indicates additive gene action, while a low ratio indicates specific gene action.

3.5.3 Genetic correlation

Genetic correlation (rA) can be obtained by :

$$rA = COVxy/\sqrt{(Varx\ Vary)}$$

where : COVxy = covariance of the character x and y.

Varx = variance of character x

Vary = variance of character y

Simple genetic correlation between characteristics was computed from GCA effect, using AGROBASE 98 sub-menu Statistic corr. command. The analysis provides both positive and negative correlation coefficients estimates together with their probabilities, such that a probability near zero indicates significant correlation, and near 1.00 indicates no correlation (AGROBASE, 98).

3.5.4 Heritability

Heritability is defined as the ratio of the genotypic variance (σ^2g) to the phenotypic variance (σ^2p), thus the genotypic variance is the variation of genetic differences among individuals. Heritability can be expressed in a broad-sense or a narrow-sense. Broad-sense heritability expresses the extent to which an individual's phenotypes are determined by their genotypes. Therefore broad-sense heritability is estimated from the ratio of the total genetic variance to the phenotypic variance. Narrow-sense heritability expresses the extent to which

phenotypes are determined by the genes transmitted from the parents. Narrow-sense heritabilities are estimated from the ratio of the additive portion of the genetic variance to the phenotypic variance. Heritability was computed from genetic components of the Line x Tester analysis using the AGROBASE 98 computer program.

Broad-sense heritability was calculated from the formula :

$$h^2b = \sigma^2A + \sigma^2D / \sigma^2A + \sigma^2D + MSEgca$$

Narrow-sense heritability was calculated from the formula :

$$h^2n = \sigma^2A / \sigma^2A + \sigma^2D + MSEgca$$

Where : σ^2A = additive genetic variance

σ^2D = dominance genetic variance

MSEgca = mean square error

3.5.5. Heterosis

Heterosis is a function of the degree of dominance and the difference in gene frequency between the parent lines. The level of heterosis was determined for yield and related quality characteristics. Two types of heterosis were calculated based on mean values of the genotypes.

Mid-parent heterosis

This is measured as the deviation of the offspring from the mid-parent value, often expressed as a percentage of mid-parent value. Mid-parent heterosis can be calculated from the formula :

$$HF1 = \frac{(F1 - mp)}{mp} \times 100\%$$

Where :

HF1 = Heterosis for F1 cross

F1 = Mean value of F1 cross

mp = mean mid-parent value

High parent heterosis

This was calculated from the mean values of the F1 cross and high parent, using the formula :

$$HF1 = \frac{F1 - hp}{hp} \times 100\%$$

Where :

HF1 = Heterosis for F1 cross

F1 = Mean value of F1 cross

hp = mean value of high parent

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Analysis of variance

4.1.1 Yield characteristics

The results of the analysis of variance done on yield and various yield components are given in Table 4.1. The mean squares of all the parents for yield and yield components were significantly different. Significant differences were found between the crosses except for unmarketable yield. No significant differences for unmarketable yield, average fruit mass and average fruit size for parents vs. crosses were recorded. There were highly significant differences between most of the lines for yield characteristics measured, except for unmarketable yield and average fruit size. No significant differences were found between the testers. Only unmarketable yield was not significantly different for the line x testers.

Table 4.1 Analysis of variance for yield and yield components

Source	d.f	Total yield	Marketable yield	Unmarketable yield	Average fruit mass	Average fruit size
Replications	2	857497.98**	888216.84**	40425.00**	7756.43**	892.73**
Treatments	26	21139.50**	22831.73**	53.99	112.81**	16.42**
Parents	8	19177.03**	23734.39**	589.88*	125.79**	35.26**
Crosses	17	21906.18**	21598.68**	411.05	112.07**	7.69*
Par.vs crosses	1	23805.86*	36572.50**	96.88	21.41	4.01
Lines	5	42596.78**	45287.99**	1.33	158.39**	8.10
Testers	2	5334.95	4405.36	85.19	34.95	0.31
Line x Testers	10	14875.12**	13192.75**	81.08	104.34**	8.96*
Residual	52	4828.21	3738.40	276.33	36.33	3.86
Total	80					

** significant at level 0.01 and * significant at level 0.05.

4.1.1.1 Total yield

The total yield of the parental lines and their F1 hybrids are illustrated in Figure 4.1. The highest ranking parent was RIN 2, followed by RDP 2 and RIN 3. RIN 2 differed significantly from KOM 1, F1B, RIN 1, RDP 1 and R 1. R 1 was the lowest ranking parent. Although RIN 2 ranked first of the parents, it was out yielded (insignificantly) by four hybrids (KOM 1 x RIN 1, F1B x RIN 1, RDP 1 x RIN 2 and KOM 2 x RIN 3). KOM 1 x RIN 1 had the highest yield of all the entries and yielded significantly higher than 12 other hybrids. R 1 x RIN 3 and R 1 x RIN 1 had the lowest and second lowest yields respectively of the hybrids.

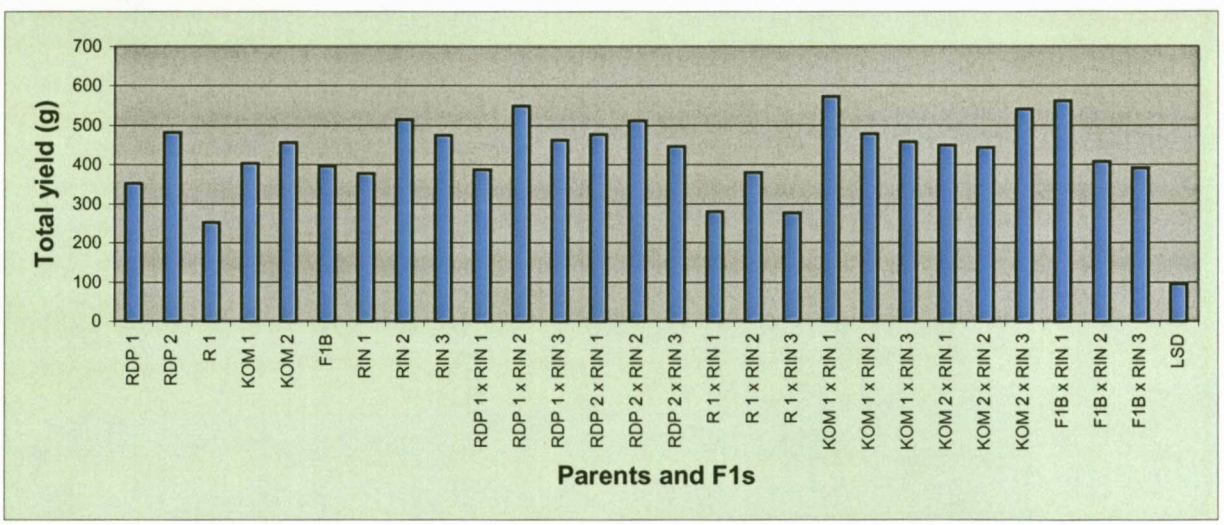

Fig 4.1 Total yield of the F1 hybrids and their parents

4.1.1.2 Marketable yield

Marketable yield for the parents and hybrids are illustrated in Figure 4.2. Significant differences were found between the parental lines as well as between the hybrids. RIN 2 and RDP 2 had the highest marketable yield and were significantly different from F1B, KOM 1, RDP 1, RIN 1 and R 1. R 1 had a significantly lower marketable yield than all the other parents. The F1 hybrid KOM 1 x RIN 1 ranked first, overall with hybrid F1B x RIN 1 in second place. Both these hybrids performed significantly better than most of the parental lines and significantly better than 13 other hybrids. R 1 x RIN 1 had the lowest marketable yield of all the hybrids.

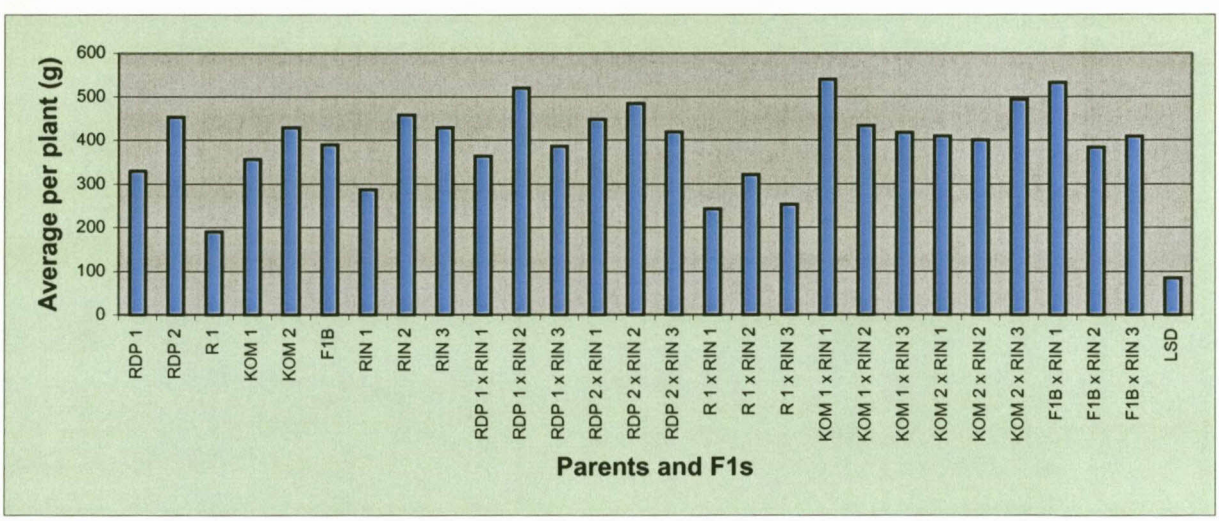

Fig 4.2 Marketable yield of the F1 hybrids and their parents

4.1.1.3 Unmarketable yield

Unmarketable yield for the parents and hybrids are illustrated in Figure 4.3. F1B had the lowest unmarketable yield for both the parents and the hybrids. F1B were significantly different from the other parental lines RDP 2, R 1, RIN 1, RIN 3, KOM 1 and RIN 2. The best hybrid was F1B x RIN 2, followed by RDP 1 x RIN 1 and they were significantly different from five other hybrids namely KOM 1 x RIN 3, KOM 2 x RIN 3, F1B x RIN 3, RDP 1 x RIN 3 and R 1 x RIN 2. R1 x RIN 2 had the highest unmarketable yield.

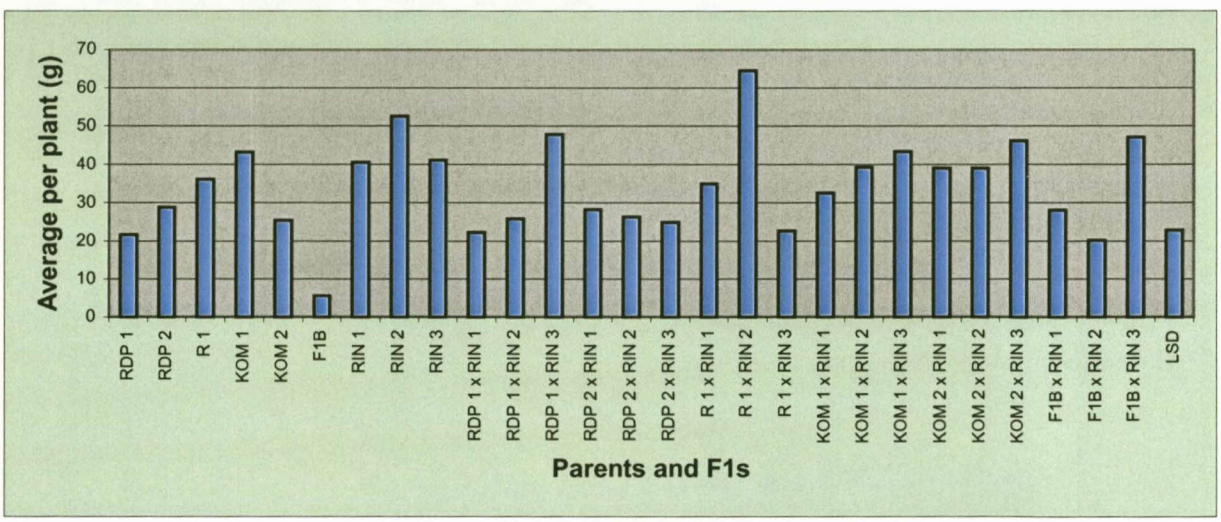

Fig 4.3 Unmarketable yield of the F1 hybrids and their parents

4.1.1.4 Average fruit mass

The average fruit mass of both the parental and F1 hybrids are illustrated in Figure 4.4. The highest average fruit mass between the parental lines was recorded for RIN 3, which was also the highest entry, followed by F1B. KOM 1

had the lowest average fruit mass for the parents. RIN 3 had a significantly higher average fruit mass than parental lines RIN 2, RDP 1, R 1, RIN 1, KOM 2 and KOM 1. Hybrid F1B x RIN 1 had the highest average fruit mass, but did not differ significantly from RDP 2 x RIN 1, F1B x RIN 3 and RDP 1 x RIN 2. Hybrid RDP 1 x RIN 3 had the lowest average fruit mass. Parent line RIN 3 had a significantly higher average fruit mass than 13 hybrid lines.

