QTL ANALYSIS FOR FRUIT TRAITS IN *Solanum pimpinellifolium* INBRED BACKCROSS LINES

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Nilüfer TAMER

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We approve the thesis of **Nilüfer TAMER**

Prof. Dr. Anne FRARY
Supervisor

Assoc. Prof. Sami DOĞANLAR
Committee Member

Assoc. Prof. Bahattin TANYOLAÇ
Committee Member

28 June 2010

Assoc. Prof. Sami DOĞANLAR
Head of the Department of
Molecular Biology and Genetics

Assoc. Prof. Talat YALÇIN
Dean of the Graduate School of
Engineering and Sciences

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ABSTRACT

QTL ANALYSIS FOR FRUIT TRAITS IN *Solanum pimpinellifolium* INBRED BACKCROSS LINES

Tomato is one of the most economically and nutritionally important crops. It contains antioxidants such as lycopene, phenolics, vitamins E, C, and β-carotene at high levels. These traits are of interest to consumers and plant breeders for their health-related contributions.

The main aim of plant breeding is to improve agronomically relevant traits by combining characters from different parental lines or their relatives. Genetic markers reveal these characters and other genetic differences between organisms. In this study both health-related and agronomically important traits were phenotypically identified by using 120 BC_2F_7, BC_2F_8, and BC_2F_9 IBLs. Also the lines were genotypically identified using the BC_2F_{10} IBL population.

A total of 66 COSII and 11 COS markers were positioned on the IBL map. A total of 103 QTLs were identified. Of these QTLs, 25 loci were identified for antioxidant traits: total water soluble antioxidant capacity, vitamin C content, lycopene and phenolic content. In addition, 78 QTLs were identified for agronomic traits: fruit weight, fruit shape, fruit firmness, stem scar size, external and internal color, locule number, fruit wall size, and soluble solid content. For most of the antioxidant QTLs, alleles from the *S. pimpinellifolium* parent were favorable. This result indicates that *S. pimpinellifolium* can be used as a source of high nutritional traits in order to improve elite tomato lines.

ÖZET

Solanum pimpinellifolium SAF DÖL GERİMELEZ HATLARINDA MEYVE KARAKTERLERİ İÇİN KANTİTATİF KARAKTER LOKUS ANALİZLERİ

Domates ekonomik açıdan ve besin kaynağı olarak insanlar için önemli bir tarımsal üründür. Domates, likopen, fenolik, vitamin E, C ve β-karoten gibi antioksidantları yüksek miktarda içerir. Bu kalıtsal özellikler insan sağlığı açısından önemli oldukları için birçok bitki ıslahçısının odak noktası olmuştur.

Bitki ıslahının ana amacı farklı ebeveyn hatlarının veya akrabalarının sahip olduğu çeşitli karakterlerin birleştirilerek tarımsal açıdan önem taşıyan istenilen özelliklerdeki bireylerin oluşturulmasıdır. Genetik işaretleyiciler bu karakterleri ve organizmalar arasındaki diğer genetik farklılıkları ortaya çıkarırlar. Yapılan çalışmada, 120 bireyden oluşan BC_2F_7, BC_2F_8, and BC_2F_9 saf döl gerimelez hatlarında hem sağlık hem de tarımsal açıdan önem teşkil eden fenotipik özellikler karakterize edilmiştir. Aynı zamanda 120 bireyden oluşan BC_2F_{10} saf döl gerimelez hatlarında ise genotipik karakterizasyonlar yapılmıştır.

66 COSII ave 11 COS işaretleyici saf döl gerimelez hatları genotip haritasında pozisyonlandırılmıştır. Toplamda 103 QTL elde edilmiştir. 103 QTL'den 25'i suda çözünen toplam antioksidant aktivitesi, C vitamini, likopen ve fenolik içeriği olmak üzere dört antioksidant karakteri için belirlenmiştir. Diğer 78 QTL ise meyve ağırlığı, şekli, sertliği, gövde izi, iç ve dış rengi, lokul sayısı, perikarp kalınlığı ve suda çözünebilir madde mitarı olmak üzere dokuz tarımsal açıdan önem taşıyan karakter için belirlenmiştir. Antioksidant karakterler için elde edilen QTL'lerin çoğunun kaynağı *S. pimpinellifolium* ebeveynidir. Bu sonuç göstermektedir ki; *S. pimpinellifolium* birinci sınıf kültür domates hatları geliştirmek için zengin besin karakterleri kaynağı olarak kullanılabilir.

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CHAPTER 1

INTRODUCTION

1.1. Tomato

Tomato, *Solanum lycopersicum* (synonym: *Lycopersicon esculentum*), is one of the most important members of the Solanaceae or nightshade family, which possesses more than 3000 species such as potato, tobacco, pepper, eggplant and petunia. Tomato comes second after potato as the most consumed vegetable in the family.

The origin of tomato is western South America, the Andean region, through Chile, Bolivia, Ecuador and the coastal areas of Peru. Although the exact time and place of tomato's domestication remain unclear, the records show it reached a certain level of cultivation in Mexico before its first transportation to Europe in 1554 (Tucker et al., 2007). After the renewal of phylogenetic classification, genus *Lycopersicon* has become a section of the genus *Solanum* with 13 species including *S. pimpinellifolium*, *S. pennellii*, *S. habrochaites*, *S. peruvianum*, *S. chmielewskii* and, the only domesticated species, *S. lycopersicum* (Peralta et al., 2006). With domestication of tomato a number of different morphological and physiological traits have been altered, these traits are called the domestication syndrome. In tomato important traits that are studied are plant height and earliness, self-pruning, fruit set, fruit size, fruit shape, fruit color, fruit morphology, quality, flavor, yield and heterosis, and disease and stress resistance. Qualitative genes and quantitative trait loci (QTLs) for these characteristics that have been identified in tomato have had huge importance to breeders (Bai and Lindhout, 2007).

Now grown worldwide, tomato is one of the most economically important crops with 130 million tons of total world production from 5.2 million hectares. Turkey ranks third in production with 11 million tons of production on 300,000 hectares and follows the world leaders China and the United States. With Mexico leading exports, Turkey ranks sixth in world tomato exports with 372,094 tons (FAO 2008).

Consumed in high amounts, tomato fruit has an important place in the human diet as it contains antioxidants such as lycopene, phenolics, vitamins E, C, and β-

carotene in high levels (Tucker et al., 2007). Lycopene is the most abundant carotenoid present in tomato and comprises more than 90% of the total carotenoids (Dorgan et al., 1998). Research shows that lycopene can inhibit human cancer cell growth, especially in prostate and breast cancer cells, reduces cellular DNA injuries, and prevents liver fibrosis (Heber and Lu, 2002; Zhou et al., 2008; Kitade et al., 2002). β-carotene is the precursor of vitamin A and is an antioxidant that reduces cellular or tissue damage, prevents cardiovascular disease and the major cancers (Mantzouridou et al., 2001; Zhang and Omaye, 2001). Tomato is also a very good source of molybdenum, iron, phosphorus, magnesium, niacin and potassium which all have health benefits for humans such as lowering high cholesterol levels and high blood pressure.

Tomato's role is not only economical; it is also an important model system for genetic studies in plants. As a simple diploid (2n=24), it is one of the most well-studied crop species. It is one of the first plants for which a high-density DNA-based molecular map was constructed (Tanksley et al., 1992). Also tomato is the first plant for which QTL mapping for a complete genome was conducted in a single segregating population (Paterson et al., 1988). The first plant resistance gene and first plant QTL that were cloned in plants were in tomato (Martin et al., 1993; Frary et al., 2000). Now the International Tomato Sequencing Project has reached 57% completion (Mueller et al., 2005).

1.2. Genetic Markers

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.

Characteristics that occur in a population with more than one trait and reveal the difference between individuals in this population are called polymorphic genetic markers. Polymorphic markers can also be divided into two categories: dominant and codominant. Codominant markers can separate heterozygote and homozygote individuals from each other, whereas dominant markers cannot (Collard et al., 2005).

Genetic markers are generally classified into two major groups. The first group is morphological markers which can be observed visually without specialized biochemical or molecular techniques. Such markers are color, height or shape. Morphological markers have some disadvantages as they are limited in number and affected by environmental changes. Also they are inefficient in distinguishing heterozygous and homozygous individuals (Kumar, 1999). The second group of markers is molecular markers which 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). On the other hand DNA markers have eliminated these disadvantages and become the most widely used type of markers.

DNA markers originate from DNA mutations such as point mutations, insertions or deletions that generally occur in non-coding regions (Collard et al., 2005). They can be classified into two categories; hybridization-based and PCR-based polymorphisms. Hybridization-based polymorphisms, including RFLPs (restriction fragment length polymorphisms) and VNTR (variable number tandem repeats), were the first techniques used for DNA profiling. However their slowness, difficulty, requirement for high amounts of DNA and combination of different processes led to the generation of new techniques based on PCR (Kumar, 1999). RAPDs (random amplified polymorphic DNAs) was one of the first PCR-based methods and it was followed by SPARs (single primer amplification reactions), AFLPs (amplified fragment length polymorphisms), SRAPs (sequence-related amplified polymorphisms), SNPs (sequence nucleotide polymorphisms), SSRs (simple sequence repeats), and CAPs (cleaved amplified polymorphisms).

Due to advantages over other markers, CAPs is one of the most preferred marker systems in recent years. First, because it is a codominant system, relatively good map positions can be obtained with a small number of plants and that is a great advantage for populations whose phenotypic characters are difficult to observe. With the use of PCR (polymerase chain reaction), a small amount of DNA is adequate for determining a map position. Another benefit of CAPs is the cleaved and uncleaved products highly differ in size, leading to an easy detection with agarose gel electrophoresis. Another advantage of this system is that CAPs are fast and simple without need for any other processes (Glazebrook et al., 2008).

CAPs utilize DNA fragments, which are amplified by PCR and then digested with a restriction endonuclease in order to display a restriction site polymorphism after separation by agarose gel electrophoresis (Figure 1.1). COS (conserved ortholog set) and COSII (conserved ortholog set II) markers are the most commonly used CAPs markers for tomato. These markers were developed by computationally comparing the Arabidopsis genomic sequence with the ESTs (expressed sequence tags) database of tomato to identify putatively conserved orthologous sequences and design primers for these sequences (Fulton et al., 2002, Wu et al. 2006).

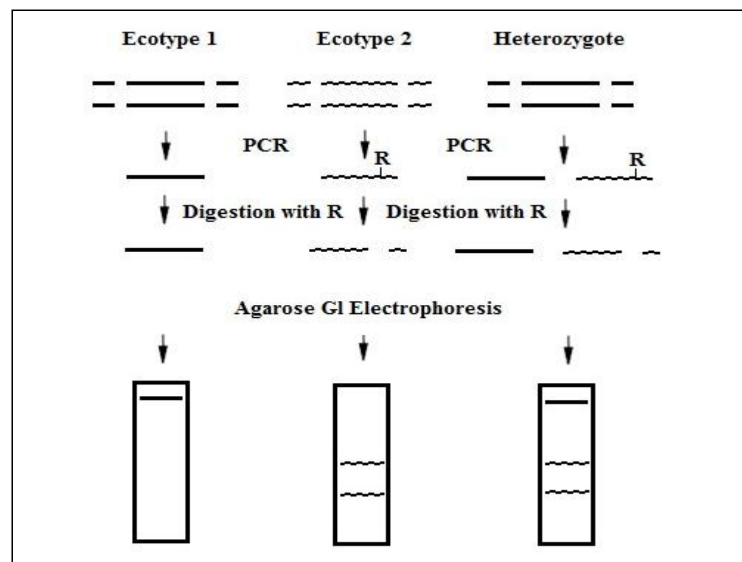

Figure 1.1. Schematic representation of CAPs.

One of the applications of these DNA markers is construction of molecular marker maps. In a molecular marker map all these polymorphisms are placed in genomic locations on plant chromosomes.

1.3. Molecular Marker Mapping

For localizing important genes controlling both qualitative and quantitative traits in plants, molecular marker linkage maps are very useful (Dirlewanger et al., 1998). Along the chromosome the distance between mapped markers are expressed in

centimorgans (cM). Centimorgans represent the recombination rates of the loci on the map (Kumar, 1999).

Construction of a segregating mapping population is the first step for developing a molecular marker map. A mapping population can be grown by crossing two parents that show differences in one or more traits for detection of polymorphisms by markers. Several different populations can be constructed. In most studies, balanced mapping populations are used in which both parental alleles are in high frequency (Doganlar et al., 2002). On the other hand unbalanced populations were developed, in which the alleles from one parent show higher frequency than the other one. One type of unbalanced populations is inbred backcrosses that were first introduced by Wehrhahn and Allard in 1965. These inbred backcross lines (IBLs) are produced by at least one backcross of the F_1 population to the recurrent parent and then advanced by single seed descent until the requested loci is fixed and the population reaches a certain level of homozygosity (Mulitze and Baker, 1985; Doganlar et al., 2002). Figure 1.2 shows the construction of IBLs. The population is more similar to the recurrent parent which is an advantage of IBLs, because they allow examination of the effect of multiple donor alleles in the elite background of the recurrent parent.

The second step for developing a molecular marker map is identification of markers that show polymorphism between parents. Polymorphic markers are selected and screened on the whole mapping population one by one.

In order to construct a molecular marker map, the last step is linkage analysis of markers. By using a maximum likelihood method, recombination frequencies and their standard errors are calculated. These marker analyses can be performed manually, but with the high number of markers needed for maps, computer programs such as Linkage 1 and MAPMAKER are used (Collard et al., 2005; Kumar, 1999).

Once a genetic map is constructed it can be used for many applications. One of these is comparative mapping analysis in which the mapping information of populations is used to identify relationships between these populations. Another use is identifying genes controlling economically important traits like yield, quality or resistance to many biotic and abiotic stresses. Marker assisted selection (MAS) and map based cloning are other areas in which molecular marker maps are being used (Collard et al., 2005).

