QUANTITATIVE TRAIT ANALYSIS IN *Solanum lycopersicum* x *Solanum peruvianum* POPULATION

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ABSTRACT

QUANTITATIVE TRAIT ANALYSIS IN *Solanum lycopersicum X Solanum peruvianum* POPULATION

Tomato is an important vegetable for both the economy and the human diet and it is a good model system for genetic studies. Because of tomato's commercial importance, agronomic traits such as yield, fruit weight, size, color and firmness are very significant for the tomato processing industry and fresh consumption. However with increased attention on health, plant breeders also consider the improvement of health related traits of tomato such as antioxidant characters. Improvement of these desired traits is very difficult because many plant traits are controlled by more than one gene.

In this study both health-related and agronomically important traits were characterized in an BC_2F_2 *S. peruvianum* mapping population of 118 individuals. All plants were phenotypically characterized for total water-soluble antioxidant activity, phenolic and vitamin C contents as well as several agronomic traits including fruit weight and shape, color and firmness. All antioxidant traits showed good variation in the population with the *S. peruvianum* parent having significantly higher values for all three antioxidant traits. Based on trait distributions and transgressive segregation in the population, it was expected that some alleles from the wild species *S. peruvianum* had the capacity for improvement of both antioxidant and agronomic traits of cultivated tomato. Both parents were genotypically characterized with 169 genetic markers including 96 COSII and 73 SSR markers. Good levels of polymorphism were identified with both types of marker. Thus, it was shown that the population contains sufficient trait and genotypic variation for efficient mapping of quantitative trait loci.

ÖZET

Solanum lycopersicum X Solanum peruvianum POPULASYONUNDA KANTİTATİF KARAKTER ANALİZLERİ

Domates hem ekonomi için hemde insan beslenmesi için önemli bir sebzedir. Ayrıca zengin genetik kaynağı ile genetik çalışmalar için iyi bir model sistemdir. Domatesin ticari önemi nedeniyle, verimlilik, meyve ağırlığı, meyve büyüklüğü, meyve rengi ve meyve sertliği gibi tarımsal açıdanda önem teşkil eden birçok karakter domates işleme endüstrisi ve taze tüketim için çok mühimdir. İnsan sağlığına verilen değerin artmasıyla beraber, bitki ıslahçıları artık domates antioksidant karakterleri gibi sağlıkla ilişkili özelliklerinde geliştirilmesini dikkate almaktadırlar. Birçok bitki karakterinin birden fazla gen tarafından kontrol edilmesinden dolayı, istenilen özelliklere sahip bitkilerin ıslahı oldukça zordur.

Yapılan bu çalışmada, 118 bireyden oluşan BC_2F_2 *S. peruvianum* populasyonu kullanılarak hem sağlık açısından, hemde tarımsal açıdan önem teşkil eden özellikler karakterize edilmiştir. Populasyondaki tüm bireyler fenotipik olarak suda çözünen toplam antioksidant aktivitesi, C vitamini ve fenolik içeriği için hem de meyve ağırlığı, şekli, meyve rengi ve meyve sertliğini içeren tarımsal açıdan önemli özellikler için karakterize edilmiştir. *Solanum peruvianum* ebeveyninin önemli derecede yüksek miktarda bu üç antioksidant özelliği içermesi ile populasyon antioksidant karakterler açısından iyi bir varyasyon göstermektedir. Populasyondaki karakter dağılımları ve farklı ayrımlara dayanılarak, *S. peruvianum*dan gelen bazı allellerin kültür hatta bulunan antioksidant karakterleri ve tarımsal öneme sahip bazı karakterleri geliştirebilcek kapasiteye sahip olduğu düşünülmüştür. İki ebeveyn aynı zamanda 96' sı COSII ve 73' ü SSR marker olan 169 genetik marker ile genotipik olarak karakterize edilmiştir.Her iki tip marker sistemi ile iyi derecede polimorfizim belirlenmiştir.Böylece populasyon yeterli özellik ve kantitatif karakter lokuslarının haritalanması için yeterli genotipik varyasyon göstermektedir.

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CHAPTER 1

INTRODUCTION

1.1 Tomato

Solanaceae is an economically important plant family and serves mankind with important members such as potato, pepper, tomato, eggplant and tobacco. After potato, tomato is the most consumed vegetable of this family. Tomato (*Solanum lycopersicum* L., synonym: *Lycopersicon esculentum*) is also an excellent model system for molecular genetic analysis.

The wild ancestral tomato species originated from the Andean region which is encompassed by Ecuador, Colombia, Bolivia and Peru. Tomato species are also native to South America from Chile to southern North America (Rick 1973). Tomato species consist of 13 wild relatives and *S. lycopersicum* which includes cultivated tomato and the wild variant cerasiforme. The genus *Lycopersicon* which has now been renamed and included in genus *Solanum*, was originally divided into two subgenera: Eulycopersicum with red coloured fruits and Eriopersicon with green-fruits (Luckwill 1943). *Solanum lycopersicum* L.Mill. and *S. pimpinellifolium* Mill. species belong to Eulycopersicum while species *S.peruvianum* L. Mill. *S. chilense* Dun and *S. habrochaites* Humb. and Bonpl. belong to subgenus Eriopersicon. Not only morphological differences separate species but also major barriers to hybridization divide the genus into two groups, the 'esculentum complex' and the 'peruvianum complex'. The esculentum complex consists of *S. pimpinellifolium* L., *S. cheesmaniae*, *S. galapagense, S. neorickii*, *S. chmielewskii*, *S. habrochaites*, and *S. pennellii*. The peruvianum complex includes *S. chilense*, *S. peruvianum*, *S. N. peruvianum var. Humifusum*, *S. N. peruvianum var. Marathon, S. N. peruvianum var. Lomas* and *S. N. peruvianum var. Chotano-Yamaluc* (Peralta and Spooner 2000). The incompatibility barriers between these two groups can be overcome by various embryo rescue techniques and this allows the transfer of much

valuable genetic information to *S. lycopersicum*. Wild tomatoes are very valuable because they contain genes for resistance to diseases, abiotic stresses, improved color and fruit quality (Rick 1978). Interspecific hybridization can also lead to yield increases and has been important in the development of new cultivars.

The evolution of tomato as a crop started with domestication in America in the 16th century (Peralta and Spooner 2007) and domestication led to the modification of a wide range of physiological and morphological traits. These characteristic traits are called the domestication syndrome and distinguish domesticated crops from wild species. These traits are controlled by both qualitative and quantitative genes and have been further manipulated by plant breeders over the last century. Additionally, plant breeders have targeted agronomic, appearance, nutritional and processing traits such as disease resistance, fruit size, fruit shape, fruit weight, external and internal fruit colour, firmness, stem scar size, fruit locule number, fruit wall thickness and soluble solids content (Grandillo and Tanksley 1996, Doganlar, et al. 2000, Frary and Doganlar 2003, Tanksley 2004).

Tomato is currently grown in almost every country of the world. High production and consumption of tomato have also led to the tomato processing industry. Worldwide tomato production reached 126 million tonnes in 2007 (FAOSTAT 2007) and occupied 4.6 million ha (FAO 2007) with an average yield of 27.2 ton/hectare (FAOSTAT 2007). Asia ranks first with 50% of total production, Europe ranks second with 17.5% of production, followed by Central and North America with 12.3%, Carribean with 7.8% and Oceania with 0.05% . China is the first tomato producer in the world with the five next important countries being: USA, Turkey, India, Egypt and Italy (USDA-FAS 2005). Mexico ranks first in tomato exports and Turkey ranks second while USA ranks first in tomato imports. Tomato breeders' goal around the world is to develop cultivars with excellent fruit quality and yield. Moreover, long shelf-life is an important trait for fresh types and soluble solids content is important for processing types.

Tomato is not only important for the economy, but also prominent in the human diet providing essential vitamins and antioxidants. Although the nutritive value of tomato is not very high, it is a highly consumed vegetables and, therefore, an important source of vitamins and minerals. As the popularity of healthy eating grows with consumer awareness, tomato has gained special attention mainly because it is rich in antioxidants such as lycopene, vitamins E and C, β-carotene and phenolic compounds

(Adalid, et al. 2004). Between 90-95 % of the carotenoids present in ripened tomatoes and lycopene is the most abundant carotene in red tomato (Adalid, et al. 2005). 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). β-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 potassium, thiamine, riboflavin, folate, magnesium, phosphorus, iron, and niacin (Lachance 1998). Tomato pericarp tissue contains cellulose and lignin and tomato only includes about 0.3% fat. Additionally, tomato is a cheap ideal food supplement and people throughout the world consume it fresh, in salads, uncooked or cooked in many recipes, as juice, soup, and as processed puree, paste, concentrate, diced or whole peeled tomatoes, and sauce. Tomato is a simple diploid with twelve chromosomes (2n=24), can be cultured easily and can be used to detect an array of hereditary variations. For these reasons, tomato ranks among the best mapped of the flowering plants with numerous previous and on going basic and applied molecular research. In fact, tomato is one of the first crops for which molecular breeding methods were developed (Rick and Fobes 1974, Tanksley and Rick 1980). Genetic improvement of tomato can be achieved by using the abundant genetic variation which has been provided by nature. Although humans have been utilizing this natural variation over 10.000 years by domestication, selection was only based on the phenotype of desired traits such as large fruit and seed size, pleasant aroma, sweet flavor, brilliant colour or undesired traits including seed shattering and unpleasant aroma. After discovery of the genetic basis of these traits, 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). Moreover, this reduction of genetic diversity makes tomato susceptible to diseases and also interferes with the identification of new combinations of genes. On the other hand, exotic germplasm, including wild relatives are a huge genetic resource with the potential to broaden the genetic base of modern varieties. Because of the value of these resources, Nikolai Vavilov (1887-1943) and Jack Harlan (1917- 1998) set up plant collections.

The exotic germplasm in gene banks has traditionally been used as sources of genes for resistance to diseases and insects (Simmonds 1976). However, breeders can also exploit this preserved germplasm for the improvement of complex traits important

to agriculture, nutritional quality, yield and stress tolerance. Improvement of such quantitative traits using exotic genetic resources has some problems such as inheritance problems and the fact that undesirable genes can be carried into elite lines along with the gene of interest (linkage drag). These problems have been overcome with the development of molecular marker technology. Molecular markers are genetic markers which have been increasingly applied for trait improvement over the past 30 years.