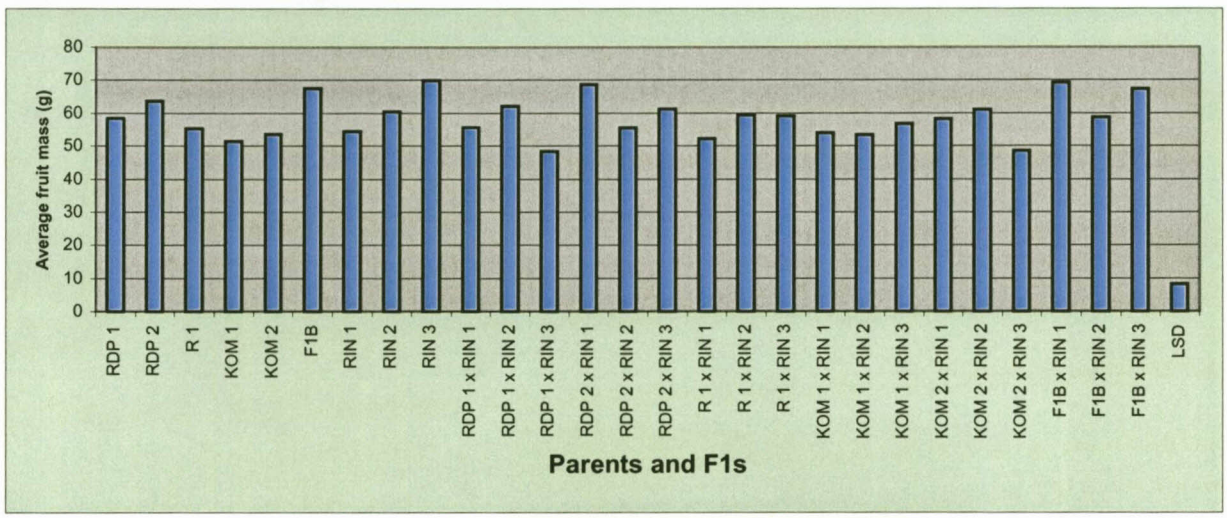

Fig 4.4 Average fruit mass of the F1 hybrids and their parents

4.1.1.5 Average fruit size

The average fruit size of the parents and hybrids are illustrated in Figure 4.5. Significant differences were found between the parents and hybrids. The parent, RIN 3, differed significantly from the rest of the parents and was the highest entry. RIN 1 had the lowest average fruit size and differed significantly from the other parents except KOM 1. F1B x RIN 3 ranked the highest among the hybrids

and differed significantly from nine other hybrids. RDP1 x RIN 3 and KOM 2 x RIN 3 ranked the lowest and second lowest respectively. The parental line RIN 3 had a significantly higher average fruit size than all the hybrid lines.

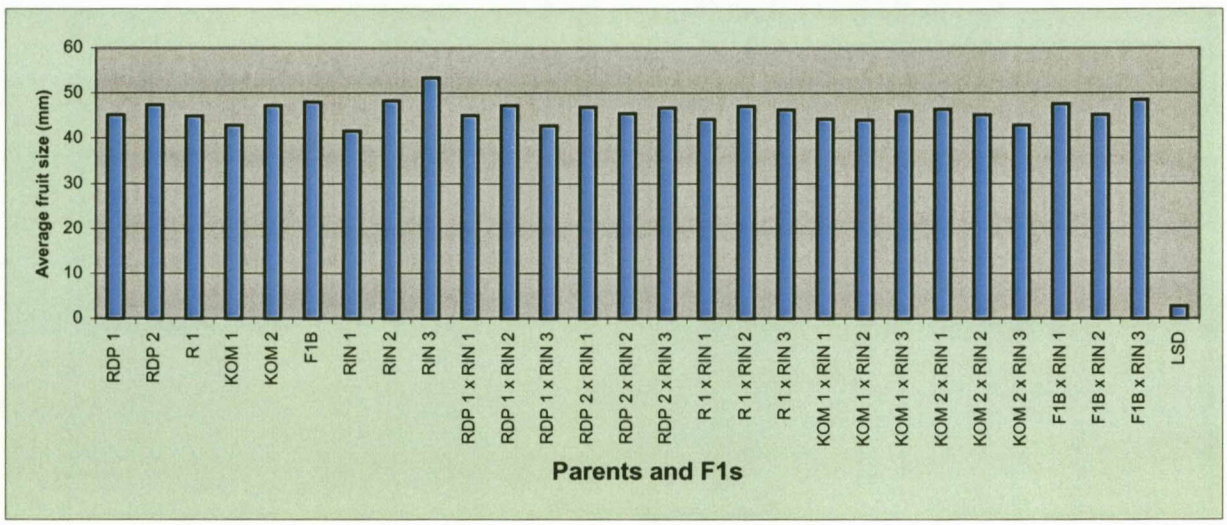

Fig 4.5 Average fruit size of the F1 hybrids and their parents

4.1.2 Quality characteristics

The results of the analysis of variance for fruit quality characteristics are given in Table 4.2 to Table 4.4. Significant differences were found between the parents, crosses, parents vs. crosses, lines, testers and line x testers for shelf life. Significant differences between the parents and between the crosses for sugar-content were found. Significant differences between parents, crosses, parents vs. crosses and lines for fruit pH were also recorded. In general the testers and line x testers showed less differences for both sugar-content and fruit pH over the different days.

Table 4.2 Analysis of variance for shelf life

Source	d.f	Shelf life
Replications	2	328.99*
Treatments	26	504.14**
Parents	8	632.47**
Crosses	17	404.88**
Par.vs crosses	1	1164.83**
Lines	5	669.11**
Testers	2	312.70*
Line x Testers	10	291.20**
Residual	52	85.45
Total	80	

** significant at level 0.01 and * significant at level 0.05.

Table 4.3 Analysis of variance for sugar-content

Source	d.f	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20
Replications	5	1.36	0.96	0.39	4.71	10.17	0.87
Treatments	26	2.52**	29.28**	2.55**	15.61**	12.13**	8.01**
Parents	8	3.30*	85.27**	1.62**	31.21**	16.98**	15.83**
Crosses	17	1.88*	4.10**	3.13**	7.84**	10.57**	4.05**
Par.vs crosses	1	6.93*	9.47**	0.13	22.93**	0.00	12.96**
Lines	5	4.45**	8.55**	5.11**	15.12**	0.87	5.51**
Testers	2	0.55	6.72**	3.79**	4.84	10.80	2.76*
Line x Testers	10	0.87	1.36	2.00**	4.81	15.38**	3.57**
Residual	130	1.02	0.82	0.42	3.00	3.96	0.69
Total	161						

** significant at level 0.01 and * significant at level 0.05.

Table 4.4 Analysis of variance for fruit pH

Source	d.f	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20
Replications	5	0.01	0.02	0.01	0.01	0.10*	0.06**
Treatments	26	0.06**	38.08**	0.05**	0.08**	0.12**	0.05**
Parents	8	0.13**	113.46**	0.06**	0.13**	0.07**	0.07**
Crosses	17	0.03**	0.05**	0.04**	0.04**	0.12**	0.04**
Par.vs crosses	1	0.17**	81.69**	0.08**	0.33**	0.52**	0.15*
Lines	5	0.03*	0.09**	0.08**	0.06*	0.09	0.11**
Testers	2	0.07*	0.05**	0.05**	0.01	0.14*	0.01
Line x Testers	10	0.03	0.03**	0.01	0.04*	0.14**	0.02
Residual	130	0.01	0.01	0.01	0.02	0.04	0.02
Total	161						

** significant at level 0.01 and * significant at level 0.05.

4.1.2.1 Shelf life

The shelf life of the parental lines and their F1 hybrids are illustrated in Figure 4.6. The parental line RIN 3 had the longest shelf life of all the entries evaluated and differed significantly from the other parental and hybrid lines. R 1 had the shortest shelf life of all the entries and differed significantly from the other parental lines. F1B x RIN 3 ranked the highest between the hybrids and had a significant longer shelf life than 11 other hybrids. Hybrid R 1 x RIN 3 had the shortest shelf life.

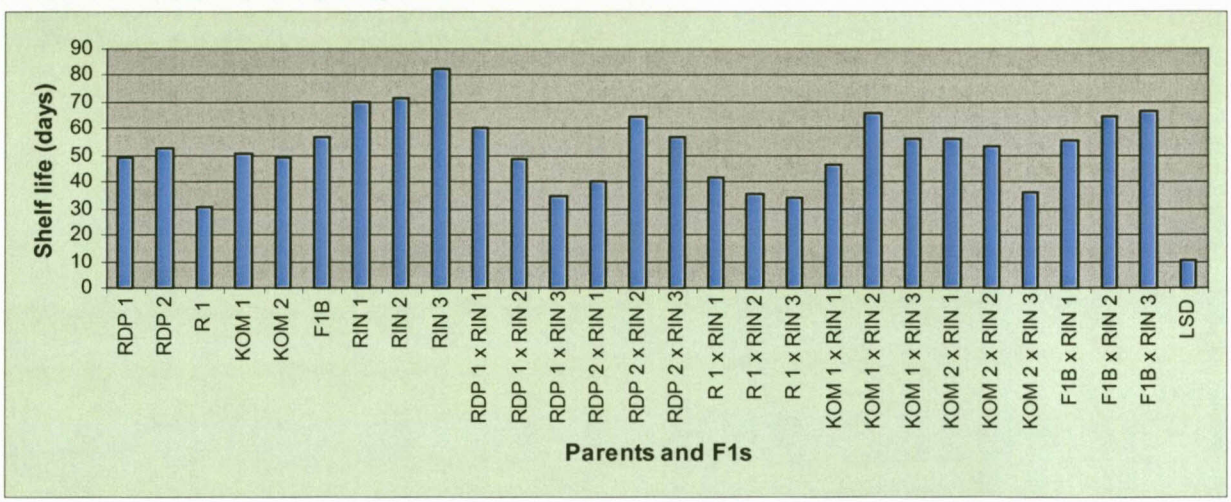

Fig 4.6 Shelf life of the F1 hybrids and their parents

4.1.2.2 Sugar-content

Day 1

Differences regarding the sugar-content (day 1) between the various parents and their F1 hybrids are illustrated in Figure 4.7. R 1 ranked the highest of all the genotypes and had a significantly higher sugar-content than all the other parental lines. RIN 2 ranked the lowest of the parental lines and differed significantly from the other parents except from F1B, RDP2 and RIN 1. R 1 x RIN 1 had the highest sugar-content of the hybrids and differ significantly from all the other hybrids. R 1 x RIN 1 had a significant higher sugar-content than RIN 1, RDP 2, F1B and RIN 2. F1B x RIN 2 had the lowest value for sugar-content and differ significantly from seven other hybrids.

