QUANTITATIVE TRAIT LOCI ANALYSIS (QTL) OF FRUIT CHARACTERISTICS IN TOMATO

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ABSTRACT

QUANTITATIVE TRAIT LOCI ANALYSIS (QTL) OF FRUIT CHARACTERISTICS IN TOMATO

Tomato has a crucial part in the human diet. Therefore, many plant breeders have tried to improve horticulturally important traits such as yield, fruit size, shape and color. With increased attention on human health, plant breeders also consider the improvement of health-related traits of fruits and vegetables such as antioxidant characters. However, because most plant traits are controlled by more than one gene, improvement of crops that possess the desired traits is very difficult.

Development of molecular marker techniques makes these processes feasible for plant breeders. In this study both health-related and horticulturally important traits were characterized for identificaton of their locations in the tomato genome using 152 *Lycopersicon hirsutum* BC_2F_2 mapping individuals. For this aim, all plants were phenotypically and genotypically characterized. It was expected that some alleles from the wild species *L.hirsutum* had the capacity for improvement of both antioxidant and agronomically important traits of elite lines.

A total of 75 QTLs were identified for all traits. Of the 75 QTLs, 28 were identified for five antioxidant traits including total water soluble antioxidant capacity, vitamin C, phenolic, flavonoids and lycopene content and 47 QTLs were identified for 8 agronomic traits including external and internal fruit color, fruit weight, firmness, fruit shape, stem scar size, locule number and wall thickness. Seventeen of these QTLs were also identified by previous studies. Markers linked with these QTLs can be used in Marker Assisted Selection (MAS) for improvement of elite tomato lines.

ÖZET

DOMATESTE MEYVE KARAKTERLERİ İÇİN KANTİTATİF KARAKTER LOCUS ANALİZLERİ

Domatesin insan beslenmesinde çok önemli bir yeri vardır. Bundan dolayı birçok bitki ıslahçısı bugüne kadar domatesin tarımsal açıdan önem teşkil eden, verimlilik, meyve büyüklüğü, şekli ve rengi gibi birçok karaterin geliştirilmesi için çaba sarfetmişlerdir. İnsan sağlığına verilen değerin artmasıyla beraber, bitki ıslahçıları artık meyve ve sebzelerde antioksidant karakterleri gibi sağlıkla ilişkili özelliklerin de geliştirilmesini dikkate almaktadırlar. Ne yazıkki, birçok bitki karakterinin birden fazla gen tarfından kontrol edilmesinden dolayı, istenilen özelliklere sahip bitkilerin ıslahı oldukça zordur.

Moleküler markör sistemlerinin geliştirilmesi bitki ıslahçılarının birden fazla genle kontrol edilen bu karakterlerin ıslahını olası hale getirmiştir. Yapılan bu çalışmada, 152 bireyden oluşan BC_2F_2 *L.hirsutum* populasyonu kullanılarak, hem sağlık açısından hem de tarımsal açıdan önem teşkil eden özellikler domates genomu üzerindeki yerlerinin belirlenmesi için karakterize edilmiştir. Bu amaç doğrultusunda, populasyondaki bütün bireyler fenotipik ve genotipik olarak karakterize edilmişlerdir. Yabani bir tür olan *L.hirsutum*'dan gelen bazı allellerin kültür hatta bulunan antioksidant ve tarımsal öneme sahip bazı karakterleri geliştirebilecek kapasiteye sahip olduğu düşünülmüştür.

Analiz edilen bütün karakterler için toplamda 75 QTL (genetic lokus) belirlenmiştir. Bu 75 QTL içerisinden, suda çözünen toplam antioksidant aktivitesi, C vitamini, toplam fenolic, flavonoid ve likopen miktarını da içerisine alan beş antioksidant karakteri için 28 adet, tarımsal açıdan önem taşıyan dış ve iç meyve rengi, meyve ağırlığı, sertliği, şekli, stem scar, lokul sayısı ve perikarp kalınlığı gibi sekiz karakter içinse toplamda 47 QTL belirlenmiştir. Bu QTL'lerin 17 tanesi daha önceden yapılmış olan bazı çalışmalarda da belirlenmiştir. Belirlenen bu QTL'lerle ilişkili olan markörler, markör dayalı seleksiyon da (MAS) kullanılmak suretiyle birinci sınıf kültür domates hatları geliştirilebilir.

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CHAPTER 1

QTL MAPPING

1.1. Introduction

The innovation of new molecular techniques drastically increases the importance and application of biotechnology in agriculture. Biotechnology is 'any technique that uses living organisms or substances from those organisms, to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses' (Kumar 1999). Tissue culture, genetic engineering and using molecular markers in conventional plant breeding for improvement of crops are the main biotechnological applications that are used in agriculture (Kumar 1999).

Since humans changed their lifestyle from hunting-gathering to agrarian societies, approximately 10000 years ago, agriculture has played a significant role for human life. The main objective of conventional breeding for improvement of existing crops is transfer of desired traits by crossing cultivars that do not possess such favorable traits with cultivars that have them. Desired traits such as high quality and yield, fruit size, shape and color, disease and insect resistance and high nutrient quality, have been selected during domestication and breeding. In addition, undesired traits such as shattering of seeds, non-compact growth habit, and germination inhibition have been eliminated from cultivated plants (Tanksley and McCouch 1997). However, the conventional breeding procedure is laborious and time consuming, requiring approximately 10-12 years to produce a new cultivar. This is because when two lines are crossed their whole genomes are combined, thereby the selection of desired recombinants, which contain desired traits, requires several crosses, several generations and careful phenotypic selection in the segregating population. Also the existence of unexpected plants with undesired traits that are tightly linked with desired traits decreases the success of this approach (Kumar 1999). The use of recombinant DNA

technology and genetic engineering overcomes many limitations that are faced in conventional plant improvement. However, these techniques also have some disadvantages such as the availability of a limited number of cloned genes and the difficulty of transformation of polygenic traits to plant. Also genetically modified organisms are hot ethical issues that are still debated in society (Kumar 1999).

1.2. Genetic Markers and Mapping

The main source of genetic variation or polymorphism among individuals, species, and other taxonomic groups stems from mutation. Mutation occurs in all organisms as a result of normal cellular mechanisms or interactions with the environment (exposure to UV radiation, mutagens, chemicals, etc.). There are many types of mutation at the DNA level including base substitutions, insertion or deletion of nucleotides and inversion of DNA segments. Accumulation of different types of mutation at different ratios in a species defines the genetic variation among individuals in the species and among different species. These phenotypic or genotypic variations can be used as markers for several genetic approaches such as characterization of germplasm, estimation of genetic distances between populations, construction of genetic maps, identification of monogenic and polygenic traits and so on. To use this variation as markers in genetic analyses, it must be heritable and recognizable whether in phenotype or at the molecular level of DNA or protein via gel electrophoresis (Liu and Cordes 2004). There are two main marker types: 1) Morphological markers and 2) Molecular markers (Tanksley 1993, Staub, et al. 1996, Kumar 1999).

1.2.1. Morphological Markers

Morphological markers are single gene mutations whose expression can be visualized in phenotypes such as dwarfism, anthocyanin production and leaf veins in plants. Morphological markers are affected by environment, thereby their reliability and

reproducibility can be low. In addition, there are a limited number of morphological markers in nature. Because the formation of morphological markers depends on gene mutations, the presence of single or multiple mutations may interfere with plant health and result in death (Staub, et al. 1996) .

1.2.2. Molecular Markers

Restricted usage of morphological markers led geneticists to find new approaches to identify variation among organisms. Molecular markers are genetic loci for which different alleles reveal sequence variation at the DNA level. Molecular markers may be gene-coding or non-coding pieces of DNA. Virtually all molecular markers have neutral effect on phenotype, thereby they cannot be visualized in phenotype. In addition, they are very abundant and stable markers that are easily detectable with molecular techniques (Tanksley 1993).

Molecular markers have several advantages over morphological markers. Unlike morphological markers, molecular markers do not cause any visible changes in phenotype, thereby there are more molecular markers available than morphological markers (Tanksley 1993). Variation occurring at the DNA level, such as a nucleotide difference or insertion/deletion of DNA pieces, is the main source of molecular markers. Polymorphism among individuals is detected by electrophoretic techniques. There are many types of molecular markers that are popular with molecular biologists.

The era of molecular markers was started with the discovery of isozymes. Isozymes are different allelic forms of enzymes produced by a single gene locus. It is supposed that any alteration that occurs at the DNA level may change the amino acid sequence of enzymes/proteins. These amino acid alterations result in the formation of differently charged or sized enzyme molecules that have the same function. The variation between these isozymes can be determined by using electrophoretic techniques which separate molecules in terms of their charge or size. The major drawbacks of using isozymes include: limited number of these marker types and their heterozygote deficiencies. In addition, post translational modification of proteins, which is not related with genetic

variation, restricts the usage of isozymes (Staub, et al. 1996, Tanksley and Nelson 1996).

Development of DNA-based molecular markers has enormously enhanced the potential usefulness of molecular marker types in genetics because of their ability to reveal more polymorphisms at the DNA level and their abundance. RFLP, restriction fragment length polymorphism, was the first type of DNA hybridization-based molecular marker that was developed in the 1980s. In this technique, genomic DNA is digested with a particular restriction enzyme at specific nucleotide sequences. Each different restriction enzyme recognizes a specific DNA sequence. Thus, any changes that occur in these restriction sites can create or eliminate restriction sites for a specific enzyme. Therefore, digestion of genomic DNA with an appropriate restriction enzyme can reveal variable sizes and numbers of DNA fragments among individuals or species. The Southern blotting method is applied with a specific probe to visualize these DNA fragments (Figure 1). RFLP markers are codominant markers, thereby allowing discrimination between homozygous and heterozygous individuals. However, RFLP markers have low levels of polymorphism and also require prior DNA sequence information and radioactive probes. These characteristics make this method more expensive and laborious (Staub, et al. 1996, Tanksley and Nelson 1996).

The next advance in molecular markers was development of DNA amplification-based molecular markers. RAPD, randomly amplified polymorphic DNA markers are derived from PCR (Polymerase Chain Reaction). In this technique, homologous arbitrary sequences in the genome are randomly amplified by PCR using 8-10 bp length single primers. Because of the short length and low annealing temperature (36-40 ^{o}C) of these primers, they can bind and amplify many DNA segments throughout the genome. Primers can amplify 200-2000 kb long pieces of DNA. The PCR products of RAPD primers can be separated by agarose or polyacrylamide gel electrophoresis and observed by staining with ethidium bromide or silver. The polymorphism of RAPD markers derives from sequence variation among the genomes that alter the primer binding sites. Thus, not all RAPD marker bands are amplified in all individuals using the same primer. RAPD markers are dominant markers and polymorphism is defined as presence or absence of particular RAPD bands (Figure 1.1). This is one of the shortcomings of RAPD markers, because they cannot distinguish between individuals homozygous for band presence and heterozygous individuals (Staub, et al. 1996, Jones, et al. 1997).

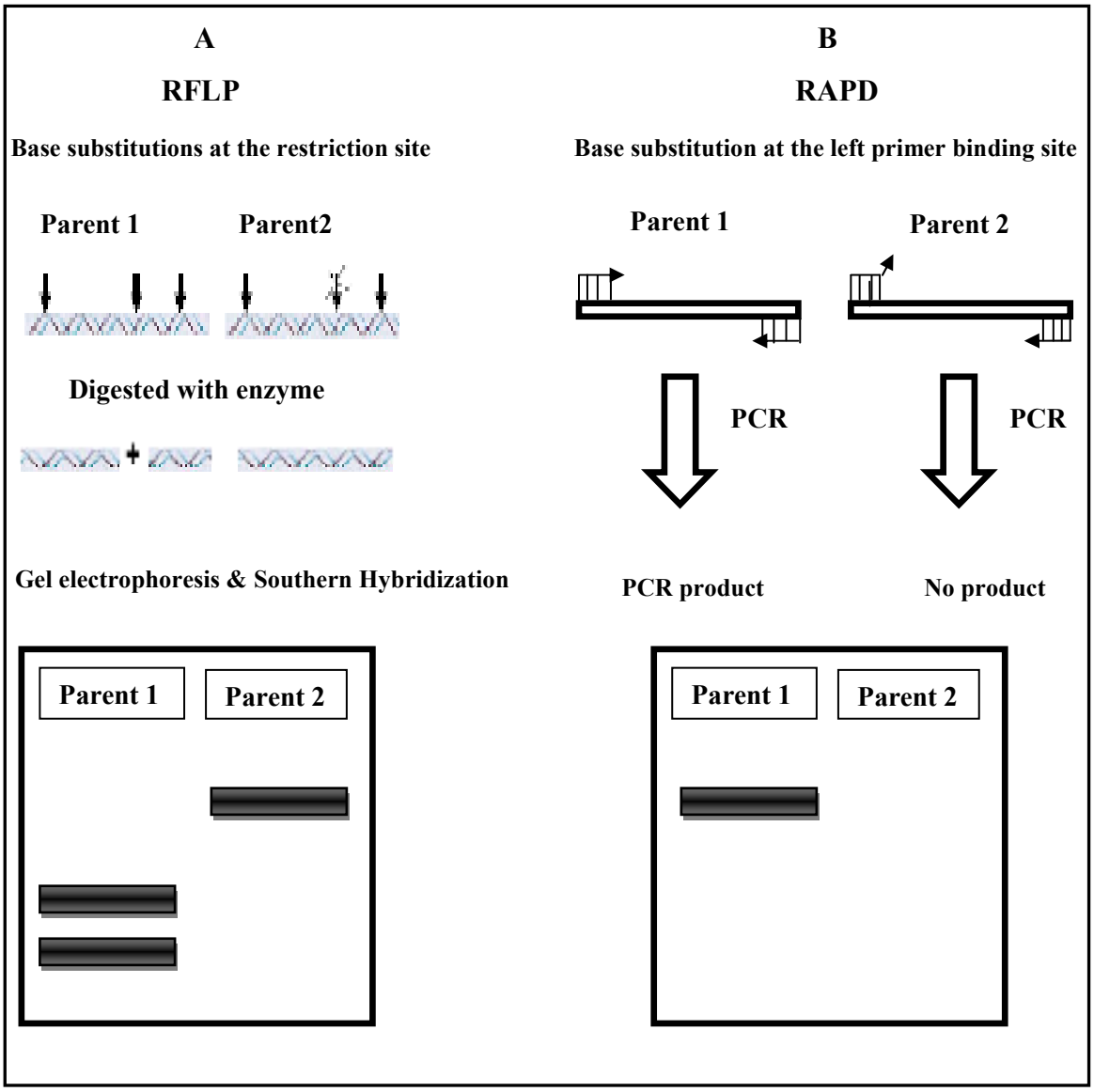

Figure 1.1. Schematic depiction of (A) restriction fragment length polymorphism (RFLP) and (B) randomly amplified polymorphic DNA (RAPD) markers

Unlike RFLP, RAPD does not require prior knowledge of DNA sequence and radioactive probes. These advantages make RAPD cheaper than RFLP. However, poor reliability and reproducibility of RAPD markers and their high sensitivity to environmental conditions decrease the usage of this technique (Staub, et al. 1996, Jones, et al. 1997).

CAPs, cleaved amplified polymorphic sequences, are PCR-based molecular markers that are analogous to RFLP markers. In this technique, sequence-specific primers are used to amplify a specific DNA region that contains restriction sites. After amplification

of this DNA region, the incidence of variation/polymorphism is enhanced by using particular restriction enzymes that cleave the PCR products (Staub, et al. 1996). They are highly polymorphic, codominant, phenotypically neutral and abundant molecular markers, thereby they are commonly used in mapping studies.

1.3. Molecular Marker Mapping

Molecular marker analysis has several important applications in plant biology, but the construction of molecular marker maps is one of the most useful. Molecular marker mapping can be described as placing markers in their correct order along different linkage groups (Jones, et al. 1997). This technique depends on segregation of different genotypes, linkage between close markers and recombination between markers that are not closely linked. The relative genetic distance between markers is expressed in centimorgans (cM) and represents the rate of recombination between them (1% recombination = 1 cM). Because the incidence of recombination varies along the chromosome, markers that are far away from each other may be defined as close markers if they are located in a chromosome region where recombination is suppressed. Therefore, the distance between two markers in genetic mapping (cM) and in physical mapping (expressed in base pairs) is not equal (Jones, et al. 1997, Kumar 1999).

The construction of a molecular marker map depends on development of an appropriate mapping population, estimation of recombination frequencies of marker loci in this population, establishment of linkage groups of markers and determination of map distance and order of markers (Staub, et al. 1996).

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

reason, recombination frequency can be used to define appropriate distances between two loci along the chromosome (Jones, et al. 1997). By using computer programs such as MapManager, Joinmap and MAPMAKER that determine the linear arrangement of molecular markers by estimating recombination frequencies, a linkage map can be easily constructed (Staub, et al. 1996).

Once a genetic linkage map, based on molecular markers, has been constructed, it can be used for identification of gene location, positional gene cloning, comparative mapping and marker assisted selection in plant breeding. The ability of markers to act as a landmarks leads us to genes of interest along the chromosome (Jones, et al. 1997). By using molecular marker maps, both qualitatively and quantitatively inherited traits can be mapped.

A qualitative character is a trait that is controlled by a single gene with major phenotypic impact such as flower color in pea and some types of disease resistance in plants. There is little environmental effect on the phenotype that exhibits discrete variation. Therefore, mapping of such qualitative genes, which are inherited in a Mendelian manner, is very simple (Tanksley 1993, Jones, et al. 1997). In order to detect the location of the gene of interest on the molecular marker map, the mapping population must possess phenotypic variation for the desired tarit. The assumption is that if one or more of the markers and alleles at the gene locus have linkage between each other, they will segregate together. Finally, the location of the gene can be identified (Tanksley 1993, Jones, et al. 1997).

Quantitative traits are controlled by more than one gene with great environmental effect. The locations of genes that contribute to the expression of a polygenic trait in the genome are called quantitative trait loci (QTL). Many agronomically important traits such as yield, plant height, stress tolerance, nutritional quality and antioxidant production are controlled by QTL with great environmental effect. In contrast to qualitative traits, quantitative traits show continuous phenotypic variation for the trait in question. Therefore, mapping QTLs is not as simple as mapping major genes. However, the development of a molecular linkage map makes it feasible to study quantitatively inherited complex traits (Tanksley 1993, Jones, et al. 1997, Tanksley and McCouch 1997).

QTL analysis of traits of interest involves several requirements. The first requirement is the development of an appropriate mapping population. The mapping population must exhibit sufficient polymorpism for both molecular markers and desired traits. Without any polymorphism among the progeny, a gene cannot be mapped. The best approach for enhancement of genetic variation in a population is to cross two parent lines that are divergent for the desired trait and also for markers. For this reason, use of interspecific populations is often preferred. The use of two cultivated lines as parents reduces variation because during domestication the variation among cultivated crops has been decreased dramatically and lower genetic variation reduces the combination of new and useful alleles in progeny (Tanksley and McCouch 1997). Thus, using a wild species as one of the parents is an effective way to get an appropriate mapping population (Tanksley 1993, Jones, et al. 1997, Kumar 1999). There are several types of populations available for QTL mapping. Some important ones are: F_2 populations, backcross (BC) populations, recombinant inbred lines (RIL) and double haploid lines (DHL). Each population has strengths and weaknesses. Figure 1.2 shows some population types that are used in mapping studies.

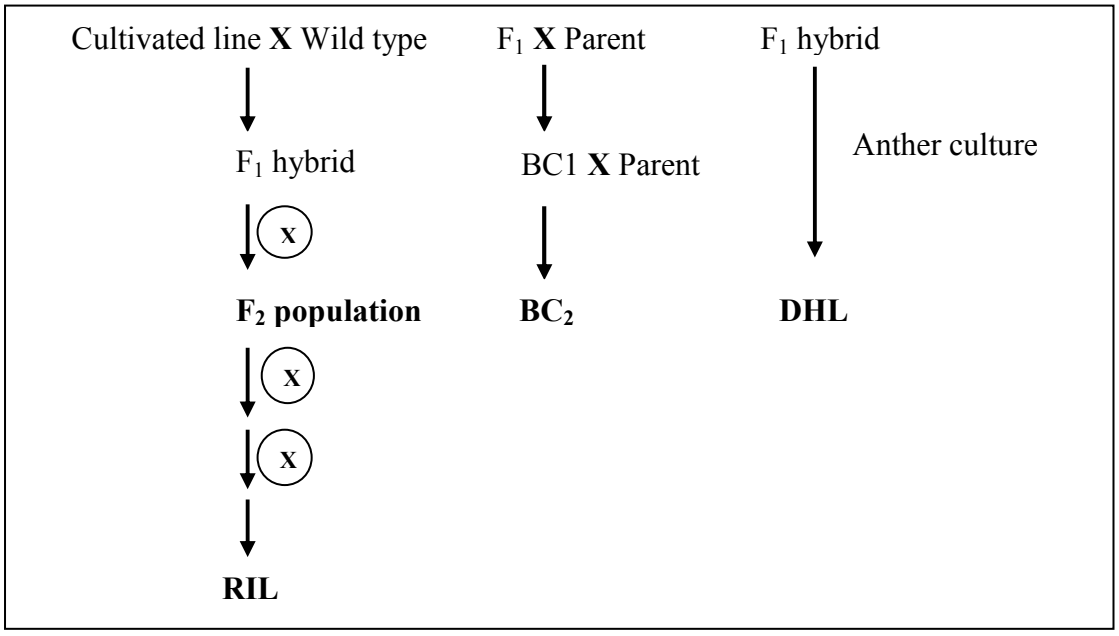

Figure 1.2. Commonly used population types in mapping studies. F_2 population, recombinant inbred lines (RIL), backcross (BC), and double haploid lines (DHL)

Secondly, a complete molecular marker linkage map for the studied population must be developed as described above. The presence of linkage disequilibrium between alleles of the molecular markers and alleles of the QTL is essential for molecular mapping analysis. Linkage disequilibrium is the nonrandom association of alleles at different loci in a population (Tanksley 1993). Physical linkage of loci that are located on the same chromosome is the main source of linkage disequilibrium. Linkage disequilibrium is inversely proportional to the distance between two loci. That means closer loci, have higher linkage disequilibrium (Tanksley 1993).