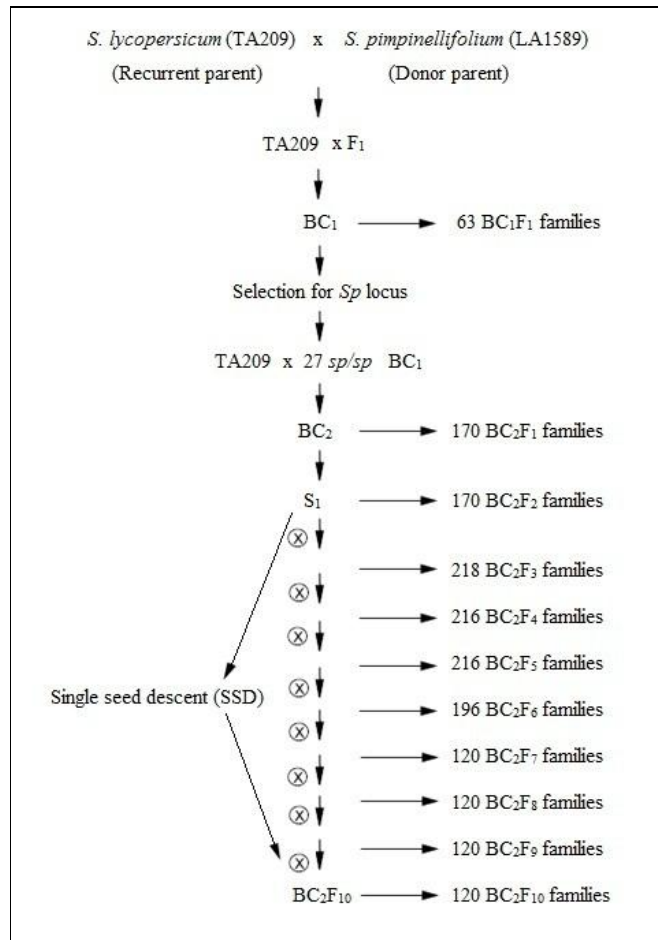

Figure 1.2. Construction of IBLs.

The traits for which genes are to be identified can be qualitative or quantitative. Qualitative traits are controlled by a single gene whereas quantitative traits are controlled by multiple loci. Quantitative trait loci (QTL) are the regions on the genome that control different quantitative traits of interest. QTL analysis is based on detection of association between phenotype and the genotype of markers (Doerge, 2002; Collard et al., 2005). In order to detect QTL; first the population is divided into different genotypic classes according to genotypes at the marker locus, then these groups are analyzed whether individuals of these groups also show phenotypic differences for the trait being measured. If there is a significant difference, it means that a gene or genes that affect the trait is/are linked to that marker (Tanksley, 1993).

The simplest way to detect a QTL is using single-marker test (single point analysis), which analyzes the markers one by one. Single-marker analysis has an

advantage as it does not require a complete molecular linkage map. However because the crossing-over probability increases as the distance between the marker and QTL increases, there is a lower chance of detecting the QTL using single marker analysis that with other approaches. Using a high number of molecular markers that cover the whole genome is the solution. Once the markers are set on the map, the relationship between markers will be revealed (Doerge, 2002; Tanksley, 1993).

1.4. Free Radicals and Antioxidants

It has been reported that the risk of many chronic diseases, such as coronary heart disease and most types of cancer are decreased with the help of antioxidants. Tomato and many other fruits and vegetables are known to contain high levels of antioxidants; therefore high intake of these products will be for the benefit of human health (Weisburger, 1999). Since antioxidants have importance for the human diet, plant breeders are interested in enhancement of their production.

Free radicals are atoms, molecules or ions that have one or more highly reactive unpaired electrons in their outer orbital with high capacity for participation in chemical reactions. When a free radical and a nonradical react with each other, a new radical forms and that leads to a chain reaction of other new radicals (Halliwell, 2006). These free radicals carry the potential to interact with different tissue components resulting in dysfunction of DNA, lipids and proteins (Kehrer, 1993).

Of the many types of free radicals, the most important ones are the oxygen free radicals, also called reactive oxygen species (ROS), and reactive nitrogen species (RNS). These radicals are normally the products of cellular metabolism, and have benefits when produced in low concentrations. ROS can also be produced from exogenous substances such as environmental agents, xenobiotics, metal ions, radiation and barbituates. ROS have roles in cellular responses, like defense against infectious agents, and also in cellular signaling systems and induction of mitosis. Molecular oxygen (dioxygen) is a radical, and with the addition of one electron, it forms the superoxide anion radical. Superoxide anion is the primary ROS that generates secondary ROS when it reacts with other molecules (Valko et al., 2007; 2006). When ROS are produced in high amounts, they cause biological damage called oxidative stress (Halliwell, 2006). Oxidative stress may lead to cell death, necrosis, cancer, DNA

damage, aging, diabetes, atherosclerosis, neurodegenerative diseases, telomerase shortening and several other diseases (Dröge, 2002). In order to prevent oxidative stress, antioxidants play a great role.

Antioxidants are molecules that prevent the oxidation of other molecules by being oxidized themselves (Halliwell, 2006). Antioxidants can be categorized as water soluble and lipid soluble, or endogenous and exogenous, or enzymatic and non-enzymatic antioxidants. We will be interested in non-enzymatic antioxidants.

Non-enzymatic antioxidants can be both water-soluble and lipid-soluble compounds. Vitamin C is the most abundant water soluble antioxidant in the body and is an electron donor, so it is a reducing agent. Also it promotes the formation of collagen in the body. Tomato, potato, orange and mango are some of the richest vitamin C sources. Vitamin E is one of the most important lipid-soluble antioxidants, and protects fatty acids from oxidative damage in lipid membranes (Byers and Perry, 1992; Padayatty et al., 2003). Other lipid-soluble antioxidants are carotenoids. Carotenoid pigments are highly abundant in many vegetables and fruits. β-carotene has been studied as cancer suppressor and a vitamin A precursor. Lycopene, the most abundant carotenoid in tomato, can be converted to β-carotene with the help of lycopene cyclase enzyme (Heber and Lu, 2002).

Phenolic compounds are secondary metabolites in plants and roles as being anti-allergenic, anti-inflammatory, anti-microbial, antioxidant. Also they are responsible for plants' taste, aroma and color. Phenolic acids, flavonoids and simple phenolics are the most important classes of phenolic compounds in plants (Balasundram et al., 2006). Flavonoids are a huge family of plant secondary metabolites including anthocyanins, flavonols, flavones, catechins, and flavonones (Crozier et al., 1997). They give orange, red, and blue colors to vegetables, fruits, and flowers. Anthocyanins are the main attractors of animals leading to pollination. Flavonoids also regenerate vitamin C, which then regenerates vitamin E (Merken and Beecher, 2000). Phenolic compounds are highly consumed in the human diet and have important benefits for human health.

1.5. Goals of This Study

The purpose of this study was to construct and characterize a permanent inbred population and identify the genes that control nutritionally important traits in tomato

such as total water soluble antioxidant activity, total vitamin C content, total phenolic content, total flavonoid content, and lycopene content. Also several horticultural traits were measured. The QTLs for these traits were determined with genetic markers. In future studies these alleles can be used to improve the quality of new tomato hybrids through marker-assisted selection.

CHAPTER 2

MATERIALS AND METHODS

2.1. Plant Material

The BC_2F_7, BC_2F_8, BC_2F_9, and BC_2F_{10} mapping populations were developed by Sami Doganlar, by crossing the cultivated recurrent parent *S. lycopersicum* (TA209) with wild donor parent *S. pimpinellifolium* (LA1589). In order to increase the amount of *S. lycopersicum* genome in the population, one F_1 hybrid was backcrossed with the recurrent parent. A total of 27 BC_1 plants were selected by RFLP marker TG279 for homozygous *S. lycopersicum* alleles at the *sp* locus on chromosome 6, which is responsible for determinate growth habit. These selected plants were then backcrossed to *S. lycopersicum* to produce BC_2 population. To fix the population genotypes BC_2F_1 individuals were selfed for six generations, resulting in a population of 120 BC_2F_7 IBLs. Again selfing of this population led to BC_2F_8, then BC_2F_9 and BC_2F_{10} populations.

Each individual of the BC_2F_7, BC_2F_8, and BC_2F_9 populations was transplanted to the field in Menemen by Aegean Agricultural Research Institute (ETAE) in summer 2004, 2005, and 2006 respectively. The BC_2F_{10} population was transplanted to the field in Antalya by MULTİ Tarım Seed Company in 2009 and, as controls, recurrent and donor parents were planted at İYTE in April 2009.

2.2. Phenotypic Characterization

In this study 13 agronomically and nutritionally important traits were analyzed for QTL identification on BC_2F_7, BC_2F_8, and BC_2F_9 populations. Agronomically important traits were: fruit weight (FW), internal (IC) and external fruit color (EC), fruit firmness (FIRM), soluble solid content (SSC), fruit shape (FS), fruit stem scar size (SCAR), fruit locule number (LOC), and wall thickness (WALL). As nutritionally important traits; total water soluble antioxidant activity (AUC), total vitamin C content (VITC), total phenolic content (PHEN), and lycopene content (LYCO) were determined by biochemical assays.

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 July in 2004, 2005, and 2006. Each year after being washed, about one kilo fruit from each sample were cut into slices and mixed. Until the time of analysis, these mixtures were packed and stored at -20 ^{o}C. Within four months of harvest all analyses were performed as described below.

2.2.2. Determination of Total Water Soluble Antioxidant Activity

For the determination of total water soluble antioxidant activity, approximately 200 g fruit was homogenized with 100 ml distilled water in a Waring blender that had a 1 L double walled stainless steel jar at +4 ^{o}C. The homogenization proceeded at low speed for 2 minutes. From the homogenate 10 g sample was taken to be diluted with 15 ml cold water. This diluted homogenate was filtered into two 15 ml falcon tubes through 4 layers of nylon cloth. In order to clear the supernatants, samples were centrifuged at 3000 x g for 10 min at 4 ^{o}C. Supernatants of two tubes were mixed in a 50 ml falcon tube by being filtered through 3 layers of nylon cloth. The sample was kept on ice during the measurement.

To measure the antioxidant activity of tomato fruits spectrophotometrically (Shimadzu, 1700 UV Visible Spectrophotometer, Japan), the ABTS [2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] decolorization assay of Re et al. (1999) was used. The absorbance of ABTS radical cation decreases when reduced by an antioxidant. The ABTS radical cation stock solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate and was stored in dark for 12-16 hours. To adjust the absorbance of ABTS radical cation to 0.70 at 734 nm, stock solution was diluted with phosphate buffered saline (PBS) at pH 7.4. After preparation, 2.5 µl of tomato supernatant were mixed to 2 ml ABTS radical cation solution then the decolorization of blue-green ABTS radical cation solution was monitored kinetically at 734 nm for 6 min at 30 ^{o}C. The test was repeated three times then the same measurements were carried out with 5 and 7.5 µl of tomato supernatant again with three replicates of each. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard. The results expressed as µmol Trolox/kg fresh weight of tomato

fruits were calculated as area under the curve (AUC). The percent inhibition /concentration values for Trolox and the extracts were plotted over 1, 3, and 6 min test periods. The ratio areas were used to calculate the AUC value.

2.2.3. Determination of Vitamin C Content

Vitamin C content was analyzed by the AOAC 967.21 titrimetric method. As reactive substance 2,6-dicloroindophenol was used (Nielsen, 2003). First 100 g of tomato was homogenized with 115 ml acetic acid-metaphosphoric acid extraction solution. The homogenization was carried out in a Waring blender at +4 ^{o}C and low speed for 2 min. A 100 ml dilution was prepared with 25 g of homogenated extract and cold extraction buffer. Then this dilution was passed through filter paper. A 15 ml sample was taken and titrated against 2,6- dicloroindophenol dye solution. The test was repeated three times for each extract. For calibration, commercial L-ascorbic acid was used and the results were expressed as mg ascorbic acid/kg fw of tomato fruit.

2.2.4. Determination of Total Phenolic Compounds

Tomatoes total phenolic compounds (PHEN) were measured according to the method of Singleton and Rossi (1965). In this spectrophotometric procedure, Folin-Ciocalteau was used as a reactive agent and gallic acid was used for generation of a standard curve. A 100 g tomato sample was blended with 200 ml distilled water in a Waring blender for 2 min at low speed and +4 ^{o}C. Then 2.5 g homogenate and 20 ml cold distilled water were mixed for dilution and the sample centrifuged at 3000 x g for 10 min at +4 ^{o}C in a refrigerated centrifuge (Eppendorf). Next 2 ml supernatant and 10 ml 2 N (10%) Folin-Ciocalteau were mixed. After 3 minutes of incubation, 8 ml 0.7 M Na_2CO_3 was added and left to incubate at room temperature for 2 hours. The absorbance of the mixture was measured in a spectrophotometer (Shimadzu, 1700 UV Visible Spectrophotometer, Japan) at 760 nm. Each sample was repeated three times. The total phenolic content of samples was interpreted as gallic acid equivalents (mg/kg fresh weight).

2.2.5. Determination of Lycopene Content

Lycopene content (LYCO) of tomato fruit was determined by using the procedure of Sadler et al. (1990). A total of 100 g tomato and 200 ml distilled water were homogenized in a Waring blender at +4°C, low speed for two min. Then 3 g homogenate was diluted with 50 ml hexane-acetone-ethanol (2:1:1; v:v:v) extraction buffer in a brown volumetric flask. Samples were shaken on a rotary mixer for 30 min at 150 rpm at room temperature in dark. Then samples were transferred into separation funnels. In order to separate polar and non-polar phases, after addition of 10 ml distilled water to the mixture, samples were left for 4 hours at dark. Lycopene that dissolved on the top was taken and measured by spectrophotometer (Shimadzu, 1700 UV Visible Spectrophotometer, Japan) at 472 nm. The results were expressed as mg/kg fresh weight based on a lycopene standard curve.