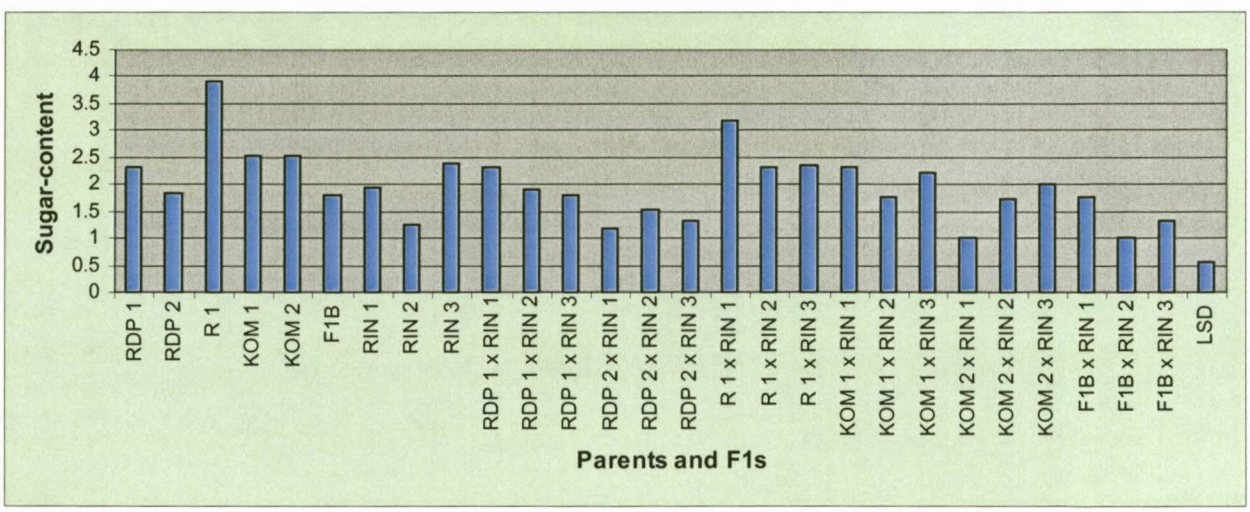

Fig 4.7 Sugar-content (Day 1) of the F1 hybrids and their parents

Day 4

The sugar-content of the parental lines and their hybrids (day4) are illustrated in Figure 4.8. RDP 1 ranked the highest of all the entries and differed significantly from the other parents. RIN 1 ranked the lowest of the parental lines. No significant differences were found between the other parents. R1 x RIN 3 ranked the highest between the hybrids and differ significantly from all other hybrids except R 1 x RIN 2. KOM 2 x RIN 1 and F1B x RIN1 ranked the lowest and second lowest, respectively.

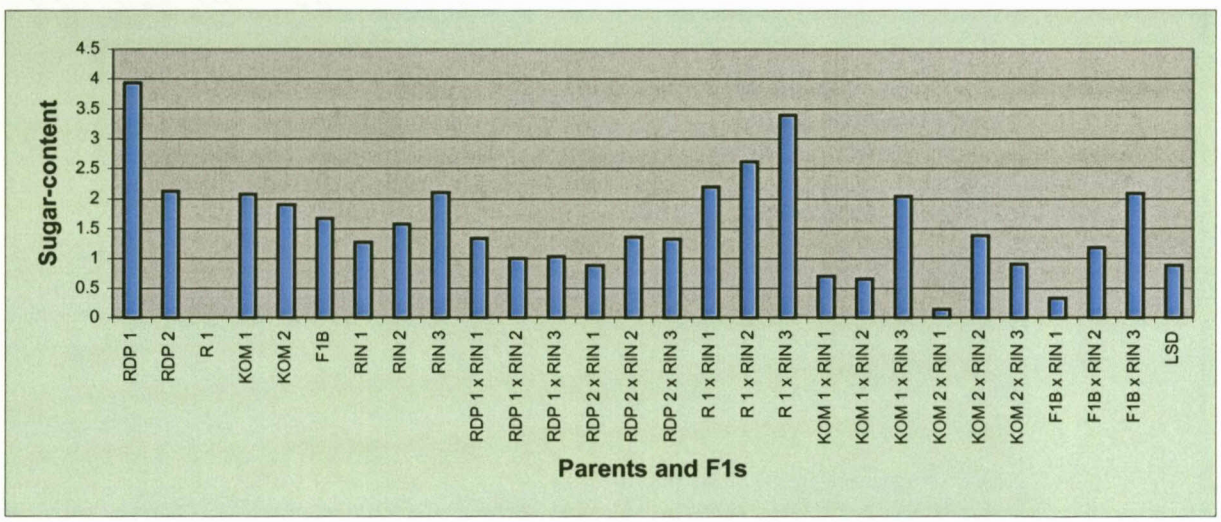

Fig 4.8 Sugar-content (Day 4) of the F1 hybrids and their parents

Day 8

The sugar-content of the parents and the hybrids are illustrated in Figure 4.9.

The parental line RIN 3 had the highest sugar-content, followed by R 1. RIN 3 was significantly different from the other parents, except from R 1 and F1B.

Although RIN 3 ranked the highest of the parents, it was outranked by two hybrids. The hybrids were R 1 x RIN 3 and RDP 1 x RIN 1. Hybrid R 1 x RIN 3 ranked the highest of all the entries and differed significantly from the rest of the hybrids. KOM 1 x RIN 2 had the lowest sugar-content, which was significantly lower than both its parents.

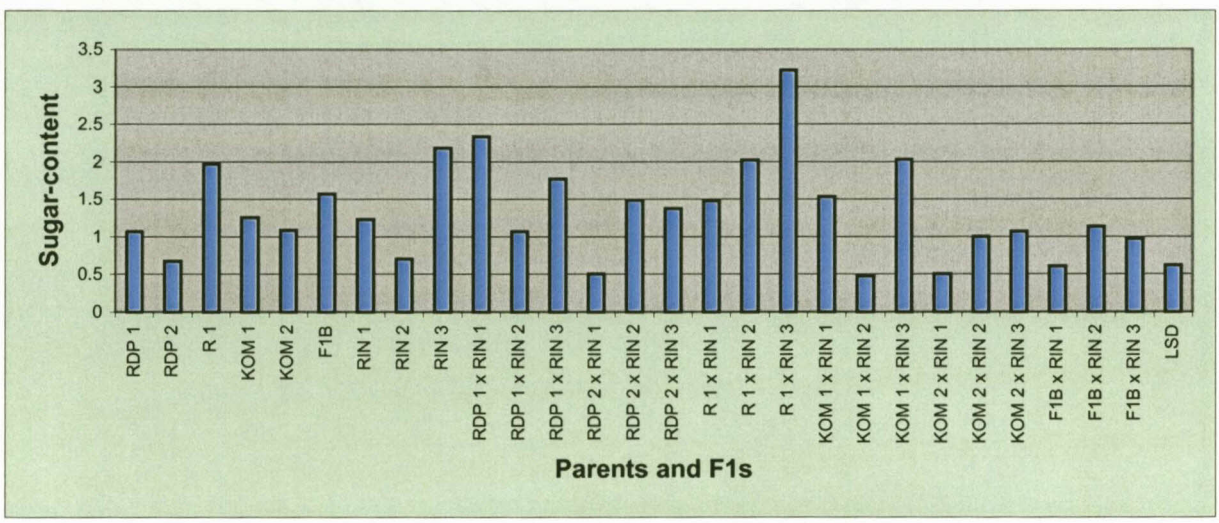

Fig 4.9 Sugar-content (Day 8) of the F1 hybrids and their parents

Day 12

The sugar-content of the parents and the hybrids are illustrated in Figure 4.10. There were no significant differences between RIN 3 and R 1, which ranked first and second, respectively. However, RIN 3 had a significant higher sugar-content than RDP 2, KOM 1 and F1B. R 1 x RIN 2 ranked the highest of all the entries and differed significantly from all the other hybrids and the parents. RDP 2 x RIN 1 was the hybrid with the lowest sugar-content.

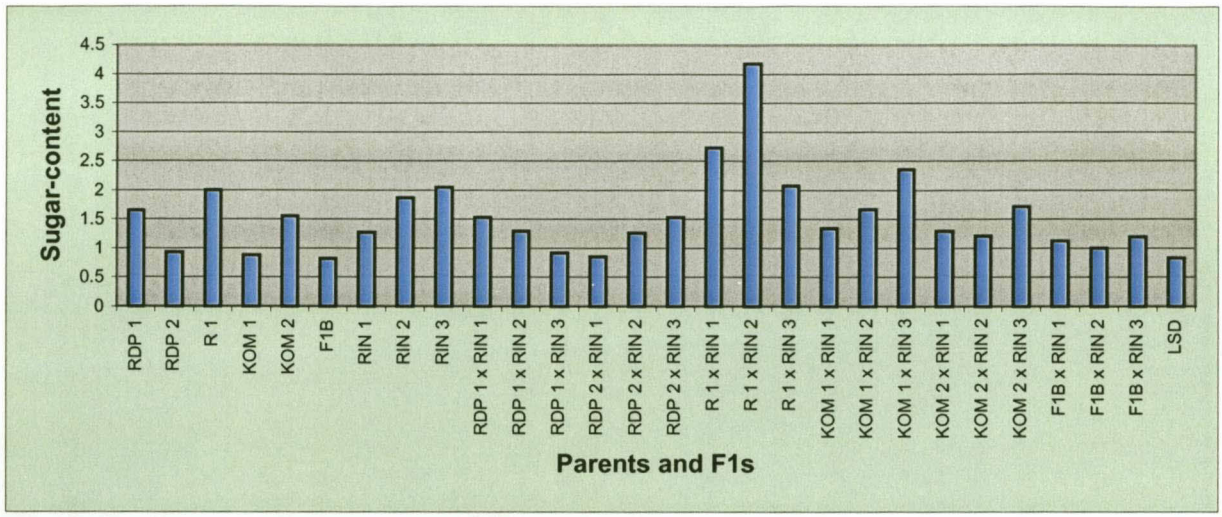

Fig 4.10 Sugar-content (Day 12) of the F1 hybrids and their parents

Day 16

The sugar-content of the parents and the hybrids are illustrated in Figure 4.11. The parental lines F1B and R 1 were the parents with the highest sugar-content and differed significantly from the other parents. KOM 1 had the lowest sugar-content of the parents, but did not differ significantly from KOM 2 and RDP 2. The highest ranking hybrid, R 1 x RIN 1, differed significantly from the other hybrids and had the highest sugar-content of all the entries. F1B x RIN 3 ranked last and differed significantly from both its parents.

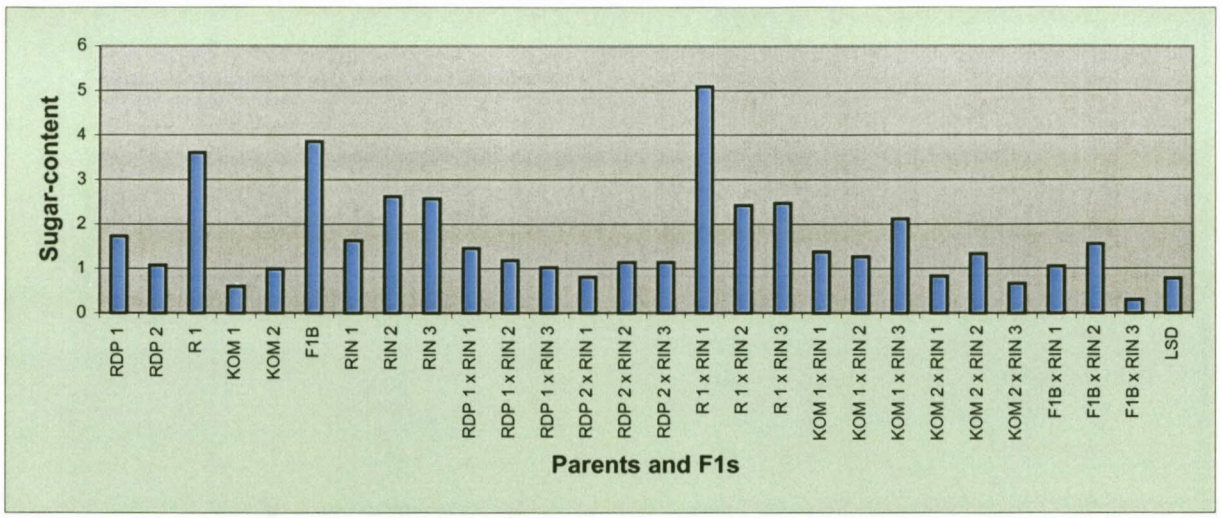

Fig 4.11 Sugar-content (Day 16) of the F1 hybrids and their parents

Day 20

The parent, R 1, ranked the highest of all the entries and had a significantly higher sugar-content than all the other parents. Among the parental lines, R 1 was followed by RIN 1, RIN 3 and RIN 2. The hybrid with the highest sugar-content, KOM 1 x RIN 1, differed significantly from all the other hybrids except from R 1 x RIN 1.