Unlike qualitative traits, quantitative traits controlled by polygenes have continuous phenotypic distribution in a population. Thus, QTL mapping relies on different statistical strategies including analysis of variance (ANOVA) and linear regression analysis. These statistical analyses can reveal significant associations between markers and traits, the approximate number of loci that affect the trait, the average gene action along with the level of interaction between polygenes and also between environments (Tanksley 1993).

The simplest way to detect QTL is to analyse the data using one marker at a time. One fundamental example is giving in Table 1. In this approach, firstly all polymorphic markers, these are Marker 1 and Marker 2 in this example, are tested on all individuals of the mapping population. Then the population is genotypically divided into three groups (homozygous like parent A, homozygous like parent B and heterozygous) based on each marker genotype. The phenotypic mean for the desired trait, in the example the trait is plant yield, is calculated for each genotypic group. Lastly, the association between marker and variation for yield is determined by testing significant differences among the means (ANOVA is used). If there is a significant difference among the phenotypic means for a marker, it can be said that this marker is linked to QTL for yield (Table 1.1).

Table 1.1. Basic strategy for QTL identification.

Marker	Genotype	Mean Yield	Conclusion
Marker 1	AA	50	No significant difference among means, no yield QTL linked to marker 1
	AB	51	
	BB	48	
Marker 2	CC	80	Significant difference among means, yield QTL linked to marker 2
	CD	60	
	DD	40	

1.4. Application of QTL Mapping

Before construction of molecular marker maps, it was believed that quantitative traits were controlled by several genes that contribute equally to the expression of the trait in question. However, QTL studies have revealed that this assumption is not true. Polygenic traits are controlled by a large number of loci that each possess weak or strong effects on the final phenotypic value of the trait (Tanksley and McCouch 1997). Thus, to find a marker tightly linked with a gene that has a large effect on the trait allows marker assisted selection in plant breeding and map-based cloning of this gene.

Marker Assisted Selection (MAS) is based on the concept of the presence of an association between the marker and the gene of interest. If they are tightly linked to each other the possibility for the marker and locus to be transmitted together to the progeny will be very high due to low recombination frequency. Thus, screening of the population with a marker linked to the desired trait makes it feasible to select individuals that have the desired traits without phenotypic characterization . In addition, MAS can also be used for negative selection which means that undesired traits can be eliminated in the population. Conventional breeding processes require dramatically more time, labour and space. In conventional breeding, when two lines are crossed thousand of progeny that contain both desired and undesired alleles are formed. Therefore, the selection of progeny that possess the traits of interest is extremely difficult. MAS is an alternative way to overcome these obstacles. In contrast to conventional breeding, MAS does not require a completely mature plant, thereby

selection can be done at the seedling stage with a higher efficiency of selection. By doing this, requirements for time, space and labour are greatly decreased (Kumar 1999).

Map-based cloning is a powerful technique for isolation of a gene of interest. As opposed to other gene cloning strategies, map-based cloning does not require prior knowledge about the gene products (Tanksley and Nelson 1996, Kumar 1999). The major necessity for map-based cloning is knowledge about the chromosomal location of the gene. Therefore, the identification of markers that are tightly linked to the desired gene is the first step in the map-based cloning strategy. If the gene region is sufficiently saturated with markers, the gene can be cloned by chromosome walking or chromosome landing. Production of a genetic library that is formed via cloning of large fragment of genomic DNA to yeast artificial chromosomes (YACs) or bacterial artificial chromosomes (BACs) makes chromosome walking possible to identified the exact location of the desired gene (Tanksley and Nelson 1996, Jones, et al. 1997). By hybridization with appropriate probes, the YAC or BAC clones that carry the markers linked to the desired gene can be identified. Analysis of the overlapping clones allows identification of the most likely position of the target gene (Kumar 1999). Finally, sequence and/or complementation analysis are used to confirm that the correct gene has been isolated.

CHAPTER 2

ANTIOXIDANTS

2.1. Free Radicals and Antioxidants

Recently, there is convincing evidence of a link between diet and human health. Therefore, there is great interest about food, food components and the positive effects of these components that improve health. Many reports demonstrate that fruits and vegetables contain some basic nutritives as well as biologically important substances, such as, vitamins, minerals and antioxidant components that have benefical effects on human health (Jones 2002, Rodriguez, et al. 2006). Since plants are rich in many types of vitamins and phytochemicals, high consumption of plant products may decrease the risk of several diseases such as atherosclerosis, cardiovascular diseases and many types of cancer (Yao, et al. 2004, Podsedek 2007). For that reason, in addition to improvement of agronomically important traits (yield, disease resistance, size, etc.), enhancement of the nutritional content of fruits and vegetables is now favored among plant breeders for improvement of human life expectancy.

Antioxidants are capable of inhibiting free radical formation and protecting organisms against oxidative stress-mediated damage (Nordberg and Arner 2001, Somogyi, et al. 2007). Therefore, antioxidants are vital for maintaining an organism's health and well-being. To appreciate the importance of antioxidant defense systems, it is essential to understand how free radicals are formed and how they damage cellular components in organisms.

2.2. Free Radicals

A free radical is any electrically charged atom, molecule or compound that contains one or more unpaired electrons. An unpaired electron is one that occupies an atomic or molecular orbital by itself. Because of their unpaired electron, free radicals are very unstable and reactive and seek out and pull electrons from other substances to make a new pair. Although pairing of electrons causes a free radical to become neutralized, this process initiates a chain reaction that results in formation of new free radicals (Halliwell 2006). Free radicals have many harmful effects on biologically important macromolecules such as DNA, lipids and proteins. They may disturb the normal structures and functions of these cellular components. Therefore, the presence of a high level of free radicals in living cells may contribute to a variety of disorders in both animals and plants. In animals, free radicals are major contributors to ageing and many of the degenerative diseases of ageing, including cardiovascular disease, many types of cancer, cataracts, age-related immunodeficiencies and degenerative diseases of the nervous system. In plants, free radicals may be responsible for membrane leakage, senescence, chlorophyll destruction and thereby decrease photosynthesis and yield (Percival 1998, Vichnevetskaia and Roy 1999, Devasagayam, et al. 2004, Singh, et al. 2004). There are many types of free radicals in biological systems, but radicals that are derived from oxygen and nitrogen represent the most important classes. These are called reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Percival 1998, Devasagayam, et al. 2004).

Oxygen is essential to all aerobic organisms for efficient energy production and survival. However, when living things are exposed to high oxygen concentrations they suffer from oxygen toxicity. Oxygen has two unpaired electrons, therefore it is a kind of free radical. Oxygen can also be converted to more reactive forms which are called reactive oxygen species (ROS). ROS is a term that includes all reactive oxygen-containing molecules, including free radicals (Percival 1998). The most important ROS are the hydroxyl radical ($HO^{\bullet}$), the superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), nitric oxide radical (NO), singlet oxygen ($^1O_2$) and various lipid peroxides (Nordberg and Arner 2001, Halliwell 2006).

The superoxide anion radical ($O_2^{\bullet-}$) can be generated by one electron reduction of molecular oxygen or one electron oxidation of hydrogen peroxide (Reaction 1). Formation of $O_2^{\bullet-}$ occurs spontaneously during normal aerobic respiration in the mitochondria. $O_2^{\bullet-}$ is also produced by reactions catalyzed by enzymes such as xanthine oxidase, lipoxygenase and the NADPH-dependent oxidase of phagocytic cells (Nordberg and Arner 2001, Halliwell 2006).

- $O_2 \xrightarrow{e^- \text{ reduction}} O_2^{\bullet-}$ (superoxide anion) Reaction 1

Hydrogen peroxide (H_2O_2) is formed by two electron reduction of molecular oxygen (Reaction 2). There is no unpaired electron in H_2O_2 orbitals, for that reason it is not a free radical. In spite of the fact that it is not a radical, it is a very crucial ROS because of its stability, ability to penetrate biological membranes and involvement in intracellular signaling. H_2O_2 also has an important role as an intermediate molecule in formation of hypochlorous acid (HOCl) and the hydroxyl radical which are both highly reactive free radicals. H_2O_2 is also produced as a result of normal functions of some enzymes, such as xanthine oxidase and amino acid oxidases (Nordberg and Arner 2001, Halliwell 2006).

- $O_2 + 2H^+ \xrightarrow{2e^- \text{ reduction}} H_2O_2$ (hydrogen peroxide) Reaction 2

The hydroxyl radical ($HO^{\bullet}$) is the most reactive free radical due to its highly unstable structure. It can attack any biological molecules that are in its vicinity. Therefore, it causes more damage to biological systems than other ROS. Hydroxyl radicals are produced as a result of ionizing radiation and also from H_2O_2 via the Fenton reaction. The Fenton reaction is catalyzed by transition metals such as Fe^{2+} and Cu^+ (Reactions 3-4) (Nordberg and Arner 2001).

- $O_2^{\bullet-} + Fe^{3+}/Cu^{2+} \longrightarrow O_2 + Fe^{2+}/Cu^+$ Reaction 3
- $H_2O_2 + Fe^{2+}/Cu^+ \longrightarrow OH^- + HO^{\bullet} + Fe^{3+}/Cu^{2+}$ Reaction 4

The nitric oxide radical (NO) has one unpaired electron, therefore like the superoxide anion radical, it is not highly reactive. However, when concentrations of both NO and $O_2^{\bullet-}$ increase in the cell, the two can combine with each other to generate another toxic reactive oxygen species known as peroxynitrite ($OONO^-$) (Reaction5). Peroxynitrite causes lipid peroxidation and nitration of tyrosyl hydroxyl groups of proteins that are located in the membrane (Nordberg and Arner 2001).

- $O_2^{\bullet-} + NO \longrightarrow OONO^-$ (peroxynitrite) Reaction 5

ROS are generated in a number of ways in organisms as a consequence of normal metabolic processes or as a result of environmental effects. The main source of ROS in organisms is mitochondria. In normal aerobic respiration, four electrons are transferred to molecular oxygen through the electron transport system (ETS) in order to reduce molecular oxygen to water. But during these reactions many types of ROS are generated. For example, the major site of superoxide radical formation is in the mitochondria. The chloroplast is another source of ROS in plants due to its high energy reactions and high oxygen concentration. Phagocytes are immune cells that kill bacterial and viral pathogens and also degrade foreign proteins via production of superoxide anions, hydrogen peroxide and hydroxyl radicals. However, after decomposition of phagocytes these ROS leak into the body plasma. Another way that ROS may be formed is xenobiotic metabolism; which is required for detoxification of toxic substances such as drugs and pesticides (Percival 1998).

Environmental factors may also contribute to formation of ROS. Cigarette smoke is a source of a large amount of ROS, it contains nitric oxide and nitrogen dioxide that are known as active oxidants (Devasagayam, et al. 2004). Environmental pollutants, certain drugs, pesticides, anaesthetics, industrial solvents, ionizing radiation such as X-rays and γ-rays, and ultraviolet (UV) light also increase formation of free radicals (Madhavi, et al. 1996).

As previously mentioned, ROS are highly reactive molecules and tend to be harmful for many organic molecules including DNA, lipids and proteins which have crucial roles in biological systems. Lipid peroxidation is one of the most important issues in redox biology. Lipids containing polyunsaturated fatty acids (PUFAs) are prone to be oxidized by ROS due to their multiple double bonds (Reactions 6-7). Peroxidation of PUFAs results in formation of peroxide and many other toxic

byproducts that have higly deleterious effects on both the structure and function of the cell membrane. ROS can also oxidize cholesterol to cholesterol oxide and low density lipoproteins (LDL) that are associated with atherosclerosis and cardiovascular diseases (Nordberg and Arner 2001, Ferrari and Torres 2003, Devasagayam, et al. 2004). In addition to ROS, transition metals such as Fe^{2+} and Cu^{+} can also oxidize lipids (Reaction 8-9). As a result of these oxidation reactions, alkoxyl ($LO^{\bullet}$) and peroxyl ($LOO^{\bullet}$) radicals, which lead to loss of membrane integrity, are formed (Madhavi, et al. 1996, Halliwell 2006).

- $LH + HO^{\bullet} \longrightarrow L^{\bullet}$ (Lipid radical) $+ H_2O$ Reaction 6
- $L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$ (Peroxyl radical) Reaction 7
- $LOOH + Fe^{2+}/ Cu^{+} \longrightarrow Fe^{3+}/ Cu^{2+} + LO^{\bullet} + OH^{-}$ Reaction 8
- $LOOH + Fe^{3+}/ Cu^{2+} \longrightarrow Fe^{2+}/ Cu^{+} + LOO^{\bullet} + H^{+}$ Reaction 9

The ability of ROS to react with DNA makes them very dangerous or even lethal for all organisms. ROS, especially $HO^{\bullet}$, have been shown to react with DNA. The initial attack results in several DNA alterations, such as cleavage of DNA, DNA-protein cross links and purine oxidation. Unless DNA repair systems are able to regenerate DNA, these DNA alterations may cause mutation along with a high incidence of cancer, (Percival 1998, Nordberg and Arner 2001, Singh, et al. 2004). Another issue that makes ROS important is that they also damage mitochondrial DNA whose activity is thought to be associated with ageing (Nordberg and Arner 2001).

Interaction of ROS with amino acid residues, especialy sulfur or selenium-containing amino acid residues, of proteins can cause loss of protein function and inactivated proteins that are degraded by proteolytic enzymes. Current research has revealed that cataract formation may stem from alteration of the lens proteins by ROS molecules. ROS cause the lens to lose its transparency (Percival 1998, Nordberg and Arner 2001).

Despite their negative impacts, some level of ROS is beneficial for living things. For example, production of ROS molecules ($O_2^{\bullet-}$, H_2O_2, $HO^{\bullet}$) in phagocytes helps an organism to kill infectious bacteria and viruses or to denature foreign antigens. They are also responsible for apoptosis of defective cells. Another positive function of ROS is

that they have important roles in signal transduction by altering the conformation or activity of all sulphydryl-containing molecules and also in the formation of regulatory enzymes including cyclo-oxygenases and lipoxygenases. Finally all aerobic organisms need ROS for efficient energy production, they have crucial functions in production of ATP from ADP via oxidative phosphorylation (Nordberg and Arner 2001).

In recent years, several studies have indicated that accumulation of high concentrations of free radicals in humans is associated with an increased risk of a number of diseases that were previously mentioned. This relationship can be explained by 'oxidative stress'. In a normal cell, there is an appropriate free radical and antioxidant balance that is maintained by the antioxidant defense systems. However, when this balance shifts towards the free radical as a consequence of high production of ROS or loss of antioxidants, organisms are exposed to oxidative stress. It has been shown that oxidative stress has a role in over 100 types of human diseases and in ageing (Devasagayam, et al. 2004).

The problem with ROS molecules is that high concentrations of them are dangerous for living things, because of their ability to damage cell components. Fortunately, antioxidants help to restore a balance of ROS.

2.3. Antioxidants

An antioxidant is a substance that is capable of delaying or inhibiting oxidation processes caused by free radicals. If it were not for antioxidant defence systems, the balance between pro-oxidant and antioxidant would be shifted in favour of free radicals. As a result, oxidative stress threatens the health and survival of organisms. Fortunately, antioxidant compounds effectively help maintain this balance and protect organisms from oxidative stress-mediated damages. Thus, antioxidants decrease the risk of a number of diseases that are associated with oxidative stress (Percival 1998).

There are numerous types of molecules that play a role in the antioxidant defence system; therefore, antioxidants can be classified in different ways. One criterion for antioxidant classification is based on their solubility: i) water-soluble antioxidants

and ii) lipid-soluble antioxidants. Another classification of antioxidants depends on their origin: i) endogenous antioxidants, which are internally synthesized by an organism and ii) exogenous antioxidants, which are obtained by an organism from its diet (Vichnevetskaia and Roy 1999). Some researchers have also divided antioxidants according to their enzymatic functions into two groups: i) enzymatic antioxidants and ii) non-enzymatic antioxidants (Somogyi, et al. 2007). Some of the antioxidants that fall into each of these groups are described below.

2.3.1. Enzymatic Antioxidants

Organisms that are exposed to the deleterious impacts of oxidative stress have inevitably envolved some defence systems against ROS to maintain their well-being. The endogenous enzymatic antioxidants are primarily defence systems that are responsible for scavenging or quenching of ROS in living systems. The most important enzymatic antioxidants are superoxide dismutase (SOD) (E.C.1.15.1.1), catalase (CAT) (1.11.1.6), and glutathione peroxidase (GPx) (1.11.1.9). All of these enzymes have one thing in common: they all require metal cofactors such as iron, copper, manganese, zinc and selenium for optimum catalytic activity (Nordberg and Arner 2001).

Among the antioxidant enzyme systems, superoxide dismutase (SOD) (E.C.1.15.1.1) is the first line of defense that is responsible for dismutation. The major function of SOD in organisms is to metabolize $O_2^{\bullet-}$ to H_2O_2 (Reaction 1). SOD enzymes are members of a family of metalloenzymes and are present in virtually all aerobic organisms. Eukaryotic cells contain a Cu/Zn-containing form of SOD in their cytosol and in the mitochondrial intermembrane space while the Mn-containing form is located in the mitochondrial matrix. In addition to these two forms, plants also have a Fe-containing SOD in the chloroplast. Bacteria have a wide variety of SOD types, such as Mn, Fe, Cu, Ni and Zn-containing SOD forms (Nordberg and Arner 2001, Halliwell 2006). Regardless of the metal cofactor that the SOD contains, all SODs catalyze the following reaction: scavenging of $O_2^{\bullet-}$. Mitochondria are the main source of $O_2^{\bullet}$ due to leakage of electrons from the respiratory chain.

- $2O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$ Reaction 1

Catalase (CAT) (1.11.1.6) is another widely distributed antioxidant enzyme that contains a heme group in its structure. Virtually all catalase enzymes are located in peroxisomes, where catalase converts H_2O_2 to H_2O and molecular O_2 (Reaction 2). By catalyzing this reaction, catalase prevents the formation of the hydroxyl radical, the most dangerous ROS, via the Fenton-reaction (Nordberg and Arner 2001). Catalase catalyzes the following reaction:

- $2\ H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$ Reaction 2

Organisms contain several glutathione peroxidase (GPx) (1.11.1.9) enzymes. All of them contain seleno GPx an unusual amino acid. Selenocysteine is an analog of cysteine that contains selenium in place of sulfur. Antioxidant activity of GPx stems from reduction of H_2O_2 and other peroxides using glutathione as a substrate (Reaction 3) (Nordberg and Arner 2001).

- $LOOH + 2GSH \longrightarrow LOH + GSSH + H_2O$ Reaction 3

Antioxidant enzyme systems are major defense mechanisms against free radical-mediated cell damage in biological systems. However, these enzyme systems are not sufficient for efficient protection of organisms from free radicals. In addition to these systems, other biological compounds such as vitamin C, vitamin E, carotenoids and phenolics are important in antioxidant activity. Although these are non-enzymatic antioxidants, contribution of these antioxidant types to an organism's health should not be underestimated. In the next section, non-enzymatic antioxidant will be discussed.

2.3.2. Non-Enzymatic Antioxidants

Vitamin C or L-Ascorbic acid is an α-keto lactone with an almost planar six-membered ring (Figure 2.1). Vitamin C is the simplest vitamin based on its chemical

structure, therefore, it was the first isolated and characterized vitamin. Synthesis of vitamin C in organisms is very common. Plants especially synthesize great amounts of vitamin C in their leaves and fruits. Also most mammals, with the exception of humans and other primates, guinea pigs and fruit bats, produce vitamin C for their well-being. Vitamin C synthesis requires glucuronic acid and galactonic acid that are derived from glucose. However, because humans and other primates lack gulono-γ-lactone oxidase enzymes, they cannot oxidase L-gulonolactone to 2-keto-L-gulonolactone which is then spontaneously converted to L-ascorbic acid. Therefore these organisms must obtain a sufficient amount of vitamin C through their diet (Madhavi, et al. 1996).

Vitamin C is one of the most important antioxidants with an electron reduction potential of + 0,28V. Vitamin C is an electron donor and therefore a reducing agent. Due to its water-soluble nature, it can react rapidly with ROS and protect macromolecules from the degenerative effects of oxidative stress. Vitamin C can detoxify $HO^{\bullet}$, $O_2^{\bullet-}$, peroxyl radicals and also scavenge singlet oxygen. After these oxidation-redution reactions, vitamin C donates its electrons to ROS and quenches them. In so doing, ascorbic acid becomes an ascorbyl radical that is less reactive. Then this ascorbyl radical can be reduced back to ascorbate or oxidized to form dehydroascorbic acid (Figure 2.1). Dehydroascorbic acid is unstable at physiological pH and it is degraded spontaneously to 2,3-diketo-gulonic acid. To prevent this degradation, dehyroascorbic acid can be reduced back to ascorbate by GSH or NADPH from the hexose monophosphate shunt (Madhavi, et al. 1996).