2.2.6. Visual Score of Agronomically Important Traits

Nine agronomically important traits were scored visually for each individual of the BC_2F_7, BC_2F_8, and BC_2F_9 populations and the controls.

Fruit weight (FW) was measured as the average weight of 20 ripe fruits of each plant. Fruit shape (FS) determined by the ratio of fruit length to fruit diameter. The scale of 1 to 5 was used on each line. Internal (IC) and external fruit color (EC) was scaled as 1 = low color, 5 = more intense red color. Fruit firmness (FIRM) was determined by hand squeezing fruit and scored as 1 = soft, 5 = very firm. Soluble solid content (SSC) was measured on puree from five randomly chosen fruits per plant in degrees Brix, using a refractometer. Fruit stem scar size (SCAR) was measured as the approximate mean diameter of the stem scar on 20 fruit. Fruit wall (WALL) thickness was scaled as 1 = thin, 5 = very thick using transverse sections of fruits. Locule number (LOC) was determined by counting the locules of cross-wise cut tomato fruit.

2.3. Genotypic Characterization

In order to identify markers that can be used to map QTLs for health related and agronomically important traits, genotypic characterizations were carried out on BC_2F_{10} population and parents. In order to map the QTLs, a previously constructed RFLP map was used (Doganlar et al., 2002).

2.3.1. DNA Extraction

DNA was extracted from the leaves of tomatoes by the procedure described by Bernatzky and Tanksley (1986). Tomato leaves were collected from the field in two eppendorf tubes separately for each IBL and then transported to Izmir Institute of Technology, where DNA extraction was performed. The DNA concentration and quality was measured with nano-drop (ND-1000) spectrophotometer. To be used for PCR, each sample of DNA was diluted to ~ 55 ng/µl with distilled water.

2.3.2. Molecular Marker Analysis

For molecular characterization and in order to construct a map, CAPs (Cleaved Amplified Polymorphic Sequence) marker analyses were performed using COS and COSII molecular markers. At first, for the identification of an adequate number of polymorphic markers, parental surveys were carried out. The two parents (TA209 and LA1589) were tested with each marker. For the CAPs procedure, 25 µl of PCR mixture was prepared including; 2.5 µl 10X PCR buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, pH: 8.3), 0.5 µl dNTP (0.2 mM), 0.5 µl forward and 0.5 µl reverse primers (10 pmol), 0.25 µl Taq polymerase (0.25 U), 18.75 µl sterile distilled water, and 2 µl DNA (~55 ng/µl). Samples were amplified in a thermocycler (GeneAmp® PCR System 9700, Applied Biosystems; Authorized Thermal Cycler, Mastercyler epgradientS, Eppendorf; C1000 Thermal Cycler™, BIO-RAD) using the PCR program in figure 2.1.

After PCR amplification, samples were digested by using different restriction enzymes (Table 1). The enzyme digestion mixture contained 25 µl PCR product plus 3 µl 10X digestion buffer, 0.5 µl enzyme (10 u/µl) and 1.5 µl sterile distilled water.

Samples were incubated at the appropriate temperature for the enzyme for at least 3 hours. After incubation the samples were loaded on 2-3% agarose gels in 1X TAE buffer (0,25 M Tris base, 12,75 M EDTA adjusted to 1 L with distilled water and pH: 8.3 with acetic acid). Samples were run at 110 V for at least 2 hours. Staining the gels with ethidium bromide allowed the identification of marker bands under UV light. Polymorphic markers were selected and then applied to whole population.

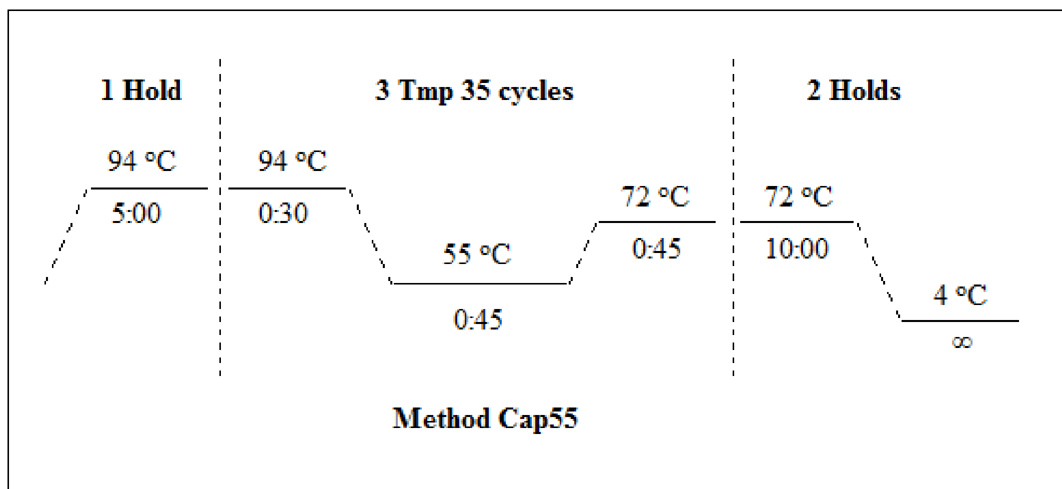

Figure 2.1. PCR profile for CAP55 procedure.

2.4. Statistical Analysis

Student's t-test, chi-square analysis, correlation analysis between traits and statistical analyses were performed in Excel 2010 computer program. QTL mapping was performed with QGENE software program (Nelson, 1997).

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Phenotypic Characterization

Phenotypic characterization of agronomically and nutritionally important traits showed continuous distribution throughout the population. These distributions are expected because two distinct parents were used for population development and the traits of interest are all quantitative. With the selected parents, the BC_2F_7, BC_2F_8, and BC_2F_9 populations displayed an enhanced variation that is favorable for genetic mapping and identification of QTLs.

3.1.1. Total Water Soluble Antioxidant Capacity

Total water soluble antioxidant (AUC) activities of the parents and 150 BC_2F_7, BC_2F_8, and BC_2F_9 lines for 2004, 2005 and 2006 were measured. Means of the antioxidant traits, standard errors and ranges for the IBLs of 2004, 2005, 2006, and all years' average are presented in Table 3.1. Means of the antioxidant traits, standard errors and ranges for the parents are presented in Table 3.2. AUC activities of the IBLs showed no significant differences in all years. The three year averages of AUC activity in the population ranged from 3548 to 7135 µmol Trolox/kg fresh tomato indicating good variation with 2-fold variation. This variation is typical for quantitative traits.

A distribution histogram for total water soluble antioxidant activities is presented in figure 3.1. This graph shows a nearly normal distribution for the trait. *S. pimpinellifolium* AUC activity was 1,4 fold higher than *S. lycopersicum* indicating no significant differences between them with P=0,10. As seen in the table both parents have extreme levels of AUC activity with higher values than most of the population. In fact, 99% of the population had lower antioxidant activity than both parents. This is the result of transgressive segregation which is caused by complementary action of genes

from parents or unmasking of recessive genes coming from parents (Vicente and Tanksley, 1993).

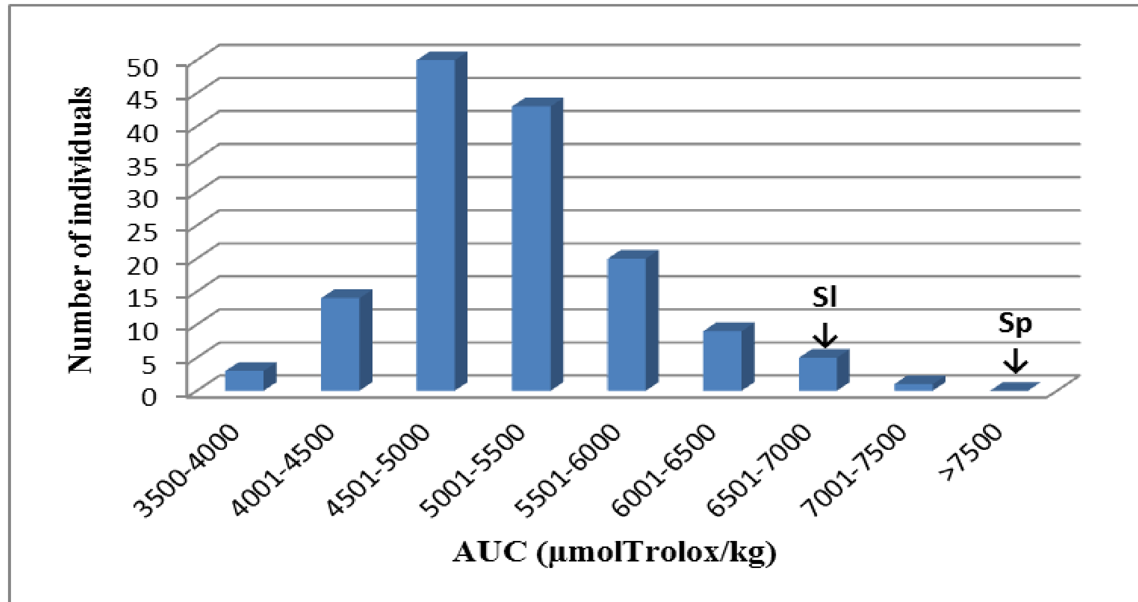

Figure 3.1. Distribution histogram for total water soluble antioxidant activities. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

3.1.2. Vitamin C Content

BC_2F_7, BC_2F_8, and BC_2F_9 populations showed similar vitamin C contents over three years with an average of 245,54 ± 3,23 mg/kg of fresh fruit. Figure 3.2. shows the distribution histogram for vitamin C content on populations' average of all years. The range for vitamin C content was between 161 and 410 mg/kg, displaying 2,5-fold difference between the highest and the lowest values in the population. Vitamin C content between individuals was normally distributed as expected. As represented in Figure 3.2, *S. lycopersicum* had moderate vitamin C content value. *S. pimpinellifolium* on the other hand, had the highest value among the population and 2-fold higher difference than the recurrent parent (P=0,06). Sixty seven percent of the population had lower values than both parents. The individuals outside the ranges of two parents are the results of transgressive segregation, different alleles from two parents leading to a decreased value for progeny.

Table 3.1. Antioxidant traits mean values, standard errors and ranges of BC_2F_7, BC_2F_8, and BC_2F_9 lines for 2004, 2005, 2006.

	2004		2005		2006		All Years	
Trait	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**
Antioxidant (µmolTrolox/kg)	4936,97 ± 58,81	3450,75-7134,92	5602,05 ± 126,31	3700,68-10637,18	4880,32 ± 60,26	3179,04-6617,14	5132,74 ± 53,01	3548,39-7134,92
VitaminC (mg/kg)	243,42 ± 4,06	137,64-420,04	207,01 ± 3,43	104,02-380,16	293,14 ± 5,23	178,50-493,10	245,54 ± 3,23	160,48-410,35
Lycopene (mg/kg)	208,3 ± 3,39	113,77-296,32	220,89 ± 3,54	126,36-343,01	-	-	214,45 ± 2,89	141,29-305,49
Phenolic (mg/kg)	496,83 ± 4,68	356,94-652,19	365,18 ± 7,88	227,55-666,18	534,10 ± 6,40	384,50-773,60	467,46 ± 4,53	339,08-683,55

Table 3.2. Antioxidant traits mean values of parents for 2004, 2005, 2006.

	2004		2005		2006		All Years	
Trait	**TA209**	**LA1589**	**TA209**	**LA1589**	**TA209**	**LA1589**	**TA209 Mean ± SE**	**LA1589 Mean ± SE**
Antioxidant (µmolTrolox/kg)	6416,77	9337,33	8405,22	13385,61	6029,41	6989,07	6950,47 ± 749,8	9904 ± 1903,36
VitaminC (mg/kg)	244,69	393,84	205,97	-	315,23	325,42	255,29 ± 32,59	359,63 ± 123,78
Lycopene (mg/kg)	214,29	331,08	225,12	327,53	-	-	219,71 ± 4,5	329,31 ± 111,84
Phenolic (mg/kg)	474,45	1199,35	422,16	723,82	525,68	843,84	474,1 ± 30,45	922,33 ± 145,47

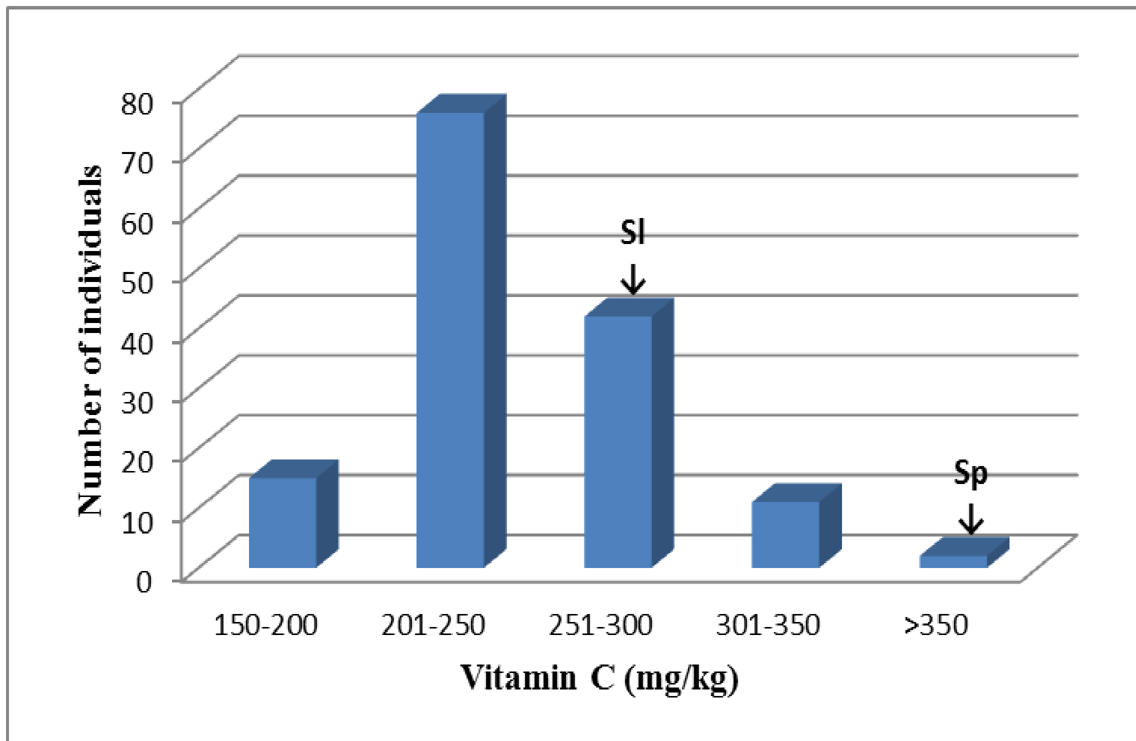

Figure 3.2. Distribution histogram for vitamin C content. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

3.1.3. Total Phenolic Content

Over three years, the total phenolic content of the population ranged between 340 and 684 mg/kg with a 2-fold difference. Figure 3.3. shows the distribution of phenolic compound content in the population. *S. lycopersicum* displays a moderate total phenolic compound with a value of 474,1 ± 30,45 mg/kg. Also 37% of the mapping population showed lower values than *S. lycopersicum* due to transgressive segregation. As in vitamin C content, *S. pimpinellifolium* exhibited the greatest value with a mean value of 922,33 ± 145,47 mg/kg. The 2-fold range in phenolic content between parents was significantly different (P=0,02).

