UNIVERSITY OF NIGERIA, NSUKKA
DEPARTMENT OF CROP SCIENCE

POSTGRADUATE RESEARCH SEMINAR

(FINAL SEMINAR)

STUDIES ON THE GENETIC PATHWAY AND SELECTION RESPONSE FOR INCREASED FRUIT SIZE AND YIELD IN TOMATO (*SOLANUM* SPECIES) USING A MODIFIED THREE - WAY CROSS.

BY

NNUNGU, STEPHEN ISSA
(PG/PHD/12/64064)

SUPERVISOR: PROFESSOR M.I. UGURU

DECEMBER, 2015

ABSTRACT

Modified three - way crosses involving advanced interspecific tomato hybrids (F_{12}) and commercially cultivated tomato varieties namely Supersteak (S), Beef (Florida) (BF) and Plumb (Rio grande) (PR) were made to generate F_1 hybrids. The F_1 hybrids were crossed to both the pollen and seed parents to produce the backcrosses and were also allowed to random mate to produce the F_2 and F_3 populations. The parents, F_1s, F_2s, F_3s and backcrosses were evaluated under rainfed conditions. Floral trait analysis and genomic analysis using Single Nucleotide Polymorphisms (SNPs) markers of the quantitative trait loci (QTL) underlying fruit size in tomato were also performed. The Analysis of Variance (ANOVA) on agronomic, yield, floral and fruit traits showed significant differences (P = 0.05) among the tomato genotypes. The cross, S x (W x R) was the most promising three - way hybrid that can be exploited for increased fruit size and yield in the humid tropics. Mean fruit weight had significant and positive correlation with all the floral traits with the exception of flower and style lengths. The number of locules per fruit had the highest correlation value (r = 0.984**) with fruit size. Path coefficient analysis revealed that number of locules per fruit had the highest positive direct effect (p = 0.8086) on fruit size. This was closely followed by ovary diameter (p = 0.7942) and stigma diameter (p = 0.7685). On the other hand, style length had the highest negative direct effect (p = -0.9147) on fruit size. The fruit shape index showed significant positive correlation with the ovary shape index (r = 0.835**) and seed shape index (r = 0.718**). However, fruit shape index was negatively and significantly correlated with ovary diameter (r = -0.601*), fruit diameter (r = -0.576*) and seed diameter (r = -0.519*). The structure analysis revealed that the tomato genotypes studied had three sub-populations. The association mapping using 25 SNPs markers detected 9 markers with significant association with mean fruit weight, fruit length, fruit diameter, number of locules per fruit and fruit shape index. The SNP marker, Solyc11-17 exhibited significant association with both fruit diameter , number of locules per fruit and fruit shape index. The variation in fruit diameter explained by the marker, Solyc11-17 was higher than the variations in number of locule and fruit shape index (141.5%, 23% and 18.3%) respectively. All the 9 markers detected are recommended for fruit size improvement breeding programe in tomato.

INTRODUCTION

Tomato (*Solanum lycopersicum. L*) is an important horticultural crop worldwide and is the second most consumed vegetable after potato (FAO, 2005). Tomato production is considered one of the main agricultural enterprises as it employs people in farms, processing industries and provides higher income per hectare to small holder farmers than most staple crops (AVRDC, 2006). It also plays a key role in human health as a source of vitamins A, C and micronutrients (Tindall, 1983; Peralta and Spooner, 2001). Tomato fruits contain lycopene, an anti-oxidant known to reduce the incidence of cancer, heart and age related diseases (AVRDC, 2003).

Tomato production is an economically important venture in Africa, but is not profitably produced in the humid zones due to excessive precipitation and the associated high relative humidity diseases. Breeding of tomato cultivars that are high yielding with acceptable market fruit size and some level of tolerance to high humidity conditions will open up a new production opportunity for the poor resources farmers in the rain forest ecologies.

An increase in the tomato growing areas will minimize the need for long distance transportation of tomato from the drier parts to the more humid regions. This will reduce the transport induced damage or deterioration and the subsequent reduction of the market price of tomato fruits. The expansion of the production areas would also create additional employment in the sector and generate income. Increased production at a reduced cost would also benefit the industrial sector especially those making tomato based products because of increased availability of raw materials for processing. These benefits can be achieved if home-based cultivars with adequate adaptation to the humid environments are developed through organized breeding.

Uguru and Atugwu (2001) reported that exotic cultivars perform poorly in terms of yield and quality under high humidity conditions. The wild tomato, *Solanum pimpinellifolium,* is tolerant to high humidity diseases and is capable of producing up to 743 tiny fruits per plant (Tanksley *et al.*,1996; Foolad and Lin, 1999; Uguru and Atugwu, 2001). The tiny fruits are generally unacceptable in urban and local markets. Crosses between the commercially acceptable but poorly adapted cultivars including Roma VF, Tropica and Nsukka local and the wild tomato variety have produced promising genotypes endowed with prolific fruiting and reduced fruit rot. Successive evaluations of the progenies at different filial generation from F_1 to F_{12} showed reliable evidence of increased fruit yield particularly in terms of number of fruits (Uguru and Umukoro, 2005; Atugwu and Uguru, 2012) and increased disease resistance (Uguru and Igili, 2002). However, the average fruit size is yet to attain a level of full acceptability in the local market. The process of tomato fruit incremental pattern as monitored from F_1 to F_{12} using interspecific hybrids of the wild tomato and the commercially cultivated varieties showed that the rate of fruit size increment was minimal from F_1 to F_5, rapid from F_5 to F_9 and less rapid from F_9 to F_{12} (Atugwu and Uguru, 2012). The authors were able to provide sufficient proof of the exhaustion of genetic variability and substantial decline in the effectiveness of any selection beyond these generations in the improvement of fruit size.

Other workers implicated multiple loci in the inheritance of fruit size in tomato (Ibarbia and Lamberth, 1969). The authors reported as high as 10 to 20 loci for the control of fruit size in tomato and speculated that genes behave in an additive manner in different fruit developmental pathways, each contributing to the final fruit size. For example developmental studies revealed

that tomato size is determined by the number of ovary cells before fertilization, number of successful fertilizations, number of cell divisions that occurred within the developing fruit after fertilization and the extent of cell enlargement (Bohner and Bangerth, 1981; Gillapsy *et al*., 1993). Some of the loci exerted their effects through modulation of the size of the carpel and number of locules, fruit length, fruit diameter and number of seeds (Nitsch, 1970). The major six fruit size QTL namely; *fw1.1*, *fw1.2*, *fw 2.1, fw2.2, fw3.1/fw3.2* and *fw11.3* are located on chromosomes 1, 2, 3 and 11 (Lippman and Tanksley, 2001). The combination and order of magnitude of these loci would determine the level of success in the improvement of fruit size in tomatoes during selection. Any selection process that is able to assemble the six major QTL for fruit size in a single population would produce large fruited tomato variety.

Large fruit size is a desirable horticultural characteristic in tomato improvement and an important feature in crop breeding. Fruit size is quantitatively inherited and large members of QTLs have been identified in tomato that are associated with fruit development, size, shape, colour, ripening, organoleptic quality and yield (Causse *et al*., 2002; Van der Knaap and Tanksley, 2003). The inheritance studies of tomato fruits (Atugwu and Uguru, 2012) have concentrated the genes for profused fruiting in tomatoes under high rainfall conditions. The contending issue at present is on making additional progress in fruit size increment in order to exploit the prolific fruiting in the interspecific hybrids to an advantage. This would necessitate further crosses between the hybrids with exotic breeds with giant fruit size and selection from the segregating population. This improvement stratergy was adopted in the present study in crosses between one of the largest fruited tomato variety, *Solanum lycopersicum* cv, supersteak and the

advanced hybrids endowed with prolific fruiting. The objectives of the present study were therefore:

1. to identify the major developmental pathway for large fruit size in tomato as ordered by the relevant loci, using crosses between the large fruited tomato variety and the interspecific hybrids.

2. to establish the number, magnitude of effects and the interaction of the QTL in the determination of fruit size.

3. to identify and select acceptable market fruit size tomato variety with excellent adaptation to high humidity conditions of south eastern Nigeria.

LITERATURE REVIEW

Botanical description and production

Tomato belongs to the family Solanaceae with more than 3000 species such as potato, tobacco, pepper, eggplant and petunia. Previously, tomato was classified as *Solanum lycopersicum* alongside with potato. It was moved to *Lycopersicon esculentum* because tomato leaves are markedly different from any other *Solanum* and many of the alkaloids common to solanum species are conspicuously absent in tomato. However, recent genetic evidence reclassified tomato as *Solanum*. It grows best in well drained soils with optimum pH of 5.5ï 6.8 (Tanzania-German IPM Project, 2000) and temperatures between 20 °C and 27 °C (Peet *et al.,* 1998). Most tomato varieties are adapted to dry conditions and share common disease and pests with other Solanaceous crops.

Tomato is currently grown in almost every country of the world. Worldwide tomato production reached 130 million tonnes in 2008 and occupied 5.2 million ha (FAOSTAT 2008). Tomato is not only important economically, but also prominent in the human diet providing essential vitamins and antioxidants. It is the most eaten vegetables in the world and their popularity stems from the fact that they can be eaten fresh or in a multiple of processed forms. 90% of Nigerian homes consume tomato in form of stew and salad vegetables. Tomato fruit has an important place in the human diet as it contains antioxidants such as lycopene; vitamins E and C, ɓ -carotene and phenolic compounds (Tuckers *et al*., 2007). Lycopene is important for human health, decreasing the risk of cardiovascular diseases, heart attacks and several types of cancer, including prostate and cervical cancer (Dorgan *et al*., 1998, Clinton 2005). ɓ-carotene is provitamin A and deficiency of it causes blindness, xerophtalmia (severe drying of the eyes) and even premature death (Laquatra *et al*., 2005). Tomatoes also include many trace elements such as

molybdenum, iron, phosphorus, magnesium, niacin and potassium, thiamine and riboflavin which all have health benefits for humans such as lowering high cholesterol level and high blood pressure (Lachance, 1998).

Tomato production constraints in southern eastern Nigeria

The major tomato production constraints reported in south eastern of Nigeria include high temperature, high relative humidity and rainfall which are usually associated with tomato diseases such as damping off, fusarium wilt, bacterial wilt, leaf curl, leaf spot and blossom end rot, and finally lack of well adapted, high yielding varieties.

High temperature, relative humidity and rainfall

The bulk of tomato production in Nigeria comes from the northern part of the country, due to the optimum environmental condition for growing tomatoes such as adequate rainfall, diurnal range of temperature, relative dry climate, low relative humidity and low incidence of pests and diseases. Tomato production along southern part of Nigeria is limited to the short cool hamatan period, but, this period attract much insect pests to the crop causing heavy damages and high economic loss (Izge and Garba, 2012). Picken (1984) reported that high temperature, relative humidity and rainfall cause severe flowers abortion in tomato that result in discernible yield decline. Similarly, Weerakkody and Peiris (1997) documented that the major effect of rainfall was on the yield of tomato as a result of reduction of number of flowers due to abortion. Also, they reported that high rainfall affects fruit quality as rainfall was positive correlated with the fruit cracking. Furthermore, heavy rainfall and high relative humidity encourage the growth of mould which results in fruit rot. At high temperature and high humidity, foliage diseases are

rampant and these often result in losses in terms of yield (Uguru and Igili, 2002). High relative humidity also creates room for many diseases like damping off, fusarium wilt, tomato leaf curl and blossom end rots, decrease in plant transpiration and nutrient absorption (Dorais *et al*., 2001).

A number of explanations have been given for the poor performance of tomatoes at high temperature. These include reduced or abnormal pollen production, abnormal development of female reproductive tissues, hormonal imbalance, and low level of carbohydrate and lack of pollination (Peet *et al*., 1997). Dinar and Rudich (1985) reported that in tomato plants, high temperature affect several physiological and biochemical processes such as photosynthetic enzymes activity, membrane intergrity, phosphorylation and electron transport in chloroplast, stomatal conductance to CO2 diffusion and photoassimilate translocation in tomato. However, the impact of high temperature to plant is not limited only to flowering and fruit set but also affect other subsequent development and maturity of the fruits.

Genetic variation and improvement in tomato

The cultivated tomato (*Solanum lycopersicum*) reached its present form and place after a long period of domestication. The evolution of tomato as a crop started with domestication in America in the 16^{th} century (Peralta and Spooner, 2007) and domestication led to the modification of a wide range of physiological and morphological traits. Initially, development was probably from selection for preferred genotypes. Moreover, the genetic variation tend to decrease even without selection, Lack of diversity was also confirmed using DNA technology where very few polymorphisms were identified. However, lack of diversity within *Solanum lycopersicum* is not a barrier to the breeding progress, due to the variation readily available from the wild relatives (Heuvelink, 2005). Wild tomatoes are very valuable because they contain

genes for resistance to diseases, abiotic stresses and improved colour and fruit quality (Rick, 1978).

The wild tomato, *S. pimpinellifolium* possesses a number of desirable horticultural characteristics including abiotic stress tolerance, diseases resistance and high fruit quality (Chen and Foolad, 1998). Moreover, the natural habitat of wild tomatoes are highly variable, from very dry to very wet and from coastal to mountainous areas of more than 3300m elevation (Warnock, 1988). This diversity in habitat has undoubtedly contributed to the great variation in wild tomato. *S. pimpinellifolium* was also reported to be resistant to many tomato diseases that are associated with high rainfall condition like fusarium wilt, bacterial spot, bacterial wilt, bacterial canker and tomato leaf curl virus (TYLCV) (Tanksley *et al*., 1996; Uguru and Igili, 2002). However, the wild tomato possesses some undesirable horticultural traits such as small fruit size (Rick and Buttler, 1956; Allard, 1999).

Genetic improvement of tomato can be achieved by using the abundant genetic variations in the wild tomato relative. Plant breeders have intensively used elite lines crosses to develop new inbred lines and hybrid cultivars and, thus the genetic base in tomato and other crops has eroded (Tanksley and McCouch, 1997).

Fruit size variation in tomato

Large fruit size is a desirable horticultural characteristics in tomato improvement and an important feature in crop breeding and market acceptability. The wild species, *Solanum pimpinefollium* harbours numerous desirable horticultural and agronomic characteristics, including diseases resistance and abiotic stresses tolerance (Tanksley *et al*., 1996; Chen and

Foolad, 1998), but it produces very small fruits. Cultivated tomatoes vary tremendously in fruit size and shape but have little genetic variation in their genome. Conversely, wild tomatoes contain tremendous genetic variation in their genome but show little variation in fruit size and shape (Foolad, 2007). Improvement in tomato fruit size has been achieved since cultivated tomato was first domesticated from their wild progenator, *Solanum pimpinefollium* (Rick, 1976). As a result a tremendous variability in fruit size exists within the solanum species, from the extremely small fruited wild species *Solanum pimpinefollium* (1 to 2 g) to *Solanum lycopersicum*, some of which produce fruit that reach 1000g.

Fruit development can be divided into four distinct phases; ovule fertilization and fruit set, cell division, cell expansion and ripening (Gillapsy *et al*., 1993). The first step occurs within 2 to 3 weeks period between floral initiation and production of mature flower, during which the identity, number and shape of all floral organ are determined. Cell division phase begins at anthesis and continue for 2 weeks after fertilization. This phase is characterized by intense cell division during which the final number of cells in the pericarp is almost determined. The duration and intensity of this phase has been shown to be related to the final fruit size (Bohner and Bangerth, 1988). Cell expansion phase is related to the accumulation of water, organic acid and minerals in fruit cell vacoules (Coombe, 1976). It begins toward the end of cell division stage and continues until 1 week before the onset of ripening (Bergervoet *et al*., 1996). The early stages of fruit development are important in the latter characteristics of the mature fruit including fruit size, weight and composition.

By definition, fruit size is a volumetric trait that is determined as the product of diameter, height and depth (Powers, 1951). Genetic studies have established that tomato fruit size and shape are largely quantitatively inherited. Quantitative trait are controlled by more than one gene with great environmental effects. Also the inheritance studies reveal that tomato fruit size is quite complex and determined by multiple loci (Ibarbia and Lambeth, 1969). Similarly, molecular mapping studies have revealed the presence of dozens of QTL for fruit size in tomato some of which have very large effects (Chen *et al*., 1999).

A classical genetics have suggested that at least 5 - 6 genes govern the inheritance of fruit size trait (Powers, 1951). Also QTL analysis studies (Lippman and Tanksley, 2001) involving crosses between wild tomato, *S. pimpinefollium* and giant heirloom tomato, *S. lycopersicum* revealed that the majority (67%) of phenotypic variation in fruit size could be attributed to six major loci localized on chromosome 1, 2, 3 and 11. However, these genes are involved in a variety of distinct fruit developmental pathways, each contributing to final fruit size. Mutation is reported to have occurred in six loci found to be essential in transforming the small, inconspicuous berries of the wild tomatoes to the extremely large fruits which are now associated with modern cultivars. Six QTLs for fruit weight were identified on chromosome 1 (*fw1.1* and *fw1.2*), chromosome 2 (*fw2.1* and *fw2.2*), chromosome 3 (*fw3.1/fw3.2*) and chromosome 11 (*fw11.3*) (Grandillo *et al*., 1999). Van der knaap and Tanksley (2003) reported that *fw1.1, fw2.2* and *fw11.3* are the most significant QTLs for the fruit size. Natural allelic variation at these loci especially *fw 2.2* has a major impact on fruit size and can change the final fruit mass by as much as 30%. *fw2.2* control cell division in carpel/fruit development rather specifying carpel number. It was also reported to be associated with locule number. Similarly *fw2.1* and *fw11.3* appeared to

exert their effect on fruit size through modulation of carpel and locule number. Similarly, seven QTL for fruit length were found to be distributed on chromosome 1 to 4, 9 and 11, moreover, the most significant QTL were detected in chromosome 2 (*fl2.1*) and 11 (*fl11.1*).

Component of fruit size and shape such as number of locules per fruit, average weight per locule and fruit length/diameter ratio are important concern when breeding for fresh market tomatoes. Powers (1945) concluded that the relationship between number of locule and locule weight in such that increments in fruits weight could be attained by simultaneous selection for large number of locules for fruit and for higher weight per locule. The *fw 2.2* locus affects fruit mass but not the overall structural organization or shape of the fruit. However, final fruit mass can also be affected by changes in the shape and architecture of the ovaries produce flower with gynoecium containing two to four carpels and after fertilization each carpel develop into the locule in the fruit. However, some varieties produce fruits with more locule often resulting in larger wider fruits (Lippman and Tanksley, 2001; Van der Knaap and Tanksley, 2003). Two loci, fasciated at chromisome 11 and locule number at chromosome 2 have been identified as causing changes in fruit size via changes in the number of carpel in the flower. All of the very large fruited, multilocular fresh market tomatoes tested to date carry mutations in one or both of these genes (Lippman and Tanksley, 2001).

The majority of studies of inheritance on fruit size in tomatoes indicated that there is hardly ever heterosis for this trait and that fruit size in the hybrid is usually smaller than the parental arithmetic mean (Larson and Currence, 1994; Maluf *et al*., 1982; Melo, 1988).

Crossing Methods

Diallel analysis is one of the mating designs used in predicting combining ability of the parents and the type of gene action involved in the expression of traits (Baloch *et al*., 1995). Plant breeders and geneticists often use Diallel methods to test for general and specific effects behind quantitative traits. Diallel methods include partial diallel with or without parents, full diallel usually required when maternal effects are expected to be large. Diallel analysis can also be used in studying the heterosis, gene action, general and specific combining ability (GCA and SCA). Superiority of F_1 over parents is called heterosis. Heterosis can be expressed when the parents of the hybrid have different alleles at a locus and there is some level of dominance or epistasis among the alleles (Falconer and Mackay, 1996). Exploitation of heterosis provide an excellent opportunity for deciding efficient traits in crops like tomato. It is reported that heterosis in tomato resulted in increased yield of 20% to 50% (Chowdhury *et al*., 1965). Combining ability is the ability of transferring genes to the hybrid when the parents combine (Sprague and Tatum, 1942). Combining ability helps the breeder to study and compare the performance of lines in a hybrid combination and understanding the genetic potential of both parents and hybrids (Griftings, 1956; Singh and Asati, 2011). General combining ability is determined by the additive genes while the specific combining ability is determined by the dominant genes. For example, crosses may deviate from the expected values of the GCA of the parental lines to a greater or lesser extent. This deviation is the specific combining ability. Combining ability studies provide useful information regarding selection of suitable genotypes for effective hybridization and at the same time to elucidate the nature and magnitude of different types of generation. If variance of GCA is more than that of SCA for a particular trait, it would imply predominance of additive generation over other dominance, epistasis and gene action.

Genetic markers and Mapping

The main aim of plant breeding is to improve agronomically relevant traits by combining characters from different parental lines or their relatives (Winter and Kahl, 1995). Genetic markers reveal these characters and other genetic differences between organisms. Markers are specific locations on a chromosome that serve as indicators for genome analysis. Genetic markers are generally classified into morphological and molecular markers.

Morphological markers are visually characterized phenotypic characters such as colour height and shape (Winter and Kahl, 1995). Morphological markers have some disadvantages as they are limited in number and affected by environmental changes, therefore, their reliability and reproducibility is very low. Also they are inefficient in distinguishing heterozygous and homozygous individuals (Kumar, 1999). In addition, there are a limited number of morphological markers in nature.

Molecular markers include biochemical markers and DNA markers. Biochemical markers reveal polymorphism at the protein level and are also called isozymes. They are proteins that can be identified by electrophoresis. However, their limited number and dependence on post-translational modifications constrain the use of isozymes (Staub *et al*., 1982). Also as morphological markers, they are easily influenced by environmental factors or the developmental stage of the plant. Besides these drawbacks these types of markers are useful to plant breeders (Eagles *et al*., 2001, Weeden *et al*., 1994). Molecular markers as a DNA markers are genetic loci for which different alleles reveal sequence variation at the DNA level and may be may be gene-coding or non-coding pieces of DNA. Also DNA markers originate from DNA mutations such as point mutations, insertions or deletions that generally occur in non-coding regions (Collard *et al*.,

2005). Unlike morphological and biochemical markers, molecular markers have several advantages. They are virtually unlimited in number and are stable markers so they are easily discovered using molecular techniques. They are not affected by environmental factors of DNA markers, they are used in many applications in plant breeding such as evaluation of the level of genetic diversity in germplasm, cultivar identification and linkage mapping (Baird *et al*., 1997, Henry 1997, Jahufer *et al*., 2003).

Until now, there are many types of DNA markers have evolved such as restriction fragment length polymorphism (RFLPs) (Botstein *et al*., 1980), randomly amplified polymorphic DNAs (RAPDs) (Williams *et al*., 1990), amplified fragment length polymorphism (AFLPs) (Vos *et al*., 1995), variable number of tandem repeats (VNTRs or minisatellites) (Jeffreys, *et al*., 1985), simple sequence repeats (SSRs or microsatellites) (He *et al*., 2003), cleaved amplified polymorphic sequences (CAPS) (Konieczny and Ausubel 1993), sequence characterized amplified regions (SCARs) (Paran and Michelmore 1993), expressed sequence tags (ESTs) (Adams *et al*., 1991), conserved ortholog set (COS) markers (Fulton *et al*., 2002), singlenucleotide polymorphisms (SNPs) and insertion-deletion (InDels) markers (Landegren *et al*., 1998). Currently, the use of RFLP for breeding purposes is limited because it requires the use of radioactivity and is labour intensive. RAPD and AFLP markers either identify only dominant alleles or are sensitive to PCR amplification conditions.

The most popular DNA marker type in tomato researches has been SSR (Fulton *et al*., 2002, Frary *et al*., 2005). SSR markers are a well established and traditional form of molecular marker (Tautz and Renz 1984). SSRs or microsatellites are short (usually 2-4 base pair), tandem repeat

DNA sequences. Replication slippage and unequal crossing over during meiosis cause variation or polymorphisim of SSRs (Levinson and Gutman 1987). SSR markers are commonly used molecular markers in crop breeding because of their useful properties: codominant inheritance, high abundance, enormous extent of allelic diversity and the ease and reproducibility of assessing SSR size variation by PCR with pairs of flanking primers. Moreover, they are practical and useful for genetic mapping, diversity studies and marker assisted selection.

Single Nucleotide Polymorphisms (SNPs) have emerged as the most widely used genotyping markers due to their abundance in the genome and the relative ease in determining their frequency in a cost-effective and parallel manner in a given panel of individuals (Deschamps, 2012). SNPs are a predominant form of sequence variation among individuals representing as much as 90% of the genetic variation in any species. SNPs are distributed throughout a genome, they provide stable marker for genetic analysis and the detection is amenable to automation. In many crop plants, SNPs are present with sufficient frequency to offer an alternative for genetic mapping and marker assisted selection. SNPs associated with traits have been discovered in tomato, rice, soybean and onion (Gupta *et al*., 2002). Recently, 62,576 non-redundant SNPs were identified based on trascriptome sequence for six tomato accessions. An advantage to using SNPs in plant breeding application is that, genotyping can be automated using single nucleotide primer extension assays (Giordano *et al*., 1999) thus offering potential to increase both efficiency and throughputs.

Molecular marker mapping can be described as placing markers in their correct order along different linkage groups (Jones *et al*., 1997). For localizing important genes controlling both

qualitative and quantitative traits in plants, molecular marker linkage maps are very useful (Dirlewanger *et al*., 1998). Linkage maps are used to identify chromosomal locations that contain genes and QTLs for traits of interest. Genetic mapping is based on the fact that genes or markers which are close together or tightly-linked are transmitted together from parent to progeny more frequently than genes or markers which are located further from each other. For mapping, segregating populations consisting of parental and recombinant genotypes are used. The frequency of recombinant types in the population is used to calculate recombination fraction which is used to determine genetic distance between markers. Markers that are close together will have less recombination than those that are further apart on a chromosome. For mapping purposes recombination fractions are converted into map units called centiMorgans (cM). When map distances are small (< 10 cM), the map distance equals the recombination frequency but this relationship does not apply for map distances greater than 10 cM (Hartl and Jone 2001). The construction of a molecular marker map depends on development of an appropriate mapping population, estimation of recombination frequencies of marker loci in this population, establishment of linkage groups of markers and determination of map distance and order of markers (Staub *et al.*, 1996).

To develop an appropriate mapping population two homozygous parent lines that show polymorphism for the markers in question are crossed to get a heterozygous F_1 (filial) hybrid, and the F_1 hybrid can be used to produce a segregating population. Recombination frequency is expressed as the percentage of recombinant progeny (for each marker) in the segregating population. Recombination frequency is directly proportional to the genetic distance between two loci. That means recombination between loci that are close to each other is lower than loci

that are far apart. For that reason, recombination frequency can be used to define appropriate distances between two loci along the chromosome (Jones *et al*., 1997). By using computer programs such as MapManager, Join map and MAPMAKER that determine the lineararrangement of molecular markers by estimating recombination frequencies, a linkage map can be easily constructed (Staub *et al*., 1996). Once a genetic linkage map, based on molecular markers, has been constructed, it can be used for identification of gene location, positional gene cloning, comparative mapping and marker assisted selection in plant breeding. The ability of markers to act as a landmarks leads us to genes of interest along the chromosome (Jones *et al*., 1997). By using molecular marker maps, both qualitatively and quantitatively inherited traits can be mapped.

Understanding the molecular genetics control of phenotypic variation is a major task in the study of natural and cultivated plant populations. This challenging objective becomes much more complicated when traits controlled by quantitative loci are considered, as for most of the features underlying crop yield and quality. Along with the classical analysis of quantitative trait loci (QTLs) through the genotyping of suitable segregating populations, new approaches have recently been proposed, where the identification of QTLs for important traits is addressed through association genetics after the phenotypic and genotypic characterization of collections of diverse materials (Lynch and Walsh 1997; Flint-Garcia *et al*., 2005). Association mapping has proven to be a reliable tool to highlight marker-trait associations in a number of plant species (Kraakman *et al*., 2001; Zhang *et al*., 2005; Aranzana *et al*., 2005; Breseghello and Sorrels 2006; Herrmann *et al*., 2006).

According to Gupta *et al.* (2005), association mapping refers to significant association of a molecular marker with a phenotypic trait. Association mapping generally falls into two broad categories: (i) candidate-gene association mapping, which relates polymorphisms in selected candidate genes that have purported roles in controlling phenotypic variation for specific traits; and (ii) genome-wide association mapping, or genome scan, which surveys genetic variation in the whole genome to find signals of association for various complex traits (Risch and Merikangas, 1996). For candidate-gene association mapping, information regarding the location and function of genes involved in genetic, biochemical or physiological pathways that lead to final trait variation is often required (Risch and Merikangas, 1996; Mackay, 2001). Candidate-gene association mapping requires the identification of SNPs between lines and within specific genes because SNPs offer the highest resolution for mapping QTLs and are potentially in LD with the causative polymorphism (Rafalski, 2002). Whole-genome association scans requires high-capacity DNA sequencing instruments or high-density oligonucleotide (oligo) arrays to efficiently identify SNPs at a density that accurately reflects genome-wide LD structure and haplotype diversity. As sequencing and genotyping costs continue to decrease, we expect to see more genome-wide association mapping. As reviewed by Zhu *et al.* (2008), population size for several association mapping studies is about 100, which is much lower compared to individuals used for linkage-based QTL mapping.

MATERIALS AND METHODS

Experimental sites and materials

The experiment was carried out at the Department of Crop Science Research Farm and greenhouse, University of Nigeria, Nsukka, located in the derived savannah zone (Latitude $0.6^{o}52$N, longitude $07^{o}24$E with an altitude of 447.26 m above sea level). The experimental materials comprised advanced generations of tomato lines, three cultivated tomato varieties (*Solanum lycopersicum*) namely: Roma VF, Tropica and a wild relative, *Solanum pimpinellifolium,* their progenies W x R, R x W and W x T and a large fruited inbred tomato variety, *Solanum lycopersicum* (Supersteak), Beef (Florida) (BF) and Plumb (Rio grande) (PR)

Experiment 1

Population Development

Advanced generations of interspecific hybrids of *S. pimpinellifolium* and *S. esculentum* were crossed with the large fruited inbred, *S. lycopersicum* cv. supersteak to produce hybrids using a modified three way cross. The advanced generations of the interspecific crosses produce many fruits in high rainfall regions and were used as the seed parents. The large fruited inbred served as the pollen parent in all the crosses.

Seedlings of the advanced generation hybrids and the large fruited inbred tomato variety were raised in nursery boxes filled with sterilized soil, well cured poultry manure and river sand mixed at a ratio of 3:2:1. The seedlings were transplanted into polybags arranged in a screenhouse at four weeks after planting. Crosses were made using a pair of dissecting pins, sharp forceps and scissors The anther cone of the pollen parent was removed and the pollens were manually transferred to the stigma of the pistillate parent. Flower buds were emasculated and covered with

paper bag to prevent stray pollen. In all the crosses, the interspecific hybrids served as the pistillate parent in order to preserve their integrity for tolerance to high humidity stress. The crossed flowers were tagged for easy identification. Pollinating tools were cleaned thoroughly before use for other crosses with 95% ethanol. Successful crosses were harvested and their seeds were planted to generate the F_1 plants. The F_1 hybrids were selfed to produce the F_2 populations.