1.2. QTL Mapping

Genetic markers can be divided into three major types. The first type is morphological markers, which are visually characterized phenotypic characters such as flower color and seed shape. The second type are biochemical markers, which consist of different allelic forms of enzymes or developmental stage of the plant (Winter and Kahl 1995). Because of the benefits called isozymes. Both these types of markers have disadvantages such as they are easily influenced by environmental factors or the developmental stage of the plant, in addition they are limited in number. (Winter and Kahl 1995). Besides these drawbacks these types of markers are useful to plant breeders (Eagles, et al. 2001, Weeden, et al. 1994). The third type of markers, molecular markers, reveal sites of variation in DNA (Jones, et al. 1997, Winter and Kahl 1995).In comparison with other types of genetic markers, molecular markers are the most common genetic markers due to their abundance. DNA markers arise from mutations such as substitution mutation (point mutation), rearrangements (insertion or deletions) or errors in replication of tandemly repeated DNA (Paterson 1996a). Molecular markers are generally located in non-coding regions of DNA, therefore they are neutral and can not be visualized in phenotype. 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).

Markers that reveal differences between individuals of the same or different species are called polymorphic markers. If a marker does not discriminate between genotypes, it is called a monomorphic marker. Polymorphic markers are also described as dominant or codominant, depending on if the marker can discriminate between homozygotes and heterozygotes. While codominant markers can distinguish between homozygotes and heterozygotes by differences in fragment size, dominant markers are either present or absent.

As previously mentioned, DNA based molecular markers reveal polymorphism at the DNA level. During the last two decades, 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), single-nucleotide 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.

Lately, two of the most popular marker types in tomato research have been SSR and COS markers (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. In recent years, due to the availability of whole genome sequences and large EST databases, “conserved orthologue set” (COS) markers have been developed by comparing the Arabidopsis genomic sequence with the EST database

of tomato, a distant relative (Fulton, et al. 2002). It was argued that these markers will prove useful in comparative mapping among fairly divergent genomes, and therefore, may also prove useful for taxonomic studies and in deducing phylogenetic relationships between different genera and species.A potential disadvantages of COS markers is showing low polymorphism because of their good conservation (Wu, et al. 2006). Advanced development of COS markers are COSII markers which are similar to COS markers but have additional phylogenetic evidence to support orthology of sequences. More than 550 of these COS markers have been mapped in tomato, pepper and eggplant and in future these will certainly be mapped in other crops (Fulton, et al. 2002, Rudd, et al. 2003, Wu, et al. 2009 a, b, c).

Linkage maps indicate the positions of and relative genetic distances between markers on the chromosome of a species. Linkage maps are used to identify chromosomal locations that contain genes and QTLs for traits of interest. Thus, these types of maps are called QTL or genetic maps. QTL mapping depends on segregation of genes and markers via chromosomal recombination during meiosis so their analysis can be done in progeny (Paterson 1996a). 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). For these longer distances, mapping functions are applied to the data to convert recombination frequency to map distance. Linkage maps are constructed by marker analysis of a segregating population and the mapping process occurs in three main steps. Firstly, a mapping population is produced, then polymorphisms are identified and at last marker linkage analysis is done.

Genetic mapping studies generally use from 50 to 250 individuals in a segregating plant population. Populations can be comprised of self-pollinating or cross pollinating species. For self pollinating species, parents are both highly homozygous

(inbred). On the other hand, cross pollinating populations may derive from a cross between a heterozygous parent and a homozygous parent (Wu, et al. 1992). Several types of populations can be used for mapping and they have some advantages and disadvantages (Paterson 1996a). F_2 populations are derived from F_1 hybrids and BC populations are derived from crossing F_1 hybrid with one of the parents. The main advantages of BC and F_2 populations are their quick and easy production. Thus, F_1, F_2 and BC_1 populations are used to identify desirable QTL alleles in early generations. On the other hand, these favorable QTL alleles often lose their effects when introgressed into genetic background of elite lines. This problem may be overcome by the use of different population types. For example, recombinant inbred lines (RI) are derived from F_2 plants and consist of a series of homozygous lines. Each line contains a unique combination of chromosomal segments from the original parents.The most important drawback for RI populations is that they require a long time for producing six to eight generations. Double haploid (DH) populations can also be developed. DH and RI populations' most significant advantages are that they consist of homozygous lines which can be reproduced without any genetic changes occuring. This permits replication of mapping experiments across different locations and years, therefore both types of populations provide enormous resources for QTL mapping.

Polymorphism between the two mapping population parents must be identified before the construction of linkage maps begins. Cross pollinating species have higher polymorphisim level than inbreeding species. Once polymorphic markers have been identified, all mapping population individuals and the parents are screened with each marker. Thus, the genotype of each individual is determined for each molecular marker.

The last step for construction of a linkage map is linkage analysis of markers. The molecular marker genotypes of each individual of the population are analysed by using computer programs. Linkage between markers is usually calculated with the logarithm of odds (LOD) value or LOD score (Rish 1992). If a LOD value of >3 supports linkage of two markers, these markers can be used for map construction. A LOD value of 3 between two markers indicates that linkage is 1000 times more likely than no linkage. By determining linkage between pairs and sets of markers in this way, a genetic linkage map for the whole genome is constructed.

With the availability of DNA markers, saturated genetic maps can be constructed which allow the identification and use of many sources of valuable alleles for the improvement of traits. These traits can be simply inherited (qualitative) or

controlled by several genes (quantitative). Quantitative trait analysis is more complex than qualitative trait analysis, however, molecular maps make the process relatively straight forward.

QTL analysis depends on the principle of detecting an association between phenotype and marker genotype. The main purpose of using markers is to partition the mapping population into different genotypic groups. These groups are based on presence or absence of a specific marker locus and to determine if important differences exist between groups for the trait of interest (Tanksley 1993, Young 1996). Significant differences between phenotypic means of genotypic groups for a marker indicate that the marker is linked to a gene or a QTL controlling the trait. If a marker is closely linked to a QTL, there is a low probability that recombination will occur between the marker and QTL. Because the QTL and the marker are usually inherited together in the progeny, the means of the different genotypic groups for the tightly-linked marker will differ. If the marker is unlinked to the QTL, independent segregation occurs between the marker and QTL. As a result, no significant differences between genotypic groups will occur.

There are three commonly used methods for detecting QTLs: (1) single-marker analysis, (2) simple interval mapping and composite interval mapping (Liu 1998, Tanksley 1993). The simplest method is the single-marker analysis method because it involves single markers. Statistical methods including t-tests, analysis of variance (ANOVA) and linear regression are used for this method. A whole linkage map is not required and analysis can be performed with basic statistical software programs. Although it is a simple method, there is a major disadvantage in that linkage between distant markers and QTL can not be detected easily (Tanksley 1993). The simple interval mapping (SIM) method uses linkage maps and analyzes intervals between adjacent pairs of linked markers along the chromosome (Lander and Botstein 1989). Compared to single marker analysis, the interval mapping method is statistically more powerful because using linked markers for analysis compensates for recombination between QTL and markers (Lander and Botstein, 1989, Liu 1998). Composite interval mapping (CIM) is the most complex of the three methods and is more effective and exact at mapping QTL as compared to other methods.

Identifying and mapping genes or QTL controlling complex traits in tomato using molecular markers started in the 1980s. Initial studies used morphological and isozyme markers. The first comprehensive and systematic utilization of markers to

dissect the genetic control of complex traits in tomato was done by Paterson, et al. (1988). In this study a complete RFLP map was employed to map QTLs for fruit quality. Afterwards QTL mapping studies for agronomically important traits became popular in tomato. Especially in the past decade, a molecular breeding strategy has become increasingly popular to use QTL mapping to exploit naturally available genetic resources for identification of new traits and improvement of crop performance in tomato and other species (Peleman and van der Voort 2003). This strategy, advanced backcross QTL analysis (AB-QTL), was described by Tanksley and Nelson (1996) as a new breeding strategy that integrates QTL discovery with variety development by simultaneous introgression of useful QTL alleles from wild germplasm into elite germplasm. This approach uses BC_2 and BC_3 populations derived from an interspecific cross while QTL mapping uses an early generation for identification and mapping of trait loci. The AB-QTL method has two important advantages. One advantage is that both phenotypic and molecular-marker analyses occur at a more advanced generation when the frequency of cultivated parent's alleles are much higher. The second advantage of this approach is that desirable QTL alleles are identified for various loci and only a few more marker-assisted generations are required to develop near isogenic lines (NIL_s) or introgression lines (IL_s) which can then be used for cultivar development.

Tomato was the first crop to which the AB-QTL method was applied (Tanksley, et al. 1996). *S. lycopersicum* cultivar E6203 was chosen as a recurrent parent for this project because of its wide availability as an open-pollinated processing variety and its commercial importance. There were several goals of this first AB-QTL project: firstly, testing of *S. pimpinellifolium* germplasm as a source of useful, new QTLs; secondly, testing the effect of molecular marker technology on QTL discovery; and lastly, developing new lines that would outperform elite commercial varieties for soluble solid content, while maintaining good quality for other important traits such as fruit size, yield, firmness, viscosity and color. In order to identify useful, new QTL, wild germplasms are used as donors for the AB-QTL method in tomato.

So far, several AB-QTL studies were conducted in tomato and in these studies E6203 was crossed with five different wild *Solanum* species: S. *pimpinellifolium* (LA1589) (Grandillo and Tanksley 1996b, Tanksley, et al. 1996), *S. peruvianum* (LA1708) (Fulton, et al. 1997), *S. habrochaites* (LA1777) (Bernacchi, et al.1998a.b), *S. neorickkii* (LA 2133) (Fulton et al. 2000) and *S. pennellii* (LA 1657) (Frary, et al.

2004). All of these wild *Solanum* species have many important resistance genes. *S. pimpinellifolium* was the species that was most closely related to *S. lycopersicum* in contrast with *S. peruvianum* which was the most distantly related species. In addition, *S. peruvianum* appears to be one of the best sources for resistance to disesases according to previous studies. These resistances are for diseases such as leaf mould (Kerr, et al. 1971), fusarium crown and root rot (Berry and Oakes 1987), corky root rot (Laterrot 1978), tomato mosaic virus (Laterrot and Pecaut 1969, Hall 1980), tomato spotted wilt virus (Stevens, et al. 1995), and root-knot nematode (Smith 1944).

For genotypic analysis in these AB-QTL studies, a minumum of 121 markers was used in the *S. pimpinellifolium* study and a maxiumum of 171 markers was used in the *S. peruvianum* study. Significant traits for the tomato processing industry were phenotyped with 19 traits measured in the *S. habrochaites* study and up to 35 traits identified in the *S. peruvianum* study. The studies examined traits such as yield, fruit firmness, fruit size, fruit weight, fruit shape, soluble solid content, pH, fruit colour and antioxidants. Fruit appearance including size, shape and color is an important characteristic for breeders, industry and consumers. All of these QTL studies studied fruit size because fruit size is an example of a trait altered by domestication and cultivated and wild tomato species show a wide diversity of fruit size. Thus, molecular marker research has revealed the presence of approximately 30 QTLs involved in control of this trait (Chen, et al. 1999) and a major fruit weight QTL was isolated (Frary, et al. 2000). The AB-QTL studies also examined fruit shape because great variations in fruit shape are also observed in tomato which can range from oblate to round to ovate to pear shape. In addition to the AB-QTL studies, additional work in *S. pimpinellifolium* and *S. pennellii* (van der Knaap and Tanksley 2003) has shown that much of the variation in tomato fruit shape is controlled by few major loci and that the great variation seen in cultivars is due to allelic variation at these loci (van der Knapp and Tanksley 2003).