The existence of a mechanism for recycling vitamin C demonstrates that some level of vitamin C is essential for organisms. Vitamin C is essential because, unlike other water-soluble vitamins that act as coenzymes, vitamin C has a role in enzymatic reactions as a co-substrate. While vitamin C is a good radical scavenger antioxidant, it also plays a vital role in regeneration of lipid-soluble vitamin E, an antioxidant that reduces ROS produced in lipid membranes and lipoproteins. As a result, vitamin C can be considered as both a direct and indirect antioxidant (Madhavi, et al. 1996).

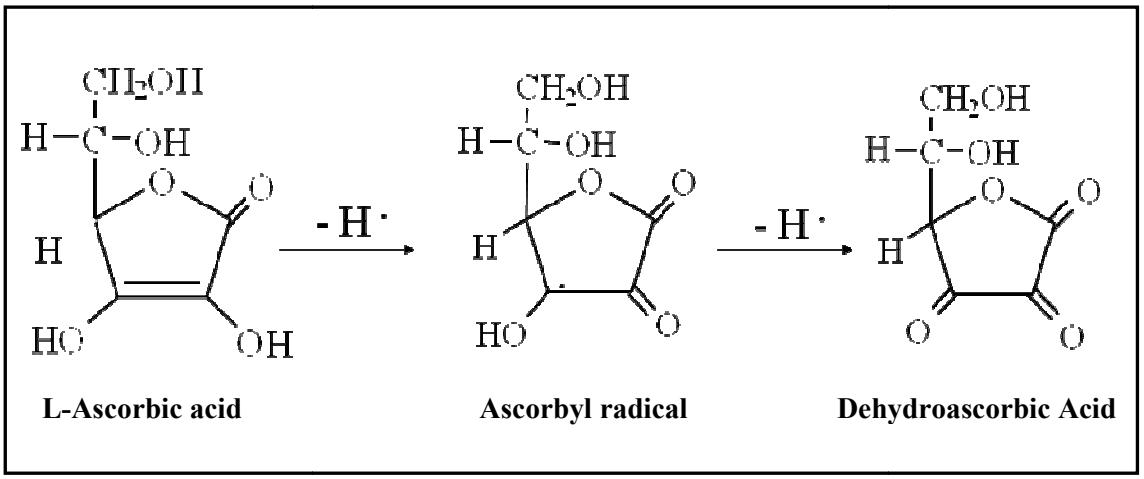

Figure 2.1. The oxidation of L-ascorbic acid to dehydroascorbic acid

Under certain conditions vitamin C can act as a pro-oxidant that helps in the formation of ROS. The pro-oxidant activity of vitamin C is derived from its ability to reduce transition metals, Fe^{3+} or Cu^{2+}. At the expense of molecular oxygen, these transition metals rapidly catalyze oxidation of vitamin C (Reactions 1-4) (Madhavi, et al. 1996).

- $AH^- + Fe^{3+}$ or $Cu^{2+} \longrightarrow A^{\bullet-} + Fe^{2+}$ or Cu^+ Reaction 1
- $AH^- + O_2 + H^+ \longrightarrow H_2O_2 + A$ Reaction 2
- Fe^{2+} or $Cu^+ + O_2 \longrightarrow O_2^- + Fe^{3+}$ or Cu^{2+} Reaction 3
- Fe^{2+} or $Cu^+ + H_2O_2 \longrightarrow OH^- + HO^\bullet + Fe^{3+}$ or Cu^{2+} Reaction 4

Vitamin E is a major lipid-soluble antioxidant that can effectively prevent lipid peroxidation in the cell membrane. Vitamin E can be classified into two groups based on their side chain structure. The first group of vitamin E is tocopherols, including the α, β, γ and δ types, which contain a phytol side chain. Similar to tocopherols the second group of vitamin E known as tocotrienols have the same structure except that they have double bonds at the 3'-, 7'- and 11'- positions of the side chain. Tocotrienols also have four types: α, β, γ and δ tocotrienols (Figure 2.2). Vitamin E is widely distributed among animals and plants. Particularly, they have been found in many plants oils, including soybean, sunflower and maize oils (Vichnevetskaia and Roy 1999).

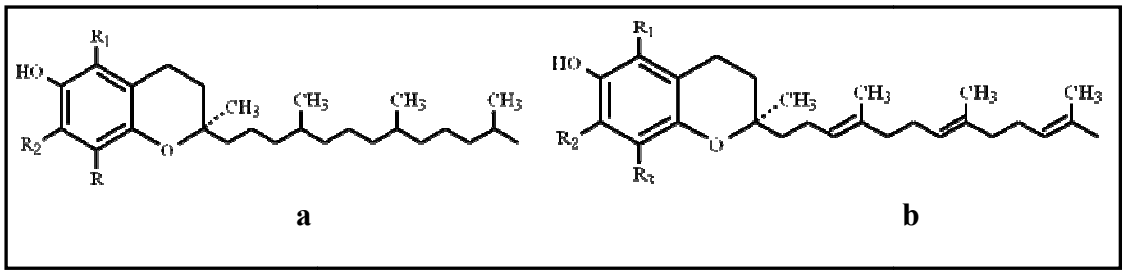

Figure 2.2 **.** The basic structure of tocopherols (a) and tocotrienols (b)

Among the eight isomers of vitamin E, the most important is α-tocopherol because of its high antioxidant activity *in vivo*. Tocopherols are hydrophobic molecules, therefore they are only found in lipid membranes and lipoproteins. For this reason, they play significant roles in protection of the cell membrane against ROS mediated damage. The antioxidant nature of tocopherols stems from the hydrogen atom of the phenolic hydroxyl group on their chromonal ring. These hydroxyl groups are labile, therefore they can easily react with lipid peroxy and alkoxy radicals in order to reduce them. Thus, tocopherols are known as the most efficient chain-breaking antioxidants because they inhibit lipid peroxidation by scavenging chain propagation radicals. By doing this, tocopherols protect cell membranes against oxidative damage (Vichnevetskaia and Roy 1999). They also have the ability to quench $O_2^{\bullet-}$ and singlet oxygen. Vitamin E exhibits protective effects against coronary heart disease due to inhibition of oxidation of low density lipoproteins (LDL) and PUFAs (Madhavi, et al. 1996).

The function of vitamin E is represented in the following reactions (Reactions 1-8). Autooxidation of a lipid starts when a hydroxyl radical takes a hydrogen atom from the lipid molecule (LH). This reaction generates a lipid radical ($L^{\bullet}$) and water. Then the lipid radical reacts with molecular oxygen to form another radical that is called a peroxyl radical ($LOO^{\bullet}$). The peroxyl radical can remove a hydrogen atom from another lipid molecule and produce a new free radical and hydroperoxide (LOOH). This step is called propagation of a chain reaction of lipid peroxidation (Madhavi, et al. 1996).

- Initiation
- $LH + HO^{\bullet} \longrightarrow L^{\bullet} + H_2O$ Reaction 1
- Reaction of radical with oxygen
- $L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$ Reaction 2
- Propagation
- $LOO^{\bullet} + LH \longrightarrow L^{\cdot} + LOOH$ Reaction 3

These lipid peroxidations cause a chain oxidation reaction that will continue throughout the fatty material until stopped by an antioxidant. Vitamin E is the main lipophilic antioxidant that inhibits this chain reaction.

- Antioxidant reaction
- $LOO^{\bullet} + EOH \longrightarrow EO^{\bullet} + LOOH$ Reaction 4

When tocopherol interacts with peroxyl radical, it donates one of its hydrogen atoms to the peroxyl radical and reduces it to hydroperoxide. After this reaction tocopherol is converted to a tocopheroxyl radical that is more stable than peroxyl radical and is a weak free radical. Regeneration of the inactive tocopheroxyl radical to active tocopherol is carried out by vitamin C and GSH. By accepting one hydrogen from vitamin C or GSH, the inactive tocopherol molecule returns to its active form. Also oxidized vitamin C and GSH can be reduced back to their normal state thanks to NADPH. This synergistic effect between vitamin E and vitamin C increases the ratio of antioxidant activity. Figure 2.3 shows the synergistic effect of vitamin E and vitamin C (Madhavi, et al. 1996).

- Regeneration
- $EO^{\bullet} + C \longrightarrow EOH + C^{\bullet}$ Reaction 5
- $C^{\bullet} + NADPH \longrightarrow C + NADP$ Reaction 6
- $EO^{\bullet} + 2GSH \longrightarrow EOH + GSSG$ Reaction 7
- $GSSG + NADPH \longrightarrow 2GSH + NADP$ Reaction 8

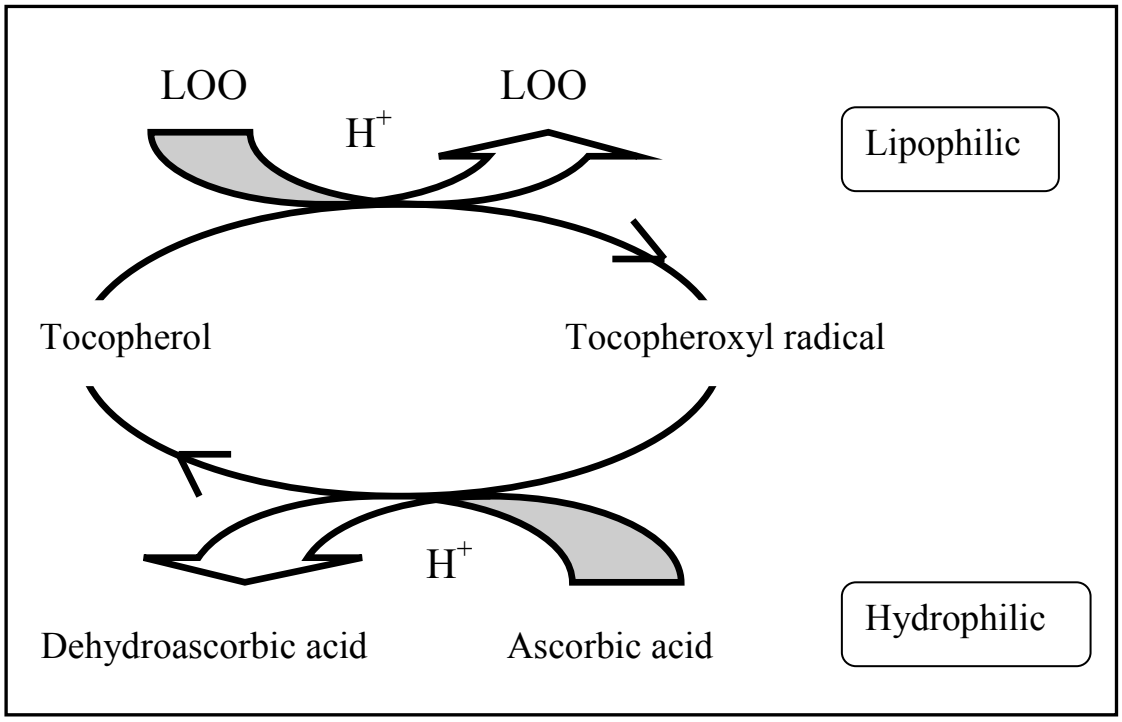

Figure 2.3. Synergistic effect of tocopherol and ascorbic acid

Carotenoids are lipid-soluble pigments that contribute to the yellow, orange and red colour of fruits and vegetables. Carotenoids are also distributed in animals such as in egg yolk, salmon and crustaceans. The major carotenoids that are found in animals are β-carotene, lutein, lycopene, β-cryptoxanthin and α-carotene. Carotenoids are synthesized from acetyl coenzyme A via a series of reactions in plants and microorganisms. Several carotenoids, especially β-carotene, are precursors of vitamin A (Madhavi, et al. 1996).

Carotenoids are accessory pigments in addition to chlorophyll in plant tissues. Carotenoids are very effective quenchers of singlet oxygen and peroxyl radicals. This is related to the number of double bonds they contain. Carotenoids are in the class of lipophilic antioxidants, therefore, carotenoids are especially important in protecting isolated lipid membranes from peroxidation, and LDL-containing lipids from oxidation (Madhavi, et al. 1996).

Carotenoids with 9, 10, and 11 conjugated double bonds are better quenchers of singlet oxygen. Carotenoids can absorb the energy from the singlet oxygen, which is then distributed over all the single and double bonds in the molecule. After that, the

energized carotenoids release the absorbed energy in the form of heat, thereby they return to their normal energy level. For that reason, carotenoids are not destroyed during the quenching of free radicals and can react with another singlet oxygen (Madhavi, et al. 1996).

Lycopene is one of the most important types of carotenoids. Lycopene is a lipid soluble pigment and mostly founds in tomato skin, watermelon and grapefruit. Lycopene is one of the strongest antioxidants, due to the abundance of conjugated bonds in its structure. Recent studies have shown that lycopene is a powerful singlet oxygen quencher among the carotenoids. Lycopene also can quench peroxyl radicals, and inhibit lipid peroxidation and the oxidation of DNA and low-density lipoprotein (LDL). It is also reported that lycopene can decrease the incidence of prostate cancer and cardiovascular diseases in humans (Arab and Steck 2000).

Phenolic compounds are the largest category of secondary metabolites that are produced by plants and are common in fruits and vegetables (Lule and Xia 2005, Podsedek 2007). They contribute to plants' taste, aroma and color. Phenolics are characterized by at least one aromatic ring that contains one or more hydroxyl groups in their structure. Flavonoids, phenols and phenolic acids are the most important phenolic compounds (Sakihama, et al. 2002).

Phenolic compounds are constitutively synthesized by virtually all plants. However, biotic and abiotic stresses; such as UV radiation, high-light condensation, low temperature, wounding and pathogen attack; enhance the accumulation of phenolics in plants (Sakihama, et al. 2002). Phenolics protect plants against these stress conditions. Recent studies have reported the antimutagenic, anticarcinogenic, antiinflammatory, antiviral and antimicrobial activity of phenolic compounds (Sakihama, et al. 2002, Lule and Wenshui 2005).

Phenolics are classified as water-soluble antioxidants whose activity greatly decreases the negative effect of ROS. The level of antioxidant activity of phenolic compounds depends on the number and position of their hydroxyl groups on their aromatic ring and/or rings. Due to their structure, the hydroxyl groups of phenolics can easily donate their H^+ to ROS in order to reduce them (Podsedek 2007). Phenolics have the ability to scavenge $O_2^{\bullet -}$, H_2O_2 and $HO^{\bullet}$ radicals and to protect organisms from their harmful effects (Sakihama, et al. 2002).

Flavonoids are one of the major categories of phenolic compounds and are commonly distributed in the epidermal cells of plant's leaves, flowers, fruits and pollen (Vichnevetskaia and Roy 1999). Flavonoids are characterized by a C_6-C_3-C_6 carbon skeleton structure. They are synthesized from phenylalanine. Flavanones, flavones, isoflavonoids and anthocyanins are particularly common types of flavonoids (Sakihama, et al. 2002) (Figure 2.4).

Flavonoids are capable of chelating transition metals and scavenging the superoxide anion by donating their hydrogen atom. The antioxidant activity of flavonoids is determined by the position and degree of hydroxylation of the B ring. The presence of hydroxyl groups at the 3', 4' and 5' positions on the B ring increases their antioxidant activity. Degradation of vitamin C is also prevented by flavonoids (Vichnevetskaia and Roy 1999). It was revealed that flavonoids have pharmacological activities such as the ability to scavenge radicals, provide resistance to pathogens, and provide anticarcinogenic and antiallergic activities (Yao, et al. 2004).

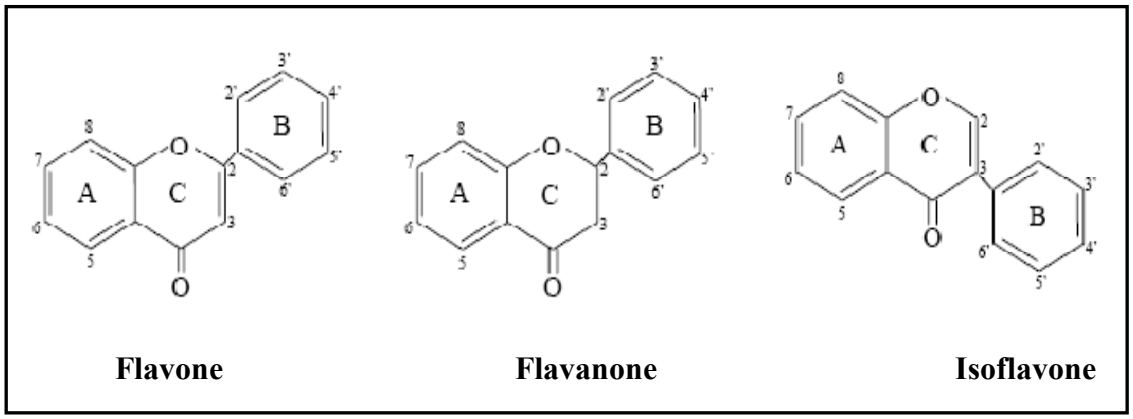

Figure 2.4. Basic structure of flavone, flavanone and isoflavone

2.4. Functional Foods

Foods are essential for human survival and plant-originated foods are located in the center of the human diet. However, statistical analysis shows that the world population will be 9 billion by the year 2050, a 50% increase in the next 50 years, thus it will be a major problem to supply the food necessities of this rapidly expanding

population (Clive 2001). In order to prevent starvation, agricultural production must expand faster than the human population; however, this is very difficult because of abiotic and biotic stresses. The world population reached 6 billion in 1999 and 1.3 billion people of this total population are suffering from hunger (Clive 2001). In addition to hunger, it has been revealed that because of inappropriate dietary habits, 160 million pre-school children suffer from malnutrition (Anderson and Cohen 2000).

Recently scientific research has demonstrated that there is a strong relationship between food consumption and disease incidence. Since then, the term 'functional food' has become popular among scientists and consumers. Functional foods are any food that prevents or reduces the risk of diseases, regulates physical and mental performance and/or slows down the aging process (Roberfroid 2000). Fruits and vegetables which provide a rich source of biologically active compounds including vitamins, antioxidants and minerals occupy a large part of the human diet. There are many reports about the positive impact of phytochemicals, especially antioxidants, on human well being. For example, flavonoids have the ability to inhibit tumor formation, formation of coronary heart disease and also have antiviral activity (Yao, et al. 2004, Podsedek 2007). Vitamin C has an important role as an enzyme cofactor and free radical scavenger (Madhavi, et al. 1996, Podsedek 2007). Vitamin E and carotenoids also decrease the incidence of cardiovascular diseases and many types of cancers, especially the preventive feature of lycopene, red colored carotenoid, aginst prostate cancer is well studied (Madhavi, et al. 1996, Bramley 2000, Rodrigez, et al. 2006, Podsedek 2007).

There is growing interest in improvement of the nutritional content of crops either by conventional breeding methods or by transgenic techniques. One of the most important transgenic studies that has been done for nutrient fortification is The Golden Rice Project. Rice represents a major contribution in the diet of developing countries. However, because of a deficiency of vitamin A in rice, approximately 500.000 children become blind and also die during childhood in these countries. In this transgenic approach, the provitamin A gene, which is responsible for production of vitamin A, was incorporated into rice endosperm which lacks this gene (Ye, et al. 2000, Al-Babili and Beyer 2005).

2.5. Tomato

Tomato, *Lycopersicon esculentum* (synonym: *Solanum lycopersicum*), 2n=24, is the second most important member of *Solanaceae* or nightshade family with potato ranking first. The *Solanaceae* family possesses several economically important crops such as potato, tomato, pepper, eggplant and tobacco. *Lycopersicon* contains several wild and cultivated tomato species such as *L.esculentum, L.hirsutum, L.peruvianum, L.penellii, L.pimpinellifolium and L.chmielewskii*. The tomato is native to Central and South America and southern North America from Mexico to Peru (Bai and Lindhout 2007). Tomato is one of the most economically important vegetable crops with over 4.5 million ha produced worldwide (FAO 2004). Turkey ranks fourth in production of tomato with 9,854,877 metric tons while China ranks first with 32,540,040 metric tons (FAO 2006). Tomato has a significant role in the human diet including in Turkey. It is widely consumed fresh, cooked, preserved and as a source of processed foods like ketchap and paste.

Because of its high production and consumption rate, plant breeders have great interest in improvement of agronomically important characters in tomato. External and internal fruit color, fruit shape, fruit weight, firmness, stem scar size, fruit locule number and fruit wall thickness are some of the most important traits that increase the value of tomato in the market place. However, like most traits in nature these characters are quantitatively inherited, thereby to improve these characters is much more difficult than for qualitatively inherited traits. But, as mention before, with the development of molecular marker technology, these difficulties have been minimized.

Besides its horticulturally important fruit characters, consumers now have great interest in tomato's nutritional content and its features that positively affect human health. Tomato fruit is a rich source of carotenes and it contains especially high amounts of lycopene and β-carotene. In addition, it is a good source of many types of vitamins such as vitamin A, vitamin C and vitamin E and also several types of minerals such as potassium and magnesium. Tomatoes are also rich in flavonoids, a type of phenolic compound (USDA Nutrient Data Laboratory 2008).