Experiment 2

Production of Backcross populations

The F_1 hybrids obtained from experiment 1 were raised in nursery boxes as in experiment 1. Seedlings were transplanted into the polybags at four weeks after planting. Crosses were made between the F_1 and the two parents to generate the backcross populations.

Experiment 3

Evaluation of F_2, BC_1 and BC_2 (Field trial during the rainy season)

Seeds of the F_1, F_2, BC_1 and BC_2 generations and their respective parents were planted in the nursery boxes with top soil, well cured poultry manure and river sand mixed in a ratio of 3:2:1 by volume. Seedlings were transplanted four weeks after planting. The experiments were laid out in a Randomized Complete Block Design with four replications at the spacing of 60 cm X 60 cm. The following data were collected on single plant basis; days to first flower bud initiation, days to anthesis, number of flowers per plant, number of trusses per plant, number of fruits per plant, number of flowers per truss, days to first fruit emergence, days to fruit ripening, single fruit weight and fruit yield per plant.

Experiment 4

Floral/Fruit traits Analysis

Parents and their progenies, F_1s were utilized for this experiment. The flowers were harvested and immediately placed in a plastic bag and taken to the laboratory for the measurement of the floral characteristics at anthesis. Flowers were cut longitudinally to expose the ovaries and other floral parts. The ovary shape index was obtained as the ratio of the length to the diameter of the ovary. Other floral characteristics that were measured were the flower length and width, stalk width, stigma diameter, length and diameter of the style, anther diameter and length, ovary diameter, length, area and perimeter. The measurements were done using moticam with Motic Images Plus 2.0 software. The fully matured fruits were harvested and cut transversely for the following observations and measurements: number of locules, fruit diameter, pericarp thickness the number and weight of seeds.

Data collection

Data were collected from parents, F_1, $F_{2,}$ BC_1 and BC_2 generations. The details of the measurements and data collection were as follows:

Days to first flower buds initiation

The days to first flowering and opening were recorded from the day of planting to the days to the first day of bud initiation.

Number of flowers per plant

Flowers of each sample plant were counted and recorded when the whole plant reached maximum flowering.

Number of fruits per truss

Fruits were counted at first fruit ripening. Fruits of three trusses of each of the sample plants were counted and recorded.

Number of fruits per plant

This was done during harvesting. All fruits from each of the sampling plant were harvested, counted and the average recorded.

Mean fruit weight of single fruits

Average fruits weight were calculated by using total weight of the fruits divide by the number of the fruits.

Fruit yield per plant

The fruit yield of the sampled plants was weighed using an electronic weighing balance and the mean recorded per plant.

Fruit length

Fruits at ripening from the sampled plants were cut longitudinally and the fruit length was measured from the point of attachment to the blossom end using a vernier calliper.

Fruit diameter

The fruit diameter were measured using vernier calliper

Number of locules per fruit

Number of locules of fruits from the sampled plant was counted after making a transverse section of the fruit.

Number of seeds per fruit

Number of seeds per fruit were counted manually after extraction from the fermented fruit

Seed weight per fruit

The seeds from each of the sampled plant was extracted, air dried and weighed with an electronic weighing balance.

Length of style

The flowers of the sampled plants were cut longitudinally to expose the style and the style length was measured using moticam with Motic Images Plus 2.0 software from the stigmatic tip to the base of the ovary.

Diameter of the style

The flowers of the sampled plants were cut longitudinally and the diameter of the cut style tube was measured using moticam with Motic Images Plus 2.0 software.

Length and width of ovary

The flowers of the sampled plants were cut longitudinal to expose the ovary and the ovary length and width were measured using ocular micrometer.

Experiment 5

Genotypic characterization

DNA extraction

Total genomic DNA was extracted using the modified minipreparation protocol described by Dellaporta *et al.* (1983) as follows: approximately 200mg (0.2g) of lyophilized leaf sample was ground into fine powder. To each tube 700ul of hot (65^{o}c) plant extraction buffer (containing 637.5ml of double distilled water, 100ml of 1M Tris-HCl (pH 8.0), 100ml of 0.5Methylenediaminetetraacetic acid (EDTA) (pH 8.0), 100ml of 5M $Nacl_2$ and 62.5ml of 20% sodiumdodecylsulphate (SDS)) was added. One percent b-mercaptoethanol was added to the pre-warmed PEB just before use. The tubes were capped and inverted gently 6-7 times to mix the sample with buffer.

The solution was incubated at 65°C in water bath for 20mins with occasional mixing to homogenize the samples. After 20mins samples were removed from the water bath and uncapped. The tubes were allowed to cool at room temperature for 2minutes. After which 500ul of 5M of potassium acetate was added to each tube and recapped. The tubes were mix inverted 6-7 times and incubated on ice for 20minutes. After 20minutes of incubation on ice tubes were spun at 12,000 rpm for10minutes at 4°c. The supernatant was transferred into new1.5ml eppendorf tubes using wider bore pipette tips (1000 µl) and making sure debris were not taken along with the supernatant. 700-µl chloroform isoamyl alcohol was added to the supernatant and spun at 10,000 rpm for 10 minutes.

The supernatant was transferred again to a new correspondingly labeled tubes and 700-µl ice-cold isopropanol was added to each tube and mixed by gently inverting the tubes 6-10 times. The tubes were allowed to stand undisturbed in a rack and stored in a freezer (-20°C) for at least 1 hour or overnight to precipitate the DNA. After 1-hour precipitation in the freezer, the tubes were centrifuged at 12,00rpm for 10 minutes at 4°c. The supernatant was carefully discarded with great care to disallow the pellet from dislodging from the bottom of the tube. The tubes were allowed to drain inverted on clean paper towels for 1 hour or more.

The DNA pellets were washed twice in 100µl, cold 70% ethanol for 20 minutes and air dried completely. After drying, 100µl of 1×TE [10mM Tris-HCL (pH 8.0), 1mM EDTA (Ph8.0)] was added to the pellets, followed by 2µl of 10ng/ml Rnase to remove the RNA. The solution was incubated for 40 minutes at 37°C with gentle mix at 10 minutes intervals and finally stored at -20°C. The DNA extraction was done in the Bioscience Department, IITA, Ibadan.

Single Nucleotide Polymorphism (SNPs) marker analysis

Tomato genotypes were genotyped with 45 SNP markers. The SNPs markers were downloaded from the Tomato SNPs Database (SolCAP tomato collection). SNPs markers selection and assay design were performed according to previously described procedures (Chao *et al*., 2010). The SNPs were selected mainly from chromosomes that are related with fruit size and shape (chromosome 2, 3 and 11).

A total of 250ng of genomic DNA per genotype was used for the Illumina SNP genotyping at the Inqaba Biotech, Pretoria, South Africa using the Sequenom Mass Array Iplex Platform following the manufactorer's protocol (Gabriel *et al*., 2009). Genotype score were called using Illumina's Genome studio V2010.3.

Statistical Analysis

Evaluation and characterization of parents

Statistical analysis to determine the means, standard deviation, variance and frequency distribution of the single fruit weights were done using SPSS software computer package version 20.0. Data collected on replicated yield trial were subjected to analysis of variance (ANOVA) and the significant difference (F - LSD) at 5% level probability according to Obi (2002). Data collected were also analysed to determine the extent of variation within and between generations. The fruit size incremental rate will be estimated in each generation. Genstat Release 10.3DE Discovery, 4th Edition software was used for the analysis (GenStat, 2010). This tested genotypic effect and estimated error variance of the traits using the linear additive model as it is indicated in Table 1.

Table 1: **Analysis of Variance Table of the Experiment in Randomized Complete Block Design (RCBD) showing the general and specific degrees of freedom**

Sources of variation	**General d.f**	**Specific d.f**
Block	r = 1	3 - 1 = 2
Treatment	t =1	6 - 1 = 5
Error	(r - 1) (t - 1)	(3 - 1) (6 - 1)
Total	rt - 1	

The linear additive model

$$X_{ij} = \varepsilon + \ddot{Q} + \acute{o}_j + E_{ij}$$

Where,

X_{ij} = Individual Observations;

ε = Population Mean;

$\ddot{Q}$ = Genotype Effect, where i = P_1, P_2, F_1, F_2, BC_1, BC_2;

$\acute{o}_j$ = Block Effect, where j = Block 1, 2, & 3;

E_{ij} = Experimental Error.

Estimation of Heterosis and Inbreeding depression

According to Allard (1960) and Uguru (2005), heterosis was estimated as a mid parent heterosis and better parent heterosis:

Heterosis over the Mid Parent (%) = $\frac{\bar{F}_1 - \overline{MP}}{\overline{MP}} X \frac{100}{1}$

Heterosis over Better Parent (%) = $\frac{\bar{F}_1 - \overline{BP}}{\overline{BP}} X \frac{100}{1}$

Where,

$\overline{MP}$ = Mean of Mid Parent value;

$\overline{BP}$ = Mean of Better Parent value;

$\bar{F}_1$ = Mean of F_1ôs.

Test of significance was done using Kumar et al. (2011);

$$CD = \sqrt{\frac{2me}{r}} \times t$$

Where;
CD = Critical Difference

t = *t* tabulated at 5% probability

me = error mean square (obtained from ANOVA)

r = number of replications

Chi Square statistic

The genetic ratio test was carried out on the F_2 using chi square statistic to test for the inheritance pattern of fruit size related traits such as fruit shapes and number of locule per fruit using the Yates correction for continuity of chi square (Stansfield, 1969).

$$x^2 = \sum_{[i=1]}^{k} \left(\frac{Oi - Ei}{Ei}\right)^2$$

Where;

O = observed frequency

E = expected frequency

k = number of classes

i = 1 - k

Estimation of Gene action on fruit size and yield characters

The estimation of the gene effect of fruit size trait were determined using the mean data from the parental lines, F_1 and BC population as described by Yang *et al*. (1997).

$m = \bar{F}_2$

a = $\bar{B}_1$ ï $\bar{B}_2$

d = $\bar{F}_1$ ï $4\bar{F}_2$ - $\left(\frac{1}{2}\right)\bar{P}_1$ - $\left(\frac{1}{2}\right)\bar{P}_2$ + $2\bar{B}_1$ + $2\bar{B}_2$

aa = $2\bar{B}_1$ + $2\bar{B}_2$ ï $4\bar{F}_2$

ad = $\bar{B}_1$ - $\left(\frac{1}{2}\right)\bar{P}_1$ ï $\bar{B}_2$ + $\left(\frac{1}{2}\right)\bar{P}_2$

dd = $\bar{P}_1$ + $\bar{P}_2$ + $2\bar{F}_1$ + $4\bar{F}_2$ ï $4\bar{B}_1$ ï $4\bar{B}_2$

t value of effect, $\pm t = \frac{gene\ effect}{\sqrt{variance\ of\ gene\ effect}}$

Where;

m = mean

a = additive effect

d = dominance effect

aa = additive x additive effects

ad = dditive x dominance effects

dd = dominance x dominance gene effects

$\overline{B}_1$ = Mean of backcross to Parent 1

$\overline{B}_2$ = Mean of backcross to Parent 2

$\overline{P}_1$ = Mean of Parent 1

$\overline{P}_2$ = Mean of Parent 2

$\overline{F}_1$ = Mean of first filial generation

$\overline{F}_2$ = Mean of second filial generation

SE = Standard Error

Effects = Dominant effects

Estimation of Heritability in Broad Sense and (H_{bs}) and Narrow Sense (H_{ns})

The estimates of the genetic and phenotypic variances of the quantitative traits were determined using the variance estimate method as described by Uguru (2005) and Acquaah (2007) as follows:

$$H_{bs} = \frac{\sigma_g^2}{\sigma_p^2} X\,100$$

$$H_{ns} = \frac{\sigma_a^2}{\sigma_p^2} X\,100$$

But,

$$\sigma_e^2 = \frac{P_1 + P_2 + F_1}{3}$$

$$\sigma_a^2 = 2F_2 - (BC_1 + BC_2);$$

$$\sigma_d^2 = \left((BC_1 + BC_2 - F_2 - (P_1 + P_2 + F_1)\right);$$

$$\sigma_p^2 = \sigma_e^2 + \sigma_a^2 + \sigma_d^2;$$

$$\sigma_g^2 = \sigma_a^2 + \sigma_d^2$$

Where;

σ_e^2 = Environmental Variance;

σ_a^2 = Additive Variance;

σ_d^2 = Dominance Variance;

σ_p^2 = Phenotypic Variance;

σ_g^2 = Genotypic Variance;

H_{bs} = Broad Sense Heritability;

H_{ns} = Narrow Sense Heritability.

Correlation and Path Coefficient Analysis

The floral and fruit size and shape related traits were subjected to correlation analysis using the computer statistical software package, SPSS version 20. Path coefficient analyses was carried out to show direct and in direct effects (magnitude and significance) of floral and fruit traits to the fruit size using SASS statistical package.

Statistical Analysis of genotypic data

PowerMarker V3.25 software was used to estimate the allele frequency of all the SNPs markers. The markers with allele frequency less than 10% minimum allele frequency (MAF) was called off from the data set (Berger *et al*., 2012). The following genetic diversity parameters were measured: polymorphism information content (PIC), number of alleles per locus (A^o), expected heterozygosity (H_e), observed heterozygosity (H_o).

SNP Polymorphism

PIC values provide an estimate of the probability of finding polymorphism between two random samples of the germplasm. To verify the potential of chosen markers to discriminate among the parents and progenies population, the polymorphism information content (PIC) was calculated for each marker as described by Botstein *et al*. (1980) and Anderson *et al*. (1993).

Number of Alleles per Locus (A)

The number of alleles per locus (A^o) is defined as the number of alleles with non zero frequency. It is the total count of different alleles in a sample.

Effective Number of Alleles per Locus (A^o_e)

This is a measure of the effective number of alleles maintained in the population, which in general is less than the actual number of alleles per locus. It is reciprocal or inverse of homozygosity (Hartl and Clark, 1989). Therefore, as the effective number of allele per locus get larger, the more individual become heterozygous.

Allele Frequency

Allele frequency for co-dominant data estimates gene frequencies at each locus from raw data. Missing values are excluded from such estimation with the formula;

$$F_x = \frac{2N_{xx} + N_{xy}}{2N}$$

where N_{xx} is the number of xx homozygous individuals, N_{xy} is the number of heterozygous individuals and y can be any other allele. N is the number of samples and can be determined by direct count of the proportion of different alleles.

Expected Heterozygosity (H_e)

This is often referred to as gene diversity, and defined as the probability that two randomly chosen alleles from the population are different (Nei, 1972). It is an estimate of the proportion of expected heterozygotes under random mating for co-dominant markers. It was calculated for each SNP locus according to the formula;

$$H_e = 1 - \times (Pi)^2$$

Where, P = frequency of the i^{th} allele for the population and $\times (Pi)^2$ is the sum of squared population allele frequencies.

Observed Heterozygosity (H_o)

Observed heterozygosity (H_o) is the proportion of observed heterozygotes at a given locus for co-dominant markers. It was estimated by dividing the number of heterozygous individuals by the total number of individuals sampled;

$$Ho = \frac{\text{Number of Heterozygous individuals}}{\text{Total number of individual sampled}}$$

Genetic Relatedness among Tomato progenies based on the Genetic Distance

Genetic distances among the parents were calculated according to Nei (1972) frequency-based distance;

$$\hat{D}_m = \left(\frac{\hat{J}_X + \hat{J}_Y}{2} \right) - \hat{J}_{XY}$$

Where pij and qij are the frequencies of i^{th} allele at the j^{th} locus in the population X and Y respectively, while aj is the number of alleles at the j^{th} locus and m is the number of loci examined. The matrix was subjected to cluster analysis to produce hierarchical representation of

the relationships among samples or group of samples or group of samples. The most commonly applied method for DNA based cluster analysis is un weighed pair group mean algorithm (UPGMA) (Nei, 1983). The level of relatedness were visualized in a dendrogram. All genetic distance calculations and construction of dendrograms were performed using PowerMarker software version 3.25 (Liu and Muse, 2005) and MEGA software version 4.0 (Tamura *et al*., 2007).

QTLs Association Mapping

QTL association mapping or mapping analysis were done using a Trait Analysis by Association, Evolution and Linkage (TASSEL 3.0 version software). The QTLs underlying fruit size such as single fruit size, fruit length, fruit width, locule number and fruit shape index were tagged. The population structure was estimated with the model-based (Bayesian) cluster software, STRUCTURE 2.33 version.

RESULTS

Meteorological Information during field research work

The meteorological data of the research environment showed that there was variation in the meteorological parameters such as rainfall, temperature and humidity (Table 2). The rainfall distribution pattern in both years revealed that the rainfall was higher in 2014 than 2015. The highest rainfall recorded was in September, 2015 with a mean of 434.5mm and September, 2014 with a mean of 401.99 (Table 4). The average relative humidity was 72.20% at 10 am and ranged between 69.93% to 73.80% while at 4pm the average relative humidity was 71.98 and ranged between 70.06% to 73% in 2014. The average relative humidity was lower in 2015, 70.97% and 69.3% average relative humidity was observed at 10am and 4pm respectively. The minimum air temperatures were recorded in December, 2014 (19.3^{o}C) and January, 2015 (20.5^{o}C). The maximum air temperatures were detected in April, 2014 (31.3^{o}C) and March, 2015 (32.3^{o}C).

The field research works started in April, 2013 in the screenhouse for the tomato population development. For the evaluation during rainy season, tomato genotypes used in the study were planted twice in April to August and September to December, 2014. Tomato genotypes were further selected and evaluated from April to August, 2015.

Table 2: **Meteorological data showing mean monthly rainfall (mm), temperature (^{0}C) and relative humidity (%) for 2014 and 2015 during the experimental period in Nsukka**

2014

Month	Rainfall (mm)	Temperature (^{0}C) Min	Max	Relative Humidity (%) 10am	4pm
April	105.16	22.30	31.30	69.93	70.53
May	241.14	21.06	28.29	72.26	72.26
June	271.79	20.87	29.13	72.00	72.00
July	195.81	20.90	27.74	72.19	72.19
August	92.36	20.71	27.29	73.00	73.00
September	401.99	20.33	27.90	73.00	73.00
October	211.08	20.84	28.90	73.00	72.77
November	77.22	21.00	30.07	73.80	71.97
December	4.83	19.03	30.65	70.58	70.06
Total	**1601.38**	**187.04**	**261.27**	**649.7**	**647.78**
Mean	**177.93**	**20.78**	**29.03**	**72.20**	**71.98**

2015

Month	Rainfall (mm)	Temperature (^{0}C) Min	Max	Relative Humidity (%) 10am	4pm
January	Nil	20.52	30.32	61.42	59.58
February	56.64	22.68	32.04	70.11	64.21
March	34.80	22.61	32.29	70.61	70.19
April	39.63	22.40	31.47	71.03	67.67
May	267.98	21.81	30.71	71.65	71.42
June	121.43	21.17	29.07	76.00	76.00
July	110.49	20.61	27.87	76.00	76.03
August	410.4	20.43	27.69	76.00	76.10
Total	**630.97**	**151.80**	**213.77**	**496.82**	**485.1**
Mean	**90.14**	**21.69**	**30.54**	**70.97**	**69.30**

Source: Meteorological Station, Department of Crop Science, University of Nigeria, Nsukka.

Agronomic Performance of the tomato parents

The parents evaluated varied considerably in performance and yield components. The analysis of the variance revealed that variety, supersteak (S) had higher number of days to flowering, fruiting and ripening (28.6, 37.7 and 68.6). It was followed very closely with plumb (rio grande) (PR) which had days to flowering, fruiting and ripening of 27.2, 37.9 and 68.3 respectively. Both Supersteak (S) and Plumb (Rio grande) (PR) had the days to flowering, fruiting and ripening higher than the parent population mean. On the other hand, W x T parent showed a significantly ($p = 0.05$) lower days to flowering, fruiting and ripening of 19.8, 31.5 and 60 respectively as shown in Table 3.

The tallest plant were produced by W x R (113.7cm), followed by R x W (93.6cm) and W x T (92.5cm). Both W x R, R x W and W x T parents had the height higher than parents population mean of 90.4cm. The shortest plants were produced by supersteak (75cm). The results showed that W x R produced more branches (33.9) than all the parents used in this study. Supersteak parent had the fattest stem girth (42.1) than the other parents.

The advanced hybrids, W x R, W x T and R x W parents had higher number of trusses/plant, flowers/plant, that differed significantly from those recorded for the cultivated varieties such as supersteak, PR and BF. The results of the fruit yield among the parents studied indicated that W x R had highest yield per hectare.

Table 3: Mean values, and least significant difference of the agronomic and yield component traits of the tomato parents studied

Tomato varieties	dfwl	dfrt	dripe	B	H (cm)	SG(cm)	flw/P	trs/P	frt/P	FY (t/ha)
W x R	21.1	32.1	58.1	27.4	113.7	33.9	239.8	46.5	120.5	25
R x W	20.1	30.1	59.4	20.6	93.6	30.4	127.5	29	43.3	11.4
W x T	19.8	31.5	60	24	92.5	32.3	171.5	37.3	66.5	14.2
S	28.6	37.7	68.6	4.8	75.6	42.1	17.5	4.8	5.3	22.6
BF	25.2	34.1	62.1	11.6	82.7	37.4	100.3	10.3	23.8	20.7
PR	27.2	37.9	68.3	7.8	84.4	37.3	77	7.8	17.5	13.1
GRAND MEAN	23.7	33.9	62.8	16.0	90.4	35.6	121.7	22.6	52.8	17.8
LSD (P = 0.05)	0.57	0.63	0.78	0.73	12.72	2.73	11.91	6.02	16.4	4.36

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande), **dflw** = days to flowering, **dfrt** = days to first fruit appearance, **dripe** = days to fruit ripening, **B** = number of branches, **H** = height at ripening, **SGT** = stem girth, **flw/trs** = number of flower per truss, **trs/plt** = number of truss per plant, **frt/P** = number of fruit per plant, **FY** = fruit yield and **AFW** = average fruit weight.

Evaluation of the tomato parents for floral characters

Table 4 contains information on the floral traits such as flower length, flower width, stalk width, style length and diameter, stigma length and diameter, ovary length, diameter, area and perimeter. There is considerable variation among the tomato parents for these characters.

Supersteak had higher mean values for all the floral characters under the study except for the style length (0.425cm). On the other hand W x R had a very long style (0.433cm) and narrow style diameter (0.016cm). The hybrids with the wild parent (W x R, W x T and R x W) followed a similar trend for almost all the floral characters while the two commonly cultivated tomato varieties (BF and PR) also found to follow the similar trend in the mean values of the floral characters except for the ovary diameter of 0.110 and 0.096cm respectively.

Table 4: Mean values and least significant differences of the floral traits of the tomato parents used in this study

Tomato varieties	FL	FW	SW	SL	SLD	SGD	SGL	OD	OL	OA	OP
W x R	0.523	0.133	0.046	0.433	0.016	0.022	0.012	0.084	0.101	0.078	0.348
R x W	0.497	0.132	0.045	0.401	0.016	0.02	0.009	0.082	0.095	0.07	0.34
W x T	0.48	0.13	0.041	0.391	0.015	0.021	0.011	0.082	0.096	0.072	0.344
S	0.742	0.245	0.102	0.425	0.109	0.119	0.011	0.243	0.188	0.441	0.81
BF	0.441	0.162	0.055	0.347	0.026	0.034	0.015	0.11	0.122	0.121	0.444
PR	0.437	0.166	0.061	0.346	0.031	0.035	0.015	0.096	0.125	0.125	0.439
GRAND MEAN	0.520	0.161	0.058	0.391	0.036	0.042	0.012	0.116	0.121	0.151	0.454

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande), **FL** = flower length, **FW** = flower width, **SW** = stalk width, **SL** = style length, **SLD** = style diameter, **SGD** = stigma diameter, **SGL** = stigma length, **OD** = ovary dimater, **OL** =ovarylength,**OA**=ovaryareaand**OP**=ovaryperimeter

Evaluation of the fruit characteristics of the tomato varieties

The results of the fruit characteristics among the parent studied showed that supersteak had significantly higher single fruit weight, fruit length and fruit width (218g, 9.6cm and 7.5cm) respectively. This was followed by the Beef (Florida) (BF) which has the Mean fruit weight, fruit length and fruit width (61g, 5.6cm and 5.5cm), respectively. On the other hand (W x R) was significantly lower in average fruit weight, fruit length and fruit width (31g, 4.4cm and 4.2cm), respectively.

The result revealed that supersteak had a fruit shape index (FSI) of less than 1 (0.775) which though differed significantly from Plumb (Rio grande) (PR) which had a fruit shape index of greater than 1 (1.461). However, all the remaining parents (MR, W x R, R x W and W x T) had fruit shape index around 1 (1.093, 1.043, 0.996 and 1.005).

The parent, PR was significantly higher in the pericarp thickness, days to the first fruit spoilage, 50% and 100% fruit spoilage.

Table 5: Mean values and least significant differences of the fruit characteristics of the tomato varieties used for the study

Tomato variety	MFW (g)	FL (cm)	FW (cm)	FSI	LC	PT	%M	NS	100 SW	1st SP	50% SP	100% SP
W x R	26.98	4.44	4.2	1.07	2.22	4.2	90.5	64.33	0.23	7	11.3	15.7
R x W	29.56	4.52	4.33	1.05	2.44	3.8	90.24	73.56	0.25	6.2	17.5	23.4
W x T	29.37	4.76	4.29	1.13	2.33	4.4	90.36	74	0.26	9.9	19.8	25.5
S	170.29	6.84	8.2	0.69	10.67	4.5	92.67	48.22	0.3	7.9	15.1	21.4
BF	75.09	4.91	5.63	0.89	4.89	5	90.48	91.78	0.26	13.6	22.4	34
PR	39.54	4.8	3.97	1.25	3.33	5.9	89.24	43.22	0.25	12	20.9	30.3
GRAND MEAN	61.83	4.88	5.1	1.1	4.32	4.63	90.58	65.90	0.26	9.43	17.83	25.05
LSD (p = 0.05)	58.36	0.62	1.42	0.12	0.74	0.68	0.75	10.87	0.09	3.89	5.01	6.31

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande), **MFW** = Mean fruit weight, **FL** = fruit length, **FW** = fruit width, **FSI** = fruit size index, **LC** = locule number, **PT** = pericarp thickness, **%M** = percent moisture content, **NS** = number of seeds, **100 SW** = weight of 100 seeds, **1st SP** = days to first spoilage, **50%SP** = days to 50% spoilage and **100% SP** = days to 100% fruit spoilage.

Agronomic Performance of the tomato progenies (F_1)

The results on the agronomic yield and yield components of the F_1 and the parents showed significant differences. The population mean days to flowering, fruiting and ripening was 22.97, 32.9 and 61.3 respectively. The crosses, S x (W x R) was significant higher in days to flowering, fruiting and ripening (33.67, 36.7 and 62.67) respectively as indicated in Table 6. The W x R and supersteak parents were also significant higher in the three traits.

The results also revealed that the crosses, (W x R) x S had the highest mean value for plant height (122cm), followed by PR x (R x W) and PR x (W x T) (101cm and 105cm), respectively. On the other hand S x (W x R) had the lowest mean values for the height (89cm). The cross, S x (W x R) was significant higher in number of branches and stem girth (35.65). A supersteak (S) parent was significantly lower in number of branch but higher in the mean value for the stem girth (42.52cm).

The results also showed that S x (W x R) had higher number of trusses/plant, flowers/plant and fruits/plant. The cross, S x (W x R) was also significantly higher in fruit yield.

Table 6: Agronomic and yield component traits of the F_1 and the parents of the tomato varieties used in this study

Tomato varieties	dfwl	dfrt	dripe	B	H (cm)	SG(cm)	flw/P	trs/P	frt/P	FY (t/ha)
W x R	21.1	32.1	58.1	27.4	113.7	33.9	239.8	46.5	120.5	25
R x W	20.1	30.1	59.4	20.6	93.6	30.4	127.5	29	43.3	11.4
W x T	19.8	31.5	60	24	92.5	32.3	171.5	37.3	66.5	14.2
S	28.6	37.7	68.6	4.8	75.6	42.1	17.5	4.8	5.3	22.6
BF	25.2	34.1	62.1	11.6	82.7	37.4	100.3	10.3	23.8	20.7
PR	27.2	37.9	68.3	7.8	84.4	37.3	77	7.8	17.5	13.1
S x (W x R)	22.5	32.3	59.4	18.3	120.2	38.5	145.3	39.3	86.8	26.1
PR x (R x W)	22.8	32.2	62.8	16	102.5	37.7	122.3	20	58.3	23.4
PR x (W x T)	23.8	34.5	64	19.2	115.8	37.1	133.8	30.5	92.8	22.6
(W x R) x S	21.1	31.1	57	21.3	99	35.9	166.3	45.3	108.5	19.3
PR x (W x R)	23.5	33.3	62.8	20	113	36.7	122.3	24.8	52.3	23.1
BF x (W x T)	20.1	28.5	53.5	19.2	112.4	35.9	137	36.5	84.3	14.9
GRAND MEAN	22.97	32.9	61.3	17.5	100.5	36.3	130.1	27.7	67.5	19.7
LSD (p = 0.05)	0.872	0.97	1.42	0.69	17.65	2.46	13.46	5.86	11.5	5.23

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande) , **dflw** = days to flowering, **dfrt** = days to first fruit appearance, **dripe** = days to fruit ripening, **B** = number of branches, **H** = height at ripening, **SGT** = stem girth, **flw/trs** = number of flower per truss, **trs/plt** = number of truss per plant, **frt/P** = number of fruit per plant, **FY** = fruit yield and **AFW** = average fruit weight

Mean performance of the tomato progenies on floral characters

The population mean with respect to flower length, flower width and stalk width were 0.5204cm, 0.154cm and 0.055cm, respectively. The supersteak parent had higher mean values in the flower length, flower width and stalk width (0.74271cm, 0.24523cm and 0.101), respectively than all the other tomato genotypes. The F_1, BF x (W x T), recorded higher mean values in flower length and flower width than the population mean. F_1 (PR x (R x W) had a higher stalk width (0.057cm) than the population mean. The results are presented in Table 7.

On the mean style length and diameter, the W x R parent recorded the longest style length (0.43cm) while supersteak parent recorded the highest style diameter (0.109cm). The F_1, MR x (W x T) had the longest style among the F_1 progenies followed closely by the S x (W x R). The hybrid, PR x (W x R) had the largest style diameter (0.210cm) which was however, less than the population mean for style diameter (0.277cm).

The Supersteak (S) parent had the largest stigma diameter (0.1192cm) while Plumb (Rio grande) (PR) parent had the longest stigma length (0.0151cm). Both stigma diameter and stigma length were greater than their population means (0.0346cm and 0.0121cm). The hybrid, PR x (W X R) showed significant higher value in both stigma diameter and stigma length (0.0317cm and 0.0133cm) than the other tomato progenies in this characters.

The population mean of ovary length, diameter, area and perimeter were 0.1161cm, 0.1045cm, 0.1245cm and 0.4263cm respectively. Supersteak (S) parent had the highest ovary length, diameter, area and perimeter (0.1883cm, 0.2434cm, 0.4407cm and 0.8097cm) respectively. The

F_1, S x (W x R) showed the highest ovary diameter (0.1009cm) while PR x (W x T) had the highest ovary length (0.1275) than the other tomato progenies in this characters.