Another significant quality studied in the AB-QTL populations is fruit color. The red color of tomato is due to lycopene and consumer interest in the benefits of fruit and vegetable consumption on human health is increasing day by day. As a result there has been much interest in the genetic control of this trait. For general fruit color as determined by appearance, QTL analysis has been performed in AB-QTL populations obtained by crossing *S. lycopersicum* with *S. peruvianum* (Fulton, et al. 1997), *S. pennellii* (Frary, et al. 2004b), *S. chimielewskii* (Frary, et al. 2003), and *S. habrochaites*

(Monforte and Tanksley 2000a). In addition, the content of lycopene and other carotenoids (e.g. beta-carotene) has been identified and mapped in tomato (Wann and Jourdain 1985). For fruit color as determined by lycopene and beta-carotene content, *S. cheesmannii*, *S. pennellii* (Ronen, et al. 2000, Zhang and Stommel 2000, Rousseaux, et al. 2005), *S. parviflorum* (Fulton, et al. 2000) and *S. pimpinellifolium* (Chen, et al. 1999) populations have been used.

In all of the AB-QTL studies, total yield, red yield and other important fruit quality characteristics were measured. In this way, favorable wild QTL alleles were identified in the five interspecific AB-QTL populations. The percentage of desirable wild alleles was estimated between a minumum 3% for total red yield to a maximum of 88% for soluble solids content (Grandillo and Tanksley 2005). With reference to these results, approximately 30% of wild species QTL alleles can be superior to the cultivated parent allele. The rate of discovery of new, useful QTL alleles was calculated as 50% after sampling of many wild species genomes (Fulton, et al. 2000, Frary, et al. 2004). These results suggest that there are still new QTL alleles to be discovered in wild species germplasm.

1.3. Antioxidants

Consumption of tomato and its products has been associated with decreased risk of chronic diseases. Epidemiological studies confirm the effects of various antioxidants in tomato on human health (Binoy, et al. 2003). Not only tomato but also other fruits and vegetables contain high levels of phytochemicals and their consumption has been recommended to prevent chronic diseases related to oxidative stress in the human body. Antioxidants are crucial for maintaining an organism's health because of their significant role in the defence system against free radicals such as reactive oxygen species.

1.3.1 What are Free Radicals?

Free radicals are charged atoms, molecules or molecular fragments that have one or more unpaired electrons. These unpaired electron(s) give free radicals a high level of reactivity. Free radicals are able to receive electrons from or give unpaired electrons to other molecules (Hallwell and Gutteridge 2006). As a results of these pairing processes, new free radicals (some bad, some good and some with both characteristics) are formed. A high level of free radicals in cells is an undesirable situation because free radicals have harmful effects on DNA, lipids and proteins. In animals and humans, free radicals may cause ageing, many types of ageing diseases such as age-related immunodeficiencies as well as many types of cancer, cardiovascular disease and degenerative diseases of nervous system. In plants, free radicals may cause senescence, membrane leakage, chlorophyll destruction, decreased photosynthesis and yield (Vichnevetskaia and Roy 1999, Devasagayam, et al. 2004, Percivall 1998, Singh, et al. 2004).

The most important free radicals are those derived from oxygen and nitrogen and are called reactive oxygen species (ROS) and reactive nitrogen species (RNS), respectively (Devasagayam, et al. 2004, Percivall 1998). Radicals derived from oxygen are a class of radical species generated in living systems (Miller, et al. 1990, Halliwell 2006). Molecular oxygen (dioxygen) has a unique electronic configuration with two unpaired electron and is itself a radical. The simplest free radical is atomic hydrogen and the most reactive one is hydroxyl. The main source of ROS is mitochondria and, in plants, chloroplasts (Percivial 1998). Besides formation of ROS in the cell, environmental effects can contribute to the occurence of ROS. Cigarette smoke is a causative agent of free radicals as it contains nitric oxide and nitrogen dioxide which are active oxidants (Devasagayam, et al. 2004). Other external sources can be environmental pollutants, industrial solvents,certain drugs, pesticides, anaesthetics, X-rays and ultraviolet (UV) light (Madhavi, et al. 1996).

ROS have harmful effects on many organic molecules with their high reactivity. One of the most important harmful effects of ROS is on lipids and is called lipid peroxidation. Lipid peroxidation can be very harmful to the cell membrane. In addition, ROS can oxidize cholesterol which is associated with cardiovascular diseases and

atherosclerosis (Nordberg and Arner 2001, Devasagayam, et al. 2004, Ferrari and Torres 2003). Dangerous effects of ROS are also observed on DNA and such effects can be lethal for all organisms. Especially hydroxyl groups react with DNA and can cause many alterations, such as DNA-protein crosslinkage, cleavage of DNA and purine oxidation. These DNA alterations can result in mutations together with high incidence of cancer (Singh, et al. 2004, Percival 1998, Nordberg and Arner 2001). In addition, ROS damage mitocondrial DNA whose activity is thought to be associated with ageing (Nordberg and Arner 2001).

Although the bad effects of ROS are usually emphasized, there are some beneficial ROS that have positive effects. For instance, ROS molecules help in phagocytosis and have important roles in signal transduction (Nodeberg and Arner 2001). In a normal cell, free radicals and antioxidants are in balance and this position is maintained by the antioxidant defence system. If the balance is upset between ROS and antioxidants, oxidative stress can result. Thus, the occurrence of too many ROS in relation to the available antioxidants is called oxidative stress. Oxidative stress has been linked to a hundred types of human diseases and ageing (Devasagayam, et al. 2004). Luckily, antioxidants help to control the free radicals balance in cells.

1.3.2. Antioxidants

Antioxidants are substances that serve to control the levels of free radicals, allowing them to perform useful biological functions without too much damage (Halliwell and Gutteridge 2006). Therefore, antioxidants reduce the risk of several diseases related with oxidative stress (Percival 1998).

The antioxidant defence system includes many types of molecules, hence, antioxidants are classified in many ways. One way is based on their solubility: i) water-soluble antioxidants and ii) lipid-soluble antioxidants. Another way to classify them is by their origin: i) endogenous antioxidants, which are synthesized in the organism, ii) exogenous antioxidants, which are obtained from outside (Vichnevetskaia and Roy 1999). Antioxidants are also categorized relative to their enzymatic functions into two groups: i) enzymatic antioxidants and ii) non-enzymatic antioxidants (Somogyi, et

al.2007). Because the focus of this thesis is non-enzymatic antioxidants, only these types of compounds will be discussed here.

1.3.3. Non-enzymatic Antioxidants

Non-enzymatic antioxidants include water-soluble and lipid soluble compounds. Vitamin C or ascorbic asid is a water-soluble vitamin with a considerable capacity for electron reduction. It is a reducing agent because it is an electron donor. Vitamin C easily reacts with ROS and prevents the degeneration of macromolecules due to oxidative stress. Plants synthesize great amounts of vitamin C. Vitamin C also acts as a co-substrate in enzymatic reactions and plays a role in regeneration of lipid–soluble vitamin E which controls ROS production in lipid membranes and lipid proteins (Madhavi, et al. 1996). Vitamin E is especially rich in many plant oils, including soybean,sunflower and maize oil (Vichnevetskaia and Roy 1999). They have an important role in the cell membrane protecting against ROS damage. Vitamin E is a hydrogen donor and the most efficient chain breaking antioxidant. Carotenoids are also lipid-soluble. They are pigments and provide the color of yellow, orange and red fruits and vegetables. Also some animals such as salmon, crustaceans and egg yolk have carotenoids. Some studies have revealed the beneficial effect of carotenoids on some diseases; for example, certain types of cancer, age-related muscular degeneration and artherosclerosis (Valko 2005). β carotene is a precursor of vitamin A. Lycopene is a lipid soluble pigment and is the strongest antioxidant and the most significant type of carotenoids. Tomato skin, watermelon and grapefruit have significant amounts of lycopene. Lycopene is associated with prevention of prostate cancer and cardiovascular diseases in the human body (Arab and Steck 2000).

Phenolic compounds are water-soluble molecules that are produced by plants and are widely found in fruits and vegetables (Podesek 2007). Flavonoids, phenols and phenolic acid are the most important phenolic compounds. Their effects are observed in plants' aroma, taste and color (Sakihamaet, al. 2002). Almost all plants fundamentally synthesize phenolic compounds against the negative effects of ROS. In addition to ROS, phenolic compounds defend plants against stress conditions, such as abiotic and

biotic stress. According to studies, phenolic compounds have antimutagenic, anticarcinogenic, antiviral and antimicrobial impacts (Sakihama, et al. 2002, Lule and Xia 2005). Flavonoids are a main class of phenolic compound and have an important role in biological process. Besides their function as pigments in flowers and fruits to attract pollinators and seed dispersers, flavonoids are involved in fertility, disease resistance and UV-scavenging. They include anthocyanins, flavanols, flavons, catechins, and flavonones (Harborne and Williams 2000). Flavonoids are integral parts of the human diet due to their effect on human health (Elio, et al. 2004).

1.4. Goals of This Work

The goals of this work were to assess the usefulness of *S. peruvianum* as a donor for improvement of antioxidant traits in cultivated tomato and to identify polymorphic markers for use in mapping genes for these traits. Thus, total water soluble antioxidant content, total phenolic content and vitamin C content were determined for *S. peruvianum* accession LA2172, *S. lycopersicum* accession TA496 and 115 individuals of an advanced backcross population. In addition to these nutritional quality traits, several horticultural traits were also measured: fruit weight and shape, internal and external color, locule number, firmness and stem scar size. Molecular markers analysis techniques were then used to identify markers that were polymorphic in the population.

CHAPTER 2

MATERIALS AND METHODS

2.1. Plant Material

The advanced backcross BC_2F_2 population was developed by crossing *S. lycopersicum* (TA496), the cultivated recurrent parent, with S. *peruvianum* (LA2172), the wild donor parent. The BC_2F_2 mapping population was developed by Dr. Sami Doganlar in the following stages: i) A simple cross was made between TA496 and LA2172 using the wild accession as a maternal parent and the cultivated genotype as a pollen source; ii) The F_1 hybrids were backcrossed to the recurrent parent (TA496) in order to obtain a BC_1F_1 population; iii) Second backcrosses were made between the BC_1F_1 individuals and the recurrent parent which was used as the female parent. In this way, the BC_2F_1 population was obtained. The resulting BC_2F_2 population consisted of 118 lines.