There are many studies on the benefits of tomato on human health; for example, there are many reports about therapeutic effect of lycopene against prostate cancer (Madhavi, et al. 1996, Bramley 2000, Sapuntzakis and Bowen 2005). Tomato also includes some nutrients such as flavonoids that are associated with reduction of low density lipoprotein, thereby regular consumption of tomato decreases or inhibits the risk of cardiovascular diseases and coronary heart diseases (Bramley 2000, Willcox, et al. 2003, Rein, et al. 2006).

2.6. Goals of Study

Our main purpose in this study was to identify genomic regions that have roles in controlling the antioxidant capacity and also agronomically important characters in tomato fruit. This was done by identifying QTLs for the nutritional quality traits: total water soluble antioxidant activity, vitamin C, total phenolic content, total flavonoid content and lycopene content and the agronomic traits: external and internal fruit color, fruit weight, firmness, fruit shape, stem scar size, fruit locule number and fruit wall thickness. After determination of genetic markers that were tightly linked with QTLs for these fruit characters, alleles related with nutritional and agronomic traits can be used for improvement of high quality new tomato hybrids with marker assisted selection.

CHAPTER 3

MATERIALS AND METHODS

3.1. Plant Material

The BC_2F_2 mapping population used in this project was developed by Sami Doğanlar by crossing *Lycopersicon esculentum* (syn: *Solanum lycopersicum*) (TA1166) as a recurrent parent with *L.hirsutum* (syn: *S.habrochaites*) (LA1223) as a donor parent for both antioxidant and agronomic traits. F_1 hybrids were backcrossed to the recurrent parent in order to obtain a BC_1F_1 population, then BC_1F_1 individuals were backcrossed one more time with the recurrent parent to produce a BC_2F_1 population. The reason for backcrossing the F_1 hybrids with the recurrent parent was to increase the amount of *L.esculentum* genome in the population. Lastly, to fix the population genotypes the BC_2F_1 individuals were selfed and a BC_2F_2 population was obtained (Figure 3.1). For this project, ten tomato plants from each of the 152 individuals of the BC_2F_2 population were planted in the field in Antalya by MULTİ Tarım seed company in April 2007.

3.2. Phenotypic Characterization

In this study 13 health related and agronomically important traits were analyzed for QTL identification. The health related fruit traits were: total water soluble antioxidant activity, total vitamin C content, total phenolic content, total flavonoids content and lycopene content and were determined using biochemical assays.

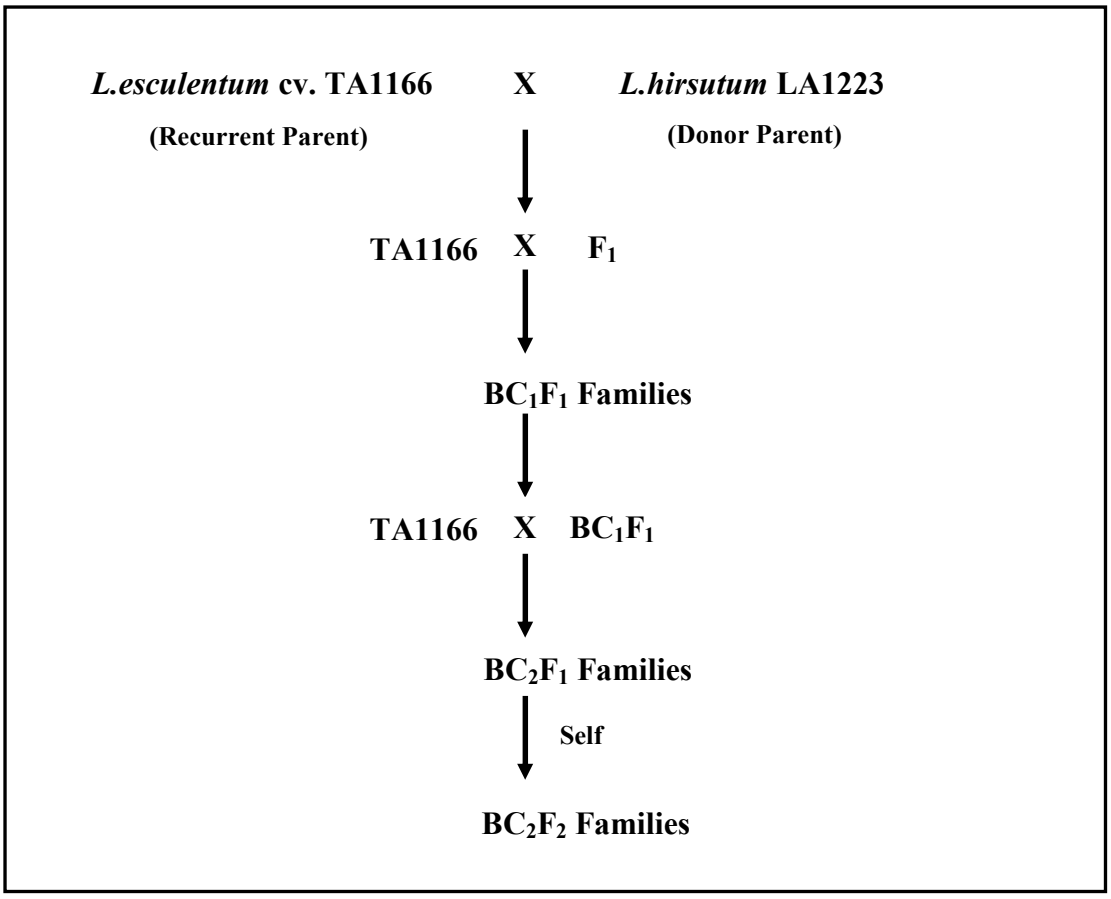

Figure 3.1. Development of BC_2F_2 mapping population by crossing *L.esculentum* (TA1166) and *L.hirsutum* (LA1223)

3.2.1. Sample Preparation for Antioxidant Traits Analysis

For the evaluation of total water soluble antioxidant activity, vitamin C content, total phenolic compounds, flavonoids content and lycopene content, tomato fruits were harvested from ten plants for each line at the normal market stage in July 2007. After the fruits were washed, about one kilo of fruits of each sample were cut into pieces and well mixed. Then, tomato fruit mixtures were packed and stored at -20^{o}C until biochemical analyses were performed. It has been reported that there is no significant difference in antioxidant content of fresh and frozen tomato fruits (Toor, et al. 2006). The tomato fruits were analyzed for total water soluble antioxidant activity, vitamin C content, total phenolic compounds, flavonoids and lycopene content as described below. All analyses were performed within four months of harvest.

3.2.2. Determination of Total Water Soluble Antioxidant Activity

For the antioxidant activity assay, approximately 200 g of fruit was homogenized with 100 ml cold distilled water for 2 min at low speed in a Waring blender equipped with a 1L double walled stainless steel jar at +4^{o}C. Then, 10 g of extract was taken from the homogenate and diluted with 15 ml cold distilled water. The resulting mixtures were homogenized for one minute by using a tissue crusher. Homogenized samples were then filtered through 4 layers of nylon cloth into two 15 ml falcon tubes. The filtrates were centrifuged at 3000 x g for 10 min at +4^{o}C in a refrigerated centrifuge (Eppendorf). After centrifugation, supernatants were merged into a single 50 ml falcon tube after filtration through 3 layers of nylon cloth to get a clear filtrate. Tomato filtrate was kept on ice until it was used for measurement of total water soluble antioxidant activity.

The total water soluble antioxidant activity of tomato fruits was measured spectrophotometrically (Shimadzu, 1700 UV Visible Spectrophotometer, Japan) using the ABTS [2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] decolorization assay of Re et al. (1999). ABTS radical cation (ABTS$^{\bullet}$+) is a free radical that absorbs at 734 nm. When this compound is reduced by antioxidant species its absorbance decreases. The ABTS radical cation stock solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persufate and was stored in the dark for 12-16 hours. Before use, the ABTS$^{\bullet}$+ stock solution was diluted with phosphate buffered saline (PBS) at pH 7.4 to adjust its absorbance to 0.700 (± 0.02) at 734 nm. Then, 2.5, 5 and 7.5 µl aliquots of tomato supernatant were mixed separately with 2 ml ABTS radical cation solution and decolorization of blue-green ABTS$^{\bullet}$+ solution was kinetically monitored at 734 nm for 6 min at 30ºC. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard for construction of a standard graph (Figure 3.2). Each assay was repeated to give three replicates for each aliquot volume. The results were calculated as area under the curve (AUC) and expressed as µmol Trolox/kg fresh weight of tomato fruits. To calculate AUC, the percent inhibition/concentration values for the extracts and Trolox were plotted separately against the test periods (1, 3, 6 min) and the ratio of the areas of curves for extracts and Trolox was used to calculate the AUC value.

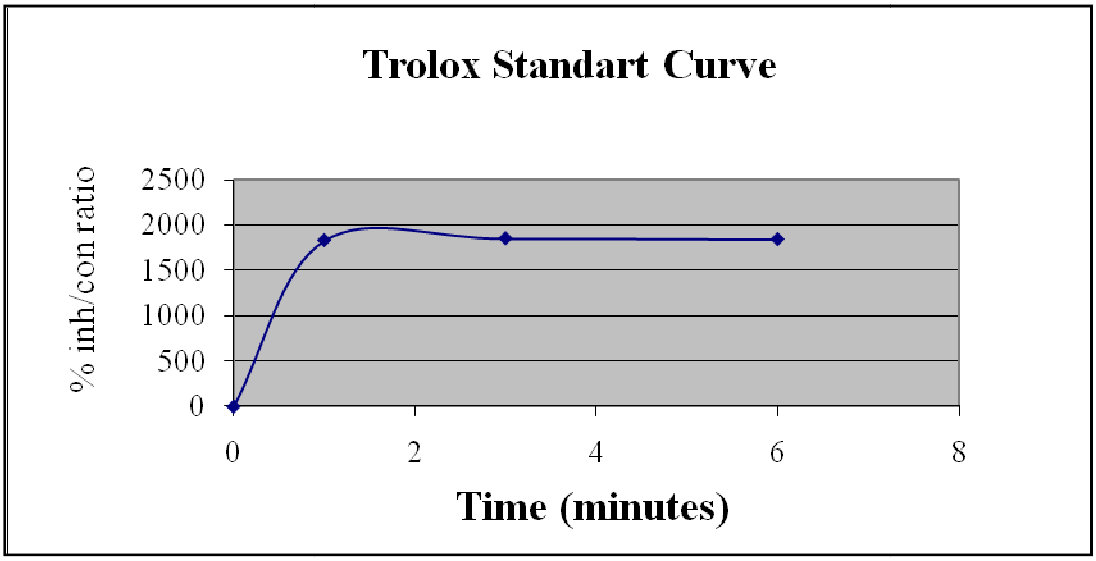

Figure 3.2. Percent inhibition vs. concentration plot of Trolox standard at 1st, 3rd and 6th minutes used to measure the Area Under Curve (AUC)

3.2.3. Determination of Vitamin C Content

Vitamin C content of tomato was measured by AOAC 967.21 titrimetric method using 2,6-dicloroindophenol as reactive substance (Nielsen 1994). The extractions were prepared by homogenization of 100 g tomato with 115 ml acetic acid-metaphosphoric acid extraction solution for 2 min at low speed in a Waring blender at +4°C. Afterwards, 25 g of extract was taken from the homogenate and diluted to 100 ml with cold extraction buffer. Then, each homogenate was passed through filter paper and 15 ml diluted sample was titrated against a 2,6-dicloroindophenol dye solution. For each tomato extract, the vitamin C content of three replicate samples was measured. The titrator was calibrated using commercial L-ascorbic acid and the results were expressed as mg ascorbic acid/kg fw of tomato fruit.

3.2.4. Determination of Total Phenolic Compounds

The total phenolic content of tomato fruits was spectrophotometrically measured using Folin-Ciocalteau as a reactive reagent adapted from the method of Singleton and Rossi (1965). In this procedure, gallic acid was used for generation of a standard curve. Homogenates were prepared by blending 200 ml cold distiled water with 100 g tomato sample for two min at low speed in a Waring blender at 4^{o}C. Then, 2.5 g homogenate was 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). The clear supernatant was used for the determination of total phenolic content. For this, 2 ml of the supernatant was mixed with 10 ml 2 N (10%) Folin-Ciocalteu and incubated for 3 min, then 8 ml 0.7 M Na_2CO_3 was added. After 2 hours of incubation at room temperature, the absorbance of the reaction mixture was measured at 765 nm in a spectrophotometer (Shimadzu, 1700 UV Visible Spectrophotometer, Japan). There were three replicates for each sample. The total phenolic content was expressed as gallic acid equivalents (mg/kg fresh weight) based on a gallic acid standard curve.

3.2.5. Determination of Flavonoids Content

The total flavonoids content of tomato fruits was spectrophotometrically measured using the method described by Zhishen et al.. (1999). In this procedure, epicatechin was used for generation of a standard curve. Tomato homogenates were prepared by blending 100 g tomato sample with 200 ml cold distilled water for two min at low speed in a Waring blender at 4^{o}C. Then, 2.5 g homogenate was 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). Then, 1250 µl clear supernatant was used for the measurement of total flavonoids content. For this, 75 µl 5% $NaNO_2$ was mixed with 1250 µl tomato supernatant and then the mixture was incubated for 5 min. After that, 75 µl 10% $AlCl_3$ was added to the mixture. After one minute, 0.5 ml 1 M NaOH and 0.6 ml distilled water were added to the reaction mixture and the absorbance was measured at 510 nm in

a spectrophotometer (Shimadzu, 1700 UV Visible Spectrophotometer, Japan). There were three replicates for each sample. The total flavonoids content was calculated based on an epicatechin standard curve (mg/kg fresh weight) (Figure 3.3).

3.2.6. Determination of Lycopene Content

Lycopene content of tomato fruits was evaluated by using the method developed by Sadler et al. (1990). In this assay, tomato homogenate was prepared by blending 100 g tomato fruit with 200 ml cold distilled water for two min at low speed in a Waring blender at 4^{o}C. Three replicate 3 g tomato homogenates were diluted with 50 ml hexane-acetone-ethanol (2: 1: 1; v: v: v) extraction buffer in a brown volumetric flask. Then, these extractions were shaken on a rotary mixer for 30 min at 150 rpm at 25ºC in the dark. After agitation, samples were transferred into separation funnels and 10 ml distilled water was added to the extract and the samples were left for 4 hours in the dark to separate polar and non-polar phases. Lycopene dissolved in the top, hexane layer. The top layer was taken and its absorbance was measured at 472 nm using a quartz cuvette in a spectrophotometer (Shimadzu, 1700 UV Visible Spectrophotometer, Japan). The lycopene content was expressed as mg/kg fresh weight based on a lycopene standard curve (Figure 3.4).

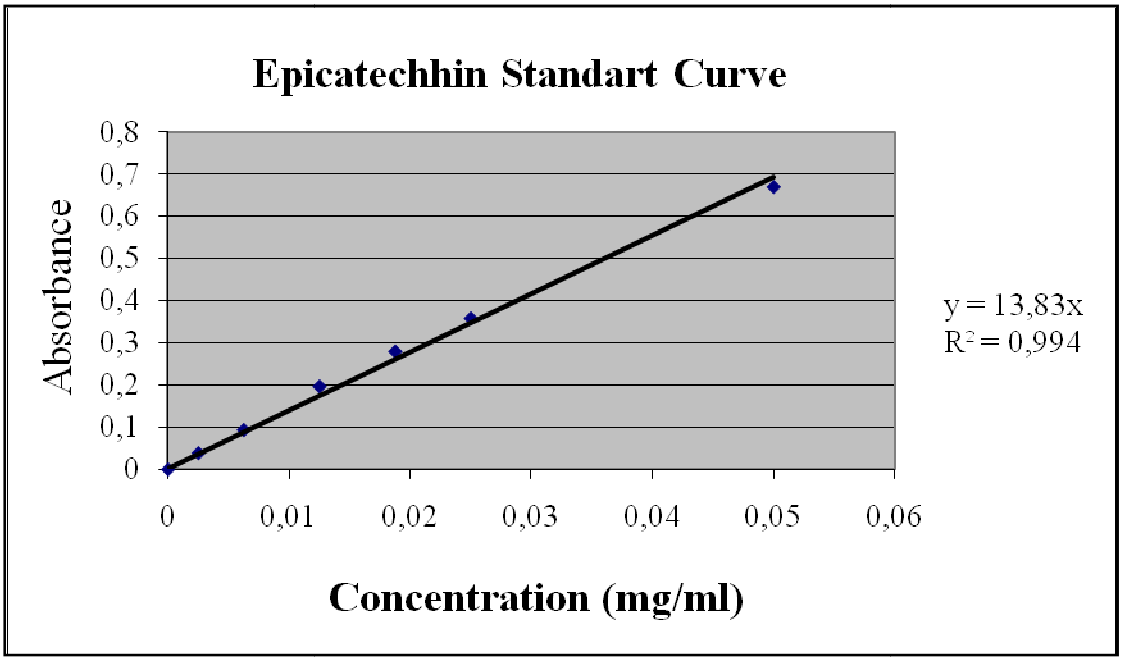

Figure 3.3. Calibration curve of epicatechin standard which was used for expression of total flavonoid contents as epicatechin equivalents

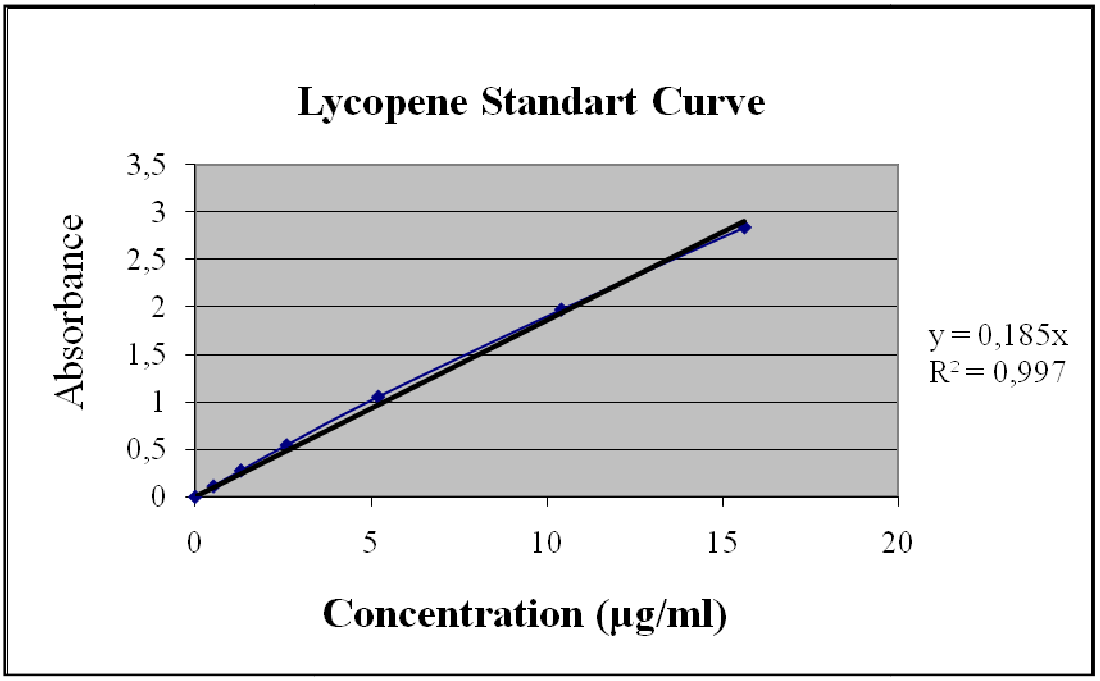

Figure 3.4. Calibration curve of lycopene standard which was used for expression of total lycopene contents as lycopene equivalents

3.2.7. Determination of Agronomically Important Traits

Eight agronomically important fruit traits were visually scored, except fruit weight, for each progeny of BC_2F_2 population. These were external and internal fruit color, total fruit weight, fruit firmness, fruit shape, stem scar, locule number and fruit wall.

External (EC) and internal fruit color (IC) were visually assessed for each line using a scale from 1 to 5 (1 = yellow or orange, 5 = most intense red). Fruit weight (FW) was determined by taking the average weight of 10 mature tomato fruits. Fruit firmness (FIRM) was measured by hand squeezing of ripe tomato fruits using a scale of 1 to 5 (1 = soft, 5 = very firm). 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 stem scar size (SSC) was measured based on fruit stem scar diameter (1 = small, 5 = very large). Locule number (LN) of tomato fruit was determined by counting the locules of tomato fruit after transversely cutting the fruit. Fruit wall (WALL) or pericarp thickness was also determined using transverse sections of fruits using a scale from 1 to 5 (1 = thin, 5 = very thick).

3.3. Genotypic Characterization

3.3.1. DNA Extraction

DNA was isolated from leaves of tomato seedlings using the protocol described by Bernatzky and Tanksley (1986). With this DNA extraction method large amounts (~5000 ng/µl) of pure and high molecular weight DNA were obtained. Tomato leaf samples were collected in the field from ten plants for each line and samples were immediately frozen in liquid nitrogen for transport to Izmir Institute of Technology. Tomato leaf samples were stored at -80^{o}C until DNA extraction was performed. The concentration and quality of the isolated DNA was measured with nano-drop ND-1000

spectorophotometer. To prepare DNA for PCR, each genotype's DNA was diluted at a 1/100 ratio (to ~50 ng/µl) with sterile distilled water.