Table 7: Floral traits of the F_1 and parents of the tomato varieties used in this study

Tomato varieties	FL	FW	SW	SL	SLD	SGD	SGL	OD	OL	OA	OP
W x R	0.5226	0.1331	0.0455	0.4333	0.0156	0.0219	0.0120	0.0836	0.1008	0.0776	0.3478
R x W	0.4969	0.1316	0.0446	0.4005	0.0158	0.0204	0.0095	0.0819	0.0947	0.0701	0.3402
W x T	0.4797	0.1302	0.0408	0.3914	0.0150	0.0210	0.0111	0.0824	0.0958	0.0717	0.3440
S	0.7427	0.2452	0.1019	0.4252	0.1093	0.1192	0.0113	0.2434	0.1883	0.4407	0.8097
BF	0.4411	0.1618	0.0548	0.3466	0.0265	0.0341	0.0146	0.1102	0.1222	0.1209	0.4436
PR	0.4366	0.1656	0.0613	0.3458	0.0314	0.0353	0.0151	0.0963	0.1253	0.1249	0.4388
S x (W x R)	0.5269	0.1528	0.0531	0.4307	0.0198	0.0256	0.0105	0.1009	0.1000	0.1109	0.4049
(W x R) x S	0.5312	0.1424	0.0514	0.4352	0.0200	0.0258	0.0115	0.0900	0.0905	0.0918	0.3811
PR x (R x W)	0.5106	0.1454	0.0573	0.4157	0.0182	0.0249	0.0132	0.0862	0.1178	0.0917	0.3956
PR x (W x R)	0.5143	0.1454	0.0476	0.4160	0.0210	0.0317	0.0133	0.0936	0.1185	0.1048	0.4116
PR x (W x T)	0.4988	0.1410	0.0523	0.4017	0.0200	0.0288	0.0125	0.0847	0.1275	0.1044	0.4157
BF x (W x T)	0.5442	0.1549	0.0516	0.4482	0.0204	0.0263	0.0106	0.1007	0.1055	0.0975	0.3922
GRAND MEAN	0.5205	0.1541	0.0552	0.4075	0.0277	0.0346	0.0121	0.1045	0.1161	0.1245	0.4263

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande), **FL** = flower length, **FW** = flower width, **SW** = stalk width, **SL** = style length, **SLD** = style diameter, **SGD** = stigma diameter, **SGL** = stigma length, **OD** = ovary diameter, **OL**=ovarylength,**OA**=ovaryareaand**OP**=ovaryperimeter.

Mean Performance of the tomato progenies in the fruit traits

The crosses, S x (W x R) was significantly (p = 0.05) higher in average fruit weight, fruit length and fruit width (41.79g, 5.18cm and 4.88cm) respectively. The W x R parent was significantly lower in mean fruit weight (MFW), fruit length (FL) and fruit width (FW) (26.98g, 4.44cm and 4.20cm) respectively while supersteak was higher in AFW, FL and FW (170.2g, 6.8cm and 8.9cm).

The cross S x (W x R) was also significantly higher in the locule number per fruit (4 locules). The fruit shape index (FSI) was greater than 1 in PR x (R x W) and PR x (W x T). The other remaining crosses S x (W x R), (W x R) x R and BF x (W x T), The FSI was found around 1 (1.08, 1.06 and 1.01). The fruit shape index for the parents ranged from 0.775 (supersteak parent) to 1.888 (Oval medium size parent).

Table 8: Fruit characteristics traits of the F_1 and parents of the tomato varieties used in the study

Tomato variety	MFW (g)	FL (cm)	FW (cm)	FSI	LC	PT	%M	NS	100 SW	1st SP	50% SP	100% SP
W x R	26.98	4.44	4.2	1.07	2.22	4.2	90.5	64.33	0.23	7	11.3	15.7
R x W	29.56	4.52	4.33	1.05	2.44	3.8	90.24	73.56	0.25	6.2	17.5	23.4
W x T	29.37	4.76	4.29	1.13	2.33	4.4	90.36	74	0.26	9.9	19.8	25.5
S	170.29	6.84	8.2	0.69	10.67	4.5	92.67	48.22	0.3	7.9	15.1	21.4
BF	75.09	4.91	5.63	0.89	4.89	5	90.48	91.78	0.26	13.6	22.4	34
PR	39.54	4.8	3.97	1.25	3.33	5.9	89.24	43.22	0.25	12	20.9	30.3
S x (W x R)	41.79	5.18	4.88	1.08	4	5.61	92.25	62.33	0.3	13.2	21.5	33.1
(W x R) x S	23.39	4.06	3.84	1.06	2.22	4.1	90.89	52.67	0.23	14.6	20.3	35.7
PR x (R x W)	39.29	5.9	4.41	1.42	2.67	5.5	93.1	61.56	0.25	14.23	24.9	29
PR x (W x R)	29.27	5.11	4.24	1.24	2.89	5	89.55	56.11	0.3	10.4	17.2	27.3
PR x (W x T)	33.93	6.06	4.22	1.52	2.67	4	90.24	83.67	0.3	7.3	13.8	23.9
BF x (W x T)	28.29	4.34	4.27	1.01	2.22	4.47	94.6	72.33	0.24	15.6	21.9	31.4
GRAND MEAN	47.21	4.99	4.74	1.12	3.53	4.71	91.18	65.33	0.26	10.99	18.88	27.56
LSD (p = 0.05)	38.29	0.63	0.93	0.12	0.55	0.71	0.79	13.62	0.077	3.96	5.12	5.97

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande) , **MFW** = mean fruit weight, **FL** = fruit length, **FW** = fruit width, **FSI** = fruit size index, **LC** = locule number, **PT** = pericarp thickness, **%M** = percent moisture content, **NS** = number of seeds, **100 SW** = weight of 100 seeds, **1st SP** = days to first spoilage, **50%SP** = days to 50% spoilage and **100% SP** = days to 100% fruit spoilage.

Estimates of Better Parent Heterosis (BPH) and Mid-Parent Heterosis (MPH) of agronomic and yield characters

The results of the better parent heterosis (BPH) and mid parent heterosis (MPH) of the agronomic, yield and yield component traits of the tomato varieties studied are presented in Tables 9 and 10. The negative heterotic values were on days to flowering, days to first fruit appearance and days to fruit ripening in all the F_1 hybrids. The cross S x (W x R) showed the highest negative values in days to flowering, fruit appearance and ripening (-26.5, -17.5 and 16.9), respectively for the better parent heterosis while the cross PR x (W x T) showed a positive value in only days to flowering for the mid parent heterosis.

All the crosses had negative better parent heterosis in number of branches. The cross S x (W x R) had a higher negative value of -33.2 while PR x (W x R) had the lowest heterotic value of -2.9 in the number of branches. All the crosses had positive mid parent heterosis in number of branches. The cross (W x R) x S had a higher value of 32.3 and BF x (W x R) had the lowest heterotic value of 7.73. Positive better parent values were observed for the S x (W x R), PR x (R x W), PR x (W x T) and BF x (W x T) in plant height at ripening. The cross PR x (R x W) had the highest positive value of 25.2. All the crosses had positive mid parent heterosis in plant height at ripening. A negative better parent heterosis was observed in all crosses in stem girth at ripening except for PR x (R x W), while a positive mid parent heterosis was observed in all the crosses except (W x R) x S.

Estimate of heterosis showed that the cross PR x (W x T) had the highest better parent heterosis in fruit number per plant (39.5) while PR x (R x W) had the highest in fruit yield (78.6ton/ha).

Table 9: Better Parent heterosis of the agronomic and yield component traits of the F_1 hybrid of tomato used for the study

Tomato variety	dfwl	dfrt	dripe	B	H (cm)	SG(cm)	flw/P	trs/P	frt/P	FY (t/ha)
S x (W x R)	-21.3	-14.3	-13.4	-33.2	5.7	-8.6	-39.4	-15.5	-39.7	4.4
PR x (R x W)	-16.2	-15.0	-8.1	-22.3	9.5	1.1	-4.1	-31.0	34.6	78.6
PR x (W x T)	-12.5	-9.0	-6.3	-20.0	25.2	-0.8	-21.8	-18.2	39.5	59.2
(W x R) x S	-26.2	-17.5	-16.9	-22.3	-12.9	-14.7	-30.7	-2.6	-32.4	-22.8
PR x (W x R)	-13.6	-12.1	-8.1	-2.9	-0.6	-1.9	-4.1	-46.7	-67.4	-7.6
BF x (W x T)	-20.2	-16.4	-13.8	-20.0	21.5	-3.8	-20.1	-2.7	26.8	-28.0
cd 5%	1.23	1.67	1.99	4.12	9.78	3.68	2.31	3.60	6.2	0.63

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande), **dflw** = days to flowering, **dfrt** = days to first fruit appearance, **dripe** = days to fruit ripening, **B** = number of branches, **H** = height at ripening, **SGT** = stem girth, **flw/trs** = number of flower per truss, **trs/plt** = number of truss per plant, **frt/P** = number of fruit per plant, **FY** = fruit yield and **AFW** = average fruit weight.

Table 10: Mid Parent heterosis of the agronomic and yield component traits of the F_1 hybrid of tomato used for the study

Tomato variety	dfwl	dfrt	dripe	B	H (cm)	SG(cm)	flw/P	trs/P	frt/P	FY (t/ha)
S x (W x R)	-9.40	-7.29	-6.20	13.18	27.04	1.37	12.93	53.17	16.74	9.61
(W x R) x S	-15.10	-10.87	-10.01	32.30	4.61	-5.38	29.25	76.59	30.92	-18.97
PR x (R x W)	-3.35	-5.39	-1.63	12.94	15.20	11.33	19.56	8.84	91.77	90.79
PR x (W x R)	-2.59	-5.00	-0.74	13.74	14.14	3.10	-22.81	-8.76	-41.29	21.02
PR x (W x T)	1.42	-0.60	-0.26	20.73	30.92	6.62	7.65	35.56	120.83	65.48
BF x (W x T)	-10.74	-13.09	-12.35	7.73	28.25	3.04	0.83	53.68	86.70	-14.63
cd 5%	1.23	1.67	1.99	4.12	9.78	3.68	2.31	3.60	6.2	0.63

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande), **dflw** = days to flowering, **dfrt** = days to first fruit appearance, **dripe** = days to fruit ripening, **B** = number of branches, **H** = height at ripening, **SGT** = stem girth, **flw/trs** = number of flower per truss, **trs/plt** = number of truss per plant, **frt/P** = number of fruit per plant, **FY** = fruit yield and **AFW** = average fruit weight.

Estimates of Better Parent Heterosis (BPH) of floral traits in tomato hybrids

The results with respect to better parent heterosis (BPH) and mid parent heterosis (MPH) for the floral traits of tomato varieties studied are summarized in Tables 11 and 12. A significant negative heterosis was obtained for the style diameter in all the six hybrids and the values ranged from -81.9% to -20.6% and -68.3% to -1.6% over the better and mid parents, respectively. Similarly, a significant negative heterosis was obtained for the stigma diameter that ranged from -81.9% to -20.6% over the better parent. Positive significant heteroses (2.4 and 10.9%) were obtained in two hybrids, PR x (W x R) and PR x (W x T) over the mid parents. All cross combinations in this study showed a significant negative heterosis in stigma length except for the BF x (W x T). Significant negative heteroses were obtained for the ovary diameter in all the hybrids except PR x (W x R). The range for the negative heterosis was -58.6 to -2.9% and -38.3 to -3.2% over better and mid parents, respectively.

The heterotic values with respect to ovary length and ovary area are also contained in Table 11. The range for the negative heterosis was -27.5 to -6.4% and -17.7 to -1.7% over better and mid parents, respectively. For the ovary length, only the hybrid (PR x (W x T)) was found with significant positive heterosis (1.7%) over the better parent. Three hybrids, PR x (W x R), PR x (R x W) and PR x (W x T) had positive heterosis (7.1, 4.8 and 15.3%, respectively) over the mid parent. Negative heteroses were obtained for the ovary area in all the six hybrids and the values ranged from -77.7 to -16.0% over the better parent. Three hybrids (PR x (W x R), (PR x (W x T) and (BF x (W x T) had positive heteroses (3.6, 6.2 and 1.2%) over the mid parent.

Table 11: Better Parent Heterosis of the floral traits of the F_1 hybrids of tomatoes used for the study

Tomato varieties	FL	FW	SW	SL	SLD	SGD	SGL	OD	OL	OA	OP
S x (W x R)	0.823	-37.677	-47.846	-0.594	-81.873	-78.539	-6.398	-58.564	-43.739	-77.717	-51.223
(W x R) x S	1.642	-41.915	-49.541	0.433	-81.685	-78.347	-3.784	-63.030	-51.937	-79.168	-52.932
PR x (R x W)	8.258	-12.221	-6.545	8.224	-42.100	-29.383	-12.826	-10.461	-3.601	-26.601	-9.846
PR x (W x R)	-1.582	-12.230	-22.335	-3.985	-20.575	-10.199	-11.802	-2.879	-5.414	-16.067	-6.187
PR x (W x T)	3.970	-14.885	-14.651	2.641	-24.287	-18.393	-17.112	-12.062	1.726	-16.431	-5.260
BF x (W x T)	13.445	-4.263	-5.932	29.306	-34.906	-23.098	-27.565	-8.638	-13.649	-19.391	-11.591
cd 5%	0.04	0.01	0.019	0.046	0.005	0.006	0.012	0.019	0.016	0.041	0.045

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande), **FL** = flower length, **FW** = flower width, **SW** = stalk width, **SL** = style length, **SLD** = style diameter, **SGD** = stigma diameter, **SGL** = stigma length, **OD** = ovary diamater, **OL** =ovarylength,**OA**=ovaryareaand**OP**=ovaryperimeter.

Table 12: Mid Parent Heterosis of the floral traits of the F_1 hybrids of tomatoes used for the study

Tomato varieties	FL	FW	SW	SL	SLD	SGD	SGL	OD	OL	OA	OP
S x (W x R)	21.92	-19.20	-27.90	43.48	-68.29	-63.73	-9.33	-38.32	-26.71	-62.10	-31.76
(W x R) x S	22.91	-24.69	-30.23	44.27	-67.96	-0.63	-0.77	-44.96	-37.39	-64.57	-34.16
PR x (R x W)	7.24	-2.66	-10.85	6.80	-10.55	10.93	-1.70	3.97	4.84	3.56	4.66
PR x (W x R)	8.86	-4.70	2.44	8.98	-13.63	2.38	-4.38	-5.23	15.31	6.19	6.21
PR x (W x T)	18.20	6.10	7.81	21.46	-1.61	-4.71	-17.66	4.55	-3.18	1.23	-0.41
BF x (W x T)	9.39	-2.17	8.23	11.39	-22.95	-10.48	7.11	-3.22	7.10	-6.00	1.56
cd 5%	0.04	0.01	0.019	0.046	0.005	0.006	0.012	0.019	0.016	0.041	0.045

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande)**, FL** = flower length, **FW** = flower width, **SW** = stalk width, **SL** = style length, **SLD** = style diameter, **SGD** = stigma diameter, **SGL** = stigma length, **OD** = ovary diamater, **OL** =ovarylength,**OA**=ovaryareaand**OP**=ovaryperimeter.

Estimates of Better Parent Heterosis (BPH) of fruit characteristics in tomato hybrids

The heterotic values with respect to the fruit characters namely; fruit length, fruit diameter, number of locules per fruit and number of fruits per plant are presented in Tables 13 and 14. Two hybrids (PR x (R x W) and (PR x (W x R) recorded positive heteroses of 1.9 and 5.1% over the better parent in fruit length. Similarly, three hybrids (PR x (R x W), PR x (W x R) and PR x (W x T) had positive heteroses (23.6, 27.4 and 4.6%) over the mid parent. Negative heteroses were recorded in all the hybrids for the fruit diameter and the values ranged from -37.3 to -15.1% over the better parent.

All the cross combinations studied showed negative heterosis for the mean fruit weight that ranged from -67.7 to -19.5% and -43.6 to -11.2% over better and mid parents, respectively except for the hybrid (PR x (W x R) which had positive heterosis (8.8%) over the mid parent only. However, only one hybrid, PR x (W x R) had positive heterosis of 5.7%. Negative heteroses were obtained for the number of fruits per plant in all the six hybrids that ranged from -7.56 to 49.76%. The MPH results showed that S x (W x R) had the highest positive MPH of 55.5% in number of fruits per plant.

Table 13: Better Parent Heterosis of the fruit characteristics of the F_1 hybrids of tomatoes used for the study

Table 14

Tomato variety	MFW (g)	FL (cm)	FW (cm)	FSI	LC	PT	%M	NS	100 SW	1st SP	50% SP	100% SP
S x (W x R)	-75.46	-24.27	-40.49	0.93	-62.51	27.50	-0.45	-3.11	0.00	67.09	42.38	54.67
(W x R) x S	-86.26	-40.64	-53.17	-0.93	-79.19	-6.82	0.43	-18.13	-23.33	84.81	34.44	66.82
PR x (R x W)	-0.63	22.92	1.85	13.60	-19.82	-6.78	3.17	-16.31	0.00	18.58	19.14	-4.29
PR x (W x R)	-82.81	-25.29	-48.29	-0.80	-13.21	-15.25	-1.05	-12.78	20.00	-13.33	-17.70	-9.90
PR x (W x T)	-14.19	26.25	-1.63	21.60	-19.82	-32.20	-0.13	13.07	15.38	-39.17	-33.97	-21.12
BF x (W x T)	-62.33	-11.61	-24.16	-10.62	-54.60	-10.60	4.55	-21.19	-7.69	14.71	-2.23	-7.65
cd 5%	31.32	0.71	4.75	5.76	0.53	0.57	2.18	6.45	0.28	14.42	18.82	20.2

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande), **MFW** = mean fruit weight, **FL** = fruit length, **FW** = fruit width, **FSI** = fruit size index, **LC** = locule number, **NS** = number of seeds, PT = pericarp thickness, %M = percent moisture content, 20 seed wt = weight of 20 seeds, 1st SP = days to first spoilage, 50% = days to 50% spoilage and 100% SP = days to 100% fruit spoilage.

Table 14: Mid Parent Heterosis of the fruit characteristics of the F_1 hybrids of tomatoes used for the study

Tomato variety	MFW (g)	FL (cm)	FW (cm)	FSI	LC	PT	%M	NS	100 SW	1st SP	50% SP	100% SP
S x (W x R)	-57.63	-8.17	-21.33	22.65	-37.93	28.97	0.72	10.76	13.21	77.18	62.88	78.44
(W x R) x S	-76.29	-12.99	-37.99	20.63	-65.52	-5.75	-0.76	-6.42	-13.21	95.97	53.79	92.45
PR x (R x W)	13.72	26.58	6.29	22.74	-7.69	13.40	3.74	5.42	0.00	56.37	29.69	8.01
PR x (W x R)	-12.01	10.58	3.95	6.74	4.00	-0.99	-0.36	4.34	25.00	9.47	6.83	18.70
PR x (W x T)	-1.52	26.74	2.29	27.62	-5.88	-22.33	0.49	42.75	17.65	-33.33	-32.19	-14.34
BF x (W x T)	-45.84	-10.11	-13.95	0.33	-38.46	-4.89	4.62	-12.73	-7.69	32.77	3.79	5.55
cd 5%	31.32	0.71	4.75	5.76	0.53	0.57	2.18	6.45	0.28	14.42	18.82	20.2

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande) **MFW** = mean fruit weight, **FL** = fruit length, **FW** = fruit width, **FSI** = fruit size index, **LC** = locule number, **NS** = number of seeds, PT = pericarp thickness, %M = percent moisture content, 20 seed wt = weight of 20 seeds, 1st SP = days to first spoilage, 50% = days to 50% spoilage and 100% SP = days to 100% fruit spoilage.

Mean Performance of F_2 segregating crosses of the tomato used for the study

The population means days to flowering, fruit appearance and ripening for the F_2 tomato hybrids used for this study were 21.1, 31.2 and 59.9 respectively. The cross BF x (W x T) was significantly ($p = 0.05$) lower in days to flowering, first fruit appearance and ripening (19.67, 28.67 and 54.67) respectively. The cross, PR x (W x T) produced the highest number of branches (14.78) among the F_2 tomato genotypes. The cross, PR x (W x T) was also found to produce the tallest plant of 125.6cm and fattest plant of 37.8 cm stem girth.

The crosses PR x (W x T), S x (W x R) and (W x R) x S produced higher number of fruits per plant (77.2, 69.8 and 42) respectively. However, the cross S x (W x R) had significantly higher yield (ton/ha) than the other hybrids.

Table 15: The Agronomic and yield component traits of the F_2 segregating tomato crosses and their parents

Tomato variety	dfwl	dfrt	dripe	B	H (cm)	SG(cm)	flw/P	trs/P	frt/P	FY (t/ha)
W x R	21.1	32.1	58.1	27.4	113.7	33.9	239.8	46.5	120.5	25
R x W	20.1	30.1	59.4	20.6	93.6	30.4	127.5	29	43.3	11.4
W x T	19.8	31.5	60	24	92.5	32.3	171.5	37.3	66.5	14.2
S	28.6	37.7	68.6	4.8	75.6	42.1	17.5	4.8	5.3	22.6
BF	25.2	34.1	62.1	11.6	82.7	37.4	100.3	10.3	23.8	20.7
PR	27.2	37.9	68.3	7.8	84.4	37.3	77	7.8	17.5	13.1
S x (W x R)	21.56	31.78	58.56	11.33	94.32	36.45	102.5	19	42	30.2
(W x R) x S	21.56	30.11	55.44	5.33	118.11	35.22	116	24	69.8	12.3
PR x (R x W)	21.89	32.89	60.67	8.56	87	36.16	99	17	45	18.1
PR x (W x R)	21.44	30.33	56.56	12.67	118.78	37.72	96.3	22.33	40	18.7
PR x (W x T)	20.67	30.67	61.44	14.78	125.61	37.81	121	30.3	77.2	28.2
BF x (W x T)	19.67	28.67	54,.67	11.89	89.22	35.21	89.1	20.3	48.67	15.6
GRAND MEAN	21.1	31.2	59.9	13.4	98.0	36.0	113.1	22.4	53.3	19.2
LSD (p = 0.05)	0.63	0.73	1.25	1.06	10.9	1.93	12.71	6.34	10.9	6.02

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande) , **dflw** = days to flowering, **dfrt** = days to first fruit appearance, **dripe** = days to fruit ripening, **B** = number of branches, **H** = height at ripening, **SGT** = stem girth, **flw/trs** = number of flower per truss, **trs/plt** = number of truss per plant, **frt/P** = number of fruit per plant, **FY** = fruit yield and **AFW** = average fruit weight

Table 16: Fruit characteristics of the F_2 segregating tomato crosses and their parents

Tomato variety	MFW (g)	FL (cm)	FW (cm)	FSI	LC	PT	%M	NS	100 SW	1st SP	50% SP	100% SP
W x R	26.98	4.44	4.2	1.07	2.22	4.2	90.5	64.33	0.23	7	11.3	15.7
R x W	29.56	4.52	4.33	1.05	2.44	3.8	90.24	73.56	0.25	6.2	17.5	23.4
W x T	29.37	4.76	4.29	1.13	2.33	4.4	90.36	74	0.26	9.9	19.8	25.5
S	170.29	5.84	8.2	0.69	10.67	4.5	92.67	48.22	0.3	7.9	15.1	21.4
BF	75.09	4.91	5.63	0.89	4.89	5	90.48	91.78	0.26	13.6	22.4	34
PR	39.54	4.8	3.97	1.25	3.33	5.9	89.24	43.22	0.25	12	20.9	30.3
S x (W x R)	58.16	4.94	5.4	0.91	4.22	4.91	89.05	95	0.31	11.7	20.3	31.5
(W x R) x S	21.78	4.14	4.04	1.06	2.44	4.61	90.43	77.44	0.21	10.4	15.6	20.3
PR x (R x W)	28.2	4.94	4.33	1.17	2.67	6.28	89.93	58.89	0.26	17.7	25.3	31.6
PR x (W x R)	31.06	5.22	3.94	1.4	2.44	5.24	90.89	40.56	0.26	9.3	15.6	20.6
PR x (W x T)	32.13	5.8	3.99	1.55	2.44	5.49	88.23	63.33	0.25	8.5	15.7	22.7
MR x (W x T)	26.77	4.83	4.1	1.22	2	5.28	91.67	70.33	0.23	19.5	28.6	41.1
GRAND MEAN	47.41	4.93	4.70	1.12	3.51	4.97	90.31	66.72	0.26	11.14	19.01	26.51
LSD (P = 0.05)	12.42	0.157	0.246	0.113	0.678	0.196	2.241	7.42	0.003	4.42	5.72	6.35

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande), **MFW** = mean fruit weight, **FL** = fruit length, **FW** = fruit width, **FSI** = fruit size index, **LC** = locule number, **PT** = pericarp thickness, **%M** = percent moisture content, **20 SW** = weight of 20 seeds, **1st SP** = days to first spoilage, **50% SP** = days to 50% spoilage and **100% SP** = days to 100% fruit spoilage.

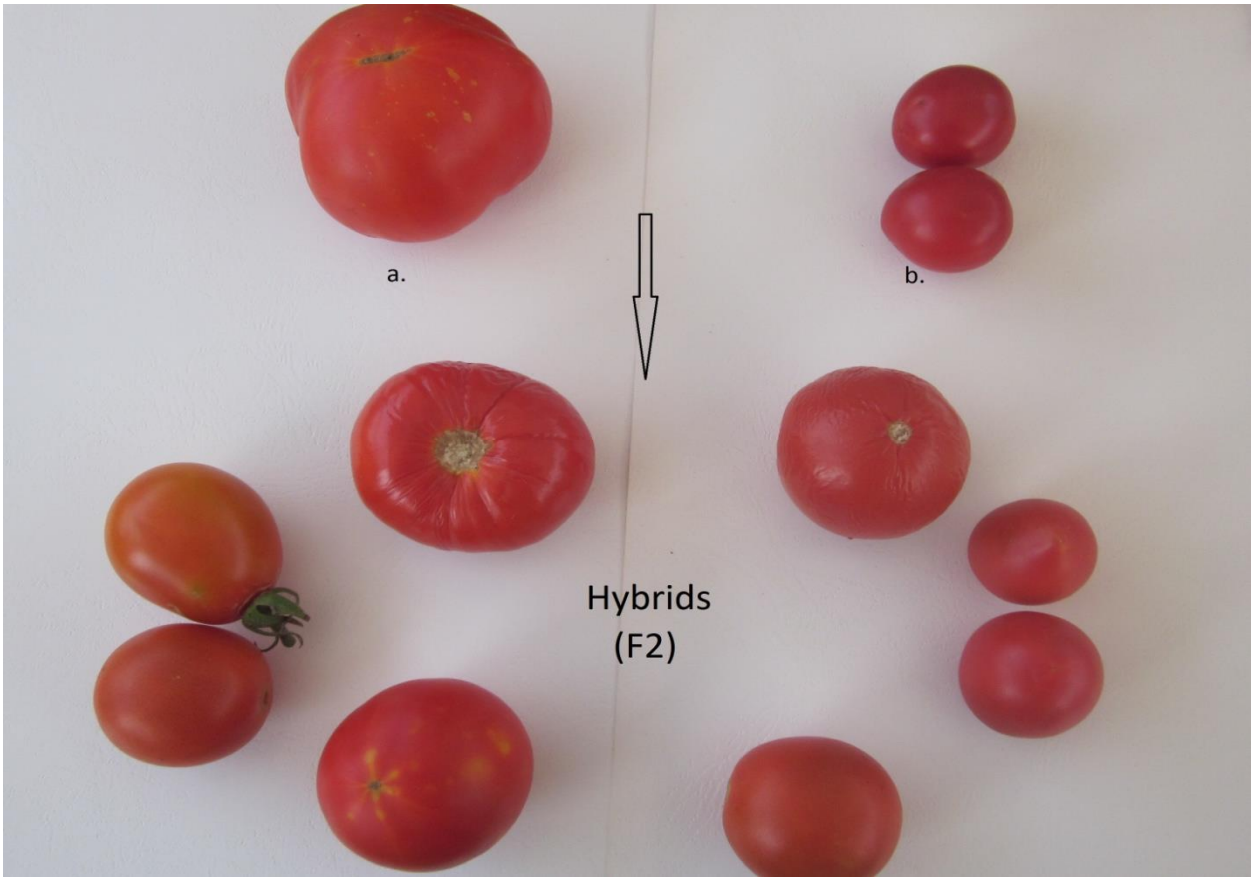


Plate 1: Tomato parents and their progenies in F_2, a. Parent $_1$, Supersteak (S), b. Parent $_2$, Wild x Roma (W x R), advanced generation and their 8 F_2 Hybrids varied in size and shapes

Generational (Parents, F_1, F_2, BC_1 and BC_2) Means of the agronomic, yield and fruit characters

The cross, BF x (W x T) of the F_1 generation showed significantly higher (p = 0.05) number of days to first flowering (17.9) and the first fruit appearance. The parent, supersteak with significantly higher days to flowering and fruit appearance was statistically similar to parent PR and BF, also with BC_1 (S x (W x R). The cross, (W x R) x S of the F_2 that ripened at 52.3 days showed significantly lower value in days to fruit ripening and was statistically similar to the BF x (W x T) of F_1 and F_2.

The hybrid S x (W x R) of BC_2 was significantly higher (p = 0.05) in plant height at ripening (139.7cm) and was statistically similar to PR x (W x T) and S x (W x R) of F_2. The hybrid, S x (W x R) of F_1 had significant higher value in stem girth among all the crosses (38.5cm) and was statistically similar to PR x (R x W) and PR x (W x T) of F_1.

The parent, W x R was significantly higher in number of branches/plant (27.4), number of trusses/plant (46.5), number of flower/plant (239.8) and number of fruit/plant (160.5). On the other hand, the parent, supersteak had a significant lower value in number of branches/plant (4.8), number of trusses/plant (4.8), number of flowers/plant (17.5) and number of fruit/plant (5.9). The cross S x (W x R) of BC1 was significantly lower in number of flowers/plant, number of trusses/plant and number of fruits/plant (25.9, 7.1 and 12.4) respectively. The hybrid, (W x R) x S) was significantly higher in number of fruit/plant (108.5) and was statistically similar to S x (W x R) of BC_2, S x (W x R) of F_1 and PR x (W x T) of F_1.