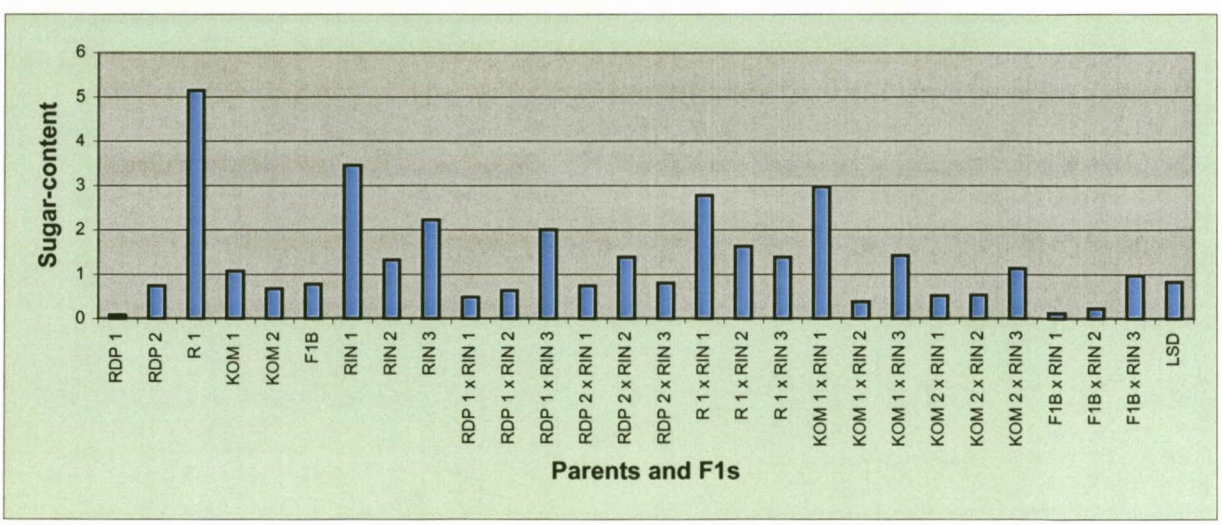

Fig 4.12 Sugar-content (Day 20) of the F1 hybrids and their parents

4.1.2.3 Fruit pH

Day 1

The fruit pH of the parents and hybrids are illustrated in Figure 4.13. Among the parental lines, R 1 had the highest fruit pH, followed by KOM 2, RIN 2, F1B, RIN 1, RIN 3, RDP 2, RDP 1 and KOM 1. R 1 differed significantly from the other parents. Hybrids R 1 x RIN 1 and RDP 1 x RIN 2 ranked first and second respectively and differed significantly from the other hybrids. KOM 1 x RIN 3 had the lowest fruit pH and differ significantly from the other hybrids. R 1 was the entry with the highest fruit pH and differed significantly from the other parents and hybrids.

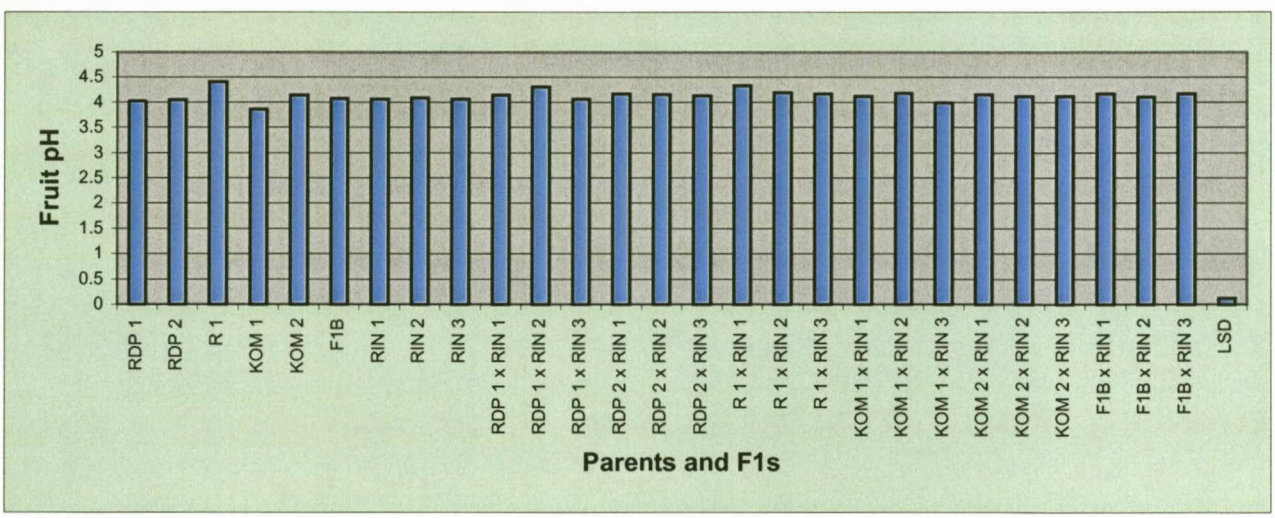

Fig 4.13 Fruit pH (Day 1) of the F1 hybrids and their parents

Day 4

The fruit pH of the parental lines and their hybrids are illustrated in Figure 4.14. RIN 2 and RIN 1 ranked first and second respectively of the parents, but no significant difference were found between the two. Although RIN 2 ranked first of the parents, it was outranked (insignificantly) by four hybrids and significantly by RDP 2 x RIN 3. RDP 2 x RIN 3 was the hybrid and entry with the highest pH and it differed significantly from the other hybrids, except R 1 x RIN 2, F1B x RIN 2, R 1 x RIN 3 and F1B x RIN 3.

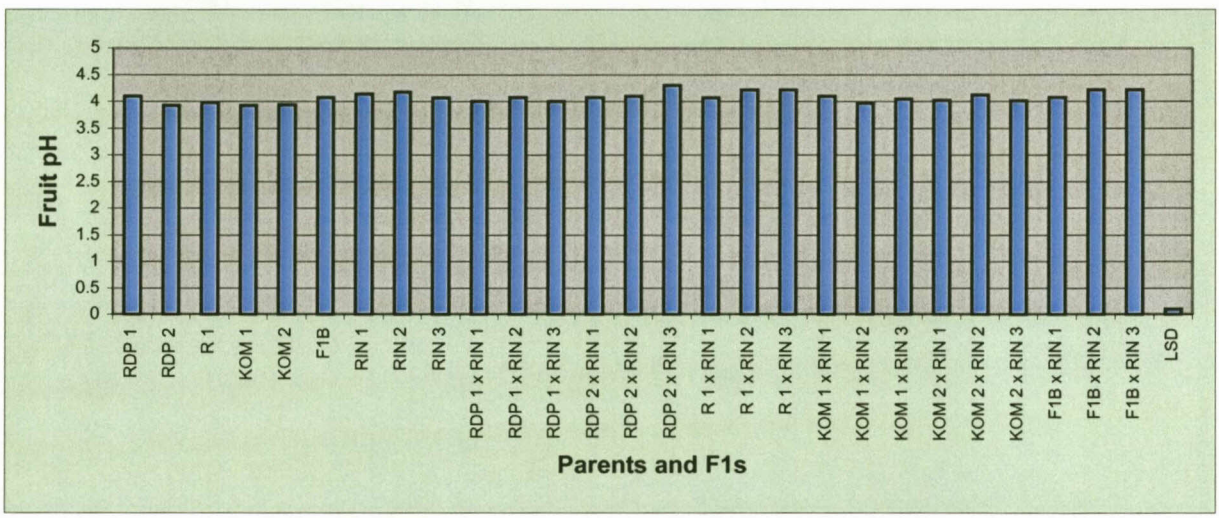

Fig 4.14 Fruit pH (Day 4) of the F1 hybrids and their parents

Day 8

The fruit pH of the parents and hybrids for day 8 are illustrated in Figure 4.15. There were significant differences between the parents and the crosses. The parent with the highest fruit pH was R 1, followed by RIN 3 and RIN 2, but with

no significant difference between them. R 1 was significantly different from parents RIN 1, KOM 1, KOM 2, RDP 1, F1B and RDP 2. Between the hybrids, R 1 x RIN 1 had the highest fruit pH, followed by R 1 x RIN 3 and F1B x RIN 1, with no significant difference between them. The three hybrids were, however, significantly different from all the other hybrids. KOM 1 x RIN 3 had the lowest pH of the hybrids followed by KOM 2 x RIN 3.

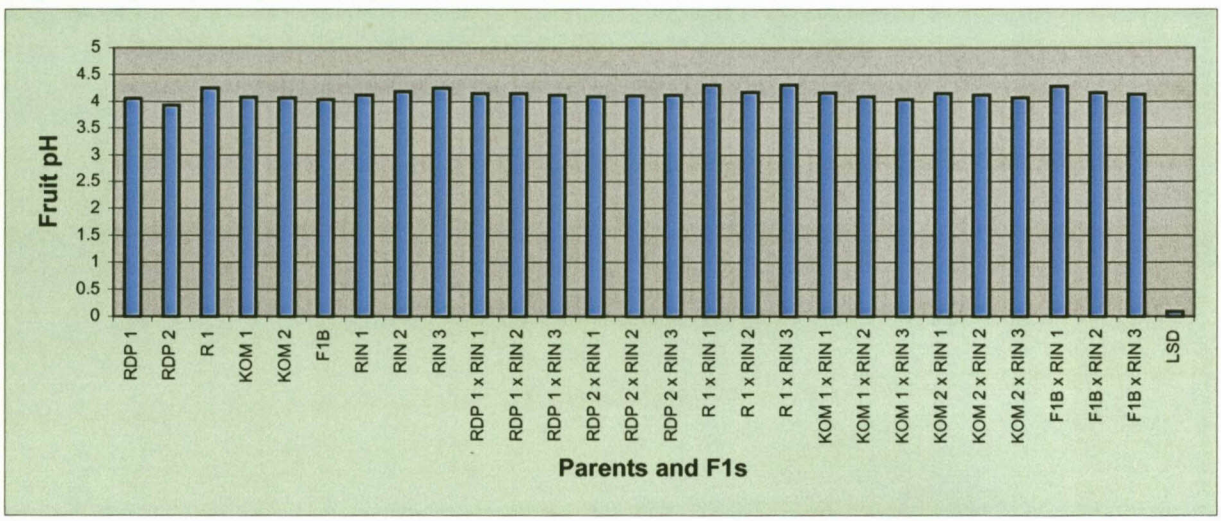

Fig 4.15 Fruit pH (Day 8) of the F1 hybrids and their parents

Day 12

The fruit pH (day 12) of the parents and hybrids are illustrated in Figure 4.16. R 1 was again the highest ranking parent for fruit pH as in day 1 and day 8. Significant differences were recorded between R 1 and the other parents. RDP 1 was the parent with the lowest rank, followed by RIN 2, RIN 1 and KOM 1. The hybrid R 1 x RIN 2 was the hybrid with the highest fruit pH and differed

significantly from eleven other hybrids. RDP 1 x RIN 3 was the lowest ranking hybrid. R 1 had a significantly higher fruit pH than 14 hybrids.

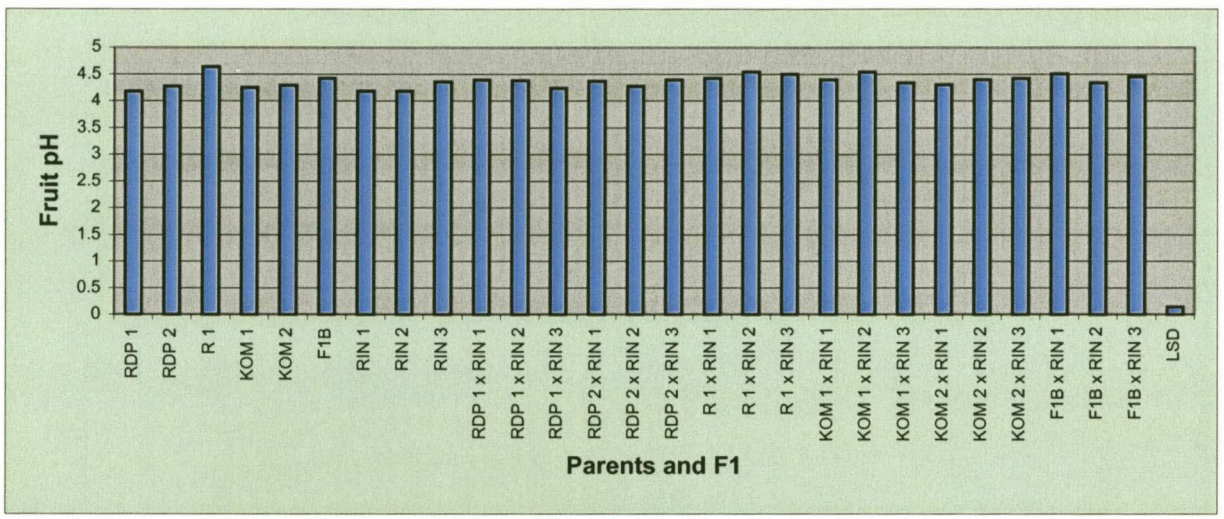

Fig 4.16 Fruit pH (Day 12) of the F1 hybrids and their parents

Day 16

The fruit pH (day 16) of the parental lines and their hybrids are illustrated in Figure 4.17. RIN 3 had the highest fruit pH of the parental lines, followed by RIN 1, R 1, KOM 2, RDP 1, RDP 2, RIN 2, KOM 1 and F1B. The parent, RIN 3, differed significantly from KOM 1 and F1B. The highest fruit pH was recorded by F1B x RIN 2, followed by KOM2 x RIN 3. F1B x RIN 2 differed significantly from all the other hybrids. Six hybrids had a higher (insignificantly) fruit pH than RIN 3.