3.3.2. Molecular Marker Analysis

For molecular characterization and QTL mapping of antioxidant traits, CAPs (Cleaved Amplified Polymorphic Sequence) marker analyses were performed. In the first step, parental surveys were done to identify a sufficient number of polymorphic markers (~100) for mapping. For this purpose, each marker was first tested on the two parental DNAs (TA1166 and LA1223). For CAPs assays, 25 µl PCR reaction mixture was prepared and amplified in a thermocycler (GeneAmp® PCR System 9700, Applied Biosystems). PCR components included: 2 µl DNA (~50 ng/µl), 2.5 µl 10X PCR buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl2, pH: 8.3), 0.5 µl dNTP (0.2 mM), 0.5 µl of each forward and reverse primers (10 pmol), 0.25 µl Taq polymerase (0.25 U) and 18.75 µl sterile distiled water. The CAPs markers were amplified using the PCR profile shown in Figure 3.5.

After amplification of CAPs markers, the PCR products were checked for amplification by electrophoresis through 2-4% agarose gels in 1X TBE buffer (0.9 M Tris, 0.002 M Na_2EDTA, 0.9 M boric acid, pH 8.3). Then amplified CAPs marker products were digested with different restriction enzymes that depicted in Table 1 for at least 3 hours at the appropriate temperature. Enzyme digestion mixture for 25 µl PCR product consisted of: 3 µl 10X digestion buffer (1X), 0.5 µl enzyme and 1.5 µl sterile distiled water. Finally, all of the samples were run on 2-4% agarose gels in 1X TBE buffer. Staining of the gels with ethidium bromide and visualization under UV light allowed identification of polymorphic CAPs marker bands. Polymorphic markers were then applied on the complete mapping population using the appropriate primer and restriction enzyme combinations.

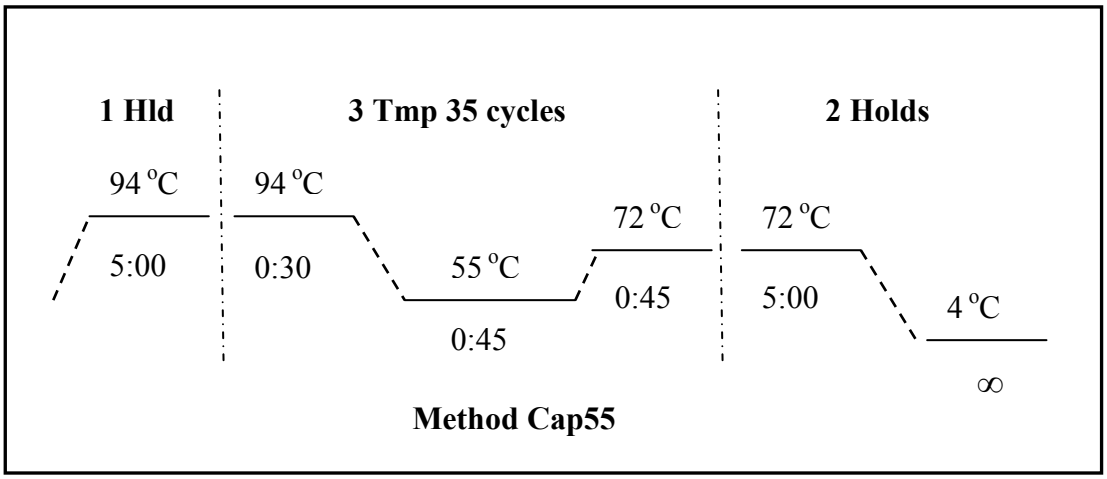

Figure 3.5. PCR profile for CAP55 method

3.4. Statistical Analysis

Analysis of variance (ANOVA) and Fishers PLSD were used for statistical analysis of the data. Significance was determined at $P<0.05$. Evaluation of correlation between the traits was done using the QGENE software program (Nelson 1997). Chi-square analysis was performed in Excel 7. Single point regression analysis was performed to determine the association between molecular markers and each trait using the QGENE software program (Nelson 1997). The effect of *L.hirsutum* alleles was calculated by subtracting the trait mean for individuals with at least one wild allele from the trait mean for individuals that were homozygous for *L.esculentum* alleles and dividing by the *L.esculentum* mean.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Phenotypic Characterization

For phenotypic characterization all characters that were analyzed exhibited a high range of variation and many of them showed continuous distribution for the trait. These results were expected because in the development of the BC_2F_2 population, two highly distinct parents were used in order to enhance the variation in the tomato mapping population. As previously mentioned, continuous distribution of trait values in the mapping population was very important for identification of QTLs.

4.1.1.Total Water Soluble Antioxidant Capacity

Total water soluble antioxidant (WAOX) activities of the 152 BC_2F_2 lines, and their parents are given in Appendix A (Table A-1). Means of the antioxidants traits, standard errors and ranges for the two parental lines and BC_2F_2 population are displayed in Table 4.1. WAOX activity of fruit from *L.hirsutum* was 1.5-fold higher than the WAOX activity of *L.esculentum*. This difference was statistically significant at $P<0.05$. While WAOX activity of *L.hirsutum* was calculated as 3925 ± 11 µmol Trolox/kg fresh tomato, the *L.esculentum* AOX activity was determined to be 2575 ± 123 µmol Trolox/kg fresh tomato. The mean value of WAOX activity of the BC_2F_2 population was 3430 ± 49 µmol Trolox/kg (Table 4.1) and the values of WAOX activity in the population ranged from 1618 to 5092 µmol Trolox/kg fresh tomato. This mean value was closer to *L. hirsutum* WAOX activity. The differences between the highest and

lowest value of WAOX activity in the population was 3.2 fold indicating good variation for the WAOX trait (Table 4.1).

Figure 4.1 depicts the distribution of WAOX capacity in the BC_2F_2 mapping population. The graph shows continuous variation for WAOX capacity and thereby it fits the normal distribution expected for a quantitative trait. As observed in Figure 4.1, there are some extreme individuals that exceeded both parental phenotypes. A total of 18% of the mapping population had higher WAOX capacity than *L.hirsutum*'s WAOX value. This was because of the transgressive segregation. Due to the additive property of polygenes, some individuals had alleles combinations that increased their WAOX activities beyond that observed in either parent.

Table 4.1. Mean values and standard errors of parental lines and BC_2F_2 population for antioxidant traits. Values followed by differentletters means are significantly different between the two parental lines ($P < 0.05$)

	L.esculentum	***L.hirsutum***	**BC_2F_2 Population**	
Trait	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Range**
WAOX (µmolTrolox/kg)	2575 ± 123 a	3925 ± 11 b	3430 ± 49	1618-5092
VitaminC (mg/kg)	170 ± 13 a	160 ± 10 a	200 ± 4	80-320
Phenolic (mg/kg)	207 ± 0.7 a	303 ± 1.4 b	247 ± 4.3	140-454
Flavonoid (mg/kg)	54 ± 0.4 a	83 ± 2.9 b	91 ± 3.6	47-250
Lycopene mg/kg	87 ± 1.3 a	4 ± 0.1 b	69 ± 2.5	4-172

4.1.2. Vitamin C Content

In both parental lines, the vitamin C content (VitC) was found to be nearly equal. While the vitamin C value of *L.hirsutum* was 160 ± 10 mg/kg, *L.esculentum* vitamin C content was 170 ± 13 mg/kg (Table 4.1). In spite of the similarity of the two parental lines in vitamin C content, the BC_2F_2 population showed distinct segregation for the trait. The vitamin C content of the BC_2F_2 population ranged from 80 to 320 mg/kg (Table 4.1), a 4-fold difference. Along with the wide range of variation for this trait, there was a normal distribution for vitamin C content of the BC_2F_2 population

(Figure 4.2). Figure 4.2 showed a very interesting distribution. While two parental lines were similar for their vitamin C values many progeny had higher vitamin C content. In fact, 84% of the population had higher vitamin C content than *L.hirsutum* and also 73% of progeny had higher values than *L.esculentum*. This was a result of the transgressive segregation, so different alleles that came from the parents could enhance the value of these progeny. The mean value of vitamin C content of the BC_2F_2 population was 200 ± 4 mg/kg, slightly higher than the two parental lines. Appendix A (Table A-1) shows vitamin C content for the individuals of the whole population.

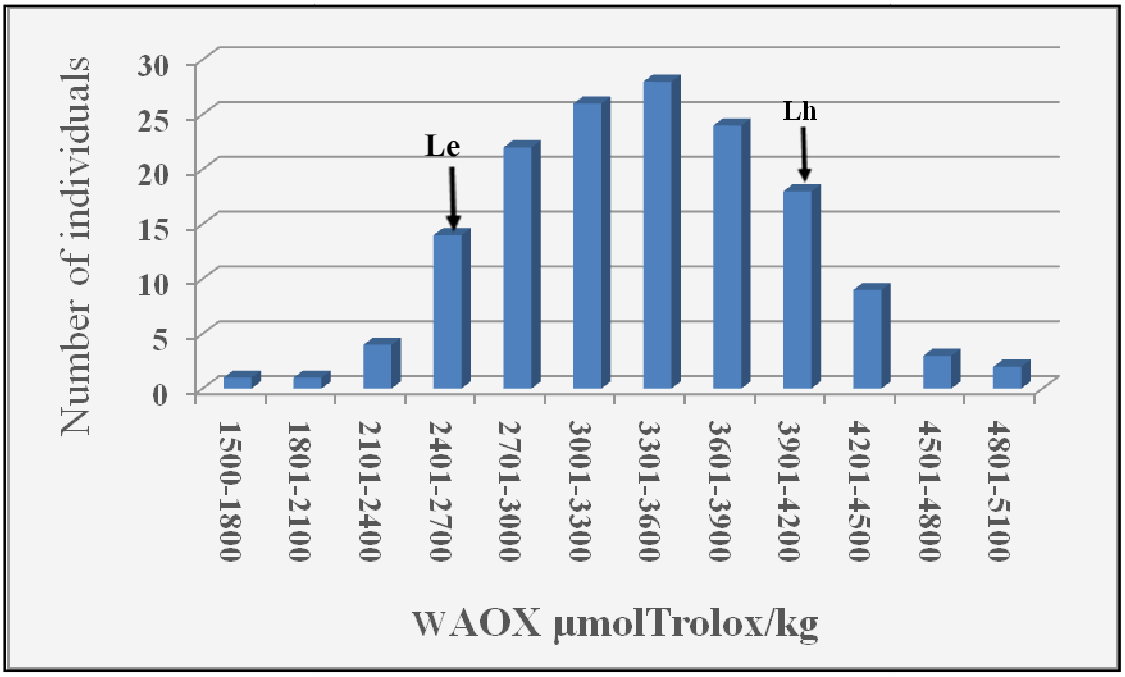

Figure 4.1. Distribution histogram for total water-soluble antioxidant activities. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

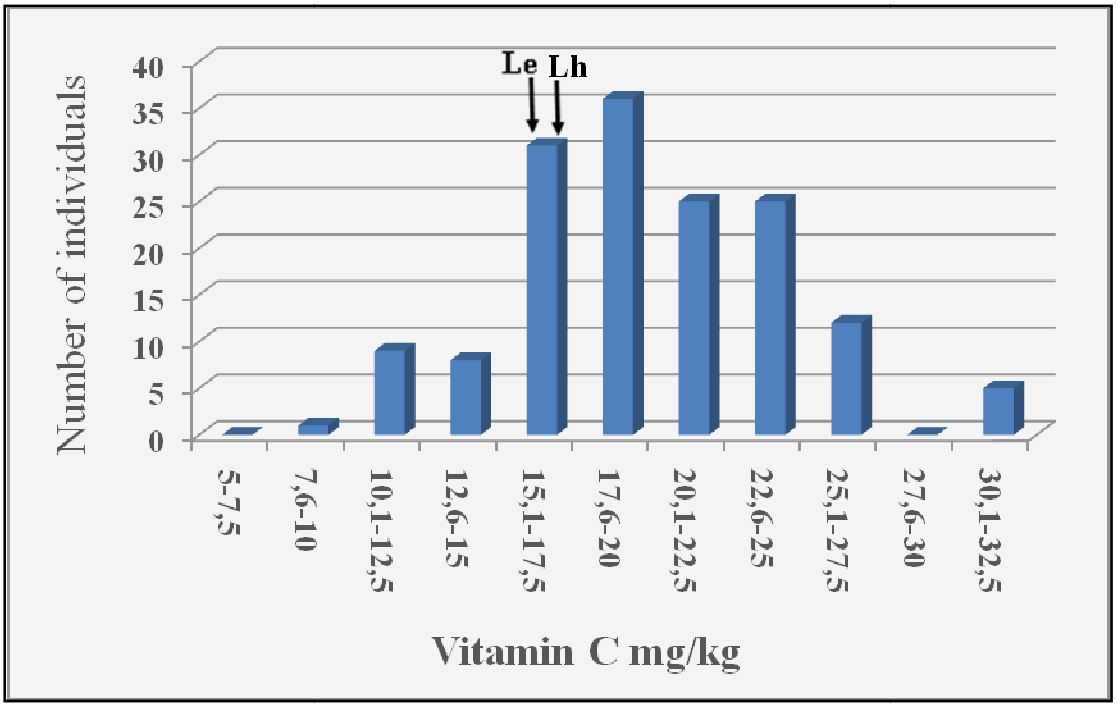

Figure 4.2. Distribution histogram for Vitamin C content. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

4.1.3. Total Phenolic Content

Total phenolic content (PHE) for the BC_2F_2 population is given in Appendix A (Table A-1). There was great variation for phenolic compounds in the population ranging from 140 to 454 mg/kg. The mean value of total phenolic compounds for the population was 247 ± 4.3 mg/kg. There was a 1.5-fold difference between phenolic content of the two parental lines whose values were 207 ± 0.7 mg/kg for *L.esculentum* and 303 ± 1.4 mg/kg for *L.hirsutum* (Table 4.1). This difference between the parents was statistically significant. Figure 4.3 shows the distribution histogram for total phenolic compounds in the BC_2F_2 population. There was continuous variation for this trait and the mean value of phenolics for the BC_2F_2 population was intermediate between the values for *L.esculentum* and *L.hirsutum*. In addition, 15% of the mapping population had higher phenolic content than the donor parent (*L.hirsutum*) due to transgressive segregation.

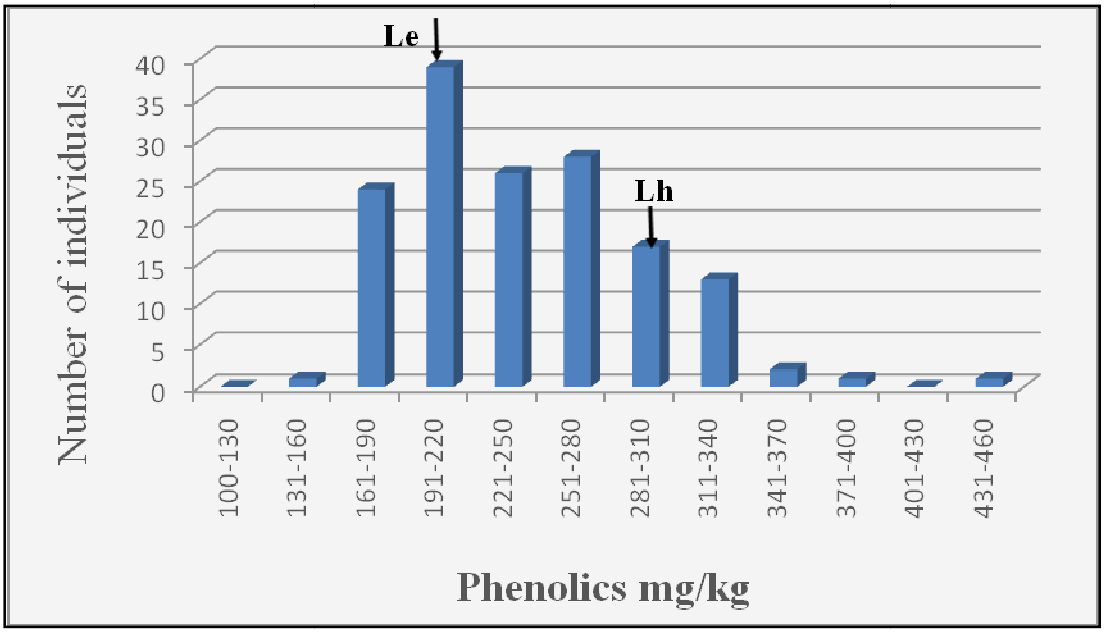

Figure 4.3. Distribution histogram for total phenolic content. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

4.1.4. Total Flavonoids Content

For total flavonoids content (FLAV), the lowest and the highest values of the BC_2F_2 population ranged from 47 to 250 mg/kg, a 5.3 fold difference (Table 4.1). The BC_2F_2 population showed wide varition with regard to flavonoid content which made QTL analysis feasible. The flavonoids content of *L.hirsutum* was 83 ± 2.9 mg/kg, while *L.esculentum* had 54 ± 0.4 mg/kg (Table 4.1). Thus, *L.hirsutum* had 1.5-fold higher flavonoids content. Appendix A (Table A-1) shows total flavonoids content of the whole population. The mean value of flavonoids content for the BC_2F_2 population was 91 ± 3.6 mg/kg and this value was closer to *L.hirsutum*'s flavonoids content than *L.esculentum*'s. Figure 4.4 shows the distribution histogram for total flavonoid content in the BC_2F_2 population. In this histogram, many individuals were located in an extreme region with higher values then both parental lines. In terms of flavonoids content, 32% of the BC_2F_2 mapping population exceeded *L.hirsutum* which was used as a donor parent for this trait. This result stemmed from transgressive segregation of the flavonoid alleles.

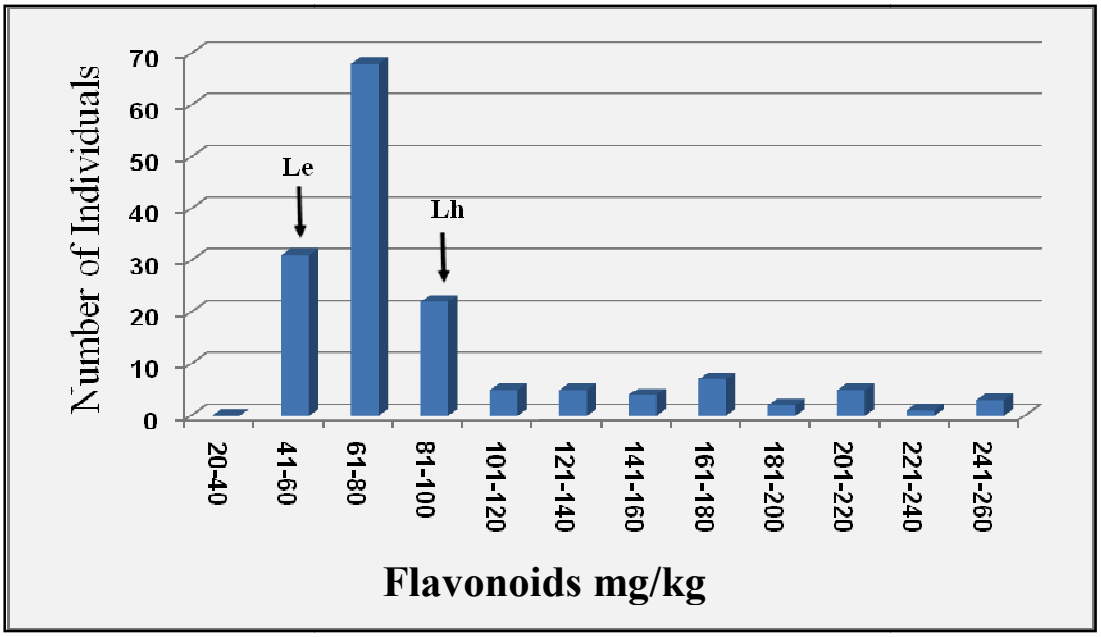

Figure 4.4. Distribution histogram for total flavonoids content. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

4.1.5. Lycopene Content

In terms of lycopene content (Lyc), the BC_2F_2 population exhibited the highest variation among the antioxidant traits (Appendix A, Table A-1). Lycopene content ranged from 4 to 172 mg/kg, a 43-fold difference (Table 4.1). The red color of tomato fruit stems from lycopene content, therefore *L.hirsutum* which has green fruits even in their mature stage had the lowest lycopene content. While *L.esculentum* contained 87 ± 1.3 mg/kg lycopene, *L.hirsutum* contained only 4 ± 0.1 mg/kg and the mean value of the population was 69 ± 2.5 mg/kg (Table 4.1). Figure 4.5 depicts the distribution histogram for lycopene content. There was continuous variation for that trait. However 76% of the BC_2F_2 population had lower lycopene content than *L.esculentum*. That meant that, as expected, most of the *L.hirsutum* alleles decreased lycopene content in the mapping population. Despite this, 24% of the BC_2F_2 population had higher lycopene content than *L.esculentum*. Thus in spite of its green fruit color, *L.hirsutum* also has some alleles that could improve elite tomato fruit color because of transgressive segregation. Therefore, the appearance of exotic germplasm does not always reflect its the genetic potential.