Table 17: Mean of the agronomic, yield and yield component traits of the parents, F_1, F_2, BC_1 and BC_2 of the parents and the crosses studied

Tomato variety	dfwl	dfrt	dripe	B	H (cm)	SG(cm)	flw/P	trs/P	frt/P	FY (t/ha)
W x R	21.1	32.1	58.1	27.4	113.7	33.9	239.8	46.5	120.5	25
R x W	20.1	30.1	59.4	20.6	93.6	30.4	127.5	29	43.3	11.4
W x T	19.8	31.5	60	24	92.5	32.3	171.5	37.3	66.5	14.2
S	28.6	37.7	68.6	4.8	75.6	42.1	17.5	4.8	5.3	22.6
BF	25.2	34.1	62.1	11.6	82.7	37.4	100.3	10.3	23.8	20.7
PR	27.2	37.9	68.3	7.8	84.4	37.3	77	7.8	17.5	13.1
F1										
S x (W x R)	22.5	32.3	59.4	18.3	120.2	38.5	145.3	39.3	86.8	26.1
(W x R) x S	21.1	31.1	57	21.3	99	35.9	166.3	45.3	108.5	19.3
PR x (R x W)	22.8	32.2	62.8	16	102.5	37.7	122.3	20	58.3	23.4
PR x (W x T)	23.8	34.5	64	19.2	115.8	37.1	133.8	30.5	92.8	22.6
PR x (W x R)	23.5	33.3	62.8	20	113	36.7	122.3	24.8	52.3	23.1
BF x (W x T)	20.1	28.5	53.5	19.2	112.4	35.9	137	36.5	84.3	14.9
F2										
S x (W x R)	21.6	31.8	58.6	11.3	94.3	36.4	102.5	26.4	42	30.2
(W x R) x S	21.6	30.1	52.4	5.3	118.1	35.2	116	21	69.8	12.3
PR x (R x W)	21.9	32.9	60.7	8.6	87	36.2	99	17	45	18.1
PR x (W x T)	20.7	30.7	61.4	14.8	125.6	37.8	96.3	30.3	57	18.7
PR x (W x R)	19.4	30.3	56.6	12.7	118.8	37.7	121	22.3	77	28.2
BF x (W x T)	17.7	28.7	52.7	11.9	89.2	35.2	89.1	20.3	48.7	15.6
BC1										
(S x(R XW))	25.5	34.5	65.6	8.5	107	35.6	25.9	7.1	12.4	10.9
(PR x (R x W))	26.8	38	67.1	10.6	95.5	34.9	66.2	11.6	31.7	15.5
(PR x (W x T))	24.6	34.6	63.6	12.7	68.1	34.3	70.9	13.8	35.2	13.8
BC2										
(S x(W XR))	20.5	29.1	58.1	21.1	139.7	31.2	167.2	31.3	104.9	30.2
(PR x (R x W))	21.8	31.8	59.9	15.5	84	29.9	97.9	18.6	27.9	11.1
(PR x (W x T))	20	30.1	59.5	18.7	109.1	30.1	146.1	24.5	48.3	12.4
LSD (p = 0.05)	1.34	1.59	1.42	1.94	11.42	2.01	10.7	5.04	8.47	4.89

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande), **dflw** = days to flowering, **dfrt** = days to first fruit appearance, **dripe** = days to fruit ripening, **B** = number of branches, **H** = height at ripening, **SGT** = stem girth, **flw/trs** = number of flower per truss, **trs/plt** = number of truss per plant, **frt/P** = number of fruit per plant, **FY** = fruit yield and **AFW** = average fruit weight, F_1 and F_2 = first and second filial generation, BC_1 and BC_2 = back crosses.

Table 18: Mean of the fruit characteristics of the parents, F_1, F_2, BC_1 and BC_2 of the tomato parents and crosses studied

Tomato variety	MFW (g)	FL (cm)	FW (cm)	FSI	LC	PT	%M	NS	100 SW	1st SP	50% SP	100% SP
W x R	26.98	4.44	4.2	1.07	2.22	4.2	90.50	64.33	0.23	7	11.3	15.7
R x W	29.56	4.52	4.33	1.05	2.44	3.8	90.24	73.56	0.25	6.2	17.5	23.4
W x T	29.37	4.76	4.29	1.13	2.33	4.4	90.36	74.00	0.26	9.9	19.8	25.5
S	170.29	6.84	8.2	0.69	10.67	4.5	92.67	48.22	0.3	7.9	15.1	21.4
MR	75.09	4.91	5.63	0.89	4.89	5	90.48	91.78	0.26	13.6	22.4	34
MO	39.54	4.8	3.97	1.25	3.33	5.9	89.24	43.22	0.25	12	20.9	30.3
F1												
S x (W x R)	41.79	5.18	4.88	1.08	4	5.61	92.25	62.33	0.3	13.2	21.5	33.1
(W x R) x S	23.39	4.06	3.84	1.06	2.22	4.1	90.89	52.67	0.23	14.6	20.3	35.7
PR x (R x W)	39.29	5.9	4.41	1.42	2.67	5.5	93.10	61.56	0.25	14.23	24.9	29
PR x (W x R)	29.27	5.11	4.24	1.24	2.89	5	89.55	56.11	0.3	10.4	17.2	27.3
PR x (W x T)	33.93	6.06	4.22	1.52	2.67	4	90.24	83.67	0.3	7.3	13.8	23.9
BF x (W x T)	28.29	4.34	4.27	1.01	2.22	4.47	94.60	72.33	0.24	15.6	21.9	31.4
F2												
S x (W x R)	58.16	4.94	5.4	0.91	4.22	4.91	89.05	95.00	0.31	11.7	20.3	31.5
(W x R) x S	21.78	4.14	4.04	1.06	2.44	4.61	90.43	77.44	0.21	10.4	15.6	20.3
PR x (R x W)	28.2	4.94	4.33	1.17	2.67	6.28	89.93	58.89	0.26	17.7	25.3	31.6
PR x (W x R)	31.06	5.22	3.94	1.4	2.44	5.24	90.89	40.56	0.26	9.3	15.6	20.6
PR x (W x T)	32.13	5.8	3.99	1.55	2.44	5.49	88.23	63.33	0.25	8.5	15.7	22.7
BF x (W x T)	26.77	4.83	4.1	1.22	2	5.28	91.67	70.33	0.23	19.5	28.6	41.1
LSD (P = 0.05)	11.8	0.146	0.301	0.11	0.54	0.167	4.73	8.79	0.007	5.14	5.12	7.02

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande) **MFW** = mean fruit weight, **FL** = fruit length, **FW** = fruit width, **FSI** = fruit size index, **LC** = locule number and **NS** = number of seeds.

Estimation of Gene Effects for the agronomic yield and yield characters of the tomato used in the study

The gene effects on agronomic and yield characters were studied using means of several different generation. Variable gene effects were observed among the cross combinations involving a modified three way crosses. The results are presented in Tables 19 and 20.

The additive gene action was positive and significant for the S x (W x R), PR x (R x W) and PR x (W x R) in days of flowering. The dominance, additive x additive and dominance x dominance gene effects were all positive but not significant. The additive x dominance gene action was also all positive but significant only for the S x (W x R). However, the additive x dominance gene effect had a small magnitude in relation to the mean effect than the other gene effects in days of flowering.

All gene effects was positive for all the tomato hybrids in days to fruit initiation. The additive gene effect was significant in S x (W x R) and PR x (W x R). The additive x dominance gene action was also significant only for the S x (W x R). Similar results were obtained for the days to ripening.

Significant additive x additive gene action was observed in PR x (R x W) and PR x (W x R) for the number of branches. S x (W x R) and PR (R x W) showed positive significant additive x

dominance gene effect. All gene effects in number of branches were positive except the additive x dominance gene effect for the S x (W x R).

Majority of the attributes had positive dominance x dominance gene action except the average fruit weight for the cross, PR x (R x W). The plant height of the cross, PR x (W x R) had high and positive but not significant dominance x dominance gene action. It was noted that statistically dominance x dominance gene action was only significant in number of flower per plant.

Table 19: Gene effects of the agronomic, yield and yield traits of the crosses for the study

Traits	Crosses	m (F2)	a = B1 - B2	d	aa	ad	dd
dfwl	S x (W x R)	18.6	5.03*	36.46	17.71	2.57*	148.76
	PR x (R x W)	18.9	5.00*	40.80	21.51	2.92	148.47
	PR x (W x R)	20.7	4.67*	26.70	6.53	2.00	158.67
dfrt	S x (W x R)	28.8	5.37*	41.70	12.16	5.15*	228.06
	PR x (R x W)	29.9	6.20	48.43	20.18	4.57	151.26
	PR x (W x R)	30.7	4.53*	37.96	6.67	2.65	167.12
dripe	S x (W x R)	58.6	7.57*	67.35	13.18	4.65*	449.47
	PR x (R x W)	60.7	7.20	69.84	11.47	5.48	467.28
	PR x (W x R)	61.4	4.13	60.19	0.36	-0.07	485.58
B	S x (W x R)	11.3	-12.60	43.54	14.00*	-2.62	164.48
	PR x (R x W)	8.6	-4.90	40.26	17.84	3.03*	114.16
	PR x (W x R)	14.8	-6.07	30.98	41.47*	21.05*	153.46
H (cm)	S x (W x R)	94.3	-32.67	255.30	116.04	38.05*	937.71
	PR x (R x W)	87.0	11.43*	118.11	11.00	32.08*	685.22
	PR x (W x R)	125.6	-40.97	-28.23	-148.11	-73.77	1074.81

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande) , **dflw** = days to flowering, **dfrt** = days to first fruit appearance, **dripe** = days to fruit ripening, **B** = number of branches, **H** = height at ripening, **M** = F2 Mean, **A** = additive effect, **d** = dominant effect, **aa** = additive x additive effect, **dd** = dominance x dominance effect.

Table 20: Gene effects of the agronomic, yield and yield traits of the crosses for the study

Traits	Crosses	m (F2)	a = B1 - B2	d	aa	ad	dd
SG(cm)	S x (W x R)	36.4	4.40*	22.18	-12.19	0.55*	281.15
	PR x (R x W)	36.2	5.00*	19.23	-15.03	3.13	159.28
	PR x (W x R)	37.8	4.20*	12.16	-22.44	3.39*	278.23
flw/P	S x (W x R)	102.5	-141.30	-7.18	-23.80	-30.18	185.4*
	PR x (R x W)	99.0	-31.70	-47.8	-67.80	-6.45	188.60*
	PR x (W x R)	121.0	-75.20	40.5	-50.00	-49.95	88.00*
trs/P	S x (W x R)	26.4	-24.20	188.95	-28.98	-6.65	332.33
	PR x (R x W)	17.0	-7.00	23.03	-7.60	7.25*	172.75
	PR x (W x R)	30.3	-10.70	0.65	-44.60	8.10*	270.00
frt/P	S x (W x R)	42.0	-92.50	240.98	66.60*	-29.75	897.25
	PR x (R x W)	26.0	3.80	86.33	15.20	33.35*	390.05
	PR x (W x R)	57.0	-13.10	56.25	-157.60	22.80*	549.90
FY (t/ha)	S x (W x R)	30.2	-19.3	-49.31	-38.6	-18.09	56.3
	PR x (R x W)	18.1	4.4	-7.86	-19	3.55*	37.16
	PR x (W x R)	28.2	1.4	-51.445	-60.4	1.955*	80.61

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande)**, SGT** = stem girth, **flw/trs** = number of flower per truss, **trs/plt** = number of truss per plant, **frt/P** = number of fruit per plant, **FY** = fruit yield and **AFW** = average fruit weight, **M** = F2 Mean, **A** = additive effect, **d** = dominant effect, **aa** = additive x additive effect, **dd** = dominance x dominance effect

Estimation of Gene Effects for the fruit characteristics of the tomato used in the study

The gene effects on fruit characteristics were studied using means of several different generation. Variable gene effects were observed among the cross combinations involving a modified three way crosses (Table 21).

The additive gene action was positive and significant for the S x (W x R), MO x (R x W) and MO x (W x R) in mean fruit weight and locule number. A positive and significant additive gene action was observed for the S x (W x R) and MO x (R x W) in fruit length, for the MO x (R x W) and MO x (W x R) in fruit shape index and only S x (W x R) in fruit shape index. A negative but not significant additive gene action was observed in number of seeds character.

The additive x additive gene action was positive in mean fruit weight and number of locules per fruit in all crosses used in this study. It was also noted that statistically additive x additive gene action was only significant in the fruit width for the only S x (W x R). Majority of the attributes had negative additive x dominance gene action. The crosses, PR x (R x W) and PR x (W x R) had positive and significant additive x dominance gene action in single fruit weight. The cross, S x (W x R) had a positive and significant additive x dominance gene action in the fruit width.

Table 21:Gene effects of the fruit characteristics of the crosses of tomato varieties used for the study

Traits	Crosses	m (F2)	a = B1 - B2	d	aa	ad	dd
MFW	S x (W x R)	58.16	61*	-23.89	32.96*	-21.31	-17.71
	PR x (R x W)	28.20	7.5*	46.54	41.8	24.98*	-48.72
	PR x (W x R)	31.06	9.6*	19.36	19.88	29.37*	-31.51
FL (cm)	S x (W x R)	4.94	0.2*	2.2	-0.16	-2	2.2
	PR x (R x W)	4.24	0.15*	0.38	-0.86	0.02	3.08
	PR x (W x R)	4.52	-0.15	-1.82	-4.5	-0.34	7.48
FW (cm)	S x (W x R)	5.4	1.2*	-0.52	0.8*	-1.6	-1.04
	PR x (R x W)	4.33	-0.2	0.14	-0.12	-0.04	0.04
	PR x (W x R)	3.94	-0.4	0.93	0.84	-0.48	-0.94
FSI	S x (W x R)	0.91	-0.15	0.1	-3.94	0.08*	0.48
	PR x (R x W)	1.17	0.08*	-0.01	-0.28	-0.04	0.94
	PR x (W x R)	1.40	0.07*	-1.41	-1.74	0.02	2.7
LC	S x (W x R)	4.22	0.8*	0.285	2.72	-6.85	-1.43
	PR x (R x W)	2.67	0.03*	0.68	0.86	-0.39	-1.29
	PR x (W x R)	2.44	0.05*	0.78	0.94	-0.9	-0.64
NS	S x (W x R)	95.00	-21.1	-116.945	-123	-26.09	103.21
	PR x (R x W)	58.89	-22.1	-52.39	55.84	-13.86	-107.34
	PR x (W x R)	40.56	-24.9	-3.825	56.08	-19.02	-80.92

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande), **MFW** = single fruit weight, **FL** = fruit length, **FW** = fruit width, **FSI** = fruit size index, **LC** = locule number and **NS** = number of seeds, = F2 Mean, **A** = additive effect, **d** = dominant effect, **aa** = additive x additive effect, **dd** = dominance x dominance effect.

Estimates of Heritability for the agronomic, yield and fruit related characters

The estimates of broad and narrow sense heritability in the segregating population for the agronomic, yield and fruit characters are given in the Table 23 and 24. For the cross S x (W x R), the highest broad sense heritability was recorded in the number of seeds (99.51%), followed closely with the fruit width (99.11%) and number of locules per fruit (98.6%). The lowest broad sense heritability was detected in the percentage of moisture content (51.9%). Narrow sense heritability was recorded to be highest in the fruit width (56.14%) and single fruit weight (52.9%). The results also revealed that the hybrid with supersteak as one of its parents had higher narrow sense heritability in the single fruit weight and fruit width.

The highest value of broad sense heritability was observed in the days to first flowering (98.7%) and fruit length (97.7%) for the cross, PR x (R x W) while the lowest value was detected in the locule number (51.9%) and pericarp thickness (56.79%). On the other hand, narrow sense heritability was recorded to be highest in the fruit length (53.6%) and the 100 seed weight (50.12%). The hybrids with Beef (Florida) as one of the parent, had the narrow sense heritability greater than 50%.

High percentage broad sense heritability was recorded in the pericarp thickness (97.8%) for the cross PR x (W x R). The lowest broad sense heritability was recorded in the sterm girth (51.3). The narrow sense heritability was recorded to be highest in the moisture content (52.9%) and fruit length (50.9%)

Table 22. Estimates of broad Sense (Hbs) Heritability in measured plant Attributes (%)

Tomato attributes	S x (W x R)	PR X (R x W)	PR x (W x R)
Days to flowering	86.06	98.9	79.7
Days to first fruiting	81.34	68.03	72.17
Days to first fruiting ripening	77.6	69.34	66.9
Number of branches	74.9	78.8	69.6
Plant height (cm)	96.33	87.05	83.6
Stem girth	97.61	76.9	51.3
Number of flowers/plant	62.24	86.3	51.8
Number of trusses/plant	84.31	89.8	81.5
Number of fruits/plant	98.1	94.6	78.9
Fruit Yield (t/ha)	98.9	97.6	96.9
Single fruit weight	93.1	90.8	87.9
Fruit Length (cm)	88.86	97.7	96.1
Fruit Width (cm)	99.11	71.8	76.9
Fruit Shape Index	83.2	89.8	85.9
Locule number	98.6	51.9	59.9
Pericarp Thickness (mm)	61.42	56.79	97.8
Moisture content (%)	51.9	89.6	92.8
Number of seeds	99.51	67.8	79.9
100 Seed Weight	83.27	79.1	75.23
1st SP	76.89	83.23	72.3
50% SP	70.87	71.22	78.9
100% SP	61.89	74.51	68.3

Table 23. Estimates of Narrow Sense (Hns) Heritability in measured plant Attributes (%)

Tomato attributes	S x (W x R)	PR X (R x W)	PR x (W x R)
Days to flowering	32.9	39.19	43.6
Days to first fruiting	38.16	33.27	41.73
Days to first fruiting ripening	41.9	37.8	34.9
Number of branches	44.89	48.9	39.6
Plant height (cm)	46.11	36.9	27.9
Stem girth	51.8	27.9	34.9
Number of flowers/plant	34.09	47.8	42.9
Number of trusses/plant	31.9	37.6	40
Number of fruits/plant	47.9	44.9	39.9
Fruit Yield (t/ha)	48.5	45.9	41.64
Single fruit weight	52.9	50.06	47.9
Fruit Length (cm)	39.6	53.9	50.9
Fruit Width (cm)	56.14	34.6	29.8
Fruit Shape Index	46.03	48.9	49.45
Locule number	49.67	26.3	32.9
Pericarp Thickness (mm)	24.9	21.9	44.9
Moisture content (%)	51.9	42.23	52.9
Number of seeds	27.9	33.02	22.9
100 Seed Weight	31.9	50.12	36.5
1st SP	42.1	46.7	29.9
50% SP	29	31.9	24.9
100% SP	32.26	35.18	27.8

Mean Performance of the F_3 population

The results of the mean performance of the parents and their F_3 progenies are presented in Tables 24 and 25. The results showed higher segregation in tomato progenies. The comparison of the mean of agronomic, yield and fruit characters among segregating population indicated that the mean values in general were relative higher in F_3 than F_2.

Table 24: The agronomic and yield component of the F_3 segregating tomato crosses and their parents

Tomato variety	dfwl	dfrt	dripe	B	H (cm)	SG(cm)	flw/P	trs/P	frt/P	FY (t/ha)
W x R	21.1	32.1	58.1	27.4	113.7	33.9	239.8	46.5	120.5	25
R x W	20.1	30.1	59.4	20.6	93.6	30.4	127.5	29	43.3	11.4
W x T	19.8	31.5	60	24	92.5	32.3	171.5	37.3	66.5	14.2
S	28.6	37.7	68.6	4.8	75.6	42.1	17.5	4.8	5.3	22.6
MR	25.2	34.1	62.1	11.6	82.7	37.4	100.3	10.3	23.8	20.7
MO	27.2	37.9	68.3	7.8	84.4	37.3	77	7.8	17.5	13.1
S x (W x R)	23.11	34.44	68.56	14.22	127.22	37.49	113.7	20	50.78	36.75
(W x R) x S	19.33	27.33	64.22	17.44	118	33.2	136	26.4	74.33	28.07
MO x (R x W)	23.67	32.33	62.22	9.56	111.67	34.63	94.3	15.7	41.22	26.2
MO x (W x R)	21.33	31.44	60.78	5.89	101.22	30.21	78.2	12.8	21.67	11.34
MO x (W x T)	23.44	33.56	65.22	15.22	121.22	36.5	144.1	29.6	83.67	45.7
MR x (W x T)	27.22	33.67	59	16.78	123.56	35.03	123.9	25.1	60.56	30.24
GRAND MEAN	23.34	33.01	63.04	14.61	103.78	35.04	118.65	22.11	53.26	25.44
LSD (P = 0.05)	0.945	0.82	1.17	1.22	14.73	1.89	13.35	7.24	11.8	5.67

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande) , **dflw** = days to flowering, **dfrt** = days to first fruit appearance, **dripe** = days to fruit ripening, **B** = number of branches, **H** = height at ripening, **SGT** = stem girth, **flw/trs** = number of flower per truss, **trs/plt** = number of truss per plant, **frt/P** = number of fruit per plant, **FY** = fruit yield and **AFW** = average fruit weight

Table 25: Fruit characteristics of the F_3 segregating tomato crosses and their parents

Tomato variety	MFW	FL (cm)	FW (cm)	FSI	LN	PT (mm)	%M	NS	1st SP	50% SP	100% SP
W x R	26.98	4.44	4.2	1.07	2.22	4.2	90.5	64.33	7	11.3	15.7
R x W	29.56	4.52	4.33	1.05	2.44	3.8	90.24	73.56	6.2	17.5	23.4
W x T	29.37	4.76	4.29	1.13	2.33	4.4	90.36	74	9.9	19.8	25.5
S	170.29	5.84	8.2	0.69	10.67	4.5	92.67	48.22	7.9	15.1	21.4
MR	75.09	4.91	5.63	0.89	4.89	5	90.48	91.78	13.6	22.4	34
MO	39.54	4.8	3.97	1.25	3.33	5.9	89.24	43.22	12	20.9	30.3
S x (W x R)	79.2	5.32	5.32	0.85	6.33	6.36	94.5	91.67	11.94	18.12	27.23
(W x R) x S	34.1	4.06	3.93	1.03	2.3	4.67	92.31	89.45	13.25	18.71	23.2
PR x (R x W)	43.61	4.79	4.79	1.13	2.44	7.18	93.1	76	15.7	23.55	30.8
PR x (W x R)	31.9	5	3.7	1.36	2.3	5.78	93.8	84.15	12.35	17.43	25.67
PR x (W x T)	40.4	5.8	4.2	1.38	2.9	6.3	93.2	73.8	11.91	17.45	27.56
BF x (W x T)	35.71	4.43	4.43	1.16	2.11	5.98	96.05	81.33	15.6	25.5	34.9
GRAND MEAN	52.98	4.89	4.75	1.08	3.69	5.34	92.20	74.29	11.45	18.98	26.64
LSD (P = 0.05)	16.65	0.168	0.283	0.143	0.923	0.211	2.19	9.73	5.33	6.19	7.72

W x R = Wild x Roma; **R x W** = Roma x Wild; **W x T** = Wild x Tropica; **S** = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande) **MFW** = mean fruit weight, **FL** = fruit length, **FW** = fruit width, **FSI** = fruit size index, **LC** = locule number, **PT** = pericarp thickness, **%M** = percent moisture content, **20 SW** = weight of 20 seeds, **1st SP** = days to first spoilage, **50% SP** = days to 50% spoilage and **100% SP** = days to 100% fruit spoilage.

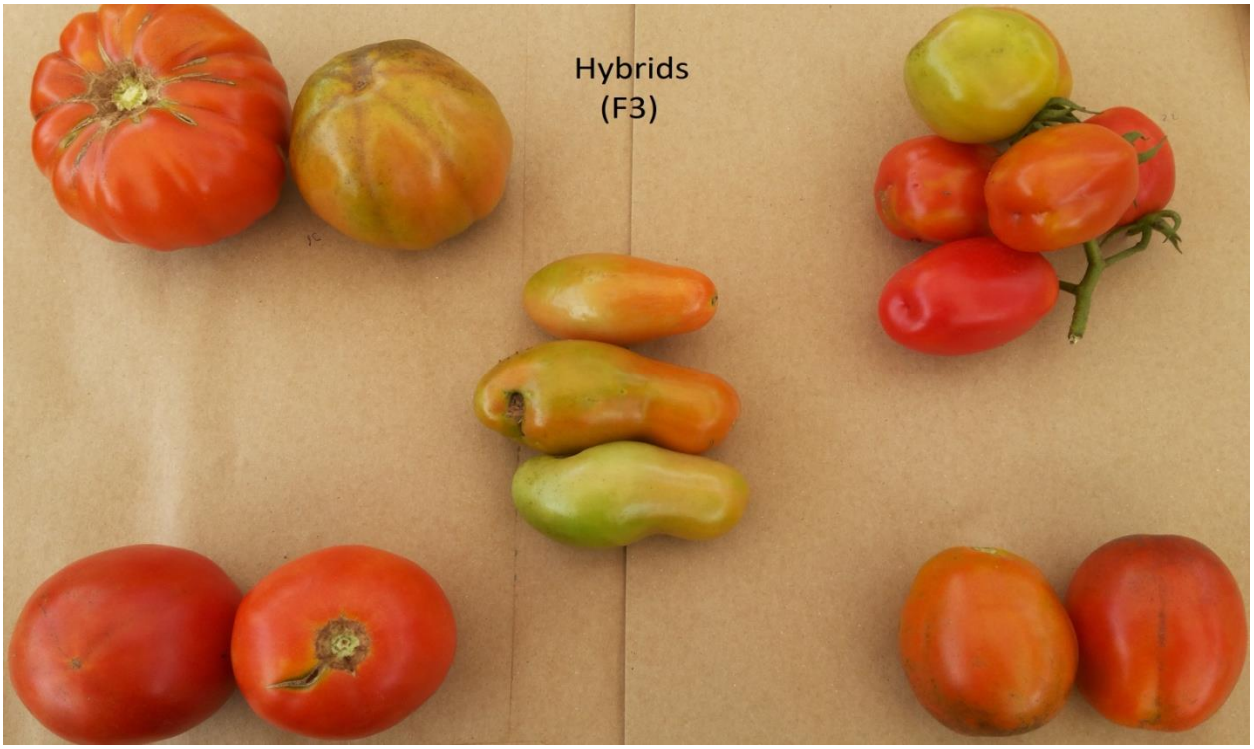


Plate 2: Variation in fruit size and shapes of the F_3 progenies develop from S x (W x R)

Means and ranks for the yield and fruit character

Fruit size is a complex quantitative trait controlled by many genes interacting with the environment. Selection of the progenies based on one character may often misleading. According to the ranking, the F_3 tomato hybrid (S x W x R), 18, 14, 16, 15 and 11 were selected as the best tomato hybrids for the further evaluation and selection in further generation during rainy season.

The F_3 hybrid, 18 had higher number of fruit per plant but was lower in single fruit weight. The hybrid, 14 had higher number of fruit, single fruit weight and the locule number. The hybrid, 16 had the highest single fruit weight and locule number but lowest number of fruit among the tomato hybrids. The hybrids, 15 had higher mean fruit weight but reasonable number of fruits per plant, similar applied for the hybrid, 11. Some of the best hybrids are displayed in Plate 3.

Table 26. Ranking of the F3 selection based on yield (kg/P), mean fruit weight and fruit number

Tomato varieties	Yield (Kg/P)	Rank	Frt/P	Rank	MFW	Rank	FL	Rank	FW	Rank	LN	Rank
P_1 (S)	1.09	22	5	28	218	1	8.5	1	9.6	1	10	1
P_2 (W x R)	2.59	6	112	1	23.1	26	3.8	28	3.9	20	2.2	28
F_3 (S x (W x R)												
1	1.48	14	27	24	54.75	8	5.2	12	6.1	6	6.5	4
2	1.00	23	42	11	23.9	25	4.5	24	4.6	16	2.9	18
3	1.44	15	35	19	41.2	12	5.2	13	5.9	7	4.6	8
5	0.80	27	37	15	21.6	27	4.15	25	3.95	19	2.21	27
6	1.75	10	32	20	54.7	9	5.6	8	5.6	8	4.5	9
7	2.22	7	59	3	37.6	15	4.65	21	5.5	9	3.5	11
11	3.03	2	51	8	59.4	5	5.5	9	5.3	10	3	16
12	1.12	20	56	4	20	28	4.1	26	3.6	24	2.5	24
13	2.70	5	49	9	55.2	7	5.3	11	5.3	13	4	10
14	3.26	1	35	18	93.1	4	5.1	16	6.7	3	6.9	3
15	2.79	3	27	23	103.2	3	6	3	6.7	4	6.2	5
16	1.15	19	6	27	191.8	2	5.8	6	8.2	2	8	2
18	2.77	4	79	2	35	19	4.8	20	4.2	17	3	15
19	1.91	9	55	5	34.7	17	5.9	5	4.1	18	2.7	21
20	1.72	11	52	6	33.1	18	5	17	4.7	15	2.5	23
21	1.10	21	42	12	26.1	23	5.9	4	3.1	27	2.4	25
22	1.23	17	52	7	23.6	24	5.5	10	3.5	26	2.9	17
23	1.20	18	45	10	26.7	22	5.2	14	3.8	23	2.2	26
24	1.32	16	38	14	34.8	16	4.9	18	4.8	14	3	14
25	1.55	13	29	22	53.6	11	5.8	7	5.3	11	3.5	12
26	0.91	26	23	25	39.4	13	6.6	2	3.5	25	2.9	19
27	1.59	12	41	13	38.9	14	4.5	22	3.8	22	3.4	13
28	1.99	8	35	16	56.8	6	5.2	15	5.3	12	6	6
29	0.99	24	35	17	28.2	21	4.5	23	3	28	2.5	22
30	0.92	25	29	21	31.7	20	3.8	27	3.9	21	2.8	20
31	0.75	28	14	26	53.7	10	4.9	19	6.3	5	6	7

Plate 3: Some of F_3 segregants for the fruit yield, size and shape

Inheritance Pattern of Fruit Characters

Inheritance of the number of locule

The results showed that the F_1 plants produced 32 fruits with 2 to 3 locules and 9 fruits with locules number ranging between 3 to 9. The back cross to the supersteak parent (BC_1) produced 10 fruits with 2 to 3 locules and 13 fruits with locules number ranging between 3 to 9. The back cross to the W x R segregated into 14 fruits with 2 to 3 locule number and 13 fruits with locule number ranging between 3 to 9. The F_2 population segregated into 34 fruit with 2 to 3 number of locule and 15 fruits with 3 to 9 locules number. The results are presented in Table 27.

The Chi- square ($G2$) analysis shown in Table 28 indicated a non significant Chi-square value of 0.823 while the corrected Chi-square was 0.364. Since the Chi square value is higher than the corrected Chi-square value, the observed ratio in F_2 and BCs generations fit the expected ratio of 3:1 and 1:1 respectively.

Inheritance of the fruit shape

The results shown in Table 31 showed that F_1's produced by the cross between supersteak (S) and W x R had the round fruit shape. F_2 genotypes with total number of 37 plants segregated into round fruit shape (27) and flat round fruit shape (10). The results indicated the chi square calculated and corrected values of 0.0810 and 0.7758 respectively, the deviation between the observed value and expected values in fruit shape were statistically significant (Table 32). The observed ratio does not match the expected ratio of 3:1.

The results presented in Table 33 showed that F_1 genotype obtained from PR x (R x W) cross produced elongated fruit shape. Out of 38 F_2 plants produced, 29 had elongated fruits and 9 had round shape. The ɢ2 analysis (Table 34) for the comparison of the observed F_2 ratio to the expected ratio of 3:1 showed a ɢ2 value of 0.035, which does not lie within the probability level, meaning that the observed ratio does not fits the expected ratio.