2.2. Phenotypic Characterization

Analyses of nutritionally and agronomically important traits were made for QTL identification. Total water soluble antioxidant activity, total vitamin C content, total phenolic content, total flavonoid content, and lycopene content were the nutritionally important traits examined in this work and they were detected by biochemical assays. Eight agronomically important traits were also determined including total fruit weight, fruit firmness, fruit shape, external and internal fruit colour, stem scar, locule number and fruit wall.

2.2.1. Preparation of Samples for Antioxidant Trait Analysis

Tomato fruits were harvested from ten plants of each line at normal market stage in August 2006. After fruits were picked, they were washed and about one kilo fruits for each sample were selected. For the biochemical analysis, each fruit sample was cut into pieces and mixed well. All tomato fruit mixtures were packed and stored at -20^{o}C until analyses were begun. All analyses were performed within four months of harvest. Biochemical analyses were performed as described below.

2.2.2. Determination of Total Water Soluble Antioxidant Activity

Total water soluble antioxidant activity of tomato fruits was measured spectrophotometrically (Shimadzu, 1700 UV Visible Spectrophotometer, Japan). In the first step tomato fruit extract was prepared. For this, approximately 200 g of fruit was weighed and then homogenized with 100 ml cold distilled water for 2 min at low speed in a Waring blender equipped with a 1 L double walled stainless steel jar at +4^{o}C. After homogenization, 10 g homogenate was taken and diluted with 15 ml cold water. Homogenized fruit pulp was then filtered through 4 layers of nylon cloth into 15 ml falcon tubes. Centrifugation of filtered samples at 3000 x g for 10 min at 4^{o}C was used to clarify supernatant. Afterwards, clear supernatants were merged into one 50 ml falcon tube and then were filtered through 3 layers of nylon cloth to obtain a clearer supernatant for determination of antioxidant activity according to the method of Re et. al.(1999).

In the second step, ABTS radical cation decolorization caused by the test samples was monitored by spectrophotometer at 734 nm. The ABTS stock solution was prepared as a mixture containing 2.45mM potassium persufate and 7mM ABTS which was then stored in the dark for 12-16 hours. Phosphate buffered saline (PBS) at pH 7.4 was used to dilute the ABTS radical cation stock solution and adjust its absorbance to 0.70 at 734 nm. After this procedure 2.5, 5 and 7.5 µl aliquots of tomato supernatant were mixed separately with 2 ml ABTS radical cation solution and decolorization of the

blue-green solution was kinetically monitored at 734 nm for 6 min at 30^{o}C. The decrease in absorbance of each sample was monitored for 6 min and tests were conducted three times at each sample volume.

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid) was used as a standard and results were calculated as area under the curve (AUC) and expressed as μmol Torolox/kg fresh weight of tomato fruits. To calculate AUC, the percent inhibition/concentration values for extracts and Trolox were plotted separately against the test periods. The ratio of the areas of curves for extracts and Trolox was used to calculate the AUC value.

2.2.3. Determination of Vitamin C

Tomato Vitamin C content was determined by the AOAC 967.21 titrimetric method using 2,6-dicloroindophenol as reactive substance (Augustin 1994). 100 g tomato and 115 ml acetic acid were put into a Warning blender and homogenized for 2 min at low speed at +4^{o}C to obtain tomato extract. Then, 25 g extract was taken and diluted with 100 ml cold extraction buffer. Each homogenate was filtered through filter paper. Then 15 ml filtered sample was titrated against a 2,6-diclorindophenol dye solution. For each tomato extract, the vitamin C content of three replicate samples was measured. Calibration of the titrator was done by using commercial L-ascorbic acid and the results were expressed as mg ascorbic acid/kg fw of tomato fruit.

2.2.4. Determination of Total Phenolic Compounds

Total phenolic content of tomato fruits assay was adopted according to Singleton and Rossi (1965). In this assay, spectrophotometric measurements were done by using Folin- Ciocalteau reactive reagent. For the standard curve, gallic acid was used. For tomato extracts, 200 ml cold distilled water and 100g tomato sample were blended with a Waring blender at 4^{o}C for two min at low speed. Then 2.5 g extract was taken from

the homogenate and diluted with 20 ml cold distilled water and centrifuged at 3000 x g for 10 min at +4^{o}C in a refrigerated centrifuge (Eppendorf). Total phenolic content was measured on the clear supernatant. In this process, 2 ml supernatant was mixed with 10 ml 2 N (10%) Follin-Ciocalteu and incubated for 3 min, then 8 ml 0.7 M Na_2CO_3 was added. After 2 hours incubation at room temperature, the absorbance of the reaction mixture was measured at 765 nm in a spectrophotometer (Shimadzu, 1700 UV Visible Spectrophotometer, Japan). Three replicates for each sample were used and the results were expressed as gallic acid equivalents (mg/kg fresh weight).

2.2.5. Visual Score of Agronomically Important Traits

According to visual appearance, fruit traits for eight agronomically important traits including total fruit weight, fruit firmness, fruit shape, external and internal fruit colour, stem scar, locule number and fruit wall were determined for each individual of the BC_2F_2 population.

Fruit weight (FW) was measured by taking the average weight of 10 tomato fruits for each sample. Fruit shape (FS) was determined by comparing the ratio of fruit length to fruit width using a scale from 1 to 5 (1 = round, 5 = elongated). Fruit firmness (FIRM) was determined by hand squeezing of ripe tomato fruits using a scale of 1 to 5 (1 = soft, 5 = very firm). External (EC) and internal fruit color (IC) were also visually evaluated for each line using scale from 1 to 5 (1 = yellow or orange, 5 = scarlet). Fruit stem scar size (SCAR) was determined by fruit stem scar diameter (1 = small, 5 = very large). Locule number (LN) of tomato fruit was evaluated by counting the locules of tomato fruit after transverely cutting the fruit. Fruit wall (WALL) or thickness of pericarp was measured by cutting fruits transversely and using a scale of 1 to 5 (1 =thin, 5 = very thick).

2.3.Genotypic Characterization

2.3.1. DNA Extraction

DNA isolation was done from leaves of tomato seedlings based on the protocol described by Bernatzky and Tanksley (1986). Tomato leaves from ten plants for each sample were collected in the field and put into liquid nitrogen to freeze immediately and then transported to Izmir Institue of Technology. The samples were stored at -80^{o}C until DNA extraction was performed. After DNA isolation, the quality and quantity of each sample DNA was measured with nano-drop ND-1000 spectrophotometer. For PCR amplification, each sample DNA was diluted to approximately 50 ng/µl with distilled water.

2.3.2. Molecular Marker Analysis

COS II (conserved orthologue set) and SSR (simple sequence repeat) marker analyses were performed in this study. Marker analyses were begun with parental surveys to identify a sufficient number of polymorphic markers for mapping. Therefore, TA496 and LA2172 parental DNAs were tested first. For both assays the same PCR mixture was prepared and amplified in two types of thermocycler (GeneAmp® PCR System 9700, Applied Biosystems; Authorized Thermal Cycler, Mastercyler epgradientS, Eppendorf). PCR mixture comprised: 2 µl DNA (~ 50ng/µl), 2.5 µl 10X PCR buffer (50 mM KCL, 10mM Tris-HCL, 1.5 mM MgCL2, pH: 8.3), 0.5 µl dNTP (0.2mM), 0.5 µl of each forward and reverse primers (10 pmol), 0.25 µl Taq polymerase (0.25 U) and 18.75 µl sterile distilled water.

For COS II assay, COS55 method was used. PCR conditions of this method were (i) 94^{o}C for 5 min followed by 35 cycles at 94^{o}C for denaturation for 30 s, (ii) 55^{o}C for annealing for 45 s and (iii) 72^{o}C for 45 s for extension and one last cycle at 72^{o}C for final extension for 5min. For SSR assay two types of method were used,one of

them was SSR55; (i) 94^{o}C for 3min followed by 35 cycles at 94^{o}C for denaturation for 30 s, (ii) 55^{o}C for annealing for 1min and (iii) 72^{o}C for 1 min for extension and one last cycle at 72^{o}C for final extension for 5min. The other method was SSR50; (i) 94^{o}C for 5 min followed by 35 cycles at 94^{o}C for denaturation for 30 s, (ii) 55^{o}C for annealing for 1 min and (iii) 72^{o}C for 1 min for extension and one last cycle at 72^{o}C for final extension for 5 min. Parents were screened with a total of 160 PCR-based molecular markers (72 SSR, 88 COSII) Polymorphism of COSII markers was surveyed using 29 restriction enzymes, including, TaqI, AluI, BamHI, Rsa, DraI, DpnII, EcoRI, EcoRV, HincII, HindIII, HinfI and KpnI at 37^{o}C, 55^{o}C, or 65^{o}C (depending on the optimum conditions for each enzyme) for 12 h with mineral oil added to prevent evaporation. Enzyme digestion mixture for 25 µl PCR product included 3µl 10 X digestion buffer (1X), 0.5 µl enzyme and 1.5µl sterile distilled water.

PCR and/or digestion products were checked by gel electrophoresis. COSII marker fragments were separated on 2% agarose gels in 1 X TBE buffer (0.9 M Tris, 0.9 M boric acid, 0.002 M NaEDTA and pH 8.3) at 110V for 2 h. For these markers, patterns were visualized under UV using ethidium bromide. SSR marker fragments were separated with capillary electrophoresis using the QIAxcel DNA Screening Kit (2400) (QIAxcel, QIAGEN).

2.4. Statistical Analysis

Student's t-test was performed with the Statview computer program with significance determined at $P<0.05$. Correlation analysis was performed with the Qgene software program (Nelson 1997).

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Phenotypic Characterization

Mean phenotypic values, standard errors and ranges for the antioxidant and horticultural traits measured in the parental lines and BC_2F_2 population are displayed in Tables 1 and 2. All of the traits analyzed exhibited continuous variation in the BC_2F_2 population, typical of quantitative traits. This continuous variation was also expected because the parents of the BC_2F_2 population were selected to be distinct from each other for the traits of interest to contribute to the enhancement of variation in the population and to allow gene mapping.