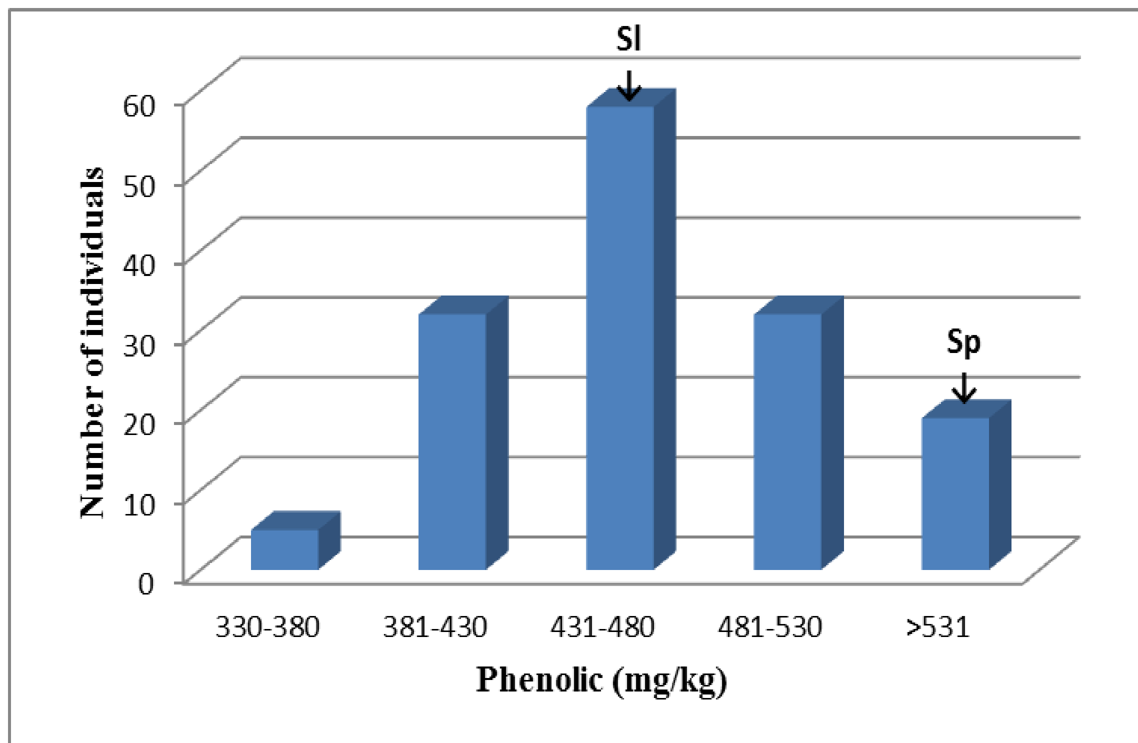

Figure 3.3. Distribution histogram for total phenolic content. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

3.1.4. Lycopene Content

Over three years, lycopene content didn't show any significant differences in populations. Lycopene content in the population ranged from 141 to 306 mg/kg with 2-fold variation. A good distribution of lycopene content exists in the population. Figure 3.4. represent this distribution as an average of years. *S. lycopersicum* displayed a moderate total phenolic compound with a value of 219,71 ± 4,5 mg/kg. However 58% of the population had lower values than *S. lycopersicum*. That again was a result of transgressive segregation. *S. pimpinellifolium* had the highest level of lycopene with a mean value of 329,31 ± 111,84 mg/kg.

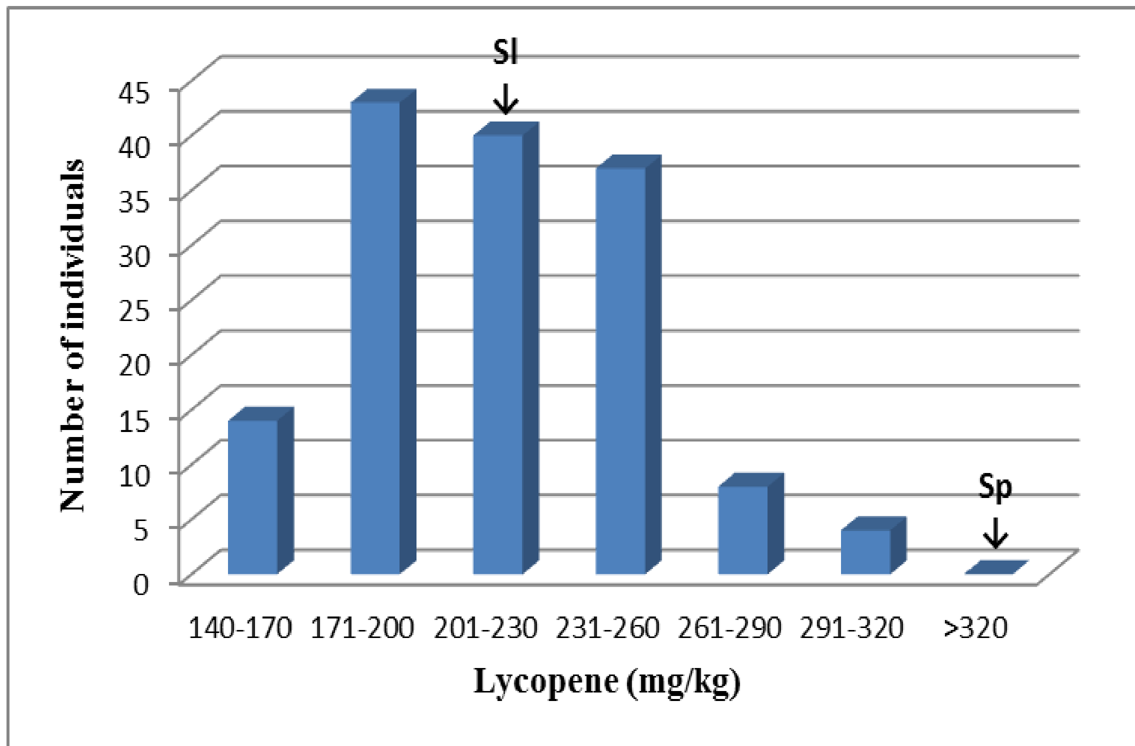

Figure 3.4. Distribution histogram for lycopene content. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

3.1.5. Average Fruit Weight

Means, standard errors and ranges for agronomic traits for the IBLs in 2004, 2005, 2006, and all years are presented in Table 3.3. Means of the agronomic traits, standard errors and ranges for the parents are displayed in Table 3.4. For fruit weight (FW) there was great variation in the population ranging from 24 to 96 g. The distribution histogram for fruit weight is displayed in Figure 3.5. The two parents showed highly different values. Weight for *S. lycopersicum* was 77,06 ± 3,45 g and for *S. pimpinellifolium* was 3,15 ± 1,68 g. These values indicated a highly significant difference between the parents (p=0,00002). *S. pimpinellifolium* showed the lowest value for the trait and only 3% of the population had heavier fruit than *S. lycopersicum.* This is also because of transgressive segregation.

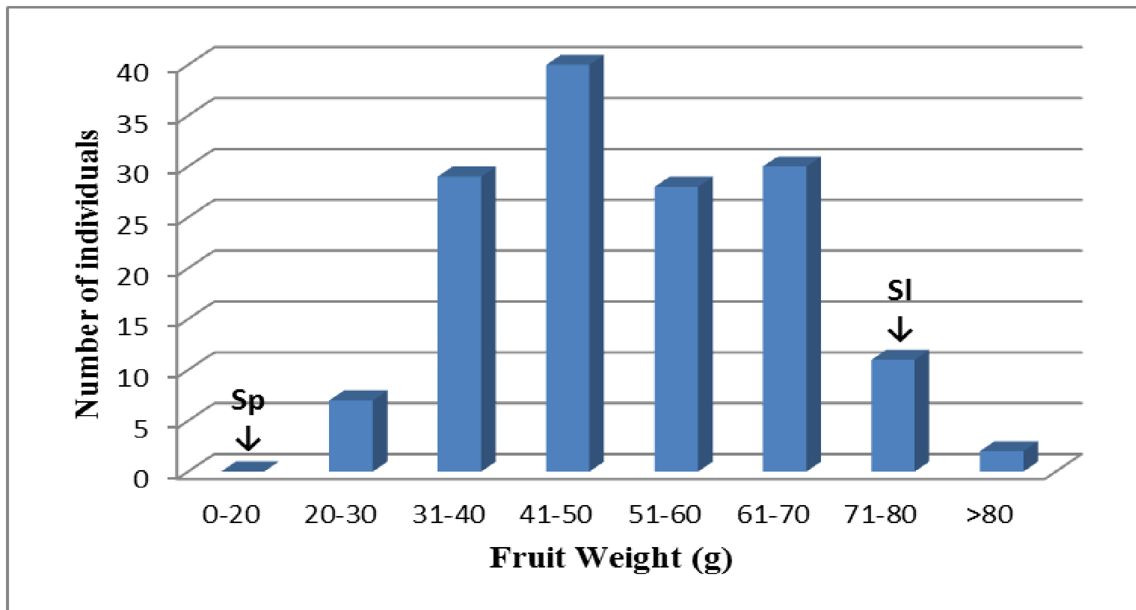

Figure 3.5. Distribution histogram for fruit weight. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

3.1.6. Fruit Shape

For fruit shape, the population and the parents were classified from 1 = round to 5 = elongated. *S. lycopersicum* classified as 4 and *S. pimpinellifolium* as 1. For the population the range was between 1 and 5. The mean value for fruit shape was 2,98 ± 0,09. Approximately 19% of the population showed higher result than *S. lycopersicum* with elongated fruits due to transgressive segregation. Most of the population had values close to *S. lycopersicum.*

3.1.7. Fruit Firmness

For the population, fruit firmness (FIRM) ranged from 1 to 5 while the parents' firmnesses were 3,83 ± 0,34 and 1,17 ± 0,17 for *S. lycopersicum* and *S. pimpinellifolium* respectively. This difference between parents was significant at P=0,001. The population displayed a continuous distribution. Figure 3.7. exhibits the histogram for fruit firmness. 1,4% of the population showed lower values than *S. pimpinellifolium* while 31% showed higher values than *S. lycopersicum*.