Table 27: Phenotypic expression in a cross between S and W x R

Phenotypes (Locule number)	Genotypes					
	P_1 (S)	P_2 (W x R)	F_1	F_2	BC_1	BC_2
2 TO 3	0	25	32	34	10	14
>3<9	0	0	9	15	13	13
>10	8	0	0	0	0	0
Total	**8**	**25**	**41**	**49**	**23**	**27**

Table 28: Chi-square estimates (χ2) of F_2 progenies in S and W x R

Phenotypes (Locule number)	Observed(O)	Expected (E)	(O - E)	(O - E)2	(O - E)2/E
2 TO 3	34	36.75	-2.75	7.563	0.206
>3<9	15	12.25	2.75	7.563	0.617
>10	0	0	0	0.000	0.000
Total	**49**	**49**	**0**	**15.125**	**0.823**

P1= first parent, P2= second parent, F1 = first filial generation, F2= second filial generation, BC1 = backcross to first parent
S = Supersteak, W = wild and R = Roma VF

Expected Ratio = 3:1

ɢ2 = 0.823

Corrected ɢ2 = 0.364

Table 29: Phenotypic expression in a cross between PR and R x W

Phenotypes (Locule number)	Genotypes					
	P_1 (MO)	P_2 (R x W)	F_1	F_2	BC_1	BC_2
2	0	42	37	31	15	26
3	19	9	13	18	19	18
>4	6	0	0	0	0	0
Total	**25**	**51**	**50**	**49**	**34**	**44**

Table 30: Chi-square estimates (χ2) of F2 progenies in PR and R x W

Phenotypes (Locule number)	Observed(O)	Expected (E)	(O - E)	(O - E)2	(O - E)2/E
2	31	36.75	-5.75	33.063	0.900
3	18	12.25	5.75	33.063	2.699
>4	0	0	0	0.000	0.000
Total	**49**	**49**	**0.000**	**66.125**	**3.599**

P1= first parent, P2= second parent, F1 = first filial generation, F2= second filial generation, BC1 = backcross to first parent

MO = Medium oval shape, W = wild and R = Roma VF

Expected Ratio = 3:1

G2 = 3.599

Corrected G2 = 0.0578

Table 31: Phenotypic expression in a cross between S and W x R

Phenotypes (fruit shape)	Genotypes			
	P_1 (S)	P_2 (W x R)	F_1	F_2
Round fruit	0	63	42	27
Slightly flat fruit	10	0	0	10
Total	**10**	**63**	**42**	**37**

Table 32: Chi-square estimates ($\chi 2$) of F_2 progenies in S and W x R

Phenotypes (fruit shape)	Observed(O)	Expected (E)	(O - E)	(O - E)2	(O - E)2/E
Round fruit	27	27.75	-0.75	0.5625	0.0203
Slight flat fruit	10	9.25	0.75	0.5625	0.0608
Total	**37**	**37**	**0**	**1.125**	**0.0810**

P1= first parent, P2= second parent, F1 = first filial generation, F2= second filial generation, BC1 = backcross to first parent
S = Supersteak, W = wild and R = Roma VF

Expected Ratio = 3:1

$\chi 2 = 0.0810$

Corrected $\chi 2 = 0.7758$

Table 33: Phenotypic expression in a cross between PR and R x W

Phenotypes (Locule number)	Genotypes			
	P_1 (MO)	P_2 (R x W)	F_1	F_2
Elongated fruit	23	0	52	29
Round fruit	0	57	0	9
Total	**23**	**57**	**52**	**38**

Table 34: Chi-square estimates (X^2) of F_2 progenies in PR and R x W

Phenotypes (Locule number)	Observed(O)	Expected (E)	(O - E)	(O - E)2	(O - E)2/E
Elongated fruit	29	28.5	0.5	0.25	0.0087
Round fruit	9	9.5	-0.5	0.25	0.02631
Total	**38**	38	0	0.5	0.03508

P1= first parent, P2= second parent, F1 = first filial generation, F2= second filial generation, BC1 = backcross to first parent

MO = Medium oval shape, W = wild and R = Roma VF

Expected Ratio = 3:1

ɢ2 = 0.03508

Corrected ɢ2 = 0.851

Correlation matrix of floral and fruit size characters on the fruit size increment

The phenotypic correlations among all floral traits and fruit size related characters are presented in Table 35. The relationship between style diameter and length, ovary size and shape and seed size and shape among tomato genotypes are shown in Plate 4. Correlation studies on 16 floral and fruit size related traits which include: length and width of the flower, stalk width, length and diameter of the style, length and diameter of the stigma, length, diameter, area and perimeter of the ovary, locule number and the length and diameter of the fruit, number of seeds, weight of 100 seeds and single fruit weight showed significant ($P<0.01$) correlation with the single fruit weight. Significant positive correlations were observed between the fruit size and the flower width, stalk width, style diameter, stigma length and diameter, diameter, length, area and perimeter of the ovary, locule number and the diameter and length of the fruit. Number of locules per fruit showed the highest positive correlation (r = 0.9844**), followed closely by ovary perimeter (r = 0.9722**), ovary diameter (r = 0.9674**), ovary area (r = 0.9578**), stigma diameter (r = 0.9535**), style diameter (r = 0.9491**). Fruit size was negative significantly correlated with the style length (r = -0.8840**), flower length (r = -0.8078**), number of seeds (r = -0.2386*). The number of locules per fruit was positive and highly significantly ($P<0.01$) correlated with the ovary perimeter (r = 0.96**), stigma diameter (r = 0.95**), ovary diameter (r = 0.954**), ovary area (r = 0.95**) and style diameter (r = 0.949**). However, the number of locules per fruit was negative and significant correlated with the style length (r = -0.85**). The ovary area was positive and highly significantly ($P<0.01$) correlated with the stigma diameter (r = 0.993**), style diameter (r = 0.990**) and ovary diameter (r = 0.990**).

Table 35: Correlation coefficients for floral traits and fruit size components among tomato varieties used in the study

	FLL	FW	SW	SL	SYD	SGD	SGL	OD	OL	OA	OP	NS	SEW	LN	FRL	FD
FLL	**1**															
FW	-0.77**	**1**														
SW	-0.78**	0.95**	**1**													
SL	0.97**	-0.87**	-0.88**	**1**												
SYD	-0.80**	0.846**	0.889**	-0.87**	**1**											
SGD	-0.79**	0.852**	0.883**	-0.86**	0.996**	**1**										
SGL	-0.18	0.063	0.213	-0.145	0.016	0.014	**1**									
OD	-0.78**	0.897**	0.888**	-0.86**	0.977**	0.981**	-0.066	**1**								
OL	-0.78**	0.924**	0.955**	-0.87**	0.894**	0.908**	0.244	0.891**	**1**							
OA	-0.80**	0.893**	0.908**	-0.88**	0.990**	0.993**	-0.024	0.990**	0.922**	**1**						
OP	-0.81**	0.936**	0.944**	-0.89**	0.968**	0.976**	0.069	0.976**	0.966**	0.98**	**1**					
NS	0.28	-0.240	-0.144	0.275	-0.351	-0.372	0.393	-0.361	-0.265	-0.371	-0.330	**1**				
SEW	-0.31	0.603*	0.490	-0.398	0.352	0.405	0.115	0.444	0.576*	0.426	0.51**	-0.002	**1**			
LN	-0.79**	0.903**	0.921**	-0.85**	0.949**	0.950**	0.117	0.954**	0.917**	0.95**	0.96**	-0.209	0.492	**1**		
FRL	-0.71**	0.846**	0.900**	-0.79**	0.786**	0.803**	0.457	0.788**	0.923**	0.81**	0.87**	-0.072	0.64**	0.872**	**1**	
FD	-0.67**	0.853**	0.794**	-0.73**	0.637*	0.642*	0.293	0.703**	0.756**	0.67**	0.74**	0.002	0.569*	0.804**	0.83**	**1**
MWF	-0.81**	0.914**	0.919**	-0.88**	0.949**	0.953**	0.117	0.967**	0.919**	0.95**	0.97**	-0.238	0.502*	0.984**	0.88**	0.83**

** Correlation is significant at 0.01 level, * Correlation is significant at 0.05 level

FLL = Flower length, FW = Flower width, SW = Stalk width, SL = Style length, SYD = Style diameter, SGD = Stigma diameter, SGL = Stigma length, OD = Ovary diameter, OL = Ovary length, OA = Ovary area, NS = Number of seeds, SEW = Seed weight, LN = Locule number per fruit, FRL = Fruit length, FD = Fruit width.

The direct and indirect effect of floral and fruit size related characters on the fruit size of the tomato

The results of the path coefficient analysis of the 15 floral and fruit size related characters are presented in Table 36. The results showed that the locule number had the highest positive direct effect (p = 0.8086) on fruit size. Similarly, ovary diameter exhibit the positive direct effect (p = 0.7942) on fruit size. On the other hand the highest negative direct effect was indicated by the style length (p = -0.9147). The highest indirect effect was exhibited by the stigma diameter and the style diameter. The residual effect was 0.0001833. Plate 5, display the relationship between the locule number and fruit size among the tomato parents and hybrids generated in this work.

Table 36: Partitioning the phenotypic correlation into direct (bold) and indirect effect of the fruit size components

	FLL	FW	SW	SL	SGL	OD	OL	OA	NS	SEW	LN	rg
FLL	**0.3774**	-0.0880	-0.1342	-0.8905	0.0243	-0.6221	-0.0522	2.2247	0.0166	0.0709	-0.6295	-0.81**
FW	-0.2922	**0.1136**	0.1634	0.7971	-0.0084	0.7130	0.0617	-2.4749	-0.0145	-0.1380	0.7303	0.91**
SW	-0.2935	0.1076	**0.1726**	0.8076	-0.0283	0.7059	0.0638	-2.5156	-0.0087	-0.1122	0.7453	0.92**
SL	0.3674	-0.0990	-0.1524	**-0.9147**	0.0193	-0.6893	-0.0582	2.4523	0.0166	0.0910	-0.6885	-0.88**
SYD	-0.3028	0.0962	0.1535	0.7995	-0.0021	0.7767	0.0597	-2.7429	-0.0211	-0.0805	0.7681	0.95**
SGD	-0.2968	0.0969	0.1525	0.7891	-0.0019	0.7797	0.0607	-2.7509	-0.0224	-0.0927	0.7685	0.95**
SGL	-0.0691	0.0072	0.0368	0.1330	**-0.1328**	-0.0527	0.0163	0.0688	0.0237	-0.0264	0.0949	0.117
OD	-0.2956	0.1020	0.1534	0.7938	0.0088	**0.7942**	0.0595	-2.7415	-0.0218	-0.1016	0.7718	0.97**
OL	-0.2953	0.1051	0.1649	0.7972	-0.0324	0.7079	**0.0667**	-2.5548	-0.0160	-0.1318	0.7420	0.92**
OA	-0.3042	0.1016	0.1568	0.8102	0.0033	0.7864	0.0616	**-2.7687**	-0.0224	-0.0976	0.7707	0.95**
NS	0.1043	-0.0273	-0.0249	-0.2524	-0.0522	-0.2872	-0.0177	1.0283	**0.0602**	0.0006	-0.1696	-0.24
SEW	-0.1170	0.0686	0.0847	0.3642	-0.0153	0.3530	0.0385	-1.1818	-0.0001	**-0.2287**	0.3980	0.50*
LN	-0.2938	0.1026	0.1591	0.7788	-0.0156	0.7580	0.0612	-2.6390	-0.0126	-0.1125	**0.8086**	0.98**

R = 0.0001833, ** Correlation is significant at 0.01 level, * Correlation is significant at 0.05 level.

FLL = Flower length, FW = Flower width, SW = Stalk width, SL = Style length, SYD = Style diameter, SGD = Stigma diameter, SGL = Stigma length, OD = Ovary diameter, OL = Ovary length, OA = Ovary area, NS = Number of seeds, SEW = Seed weight, LN = Locule number per fruit, R = Residual effect.

Correlation matrix on the floral and fruit shape related characteristics among tomato varieties

The results of the correlation coefficient analysis are presented in Tables 37 ï 38. Table 37 contain information on the fruit size and morphological related characteristics such as diameter, length, area, perimeter and shape index of the ovary, diameter, length, area, perimeter and shape index of seed and diameter, length and shape index of the fruits. The results showed variations among the tomato lines in all the shape index related characters studied.

The correlation studies on the 13 floral and fruit shape related traits which include: diameter, length, area, perimeter and shape index of the ovary, diameter, length, area, perimeter and shape index of seed and diameter, length and shape index of the fruits showed significant ($P<0.01$) correlation with the fruit shape index. The result further revealed that the fruit shape index had significant positive correlation with the ovary shape index ($r = 0.835^{**}$) and seed shape index ($r = 0.718^{**}$). However, fruit shape index was negatively and significantly correlated with ovary diameter ($r = -0.601^{*}$), fruit diameter ($r = -0.576^{*}$) and seed diameter ($r = -0.519^{*}$).

The seed shape index was positive and significantly correlated with the ovary shape index ($r = 0.785^{**}$) and the style length ($r = 0.718^{**}$). On the other hand, seed shape index was negative and significantly correlated with seed perimeter. The ovary shape index was negative and significantly correlated with the ovary diameter ($r = -0.715^{**}$), seed diameter ($r = -0.67^{**}$), ovary area ($r = -0.628^{*}$) and fruit diameter ($r = -0.622^{*}$).

Table 37: Mean values of the fruit morphological related characteristics in tomato varieties used for the study

Tomato varieties/hybrids	OD	OL	OA	OP	SD	SL	SA	SP	FL	FD	OSI	SSI	FSI
W	0.0629	0.0793	0.0439	0.2767	0.1020	0.1232	0.0102	0.3931	0.9113	0.8600	1.26129	1.20806	1.05962
R	0.0765	0.1121	0.0686	0.3614	0.1368	0.2147	0.0217	0.5763	3.4800	2.2300	1.46583	1.56882	1.56054
W x R	0.0836	0.1008	0.0776	0.3478	0.1456	0.1729	0.0209	0.5620	2.0409	1.4864	1.20527	1.18779	1.37309
R x W	0.0819	0.0947	0.0701	0.3402	0.1548	0.1612	0.0206	0.5490	2.0400	1.7300	1.15575	1.04134	1.17919
W x T	0.0824	0.0958	0.0717	0.3440	0.1580	0.1748	0.0225	0.5718	2.2700	1.6600	1.16198	1.10601	1.36747
S	0.2434	0.1883	0.4407	0.8097	0.2176	0.1737	0.0314	0.6908	3.4700	5.3400	0.77348	0.79842	0.64981
BF	0.1102	0.1222	0.1209	0.4436	0.1978	0.1607	0.0264	0.6293	3.5700	3.3340	1.10865	0.81250	1.07079
PR	0.0963	0.1253	0.1249	0.4388	0.1327	0.2259	0.0259	0.6280	3.1400	2.4500	1.30096	1.70234	1.28163
S x (W x R)	0.1009	0.1059	0.0982	0.3949	0.1753	0.1752	0.0253	0.6053	2.9150	2.6400	1.05021	0.99937	1.10417
PR x (R x W)	0.0862	0.1178	0.0917	0.3956	0.1395	0.2215	0.0238	0.6213	3.2000	1.6889	1.36576	1.58781	1.89474
PR x (W x R)	0.0935	0.1185	0.1048	0.4116	0.1597	0.2113	0.0257	0.6272	3.3000	2.0800	1.26699	1.32320	1.58654
PR x (W x T)	0.0847	0.1275	0.1044	0.4157	0.1581	0.1988	0.0245	0.6104	2.8300	1.6700	1.50494	1.25692	1.69461
BF x (W x T)	0.1007	0.1055	0.0975	0.3922	0.1803	0.1595	0.0247	0.6057	2.1400	2.0900	1.04783	0.88447	1.02392

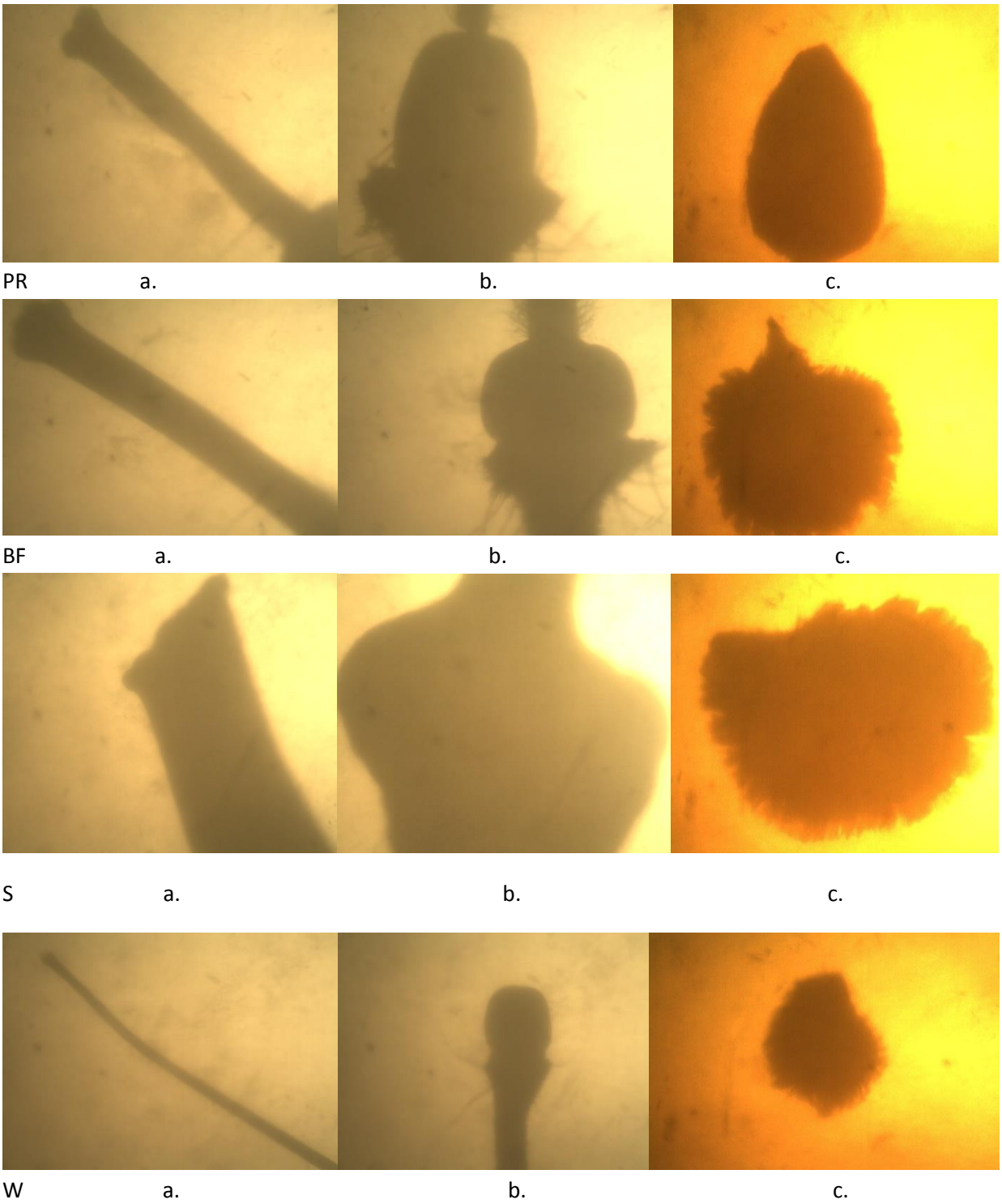
OD = Ovary diameter, OL = Ovary length, OA = Ovary area, OP = Ovary perimeter, SD = Seed diameter, SL= Seed length, SA = Seed area, SP = Seed perimeter, FL = Fruit length, FD = Fruit diameter, OSI = Ovary shape index, SSI = Seed shape index, FSI = Fruit shape index. W x R = Wild x Roma; R x W = Roma x Wild; W x T = Wild x Tropica; S = Supersteak; **BF** = Beef (florida); **PR** = Plumb (Rio grande)

Table 38: Correlation coefficient analyses between fruit morphological related characteristics among tomato varieties

	OD	OL	OA	OP	SD	SL	SA	SP	FL	FD	OSI	SSI	FSI
OD	**1**												
OL	0.898**	**1**											
OA	0.990**	0.928**	**1**										
OP	0.978**	0.968**	0.988**	**1**									
SD	0.745**	0.658**	0.680**	0.721**	**1**								
SL	-0.014	0.346	0.053	0.158	-0.027	**1**							
SA	0.650**	0.762**	0.635*	0.723**	0.818**	0.528*	**1**						
SP	0.598*	0.759**	0.591*	0.691**	0.755**	0.617*	0.988**	**1**					
FL	0.447	0.710**	0.459	0.578*	0.511	0.694**	0.777**	0.827**	**1**				
FD	0.927**	0.891**	0.908**	0.936**	0.807**	0.115	0.758**	0.711**	0.666**	**1**			
OSI	-0.715**	-0.361	-0.628*	-0.569*	-0.67**	0.497	-0.344	-0.241	0.042	-0.622*	**1**		
SSI	-0.457	-0.145	-0.363	-0.318	-0.70**	0.718**	-0.183	-0.077	0.171	-0.403	0.785**	**1**	
FSL	-0.601*	-0.275	-0.544*	-0.474	-0.52*	0.597	-0.166	-0.038	0.161	-0.576*	0.835**	0.718**	**1**

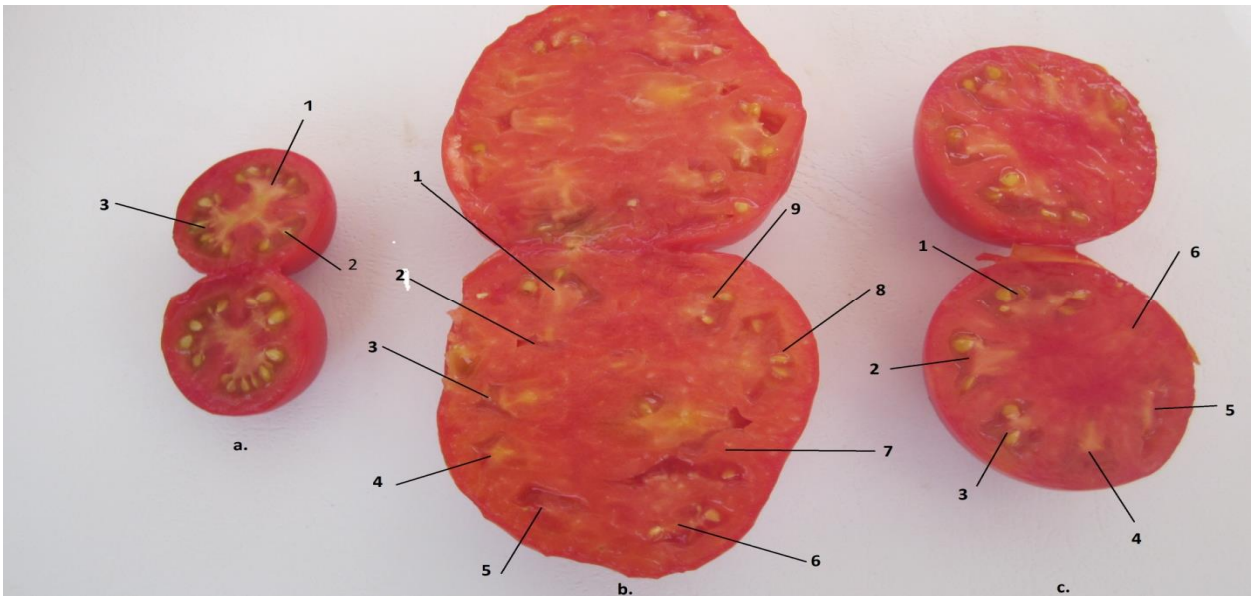
** Correlation is significant at 0.01 level, * Correlation is significant at 0.05 level

OD = Ovary diameter, OL = Ovary length, OA = Ovary area, OP = Ovary perimeter, SD = Seed diameter, SL= Seed length, SA = Seed area, SP = Seed perimeter, FL = Fruit length, FD = Fruit diameter, OSI = Ovary shape index, SSI = Seed shape index, FSI = Fruit shape index.



BF = Beef (florida); **PR** = Plumb (Rio grande) , S = Supersteak and W = Wild tomato

Plate 4: Tomato parents used in this work showing the relationship in size and shape, a. style and stigma, b. ovary and c. seed.



d.

e.

f.

g.

h.

i.

Plate 5. Fruit morphology showing locule number for tomato parents, a. W x R b. S and tomato F_3 hybrid c. 6 locules d. 3 locules e.5 locules f. 6 locules g. 7 locules h. 8 locules and i. 9 locule

Quality and Concentration of Extracted DNA

The CTAB extraction method employed for DNA extraction produced a wide range of DNA concentration and quality. However, most of the DNA found to have higher concentration of PCR inhibitors as the result of the extraction method used. Most of the sample DNA bands were clear without protein and polysaccharide contamination. A260/A280 values ranging between 1.28 to 2.14 (Table 39). The final concentration of DNA was high. Figure 1 displays the gel electrophoresis pictures of some extracted DNA samples.

Table 39. DNA concentration and Optical Density reading of parents and progenies used in this study

S/N	Parent/Hybrid code	Population	Concentration (ng/µl)	260/280
1	S x (W x R)	F3	969.2	1.5
2	S x (W x R)	F3	1302.4	1.67
3	S x (W x R)	F3	1364	1.44
4	S x (W x R)	F3	1012.4	1.68
5	S x (W x R)	F3	1030.5	1.52
6	S x (W x R)	F3	856.2	1.61
7	S x (W x R)	F3	860.3	1.65
8	S x (W x R)	F3	985.7	1.47
9	S x (W x R)	F3	1045.7	1.65
10	S x (W x R)	F3	853.9	1.37
11	S x (W x R)	F3	1293.4	1.54
12	S x (W x R)	F3	216.9	1.51
13	S x (W x R)	F3	834.2	1.71
14	S x (W x R)	F3	764.3	1.7
15	S x (W x R)	F3	1374.9	1.64
16	S x (W x R)	F3	2466	1.7
17	S x (W x R)	F3	1245	1.65
18	S x (W x R)	F3	2330.6	1.73
19	S x (W x R)	F3	2432.2	1.63
20	S x (W x R)	F3	2685.2	1.64
21	S x (W x R)	F3	766.5	1.73
22	S x (W x R)	F3	1024.6	1.7
23	S x (W x R)	F3	1732	1.68
24	S x (W x R)	F3	2038.7	1.75
25	S x (W x R)	F3	1607	1.61
26	S x (W x R)	F3	1486.6	1.52
27	S x (W x R)	F3	2309.8	1.86
28	S x (W x R)	F3	2389.9	1.92
29	S x (W x R)	F3	2654.2	1.84
30	S x (W x R)	F3	809.1	1.69

S/N	Parent/Hybrid code	Population	Concentration (ng/µl)	260/280
31	S x (W x R)	F3	952	2.01
32	S x (W x R)	F3	870.4	1.63
33	S x (W x R)	F3	659.9	1.95
34	S x (W x R)	F3	663.4	1.92
35	S x (W x R)	F3	533.3	1.59
36	S x (W x R)	F3	984.8	1.81
37	S x (W x R)	F3	985.1	1.8
38	S x (W x R)	F3	952.1	1.71
39	S x (W x R)	F3	902.6	1.74
40	S x (W x R)	F3	718.3	1.86
41	S x (W x R)	F3	505.8	1.58
42	S x (W x R)	F3	928.6	1.75
43	S x (W x R)	F3	943.7	1.51
44	S x (W x R)	F3	1762.1	1.33
45	S x (W x R)	F3	1508.7	1.45
46	S x (W x R)	F3	928.5	1.45
47	S x (W x R)	F3	216.9	1.3
48	S x (W x R)	F3	789.6	1.28
49	S x (W x R)	F3	1347.7	1.32
50	S x (W x R)	F3	1594.1	1.29
51	S x (W x R)	F3	720.2	1.72
52	W	P1	42.4	2.14
53	W x R	P4	35.4	1.3
54	S	P3	117.7	1.77
55	S x (W x R)	F3	446.8	1.27
56	S x (W x R)	F3	81.6	1.3
57	S x (W x R)	F3	47.4	1.61
58	S x (W x R)	F3	602.2	1.59
59	S x (W x R)	F3	39.4	1.36
60	S x (W x R)	F3	4698.5	1.98
61	S x (W x R)	F3	3616.4	2
62	S x (W x R)	F3	2899.3	2
63	S x (W x R)	F3	2806.2	1.98
64	S x (W x R)	F3	4994.3	2
65	S x (W x R)	F3	7016.3	1.97

S/N	Parent/Hybrid code	Population	Concentration (ng/µl)	260/280
67	S x (W x R)	F3	12033.5	2.01
68	S x (W x R)	F3	5057.5	1.96
69	S x (W x R)	F3	6408.6	1.98
70	S x (W x R)	F3	17310	1.98
71	S x (W x R)	F3	7258.2	2.02
72	S x (W x R)	F3	6586	2.05
73	S x (W x R)	F3	7800	1.94
74	S x (W x R)	F3	5875.6	1.98
75	S x (W x R)	F3	5222.5	2
76	S x (W x R)	F3	12320.4	1.95
77	S x (W x R)	F3	8527.8	2.02
78	S x (W x R)	F3	11537.8	2.03
79	S x (W x R)	F3	10741.7	2.03
80	S x (W x R)	F3	13620.1	1.95
81	S x (W x R)	F3	7333.2	2.02
82	S x (W x R)	F3	7469.4	1.99
83	S x (W x R)	F3	6888.3	2.03
84	S x (W x R)	F3	11457.5	2.05
85	S x (W x R)	F3	9325.1	1.96
86	S x (W x R)	F3	7597.7	2.06
87	S x (W x R)	F3	7198.8	2.07
88	S x (W x R)	F3	13220.2	2.07
89	S x (W x R)	F3	6492.7	2.04
90	S x (W x R)	F3	14652.9	2.01
91	S x (W x R)	F3	9305.7	1.99
92	S x (W x R)	F3	14532.9	2.01

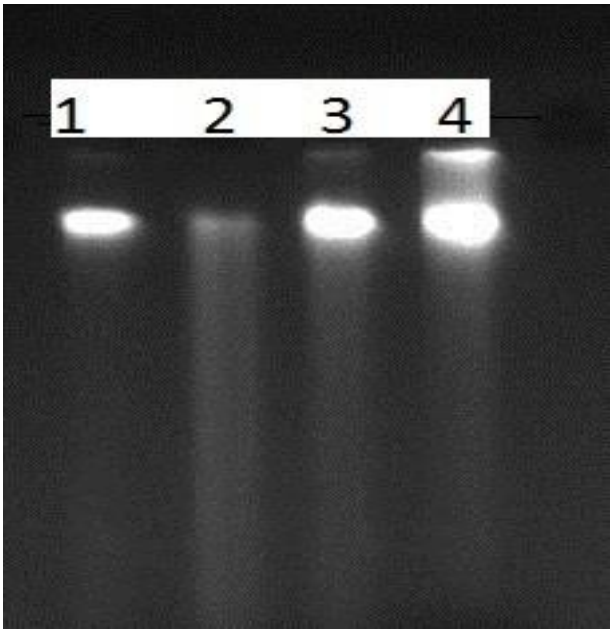


Figure 1: **Gel electrophoresis picture of DNA samples extracted from the parents used in the study. 1 and 2 = W x R, 3 and 4 = S**

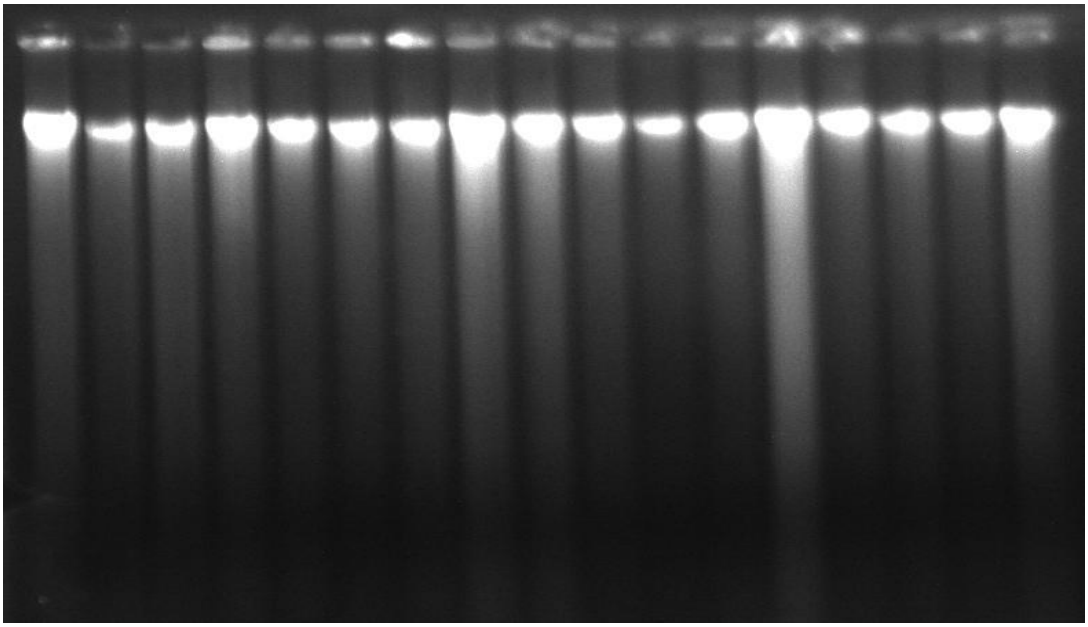


Figure 2. **Gel electrophoresis picture of DNA samples extracted from the tomato F_3 hybrids (S x (W x R) used in the study**

Single Nucleotide Polymorphisms (SNPs) diversity

Basically SNPs markers used for this works were selected randomly from specific chromosomes related to the fruit size, such as chromosome 2, 3, 4 and 11. A total of 45 SNPs were selected and used for both parents and their progenies. Out of 45 SNPs markers, 25 markers showed high level of polymorphism and good reproducibility (Table 40). The remaining 20 markers were monomorphic and discarded.