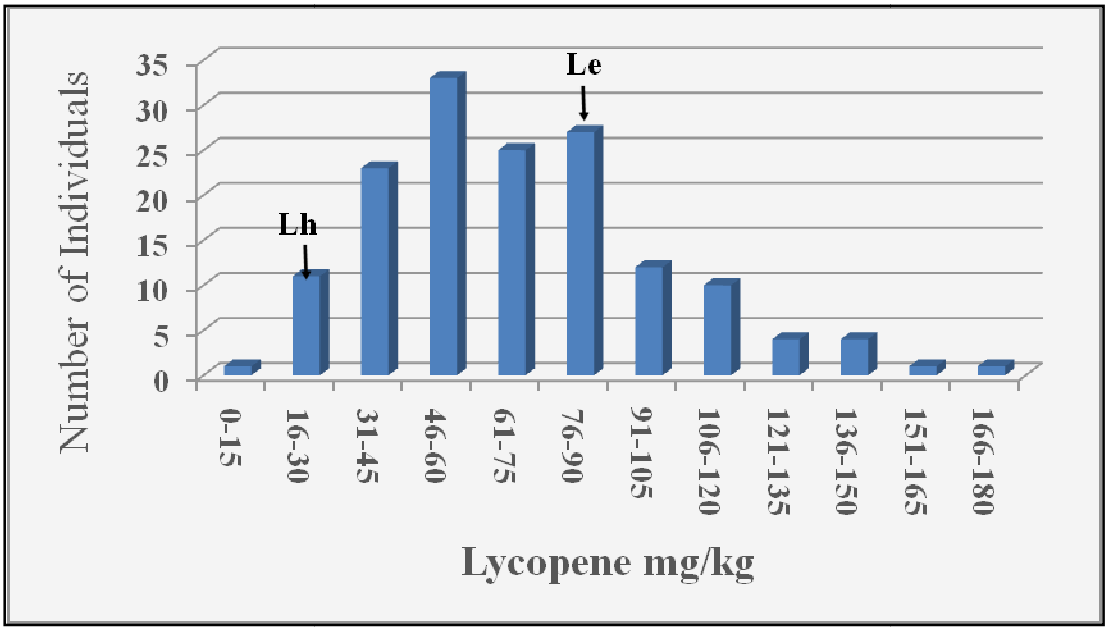

Figure 4.5. Distribution histogram for lycopene content. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

4.1.6. Correlations Between the Antioxidant Traits

Moderate, but statistically significant ($P<0.05$) positive correlations were observed between some of the antioxidant traits (Table 4.2). The strongest positive correlations for antioxidant traits were found between total water soluble antioxidant capacity (WAOX) and phenolic compounds ($r = 0.48$) and between WAOX and vitamin C ($r = 0.44$). These results were not suprising because both phenolic compounds and vitamin C have great contributions to the total amount of water soluble antioxidant activity (WAOX). An additional positive correlation was observed between vitamin C content and phenolic compounds ($r = 0.36$), this correlation was also described in pepper by Frary et al. (2008). Previous studies also indicated similar positive correlations between phenolic compounds and WAOX in tomato and pepper (Hanson, et al. 2004, Rousseaux, et al. 2005, Toor, et al. 2006, Frary, et al. 2008). However, unexpectedly there was no correlation between flavonoid content and phenolics and also between flavonoid content and WAOX. Because flavonoids are some of the main contributors to total phenolic content and also a moderate contributor to WAOX

capacity, this was an unexpected result. Lycopene content also did not have any significant correlation with other antioxidant traits. This was not suprising because lycopene is a lipid soluble antioxidant so it could not contribute to total water soluble antioxidants.

Table 4.2. Correlations between antioxidant traits in the population. *P*-value of each correlation is given in parentheses. Only correlations with *P*-value <0.05 are considered to be significant

Trait	**Vitamin C**	**Phenolics**	**Flavonoids**	**Lycopene**
WAOX	0.44 (0.0001)	0.48 (0.0001)	0.04 (0.66)	-0.16 (0.05)
Lycopene	-0.16 (0.05)	-0.005 (0.9)	-0.06 (0.5)	
Flavonoids	-0.034 (0.6)	0.08 (0.35)		
Phenolics	0.36 (0.0001)			

4.1.7. External and Internal Fruit Color

Means, standard errors and ranges for agronomic traits evaluated for the two parental lines and the BC_2F_2 population are given in Table 4.3. For both internal (INC) and external fruit color (EXC), *L.esculentum* had a moderate red fruit color of 3 while *L.hirsutum* had green fruit and was scored as 1. Appendix A (Table A-2) indicates all agronomic traits scores for the BC_2F_2 population. Means for both internal and external fruit color for the BC_2F_2 population were calculated as approximately 2 ± 0.1. There was a wide range of variation for both characters ranging from 1 to 5 (Table 4.3). Figures 4.6 and 4.7 depict the distribution histogram for internal and external fruit color. Some progeny exhibited better color (approximately 17 % of the BC_2F_2 mapping population) than *L.esculentum*, this is because of transgressive segregation. Although *L.hirsutum* had green fruit color in its mature stage, it could still have some alleles that could improve the red fruit color of next generations. This same result assocaiated with *L.hirsutum* alleles was observed by Bernacchi et al. (1998).

Table 4.3. The mean value and standard errors of parental lines and BC_2F_2 population for agronomic traits

	L.esculentum	*L.hirsutum*	BC_2F_2 Population	
Trait	**Mean**	**Mean**	**Mean ± SE**	**Range**
Internal Color	3	1	2 ± 0.1	1- 4.5
External Color	3	1	2.2 ± 0.1	1- 5
Fruit Weight (g)	262	6.5	96 ± 3.2	6.5- 262
Firmness	3.5	2	3.2 ± 0.1	1- 5
Fruit Shape	1	1	1.3 ± 0.04	1- 4.5
Stem Scar	5	1	3.7 ± 0.1	1- 5
Locule Number	6	3	4.3 ± 0.1	2- 6
Wall	4.5	1	2.8 ± 0.1	1- 5

4.1.8.Average Fruit Weight

There was great variation for fruit weight (FW) in the BC_2F_2 population; ranging from 6.5 to 262 g (Table 4.3). The two parental lines showed extremely different values for fruit weight; fruit weight was 262 g for *L.esculentum* while only 6.5 g for *L.hirsutum*, a 40-fold difference. All progeny showed intermediate values for the trait. That means the two parental lines showed extreme values and no progeny exceeded them. The mean value of fruit weight for the population was calculated as 96 ± 3.2 g. The BC_2F_2 population exhibited continuous distribution for fruit weight (Figure 4.8).

4.1.9. Fruit Firmness

For the BC_2F_2 population, fruit firmness (FIRM) ranged from 1 to 5 with an average of 3.2 ± 0.1 (Table 4.3). While *L.esculentum* was scored as 3.5, *L.hirsutum* had softer fruit and was scored as 2. Figure 4.9 shows the distribution of fruit firmness in the BC_2F_2 population. A total of 36% of the mapping population's values exceeded *L.esculentum*'s value and 9% of the population had lower values than *L.hirsutum* for firmness. This is also because of transgressive segregation.

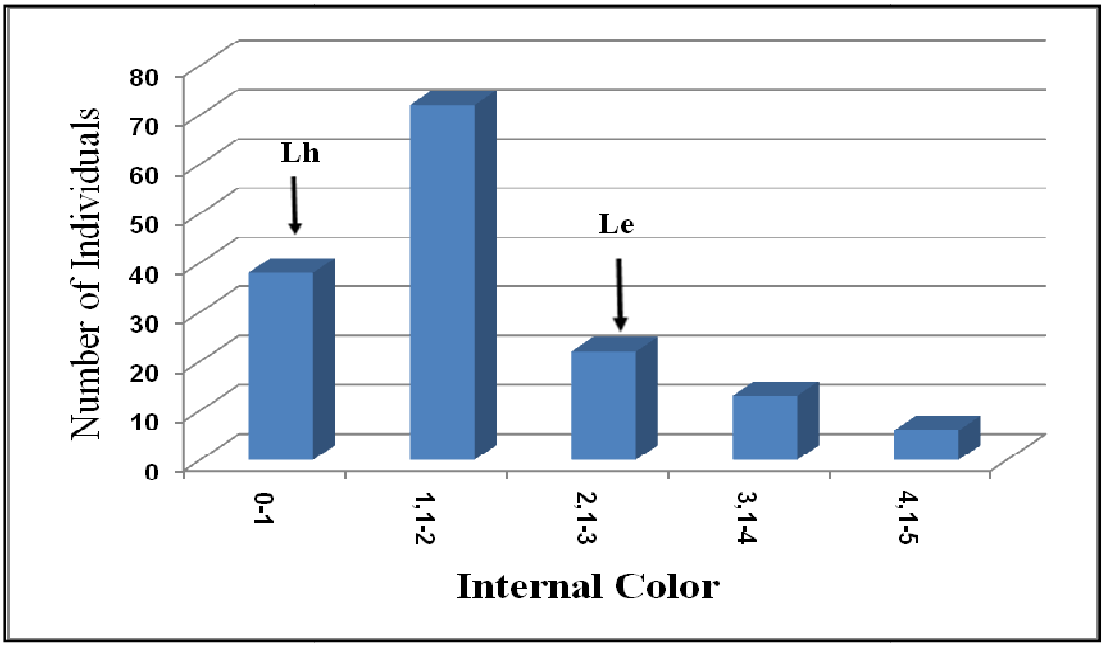

Figure 4.6. Distribution histogram for internal fruit color. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

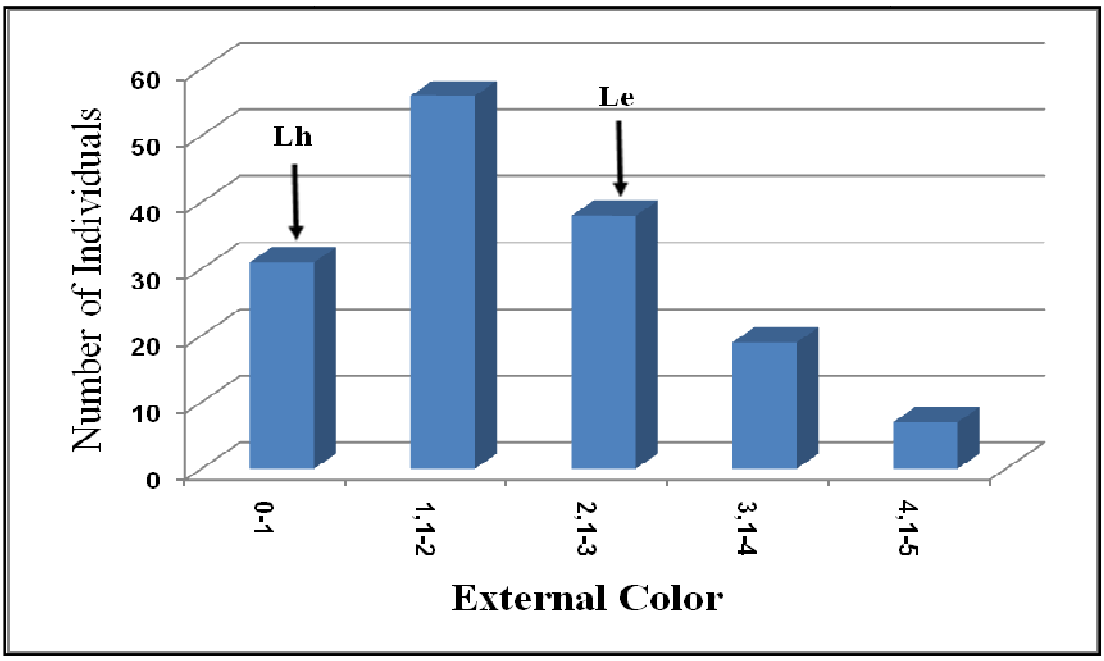

Figure 4.7. Distribution histogram for external fruit color. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

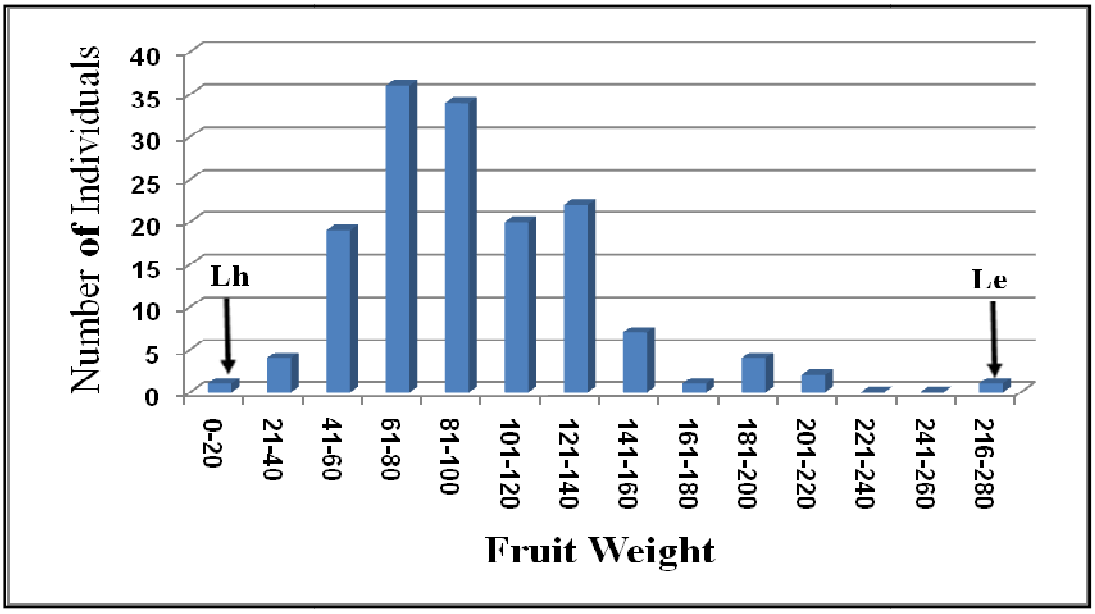

Figure 4.8. Distribution histogram for average fruit weight. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

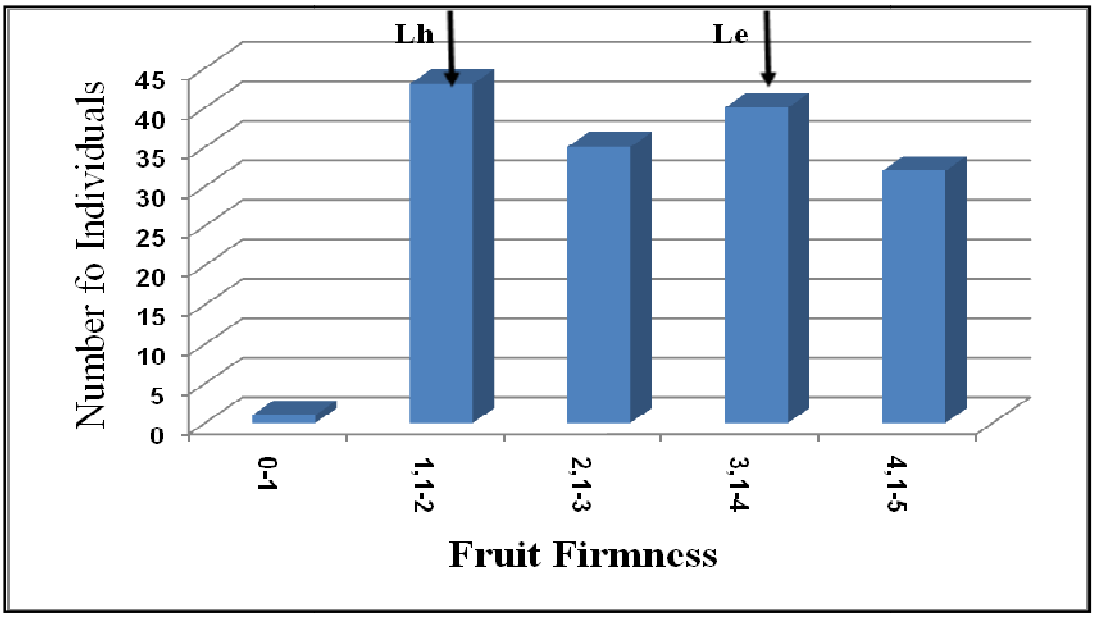

Figure 4.9. Distribution histogram for fruit firmness. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

4.1.10. Fruit Shape

In both parental lines, fruit shape (FS) was spherical (1 = round, 5 = elongated). However, there was good variation ranging between 1 and 4.5 for fruit shape in the BC_2F_2 population (Table 4.3). The mean fruit shape for population was 1.3 ± 0.04, so nearly all progeny (88% of population) had round fruit shape similar to the two parents (scored 1 or 1.5) (Figure 4.10). In contrast,12% of the mapping population had elongated fruit. That means that one or both of the parental lines might contain alleles that were responsible for formation of elongated fruit shape.

4.1.11. Stem Scar

The two parental lines were extremely different for stem scar size (SSC). There was a five-fold difference between them, *L.esculentum*'s stem scar size was very large and scored as 5 whereas *L.hirsutum*'s stem scar was very small and scored as 1 (Table 4.3). The mean value of this trait in the BC_2F_2 population was calculated as 3.7 ± 0.1. Figure 4.11 exhibits the distribution of stem scar size in the population.

4.1.12. Locule Number

Fruit of *L.esculentum* had an average of six locules whereas *L.hirsutum* had an average of three locules.. The average fruit locule number (LN) for the population was estimated as 4.3 ± 0.1 with variation from 2 to 6 locules (Table 4.3). Figure 4.12 shows the distribution histogram for locule number of the BC_2F_2 population.

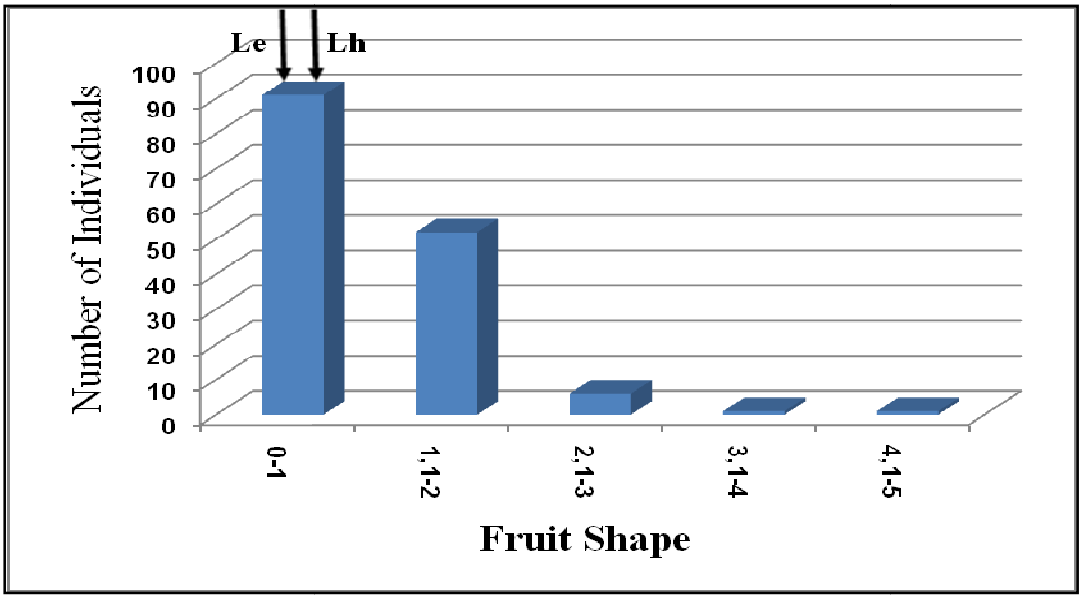

Figure 4.10. Distribution histogram for fruit shape. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

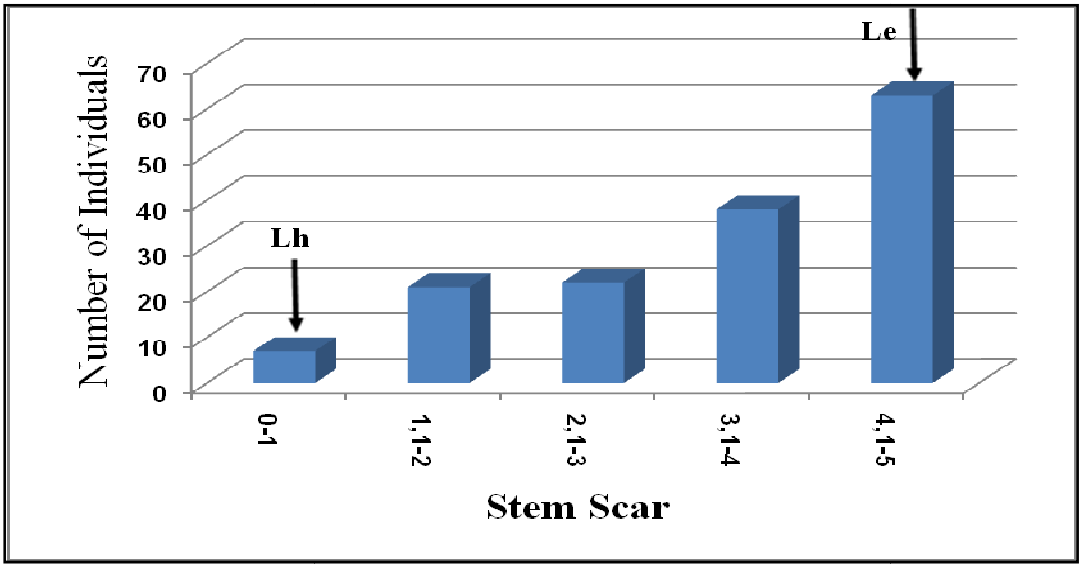

Figure 4.11. Distribution histogram for stem scar. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