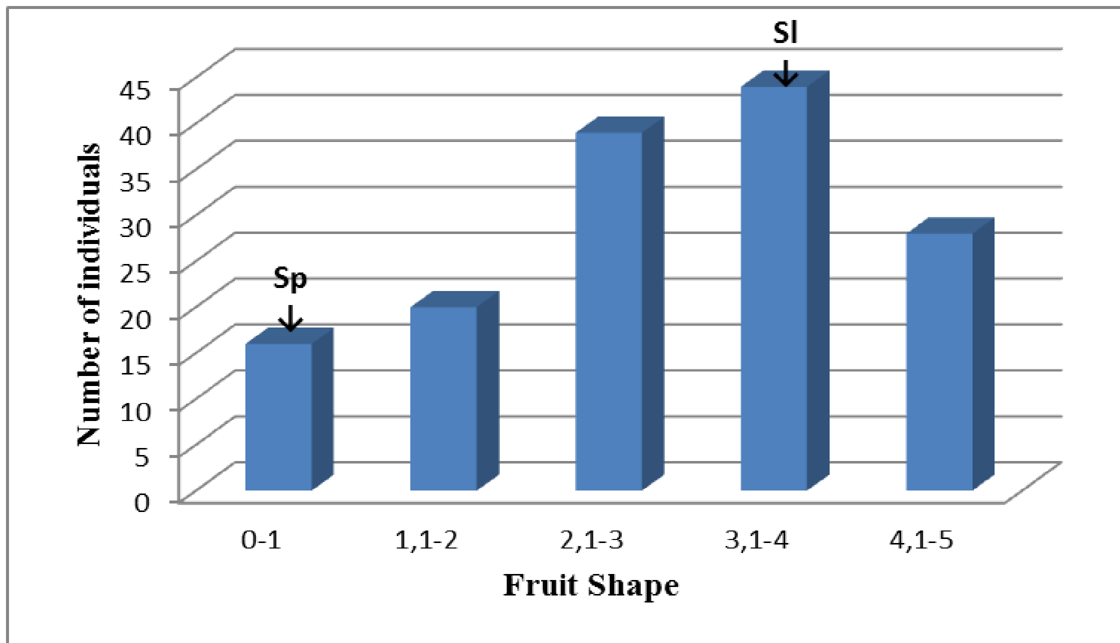

Figure 3.6. Distribution histogram for fruit shape. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

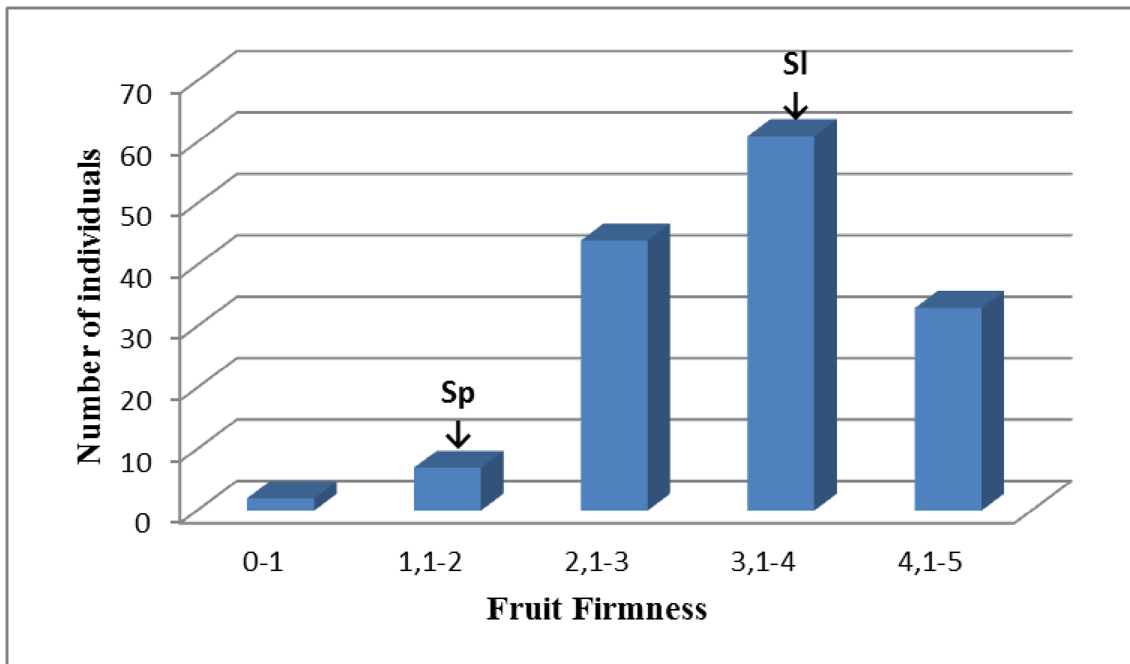

Figure 3.7. Distribution histogram for fruit firmness. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

Table 3.3. Agronomic traits' mean values, standard errors and ranges of BC_2F_7, BC_2F_8, and BC_2F_9 lines through 2004, 2005, 2006 and total.

	2004		**2005**		**2006**		**All Years**	
Trait	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**
Fruit Weight (g)	49,87 ± 1,19	21,89-85,58	48,79 ± 1,36	22,91-92,89	56,67 ± 1,50	19,70-106,56	51,34 ± 1,17	23,77-95,57
Fruit Shape	3,11 ± 0,11	1-5	2,76 ± 0,10	1-5	2,82 ± 0,11	1-5	2,98 ± 0,09	1-5
Firmness	2,97 ± 0,09	1-5	3,47 ± 0,10	1-5	3,88 ± 0,08	1,5-5	3,41 ± 0,07	1-5
Stem Scar Size	3,57 ± 0,08	1,5-5	2,55 ± 0,09	1-5	3,31 ± 0,10	1-5	3,22 ± 0,07	1,17-5
External Color	3,22 ± 0,06	2-5	3,41 ± 0,07	2-5	3,31 ± 0,07	1,5-5	3,30 ± 0,05	2-4,83
Internal Color	3,08 ± 0,08	1,5-5	3,00 ± 0,08	1-5	3,08 ± 0,07	1,5-5	3,07 ± 0,06	1,50-4,67
Locule Number	-	-	2,81 ± 0,04	2-4	2,81 ± 0,04	2-4,5	2,81 ± 0,03	2-4,5
Wall	-	-	3,19 ± 0,09	1-5	3,25 ± 0,08	1-5	3,23 ± 0,07	1,5-5
Soluble Solid Content	5,93 ± 0,05	4,5-7,5	4,55 ± 0,06	3,2-6,2	5,01 ± 0,07	3-7,8	5,24 ± 0,04	4,2-6,6

Table 3.4. Agronomic traits' values of parents through 2004, 2005, 2006 and total.

	2004		**2005**		**2006**		**All Years**	
Trait	**TA209**	**LA1589**	**TA209**	**LA1589**	**TA209**	**LA1589**	**TA209 Mean ± SE**	**LA1589 Mean ± SE**
Fruit Weight (g)	70,42	1,5	81,55	1,5	79,22	6,45	77,06 ± 3,45	3,15 ± 1,68
Fruit Shape	4	1	4	1	4	1	4 ± 0	1 ± 0
Firmness	3,5	1	3,5	1	4,5	1,5	3,83 ± 0,34	1,17 ± 0,17
Stem Scar Size	5	1	4	1	5	1	4,67 ± 0,34	1 ± 0
External Color	3,5	5	3	4	3	4,5	3,17 ± 0,17	4,5 ± 0,29
Internal Color	4	5	3	4	3	4	3,33 ± 0,34	4,33 ± 0,34
Locule Number	2,5	2	3	1	2	3	2,5 ± 0,29	2 ± 0,59
Wall Thickness	4	1	4	1	4	1	4 ± 0	1 ± 0
Soluble Solid Content	5,4	6,6	3,8	5,4	4,8	9	4,67 ± 0,48	7 ± 1,08

3.1.8. External and Internal Fruit Color

External and internal fruit colors detected similar results over population and parents as expected. While external and internal color were 4,5 ± 0,29 and 4,33 ± 0,34 for *S. pimpinellifolium*, external color was 3,17 ± 0,17 and internal color was 3,33 ± 0,34 for *S. lycopersicum*. External color showed a significant difference between the parents (P=0,008) while internal color did not (P>0,05). For the population both traits ranged between 2 and 5. For external and internal color 38 and 59% of the population, respectively, had lower values than both parents due to transgressive segregation. Internal and external fruit color histograms are displayed in Figure 3.8. and Figure 3.9. They both show continuous distribution for the population averaged over years.

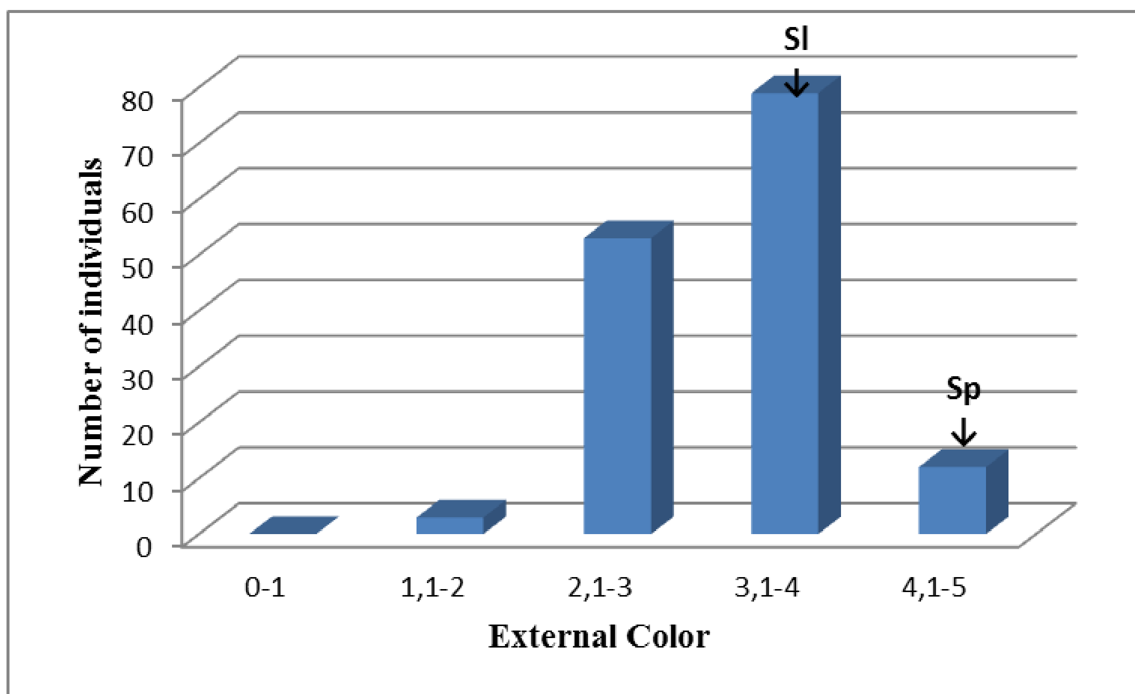

Figure 3.8. Distribution histogram for external fruit color. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

3.1.9. Stem Scar

Stem scar size (SCAR) showed a great variety between parents and also among individuals of the population. While *S. lycopersicum* averaged 4,67 ± 0,34 with a large scar, *S. pimpinellifolium* averaged as 1 ± 0 with a very small scar size. This was a highly significant difference between parents (P=0,0002). Also stem scar in the population ranged between 1 and 5 with a mean value of 3,22 ± 0,07 showing continuous variation. Figure 3.10. exhibits the distribution of stem scar in the population. Only 5,4% of the population exceeded the value of *S. lycopersicum* with a higher value due to transgressive segregation.

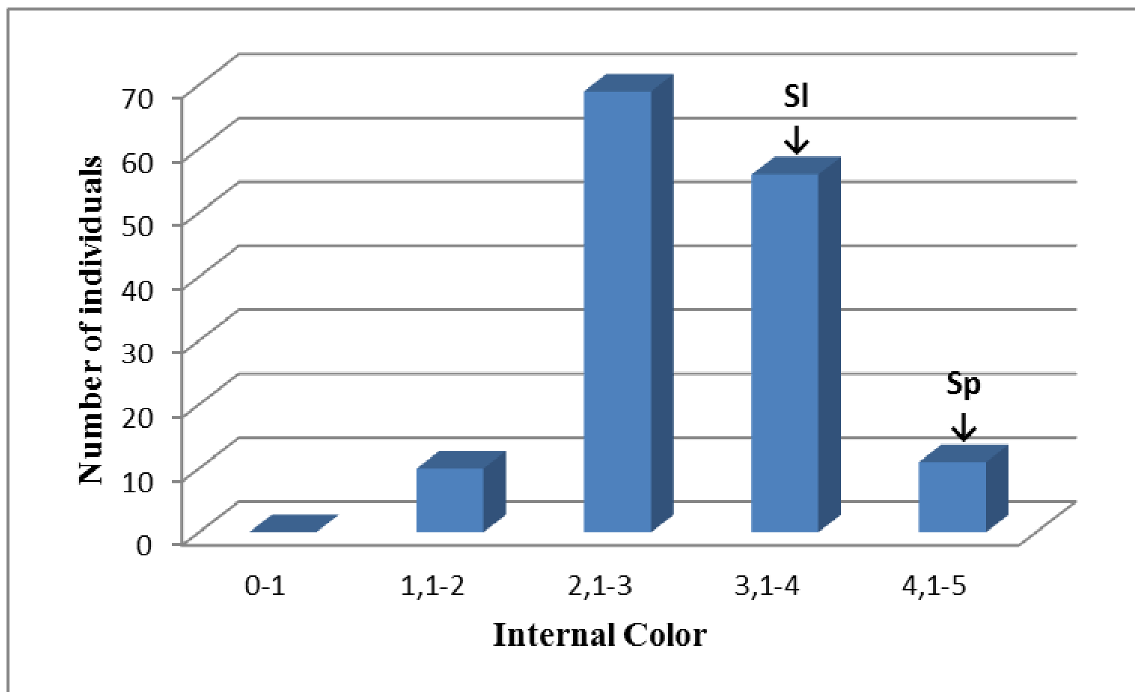

Figure 3.9. Distribution histogram for internal fruit color. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

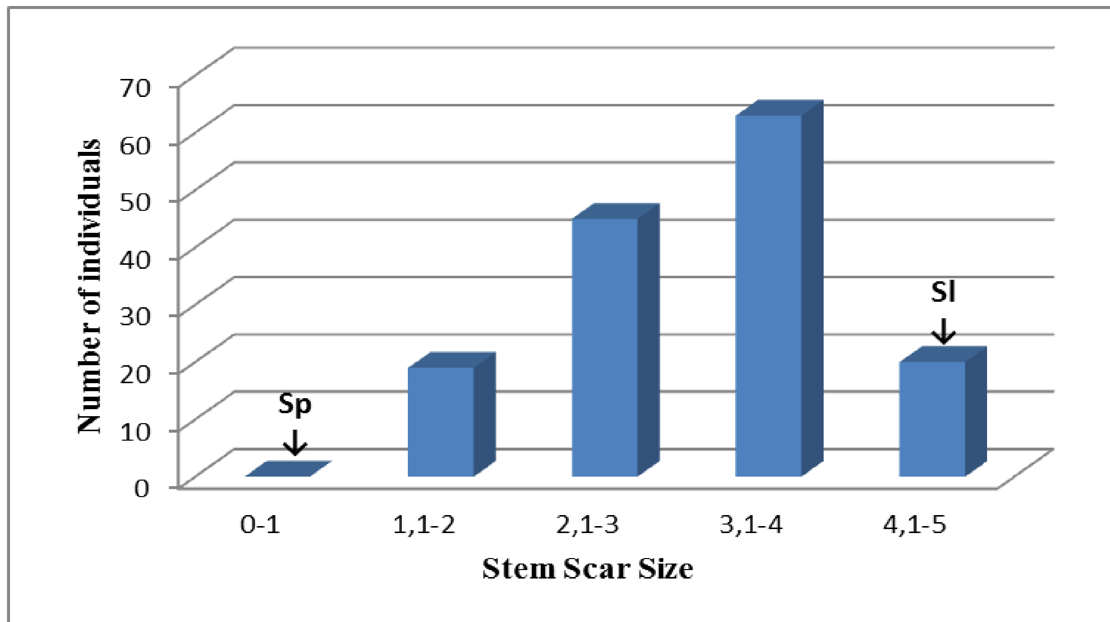

Figure 3.10. Distribution histogram for stem scar size. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

3.1.10. Locule Number

Locule number in the population ranged from 2 to 4,5 with a mean value of 2,81 ± 0,03. A total of 88% of the population had 3 locules in fruit. Figure 3.11. displays the distribution graph for locule number in the population.