3.1.1. Total Water Soluble Antioxidant Capacity

The total water soluble antioxidant activities (WAOX) for the 112 BC_2F_2 lines and their parents are displayed in Table 1. *S. peruvianum* WAOX activity of the fruit was 1.5 fold higher than *S. lycopersicum* and this difference was statistically significant at $P<0.001$. WAOX activity of the small green fruits of *S. peruvianum* was calculated as 8764 ± 110 µmol Trolox/kg weight. *S. lycopersicum* AOX activity was determined to be 4767 ± 215 µmol Trolox/kg. The mean value of WAOX activity of the BC_2F_2 population was 4798 ± 55 µmol Trolox/kg (Table 1). WAOX activity for the population ranged from 3622 to 6757 µmol Trolox/kg fresh tomato. According to the results, the mean value of the population was very similar to the value for *S. lycopersicum*. The highest mean value of WAOX activity was 1.8 fold that of the lowest mean AOX value. These results indicate that the BC_2F_2 population showed variation that is typically for

quantitative traits. Additionally, 44% of the population had higher WAOX capacity than *S. lycopersicum* (Figure 1). Moreover, 33% of the population had WAOX values less than *S. lycopersicum*. This is the result of transgressive segregation which is due to new combinations of alleles in the population that are not found in the parents. According to the distribution graph of WAOX capacity for the BC_2F_2 mapping population, the population showed a normal distribution for this trait.

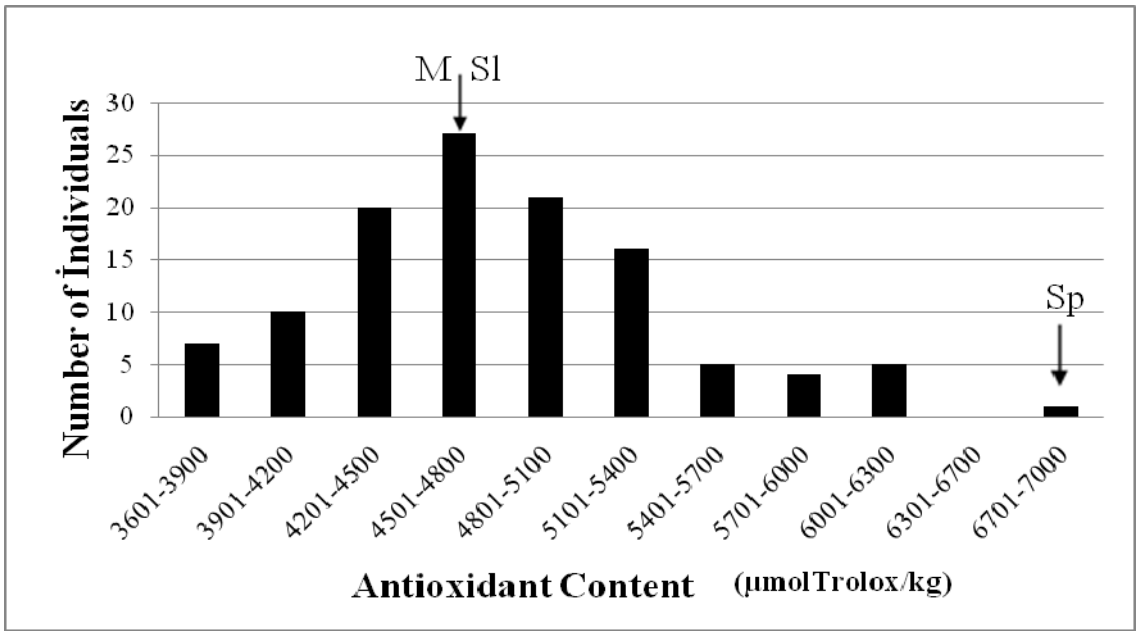

Figure 3.1. Distribution histogram for total water-soluble antioxidant activity. Sl, Sp and M indicate locations of *S. lycopersicum*, *S. peruvianum* and population means.

Table 3.1. Mean values and standard errors of parental lines and BC_2F_2 population for antioxidant traits. a and b letters means that there is a significant difference between two parental lines (P<0.05).

	S. lycopersicum	***S. peruvianum***	**BC2F2 Population**	
Trait	**Mean**	**Mean**	**Mean ± SE**	**Range**
WOAX(µmolTrolox/kg)	4767 ± 215a	8764 ± 110b	4798 ± 55	3622-6757
Vitamin C (mg/kg)	270± 0a	305± 0.8b	369 ± 0.6	177-556
Phenolic(mg/kg)	505 ± 2.2a	1619 ± 2.0b	559 ± 7.4	408-782

3.1.2. Vitamin C Content

Fruits of the BC_2F_2 population were rich in vitamin C content, containing a mean of 369 ± 0.6 mg/kg of fresh fruit (Table 1). Among the BC_2F_2 population, the fruit vitamin C content ranged between 177 and 556 mg /kg, a more than 3-fold difference for the trait and exhibited a normal distribution (Figure 2). Both parental lines had moderate vitamin C content values, which were significantly different at P<0.05. *S. lycopersicum* vitamin C content was measured as 270 ± 0 mg/kg and *S. peruvianum* vitamin C value was found to be 305 ± 0.8 mg/kg. Figure 2 depicts the distribution of vitamin C content and shows that 82% of the progeny had higher vitamin C content than *S. peruvianum* and 89% of progenies had higher values than *S. lycopersicum*. Again this result is due to transgressive segregation which is related with different alleles coming from each parent and resulting in increased value for the progeny.

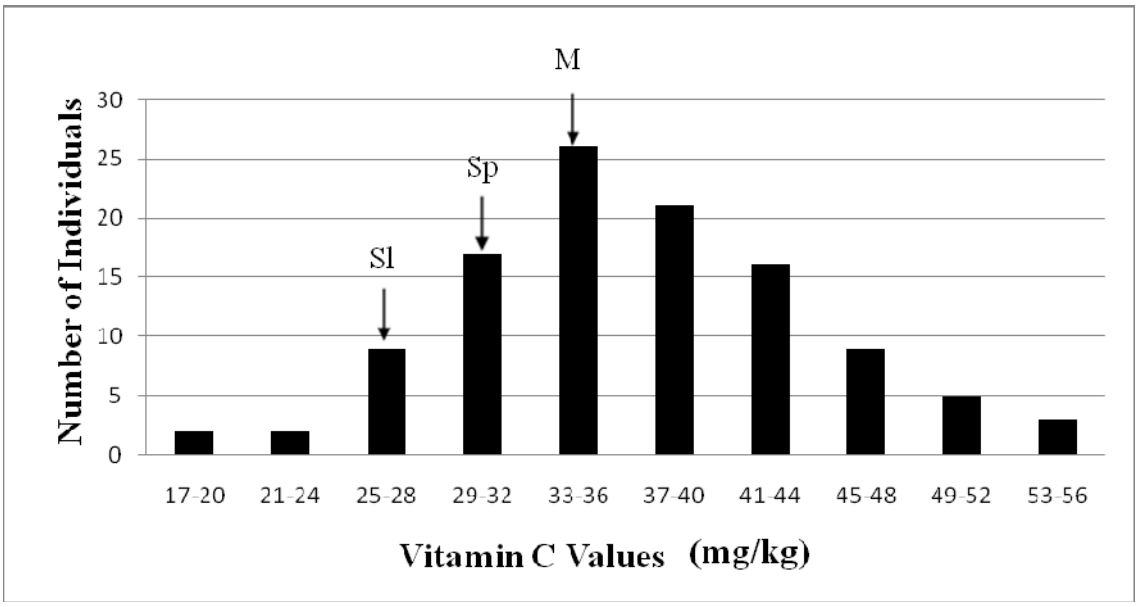

Figure 3.2. Distribution histogram for vitamin C content. Sl, Sp and M indicate locations of *S. lycopersicum*, *S. peruvianum* and population means, respectively.

3.1.3. Total Phenolic Content

In the BC_2F_2 progeny, the total phenolic content ranged from 408 to 782 mg/kg with an average of 559 ± 7.4 mg/kg (Table 1). Thus, the population showed great variation for phenolic compounds. Parental values were 505 ± 2.2 mg/kg for *S. lycopersicum* phenolic content and 1619 ± 2.0 mg/kg for *S. peruvianum* (Table 1). There was a 3.2 fold difference between the parents which was statistically significant at $P<0.0001$ and the population mostly resembled the recurrent parent for this trait. In Figure 3, continuous variation is shown for BC_2F_2 phenolic content and also 44% of the mapping population had higher phenolic compound content than the recurrent parent *(S. lycopersicum).*

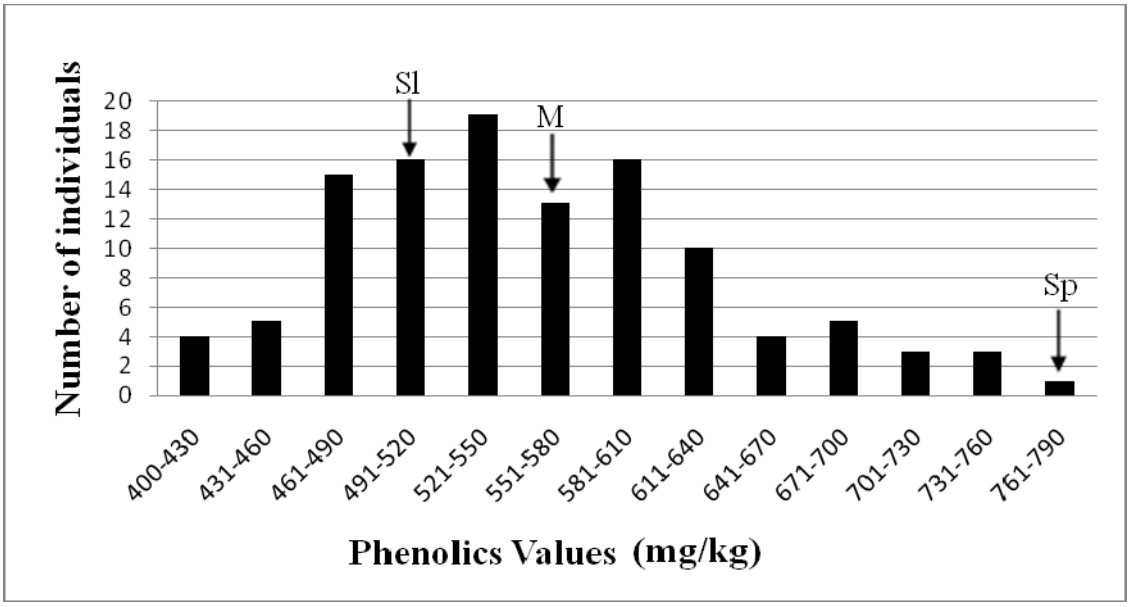

Figure 3.3. Distribution histogram for total phenolics content. Sl, Sp and M indicate locations of *S. lycopersicum*, *S. peruvianum* and population means, respectively.