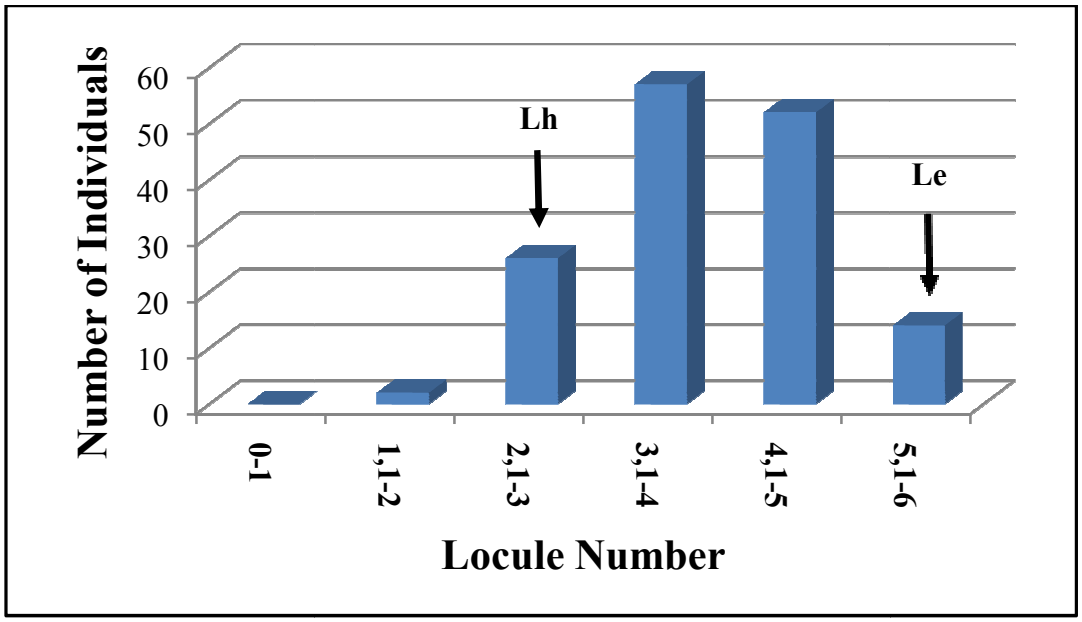

Figure 4.12. Distribution histogram for locule number. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

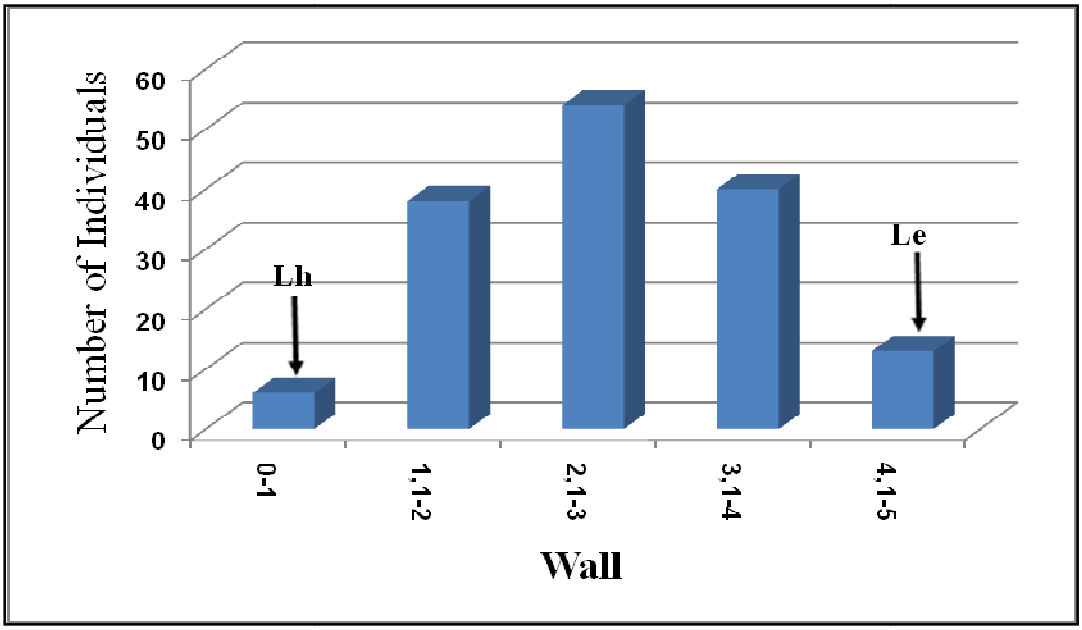

Figure 4.13. Distribution histogram for fruit wall thickness. Le and Lh indicate locations of *L.esculentum* and *L.hirsutum* means, respectively

4.1.13. Wall

Wall thickness (WALL) was evaluated as 4.5 for *L.esculentum* and 1 for *L.hirsutum* (Table 4.3). Thus, cultivated tomato had much thicker pericarp than the wild species. Among the BC_2F_2 population, wall values ranged from 1 to 5 with a mean of 2.8 ± 0.1 and showed a continuous distribution (Figure 4.13).

4.1.14. Correlation Between Agronomically Important Traits

All of the agronomically important traits that were analyzed exhibited great phenotypic variation (Table 4.3). The highest statistically significant positive correlation was observed between internal and external fruit color as expected (r = 0.88) (Table 4.4). Similar association was also identified by Fulton et al. (2000) and by Doğanlar et al. (2002). There were also significant high correlations between lycopene content and internal and external fruit color (r = 0.53 and r = 0.57, P = 0.0001). These correlations were expected because of the possibility of pleiotropic effects of the same loci for internal and external fruit color and lycopene content. There was a high positive correlation between stem scar size and fruit weight (r = 0.60). This was not surprising because large fruit was expected to have large stem scar size and a similar result was also observed by Doğanlar et al. (2002). Stem scar size also showed high correlation with fruit locule number (r = 0.58). There was also a moderate significant correlation between fruit weight and locule number (r = 0.50). Elongated fruit shape was correlated with fewer locules and smaller stem scar size (Table 4.4). Wall thickness was weakly correlated with INC, EXC, FW, FIRM, FS and SSC (Table 4.4).

Table 4.4. Correlations between agronomically important traits in the population. *P*-value of each correlation is depicted in parentheses. Only correlations with *P*-value < 0.05 are considered to be significant

Trait	In.color	Ex.color	FruitWeight	Firmness	Shape	Stemscar	Locule
Wall	0.38	0.38	0.16	0.20	0.22	0.18	-0.10
Locule	-0.003	-0.04	0.50	-0.1	-0.51	0.58	
Stemscar	0.07	0.04	0.60	0.001	-0.34		
Shape	0.20	0.20	-0.33	0.02			
Firmness	0.06	0.07	0.01				
Weight	0.02	-0.02					
Excolor	0.88						

4.2. Genotypic Characterization and QTL Mapping

To identify QTLs for both health-related and agronomically important traits, 70 CAPs and 2 SSR markers were tested on the 152 BC_2F_2 lines for genotypic characterization. Table 4.5 is a list of these CAPs and SSR markers with their amplification conditions and the sizes of restriction products after cutting with the indicated enzyme. Out of the 70 CAPs and 2 SSRs markers that were mapped in the BC_2F_2 population, 14 of the markers (19%) fit the 29/32 AA : 3/32 Aa segregation ratio expected for a dominant markers after *Chi*-square analysis ($P<0.05$). A total of 58 of the markers (81%) were skewed toward the *L.hirsutum* genotypes, but there were no markers that were skewed toward the *L.esculentum* homozygous genotype. This type of skewing is commonly observed in interspecific populations as repoted by Paterson et al. (1990).

A genetic linkage map was drawn for the 72 markers using the locations of the markers in a *L.pennellii* interspecific population as reference (Sol Genomics Network 2008). The number of markers per linkage group ranged from 3 (chromosomes 6 and 8) to 12 (chromosome 2) (Figure 4.14). The average distance between markers was 15 cM while the largest gaps between markers were 77 cM on chromosome 1 and 60 cM on chromosome 5. Overall, the map provided approximately 65% genome coverage (905 cM as compared to 1386 cM for the *L.pennellii* map). Poorest coverage was on

chromosome 6 with only 9% of the genome represented by the three markers mapped on this chromosome. Five chromosomes (1, 2, 5, 11 and 12) had at least 75% coverage with best coverage on linkage groups 2 (96%) and 5 (94%).

Single point regression analysis was performed to determine the association between molecular markers and each trait in the BC_2F_2 mapping population using the QGENE software program (Nelson 1997). If more than one contiguous marker showed significant association with the same trait, it was assumed that only one locus was involved. In this study, a total of 75 significant ($P < 0.05$) QTLs were identified for all 13 characters. Table 4.6 shows the QTLs that were identified for each trait. Of the 75 QTLs, 28 (37%) were related with antioxidant traits, while 47 (63%) were associated with horticulturally important traits. Figure 4.14 exhibits the location of each QTL on the tomato genetic map. Each chromosome had at least 2 QTLs (chromosome 10) and at most 12 QTLs (chromosome 12) (Figure 4.14). The number of QTLs detected for each trait ranged from 3 for fruit weight to 8 for lycopene content.

4.2.1. Total Water Soluble Antioxidant Capacity

Six QTLs were identified for total water soluble antioxidant (WAOX) capacity. These QTLs were located on chromosome 1 (*waox1.1*), 5 (*waox5.1*), 6 (*waox6.1*), 8 (*waox8.*1) and 12 (*waox12.1* and *12.2*) (Figure 4.14). The most significant one was *waox12.1* on chromosome 12 with $P = 0.0002$ (Table 4.6). For this locus, the *L.hirsutum* allele was associated with a 12% increase in antioxidant capacity. Rousseaux et al. (2005) also identified a QTL for the same trait in the same location on chromosome 6 in *L.pennellii* introgression lines.. For five out of the six QTLs, as expected based on the values for the parental lines, *L.hirsutum waox* alleles enhanced the WAOX capacity. On the other hand, only one *L.esculentum waox* allele (*waox1.1*) was associated with higher WAOX capacity.

4.2.2. Vitamin C Content

Vitamin C content was associated with five QTLs, *vitc1.1* on chromosome 1, *vitc2.1* and *vitc2.2* on chromosome 2, *vitc6.1* on chromosome 6 and *vitc12.1* on chromosome 12 (Figure 4.14). The most significant *vitc* QTL was *vitc6.1,* marked by CT206, with $P = 0.0005$ (Table 4.6). The wild allele for this locus was associated with a 16% increase in vitamin C. *vitc2.2* QTL region was also identified by Stevens et al. (2007). In addition, the *vitc12.1* QTL on chromosome 12 was identified in approximately the same map position in two previous studies carried out by Rousseaux et al. (2005) and Stevens et al. (2007). For *vitc1.1*, *vitc6.1* and *vitc12.1* QTLs, *L.hirsutum* alleles were associated with higher vitamin C content, while for *vitc2.1* and *vitc2.2* QTLs, *L.esculentum* alleles were responsible for higher vitamin C content. The parental lines showed no significant difference for vitamin C content.

4.2.3. Total Phenolic Content

Five QTLs were detected for total phenolic content. These *phe* QTLs were located on chromosomes 1 (*phe1.1*), 6 (*phe6.1*), 7 (*phe7.1*), 9 (*phe9.1*) and 12 (*phe12.1*) (Figure 4.14). *phe6.1* was the most significant one ($P = 0.01$) and was linked to marker CT206 (Table 4.6). All of the alleles associated with high phenolic content came from *L.hirsutum* as expected because the wild species had higher phenolic content than cultivated tomato. *phe7.1* and *phe9.1* mapped to similar locations as *phe* QTLs previously identified by Rousseaux et al. (2005). *phe7.1* was of special interest because the *L.hirsutum* allele at this locus was associated with a 17% increase in phenolic content.

Table 4.5. List of CAPs and SSR markers, their methods and sizes of restriction products after cutting with indicated enzyme

Markers	**Method**	**Enzymes**	**Size for L.hirsutum**	**Size for L.esculentum**
At1g14000	Cos55	RsaI	750+600	600
At1g20050	Cos55	RsaI	500+450+250	500+250
At1g30580	Cos55	HinfI	850+350	450+350
At1g46480	Cos55	EcoRI	300+190+175	190+175
At1g47830	Cos55	TaqI	1000+800+450	800+450
At1g50020	Cos55	HindIII	1700+825	825
At1g55870	Cos55	HinfI	750+500	750
At1g60640	Cos55	TaqI	390+350+200	350+200
At1g61620	Cos55	AluI	900+800+375	800+375
At1g63610	Cos55	AluI	450+375+300	450+375
At1g71810	Cos55	RsaI	850+600	850
At1g75350	Cos55	RsaI	200+190	150+75
At1g78690	Cos55	HinfI	850+700	450+250
At2g01720	Cos55	RsaI	450+375	450
At2g06530	Cos55	RsaI	500+300+250	300+250
At2g15890	Cos55	HhaI	850+450+400	450+400
At2g26590	Cos55	RsaI	850+750	850
At2g29210	Cos55	AluI	375+250+150	250+150
At2g32970	Cos55	AluI	375+300	300
At2g42750	Cos55	HaeIII	800+400+350	400+350
At3g06050	Cos55	AluI	425+375+350	375+350
At3g09925	Cos55	HaeIII	350	325+200
At3g13235	Cos55	RsaI	700+400+300	400+300
At3g14910	Cos55	EcoRI	800+600+200	800
At3g15430	Cos55	TaqI	450+375+225	375+225
At3g16150	Cos55	TaqI	390+250+125	250+125

(cont. on next page)

Table 4.5. (Cont.) List of CAPs and SSR markers, their methods and sizes of restriction products after cutting with indicated enzyme

Markers	**Method**	**Enzymes**	**Size for L.hirsutum**	**Size for L.esculentum**
At3g47640	Cos55	TaqI	900+700	700
At3g52220	Cos55	AluI	380+350	350
At3g57280	Cos55	HhaI	880+850	850
At4g00560	Cos55	HinfI	800+750	750
At4g03280	Cos55	HinfI	780+750	780
At4g16580	Cos55	HinfI	400+200+175	200+175
At4g21710	Cos55	HinfI	425+350+175	425
At4g22260	Cos55	HinfI	425+350	500
At4g28530	Cos55	HinfI	950+850+750	750
At4g33985	Cos55	HinfI	380+250	380
At4g35560	Cos55	HinfI	375+200+175	375
At4g37280	Cos55	TaqI	900+700	700
At5g04910	Cos55	AluI	350+300+200+1500	200+150
At5g06130	Cos55	HinfI	250+150	250
At5g06430	Cos55	PstI	1000+800	800
At5g13030	Cos55	PstI	500+410	500
At5g13640	Cos55	TaqI	550+400	550
At5g14520	Cos55	HinfI	375+225	375
At5g16710	Cos55	AluI	750+500+350	500+350
At5g20180	Cos55	TaqI	1500	800+400
At5g35360	Cos55	HinfI	425+375+150	425
At5g37260	Cos55	HhaI	500+375+250	375+250
At5g41350	Cos55	TaqI	475+225	225
At5g42740	Cos55	PstI	500+350+180	350+180
At5g49970	Cos55	HinfI	500+450	450
At5g51110	Cos55	PstI	400+360	360

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Table 4.5. (Cont.) List of CAPs and SSR markers, their methods and sizes of restriction products after cutting with indicated enzyme

Markers	**Method**	**Enzymes**	**Size for L.hirsutum**	**Size for L.esculentum**
CT138	Cos50	HhaI	800+450+375	800
CT143	Cap50	RsaI	400+380	380
CT167	Cap50	TaqI	200+190	200
CT20	Cap50	RsaI	500+380+200	500+200
CT206	Cap50	RsaI	550+400	400
CT269	Cap50	TaqI	1500+900+500	900+500
CT59	Cap50	TaqI	425+390	390
CT64	Cos50	HinfI	450+380	450
SSR32	SSR50	-	200+180	180
SSR40	SSR50	-	190+175	175
T0266	Cap50	TaqI	800+400+350	800
T0564	Cap50	TaqI	950+750	750
T0668	Cos50	HinfI	375+200+150	200+150
T0671	Cap50	TaqI	800+750	800
T1422	Cos50	AvaII	800+600	600
TG180	Cap50	RsaI	875+800	875
TG307	Cap50	RsaI	900+800	800
TG36	Cap50	RsaI	500+425	425
TG46	Cap50	TaqI	850+750+375	850+375
TG566	Cap50	RsaI	250+190+175	190+175

4.2.4. Total Flavonoids Content

For flavonoid content, four QTLs regions were identified on the molecular marker map (Figure 4.14). These were *flav2.1* (on chromosome 2), *flav3.1* (on chromosome 3), *flav5.1* (on chromosome 5) and *flav11.1* (on chromosome 11). The

most significant one was *flav11.1* QTL linked with TG36 (Table 4.6). The source of high flavonoid content for *flav5.1* and *flav11.1* loci was *L.hirsutum*, while for the other two QTL regions *L.esculentum* alleles were associated with higher flavonoids. For *flav11.1*, the *L.hirsutum* allele accounted for a 24% increase in flavonoids content.

4.2.5. Lycopene Content

Eight QTLs were identified for lycopene content (Table 4.6; Figure 4.14). These QTLs were located on chromosomes 2 (*lyc2.1*), 3 (*lyc3.1*), 7 (*lyc7.1*), 8 (*lyc8.1*), 9 (*lyc9.1*), 10 (*lyc10.1*), 11 (*lyc11.1*) and 12 (*lyc12.1*) (Figure 4.14). *lyc8.1* and *lyc12.1* were the two most significant QTLs (Table 4.6). For *lyc3.1*, *lyc7.1*, *lyc8.*1 and *lyc12.1*, *L.esculentum* alleles were associated with an increase in lycopene content, while for the rest of the QTLs, *L.hirsutum* alleles were responsible for high lycopene content. This is an interesting finding as *L.hirsutum* has green fruit. These results also support the work of Bernacchi et al. (1998) and Monforte and Tanksley (2000) who found that *L.hirsutum* alleles could be used to improve red color in tomato fruit. Of most interest were *lyc9.1* and *lyc10.1* as wild alleles at these loci were responsible for 37 and 46% increases in lycopene content, respectively. *lyc3.1* and *lyc12.1* matched loci that were identified by Rousseaux et al. (2005) in the same map region. The *Delta* mutation, which results in reddish orange fruit, maps to a similar location on chromosome 12 suggesting that *Delta* might be a candidate locus for this QTL (Rousseaux, et al. 2005). In addition, the never ripe mutant of tomato, *nor*, has been mapped to the same region of chromosome 10 as *lyc10.1* (Tanksley, et al. 1992).

4.2.6. External and Internal Fruit Color

Nine QTLs were identified for external fruit color on six different chromosomes (Figure 4.14). Chromosomes 4, 9 and 12 contained two *exc* QTLs, while chromosomes 1, 7 and 8 had one QTL each. The most significant QTL for external fruit color was

exc4.2 with P < 0.002. For *exc1.1*, *exc7.1*, *exc8.1, exc12.1* and *exc12.2 L.hirsutum* allele were associated with decreased fruit color; however, *exc4.1*, *exc4.2*, *exc9.1* and *exc9.2* alleles from *L.hirsutum* were responsible for increased red color. *exc4.2* QTL was also detected by Monforte and Tanksley (2000). The wild alleles for the two loci on chromosome 9 increased external color by 41 and 32%, respectively.

For internal fruit color, seven QTLs regions were identified. These were *inc1.1* (on chromosome 1), *inc4.1* (on chromosome 4), *inc7.1* (on chromosome 7), *inc8.1* (on chromosome 8), *inc9.1* (on chromosome 9), *inc12.1* and *inc12.2* (on chromosome 12) (Figure 4.14). *inc8.1* linked with TG307 was the most significant QTL for internal color with *P* = 0.0007 (Table 4.6). For *inc4.1* and *inc9.1 L. hirsutum* alleles were related with higher color formation with these alleles increasing red color by 22 and 30%, respectively. However, *L.esculentum* alleles increased internal red color for *inc1.1*, *inc7.1*, *inc8.1*, *inc12.1* and *inc12.2*. Monforte and Tanksley (2000) also identified the *inc4.1* QTL region for internal fruit color in their study. In addition, *inc7.1* and *inc8.1* QTLs were in similar regions as color QTL identified by Bernacchi et al. (1998). The external and internal color QTL on the top of chromosome 12 also co-localize with the *Delta* fruit color mutant of tomato. Moreover, as with lycopene, it was found that *L.hirsutum* alleles could increase the external and internal red color of fruit which again confirms the findings of Bernacchi et al. (1998) and Monforte and Tanksley (2000).

4.2.7. Average Fruit Weight

Three QTLs were identified for fruit weight and each QTL was located on different chromosomes. *fw7.1* was the most significant QTL region for fruit weight and it was marked by both At2g42750 and At3g14910 with *P* = 0.00001. *fw2.1*, located on chromosome 2, matched the location of *fw2.2,* a major fruit weight QTL that was cloned by Frary et al. (2000). The source of high fruit weight were cultivated tomato alleles as expected.