3.1.11. Fruit Wall Thickness

Wall thickness (WALL) was ranged 1,5 to 5 in the population with a mean value of 3,23 ± 0,07. *S. lycopersicum* was scored as 4 for wall thickness. Figure 3.12. shows the continuous distribution of wall thickness in the population. Approximately, 14% of the population showed higher values than *S. lycopersicum.*

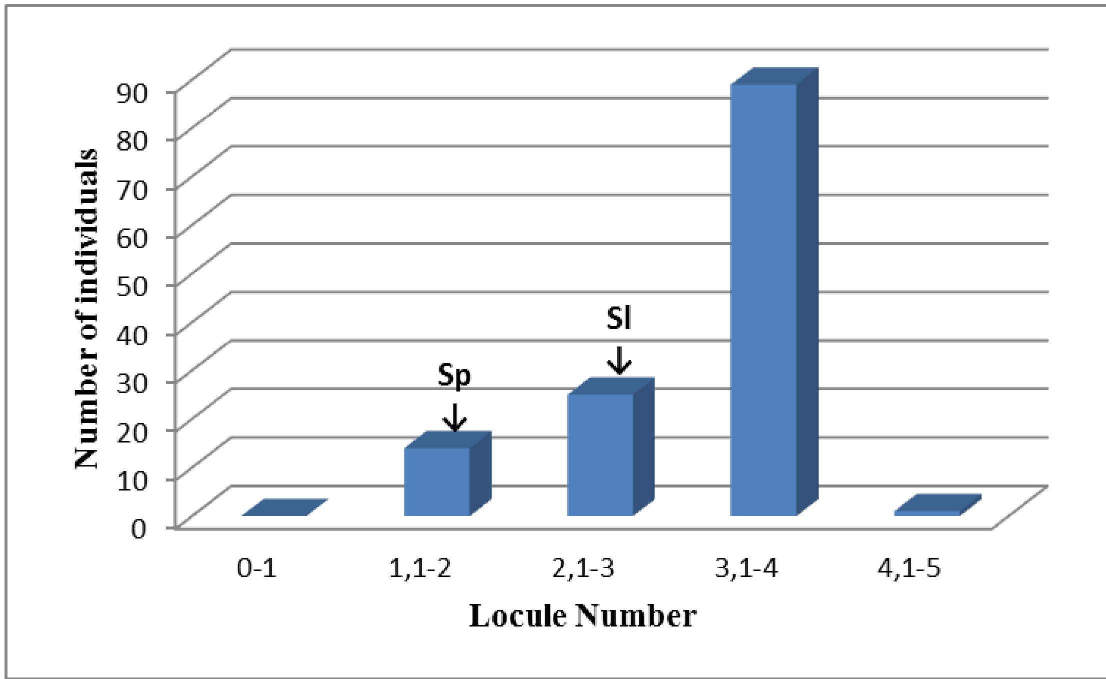

Figure 3.11. Distribution histogram for locule number. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

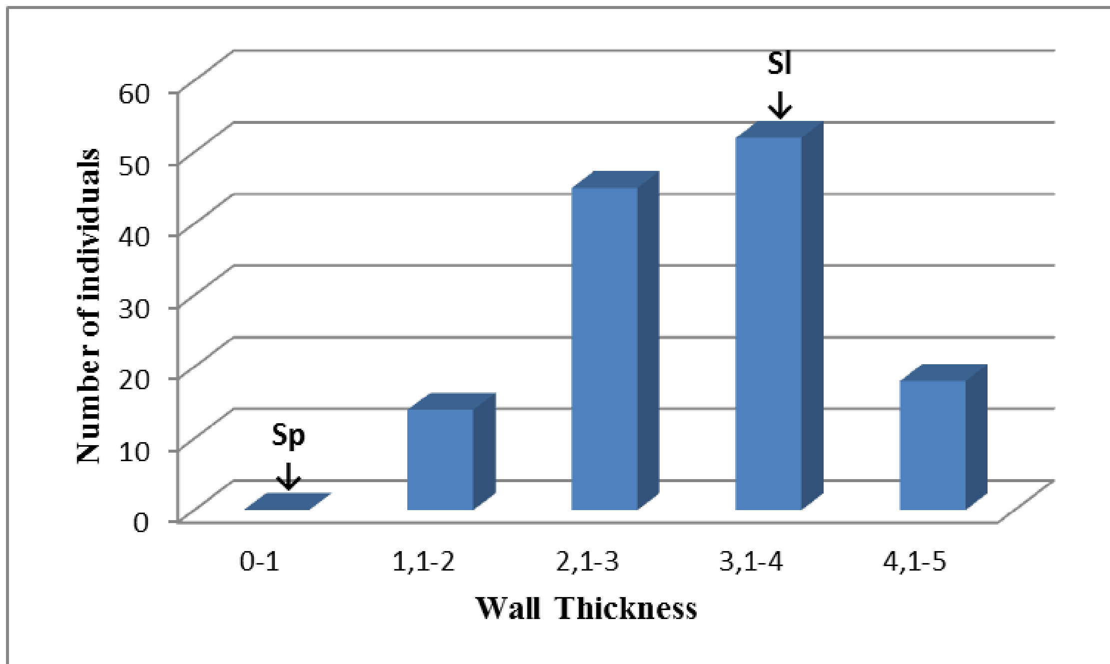

Figure 3.12. Distribution histogram for fruit wall thickness. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

3.1.12. Soluble Solid Content

Soluble solid content ranged from 4,2 to 6,6 brix over the population with a mean of 5,24 ± 0,04 brix. The average was 4,67 ± 0,48 brix for *S. lycopersicum*, while it was 7 ± 1,08 brix for *S. pimpinellifolium*. The parents showed no significant difference (P>0,05). Only 8% of the population exhibited lower values than *S. lycopersicum*. Overall, population showed continuous variation.

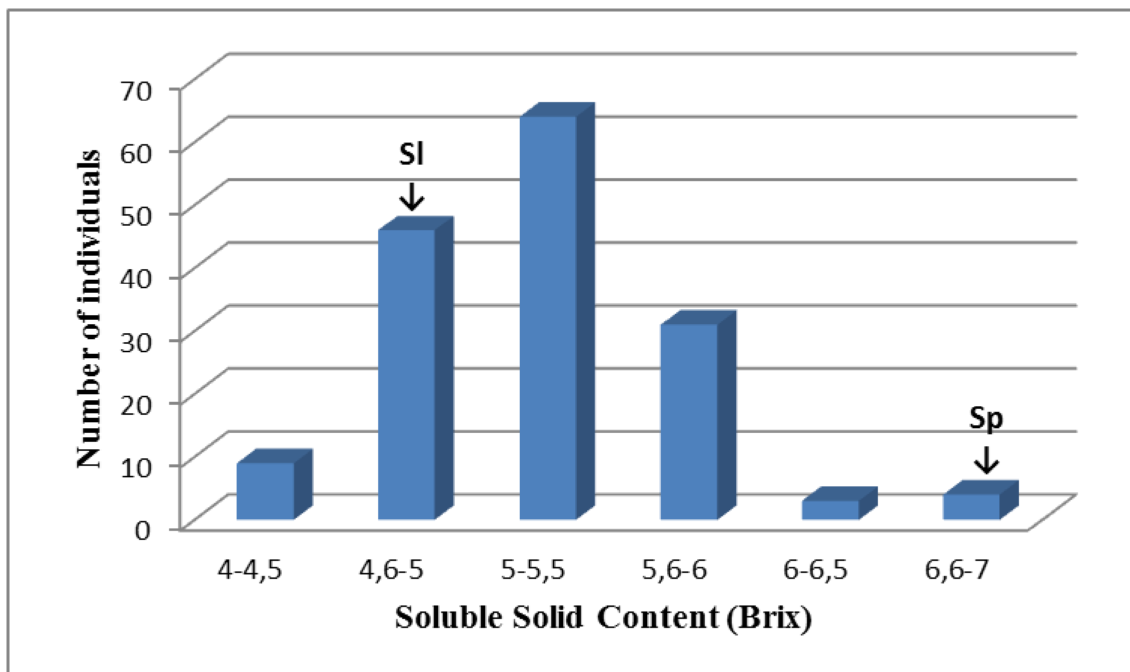

Figure 3.13. Distribution histogram for soluble solid content. Sl and Sp indicate locations of *S. lycopersicum* and *S. pimpinellifolium*.

3.1.13. Correlations Between Traits

Correlations between traits showed moderate but significant results (Table 3.5). Fruit weight was positively correlated with fruit shape, fruit firmness, stem scar size, and fruit wall (r = 0,53; r = 0,41; r = 0,56; r = 0,51 respectively). These results were expected as the bigger fruits tend to be elongated, have thicker fruit walls, bigger stem scar sizes and better firmness. All these traits are horticulturally important since they

affect yield and fruit appearance. In the same manner, fruit shape also showed correlations with firmness and wall thickness (r = 0,46; r = 0,42 respectively). This again confirms the relationship of weight and shape. Another important trait is color, identified separately as internal and external color. Internal and external fruit colors had a good correlation between them giving the highest value of all traits (r = 0,61). This result was also expected as both traits are controlled by the same pathway.

Also there was a good relationship between lycopene content and internal and external fruit color (r = 0,54 and r = 0,44 respectively). This is the result of lycopene being a carotenoid pigment that gives the red color to tomato. Another important correlation was between vitamin C and phenolic compounds (r = 0,54). This is expected because both compounds have contributions to the total amount of water soluble antioxidant activity. According to this information it was expected to see a good correlation between antioxidant activity and phenolics, but it exhibited a weak correlation (r = 0,27).

Table 3.5. Correlations between traits.

Trait	FW	FS	FIRM	SCAR	EC	IC	LOC	WALL	SSC	AUC	VitC	LYCO	PHEN
FW	1												
FS	0,53	1											
FIRM	0,41	0,46	1										
SCAR	0,56	0,19	0,08	1									
EC	-0,21	-0,22	-0,13	-0,03	1								
IC	-0,25	-0,32	-0,14	0,07	0,61	1							
LOC	0,01	-0,19	-0,11	0,17	0,06	0,18	1						
WALL	0,51	0,42	0,25	0,33	-0,02	-0,18	-0,10	1					
SSC	-0,15	-0,08	-0,17	0,16	0,16	0,26	-0,09	0,09	1				
AUC	-0,14	-0,17	-0,34	0,14	0,07	0,19	0,14	-0,02	0,17	1			
VitC	-0,19	-0,32	-0,10	-0,02	0,06	0,15	0,14	-0,12	0,15	0,24	1		
LYCO	-0,07	0,01	0,04	0,09	0,44	0,54	0,09	-0,05	0,11	0,15	0,04	1	
PHEN	-0,25	-0,31	-0,31	0,01	0,21	0,18	0,07	-0,15	0,25	0,27	0,53	0,12	1

3.2. Genotypic Characterization

In order to map the QTLs, the previously constructed RFLP map was used as framework (Doganlar et al., 2002). By using QGENE software program, single point regression analysis was carried out to identify the associations between markers and traits in the mapping population (Nelson, 1997). A total of 11 COS and 66 COSII markers that were tested on the 120 BC_2F_{10} lines for genotypic characterization were also positioned on the RFLP map. Table 3.6. and 3.7. list the COSII and COS markers with the restriction enzymes used for each.

In this research, a total of 103 significant ($p<0,05$) QTLs were identified. Table 3.8 and Table 3.9 list the identified QTLs for agronomic traits and antioxidant traits, respectively. Out of 103 loci, 25 QTLs were antioxidant trait related (24,5%). The other 78 QTLs were related to agronomic traits and accounted for 75,7% of all QTLs. Figure 3.14 exhibits the QTLs that were mapped.

For 26 of 78 QTLs for agronomic traits, favorable alleles were from *S. pimpinellifolium*. These traits mostly included internal and external fruit color and soluble solid content. This result is expected because fruits of *S. pimpinellifolium* tend to have darker red color. Favorable alleles for the other 52 agronomic traits were from *S. lycopersicum* including fruit weight, shape, firmness, stem scar size, wall thickness and locule number. This is also an expected result since the fruits of *S. lycopersicum* are much longer, heavier and firmer.

Favorable alleles for 18 out of 25 QTLs for antioxidant traits were from *S. pimpinellifolium* (72%). This high percentage indicates that as a wild tomato *S. pimpinellifolium* has more nutritionally valuable traits than *S. lycopersicum*. In order to construct elite tomato lines with high antioxidant traits, the markers that are linked to these QTLs can be used for marker assisted selection (MAS).

3.2.1. Reliability and Conservation of Loci

To confirm the reliability and conservation of the loci that were identified in this research, the results were cross-checked with previous research. Out of 25 antioxidant loci, 13 (52%) of them exhibited a match with a QTLs that were identified in the same population in 2004 (Ruscuklu 2005; Table 3.9). This indicated a good match with a

higher percentage than was seen by Rousseaux et al. (2005) when they compared antioxidant QTLs across years and found that 35% of the loci were identified in multiple years.