3.1.4. Average Fruit Weight

Means, standard errors and ranges for agronomic traits evaluated for the two parental lines and BC_2F_2 population are given in Table 2. The two parents were strikingly different in fruit weight (FW); the mean FW of the recurrent parent, *S. lycopersicum*, was 118.2 g and the mean FW of the donor parent, *S. peruvianum*, was 5.6 g. Thus there was a 21 fold difference between the parental lines. The FWs of the BC_2F_2 progenies were intermediate between two parents, ranging between 38.9 and 98.5 g. The mean FW value was calculated as 68.4 ± 1.1 g (Table 2). Figure 4 shows the continuous distribution for the fruit weight trait in the population and it is obvious that the two parents have extreme values.

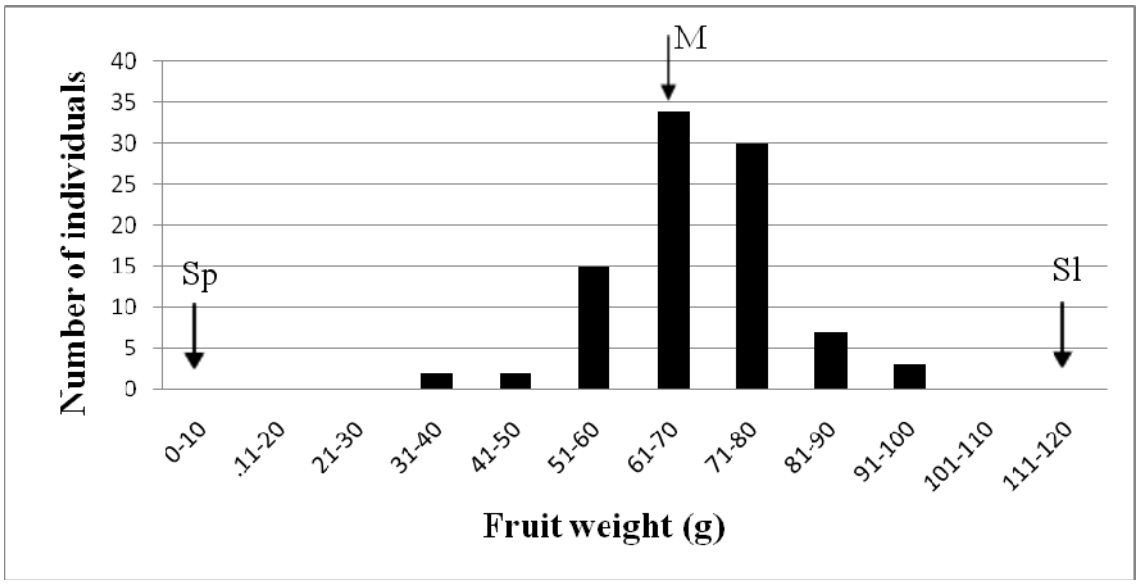

Figure 3.4. Distribution histogram for fruit weight. Sl, Sp and M indicate locations of *S. lycopersicum*, *S. peruvianum* and population means, respectively.

Table 3.2. The mean values and standard errors of parental lines and BC_2F_2 population for agronomic traits.

	S. lycopersicum	***S. peruvianum***	**BC2F2 Population**	
Trait	**Mean**	**Mean**	**Mean ± SE**	**Range**
Fruit Weight (g)	118.2	5.6	68.4 ± 1.1	38.9-98.5
Fruit Shape	4.5	1	3.5 ± 0.1	1.5-5
Firmness	4	5	3.9 ± 0.1	2-5
ExternalColor	3	1	3.3 ± 0.1	1-4.5
Internal Color	3	1	2.9 ± 0.1	1-4.5
Scar Size	4	1	3.8 ± 0.1	2-5
Locule Number	3	2	2.9 ± 0.1	2-4
Wall Thickness	4.5	1	3.6 ± 0.1	2-5

3.1.5. Fruit Shape

Fruit shapes (FS) of the two parental lines were distinctly different from each other. *S. lycopersicum* was scored as 4.5 and *S. peruvianum* was scored as 1 (1 = round, 5 = elongated). There was good variation ranging between 1 and 5 for FS in the BC_2F_2 population (Table 3). The mean value of fruit shape was scored as 3.5 ± 0.1, so generally progeny were intermediate between the parents and 40% of population had more elongated fruit than the average as can be seen in Figure 5.

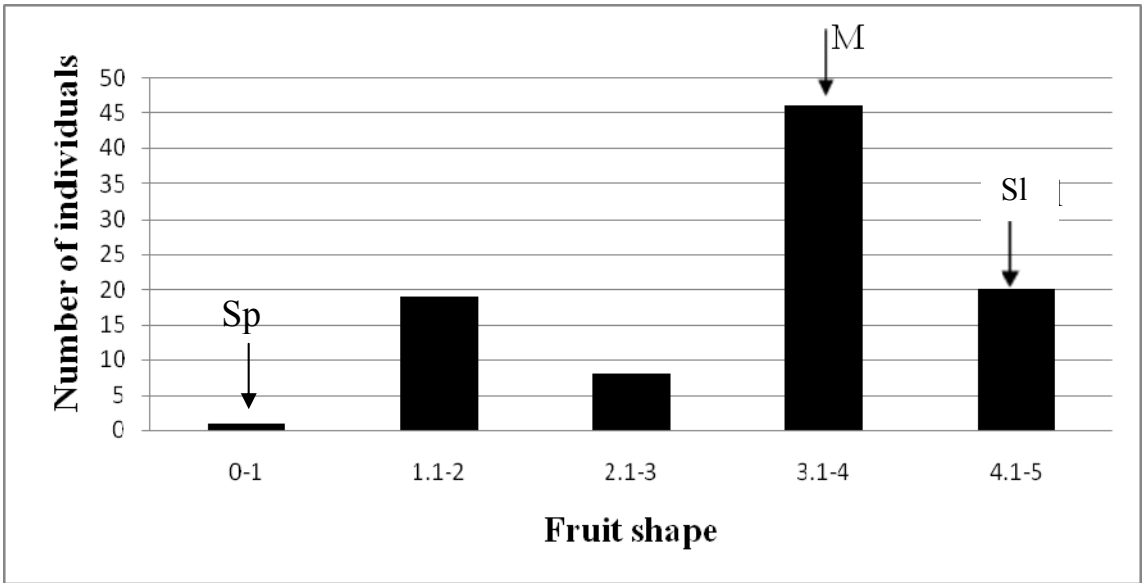

Figure 3.5. Distribution histogram for fruit shape. Sl, Sp and M indicate locations of *S. lycopersicum*, *S. peruvianum* and population means, respectively.

3.1.6. Fruit Firmness

In both parental lines, fruit firmness (FIRM) were scored similarly to each other, while *S. lycopersicum* was scored as 4, *S. peruvianum* had firmer fruit and was scored 5. Among the BC_2F_2 population, the fruit firmness was scored between 2 and 5 with a mean value of 3.9 ± 0.1 (Table 2). Figure 6 shows the distribution of fruit firmness in

the BC_2F_2 population. A total of 34% of the mapping population had lower values than both parental lines for firmness.

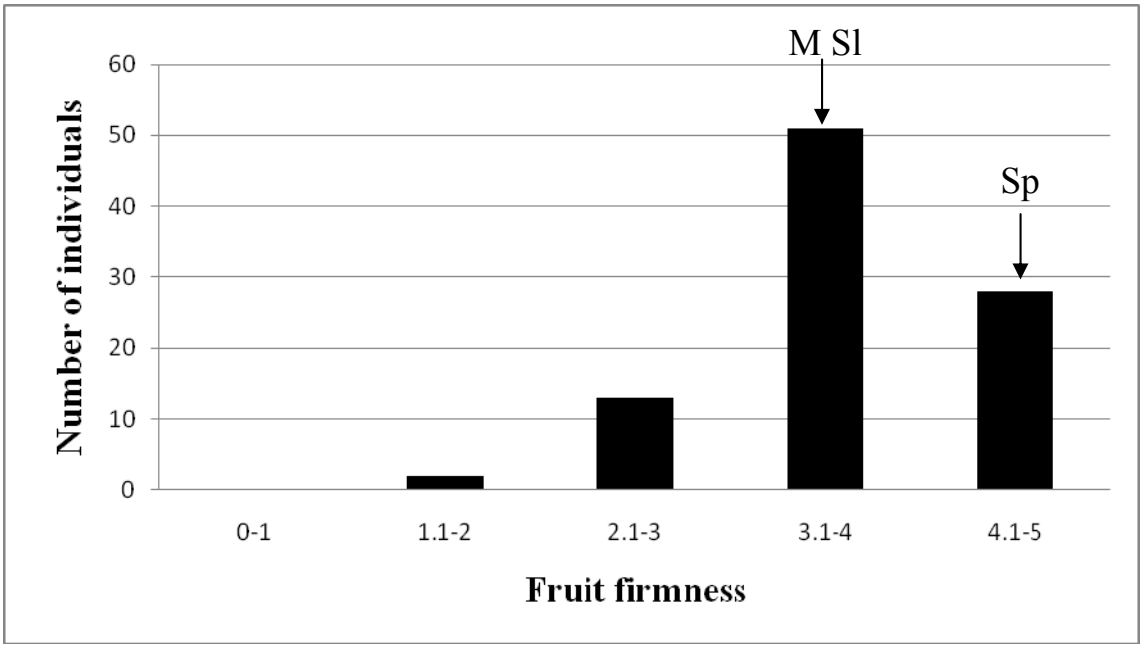

Figure 3.6. Distribution histogram for fruit firmness. Sl, Sp and M indicate locations of *S. lycopersicum*, *S. peruvianum* and population means, respectively.

3.1.7. External and Internal Fruit Color

External color (EXC) and internal color (INC) were determined as a moderate red colour of 3 for *S. lycopersicum*. *S. peruvianum* had green fruits so both EXC and INC were scored as 1. The mean external colour for the BC_2F_2 population was calculated as 3.3 ± 0.1, while internal colour was calculated as 2.9 ± 0.1 for the mapping population. There was a wide range of variation for both characters ranging from 1 to 4.5 (Table 3). Figure 7 and Figure 8 show the distribution histogram graphs for external and internal fruit color, respectively. A total of 52% of the BC_2F_2 population had better external fruit color than cultivated tomato and 48% of the population exhibit lower internal fruit color than *S. lycopersicum*. These results are also due to transgressive segregation.