The results from 25 SNPs markers used for this work on tomato fruit size detected appreciable degree of polymorphism within the set of tomato progenies used for this work. A total of 50 SNPs alleles were detected and the total number of allele detected per primer was 2. Most of the loci produced a maximum of two rare alleles. Some of the alleles may be useful as a diagnostic markers for some of the assayed tomato progenies.

Most loci were highly polymorphic as indicated by values for polymorphism information content (PIC), expected heterozygosity or gene diversity (H_e) and observed heterozygosis' (H_o). The PIC value for each marker ranged from 0.0487 for the marker detected by Solyc2 - 2 to 0.3749 for the marker detected by Solyc4 - 1. The marker PIC value greater than 0.5 were considered highly informative and markers with 0.5 >PIC>0.2 were just considered to be informative (Bostein *et al*., 1980). However, because of the bi allelic nature of the SNPs markers, the maximum PIC value is only 0.5 while SSRs markers can go beyond 0.5. The variation was significant associated with the number of alleles detected at each locus, therefore SNPs markers showed a reasonable amount of variation in the tomato genotypes in this study.

The expected heterozygosity or gene diversity (He) value per marker ranged from 0.05 at Solyc2 - 2 to 0.499 at Solyc4-1 with the mean value of 0.266. The observed heterozygosity were ranged from 0.05 at Solyc2-2 to 0.372 at Solyc4-1. Overall the expected heterozygosities were higher than the observed heterozygosities. The major alleles frequency for each marker ranged from 0.51 to 0.97, therefore the minor allele were less than 0.5 in most of the markers.

Table 40: Allelic variation of 25 SNPs loci in the tomato hybrids

Marker	MAF	G	A^o	Availability	He	Ho	PIC
Solyc04 - 1	0.5116	3.0000	2.0000	0.7167	0.4997	0.3721	0.3749
Solyc11 - 1	0.9375	3.0000	2.0000	0.9333	0.1172	0.0893	0.1103
Solyc11 - 3	0.9375	3.0000	2.0000	0.9333	0.1172	0.0893	0.1103
Solyc11 - 7	0.9167	2.0000	2.0000	0.5000	0.1528	0.1667	0.1411
Solyc11- 8	0.9286	2.0000	2.0000	0.5833	0.1327	0.1429	0.1239
Solyc11 - 9	0.8750	3.0000	2.0000	0.8667	0.2188	0.2115	0.1948
Solyc11 -10	0.8729	3.0000	2.0000	0.9833	0.2219	0.2203	0.1973
Solyc11 - 11	0.8878	2.0000	2.0000	0.8167	0.1993	0.2245	0.1794
Solyc11 - 12	0.6098	3.0000	2.0000	0.6833	0.4759	0.5366	0.3627
Solyc11 - 13	0.7045	3.0000	2.0000	0.7333	0.4163	0.3182	0.3297
Solyc11 - 14	0.7586	3.0000	2.0000	0.9667	0.3662	0.2759	0.2992
Solyc11 - 15	0.8298	3.0000	2.0000	0.7833	0.2825	0.2553	0.2426
Solyc11 - 16	0.7128	3.0000	2.0000	0.7833	0.4095	0.3191	0.3256
Solyc11 - 17	0.7364	3.0000	2.0000	0.9167	0.3883	0.3091	0.3129
Solyc11 - 19	0.7586	3.0000	2.0000	0.9667	0.3662	0.2759	0.2992
Solyc11 - 21	0.7800	3.0000	2.0000	0.8333	0.3432	0.2400	0.2843
Solyc02 - 1	0.5488	3.0000	2.0000	0.6833	0.4952	0.3171	0.3726
Solyc02 - 2	0.9744	2.0000	2.0000	0.6500	0.0500	0.0513	0.0487
Solyc11 - 2	0.9500	2.0000	2.0000	0.6667	0.0950	0.1000	0.0905
Solyc11 - 4	0.9386	2.0000	2.0000	0.9500	0.1153	0.1228	0.1086
Solyc11 - 5	0.9286	3.0000	2.0000	0.9333	0.1327	0.1071	0.1239
Solyc11 - 6	0.8600	2.0000	2.0000	0.8333	0.2408	0.2800	0.2118
Solyc11- 18	0.8191	2.0000	2.0000	0.7833	0.2963	0.3617	0.2524
Solyc11 -20	0.8491	2.0000	2.0000	0.8833	0.2563	0.3019	0.2235
Solyc11- 22	0.8426	2.0000	2.0000	0.9000	0.2653	0.3148	0.2301
Mean	**0.8188**	**2.6000**	**2.0000**	**0.8113**	**0.2662**	**0.2401**	**0.2220**

MAF = Major allele frequency, G = Genotype number, A^o = Number of allele, He = Expected heterozygosity/gene diversity, Ho = Observed heterozygosity, PIC = Polymorphism information content (PIC)

The genetic distance among the tomato progenies in this study

Based on the information obtained at the 25 SNPs loci, Nei genetic distance coefficients were estimated for all pair-wise comparison of the tomato genotypes developed from modified three way cross between advanced generation tomato hybrids and Supersteak.

The average distance between individual tomato progenies was moderate 0.56. The genetic distance among the F_2 tomato genotypes ranged from 0.0 to 0.6174 (Table 41) while for F_3 ranged from 0.000 to 0.6154 (Table 42). This value is an indication of the magnitude of diversity among the progenies studied. However, some tomato genotypes in both F_2 and F_3 showed 100% similarities.

The largest genetic distance was observed between S28 and S25 (0.6174) while the lowest one was detected between S33 and S30, S33 and S31 (0.000) for the F_2 tomato genotypes. For the F_3 tomato genotypes, the largest distance was observed between S63 and S55 (0.6154) while the lowest was detected between S64 and S55 (0.000).

Table 41: Nei (1984) Pair - wise genetic distance between F_3 tomato genotypes calculated from SNPs analysis

	S21	S22	S24	S25	S26	S27	S28	S30	S31	S32	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42	S44	S46	S48	S49
S21																								
S22	0.1464																							
S24	0.0761	0.1964																						
S25	0.3260	0.1757	0.5811																					
S26	0.0714	0.2345	0.0139	0.6227																				
S27	0.2139	0.0439	0.2472	0.2089	0.2404																			
S28	0.0345	0.1813	0.0255	0.6174	0.0146	0.1953																		
S30	0.1577	0.0325	0.1696	0.1757	0.1895	0.0325	0.1850																	
S31	0.2028	0.0325	0.1953	0.1883	0.2115	0.0462	0.2050	0.0000																
S32	0.0923	0.1760	0.0462	0.4913	0.0517	0.1760	0.0732	0.1139	0.1551															
S33	0.1953	0.0146	0.1783	0.1648	0.2050	0.0509	0.1910	0.0000	0.0000	0.1387														
S34	0.2483	0.0293	0.2038	0.1831	0.2197	0.0382	0.2165	0.0000	0.0000	0.1542	0.0127													
S35	0.2311	0.0279	0.1953	0.1723	0.2092	0.0255	0.1953	0.0146	0.0154	0.1464	0.0127	0.0000												
S36	0.0718	0.1864	0.0366	0.5811	0.0139	0.1831	0.0117	0.1757	0.2050	0.0697	0.1953	0.1953	0.1757											
S37	0.0761	0.1953	0.0382	0.6174	0.0146	0.1831	0.0117	0.1850	0.2050	0.0732	0.2038	0.2038	0.1831	0.0000										
S38	0.2899	0.0616	0.1910	0.2273	0.2050	0.0661	0.2109	0.0308	0.0293	0.1464	0.0255	0.0127	0.0122	0.1992	0.1992									
S39	0.0000	0.1895	0.0616	0.5991	0.0500	0.1953	0.0139	0.2067	0.2115	0.1105	0.2197	0.2343	0.2232	0.0139	0.0139	0.2568								
S40	0.0588	0.1696	0.0139	0.5418	0.0000	0.1813	0.0133	0.1723	0.2067	0.0617	0.1953	0.1953	0.1731	0.0000	0.0000	0.1864	0.0000							
S41	0.0345	0.1757	0.0133	0.5919	0.0154	0.2038	0.0000	0.1790	0.2004	0.0732	0.1864	0.2130	0.1910	0.0122	0.0122	0.2075	0.0146	0.0133						
S42	0.0714	0.2311	0.0000	0.6174	0.0146	0.2568	0.0279	0.1895	0.1953	0.0517	0.2050	0.2197	0.2092	0.0279	0.0279	0.1953	0.0616	0.0000	0.0146					
S44	0.1953	0.0451	0.2014	0.1577	0.1953	0.0183	0.1895	0.0000	0.0000	0.1367	0.0000	0.0000	0.0172	0.1895	0.1895	0.0345	0.2014	0.2014	0.1895	0.2014				
S46	0.1723	0.0154	0.2185	0.1367	0.2376	0.0139	0.1997	0.0163	0.0172	0.1605	0.0133	0.0279	0.0382	0.2038	0.2130	0.0721	0.2004	0.1953	0.1997	0.2530	0.0183			
S48	0.1895	0.0146	0.2247	0.1723	0.2568	0.0255	0.2038	0.0308	0.0163	0.1914	0.0255	0.0382	0.0366	0.2197	0.2165	0.0562	0.2092	0.2092	0.1997	0.2429	0.0183	0.0133		
S49	0.1883	0.0000	0.2404	0.1562	0.2530	0.0146	0.2197	0.0183	0.0163	0.2089	0.0154	0.0154	0.0146	0.2197	0.2197	0.0500	0.2050	0.2067	0.2158	0.2404	0.0183	0.0000	0.0000	
S51	0.0588	0.1542	0.0279	0.4913	0.0163	0.1674	0.0266	0.1627	0.2067	0.0462	0.1813	0.1813	0.1598	0.0127	0.0133	0.1731	0.0163	0.0133	0.0266	0.0163	0.1953	0.1813	0.1953	0.2067

Table 42: Nei (1984) Pair - wise genetic distance between F_3 tomato genotypes calculated from SNPs analysis

	S55	S56	S57	S58	S59	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71	S72	S73	S74	S75	S77	S78	S79	S81	S82	S83	S84	S85	S86
S55																														
S56	0.1065																													
S57	0.0000	0.1760																												
S58	0.0293	0.1252	0.0146																											
S59	0.4615	0.1281	0.4086	0.2165																										
S60	0.5000	0.1378	0.5180	0.3232	0.0172																									
S61	0.5066	0.1857	0.4062	0.2440	0.0439	0.1143																								
S62	0.5066	0.1794	0.3833	0.2413	0.0488	0.1206	0.0000																							
S63	0.6154	0.2806	0.4804	0.3338	0.1231	0.1887	0.0255	0.0279																						
S64	0.0000	0.1281	0.0133	0.0308	0.3458	0.4540	0.3636	0.3672	0.4525																					
S65	0.0418	0.1723	0.0399	0.0651	0.4391	0.5135	0.3833	0.3879	0.4318	0.0279																				
S66	0.0488	0.1970	0.0617	0.0732	0.3731	0.4563	0.3199	0.3232	0.3338	0.0517	0.0172																			
S67	0.0000	0.1378	0.0588	0.0881	0.4391	0.4827	0.4525	0.4609	0.4879	0.0146	0.0279	0.0761																		
S68	0.1352	0.0000	0.1760	0.1357	0.1206	0.1378	0.1857	0.1857	0.2806	0.1378	0.1895	0.2346	0.1551																	
S69	0.0000	0.1233	0.0400	0.0646	0.4031	0.4704	0.4432	0.4247	0.5143	0.0133	0.0399	0.1086	0.0133	0.1233																
S70	0.0000	0.1302	0.0000	0.0154	0.4147	0.4939	0.4247	0.4025	0.5023	0.0000	0.0279	0.0651	0.0139	0.1302	0.0000															
S71	0.0000	0.1233	0.0400	0.0646	0.4086	0.4704	0.4497	0.4310	0.5239	0.0133	0.0399	0.1143	0.0133	0.1233	0.0000	0.0000														
S72	0.0000	0.1233	0.0400	0.0646	0.4086	0.4704	0.4497	0.4310	0.5239	0.0133	0.0399	0.1143	0.0133	0.1233	0.0000	0.0000	0.0000													
S73	0.0000	0.1974	0.0000	0.0000	0.3792	0.4899	0.3607	0.3660	0.4322	0.0209	0.0418	0.0488	0.0923	0.2240	0.0667	0.0000	0.0667	0.0667												
S74	0.0000	0.1387	0.0117	0.0293	0.4228	0.4844	0.4432	0.4247	0.5143	0.0000	0.0266	0.0879	0.0266	0.1387	0.0225	0.0000	0.0117	0.0117	0.0195											
S75	0.0000	0.1172	0.0195	0.0000	0.4203	0.5291	0.4424	0.4654	0.5367	0.0000	0.0418	0.0451	0.0000	0.1757	0.0183	0.0195	0.0195	0.0195	0.0000	0.0366										
S77	0.0000	0.1281	0.0139	0.0183	0.4391	0.5095	0.4252	0.4391	0.5172	0.0000	0.0293	0.0345	0.0000	0.1562	0.0133	0.0139	0.0139	0.0139	0.0000	0.0266	0.0000									
S78	0.1953	0.0861	0.2232	0.1953	0.1627	0.1953	0.1116	0.1172	0.1395	0.2004	0.1757	0.1551	0.2050	0.0689	0.2130	0.2197	0.2232	0.2232	0.2511	0.2130	0.2301	0.2050								
S79	0.0000	0.1953	0.0000	0.0000	0.6098	0.6982	0.5464	0.5746	0.5464	0.0000	0.0000	0.0366	0.0000	0.2441	0.0266	0.0000	0.0000	0.0000	0.0000	0.0000	0.0266	0.0266	0.2636							
S81	0.0000	0.1302	0.0225	0.0000	0.3792	0.5344	0.4110	0.4325	0.5198	0.0000	0.0488	0.0799	0.0000	0.1831	0.0418	0.0225	0.0225	0.0225	0.0000	0.0923	0.0209	0.0209	0.2685	0.0909						
S82	0.0000	0.1139	0.0562	0.0718	0.3929	0.4586	0.4380	0.4525	0.5156	0.0146	0.0418	0.1143	0.0139	0.1378	0.0244	0.0133	0.0127	0.0127	0.0714	0.0661	0.0183	0.0133	0.2263	0.0909	0.0000					
S83	0.2197	0.1302	0.1953	0.1627	0.3064	0.2757	0.3030	0.3030	0.4209	0.1953	0.2441	0.2929	0.2130	0.1302	0.2028	0.1953	0.1953	0.1953	0.1831	0.1802	0.1953	0.2028	0.1709	0.1953	0.2507	0.2572				
S84	0.0000	0.1302	0.0417	0.0680	0.4586	0.4939	0.4932	0.4786	0.5674	0.0139	0.0418	0.1297	0.0139	0.1302	0.0117	0.0000	0.0000	0.0000	0.0714	0.0117	0.0366	0.0266	0.2263	0.0000	0.0923	0.0539	0.1802			
S85	0.0000	0.1302	0.0435	0.0718	0.4827	0.4939	0.5156	0.5025	0.5932	0.0146	0.0418	0.1297	0.0139	0.1378	0.0122	0.0000	0.0000	0.0000	0.0714	0.0122	0.0366	0.0266	0.2263	0.0000	0.0923	0.0539	0.1802	0.0000		
S86	0.0000	0.1206	0.0435	0.0680	0.3929	0.4673	0.4380	0.4449	0.5156	0.0139	0.0418	0.1044	0.0139	0.1302	0.0000	0.0000	0.0000	0.0000	0.0714	0.0244	0.0000	0.0000	0.2130	0.0266	0.0225	0.0127	0.2028	0.0122	0.0127	
S87	0.0000	0.1331	0.0183	0.0244	0.4408	0.5628	0.4589	0.4827	0.5473	0.0000	0.0391	0.0628	0.0000	0.1864	0.0345	0.0183	0.0183	0.0183	0.0000	0.0761	0.0183	0.0172	0.2538	0.0909	0.0000	0.0000	0.2542	0.0761	0.0761	0.0183

Genetic Diversity among tomato progenies using UPGMA based cluster analysis

Tomato progenies were separated using un-weighted pair-group mean algorithm (UPGMA) dendrogram (Sneath and Sokal, 1973) to describe their genetic relationship. The similarities among the tomato progenies hybrids reflected in the 25 SNPs alleles were estimated and grouped into four major groups. The first cluster consists of tomato progenies with large fruit size, the mean locule number per fruit greater than 5 and the fruit shape index of less than 1. This members of this clusters showed a direct relationship with a Supersteak, one of the parent used in this work.

Cluster 2 comprised of tomato progenies with locule number ranged from 2 to 3 and the fruit shape index around 1. This members of this clusters showed a direct relationship with the advanced generation hybrid that generated from wild tomato and roma VF. Cluster 3 comprised of tomato hybrid with locule number ranged between 3 to 5 and fruit shape index around 1. Apart from three major group, a number of hybrids were scattered and distributed across the cluster.

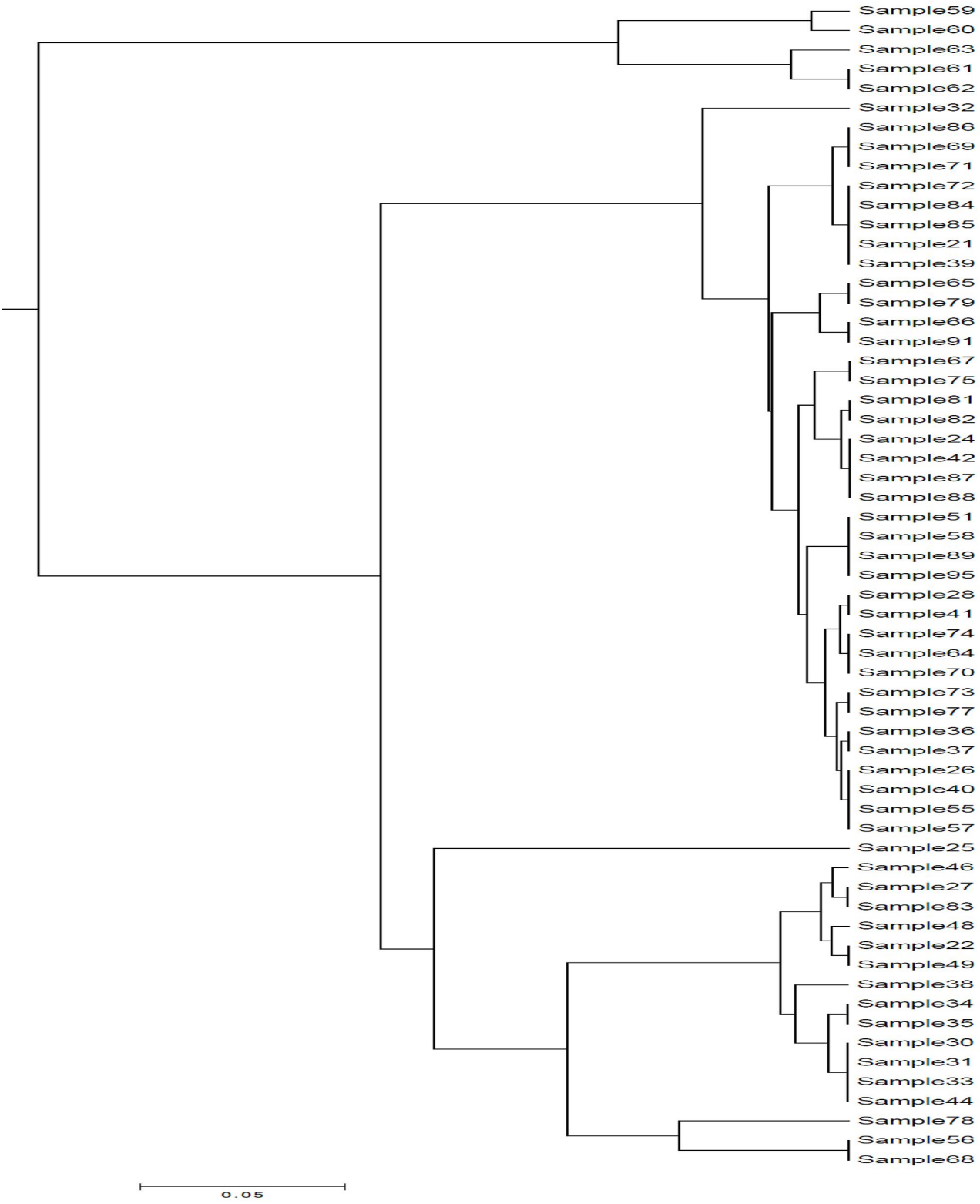


Figure 3: Dendrogram constructed using Nei similarity coefficient and UPGMA Clustering for the F_3 tomato genotypes

Population structure of the tomato progenies

Genotyping data generated using the 25 polymorphic SNP markers were used for genetic structure analysis using the Bayesian clustering model implemented in the structure software. DK was also calculated, and the result showed that DK reached the maximal value when K = 3. The model used indicated K = 3 is the best number of sub-population (hereafter referred to as Q = 3, providing support for the existence of the three distinct clusters in our association panel. The analysis of these data identified accessions into three subgroups as well, and the results were very similar to those of the clustering results (Figure 3). The Q matrix outputs of the three subpopulations were used for the association analysis.

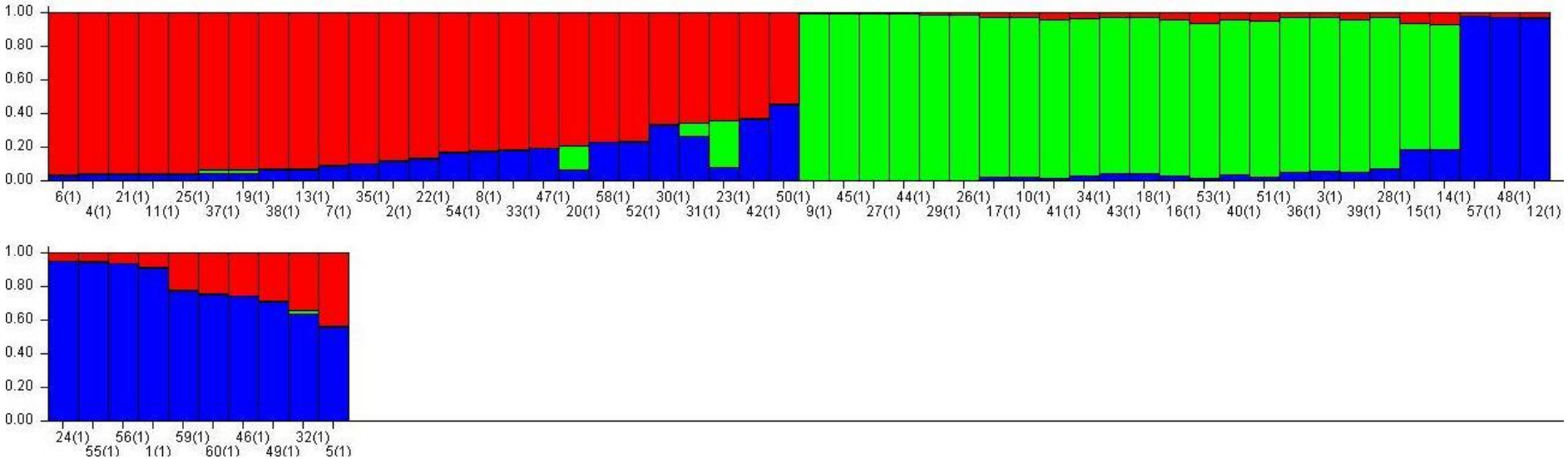


Figure 4. Each individual sample was represented by a single row broken into three-colored segments (red, green and blue), with length proportions to each of the two inferred population subgroups. Each individual corresponded to the samples in the dendrogram.

Association mapping between the fruit size QTL and the SNP markers

Association mapping or mapping analysis detects and locates QTLs based on the strength of the correlation between mapped SNPs markers and traits (Mackay and Powell, 2007). Of the 45 selected SNPs markers, 20 markers showed monomophorphism in all of the genotypes studied. These 20 markers were excluded from the association mapping analysis. Therefore, 25 SNPs were selected and used for the mapping analysis.

A total of 25 SNPs markers were used for this work and all of them were polymorphic. Association mapping results revealed that, one SNP marker, Solyc02 - 1 was significantly ($P < 0.05$) associated with mean fruit weight (Table 43). The results also indicated the location of SNPs marker on chromosome 2 at 72540 (Figure 5a). The marker explained 13% of the mean fruit weight variation.

SNP marker (Solyc11 - 21) mapping on chromosome 11 was significantly ($P < 0.01$) associated with fruit length. The marker explained approximately 89.3% of the fruit length variation. For the fruit width, the analysis revealed significant ($P < 0.01$) associations for SNPs markers, Solyc11-9, Solyc11 - 14, Solyc11 - 17 and Solyc11 - 21) located on chromosome 11. Each markers explained approximately 184%, 23%, 141.5% and 30.6%, respectively of the fruit width variation. The results are summarized in Table 44 and 45 and Figure 5b.

Only one significant SNP marker, Solyc11 - 17 was associated with the number of locules per fruit and explained 23% of locule number variation. Fruit shape index was significantly ($P < 0.05$) associated with two SNPs markers. The first marker was Solyc11-10 located on

chromosome 11 which explained about 14.4% of the fruit shape variation. The second marker, Solyc11-17 was also on chromosome 11 which explained 18.3% of the fruit shape variation. The results are presented in Tables 46 and 47 and Figure 6b.

The association mapping results confirm the interrelation among the fruit size related characters. For example SNP marker (Solyc11 -17) located on chromosome 11 was significantly association with both fruit width, locule number and fruit shape index. However, the fruit width variation explained by this marker was higher than locule number and fruit shape index (141.5%, 23% and 18.3%) respectively.