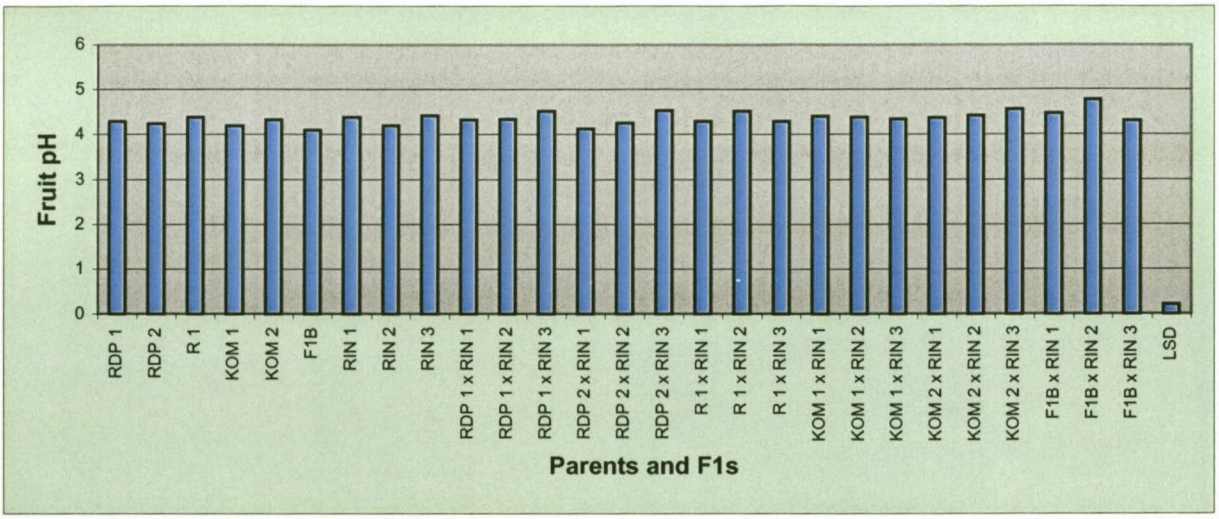

Fig 4.17 Fruit pH (Day 16) of the F1 hybrids and their parents

Day 20

The fruit pH (day 20) of the parents and hybrids are illustrated in Figure 4.18. The parental line RIN 3 had the highest fruit pH of the parents, followed by F1B. RIN 3 differed significantly from parents KOM 1 and RDP 2. Of the hybrids, F1B x RIN 2 had the highest fruit pH and differed significantly from all the parents and hybrids, except from F1B x RIN 1 and F1B x RIN 3. This means that F1B x RIN 1 had a significantly higher fruit pH than its high-parent and 15 other hybrids.

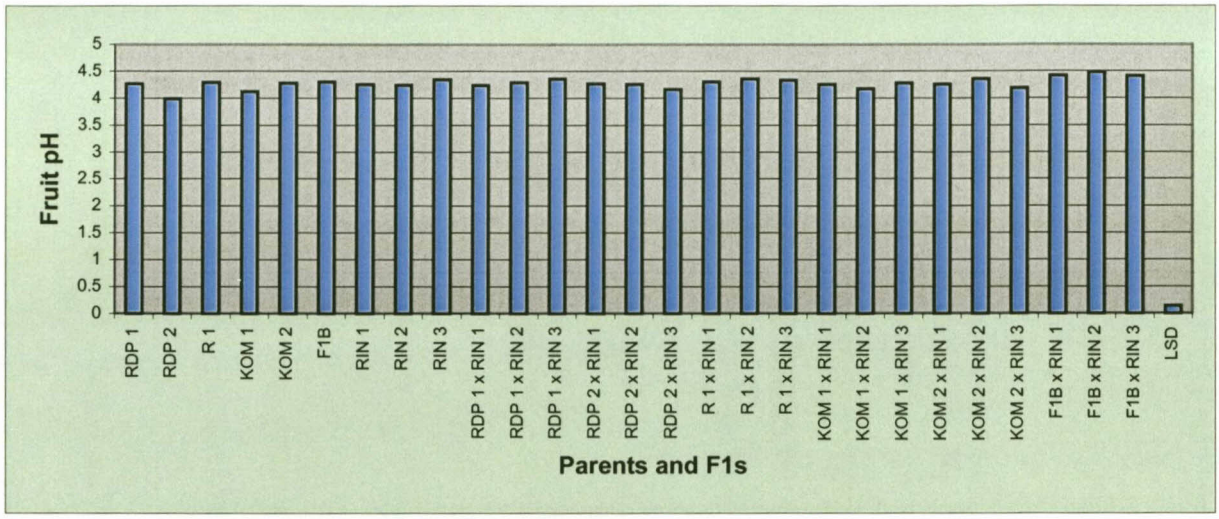

Fig 4.18 Fruit pH (Day 20) of the F1 hybrids and their parents

Discussion

The parental lines and hybrids showed significant differences for all the different characteristics measured. For unmarketable yield, average fruit mass, average fruit size, shelf life, sugar-content (day 1, 4, 8, 12 and day 20) and fruit pH (day 1, 8, 12 and day 20), the best parent produced the best crosses, thus indicating the transfer of its superiority to its offspring. Some crosses performed equal or better than their best parents indicating the presence of heterosis effects. If yield is the most important selection criteria, the hybrid KOM 1 x RIN 1 will be the best in a breeding program as it had the highest rank for both total and marketable yield. KOM 1 x RIN 1 performed better than its best parents indicating that heterosis played an major role in the enhanced total and marketable yield.

The parents (RIN 1, RIN 2 and RIN 3) containing the *rin* gene, had the longest shelf life.Of these three, RIN 3 had the longest shelf life. F1B and RIN 3 had long

shelf life's and transferred their superiority to the top cross F1B x RIN 3. Most of the hybrids had an intermediate shelf life between the parents. Similar results had been previously reported by Kopeliovitch *et al* (1979). The shelf life can therefore be further extended in a breeding program through the selection of parents with an even longer shelf life than F1B and to cross these parents with RIN 3. In both sugar-content and fruit pH, R 1 was a common parent for the top crosses over the different days, indicating the transfer of its superiority to its offspring. The sugar-content and fruit pH of the parents and the hybrids inclined or declined inconsistently over the different days. This might be caused by the plant's reactions to the stress conditions or the different expression of the characters under the large environmental effects. No significant differences were found between the parental lines and hybrids for sugar-content and fruit pH.

4.2 General and Specific combining ability

4.2.1 General combining ability (GCA)

Estimates for GCA effects of the parents are given in Table 4.5 to Table 4.8. The general combining ability of the parental line, KOM 1, was the best, but was only significantly better than R 1 for total yield. It was followed by RDP 2, KOM 2, RDP1, RIN 2 and RIN 1. It is clear that the significant GCA effect of KOM 1 for yield was associated with positive and high GCA effects for marketable yield.

The GCA effect of KOM 1 was the highest for marketable yield and was significantly better than all the other lines except RDP 2. RDP 2 had the second

highest marketable yield and was associated with high positive GCA effects for total yield, marketable yield, average fruit mass, average fruit size, shelf life and sugar-content (day 4). R 1 had a significant lower GCA effect for both total and marketable yield than all the other lines.

The parental line KOM 2 had the highest GCA effect for unmarketable yield with no significant differences between the other parents. The GCA effects of F1B were the highest for average fruit mass, average fruit size, shelf life and fruit pH (day 16 and day 20). F1B differed significantly from KOM 1 for average fruit mass and from RDP 1, KOM 2 and RIN 3 for shelf life and from all the other lines for fruit pH (day 20).

Table 4.5 General combining ability effects for yield characteristics

Parents	Total yield	Marketable yield	Unmarke-table yield	Average fruit mass	Average fruit size
RDP 1	17.5906	8.9715	-3.1767	-2.9381	-0.6689
RDP 2	29.7317	36.0981	-8.6645	3.4496	0.6833
R 1	-136.3583	-142.0796	5.6066	-1.3837	0.2344
KOM 1	54.7750	49.2659	3.3077	-3.5559	-0.8956
KOM 2	29.1861	20.4859	6.2955	-2.4281	-0.8000
F1B	5.0750	27.2581	-3.3687	6.6563	1.4467
Rin 1	6.4361	8.6737	-4.2905	1.3569	0.0967
Rin 2	13.0706	9.3854	0.7177	0.0707	0.0550
Rin 3	-19.5067	-18.0591	3.5727	-1.4276	-0.1517
$LSD_{(0.05)}$	95.0137	22.7304	22.7304	8.2421	2.6897

R 1 recorded the highest GCA effect for sugar-content for all the days, except for day 16. R 1 differed significantly from RDP 2, KOM 2 and F1B for day 1 and from

all the lines, except RDP 1 and RIN 3 for day 8. R 1 also differed significantly from all the parents for day 4 and day 12 and significantly from RDP 1, RDP 2, KOM 2, F1B and RIN 2 for day 20.

Table 4.6 General combining ability effects for shelf life

Parents	Shelf-life
RDP 1	-3.1481
RDP 2	3.0074
R 1	-13.8815
KOM 1	5.2741
KOM 2	-2.4815
F1B	11.2296
Rin 1	-0.7926
Rin 2	4.5074
Rin 3	-3.7148
$LSD_{(0.05)}$	12.6406

Table 4.7 General combining ability effects for sugar-content

Parents	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20
RDP 1	0.1815	-0.2407	0.3602	-0.1815	0.1267	-0.0739
RDP 2	-0.4852	-0.1796	-0.2454	-0.2148	-0.0756	-0.1406
R 1	0.7815	1.3759	0.8713	1.5630	-0.3033	0.8172
KOM 1	0.2537	-0.2352	-0.0176	0.3574	0.5656	0.4706
KOM 2	-0.2519	-0.5574	-0.5065	-1.2037	-0.1622	-0.3939
F1B	-0.4796	-0.1630	-0.4620	-0.3204	-0.1511	-0.6794
Rin 1	0.1259	-0.4324	-0.3065	0.0519	0.6700	0.1539
Rin 2	-0.1213	0.0009	-0.1676	0.3380	-0.8483	-0.3200
Rin 3	-0.0046	0.4315	0.3741	-0.3898	0.1783	0.1661
$LSD_{(0.05)}$	0.9668	0.8793	0.6218	0.8386	0.7824	0.8188

R 1 had the highest GCA effects for days 1, 4, 8 and 12 for fruit pH. R 1 differed significantly from KOM 1 and RIN 3 for day 1 and significantly from RDP 1, KOM 1, KOM 2 and RIN 1 for day 4. R 1 also differed significantly from RDP 1 and RDP 2 for days 8 and 12 and also significantly from KOM 1, KOM 2, RIN 2 and RIN 3 for day 8. F1B showed the best GCA effects for both day 16 and day 20 with significant differences from all the other lines for day 20.

Table 4.8 General combining ability effects for fruit-pH

Parents	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20
RDP 1	0.0170	-0.0775	-0.0129	-0.0600	-0.0021	-0.0037
RDP 2	-0.0024	0.0586	-0.0523	-0.0555	-0.0963	-0.0698
R 1	0.0781	0.0664	0.1127	0.0884	-0.0333	0.0344
KOM 1	-0.0569	-0.0653	-0.0612	0.0223	-0.0244	-0.0626
KOM 2	-0.0246	-0.0503	-0.0379	-0.0251	0.0460	-0.0334
F1B	-0.0113	0.0681	0.0516	0.0300	0.1101	0.352
Rin 1	0.0262	-0.0450	0.0430	-0.0014	-0.0720	-0.0118
Rin 2	0.0240	0.0169	-0.0209	0.0117	0.0455	0.0231
Rin 3	-0.0502	0.0281	-0.0220	-0.0103	0.0265	-0.0113
$LSD_{(0.05)}$	0.1172	0.1035	0.0865	0.1385	0.2115	0.1366

Discussion

Significant GCA effects were found between the parents for the different characteristics measured. The results indicate that the parental line, KOM 1, was the best general combiner for both total and marketable yield and can be used for the improvement of these yield characteristics. F1B was the best general

combiner for average fruit mass and average fruit size, which are main components of total yield. F1B also proved to be the best general combiner to improve the shelf life. R 1 was in general the best general combiner over the different days for sugar-content and fruit pH and can be used as a parental line to improve these two quality traits.