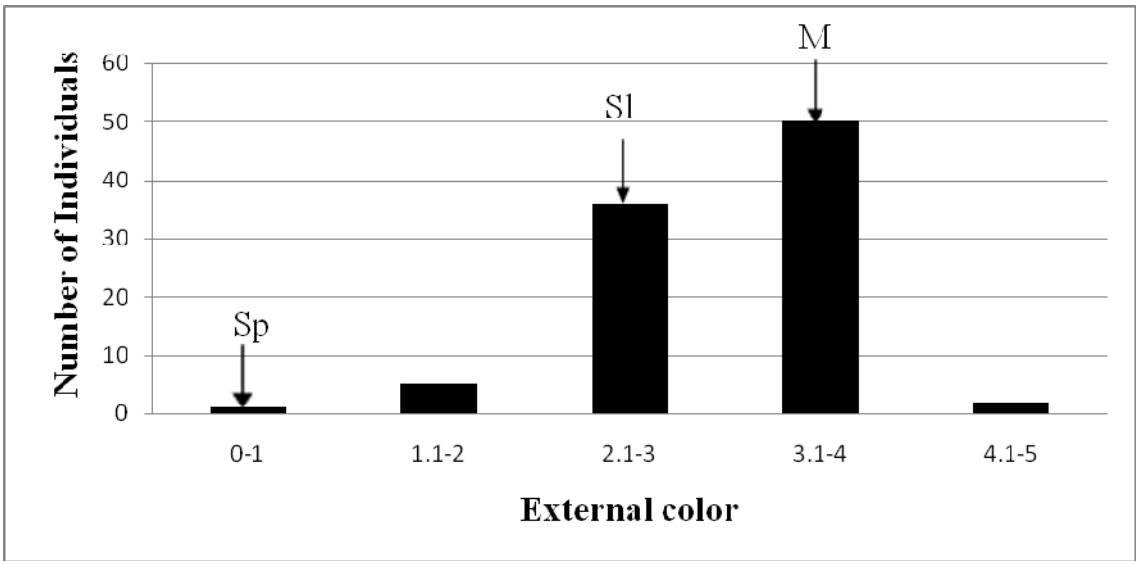

Figure 3.7. Distribution histogram for fruit external color. Sl, Sp and M indicate locations of *S. lycopersicum*, *S. peruvianum* and population means, respectively.

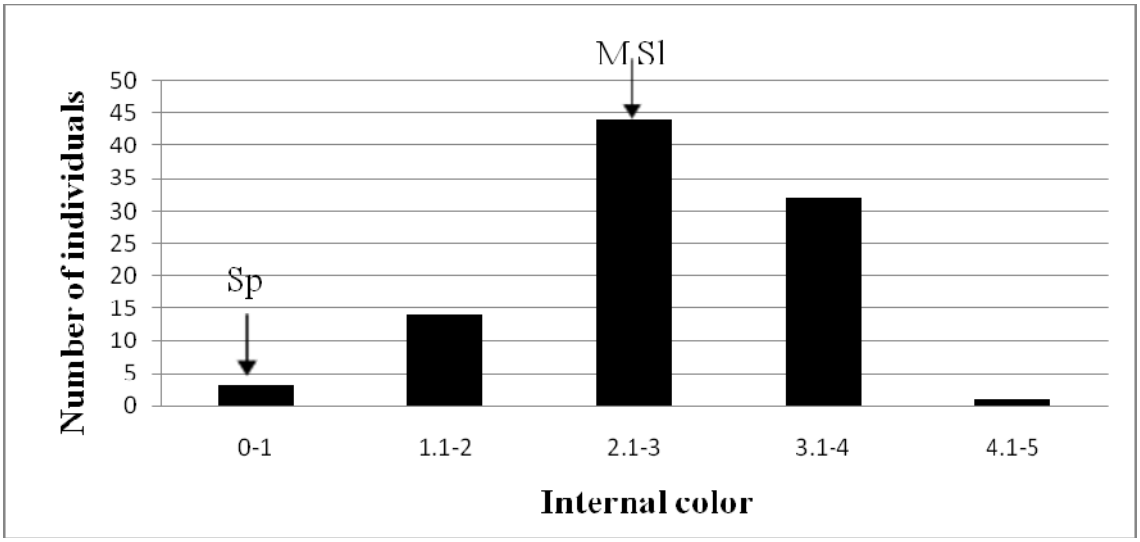

Figure 3.8. Distribution histogram for fruit internal color. Sl, Sp and M indicate locations of *S. lycopersicum*, *S. peruvianum* and population means, respectively.

3.1.8. Stem Scar

Stem scar size of *S. peruvianum* was very small and scored as 1 but *S. lycopersicum* stem scar was large and scored as 4. The two parents showed a 4-fold difference for this trait. The mean value for the population was 3.8 ± 0.1. (Table 3). Figure 9 depicts the distribution of stem scar size in the population.

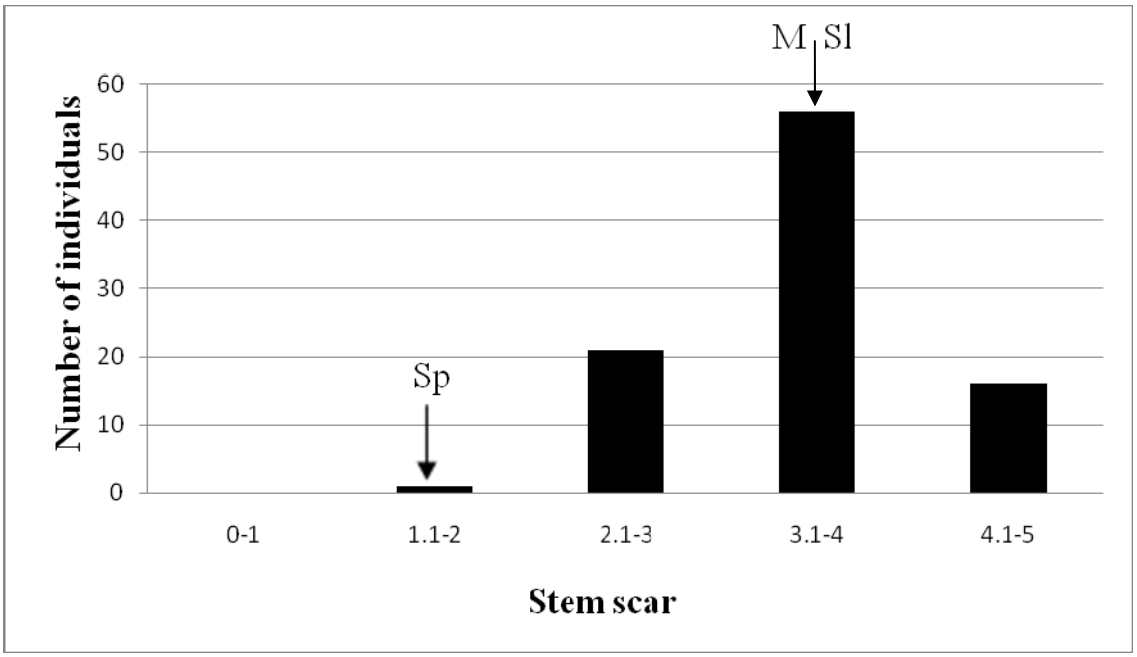

Figure 3.9. Distribution histogram for fruit stem scar size. Sl, Sp and M indicate locations of *S. lycopersicum*, *S. peruvianum* and population means, respectively.

3.1.9. Locule Number

The mean locule number (LN) of the BC_2F_2 population was found to be 2.9 ± 0.1 with variation from 2 to 4 locules. *S. peruvianum* had small fruits and had an average of 2 locules while *S.lycopersicum* had an average of 3 locules with larger

fruits. Figure 10 shows the distribution histogram of locule number of the mapping population and shows that there was little variation for this trait in the population.

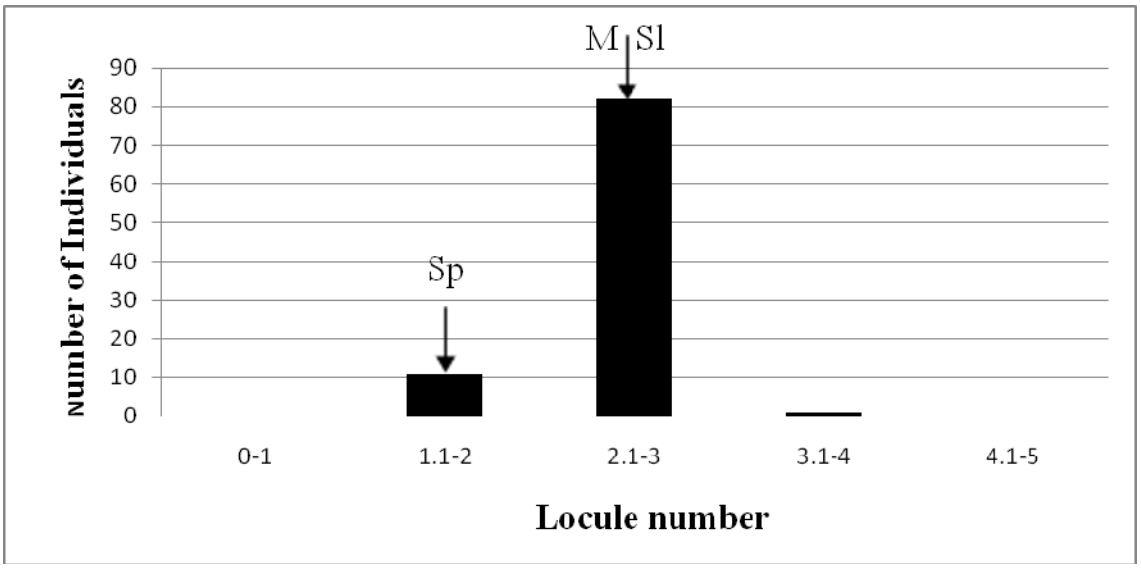

Figure 3.10. Distribution histogram for fruit locule number. Sl, Sp and M indicate locations of *S. lycopersicum*, *S. peruvianum* and population means, respectively.

3.1.10. Fruit Wall Thickness

The mean wall thickness (WALL) was scored as 3.6 among the BC_2F_2 progeny ranging between 2 and 5. *S.lycopersicum* wall thickness was evaluated as 4.5 and *S. peruvianum* was scored as 1 for wall thickness (Table 3). Therefore, *S. lycopersicum* had much thicker pericarp than *S. peruvianum* and the population was more similar to the recurrent parent for this trait (Figure 11).