In order to exhibit the conservation of loci in other wild species *S. hirsutum* and *S. pennellii* populations were crosschecked for antioxidant QTLs. Out of 25 QTLs that were identified in this research, 5 of them (20%) matched with *S. hirsutum* (Okmen, 2008). Some of the QTLs, (24%) were also QTLs identified in a *S. pennellii* population (Rousseaux et al. 2005).These results suggest that genes for antioxidant traits have been conserved during evolution of tomato.

A total of four QTLs were identified for antioxidant activity in this research and 3 of them were identified previously. That indicates a 75% match to previous work. For vitamin C, 11 QTLs were identified and 9 of them showed a match (82%). For lycopene content, 2 of 4 QTLs were identified as a match (50%). For phenolics, 4 QTLs out of 6 displayed a match with previously identified loci (67%). These high percentages support the QTLs that we identified in this research.

Table 3.6. List of polymorphic COS markers and their restriction enzymes.

Marker	**Enzyme**
TG48	DraI
TG70	HinfI
TG176	RsaI
TG180	DraI
TG183	EcoRV
TG254	DraI
TG302	AluI
TG328	HpaII (MspI)
TG342	HhaI
TG393	HinfI
TG565	BamHI

Table 3.7. List of polymorphic COSII markers and their restriction enzymes.

Marker	Enzyme	Marker	Enzyme
At1g03310	CfoI	At3g12290	ApoI (XapI)
At1g05350	ApoI (XapI)	At3g16150	HinfI
At1g05970	TaiI	At3g17040	ApoI (XapI)
At1g07080	DpnII	At3g17930	MspI
At1g07960	DraI	At3g23400	CfoI
At1g10500	HaeIII (BsuRI)	At3g24050	DraI
At1g19140	PCR	At3g44890	HinfI
At1g19530	DraI	At3g47990	BcuI
At1g29320	MvaI	At3g55360	DraI
At1g48300	ApoI (XapI)	At3g63190	TaqI
At1g53000	EcoRI	At4g00090	RsaI
At1g55870	PCR	At4g10030	HinfI
At1g71810	CfoI	At4g15530	RsaI
At1g78690	AluI	At4g22260	HinfI
At2g01490	DdeI (HpyF3I)	At4g23100	Styl (Eco 130I)
At2g01720	DraI	At4g24830	HpaII (MspI)
At2g06005	HinfI	At4g30220	HinfI
At2g16920	PCR	At4g34700	CfoI
At2g20860	DraI	At4g35250	RsaI
At2g24270	AluI	At4g39660	HinfI
At2g24390	HinfI	At5g04740	MspI
At2g25570	VspI	At5g06430	PCR
At2g26270	HinfI	At5g13240	AluI
At2g32090	HinfI	At5g13700	HhaI
At2g38730	AluI	At5g20180	CfoI
At2g39100	HhaI	At5g20350	ApoI (XapI)
At2g42750	BstUI (Bsh1236I)	At5g23120	RsaI
At2g45730	MspI	At5g41480	Hin1I (NIaIII)
At2g46820	HinfI	At5g45410	BcuI
At3g02220	HinfI	At5g47040	DraI
At3g02300	TaqI	At5g51110	MspI
At3g08030	HincII	At5g51970	DraI
At3g11830	HinfI	At5g60160	HinfI

Table 3.8. QTLs identified for agronomic traits. R square values indicate effect of each QTL to the total phenotype.

Trait	QTL Symbol	Chrm	Marker	P Value	RSq Value (%)	Source
Fruit Weight	*fw1.1*	1	CT149	0,0467	4,2	TA209
	fw2.1	2	TG167	0,0001	12,2	TA209
	fw3.1	3	TG246	0,0002	11,3	TA209
	fw3.2	3	CT141	0,0344	4,6	TA209
	fw5.1	5	CT167	0,0341	4,7	LA1589
	fw7.1	7	CD57	0,017	5,5	TA209
	fw8.1	8	CD40	0,0455	4,3	TA209
	fw12.1	12	CT211	0,0069	5	TA209
	fw12.2	12	CT156	0,0409	4,4	TA209
Fruit Shape	*fs2.1*	2	TG167	0,0031	7,7	TA209
	fs2.2	2	TG308	0,031	4,7	TA209
	fs5.1	5	CT167	0,0056	7,1	LA1589
	fs8.1	8	TG45	0,0001	29,8	TA209
	fs9.1	9	TG654	0,0068	6,8	LA1589
	fs9.2	9	CT74	0,0073	6,7	TA209
	fs11.1	11	TG546	0,0278	5,1	TA209
Firmness	*firm1.1*	1	TG460	0,0039	5,6	LA1589
	firm2.1	2	TG308	0,0123	6	TA209
	firm2.2	2	TG492	0,0226	5,2	TA209
	firm3.1	3	TG66	0,0467	4,2	TA209
	firm4.1	4	TG272	0,0179	5,5	TA209
	firm8.1	8	TG45	0,003	8	TA209
	firm10.1	10	U	0,0126	4,2	TA209
	firm12.1	12	CT156	0,0073	6,7	TA209
Stem Scar Size	*scar1.1*	1	CT191	0,0457	4,7	TA209
	scar2.1	2	TG167	0,0005	10,1	TA209
	scar3.1	3	TG242	0,0001	13,8	TA209
	scar3.2	3	CT141	0,0218	5,2	TA209
	scar4.1	4	TG483	0,0085	6,7	TA209
	scar6.1	6	CT216	0,0244	5,1	TA209
	scar8.1	8	TG330	0,0001	11,8	TA209
	scar10.1	10	CT95	0,0275	5	LA1589
	scar12.1	12	CT276	0,0148	5,7	TA209

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Table 3.8 (cont.)

Trait	QTL Symbol	Chrm	Marker	P Value	RSq Value (%)	Source
External Color	*ec2.1*	2	CT176	0,0166	5,6	TA209
	ec3.1	3	TG214	0,0166	5,6	TA209
	ec4.1	4	CT192	0,0287	4,8	TA209
	ec5.1	5	CT167	0,0008	9,8	LA1589
	ec5.2	5	CT93	0,0274	4,9	TA209
	ec5.3	5	CT118	0,0196	5,4	LA1589
	ec6.1	6	TG314	0,0211	3,6	LA1589
	ec6.2	6	TG365	0,0068	6,7	LA1589
	ec7.1	7	TG342	0,001	9,4	LA1589
	ec9.1	9	CT74	0,0143	5,8	TA209
	ec10.1	10	U	0,0206	3,7	LA1589
	ec11.1	11	TG393	0,0007	9,7	LA1589
Internal Color	*ic1.1*	1	TG460	0,0281	3,3	TA209
	ic1.2	1	TG580	0,0304	4,8	TA209
	ic2.1	2	TG492	0,0454	4,2	LA1589
	ic3.1	3	TG152	0,023	5,4	TA209
	ic5.1	5	CT167	0,0092	6,5	TA209
	ic6.1	6	TG365	0,0023	8,2	LA1589
	ic6.2	6	TG314	0,0348	3,1	LA1589
	ic7.1	7	TG342	0,0025	8,2	LA1589
	ic8.1	8	CT111	0,0127	6,7	TA209
	ic11.1	11	TG393	0,0015	8,8	LA1589
	ic11.2	11	TG57	0,0402	4,6	LA1589
	ic12.1	12	TG360	0,038	4,7	TA209
Locule Number	*loc1.1*	1	TG245	0,0044	7,1	TA209
	loc1.2	1	TG460	0,023	4	TA209
	loc6.1	6	TG590	0,019	6,1	LA1589
	loc9.1	9	TG654	0,0345	5,3	TA209
	loc12.1	12	CT211	0,0337	3,5	LA1589
Wall Thickness	*wall2.1*	2	TG151	0,0006	1,1	TA209
	wall3.1	3	TG246	0,0055	8	TA209
	wall7.1	7	CT52	0,0262	3,9	TA209
	wall11.1	11	CT182	0,0375	5,1	TA209
	wall12.1	12	TG473	0,0011	10,4	TA209
	wall12.2	12	TG111	0,0044	9,5	TA209
Soluble Solid Content	*ssc1.1*	1	TG67	0,0168	6,3	TA209
	ssc2.1	2	CT205	0,007	7,7	LA1589
	ssc3.1	3	CD51	0,023	5,9	TA209
	ssc4.1	4	TG500	0,018	6,3	TA209
	ssc5.1	5	CT167	0,0001	13,7	LA1589
	ssc6.1	6	CT206	0,0067	5,7	LA1589
	ssc6.2	6	TG356	0,0141	6,6	LA1589
	ssc7.1	7	TG342	0,0059	8	LA1589
	ssc9.1	9	TG254	0,0214	6,2	TA209
	ssc10.1	10	CT234	0,0234	5,9	LA1589

Table 3.9. QTLs identified for antioxidant traits. R square values indicate effect of each QTL to the total phenotype.

Trait	QTL Symbol	Chrm	Marker	P Value	RSq Value (%)	Source	Reference*
AUC	*auc2.1*	2	CT205	0,013	6,1	LA1589	1
	auc3.1	3	TG242	0,0429	4,4	TA209	
	auc6.1	6	TG314	0,0364	3,1	LA1589	1,2,3
	auc10.1	10	CT234	0,0218	5,3	TA209	1,3
Vitamin C	*vitc2.1*	2	CT205	0,0013	9,1	LA1589	1,2
	vitc4.1	4	TG163	0,0026	6,2	LA1589	
	vitc5.1	5	CT167	0,011	6,3	TA209	1
	vitc6.1	6	TG99	0,0001	12,8	LA1589	1,2
	vitc6.2	6	TG365	0,0048	7,2	LA1589	1
	vitc7.1	7	CD57	0,0454	4,3	LA1589	1
	vitc8.1	8	CD40	0,0001	14,5	LA1589	1
	vitc10.1	10	CT234	0,0425	4,4	TA209	3
	vitc10.2	10	CT95	0,043	4,5	LA1589	
	vitc11.1	11	TG36	0,0375	4,5	LA1589	1
	vitc12.1	12	TG360	0,0258	5,3	LA1589	3
Lycopene	*lycop6.1*	6	CT206	0,0006	7,9	LA1589	
	lycop6.2	6	TG365	0,0087	6,5	LA1589	
	lycop9.1	9	CT283	0,011	6,2	TA209	1
	lycop9.2	9	CT74	0,0199	5,4	TA209	2
Phenolic	*phen2.1*	2	TG608	0,0044	7,4	LA1589	
	phen5.1	5	CT167	0,0003	11,1	LA1589	1
	phen6.1	6	CT206	0,0004	8,5	LA1589	2
	phen6.2	6	TG365	0,0007	9,7	LA1589	1,3
	phen8.1	8	CD40	0,0045	7,5	LA1589	3
	phen8.2	8	CT111	0,0196	6,1	TA209	

*References are coded as 1=Rousseaux et al. (2005); 2=Okmen (2008); 3=Ruscuklu (2005).

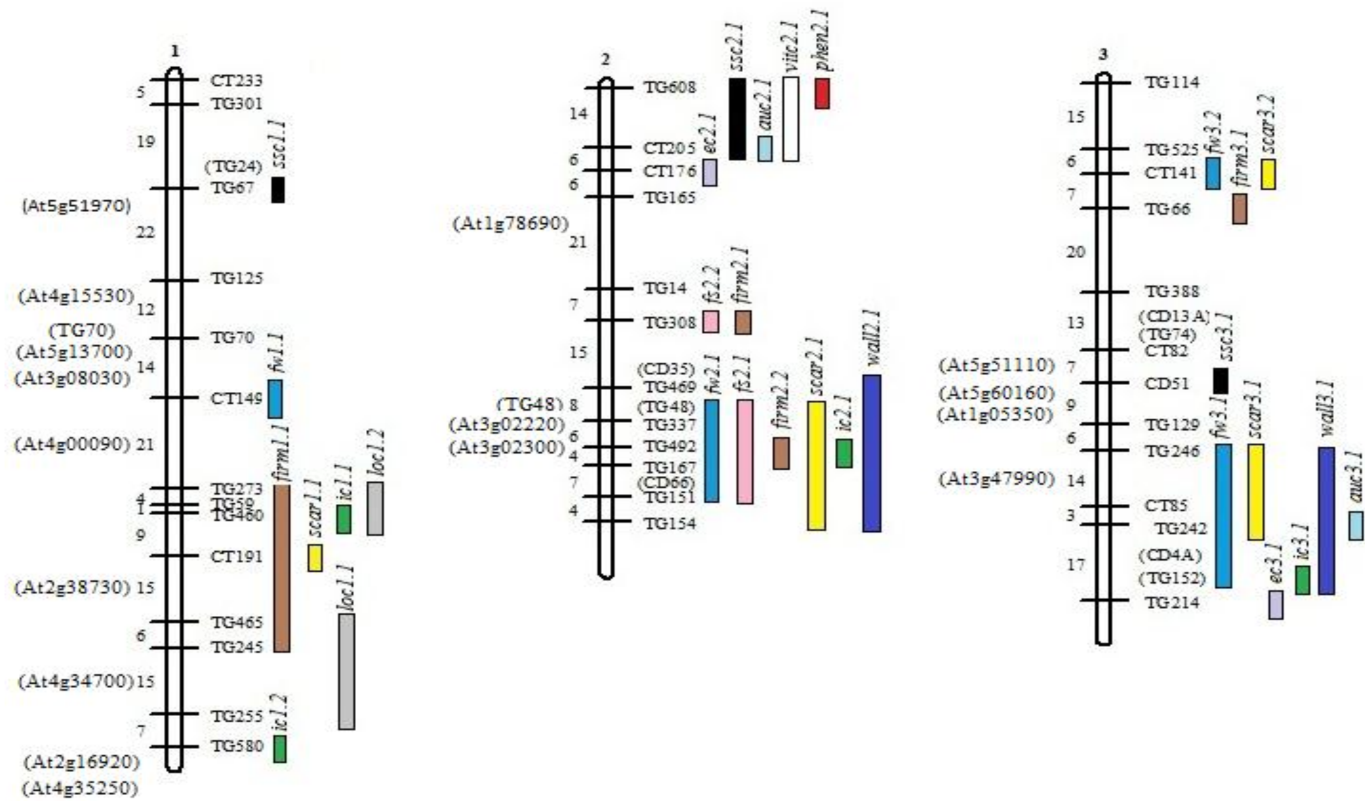

Figure 3.14. Molecular map of the tomato genome and locations of QTLs. COS and COSII markers are positioned on the left side, in parenthesis.