4.2.2 Specific combining ability (SCA)

Estimates of specific combining ability for yield and quality characteristics are given in Table 4.9 to Table 4.12. Approximately 46.7% of all the SCA values for the yield characteristics were positive in comparison to the 50.62% positive values recorded for the quality SCA values.

1. Total yield

The F1 combination F1B x RIN 1 ranked first for specific combining ability (SCA) effect for total yield, which indicates that it is the best specific combiner. It was followed by KOM 2 x RIN 3 and RDP 1 x RIN 2. There were, however, no significant differences between these three combinations. F1B x RIN 1 had a significantly better SCA effect than 11 of a total of 18 F1 combinations.

2. Marketable yield

RDP 1 x RIN 2 had the highest SCA effect for marketable yield followed by F1B x RIN1, KOM 2 x RIN 3 and KOM 1 x RIN 1. There were no significant differences between these four hybrids, but they differed significantly from all the other hybrid combinations. The SCA effect of RDP 1 x RIN 2 was mainly associated with high negative SCA effects for unmarketable yield.

3. Unmarketable yield

There were no significant differences in the SCA effects between R 1 x RIN 2, RDP 1 x RIN 3, F1B x RIN 3 and RDP 2 x RIN 1, which ranked first, second, third and fourth respectively. The SCA effect of R 1 x RIN 2 was significantly better than ten other combinations.

4. Average fruit mass

The hybrid RDP 1 x RIN 2 expressed the highest SCA effect for average fruit mass followed by RDP 2 x RIN 1, KOM 2 x RIN 2 and KOM 1 x RIN 3. There were no significant differences between these four combinations, but they differed significantly from F1B x RIN 2, KOM 2 x RIN 3, R 1 x RIN 1, RDP 2 x RIN 2 and RDP 1 x RIN 3. Ten of the 18 combinations recorded a positive SCA effect for average fruit mass.

5. Average fruit size

There were no significant difference in SCA effect between the top four combinations, namely RDP1 x RIN 2, F1B x RIN 3, KOM 2 x RIN 1 and KOM 1 x RIN 3. These four hybrids differed significantly from RDP 1 x RIN 3, KOM 2 x RIN 3 and F1B x RIN 2.

Table 4.9 Specific combining ability effects for yield characteristics.

Cross	Total yield	Marketable yield	Unmarketable yield	Average fruit mass	Average fruit size
RDP 1 x Rin 1	-84.9872	-67.7582	-5.4229	-1.0202	-0.0489
RDP 1 x Rin 2	69.5050	86.2502	-6.8911	6.6459	2.1428
RDP 1 x Rin 3	15.4822	-18.4920	12.3139	-5.6257	-2.0939
RDP 2 x Rin 1	-7.7483	-11.2015	6.0316	5.5187	0.4522
RDP2 x Rin 2	20.6006	25.0502	-0.8966	-6.3452	-0.9761
RDP2 x Rin 3	-12.8522	-13.8488	-5.1349	0.8265	0.5239
R 1 x Rin 1	-37.9083	-38.6470	-1.4995	-6.0413	-1.7856
R 1 x Rin 2	54.2506	39.4379	23.0623	2.4948	1.1938
R 1 x Rin 3	-16.3422	-0.7909	-21.5627	3.5465	0.5928
KOM 1 x Rin 1	63.1650	67.9207	-1.6106	-2.1991	-0.4989
KOM 1 x Rin 2	-37.8528	-39.5309	0.2045	-1.4229	-0.8006
KOM 1 x Rin 3	-25.3122	-28.3898	1.4062	3.6220	1.2994
KOM 2 x Rin 1	-34.5428	-33.1859	1.9316	0.9465	1.5122
KOM 2 x Rin 2	-47.5406	-44.0176	-3.0966	5.0426	0.3406
KOM 2 x Rin 3	82.0833	77.2035	1.1650	-5.9891	-1.8528
F1B x Rin 1	102.0217	82.8719	0.5698	2.7954	0.3689
F1B x Rin 2	-58.9628	-67.1898	-12.3824	-6.4152	-1.8994
F1B x Rin 3	-43.0589	-15.6820	11.8126	3.6198	1.5306
LSD(0.05)	95.0137	22.7304	22.7304	8.2421	2.6897

6. Shelf life

Estimates of specific combining ability effects for shelf life are given in Table 4.10. Ten of the combinations for shelf life had positive SCA effects. The F1

combination RDP 1 x RIN 1 had the largest SCA effect, while RDP 2 x RIN 1 had the smallest effect. RDP 1 x RIN 1 differed significantly from nine other combinations.

Table 4.10 Specific combining ability effects for shelf life

Cross	Shelf life
RDP 1 x Rin 1	13.3704
RDP 1 x Rin 2	-3.7296
RDP 1 x Rin 3	-9.6407
RDP 2 x Rin 1	-12.8519
RDP2 x Rin 2	6.2482
RDP2 x Rin 3	6.6037
R 1 x Rin 1	5.3704
R 1 x Rin 2	-6.1296
R 1 x Rin 3	0.7593
KOM 1 x Rin 1	-8.6519
KOM 1 x Rin 2	5.1815
KOM 1 x Rin 3	3.4704
KOM 2 x Rin 1	8.6370
KOM 2 x Rin 2	0.4037
KOM 2 x Rin 3	-9.0407
F1B x Rin 1	-5.8741
F1B x Rin 2	-1.9741
F1B x Rin 3	7.8482
LSD(0.05)	12.6406

7. Sugar-content

Estimates of specific combining ability effects for sugar-content are given in Table 4.11. The F1 combination R1 x RIN 1 had the highest SCA effect for sugar-content for both day 1 and day 16. R 1 x RIN 1 differed significantly only from KOM 2 x RIN 1 for day 1 and significantly from all the other combinations for day 16. RDP 1 x RIN 1 had the highest SCA effect for day 4 and day 8 and

differed significantly from RDP 1 x RIN 3, RDP 2 x RIN 3, KOM 1 x RIN 2, KOM 2 x RIN 1, KOM 2 x RIN 3 and F1B x RIN 1 for day 4 and significantly from all the combinations with negative SCA effects for day 8. The hybrid KOM 2 x RIN 1 had the best SCA effect for sugar-content (day 12) followed by KOM 1 x RIN 3 and R 1 x RIN 2. These three hybrids differed significantly from all the other combinations. KOM 1 x RIN 1 had the highest sugar-content for day 20 followed by RDP 1 x RIN 3 and RDP 2 x RIN 2 and differed significantly from all the other combinations.

Table 4.11 Specific combining ability effects for sugar-content

Cross	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20
RDP 1 x Rin 1	0.1907	0.6435	0.8176	0.2315	-0.3644	-0.7094
RDP 1 x Rin 2	0.0213	-0.1231	-0.4879	-0.2879	0.7706	-0.0856
RDP 1 x Rin 3	-0.2120	-0.5204	-0.3296	0.0565	-0.4061	0.7950
RDP 2 x Rin 1	-0.2926	0.1324	-0.4120	-0.4185	-0.9122	-0.3928
RDP2 x Rin 2	0.3046	0.1657	0.5343	-0.2879	0.9561	0.7311
RDP2 x Rin 3	-0.0120	-0.2982	-0.1241	0.7065	-0.0438	-0.3383
R 1 x Rin 1	0.4241	-0.1065	-0.5602	-0.3129	3.6422	0.6994
R 1 x Rin 2	-0.1620	-0.1232	-0.0491	0.8343	0.4321	0.0233
R 1 x Rin 3	-0.2620	0.2296	0.6093	-0.5213	1.4738	-0.7228
KOM 1 x Rin 1	0.1019	0.0046	0.3954	-0.4907	-0.9866	1.2294
KOM 1 x Rin 2	-0.2176	-0.4787	-0.7102	-0.4602	0.7316	-0.8967
KOM 1 x Rin 3	0.1157	0.4741	0.3148	0.9509	0.2550	-0.3328
KOM 2 x Rin 1	-0.6926	-0.2398	-0.1491	1.0204	-0.7922	-0.3561
KOM 2 x Rin 2	0.2713	0.5769	0.3120	0.6509	1.2261	0.1244
KOM 2 x Rin 3	0.4213	-0.3370	-0.1629	-0.2964	-0.4338	0.2317
F1B x Rin 1	0.2685	-0.4343	-0.0935	-0.0296	-0.5866	-0.4706
F1B x Rin 2	-0.2176	-0.0176	0.4009	-0.4491	1.4316	0.1033
F1B x Rin 3	-0.0509	0.4519	-0.3074	0.4787	-0.8450	0.3672
$LSD_{(0.05)}$	0.9668	0.8793	0.6218	0.8386	0.7824	0.8188

8. Fruit pH

The SCA effects for fruit pH varied to a great extend for the combinations over the different days. The F1 hybrid RDP 1 x RIN 2 had the best SCA effect for fruit pH (day 1) and differed significantly from all the other hybrids except from KOM 1 x RIN 1 and F1B x RIN 1. KOM 1 x RIN 1 had the highest SCA effect for day 4 followed by RDP 2 x RIN 3 and KOM 2 x RIN 2. They did not differed significantly from each other, but they did however differed significantly from RDP 2 x RIN 3, RDP 2 x RIN 2, R 1 x RIN 1, KOM 1 x RIN 2 and KOM 2 x RIN 3.

The hybrid R 1 x RIN 3 had the highest SCA effect for day 8 followed by F1B x RIN 1 and RDP 2 x RIN 3 with no significant differences between them. The three combinations, however, differed significantly from R 1 x RIN 2. KOM 1 x RIN 2 recorded the highest SCA effect for fruit pH (day 12). KOM 1 x RIN 2 differed significantly from RDP 1 x RIN 3, RDP 2 x RIN 2, R 1x RIN 1, KOM 1 x RIN 3 and KOM 2 x RIN 1.

Eight of the combinations had positive SCA effects for fruit pH (day 16). F1B x RIN 2 had the highest rank and differed significantly from ten other combinations. RDP 1 x RIN 3 had the highest SCA effect for fruit pH (day 20) and had a significantly higher SCA effect than KOM 1 x RIN 2 and KOM 2 x RIN 3.

Table 4.12 Specific combining ability effects for fruit-pH

Cross	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20
RDP 1 x Rin 1	-0.0523	0.0178	-0.0247	0.0541	0.0009	-0.0438
RDP 1 x Rin 2	0.1066	0.0308	0.0326	0.0311	-0.1016	-0.0269
RDP 1 x Rin 3	-0.0543	-0.0486	-0.0079	-0.0852	0.1007	0.0708
RDP 2 x Rin 1	-0.0128	-0.0317	-0.0552	0.0263	-0.1067	0.0457
RDP2 x Rin 2	-0.0189	-0.0769	0.0237	-0.0834	-0.0958	0.0041
RDP2 x Rin 3	0.0319	0.1086	0.0315	0.0570	0.2025	-0.0498
R 1 x Rin 1	0.0749	-0.0644	0.0015	-0.0642	-0.0070	-0.0169
R 1 x Rin 2	-0.0595	0.0386	-0.0729	0.0428	0.1135	0.0016
R 1 x Rin 3	-0.0154	0.0258	0.0714	0.0214	-0.1065	0.0152
KOM 1 x Rin 1	-0.0084	0.1122	0.0270	-0.0248	0.1031	0.0284
KOM 1 x Rin 2	0.0655	-0.0847	0.0093	0.1055	-0.0460	-0.0798
KOM 1 x Rin 3	-0.0571	-0.0276	-0.0363	-0.0808	-0.0571	0.0513
KOM 2 x Rin 1	0.0010	0.0106	-0.0029	-0.0724	-0.0122	-0.0024
KOM 2 x Rin 2	-0.0301	0.0569	0.0276	0.0163	-0.0780	0.0686
KOM 2 x Rin 3	0.0291	-0.0675	-0.0246	0.0561	0.0903	-0.0662
F1B x Rin 1	-0.0023	-0.0444	0.0543	0.0808	0.0219	-0.0110
F1B x Rin 2	-0.0634	0.0353	-0.0212	-0.1123	0.2079	0.0325
F1B x Rin 3	0.0657	0.0092	-0.0341	0.0314	-0.2299	-0.0214
LSD(0.05)	0.1172	0.1035	0.0865	0.1385	0.2115	0.1366

Discussion

When the SCA effects of the F1 hybrids are compared, the influence of each trait can be seen. Significant SCA effects between the crosses were found for the different yield and quality characteristics measured. The F1 combinations F1B x RIN 1 and R 1 x RIN 2 had positive SCA effects for all yield characteristics. F1B x RIN 1 had the highest SCA effect for total yield and can be used in a breeding program to enhance total yield. RDP 1 x RIN 2 were the best specific combiner for marketable yield, average fruit mass and average fruit size and can be used

to improve these yield characteristics. Most of the F1 combinations with large SCA effects for total yield had also high SCA effects for marketable yield. The F1 combination KOM 2 x RIN 2 had the most positive SCA effects for both quality and yield characteristics. KOM 2 x RIN 2 had the highest mean for SCA effects for sugar-content over the different days. RDP 2 x RIN 3 had the most positive and highest mean SCA effects for fruit pH and can be used to improve this trait. RDP 1 x RIN 1 was the best specific combiner for shelf life and can be selected to improve this quality trait.