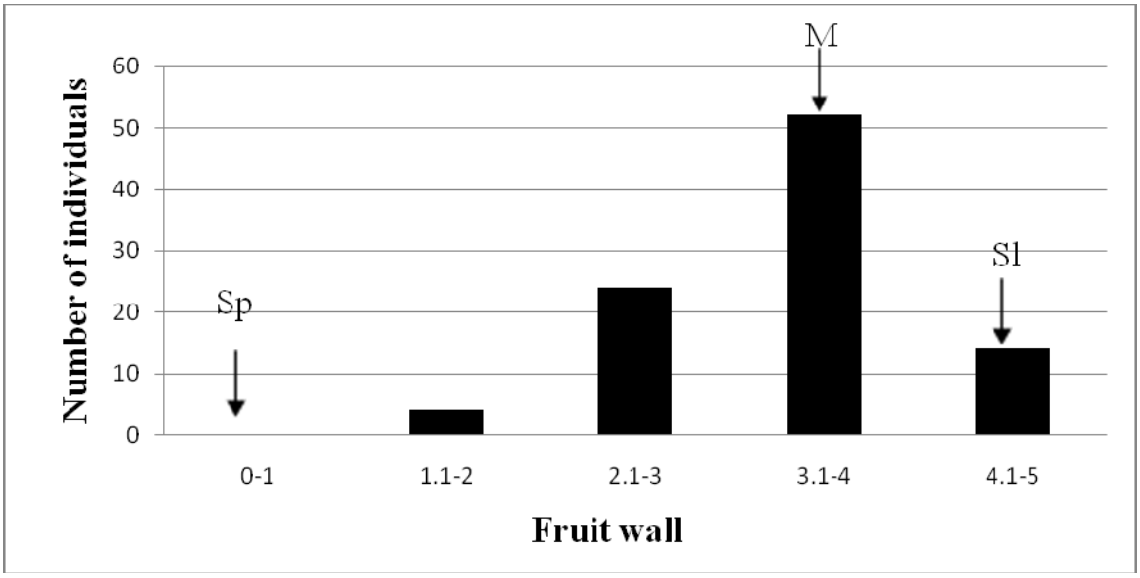

Figure 3.11. Distribution histogram for fruit wall thickness. Sl, Sp and M indicate locations of *S. lycopersicum*, *S. peruvianum* and population means, respectively.

3.1.11. Correlations Between Traits

Moderate but statistically significant correlations were observed between some of the traits (Table 3). Total water-soluble antioxidant activity was positively correlated with both phenolic content and vitamin C content (r=0.62 and r=0.43, respectively). This was expected as both phenolic compounds and vitamin C are water-soluble and therefore, make significant contributions to total water-soluble antioxidant activity. For the horticultural traits, internal and external fruit color were very strongly associated (r=0.80) as expected because both traits are controlled by the same pathway. Fruit weight is one of the most important horticultural traits because it and fruit number determine yield. It was observed that fruit weight was positively correlated with fruit shape and stem scar size. Thus, larger fruit tended to be more elongated and to have larger scars. Fruit weight was weakly negatively correlated with all threee antioxidant traits. Thus, larger fruit tended to have lower WAOX, phenolics and vitamin C contents. This is an important finding as it suggests that there may be limitations in attempts to breed very large tomato fruit with high antioxidant content.

Table 3.3. Correlation results for the antioxidant and horticultural traits. R values are given only for correlations that were significant at P<0.05.

Trait	FIRM	FS	SSC	EXC	INC	LC	WALL	WAOX	PHE	VITC
FS	-									
SSC	-	-								
EXC	0.239	0.375	-							
INC	-	0.433	0.232	0.803						
LC	-	-0.186	-	-	-					
WALL	-	0.234	0.245	-	-	-				
WAOX	-	-	-	-	-	-	-			
PHE	-	-	-	-	-	-	-	0.619		
VITC	-	-0.263	-	-	-	-	-	0.429	0.238	
FW	-	0.356	0.443	0.3	0.343	-	-	-0.218	-0.232	-0.278

3.2. Genotypic Characterization

To identify markers that can be used to map QTLs for health related and agronomically important traits, 96 COSII markers and 73 SSR markers were surveyed and 61 polymorphic (63.5%) COSII and 52 (71.2%) polymorphic SSR markers were identified as can be seen in Tables 4 and 5. Based on these results, it was evident that the IBL population has a sufficient level of DNA polymorphism for mapping and that SSR markers were slightly more polymorphic than COSII markers. The polymorphic markers will be used in future work to test the 192 BC_2F_1 population for genotypic characterization.

Table 3.4. List of polymorphic COSII markers,their methods and their restriction enzymes.

Markers	Method	Enzymes	Markers	Method	Enzymes
At1g16180	Cos 55	Mbo I	At3g24050	Cos 55	Rsa I
At1g20050	Cos 55	Alu I	At3g26060	Cos 55	Taq I
At1g29900	Cos 55	Msp I	At3g60830	Cos 55	Rsa I
At1g30580	Cos 55	Rsa I	At4g00090	Cos 55	Rsa I
At1g44446	Cos 55	Hae III	At4g00560	Cos 55	Msp I
At1g46480	Cos 55	Dra I	At4g09010	Cos 55	Hpa II
At1g53670	Cos 55	Dra I	At4g12230	Cos 55	BamH I
At1g60200	Cos 55	Mbo I	At4g18593	Cos 55	Sca I
At1g60440	Cos 55	Hae III	At4g20410	Cos 55	Msp I
At1g67730	Cos 55	Hae III	At4g26750	Cos 55	Hpa II
At1g76150	Cos 55	Msp I	At4g38630	Cos 55	EcoR V
At1g78690	Cos 55	Mbo I	At5g04910	Cos 55	Rsa I
At1g80360	Cos 55	Taq I	At5g06130	Cos 55	Eco 24 I
At2g01720	Cos 55	Hinf I	At5g11490	Cos 55	Sca I
At2g04700	Cos 55	Mbo I	At5g12200	Cos 55	Tru I
At2g25950	Cos 55	Msp I	At5g13030	Cos 55	Rsa I
At2g28490	Cos 55	Hinc II	At5g13640	Cos 55	Msp I
At2g29210	Cos 55	Vsp I	At5g16710	Cos 55	Taqı
At2g38025	Cos 55	Alu I	At5g19690	Cos 55	Mbo I
At2g43360	Cos 55	Vsp I	At5g20890	Cos 55	Msp I
At2g47580	Cos 55	EcoR V	At5g25760	Cos 55	Mbo I
At3g06050	Cos 55	Hinf I	At5g27620	Cos 55	EcoR I
At3g08760	Cos 55	Sac I	At5g38530	Cos 55	Mbo I
At3g09740	Cos 55	Alu I	At5g41350	Cos 55	Eco R I
At3g10920	Cos 55	Alu I	At5g42740	Cos 55	Alu I
At3g11210	Cos 55	Vsp I	At5g45680	Cos 55	Hha I
At3g13235	Cos 55	Tru I	At5g47010	Cos 55	Rsa I
At3g14190	Cos 55	Dpn I	At5g48300	Cos 55	Eco24 I
At3g16150	Cos 55	EcoR I	At5g51840	Cos 55	Taq I
At3g18040	Cos 55	Rsa I	At5g67370	Cos 55	Msp I
At3g18860	Cos 55	Tru I			

Table 3.5. List of polymorphic SSR markers and their methods.

Markers	Method	Markers	Methods
SSR19	SSR 50	SSR4	SSR 55
SSR26	SSR 50	SSR11	SSR 55
SSR27	SSR 50	SSR14	SSR 55
SSR32	SSR 50	SSR22	SSR 55
SSR38	SSR 50	SSR34	SSR 55
SSR40	SSR 50	SSR65	SSR 55
SSR43	SSR 50	SSR69	SSR 55
SSR45	SSR 50	SSR76	SSR 55
SSR46	SSR 50	SSR128	SSR 55
SSR66	SSR 50	SSR150	SSR 55
SSR67	SSR 50	SSR223	SSR 55
SSR70	SSR 50	SSR241	SSR 55
SSR80	SSR 50	SSR248	SSR 55
SSR85	SSR 50	SSR270	SSR 55
SSR96	SSR 50	SSR285	SSR 55
SSR111	SSR 50	SSR301	SSR 55
SSR115	SSR 50	SSR310	SSR 55
SSR117	SSR 50	SSR316	SSR 55
SSR124	SSR 50	SSR320	SSR 55
SSR134	SSR 50	SSR327	SSR 55
SSR136	SSR 50	SSR350	SSR 55
SSR146	SSR 50	SSR450	SSR 55
SSR162	SSR 50	SSR557	SSR 55
SSR188	SSR 50	SSR578	SSR 55
SSR192	SSR 50	SSR586	SSR 55
SSR478	SSR 50	SSR590	SSR 55
SSR603	SSR 50	SSR593	SSR 55
SSR605	SSR 50	SSR594	SSR 55

CHAPTER 4

CONCLUSION

Tomato is the second most consumed vegetable in the world. It is not only important for the economy but also prominent for the human diet. For these reasons, its production is very important for Turkey and many other countries. The main goals of this study were to assess the usefulness of the wild tomato species, *Solanum peruvianum* as a source for improvement of health related traits and to identify polymorphic markers for use in mapping the genes controlling agronomically important and health related traits. For this aim, antioxidant traits were measured using biochemical assays and agronomically important traits were visually scored in a *S. lycopersicum* x *S. peruvianum* IBL population. Parental surveys were done between the *S. lycopersicum* and *S. peruvianum* parents of the mapping population. For this genotypic characterization, 96 COSII markers and 73 SSR markers were surveyed.

S. peruvianum was used as a donor parent in this study. This species was used to improve both genotypic and phenotypic variation within the mapping population. *S. peruvianum* has been the source of many major resistance genes against important diseases and, as the results of this thesis show, it also has many desired antioxidant traits. These favorable antioxidant traits may be present in the wild species because antioxidant compounds have important roles in the plant's defence system and during natural selection, alleles that are responsible for production of high antioxidant compound may have accumulated in wild species. This accumulation in *S.peruvianum* may have arisen due to exposure to various abiotic and biotic stresses over a long time. In contrast, *S. peruvianum* is expected to negatively influence quality of the elite line for agronomic traits such as fruit color and fruit weight because this species has not experienced artificial selection for these domestication-related traits. *S. lycopersicum* was used as a recurrent parent in this study for its agronomically important traits such as fruit weight, fruit color, and fruit firmness. However, during domestication and breeding, cultivated tomato may have lost some desired antioxidant traits. Thus, the wild species may be useful to improve these traits in cultivated tomato.

The *S. peruvianum* x *S. lycopersicum* population showed significant differences for the studied traits, so this population was found to be appropriate for mapping. Transgressive segregation in the population showed that traits can be improved in cultivated tomato with the wild *S. peruvianum.* Thus, alleles coming from the distantly related parental types can be combined in new ways to generate progeny that can exceed both parental lines for the traits of interest. In addition, genotyping showed sufficient DNA polymorphism for QTL mapping. However, because antioxidant traits are sensitive to environmental conditions, at least two years of data are required for reliable QTL identification. Thus, in future work, the *S. lycopersicum* x *S. peruvianum* population will be re-grown and phenotyped for the traits of interest in order to reliably identify QTL and determine the influence of environment on QTL expression.

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