Table 43. SNPs Marker loci associated with single fruit weight and their explained phenotypic variation

Trait	Marker	Locus	Site	df	F	p	errordf	markerR2
SFW	Solyc02 - 1	2	72540	2	3.68445	0.03562*	39	0.13588
SFW	Solyc02 - 2	2	81500	1	0.88647	0.35328	37	0.01734
SFW	Solyc04 - 1	4	5310	2	0.34947	0.70749	40	0.12035
SFW	Solyc11 -1	11	11930	2	0.03243	0.96811	53	0.00127
SFW	Solyc11 -2	11	12060	1	0.07716	0.78287	38	0.00182
SFW	Solyc11 -3	11	12770	2	0.01822	0.98195	53	8.48E-04
SFW	Solyc11 -4	11	13290	1	0.38477	0.53788	54	0.00643
SFW	Solyc11 -5	11	13350	2	0.19135	0.82646	54	0.00705
SFW	Solyc11 -6	11	17070	1	0.49107	0.48722	47	0.00823
SFW	Solyc11 -7	11	17250	1	0.05408	0.81793	30	0.00126
SFW	Solyc11 -8	11	17470	1	2.66×10^{-4}	0.9871	33	1.59E-04
SFW	Solyc11 -9	11	18580	2	0.39887	0.67342	50	0.01368
SFW	Solyc11 -10	11	18690	2	0.01193	0.98815	56	4.96E-04
SFW	Solyc11 -11	11	18720	1	0.1692	0.68287	47	0.00308
SFW	Solyc11 -12	11	20040	2	0.15587	0.8563	38	0.00584
SFW	Solyc11 -13	11	20300	2	0.02008	0.98013	42	0.00725
SFW	Solyc11 -14	11	20720	2	0.22504	0.79928	55	0.03279
SFW	Solyc11 -15	11	20730	2	0.17209	0.84254	44	0.03156
SFW	Solyc11 -16	11	39650	2	0.08889	0.91513	45	0.01715
SFW	Solyc11 -17	11	39870	2	0.36529	0.69595	52	0.04807
SFW	Solyc11 -18	11	42440	1	0.35056	0.55705	45	0.01688
SFW	Solyc11 -19	11	43170	2	0.39386	0.67652	55	0.01414
SFW	Solyc11 -20	11	45480	1	0.75307	0.39001	50	0.014
SFW	Solyc11 -21	11	62000	2	1.01483	0.37118	47	0.28259
SFW	Solyc11 -22	11	62010	1	0.24127	0.62558	51	0.00454

***** Significant at P = 0.05

Table 44. SNPs Marker loci associated with fruit length and their explained phenotypic variation

Trait	Marker	Locus	Site	df	F	p	errordf	markerR2
FL	Solyc02 - 1	2	72540	2	0.35345	0.70505	36	0.02016
FL	Solyc02 - 2	2	81500	1	0.02963	0.86446	35	8.98E-04
FL	Solyc04 - 1	4	5310	2	0.46871	0.63017	36	0.02844
FL	Solyc11 -1	11	11930	2	-7.50×10^{-2}	NaN	49	0.00921
FL	Solyc11 -2	11	12060	1	0.21499	0.64623	34	0.00838
FL	Solyc11 -3	11	12770	2	0.43691	0.6488	49	0.03458
FL	Solyc11 -4	11	13290	1	3.49559	0.06791	50	0.07138
FL	Solyc11 -5	11	13350	2	1.27763	0.28861	50	0.04299
FL	Solyc11 -6	11	17070	1	-2.22×10^{-1}	NaN	43	0.03877
FL	Solyc11 -7	11	17250	1	0.03215	0.85927	27	0.03137
FL	Solyc11 -8	11	17470	1	0.8438	0.36645	31	0.03164
FL	Solyc11 -9	11	18580	2	2.57971	0.08805	46	0.42225
FL	Solyc11 -10	11	18690	2	2.70161	0.07752	52	0.04793
FL	Solyc11 -11	11	18720	1	3.07369	0.08743	43	0.11853
FL	Solyc11 -12	11	20040	2	0.68374	0.51268	34	0.09918
FL	Solyc11 -13	11	20300	2	1.18451	0.31857	38	0.2758
FL	Solyc11 -14	11	20720	2	2.24823	0.11707	51	0.11548
FL	Solyc11 -15	11	20730	2	-3.89×10^{-1}	NaN	41	0.01669
FL	Solyc11 -16	11	39650	2	1.48983	0.23898	41	0.07333
FL	Solyc11 -17	11	39870	2	-1.38×10^{-3}	NaN	48	0.1879
FL	Solyc11 -18	11	42440	1	-3.17×10^{-3}	NaN	41	0.50326
FL	Solyc11 -19	11	43170	2	0.20003	0.81942	51	0.0076
FL	Solyc11 -20	11	45480	1	-6.68×10^{-2}	NaN	47	0.0215
FL	Solyc11 -21	11	62000	2	14.14308	2.4×10^{-5}**	44	0.8925
FL	Solyc11 -22	11	62010	1	-1.31×10^{1}	NaN	48	5.81459

** Significant at P = 0.01

NaN = Missing information

Table 45. SNPs Marker loci associated with fruit width and their explained phenotypic variation

Trait	Marker	Locus	Site	df	F	p	errordf	markerR2
FW	Solyc02 - 1	2	72540	2	3.02256	0.06321	36	0.14614
FW	Solyc02 - 2	2	81500	1	1.49566	0.23056	35	0.03753
FW	Solyc04 - 1	4	5310	2	0.06986	0.93267	36	0.00729
FW	Solyc11 -1	11	11930	2	0.12193	0.88551	49	0.00249
FW	Solyc11 -2	11	12060	1	1.43×10^{-4}	0.99055	34	0.01842
FW	Solyc11 -3	11	12770	2	0.03245	0.9681	49	0.00486
FW	Solyc11 -4	11	13290	1	0.13898	0.71101	50	0.01356
FW	Solyc11 -5	11	13350	2	-3.77×10^{-2}	NaN	50	0.06892
FW	Solyc11 -6	11	17070	1	1.12929	0.29446	43	0.10069
FW	Solyc11 -7	11	17250	1	0.81489	0.37604	27	0.03985
FW	Solyc11 -8	11	17470	1	0.26791	0.60895	31	0.01786
FW	Solyc11 -9	11	18580	2	25.77063	5.65×10^{-8}**	46	1.84834
FW	Solyc11 -10	11	18690	2	0.90235	0.41253	52	0.38859
FW	Solyc11 -11	11	18720	1	0.18242	0.67165	43	0.00498
FW	Solyc11 -12	11	20040	2	0.47257	0.62811	34	0.02898
FW	Solyc11 -13	11	20300	2	0.41002	0.66697	38	0.19774
FW	Solyc11 -14	11	20720	2	5.50779	0.00717**	51	0.23256
FW	Solyc11 -15	11	20730	2	0.04664	0.95449	41	0.02913
FW	Solyc11 -16	11	39650	2	0.3501	0.70699	41	0.02541
FW	Solyc11 -17	11	39870	2	34.16942	1.31×10^{-9}**	48	14.15861
FW	Solyc11 -18	11	42440	1	0.0062	0.93767	41	0.01042
FW	Solyc11 -19	11	43170	2	1.24246	0.29818	51	0.07126
FW	Solyc11 -20	11	45480	1	0.01767	0.89487	47	0.15836
FW	Solyc11 -21	11	62000	2	40.39903	3.13×10^{-10}**	44	3.06444
FW	Solyc11 -22	11	62010	1	0.69871	0.40773	48	0.12958

** Significant at 0.01

NaN = Missing information

Table 46. SNPs Marker loci associated with locule number and their explained phenotypic variation

Trait	Marker	Locus	Site	df	F	p	errordf	markerR2
LN	Solyc02 - 1	2	72540	2	0.73812	0.48622	36	0.15802
LN	Solyc02 - 2	2	81500	1	1.03556	0.31673	35	0.04926
LN	Solyc04 - 1	4	5310	2	0.17621	0.83928	36	0.0782
LN	Solyc11 -1	11	11930	2	0.06051	0.94136	49	0.00586
LN	Solyc11 -2	11	12060	1	0.51615	0.47805	34	0.04149
LN	Solyc11 -3	11	12770	2	0.0443	0.95671	49	0.00355
LN	Solyc11 -4	11	13290	1	0.05963	0.80816	50	0.00352
LN	Solyc11 -5	11	13350	2	0.04374	0.95724	50	0.00494
LN	Solyc11 -6	11	17070	1	-3.04×10^{-1}	NaN	43	0.05578
LN	Solyc11 -7	11	17250	1	0.50268	0.48545	27	0.16869
LN	Solyc11 -8	11	17470	1	3.94×10^{-4}	0.98432	31	0.0465
LN	Solyc11 -9	11	18580	2	0.1731	0.84166	46	0.02261
LN	Solyc11 -10	11	18690	2	0.06257	0.93943	52	0.0462
LN	Solyc11 -11	11	18720	1	0.58681	0.44827	43	0.86242
LN	Solyc11 -12	11	20040	2	0.56095	0.57674	34	0.07653
LN	Solyc11 -13	11	20300	2	0.31334	0.73315	38	0.12661
LN	Solyc11 -14	11	20720	2	1.05746	0.35563	51	0.25385
LN	Solyc11 -15	11	20730	2	0.1713	0.84325	41	13.13178
LN	Solyc11 -16	11	39650	2	-1.59	NaN	41	284.60541
LN	Solyc11 -17	11	39870	2	14.88021	1.23×10^{-5}**	48	0.23024
LN	Solyc11 -18	11	42440	1	0.00184	0.96602	41	0.02205
LN	Solyc11 -19	11	43170	2	0.06147	0.94046	51	0.0135
LN	Solyc11 -20	11	45480	1	0.00348	0.95322	47	0.10562
LN	Solyc11 -21	11	62000	2	1.45417	0.24598	44	0.02973
LN	Solyc11 -22	11	62010	1	0.1288	0.72139	48	0.00801

** Significant at 0.01

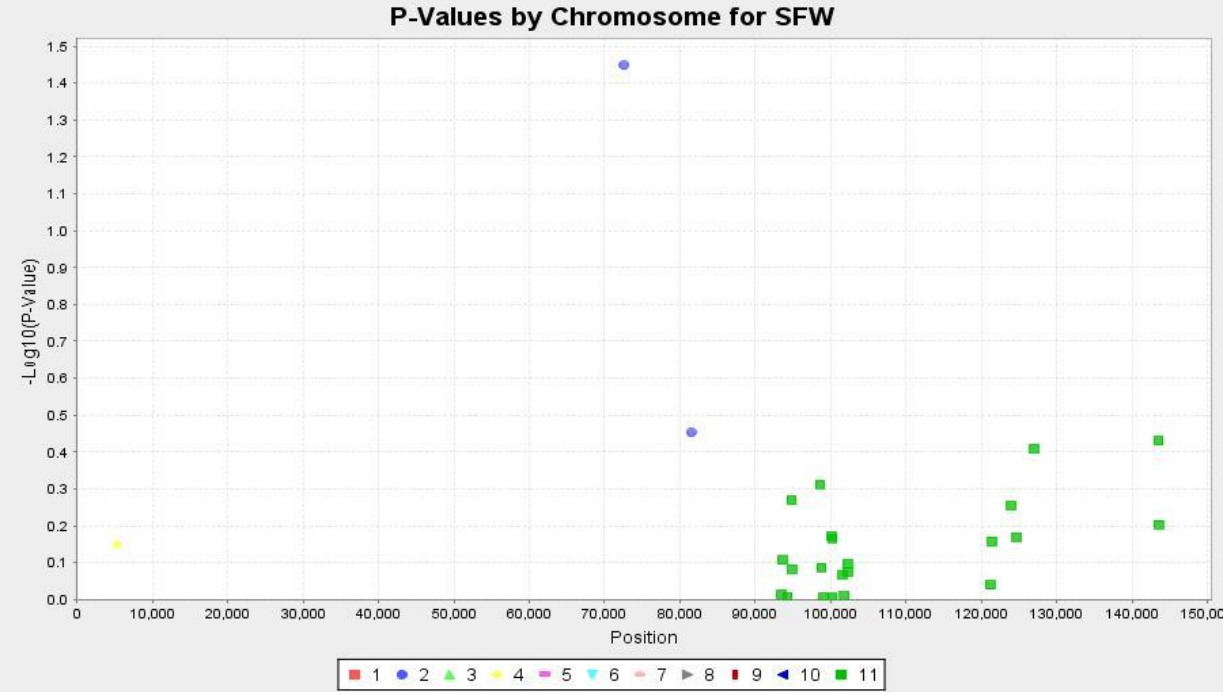
NaN = Missing information

Table 47. SNPs Marker loci associated with fruit shape index and their explained phenotypic variation

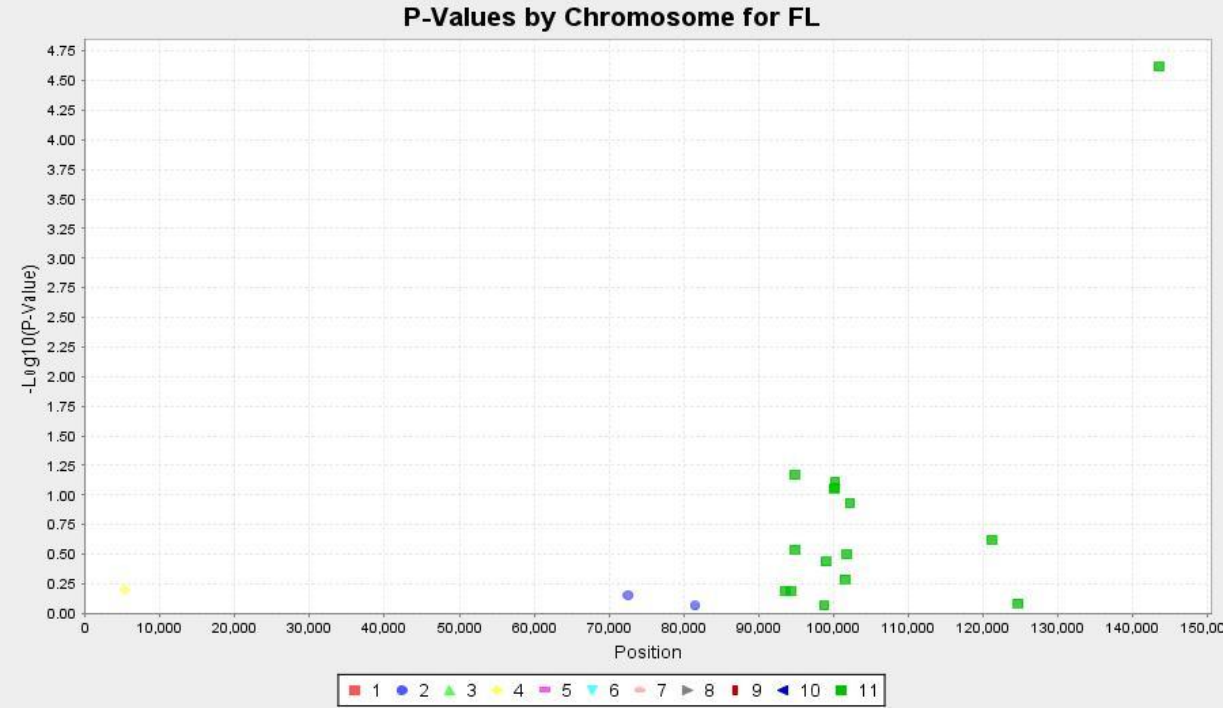
Trait	Marker	Locus	Site	df	F	p	errordf	markerR2
FSI	Solyc02 - 1	2	72540	2	2.18333	0.12969	36	0.09941
FSI	Solyc02 - 2	2	81500	1	0.75994	0.39005	35	0.02177
FSI	Solyc04 - 1	4	5310	2	0.42463	0.65776	36	0.02252
FSI	Solyc11 -1	11	11930	2	0.0029	0.99711	49	0.00159
FSI	Solyc11 -2	11	12060	1	0.01663	0.89826	34	0.00168
FSI	Solyc11 -3	11	12770	2	0.00252	0.99749	49	0.01234
FSI	Solyc11 -4	11	13290	1	0.53392	0.46867	50	0.01539
FSI	Solyc11 -5	11	13350	2	0.45681	0.63621	50	0.02623
FSI	Solyc11 -6	11	17070	1	0.34779	0.55877	43	0.75847
FSI	Solyc11 -7	11	17250	1	1.03782	0.31892	27	0.02451
FSI	Solyc11 -8	11	17470	1	0.77214	0.38731	31	0.03333
FSI	Solyc11 -9	11	18580	2	2.09657	0.13586	46	0.37538
FSI	Solyc11 -10	11	18690	2	3.86104	0.02802*	52	0.14474
FSI	Solyc11 -11	11	18720	1	0.01686	0.89735	43	0.10098
FSI	Solyc11 -12	11	20040	2	0.08299	0.92057	34	0.0128
FSI	Solyc11 -13	11	20300	2	-2.13×10^{-2}	NaN	38	2.45333
FSI	Solyc11 -14	11	20720	2	-1.85	NaN	51	0.64656
FSI	Solyc11 -15	11	20730	2	0.10914	0.8969	41	0.04268
FSI	Solyc11 -16	11	39650	2	-2.32	NaN	41	3.01393
FSI	Solyc11 -17	11	39870	2	3.27283	0.04753*	48	0.18348
FSI	Solyc11 -18	11	42440	1	3.00019	0.09159	41	0.04169
FSI	Solyc11 -19	11	43170	2	-2.77	NaN	51	0.36727
FSI	Solyc11 -20	11	45480	1	-5.04	NaN	47	5.26641
FSI	Solyc11 -21	11	62000	2	-4.02	NaN	44	0.14661
FSI	Solyc11 -22	11	62010	1	0.47638	0.49369	48	0.01235

***** Significant at 0.05 level

NaN = Missing information

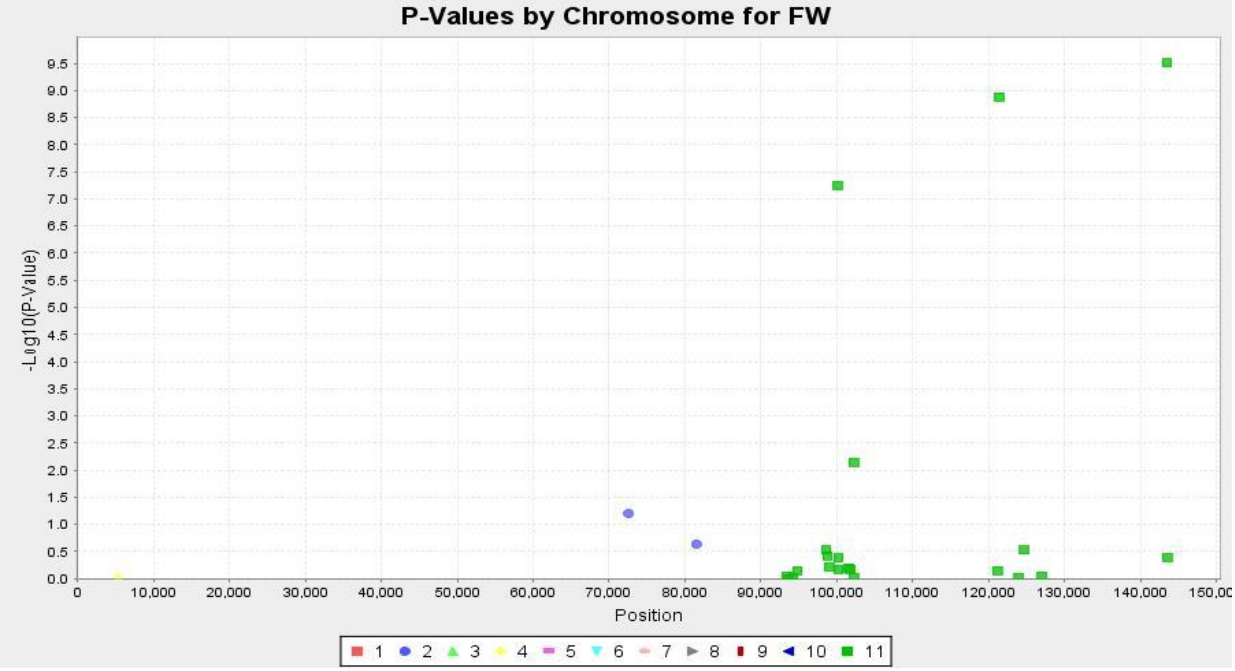


a.

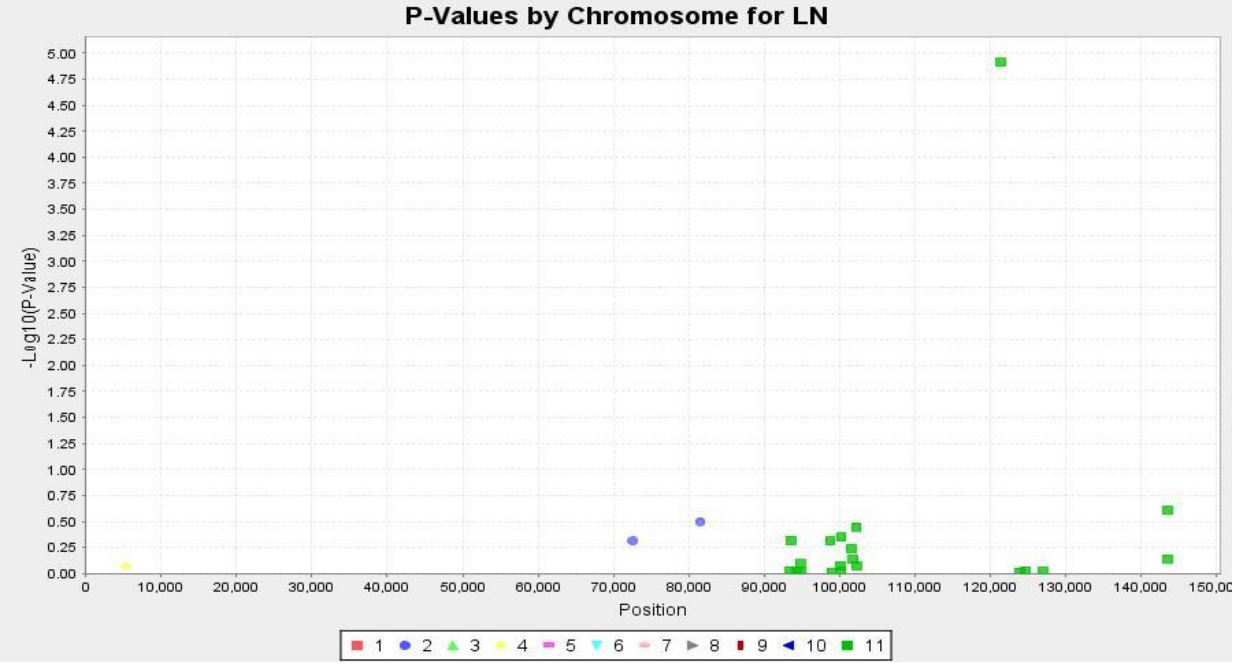


b.

Figure 5a and b. Significant association between SNP markers and their chromosome sites with the fruit size characters.



c.



d.

Figure 6a and b. Significant association between SNP markers and their chromosome sites with the fruit size characters.

DISCUSSION

Mean Performance of tomato parents and progenies

Proper and efficient use of plant genetic resources is only possible if their characteristics or attributes are known in detail and their potential utility is visualized (Jaramillo and Baena, 2000). Selection of suitable parents is of importance in a breeding program. In this study, the parent, supersteak differed significantly from the other parental materials in days to flowering, fruiting and ripening, stem girth, fruit weight, fruit length and width and the locule number. The parent, W x R revealed significant increase in plant height, number of branches, number of trusses per plant, number of flower per plant and number of fruits per plant. The observed differences in most attributes are indications of great genetic diversity among the parents. The results are in agreement with Agong *et al*. (2001) who reported large and significant variation in quantitative traits among tomato accessions.

The magnitude of variation in mean fruit weight was observed to be high, ranging from 23g for W x R for 218g of supersteak. Similar diversity in tomato fruit weight has been reported by Reddy and Reddy (1992). Grandillo *et al* .(1999) postulated that the allele for small fruit size are semi dominant over the alleles involved in the expression of bigger fruits. On the other hand polygene with dominant effects in wild species could cause fruit weight reduction (Weller *et al*., 1988).

Estimation of Heteroses

The expression of heterosis depends on the genetic divergence of the two parental varieties used in a particular cross (Rahmani Gul *et al*., 2010). If heterosis obtained from two parental varieties is high, there is every tendency that the varieties are genetically diverse. The hybrids showing high heterosis have good chances to identify desirable lines in succeeding generations as compared to hybrids having low heterotic effects (Sharif *et al*., 2001).

Heterosis for the days to flowering, fruiting and ripening have been estimated in term of earliness. The results in this study recorded negative better parent heterosis for all the crosses. Negative heterosis for the earliness has also been recorded by Dod and Kale (1992), Ahmad *et al*.(2011) and Kumar and Sharma (2011). Three crosses, S x (W x R), PR x (R x W) and PR x (W x T) exhibited positive and significant better parent heterosis in plant height. All the remaining crosses showed the negative better parent heterosis. Positive heterosis in plant height was also reported by Dod and Kaale (1992), Pujari and Kale (1994) and Ahmad *et al*. (2011).

Most of the agronomic and yield related characters in this study showed negative better parent heterosis with the exception of PR x (R x W) and PR x (W x T) for number of fruits per plant, and S x (W x R), PR x (R x W) and PR x (W x T) for the fruit yield. However, it was reported that, negative heterosis over superior plant is desirable attribute for some of the characters especially concerning fruit maturity and plant height (Saeed *et al*., 2014).

Significant efforts have been made for exploitation of heterosis in different yield contributing traits to find out the feasible cross for the production of F_1 hybrids. The hybrid showing high

heterosis have good chances to identify desirable lines in succeeding generations as compared to the hybrids having low heterotic effects (Sharif *et al*., 2001). Most of the crosses had significant and positive mid parent heterosis in majority of the traits indicating a predominance of non - additive gene action in the genetic control of the traits.

Floral traits showed significant negative heterosis over both better and mid parents except for flower and style lengths. The negative BPH and MPH could be as a result of a wide genetic distance between the exotic variety (supersteak) and the advanced generation of tomato lines that were developed from the wild tomato (Amaefula *et al*., 2014). The majority of the studies of inheritance of fruit size in tomatoes indicate that there is hardly ever heterosis for floral traits in the hybrids. Usually, the hybrids are smaller than the parental arithmetic mean (Powers, 1952, Maluf *et al*., 1982 and Melo, 1988). Generally, the hybrid vigor can be easily detected for yield by the increased number of fruits rather than by increased floral and fruit size traits (Rick and Butter, 1956).

All the hybrids had fewer locules when compared to the better parent as indicated by the negative heterotic values over the better parent. The results revealed that none of the crosses had higher number of locules per fruit over the better parent. The cross having supersteak as the pistillate parent recorded higher number of locules/fruit than the crosses involving supersteak as the staminate parent. This would appear to suggest some maternal effect in the inheritance of number of locules in tomato fruits. . Heterosis over the better and mid parents for locule number per fruit had been reported by Anbu *et al*. (1976).

The study showed negative BPH and MPH in the mean fruit weight. None of the hybrids had mean fruit weight bigger than those of the better and mid parents. This would tend to suggest the overwhelming influence of the small fruit size over the large fruit size. The finding disagrees with Larson and Currence (1994) who reported a significant positive heterosis for mean fruit weight in some tomato hybrids. It also worthy of note that the hybrids with supersteak as the pistillate parent had reasonable increment in mean fruit weight.

Estimation of Gene effect

The preponderance of positive additive type of gene action was observed for genetic determination of days to flowering, fruit set and ripening, stem girth and fruit yield in tonnes/ha. According to Gamble (1962), the additive gene effects become positive when the better performing inbred parent is used as pistillate plant. The parents, supersteak (S) and the plumb (rio grande) were used as pistillate parents and were the better performing inbreds. These finding were supported by the earlier reports by Makesh *et al*. (2002) for fruit yield. Hannan *et al*. (2007) and Cheshti *et al*. (2008) reported the prevalence of additive gene action for days to ripening and fruit yield. The prevalence of additive type of gene action suggested that the hybrids may produce transgressive segregates in the early generations which could pave the way for the development of pure lines varieties in tomato.

Additive gene action played decisive role in fruit weight inheritance for the crosses, S x (W x R), PR x (R x W) and PR x (W x R). Conti *et al*. (1984) confirmed the significance of additive gene effects in tomato fruit weight inheritance, with the prevalence of estimated additive effects.

The additive and dominance epistasis that have been detected for the number of fruits/plant and yield for the crosses, PR x (R x W) and PR x (W x R) are in accordance with the results of Zdravkovic *et al*. (2011) and Singh and Singh (1985).

Additive x additive gene effects showed greater contribution in the inheritance of mean fruit weight for all crosses. The high and positive magnitude of the additive x additive gene effects in all the crosses indicated that epistasis gene effect contributed greatly to the inheritance of fruit weight (Hayman, 1958) in the crosses. This is an indication that their inheritance is controlled by many loci.

Estimates of Heritability

The highest values of broad sense heritability were observed for the number of seeds, days to flowering, fruit width, fruit length and mean fruit weight. High heritability estimates were suggested to be useful in making selection of superior genotypes on the phenotypic performance (Khanom *et al*., 2008). Similarly, Johnson *et al*. (1955) postulated that heritability can be used in the prediction of the best individual. Higher estimate of heritability in broad sense indicated the low sensitivity of the traits to the environmental effects.

According to Amaefula *et al*. (2014), narrow sense heritability is essential to a plant breeder since it is involving the proportion of additive variance to the total variance. The additive variance is the variance that cause resemblance among relatives (Aquaah, 2007). The results detected the narrow sense heritability (>50%) in fruit width and single fruit weight (S x (W x R), fruit length and number of seeds (PR x (R x W) and moisture content (PR x (W x R). The

higher estimate of narrow sense heritability suggested the effectiveness of selection procedures for the improvement of the traits. Therefore, the above traits are highly heritable and should be selected for further studies in the crosses.

Mean fruit weight, fruit length and diameter and number of locules per fruit are the important parameters in determination of the fruit size. High estimate of broad and narrow sense heritability were observed for the mean fruit weight, fruit length and diameter and number of locules per fruit. The results are in line with Saleem *et al*. (2009) and Rajan *et al* (2012) who detected high heritability for fruit length and fruit diameter. Both characters can be improved through selection. The results are also in agreement with Khanom *et al.* (2008) who observed high heritability value for average fruit weight. According to Dar and Sharma (2011), the broad sense heritability values for fruit length and fruit width was 0.97 and 0.98 respectively. The findings in the present study is in line with report of Dar and Sharma (2011).

Inheritance pattern of the fruit characters

The production of the F_1 plant with few locule number indicates that the few locule number trait is dominant over many locule number trait. The F_2 plant segregated into the expected proportion of 3:1 while the backcrosses result revealed the expected proportion of 1:1. The results suggested that the backcross generations are inconsistent with the Mendel's law. It is also suggested that the locule number in tomatoes can be affected by environment. According to Li *et al*. (2007), the few locule number are incomplete dominant, the locule number in the backcross generation is

similar to the parent and the locule number is largely affected by environment. Similarly Younis *et al*. (1988) found that the locule number in tomatoes in the genetic model was partial dominant.

Yu *et al*. (1999) demonstrated that when tomato with two to three locules used as pistillate parent crossed with tomato with over five locules as staminate parent, the number of locules per fruit in F_1 was fewer and completely dominant. The genetic analysis of crosses between multi locule and fewer locule relative suggested that their progenies always segregated in the continous manner. With respect to fruit locule number indicating that the domestication process involved mutation at a number of different genetic loci (Ku *et al*., 1999).

The production of 32 round fruit and 9 flat round fruit in the tomato hybrid, S x (W x R) is an indication of semi dominance of the round fruit trait over the flat round shape. Zdravkovic *et al*. (2003) reported that semi dominance and gene interactions were operative for the control of fruit shape in tomato. The segregation of the F2 into 3 round and 1 flat round, further confirms the dominance of the round fruits over the flat round fruits.

Correlation matrix of the floral and fruit related characters with the fruit size

Fruit size is a complex entity associated with number of component characters including floral traits and other fruit size related components. It is part of the yield, therefore is the crucial concern of the plant breeder and also the final factor on which selection programme is based. It is marked that tomato varieties demonstrate a considerable variation with respect to fruit size and its component such as floral traits. These variations can be attributed to both ontogenic changes

in the flower traits and the environmental effect since floral traits are known to be quantitative traits (Oyiga *et al*., 2010).

A study of association of the characters related to the fruit size assist in the selection scheme for more than one character at a time. All changes in the fruit size must be accompanied by change in one or more characters (Graffius, 1964). Therefore, improvement of one character results in simultaneous improvement of all the positively related characters. In tomato fruit size improvement, the knowledge of association between the floral traits and the final fruit size is of special significance. As fruit size and shape is influenced by many factors, studying on the contributing factors based only on correlation may produce misleading results because it measures only the mutual association between two variables. On the other hand the combination between the correlation and the path coefficient analysis go further by providing an effective means of partitioning the variation into the direct and indirect causes of association.

Fruit size was significantly correlated with the fruit length, fruit width, as reported by Prashanth *et al*. (2008) and Singh (2005). Also the locule number per fruit exhibited significant positive correlation with single fruit weight. These result are in line with Power (1950), Gontijo *et al*. (1983), Singh (2005), Singh (2007) and Prashanth *et al.* (2008).