4.2.3 GCA : SCA ratio

The GCA : SCA ratios were calculated and are given in Table 4.13. The GCA : SCA ratio could be an indication of additive or non-additive gene action. The SCA variance in this study was found higher than the GCA variance for the most traits except for sugar-content. This means that a large part of the total genetic variability associated with the measured traits was the result of non-additive gene action.

The ratios were positive for most of the traits except for unmarketable yield, average fruit size and sugar-content and varied between – 1.50 : 1 for sugar-content to 0.50 : 1 for fruit pH.

All the ratios were less than one and this indicated that non-additive effects were more important than additive effects for all the traits measured. The low GCA :

SCA ratio is the result of the high SCA effects and dominant gene action and proved that the environment had a huge influence on the genetic variability.

Table 4.13 The ratios between the mean squares of general combining ability and specific combining ability.

Parameters	*GCA*	SCA	GCA : SCA
Total yield	421.61	3348.96	0.13 : 1
Marketable yield	504.05	3151.44	0.16 : 1
Unmarketable yield	-4.19	68.25	-0.06 :1
Average fruit mass	0.46	22.66	0.02 : 1
Average fruit size	-0.07	1.69	-0.04 :1
Shelf-life	6.81	68.58	0.09 : 1
Sugar-content	0.03	-0.02	-1.50 : 1
Fruit pH	0.001	0.002	0.50 : 1

4.3 Genetic correlations

Genotypic correlations were calculated between all the traits measured to determine the influence of the different yield and quality traits on each other. A genotypic correlation matrix is given in Table 4.14A and Table 4.14B.

In this study only marketable yield showed a significant positive genetic correlation with total yield (r = 0.96). Shelf life also showed a significant correlation (r = -0.67) with total yield, but it was however, negative.

Average fruit size showed a significant positive genetic correlation (r = 0.89) with average fruit mass. Shelf life showed negative genetic correlations with all the

traits except with average fruit size. Total yield and marketable yield were the only traits that were significantly correlated with shelf life.

There were no positive significant correlations found between fruit color, blossom-end rot and fruit cracks. There was however a highly significant negative correlation between blossom-end rot and fruit cracks.

Discussion

The genetic correlation is the correlation of breeding values and expresses the extent to which two measurements reflect what is genetically the same character. An increase or decrease in one character is generally associated with an increase or decrease of the other. The significant positive genetic correlation between total and marketable yield indicated that the breeder could select on the basis of marketable yield to improve total yield. Average fruit mass can be improved through selection for average fruit size as it has a significant positive genetic correlation. Shelf life had a significant negative correlation with total and marketable yield and indicate that an increase in one character will lead to a decrease in the other. Overall there were no correlations of considerable interest to the plant breeder considering the quality characteristics that were evaluated.

Table 4.14A Correlation coefficient between all variables (yield and quality characteristics)

	Total yield	Marketable yield	Unmarketable yield	Average fruit mass	Average fruit size	Shelf life	Sugar-content	Fruit pH
Total yield	1	0.9645**	0.2405	0.0363	0.0346	-0.6718**	0.2785	-0.0860
Marketable yield	0.9645**	1	0.1440	0.1374	0.1679	-0.5603*	0.2519	-0.1808
Unmarketable yield	0.2405	0.1440	1	0.0357	0.1006	-0.2483	0.2802	0.0843
Average fruit mass	0.0363	0.1374	0.0357	1	0.8927**	-0.0363	-0.4949	0.1410
Average fruit size	0.0346	0.1679	0.1006	0.8927**	1	0.2048	-0.4194	-0.0087
Shelf life	-0.6718**	-0.5603*	-0.2483	-0.0363	0.2048	1	-0.2962	-0.3821
Sugar-content	0.2785	0.2519	0.2802	-0.4949	-0.4194	-0.2962	1	0.3438
Fruit pH	-0.0860	-0.1808	0.0843	0.1410	-0.0087	-0.3821	0.3438	1

** significant at level 0.01 and * significant at level 0.05.

Table 4.14B Correlation coefficient between variables

	Fruit color	Blossom-end rot	Fruit cracks
Fruit color	1	-0.0329	-0.0251
Blossom-end rot	-0.0329	1	-0.0050**
Fruit cracks	-0.0251	-0.0050**	1

** significant at level 0.01 and * significant at level 0.05.

4.4 Heritability

The broad sense (h^2b) and narrow sense (h^2n) heritabilities were calculated for each yield and quality characteristic and can be seen in Table 4.15.

All the traits measured had relatively low broad sense heritabilities, which varied from $h^2_b = 0.49$ for marketable yield to $h^2_b = 0.01$ for sugar-content. Marketable yield (0.49) had the highest broad sense heritability, while unmarketable yield (0.18) had the lowest heritability of the yield characteristics measured. Shelf life had the highest broad sense heritability (0.46) for the quality characteristics followed by fruit pH (0.23) and sugar-content (0.01).

All the traits had very low narrow-sense heritabilities. Marketable yield (0.06) had the highest narrow-sense heritibility for the yield characteristics, while fruit pH had the highest for the quality characteristics. Unmarketable yield (-0.01) and average fruit size (-0.01) had negative heritabilities due to high negative variance components.

Table 4.15 Estimates of heritabilities for yield and quality characteristics

	Total yield	Marketable yield	Unmarketable yield	Average fruit mass	Average fruit size	Shelf life	Sugar-content	Fruit pH
σ^2_A	421.6	504.05	-4.19	0.46	-0.07	6.81	0.03	0.001
σ^2_D	3348.9	3151.44	68.25	22.66	1.69	68.58	-0.02	0.002
σ^2_E	4828.2	3738.41	276.33	36.33	3.86	85.45	1.02	0.01
h^2b	0.43	0.49	0.18	0.38	0.29	0.46	0.01	0.23
h^2n	0.04	0.06	-0.01	0.01	-0.01	0.04	0.03	0.08

Discussion

Falconer and Mackay (1996) had indicated that heritability is independent on the characteristics, the type of population, the environmental circumstances and the method of measuring the phenotype. The narrow-sense heritability is of great importance to the plant breeder because it measures the relative importance of the additive portion of the genetic variance that can be transmitted to the next generation of offspring. The narrow-sense heritability was very low for all characteristics measured with sugar-content (8%) the highest. The low narrow-sense heritability was caused by low additive effects and high dominant gene actions for all characters measured. The environment and stress conditions had also a huge influence on the expression of the different characteristics. Selection is normally less effective if the heritability is low. Selection for characters with low heritability may be more effective when the performance of progenies of the F2 plants are used.

4.5 Heterosis

Mid-parent (MP) and high-parent (HP) heterosis was calculated for all the yield and quality characteristics measured. Estimated values are presented in Table 4.16.

Eleven F1 hybrids expressed positive MP heterosis for total yield, which ranged from 2.8% to 46.9%. The F1 hybrid KOM 1 x RIN 1 expressed the highest MP

heterosis followed by F1B x RIN 1 (45.4%) and RDP 1 x RIN 2 (26.5%). Five of the 18 hybrids had positive HP heterosis for total yield. KOM 1 x RIN 1 (42.1%) had the highest rank followed by F1B x RIN 1 (41.9%) and RDP 1 x RIN 2 (6.7%).

Eleven F1 hybrids had positive mid-parent heterosis for marketable yield. KOM 1 x RIN 1 (68.0%) expressed the highest MP heterosis followed by F1B x RIN 1 (57.6%) and RDP 1 x RIN 2 (31.6%). Five hybrids had positive HP heterosis for marketable yield. KOM 1 x RIN 1 (51.6%) had also the highest rank as for total yield followed by F1B x RIN 1 (36.7%) and RDP 1 x RIN 2 (13.2%).

Eight hybrids had positive MP heterosis for unmarketable yield, which ranked from 90.1% to 0.1%. F1B x KOM 3 (90.1%) had the highest positive MP heterosis and KOM 2 x RIN 2 (0.1%) the lowest. The hybrid with the highest HP heterosis was R 1 x RIN 2 (22.6%) followed by RDP 1 x RIN 3 (16.5%).

Seven F1 hybrids expressed positive MP heterosis for average fruit mass. The hybrid RDP 2 x RIN 1 (16.1%) had the highest rank followed by F1B x RIN 1 (13.7%) and KOM 2 x RIN 1 (7.7%). Five of the 18 hybrids had positive HP heterosis. RDP 2 x RIN 1 (7.6%) had the highest amount of heterosis followed by KOM 2 x RIN 1 (6.8%) and F1B x RIN 1 (2.7%).

Eight of the F1 hybrids showed positive MP heterosis for average fruit size, which ranked from 6.1% to 1.0%. F1B x RIN 1 (6.1%) had the highest MP heterosis

followed by RDP 2 x RIN 1 (5.4%) and KOM 1 x RIN 1 (4.9%). Only one hybrid, KOM 1 x RIN 1 (3.3%) showed positive HP heterosis.

Four hybrids, KOM 1 x RIN 2 (8.7%), RDP 2 x RIN 2 (5.9%), RDP 1 x RIN 1 (3.7%) and F1B x RIN 2 (1.0%) expressed positive MP heterosis for shelf life. All the hybrids, however, recorded negative high-parent heterosis for shelf life.

Five of the 18 F1 hybrids expressed positive MP heterosis for sugar-content. RDP 1 x RIN 1 (9.1%) had the highest rank followed by R 1 x RIN 1 (8.3%). KOM 2 x RIN 1 (-54.3%) showed the highest negative MP heterosis. RDP 1 x RIN 1 (0.4%) had the best HP heterosis for sugar-content and was the only hybrid that expressed a positive HP heterosis.

Sixteen of the 18 hybrids showed positive MP heterosis for fruit pH, which was the highest for all the characteristics measured. RDP 1 x RIN 2 (6.1%) had the highest MP heterosis followed by KOM 1 x RIN 2 (5.2%) and KOM 1 x RIN 1 (3.7%). R1 x RIN 2 (-1.4%) and R 1 x RIN 3 (-1.8%) were the only hybrids that showed negative MP heterosis. Twelve F1 hybrids expressed positive HP heterosis for fruit pH. RDP 1 x RIN 2 (5.3%) had the highest HP heterosis followed by KOM 1 x RIN 2 (2.4%).

Discussion

Generally the most crosses showed a positive mid-parent (MP) and high-parent (HP) heterosis. Yordanov (1983) reported that when suitable pairs with high combining abilities are combined, the respective high heterosis effect can be expected. The heterosis effects are normally the highest in the F1-generation and cannot be predicted exactly beforehand. KOM 1 x RIN 1 showed high HP heterosis for both total yield (42.1%) and marketable yield (51.6%). Heterosis effects for total yield had been previously reported by Rick and Butler (1956). The quality characteristics had in general lower MP and HP heterosis than the yield characters with a very low MP heterosis response for shelf life. Yordanov (1983) proved that the heterosis effect is higher in tomatoes grown in glasshouses than in the field. He also proved that hybrids endure unfavorable conditions better than the parental cultivars. The environment and stress conditions had a large effect on the combining abilities of the parents, which had a large effect on the heterosis response of the different characteristics measured.