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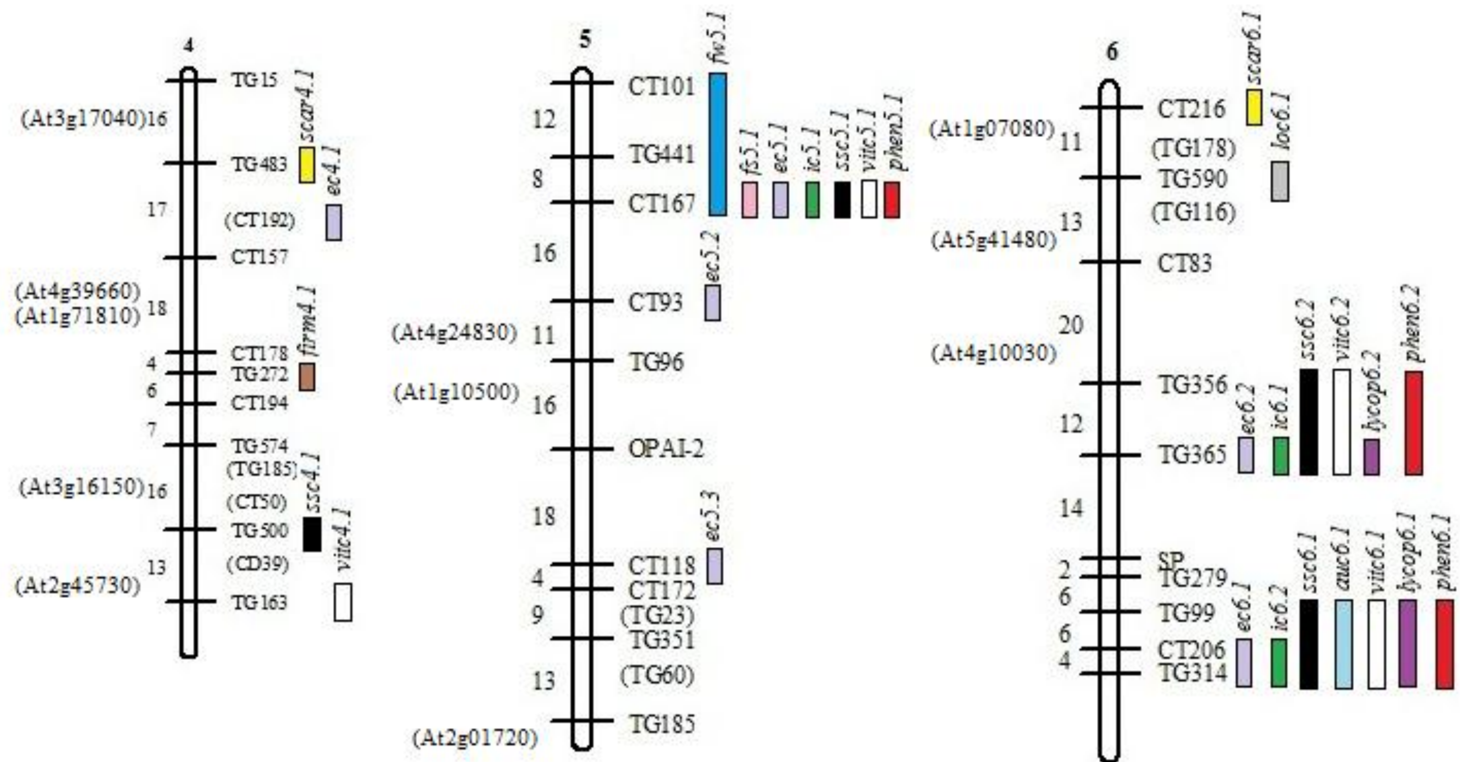

Figure 3.14 (cont.)

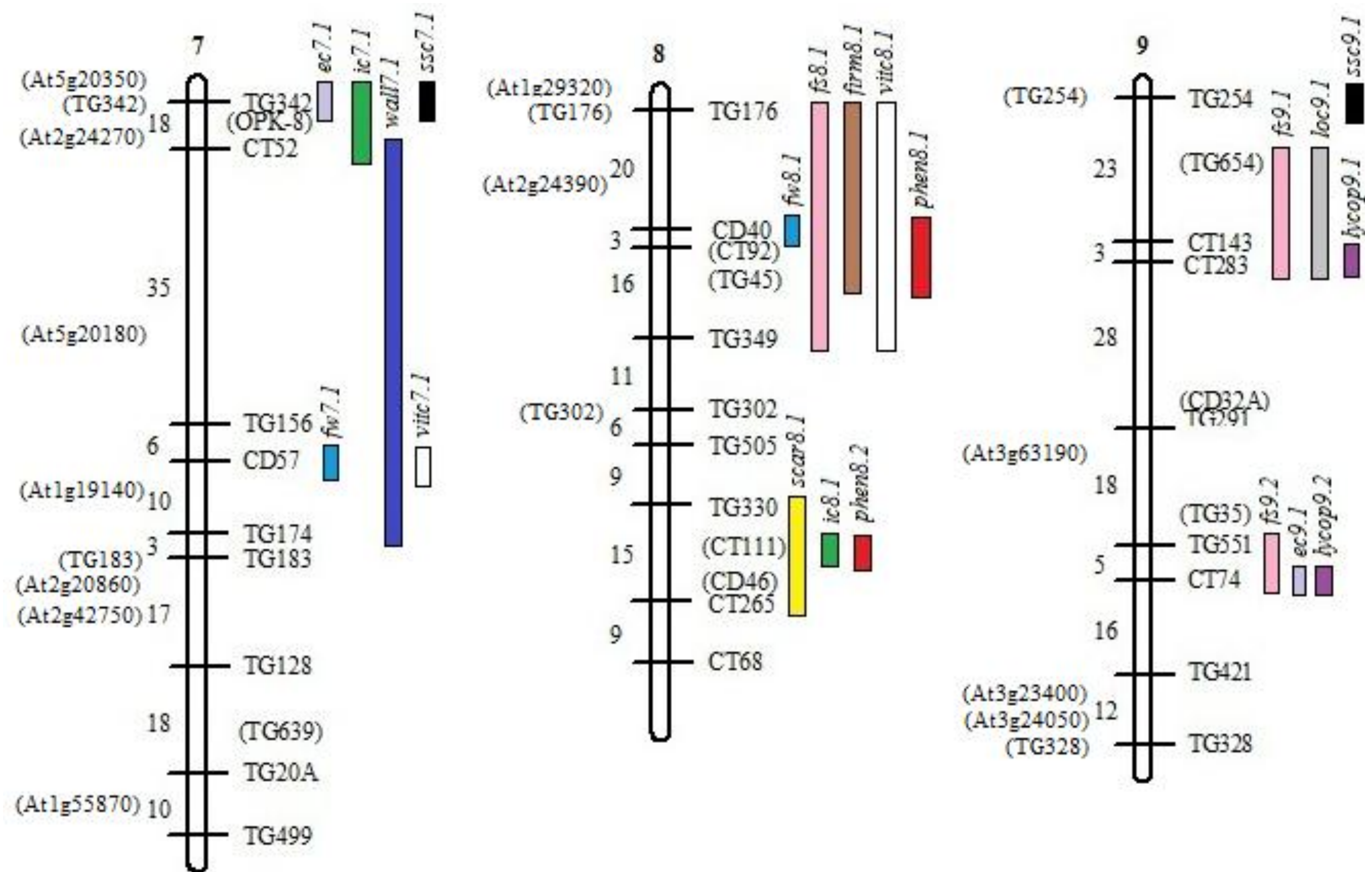

Figure 3.14 (cont.)

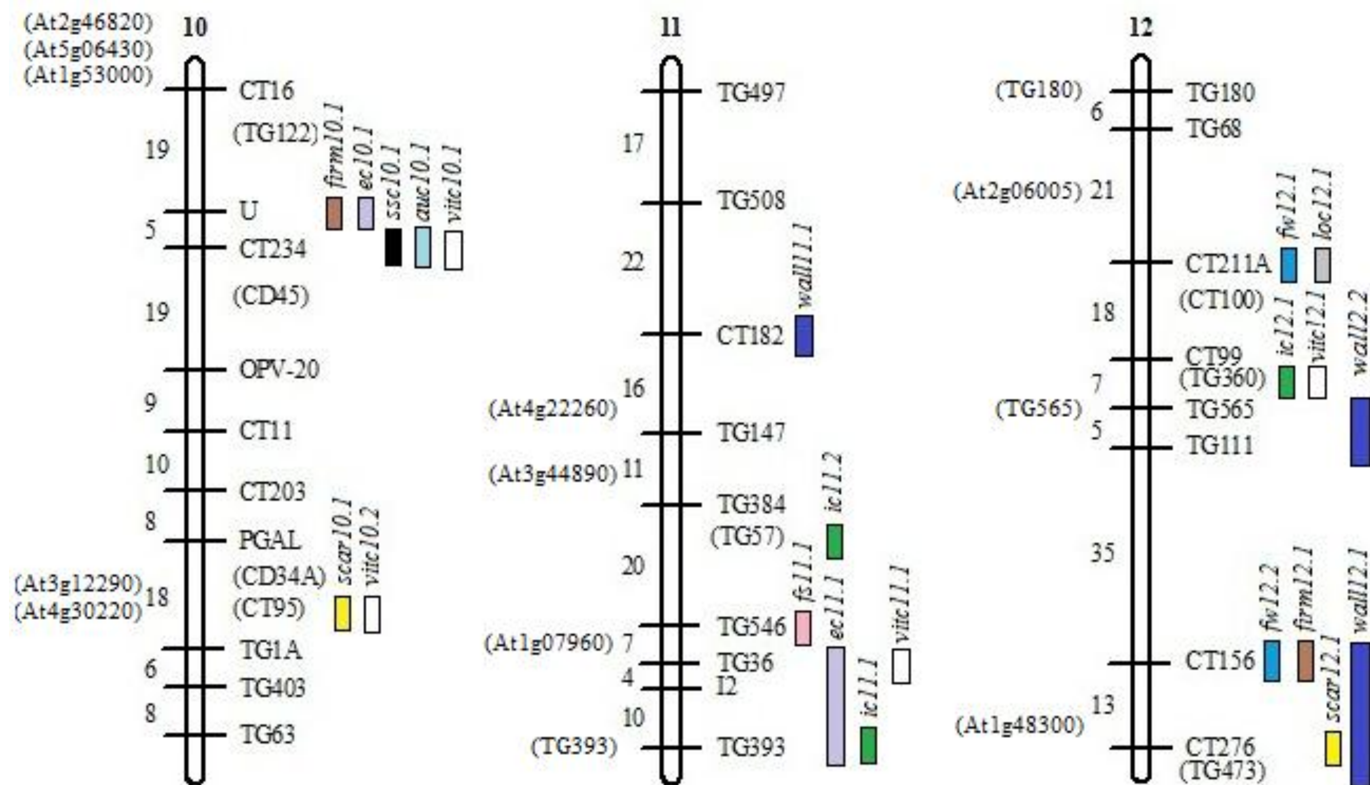

Figure 3.14 (cont.)

CHAPTER 4

CONCLUSION

Tomato is one of the most economically and nutritionally important crops and is produced and consumed in high amounts all around the world. The main goal of this study was to characterize a permanent inbred population and identify the genes that control nutritionally and agronomically important traits in tomato by identifying the QTLs for these traits with genetic markers. In order to develop a mapping population, 120IBL lines were derived from a cross between *S. lycopersicum* and *S. pimpinellifolium*. BC_2F_7, BC_2F_8, and BC_2F_9 lines were used for phenotypic characterization. Agronomic and antioxidant traits were measured visually and biochemically. For the genotypic characterization, BC_2F_{10} lines were screened with 66 COSII and 11 COS markers.

As a donor parent in this study, *S. pimpinellifolium* was shown to be a great source of antioxidant traits. For 72% of the antioxidant QTLs identified in this work, favorable alleles were from *S. pimpinellifolium.* As a recurrent parent, *S. lycopersicum* was the source of favorable alleles for agronomic traits. Thus, 67% of favorable alleles for agronomically important traits were sourced from *S. lycopersicum*. These results agree with what is expected to have resulted from the domestication of wild tomatoes. Through time agronomic traits like fruit weight, shape and firmness have been chosen while antioxidant traits have been ignored and lost. On the other hand, because of its great role in the plants' defense system, antioxidant traits may have accumulated in wild species like *S. pimpinellifolium* which have been subjected to natural selection. Using two distant parents to form a population led to great genotypic and phenotypic variance in the population. Also having different combinations of alleles from both parents resulted in progeny that exceeded both parents. This was the result of transgressive segregation. With the help of the molecular markers these potential traits can be identified and new alleles can be introgressed for the improvement of cultivated tomato.

Marker Assisted Selection (MAS) is useful for transferring of new genes and their alleles. Use of a marker identified to be linked to a trait of interest, makes it easier to select an individual that has the trait. Thus, there is no need to screen the population

for phenotypic identification. Since there is low recombination frequency between linked alleles, choosing a marker that is tightly linked to a trait will help to transfer the locus with it. Using MAS may also help saving time, energy, space and money.

For further studies, the QTLs that have been identified in this research can be transferred to improve an elite line of tomato. With an increase of antioxidant traits in tomato, healthier and more nutritional fruits can be produced. This increase contributes improving tomato plant as well as human health.

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