This study found that all floral traits measured as flower width, stalk width, style diameter, stigma length and diameter, diameter, length, area and perimeter of the ovary were positively correlated with fruit size with the exception of flower length and style length. A positive correlation shows that the changes of the two variables are in the same direction. Therefore, high

value of one variable is associated with high value of the other. For example, the positive relationship between the stigma diameter and the fruit size means that increase in stigma diameter increases fruit size. This indicate that selection of tomato varieties with large stigma diameter is one of the reliable strategies for fruit size improvement.

Among floral traits measured, the highest positive correlation was ovary perimeter (r = 0.9722**), followed by ovary diameter (r = 0.9674**), ovary area (r = 0.9578**), stigma diameter (r = 0.9535**), style diameter (r = 0.9491**). Previous researches speculated that fruit size is likely to be developmentally related to the ovary size from which the fruit develop and the ovary size may be correlated to the other floral organs (Gillaspy *et al*., 1993, Frary *et al*., 2000, Ashman and Majestic, 2006). Similarly, high positive correlations were observed between ovary (diameter, area and perimeter) and both stigma diameter and style diameter. This indicates that the increase in ovary size depends on the increase of the stigma diameter and style diameter. According to Webb and Lloyd (1986), large stigma diameter provides a larger receptive surface area for pollen deposition. Therefore, large receptive area of the stigma is an advantage as it is able to capture higher number of pollen grains. Whereas the large style diameter and shorter style tend to ease the movement of pollen grain to ovary. The stigma diameter had highly positive correlation with the style diameter, indicating that both traits could be increased simultaneously.

Fruit size was negatively correlated with the style length (r = -0.8840**), flower length (r = -0.8078**), number of seeds (r = -0.2386*). This implies that the higher the style length, the smaller the fruit size. The undesirable negative association of the style length with other fruit size contributing traits could be broken down through mutation to broaden the genetic base for

selection to improve fruit size (Arshad *et al*., 2005). A residual effect of 0.0001833, implies that 99.98197% had been determined.

Among the traits subjected to path analysis, locule number per fruit exhibited a very high direct effect upon fruit size. The direct effect of the ovary diameter and stigma diameter was also appreciably high toward the final size. The highest positive direct effect of locule number had already been documented. These characters with high direct effect on fruit size should be emphasized. It is likely that the selection for increased fruit size through selection for increased locule number, ovary size and the stigma diameter is a promising step in fruit size improvement in tomato.

Correlation matrix of the ovary, seed and fruit shapes index of the tomato

Variations in tomato fruit shape is the results of differential growth processes which probably occurs during formation of ovary, or after anthesis during the formation of the fruits (Brewer *et al*., 2006). According to Xiao *et al*. (2009), the fruit development involves some important landmarks; one to two days after anthesis, the flower will be pollinated, leading to the fusion of the maternal egg with the paternal sperm which initiate the development of the fruit. During Phase 1, rapid cell division occurs along with cell expansion, mostly in pericarp tissue, and the developing embryo increases from 4 to 16 cells. At about six to ten days post anthesis (dpa), phase 2 begins in which the developing fruit increases in size through cell expansion and the continues to increase through 20 dpa. During Phase 3 the embryo goes through its globular stage, to heart stage, torpedo stage, and finally coiled stage. From 20 to 30 dpa, a period of seed maturation occurs ending the seeds being viable for germination and then the fruit begins to

ripen at 30 dpa and becomes ripe at about 40 dpa. Therefore, it is possible to link the relationship between the ovary, seeds and fruit morphology.

Fruit shape index was significant correlated with the ovary shape index (r = 0.835**) and seed shape index (r = 0.718**). This result was in line with Perin *et al*. (2002) and Eduardo *et al.* (2007) who documented a high positive correlation between ovary and mature fruit morphology in melon. Similarly, Van dar Knaap and Tanksley (2001) and Chakrabarti *et al.* (2013) reported depending on the QTL, changes in fruit morphology such as size and shape manifest themselves either before or after anthesis, therefore there was correlation between the fruit and ovary morphology.

Fruit shape index was significantly correlated with ovary diameter (r = -0.601*), fruit diameter (r = -0.576*) and seed diameter (r = -0.519*). Similar results were reported by Monforte *et al.* (2004) and Eduardo *et al.* (2007) suggesting that fruit shape is generally also highly correlated with the length of the fruit but not with the diameter. Therefore, longitudinal growth is the major factor of the final shape (round or elongated). Fruit shape index below 1 indicating round fruit shape and fruit shape index above 1 indicating ovate fruit shape.

Correlation measure the mutual association of the character studied. A positive correlation shows that the changes of the two variables are in the same direction. Therefore, high value of one variable is associated with high value of the other. For example, the positive relationship between the fruit morphology and the ovary and seed morphology means that change in ovary or seed morphology resulting in changing the fruit morphology. This indicate that selection of

tomato varieties with a certain shape is one of the reliable strategies for fruit size improvement. Therefore, it is possible to select tomato fruit morphology at the flowering stages or before planting the seeds.

DNA Quality Assessment

The CTAB protocol has good usage and results with phenol/chloroform or chloroform followed by isopropanol precipitation is effective for a wide range of matrices. CTAB complexes out polysaccharides and proteins which reduces PCR-inhibition but the disadvantage is that it has a 260nm effect during spectrophotometric quantification. DNA measurement of absorbance (optical density) with spectrophotometer is the most common technique to determine DNA yield and purity. A good-quality DNA will have absorbance ratio (A260/A280) between 1.7 - 2.0. A reading of 1.6 does not render the unsuitable for the other application, but lower ratios indicate the presence of more contaminants (Sambrook and Russel, 2001; Aliyu *et al*., 2013). However, DNA is not the only molecule that can absorb UV light at 260nm. Since RNA also has a great absorbance at 260nm, and aromatic amino acids present in protein absorb at 280nm, both contaminants, if present in the DNA solution will contribute to the high values obtained at 260nm. This could explain the high DNA yield obtained in some of the samples with low DNA purity. The DNA solution was further concentrated through ethanol precipitation. This achieved two objectives, viz (i) it helped to further clean up the DNA and (ii) it improved the concentration of the DNA for downstream analysis such as Polymerase Chain Reaction (PCR).

SNP-Based Polymorthism and Genetic Diversity

Average Nei's gene diversity and Polymorphism Information Content (PICs) values revealed by SNP markers in this study were 0.2662 and 0.2220, respectively. This level of genetic diversity is similar to the report of Corrado *et al.* (2013) who also used SNP genotyping for the genetic diversity and detected the gene diversity and PIC values of 0.215 and 0.177, respectively. On the other hand, most of the researches involving SSR markers for genetic diversity detected high gene diversity and PICs values (Maccaferri *et al*., 2003 and Moragues *et al*., 2007).

However, the relative lower genetic variation revealed by SNP markers is expected. This is because SNP markers are mainly bi allelic and therefore, the gene diversity and PICs cannot exceed 0.5 while the multi allelic markers such as SSR can approach the maximum of 1. Similarly. Chen *et al*. (2009) and Todorovska *et al*. (2015) observed the overall genetic variation of 19.16% and 23.2% respectively across 47 SSRs and SNPs loci in 216 hybrids and elite breeding lines of tomato originating from four breeding centres in China.

The number of alleles per locus in this study was 2 allele, although this value was expected due to the bi allelic nature of the SNPs markers. Benor *et al*. (2008), reported 4.3 allele per locus and PIC value of 0.31 in tomato varieties. He *et al*. (2003) identified 2.7 alleles per locus on average and PIC value of 0.37 in the study of relationships among 17 varieties and two parental lines of tomato with 60 SSR markers. Limited allelic variation was also observed in a study of tomato populations consisting of a total of 216 genotypes from four breeding centres in China using 12 SSRs and 35 SNPs markers.

The present study revealed the genetic diversity within the F_3 tomato genotypes. The relative high polymorphism (61.7%) recorded in this study for the fruit size characters was due to the occurrence of the null allele's segregation. The genetic similarity estimated in this study according to SNPs data was scaled up to 100%, thus, suggesting the potential of SNPs markers in discriminating among tomato genotypes of close or distant genetic background.

Furthermore, it was reported that solanaceaus plants have a low frequency of polymorphism among cultivars (Nunome *et al*., 2003 and Stagel *et al*., 2008). It was also documented that cultivated tomatoes are highly monophorphic at the molecular level although they are phenotypically very diverse (Labate and Roberts, 2002).

Association mapping

A prerequisite for the association studies is a good estimation of the true population structure. The result on the population structure was highly similar to the neighbour-joining dendrogram and fruit characteristics. Both neighbour-joining dendrogram and the population structure segmented the tomato genotypes into three main groups. The result validates Ruggieri *et al*. (2014) who also segmented tomato genotypes into three major groups by using both joining dendrogram and the population structure. The number of sub-populations obtained in this study were also similar to the number of clusters observed by Mazzucato *et al*. (2007) in 61 accessions of the cultivated tomato. On the other hand, Ranc *et al*. (2008) detected only two sub-populations for the genetic structure of 90 tomato accessions using 20 SSR markers.

Association mapping is useful in populations with considerable high genetic diversity in order to ensure high mapping resolution. The three sub-populations obtained in F_3 tomato genotypes based on the fruit size and shape characteristics showed that the tomato genotypes were diverse despite the self pollinating nature of the tomato plant. This broad genetic variation reveals prospects for fruit size improvement in F_3 and subsequent generations.

Fruit size is a quantitatively inherited trait controlled by up to 28 QTLs, even though QTL analyses in previous studies revealed that most (67%) phenotypic variation in fruit size could be attributed to six major loci (*fw1.1, fw1.2, fw2.1, fw2.2, fw3.2* and *fw11.3*) localized on chromosomes 1, 2, 3 and 11 (Lippman and Tansley, 2001; Causse *et al*., 2004 and Munoz *et al*., 2011). The association mapping analysis in this study confirmed only two of the above loci (*fw 2.2* and *fw11.3*).

The results show that the fruit weight was associated with more than one SNPs markers. This is in line with finding of Ruggieri *et al* .(2014) who confirmed 6 SNPs markers associated with fruit size in tomato. Apart from Ruggieri *et al*. (2014), many association studies have been published to date for studying morphological and fruit traits in tomato. Mazzucato *et al*. (2008) studied association for 15 morphological traits in tomato genotypes using 29 SSR markers. Ranc *et al*. (2012) and Xu *et al*. (2013) investigated morphological and fruit quality trait in cultivated tomato and its wild relative by using 352 and 192 markers, respectively. Recently, Shirosawa *et al*. (2013) studied the association with fruit size and shape using an illumina golden assay for the 1536 SNPs.

Association mapping detects and locates QTLs based on the strength of the correlation between mapped SNPs markers and traits (Mackay and Powell, 2007). Association mapping related to five fruit size characters were identified to involve 25 SNPs markers. Out of 25 SNPs markers only 9 markers that were associated with SFW, FL, FW, FL, LN and FSI were detected. The results indicated that these nine loci may be stably related to the traits. Of all of the studied markers, one SNP markers was shared by three fruit size characters (FW, LN and FSI), which might be caused by the pleiotropic effects of linked genomic regions (Ge *et al*., 2013). Barrero and Tanksley reported that, the major genes controlling the locule number are on different chromosomes (*fasciated* on chromosome 11 and *locule number* on chromosome 2). Therefore, the allelic composition of Solyc11-17 may reflect a linkage with the loci, *fw11.3*.

Large populations are desirable for association mapping studies in order to obtain a high power to detect genetic effect of moderate size (Zhu *et al*., 2008 and Bernado, 2008). However, there is high cost associated with genotyping and phenotyping large population particularly for traits requiring extensive field trials. Moreover, this studies included 96 tomato genotypes and we are assumed that the size of the tomato hybrid used in this study was adequate for the association mapping studies as previous reported for beans (Galeano *et al*., 2012), peanuts (Wang *et al*., 2011), barley (Gutierez *et al*., 2011) as well as for tomato (Ranc *et al*., 2012) in analysis that involved approximately 90 tomato genotypes.

From the results, 9 markers had significant association with the mean fruit weight, fruit length, fruit width, locule number and fruit shape index. Therefore, all of these markers detected are recommended for the fruit size improvement breeding programe in tomato.

CONCLUSION

The Analysis of Variance (ANOVA) on agronomic, yield, floral and fruit traits showed significant differences (P = 0.05) among the tomato parents and their hybrids. The higher significance difference among the tomato genotypes indicated the higher genetic variance and suggested the effective scope of selection.

The cross, S x (W x R) was the most promising three - way hybrid that can be exploited for increased fruit size and yield in the humid tropics. In term of their yield and fruit size ranking in F_3 populations, S x (W x R)-14, S x (W x R)-11 and S x (W x R)-15 were ranked first, second and third, respectively.

The path coefficient analysis revealed that number of locules per fruit exhibited a very high direct effect upon fruit size. The direct effect of the ovary diameter and stigma diameter was also appreciably high toward the final size. These characters with high direct effect on fruit size should be emphasized.

The single nucleotides polymorphism (SNPs) marker analysis demonstrated the presence of high genetic variation among the F_3 tomato hybrids. The genetic diversity indicated that there are still existed substantial level of genetic variation with F_3 tomato genotypes as detected by SNPs markers. This results can be used to accelerate the tomato fruit improvement by addressing the pattern of genetic variation in the later generation through selection.

The result of the structure analysis revealed that the tomato genotypes studied had three subpopulation, indicating genetic diversity for the fruit size improvement.

The result of the association mapping of QTLs linked to fruit size detected 9 markers with significant association with mean fruit weight, fruit length, fruit diameter, number of locules per fruit and fruit shape index. All the markers detected are recommended for the fruit size improvement breeding programme in tomato.

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APPENDICES

Appendix 1: 45 SNP markers sequences used in this work

SNP_ID	Sequence
Solyc02g081640_2_1	GAGAGAGATATGATACAATCATTCCTGATTTAGATGTAGGAGAGTCTGTG[T/C]CATGTCTGCCAGATTCACAATCCGTTCATTCTGAAATGGATTTTGTGAAT
Solyc11g043170_1_1	AGTGAGTAAGATTGTGATGTTACTGGGTGCTCTTGAAAGTGGAGCTGTAG[T/G]GAGATCGGTAAGAAGGAAACCCTAGAGAGGAGTTTTTAGGGGTTTGAGAA
Solyc03g013170_2_1	TCTTTTTCTAGCTACTATAGGCGGCGAAAGACAAGAACACCAAGGACCAC[T/C]GACGAGCCTCAATTACTGGAAGCAGCGCACAACGAAGGACAGAAGTAGCG
Solyc11g064950_1_1	ATTGACATCTGCTTCTGCCTTGGTGATTTACAGAAGATACTACATCTGAT[T/C]TGTGTTCCAATTTCAATTGATTATCCACAAAGTTGGCTATAGTCCACGAT
Solyc11g039870_1_1	GTTGACAATGATGAAGGGGTTATGATTTCAGTTTGATATTTGAGAGTTCA[A/G]ACCAATGTATGGGAAAGGAAAGAGTGGAAGTATCCCCCATCCTAGATATT
Solyc02g072540_2_1	CTCTGTTCCAAACAGATTACGATTACAATGGCAATGAATCCTCAACAAAA[T/G]TAAAAAGAAAAAATAATTTGTCTCAATTGTATTGCCAATTTCTTCTTCAG
Solyc11g012060_1_1	TGCCTTTTCTGTAAAGTTTCTTCATAGTTTTTTTTATGATAAAAATGGAG[A/G]ATTTGTTTTTTTTTTGAGAGGTTTGAAGGTGGGGTAGTTAGGTAGGGTGG
Solyc11g011930_1_1	AGAACTGGAAATTCTGGTACTATAAAGAGTAATGGTGTTTGATAAAGAAA[A/G]TTTCGCCTGCGGGAGAAGCTTAAATTGTAACGAAATGGGGAAAAAATAAT
Solyc11g020730_1_1	ACACCTTGGTGTCAGAGGGAAAAAGCAGCTTCCATTTCTATTTGGAGTTC[A/C]GATCTTAGAGAAACTTGCAGCACCAATTGCTCCCTTTGTGGTTGGTCAAA
Solyc11g013290_1_1	AACCGCCATTTGAGGTGAGAAACAAGTATTTTTCTTGTATCTTGTGGAAG[A/G]TACTCCACAAATTTACCAGCTCTCCCTCTCTCCCTCTCTGCTTTGGATCT
Solyc02g076630_1_1	AGGAACTCTAATTGACGGAGTCTTATTCGTTGGTACGTCGATGATCCCTC[T/C]TCTGGCCCCATATTTTGTAACAATGCTTAAGCCTCTTGGCAGCGGCTAAG
Solyc02g081310_2_1	CCCTTTACCAGACCCAATACCTCTTCCCTTCCGAGTCTTCTGCTTCCTAG[A/C]ACCTTTGTTATCTTGCAAATCATTTAGGCTCAGAAGACTGTAAGCTCTGC
Solyc11g017070_1_1	CGACGAACAACAATCTCACTGAGAGAATCACTCCGGCGACCGACTACAAA[A/C]AACGATGAGGCCAATATTGATGAAGGGCCATGAAAGGCCGTTAACTTTTC
Solyc03g019680_2_1	TCTTAGGTTTAATTTGAAGATTGAGGTGAATAATTTTGTTTGATTNGATTGGATTGATAT[T/C]GGAGTTGGAGATGACAACGAGTCACGGAGGAGGATCCTTGTCGAGGAGGAGTTCGTTGTC
Solyc11g039650_1_1	GGTAGGGTTTTTCCTCCGATTGACGCGTAACGAACGGGAAATGATGCCGG[A/G]ATGAGAGAGATTTGAAAAGTTTTTGTGATGAGAAAAGAAAAGAAAAAAAA
Solyc11g018690_1_1	GGTTCCGGTGTAGGTGTGTCCATGGAGTCTACCTGCGAGTGCATTAAGGA[A/G]CGTTGATTTACCACTACCTGATGGTCCAAGAACGGCTAGGATTTCACCTG
Solyc03g124010_2_1	GACTATCAGCATGCTGACATGTGGTTTCAACCCCGCTGACATGCTTCAAC[T/G]TCCTCGAACAATTTTCTGGTTTCTCTGAAGTCAACAGTTTCTCCCGATCA
Solyc11g062010_1_1	AAGCTTCCTCTGTTATGGGCACAAGCAAGCAAATGAAGCCTGATCTGAGT[A/G]GTAGGAGTGGAACTGAGGCTTCAAAGGTATCACCAACTGCCTCTGCCAAT
Solyc02g091490_2_1	TGGAGAGAGGAGCAATTCCAGCTTTGCCTACAAAAGAAGTAGTGTTGAAC[A/G]CCTTGCTCAAGTCAGTTGCATAATATCCATCCGACCTATTAATCGGTTGC
Solyc11g018720_1_1	CAGAAATAATAGAAAATCAGAAAGAAAAATCAGCTTTCTAAATGGAAAAG[A/G]CGATGGCACTATGTTTGAAGTTTTAAGCAACTTTTCTGAAGTCCCAAAAG
Solyc02g081500_2_1	CGGAACAAAGCTATTGGAAACTCATTGTTTCAATCGAAAAGATATAACCC[A/G]AACAGGTACTACTTGCATATCTTAATTAATTTTTTCCATCATTATTTAAA
Solyc02g091510_2_1	TCTTTCGTTTGTATTCTCACAATTCATCATCACCGCAAAGTGTTGACCCT[A/T]AATCCAACTCTTCTGGTGGACGATAAGCACCGGACCCCTTCCCCTCACGG
Solyc11g045480_1_1	GGTTCTCATTTATAGTGCTCTTCAAGGGAGGGTAATCATAAGAAGGAATG[T/C]CAAAGCGGCCATCGGAACTCTCACCTACACTATTGTTCCGGCTAAAATAG
Solyc11g012770_1_1	CTCTGAAGTAAATTTCCTTTCTCTCTCTCTCTCTTGTCGAAACAAAAAGC[C/G]TACCTACACCTCCGGAGCTAAATGTCCTAAAACTCTCTGTTGGAACAGAA

Solyc11g017250_1_1	AAGGTGGATTGAAGAAGATGAGACATTTTTGCGAACAAATCAAGAAAAAT[T/C]GAATGATTTTAATAATGGTGAAAAGAAAAGCGTAGGTTTGTGTGTTTTTC
Solyc03g005100_2_1	AGAACAGAAAAAGAAATTAAAAATTGGATCTTTTTCTCCAATTGAATCAAGAAATCTCTA[A/G]TACCTTTCCCTTAATCACAATCCATCTTCTTAATCCTTAAACCCTAAACTATCTCTTCAT
Solyc04g005310_2_1	CTTGTGTCTTAATGTGTATGTAGTATGCCAGTACCAGCACAGTTTCTATT[A/T]GTCCTTGTTTAACCTATGCAGTCAAAACGTGTCAAATGTTGGATAGCTAT
Solyc11g017470_1_1	CCTCCTCTGTTTTCTACGTTTCAACTTTCAAAAACACCTCTTTCTCTCTG[A/T]GTAATTACAAAAACAAATTAAATCAATTCTTTTTCTTGAAGACAAAGTAA
Solyc11g039830_1_1	TGAAAGTGATAAAAATGGTATAAATGCTATCATCATTTGATGATTCACTC[T/C]TCTGCAGTCTCTGAAGAATGTAGAGGATACATCTTCAAAACATCTGCCCA
Solyc02g081430_2_1	ATGAATATTAGTCTCAAATAGAGTAACCTCCTTGTAATTCCCTTTATTTC[T/C]AGTAAGAAATGATGTCTTTTTTATGTCTGTTTTGTCATTAATATGAATTG
Solyc11g062000_1_1	TCCCTTCCTTTTCCGAAATGGAGTTGAACCACGAAAAAAGGTGGCTACTC[T/C]AAAATGGTGAAAGTCACAGAGTTTTTGAAATTGAAAGAGAGACCTTTTTC
Solyc11g020300_1_1	AAAAGGAGATATCATTTTTGCAGTTCTGATCTTCTTCTTCTGTAATAAGG[T/C]GAAGTATCAGCTAAAAATTGAGTATTTTTCAAGGTGATGGAGAACATAGG
Solyc02g090890_2_1	ATTACTTGTTTGGATATCAATCTTCCTTGCCCCACCACCATCCTCACTTT[A/C]ACCCACAAATCCCACTTTCTTTGGCCTAATTAACAATTTTTTCAATAAAT
Solyc11g042440_1_1	TAAGAAGAAGGAACATGGAGGATTAGAAACCCTAAAAAAAATCTGACATT[A/G]AAATTGAAGAAATGCCATTCAATGGAGTATTGCTGGTGTCTGTAGCTAAA
Solyc11g013350_1_1	AGGTAAGCCCTACGCGTGATGGTATCCACTGTCCAGGATTAGAAGCTGAA[T/G]TTTCTTGTGCTTTGGGGAGCATCAGGTGTCAATCTCTGTAATCAACACAC
Solyc02g090730_2_1	ACACTTGTTTTAAAGCTAAAACAACAAAAATAAGCTAAAGTAAATCGAAA[A/G]AGGCGTGAAATTTCAACTATTACCAGATTTCTTGGAAATGATGATTTAGG
Solyc11g065000_1_1	CAGATACAAAGACCCCTTTTAATCAAAATACCAACTTTTTGTGCACCAAG[A/C]TCTTTTCTTGGAAAAATCAAATCTTGATATATGAAAGAAAAGGGAAAATA
Solyc02g084030_2_1	TGGACTAGACTTAGCTGCTGCAGCTGGTGGAATAAATCTACCGGAGCTTC[T/C]GCTTTCCCTTTTTTTGCAGGGATTAATCAAAATTTTTTGTACTATCTTAA
Solyc11g018580_1_1	GCTTTTTGAGTATTCATGCAAAAAGTATCATCACAAGAAGTTACTAGGAA[C/G]TCGGAAACTGCTTTCCAGAGAGATGGAAGTATCTGCAGATGGAAGGTCGT
Solyc02g083950_2_1	GAACTAGTCTATCTTATGTTTGTAGTAAGTAAGTACTAATCTAATTTGGT[A/G]TGTGCCAAGCTATTTGGACCTTATGGTAATGTTAATTAATCTTAATCTAA
Solyc11g013810_1_1	GTTTTCCACAGATGCCGCCATTGATTTTTTTAAAATAAAATTATGAATGA[C/G]GGAATTTGGAAGAAGAAAAAAGAGATTTCTAATTAGATTTTGATGGTTCT
Solyc02g081320_2_1	CTACAAACTTGCAATGCTTGTATCTCGAATTTGCCAAAATCCGCAAGTGT[T/G]TCATAGCTGCGGGGAAACTGTTTTAGATAGGGCCACCACCTAGAACTCTT
Solyc11g064800_1_1	GAAGAAGTAAGTGCCCAACTGAGGAAAGGTCGTTATTGTAGCTGCCTTTT[T/C]TGCTTATTTTTAGCTTACAAGTCCCTCTCTCTTTGAAGAAAAAAAATTAA
Solyc11g020720_1_1	GGAAACCATAGCTTCTTCTGCGTCTATGTCAATGCAGGTGACTGGACCAT[T/C]CCCATTCGTTTCCCTGAATACAAATCTCACACGTGACACGACTAGAATAA
Solyc03g019650_2_1	CACCATTTTACCTCTCAATTTTCGATTTTGATTCTTGTTTATGCATGAAA[A/C]AATGGTAAGAGTAATACCTGGAGTAAGAGCTAAGAGTTTGATTGATCTAC
Solyc11g020040_1_1	TGATCGACAATTCTTGTTGTTGTTGAAACTCTGCAAGTGAGAGAGGGATG[T/C]ATATAGAGAAAGGATATTGGTAAAGGACAATTCTAGAAGGGTCTAGGGAA
Solyc04g007020_2_1	CTGAGGAGATTTCTGCTTTGAAAAACCTTGAAAATTTATCATCATTTTTC[A/T]GATTCACTCTCCCCCAAAATCCACACAAAAACAATGAAATACAGTTGAAA

Appendix 2: Assay summary for the 45 SNP used in this study

Assay	nAllele	Coverage	NoCall	Total	COMMON	HET	RARE	p	q	expCommon	expHet	expRare	HWp
Solyc02g081310_2_1	2	76%	18	57	57	0	0	1	0	57	0	0	1
Solyc02g081320_2_1	2	90.67%	7	68	68	0	0	1	0	68	0	0	1
Solyc02g081640_2_1	2	86.67%	10	65	65	0	0	1	0	65	0	0	1
Solyc02g084030_2_1	2	85.33%	11	64	64	0	0	1	0	64	0	0	1
Solyc02g090730_2_1	2	48%	39	36	36	0	0	1	0	36	0	0	1
Solyc02g090890_2_1	2	84%	12	63	63	0	0	1	0	63	0	0	1
Solyc02g091490_2_1	2	90.67%	7	68	68	0	0	1	0	68	0	0	1
Solyc03g005100_2_1	2	74.67%	19	56	56	0	0	1	0	56	0	0	1
Solyc03g013170_2_1	2	89.33%	8	67	67	0	0	1	0	67	0	0	1
Solyc03g019650_2_1	2	73.33%	20	55	55	0	0	1	0	55	0	0	1
Solyc03g124010_2_1	2	89.33%	8	67	67	0	0	1	0	67	0	0	1
Solyc04g005310_2_1	2	61.33%	29	46	16	17	13	0.5	0.5	13.05	22.9	10.05	0.08
Solyc11g011930_1_1	2	81.33%	14	61	55	5	1	0.9	0.1	54.2	6.6	0.2	0.06
Solyc11g012770_1_1	2	81.33%	14	61	55	5	1	0.9	0.1	54.2	6.6	0.2	0.06
Solyc11g013810_1_1	2	2.67%	73	2	2	0	0	1	0	2	0	0	1
Solyc11g017250_1_1	2	45.33%	41	34	28	6	0	0.9	0.1	28.26	5.47	0.26	0.57
Solyc11g017470_1_1	2	52%	36	39	34	5	0	0.9	0.1	34.16	4.68	0.16	0.67
Solyc11g018580_1_1	2	76%	18	57	45	11	1	0.9	0.1	44.74	11.52	0.74	0.73
Solyc11g018690_1_1	2	92%	6	69	52	15	2	0.9	0.1	51.31	16.38	1.31	0.48
Solyc11g018720_1_1	2	72%	21	54	43	11	0	0.9	0.1	43.56	9.88	0.56	0.4
Solyc11g020040_1_1	2	64%	27	48	15	25	8	0.6	0.4	15.76	23.49	8.76	0.66
Solyc11g020300_1_1	2	62.67%	28	47	26	15	6	0.7	0.3	23.88	19.24	3.88	0.13
Solyc11g020720_1_1	2	86.67%	10	65	40	18	7	0.8	0.3	36.94	24.12	3.94	0.04
Solyc11g020730_1_1	2	65.33%	26	49	34	13	2	0.8	0.2	33.47	14.05	1.47	0.6
Solyc11g039650_1_1	2	66.67%	25	50	28	15	7	0.7	0.3	25.2	20.59	4.21	0.05
Solyc11g039830_1_1	2	18.67%	61	14	14	0	0	1	0	14	0	0	1
Solyc11g039870_1_1	2	77.33%	17	58	35	17	6	0.8	0.3	32.62	21.75	3.62	0.1
Solyc11g043170_1_1	2	86.67%	10	65	41	17	7	0.8	0.2	37.7	23.61	3.7	0.02
Solyc11g062000_1_1	2	72%	21	54	37	12	5	0.8	0.2	34.24	17.52	2.24	0.02
Solyc11g065000_1_1	2	73.33%	20	55	55	0	0	1	0	55	0	0	1
Solyc02g072540_2_1	2	61.33%	29	46	17	16	13	0.5	0.5	13.59	22.83	9.59	0.04
Solyc02g076630_1_1	2	90.67%	7	68	68	0	0	1	0	68	0	0	1
Solyc02g081430_2_1	2	69.33%	23	52	52	0	0	1	0	52	0	0	1
Solyc02g081500_2_1	2	58.67%	31	44	42	2	0	1	0	42.02	1.95	0.02	0.88
Solyc02g091510_2_1	2	93.33%	5	70	70	0	0	1	0	70	0	0	1
Solyc04g007020_2_1	2	76%	18	57	57	0	0	1	0	57	0	0	1
Solyc11g012060_1_1	2	61.33%	29	46	42	4	0	1	0	42.09	3.83	0.09	0.76
Solyc11g013290_1_1	2	88%	9	66	59	7	0	1	0.1	59.19	6.63	0.19	0.65
Solyc11g013350_1_1	2	89.33%	8	67	60	6	1	0.9	0.1	59.24	7.52	0.24	0.1
Solyc11g017070_1_1	2	77.33%	17	58	42	16	0	0.9	0.1	43.1	13.79	1.1	0.22
Solyc11g042440_1_1	2	70.67%	22	53	35	17	1	0.8	0.2	35.7	15.59	1.7	0.51
Solyc11g045480_1_1	2	81.33%	14	61	42	18	1	0.8	0.2	42.64	16.72	1.64	0.55
Solyc11g062010_1_1	2	82.67%	13	62	43	18	1	0.8	0.2	43.61	16.77	1.61	0.57
Solyc11g064800_1_1	2	92%	6	69	69	0	0	1	0	69	0	0	1
Solyc11g064950_1_1	2	86.67%	10	65	65	0	0	1	0	65	0	0	1

Appendix 3: Tomato parents used in this work