Table 4.16 Heterosis (%) estimates for yield and quality characteristics

Crosses	Total yield		Marketa-ble yield		Unmarketa ble yield		Average fruit mass		Average fruit size		Shelf life		Sugar-content		Fruit pH	
	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP
RDP 1 x RIN 1	6.1	2.7	18.0	10.2	-28.5	-45.2	-1.4	-4.7	3.7	-0.3	3.7	-13.8	9.1	0.4	2.4	1.9
RDP 1 x RIN 2	26.5	6.7	31.6	13.2	-30.6	-51.0	4.3	2.6	1.0	-2.1	-12.2	-31.6	8.1	-17.2	6.1	5.3
RDP 1 x RIN 3	11.6	-2.5	1.7	-9.9	52.6	16.5	-24.7	-30.8	-13.2	-19.8	-46.7	-58.4	-23.7	-25.0	0.4	0
RDP 2 x RIN 1	11.0	-1.0	21.1	-1.1	-18.8	-30.5	16.1	7.6	5.4	-0.9	-33.5	-42.5	-37.4	-39.4	2.7	1.9
RDP 2 x RIN 2	2.8	-0.3	6.4	5.8	-35.5	-50.1	-10.6	-12.9	-4.9	-5.8	5.9	-7.6	0.3	-15.9	2.2	1.7
RDP 2 x RIN 3	-6.7	-7.4	-8.1	-7.5	-28.8	-52.7	-8.4	-12.4	-7.1	-12.4	-15.1	-31.3	-36.9	-44.5	1.9	1.7
R1 x RIN 1	-11.0	-25.6	1.6	-15.5	-8.88	-13.8	-4.7	-5.4	2.1	-1.7	-17.3	-40.6	8.3	-18.7	2.1	-1.8
R1 x RIN 2	-1.1	-26.2	-0.8	-29.9	45.4	22.6	2.8	-1.6	1.1	-2.2	-30.4	-50.1	-9.1	-40.2	-1.4	-4.9
R1 x RIN 3	-24.1	-41.8	-18.1	-41.0	-41.1	-44.6	-5.5	-15.4	-5.7	-13.1	-39.9	-58.8	-25.3	-39.7	-1.8	-5.6
KOM 1 x RIN 1	46.9	42.1	68.0	51.6	-22.4	-24.7	1.8	-1.1	4.9	3.3	-22.2	-33.2	3.5	-8.3	3.7	1.2
KOM 1 x RIN 2	4.3	-6.9	6.4	-5.4	-17.9	-25.2	-4.5	-11.7	-3.4	-8.7	8.7	-7.2	-6.9	-30.8	5.2	2.4
KOM 1 x RIN 3	4.4	-3.3	6.1	-2.8	2.99	0.3	-6.1	-18.4	-4.5	-13.9	-15.8	-32.4	-9.9	-12.2	0.7	-1.7
KOM 2 x RIN 1	7.7	-1.3	14.6	-4.4	18.3	-3.7	7.7	6.8	4.6	-1.5	-5.6	-19.6	-54.3	-59.5	1.2	2.2
KOM 2 x RIN 2	-8.5	-13.7	-9.8	-12.6	0.1	-25.8	7.1	0.8	-5.1	-6.1	-11.3	-24.8	-7.7	-31.3	0.2	-0.4
KOM 2 x RIN 3	16.2	13.9	15.0	15.0	38.8	12.4	-21.4	-30.6	-14.7	-19.6	-45.9	-56.8	-18.6	-20.6	0.2	-0.7
F1B x RIN 1	45.4	41.9	57.6	36.7	21.3	-30.9	13.7	2.7	6.1	-1.0	-12.6	-20.7	-6.6	-10.2	2.3	2.2
F1B x RIN 2	-10.4	-20.7	-9.5	-16.2	-31.1	-61.8	-8.1	-12.7	-6.0	-6.1	1.0	-8.9	-32.6	-43.3	0.6	0.4
F1B x RIN 3	-10.1	-17.5	-0.4	-5.1	90.1	14.8	-1.8	-0.1	-4.3	-9.1	-5.2	-19.8	-38.1	-45.8	2.3	2.2

MP = mid-parent
HP = high-parent

CHAPTER 5

SUMMARY

1. The objective of this study was to determine the combining ability of several tomato genotypes, the correlation and heritability between several yield and quality characteristics and the expression of heterosis in the hybrids.

2. Six parental lines and three testers were used in a Line x Tester analysis. Crosses were made in glasshouses at the University of the Free State (UFS). The F1 hybrids and their parental lines were planted in glasshouses at the UFS with three replications. Several yield and quality characteristics were measured and analysed.

3. Significant differences were found between the parents for all characteristics measured. Significant differences were recorded between crosses, except for unmarketable yield. KOM 1 x RIN 1 ranked first for total and marketable yield, while the parental line, RIN 3, ranked first for shelf life. Significant differences were found between the lines, except for unmarketable yield, average fruit size and sugar-content (day 16). Significant differences were found among the line x testers, except for unmarketable yield, fruit pH (day 1, 4 and day 12) and sugar-content (day 1, 8 and day 20).

4. The parental line KOM 1 proved to be the best general combiner for both

total and marketable yield, while F1B was the best general combiner for average fruit mass, average fruit size and shelf life. Parental line R 1 was the best general combiner for fruit pH and sugar-content over the different days.

5. Crosses F1B x RIN 1, RDP 1 x RIN 2 and KOM 2 x RIN 3 were the best specific combiners for total and marketable yield, while RDP 1 x RIN 2 was also the best specific combiner for average fruit mass and average fruit size. KOM 2 x RIN 2 proved to the best specific combiner for the quality characteristics as it had the most positive effects followed by KOM 1 x RIN 1.

6. The specific combining ability (SCA) variance of the study was found higher than the general combining ability (GCA) variance for all the traits except for sugar-content. This means that a large part of the total genetic variability was the result of non-additive gene action.

7. Marketable yield showed a significant positive genetic correlation with total yield and average fruit size with fruit mass. Shelf life showed negative genetic correlations for all the traits, except for average fruit size.

8. All the traits had low broad-sense and very low narrow-sense heritabilities. Marketable yield (h^2n = 0.06) had the highest narrow-sense heritability for

the yield characteristics and fruit pH (h^2n = 0.08) the highest for the quality characteristics.

9 Eleven hybrids expressed positive mid-parent (MP) heterosis and five hybrids expressed positive high-parent (HP) heterosis for total yield. KOM 1 x RIN 1 expressed the highest mid-parent and high-parent heterosis for total yield and marketable yield. Fruit pH had the most hybrids with positive mid-parent (16) and high-parent (12) heterosis. Heterosis was mostly negative for all the quality characteristics measured, thus indicating the effect of the environment.

HOOFSTUK 5

OPSOMMING

1. Die doel van hierdie studie was om die kombineervermoë van verskeie tamatie lyne, die korrellasie en oorerflikheid van verskeie opbrengs en kwaliteits eienskappe en die uitdrukking van heterose in die nageslag te bepaal.

2. Ses ouerlyne en drie toetsers was gebruik in 'n Lyn x Toetser analise. Kruisings was gemaak in glashuise by die Universiteit van die Vrystaat (UVS). Die ouer lyne en die F1 basters is uitgeplant in die glashuise by die UVS met drie herhalings. Verskeie opbrengs en kwaliteit eienskappe is gemeet en geanaliseer.

3. Betekenisvolle verskille is gevind tussen die ouers vir al die eienskappe. Daar is ook betekenisvolle verskille gevind tussen die kruisings, behalwe by onbemarkbare opbrengs. KOM 1 x RIN 1 het die hoogste opbrengs by totale en bemarkbare opbrengs getoon, terwyl die ouerlyn, RIN 3, die langste rakleeftyd gehad het. Betekenisvolle verskille is gevind tussen die lyn x toetsers, behalwe by onbemarkbare opbrengs, vrug pH (dag 1, 4 en 12) en suiker-inhoud (dag1, 8 en 20).

4. Die ouerlyn, KOM 1, was die beste algemene kombineerder vir beide totale en bemarkbare opbrengs, terwyl F1B die beste was vir gemiddelde

vrugmassa, gemiddelde vruggrootte en rakleeftyd. R 1 was die beste algemene kombineerder vir vrug pH en suiker-inhoud oor die verskillende dae.

5. Die kruisings F1B x RIN 1, RDP 1 x RIN 2 en KOM 2 x RIN 3 was die beste spesifieke kombineerders vir beide totale en bemarkbare opbrengs met RDP 1 x RIN 2 ook die beste spesifieke kombineerder vir gemiddelde vrugmassa en gemiddelde vruggrootte. KOM 2 x RIN 2 was die beste spesifieke kombineerder ten opsigte van die kwaliteitseienskappe met die meeste positiewe effekte gevolg deur KOM 1 x RIN 1.

6. Die spesifieke kombineervermoë (SKV) in die studie was hoër as die algemene kombineervermoë (AKV) vir al die eienskappe behalwe vir suiker-inhoud. Dit beteken dat 'n groot deel van die totale genetiese variasie die resultaat van nie-additiewe geenaksies was.

7. Betekenisvolle positiewe genetiese korrellasies tussen bemarkbare en totale opbrengs en tussen gemiddelde vrugmassa en gemiddelde vruggrootte is verkry. Rakleeftyd het negatiewe genetiese korrellasies getoon met al die ander eienskappe, behalwe met gemiddelde vruggrootte.

8. Al die eienskappe het lae breë-sin en baie lae noue-sin oorerflikheid getoon. Bemarkbare opbrengs (h^2n = 0.06) het die hoogste noue-sin oorerflikheid

van die opbrengs eienskappe getoon en vrug pH (h^2n = 0.08) die hoogste vir die kwaliteit eienskappe.

9. Elf F1 basters het positiewe middel-ouer heterose en vyf F1 basters het positiewe beste-ouer heterose getoon vir totale opbrengs. KOM 1 x RIN 1 het die hoogste middel-ouer en beste-ouer heterose getoon vir beide totale en bemarkbare opbrengs. Vrug pH was die eienskap waar die meeste basters positiewe middel-ouer (16) en beste-ouer (12) heterose getoon het. Die kwaliteit eienskappe het meestal negatiewe heterose getoon, wat dui op die groot effek wat die omgewing gehad het op die kwaliteit.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

The result of this study showed that a large amount of genetic variation existed and the environment and stress conditions had a large effect on the expression of the different characteristics. The performance of the genotypes might change in another environment.

In order to improve tomato yield and quality, identifying and selection of suitable parents which express high levels of heterosis in combination with each other and high combining ability (GCA and SCA) is extremely important. The results showed that the parental line KOM 1 was the best general combiner for total and marketable yield, while F1B x RIN 1 and KOM 1 x RIN 1 were the best specific combiners for total and marketable yield and could be used to improve these characteristics. R 1 can be used to improve the sugar-content and fruit pH, while F1B was the best general combiner for shelf life. No significant differences were found between the parental lines and hybrids for sugar-content and fruit pH, which indicates that the hybrids have acceptable taste. The high SCA effect of F1B x RIN 1 and KOM 1 x RIN 1 for total and marketable yield showed in their high heterosis response for these characters. Maximum levels of heterosis will be obtained if overdominance is present and the differences in gene frequency among the parental genotypes are high.

Significant differences were found between the hybrids and the parents for shelf life. Most of the hybrids had an intermediate shelf life between the two parents. This indicate that the choice of the parental lines to cross with the testers (RIN 1, RIN 2 or RIN 3) are very important to obtain the highest shelf life. It would be however, very difficult to improve the quality characteristics because of the low heterosis and low narrow-sense heritabilities. There were also no substantial correlations found to help the beeder to improve the quality characteristics.

The hybrid plants have produced fruit with increased utility for the fresh markets as the fruit can be harvested at a more advanced stage with a longer shelf life than the present cultivars.

ABBREVIATION LIST

ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	Analysis of Variance
AOA	N-[2-(2-amino-ethoxy)-ethenyl] glysine
AVG	(aminooxy) acetic acid
Ca	Calcium
COV	Coefficient of variance
CPTA	2-(4-chlorophenylthio) ethyldiethyammonium chloride
DF	degrees of freedom
EFE	ethylene
EMS	Expected mean squares
GCA	General combining ability
h^2	Heritability
h^2b	Heritability in broad sense
h^2n	Heritability in narrow sense
HP	High-parent
LSD	Least significant difference
Me	Mean error
MP	Mid-parent
MS	Mean squares
MSE	Mean squares for error
nor	non ripening
nr	never ripe
PE	Pectinmethylesterase
PG	Polygalacturonase
PG 1	Polygalacturonase isoform 1
PG 2A	Polygalacturonase isoform 2A
PG 2B	Polygalacturonase isoform 2B
pH	acidity
r	Correlation coefficient

rin	ripening inhibitor
SAM	S-adenosyl methionine
SCA	Specific combining ability
SE	Standard error
UFS	University of the Free State
USA	United States of America
UV	Universiteit van die Vrystaat

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