Table 4.6. QTL identified for antioxidant and for agronomic traits, their location in the tomato genome and any matches with previous studies. Table also shows the source of these QTL alleles and the effect of *L.hirsutum* alleles over the traits

Trait	**QTL symbol**	**Marker**	**Chr**	***P***	**Effect of LH allele (%)**	**Source**	**Previously identified loci[a]**
WAOX	*waox1.1*	At3g06050	chr1	0,0427	-7	LE	
	waox5.1	T564	chr5	0,0158	9	LH	
	waox6.1	CT206	chr6	0,0196	9	LH	1
	waox8.1	TG307	chr8	0,0036	9	LH	
	waox12.1	At2g06530	chr12	0,0002	12	LH	
	waox12.2	At4g16580	chr12	0,0046	9	LH	
VITC	*vitc1.1*	At4g00560	chr1	0,0505	8	LH	
	vitc2.1	SSR40	chr2	0,0067	-14	LE	
	vitc2.2	At4g37280	chr2	0,0047	-12	LE	2
	vitc6.1	CT206	chr6	0,0005	16	LH	
	vitc12.1	At2g06530	chr12	0,0199	9	LH	1,2
PHE	*phe1.1*	At2g15890	chr1	0,0362	8	LH	
	phe6.1	CT206	chr6	0,0144	11	LH	
	phe7.1	At1g55870	chr7	0,0174	17	LH	1
	phe9.1	At5g06130	chr9	0,0479	10	LH	1
	phe12.1	At2g06530	chr12	0,0177	9	LH	
FLAV	*flav2.1*	T266	chr2	0,0247	-26	LE	
	flav3.1	At1g61620	chr3	0,0487	-23	LE	
	flav5.1	At5g20180	chr5	0,0441	-9	LH	
	flav11.1	TG36	chr11	0,0166	24	LH	

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Table 4.6. (Cont.) QTL identified for antioxidant and for agronomic traits, their location in the tomato genome and any matches with previous studies. Table also shows the source of these QTL alleles and the effect of *L.hirsutum* alleles over the traits

Trait	**QTL symbol**	**Marker**	**Chr**	***P***	**Effect of LH allele (%)**	**Source**	**Previously identified loci[a]**
LYC	*lyc2.1*	At4g33985	chr2	0,042	21	LH	
	lyc3.1	At5g51110	chr3	0,0101	-26	LE	1
	lyc7.1	At2g32970	chr7	0,0077	-19	LE	
	lyc8.1	TG307	chr8	<0.0001	-30	LE	
	lyc9.1	At2g29210	chr9	0,0236	37	LH	
	lyc10.1	TG566	chr10	0,001	46	LH	3
	lyc11.1	At4g22260	chr11	0,0029	18	LH	
	lyc12.1	At2g06530	chr12	0,0001	-28	LE	1
INC	*inc1.1*	At5g13030	chr1	0,0093	-22	LE	
	inc4.1	At1g47830	chr4	0,0311	22	LH	4,5
	inc7.1	T671	chr7	0,0147	-19	LE	4
	inc8.1	TG307	chr8	0,0007	-27	LE	4
	inc9.1	At3g09925	chr9	0,0076	30	LH	
	inc12.1	TG180	chr12	0,0052	-22	LE	
	inc12.2	At2g06530	chr12	0,002	-24	LE	
EXC	*exc1.1*	At5g13030	chr1	0,0051	-23	LE	
	exc4.1	At3g16150	chr4	0,0362	20	LH	
	exc4.2	At1g47830	chr4	0,0017	31	LH	5
	exc7.1	At2g32970	chr7	0,0514	-13	LE	
	exc8.1	TG307	chr8	0,0055	-22	LE	
	exc9.1	At3g09925	chr9	0,0041	41	LH	
	exc9.2	At2g29210	chr9	0,0444	32	LH	
	exc12.1	TG180	chr12	0,0186	-18	LE	
	exc12.2	At2g06530	chr12	0,0057	-20	LE	

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Table 4.6. (Cont.) QTL identified for antioxidant and for agronomic traits, their location in the tomato genome and any matches with previous studies. Table also shows the source of these QTL alleles and the effect of *L.hirsutum* alleles over the traits.

Trait	**QTL symbol**	**Marker**	**Chr**	***P***	**Effect of LH allele (%)**	**Source**	**Previously identified loci[a]**
FW	fw2.1	At4g33985	chr2	0,0002	-30	LE	6
	fw3.1	At3g47640	chr3	0,0044	-23	LE	
	fw7.1	At2g42750	chr7	0,0001	-31	LE	
	fw7.1	At3g14910	chr7	0,0001	-29	LE	
FIRM	firm2.1	SSR40	chr2	0,0148	21	LH	
	firm2.2	T266	chr2	0,0155	21	LH	
	firm2.3	At4g37280	chr2	0,026	17	LH	
	firm3.1	At5g49970	chr3	0,0117	18	LH	
	firm4.1	At1g71810	chr4	0,0162	19	LH	
	firm5.1	CT138	chr5	0,0161	20	LH	4
	firm8.1	At5g41350	chr8	0,0422	15	LH	
FS	fs1.1	At4g00560	chr1	0,0439	14	LH	a
	fs2.1	SSR40	chr2	0,0017	31	LH	7
	fs3.1	At1g61620	chr3	0,0425	24	LH	
	fs7.1	At2g42750	chr7	0,0276	17	LH	4
SSC	ssc1.1	T1422	chr1	0,0201	-15	LE	
	ssc2.1	At4g33985	chr2	0.0001	-30	LE	
	ssc3.1	At3g47640	chr3	0,0003	-25	LE	
	ssc7.1	At2g42750	chr7	0,0006	-19	LE	
	ssc8.1	TG307	chr8	0,0331	-12	LE	
	ssc11.1	TG36	chr11	0,0424	13	LH	
	ssc12.1	TG180	chr12	0,0169	-13	LE	

(cont. on next page)

Table 4.6. (Cont.) QTL identified for antioxidant and for agronomic traits, their location in the tomato genome and any matches with previous studies. Table also shows the source of these QTL alleles and the effect of *L.hirsutum* alleles over the traits

Trait	**QTL symbol**	**Marker**	**Chr**	***P***	**Effect of LH allele (%)**	**Source**	**Previously identified loci[a]**
LN	ln2.1	At4g33985	chr2	0,0017	-15	LE	8
	ln3.1	At3g47640	chr3	0,0013	-14	LE	
	ln4.1	At1g71810	chr4	0,0285	-10	LE	
	ln7.1	At2g42750	chr7	0,006	-10	LE	
	ln10.1	At3g13235	chr10	0,0318	-7	LE	
	ln12.1	TG180	chr12	0,0123	-9	LE	
WALL	wall6.1	CT206	chr6	0,0438	-14	LE	
	wall8.1	TG307	chr8	0,0133	-14	LE	
	wall11.1	CT269	chr11	0,0147	-13	LE	
	wall12.1	At2g06530	chr12	0,0025	-16	LE	

[a] References are coded: 1=Rousseaux et al. (2005); 2=Stevens et al. (2007); 3=Tanksley et al. (1992); 4=Bernacchi et al. (1998); 5=Monforte et al. (2001); 6=Frary et al. (2000); 7=Liu et al. (2002); 8=Lippman and Tanksley (2001).

4.2.8. Fruit Firmness

For fruit firmness, there were seven QTLs identified. Three of them were located on the same chromosome (chromosome 2) while the rest were located on different chromosomes (chromosomes 3, 4, 5 and 8) (Figure 4.14). The most significant one was *firm3.1* and it was located on chromosome 3 (Table 4.6). *L.hirsutum* alleles were always associated with increased fruit firmness with effects as high as 21% for *firm2.1* and *firm2.2*. The *firm5.1* QTL region for fruit firmness was also identified by Bernacchi et al. (1998).

4.2.9. Fruit Shape

Four QTLs for fruit shape were detected in this study. All of them were located on different chromosomes. These are chromosomes 1 (*fs1.1*), 2 (*fs2.1*), 3 (*fs3.1*) and 7 (*fs7.1*) (Figure 4.14). SSR40 was associated with *fs2.1,* the most significant QTL with P = 0.002. The source of elongated fruit shape was *L.hirsutum* alleles. Liu et al. (2002) identified the *fs2.1* QTL region as *ovate*. In addition, Bernacchi et al. (1998) identified a fruit shape QTL similar to *fs7.1*.

4.2.10. Stem Scar

Seven QTLs on seven different chromosomes were associated with stem scar size (Table 4.6; Figure 4.14). The most significant QTL for stem scar size was *ssc2.1* with $P < 0.00001$. *L.hirsutum* alleles were associated with large stem scar in only one case, *ssc11.1*. For all other stem scar QTLs, the *L.hirsutum* alleles were responsible for formation of smaller stem scars. Of most interest was *ssc2.1* for which the wild allele decreased stem scar by 30%.

4.2.11. Locule Number

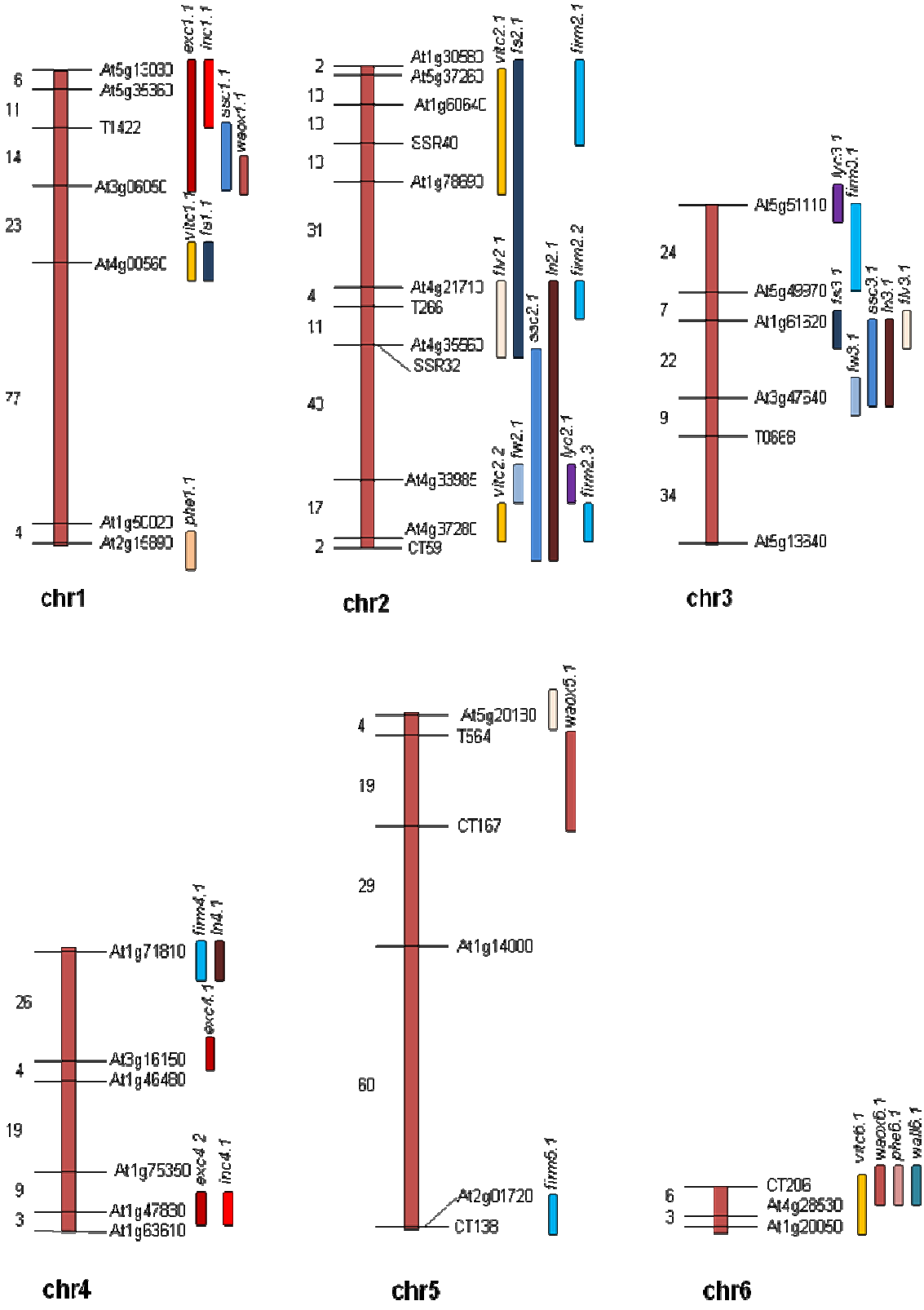
Locule number was associated with six QTLs, these were *ln2.1*, *ln3.1*, *ln4.1*, *ln7.1*, *ln10.1* and *ln12.1* (Figure 4.14). *L.esculentum* alleles were associated with higher locule number. The *ln2.1* QTL for locule number was also identified by Lippman and Tanksley (2001).

4.2.12. Wall

Four QTLs were associated with wall thickness and all of them were located on different chromosomes. These were *wall6.1*, *wall8.1*, *wall11.1* and *wall12.1* (Figure 4.14). For all of the QTLs, *L.esculentum* alleles enhanced the thickness of the pericarp. The most significant QTL for wall thickness was *wall12.1* with $P < 0.003$ (Table 4.6).

Among the 28 identified antioxidant QTLs, for 18 loci (64%) *L.hirsutum* alleles were associated with increased antioxidant trait values. This was not suprising because *L.hirsutum* had significantly higher values than *L.esculentum* for virtually all antioxidant traits, except vitamin C. On the other hand, for 10 QTLs (36%) wild alleles were responsible for reduction of antioxidant traits. The positive effects of *L.hirsutum* alleles over the antioxidant traits ranged from 8% to 46%. The *L.hirsutum* alleles for *lyc10.1* and *lyc9.1* showed the highest phenotypic effect on lycopene content. Because *L.hirsutum* has green fruit even in its ripe stage, it was unexpected to find the highest effect for lycopene content from this parent. However, some alleles located in the *L.hirsutum* genome could enhance the lycopene content of the elite line, this result is due to transgressive segregation of the lycopene alleles. On the other hand, the negative effects of *L.hirsutum* ranged from 7% (*waox1.1*) to 30% (*lyc8.1*).

Of the 47 identified agronomically important QTLs (fruit shape excluded), for 19 loci (44%) *L.hirsutum* alleles were responsible for enhancement of phenotypic values of traits and 24 wild alleles (56%) had negative effects on these traits. Thus, more than half of the QTLs wild alleles negatively impacted the elite lines for improvement of agronomic traits. This was expected, because *L. hirsutum* as a wild parent contained many undesired traits in terms of horticultural aspects such as low fruit weight and green fruit color. For example, the highest negative effect was observed in the *fw2.1* allele that came from *L.hirsutum* (with a 30% negative effect). The highest positive effect of *L.hirsutum* alleles was for *exc9.1* with a 41% increase in fruit color.



(cont. on next page)

Figure 4.14. Molecular map of the tomato genome obtained for the BC_2F_2 mapping population and locations of QTLs

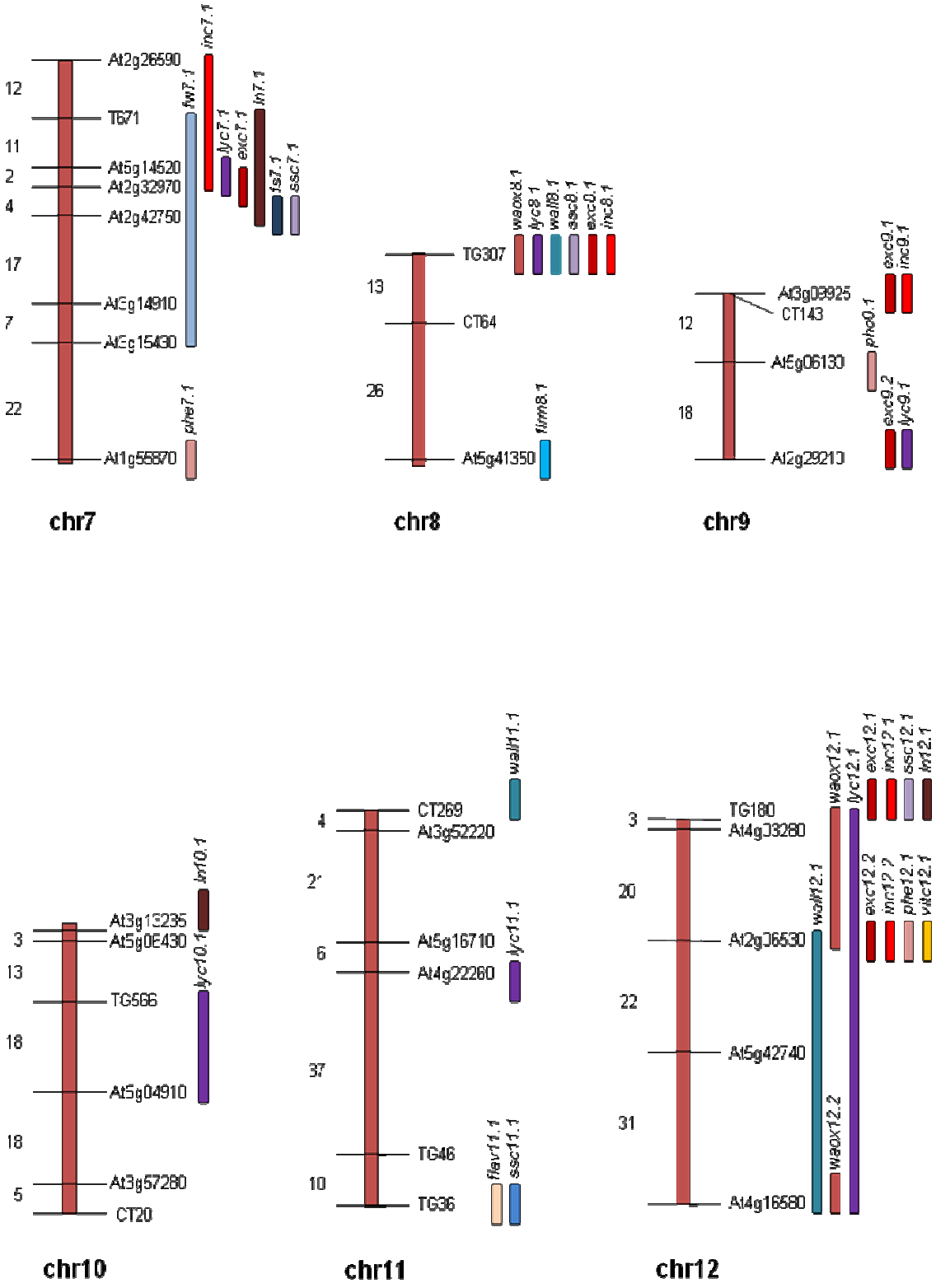


Figure 4.14. (Cont.) Molecular map of the tomato genome obtained for the BC_2F_2 mapping population and locations of QTLs

4.3. Colocalization of QTLs

A total of 75 QTLs were identified for both antioxidant and agronomically important traits on the tomato genome map. The number of QTLs per linkage group ranged from 2 (chromosome 10) to 12 (chromosome 12) (Figure 4.14). However, some of the QTLs were colocalized in the same genomic region. These QTL clusters make it possible to understand the correlation between traits that are controlled by these QTLs and also interaction between these genes. One of the most notable colocalizations was observed among internal, external color and lycopene content. All of the QTLs that were identified for internal fruit color always colocalized with external color (chromosome 1, 4, 7, 8, 9 and 12) and also *exc9.2* colocalized with *lyc9.1*. In addition, *exc7.1*, *inc7.1* and *lyc7.1*; *exc8.1*, *inc8.1* and *lyc8.1*; *exc12.1*, *inc12.1* and *lyc12.1*; and *exc12.2*, *inc12.2* and *lyc12.1* were located in same genomic regions. Because lycopene pigment concentration determines the red color of tomato fruit, most probably these three traits are controlled by pleiotropic genes. This also clarified why these traits are highly and positively correlated.

For antioxidant traits, *waox 6.1*, *vitc6.1* and *phe6.1* were located on the same chromosomal location on the sixth linkage group and *waox12.1*, *vitc12.1* and *phe12.1* were colocalized on chromosome 12. A positive correlation was also seen among these antioxidant traits. Vitamin C and phenolic compounds are water soluble antioxidants; therefore genes that enhance these traits would also be expected to increase total water soluble antioxidant capacity. Also colocalization of the vitamin C and phenolic loci could be explained in that they have similar pathways and complementary effect against reactive oxygen species (ROS).

For agronomic characterization, high significant correlations among locule number, fruit weight and stem scar size were observed. This is expected as fruit with more locules tend to be larger and have larger stem scars. Colocalization of these traits on the molecular marker linkage map (*ln2.1*, *fw2.1* and *ssc2.1*; *ln7.1* and *fw7.1*; *ln12.1* and *ssc12.1*) add support to this hypothesis. Thus these multiple QTLs may represent fewer loci with pleiotropic effects.

CHAPTER 5

CONCLUSION

Tomato is one of the most important vegetables and is widely produced and consumed all over the world including in Turkey. The main goal of this study was to identify genetic regions for health related and agronomically important traits by identification of QTLs for these traits. For this aim, 152 BC_2F_2 mapping individuals derived from a cross between *L.esculentum* and *L.hirsutum* were analysed for both phenotypic and genotypic characters. While antioxidant traits were measured using biochemical assays, agronomic traits were visually scored. For genotypic characterization, 70 CAPs and 2 SSR markers were tested on the mapping population for construction of the molecular linkage map.

In this study, *L.hirsutum* was used as a donor parent in order to increase both phenotypic and genotypic variation among the mapping population. *L.hirsutum* has many desired traits with regard to antioxidant capacity. This may be due to the fact that antioxidant compounds have crucial roles in plant defence systems and during natural selection, alleles that are responsible for production of high antioxidant compound may have accumulated in wild species. In contrast, *L.esculentum* has been artificially selected for agronomic traits and may have lost some of the favorable antioxidant alleles. As expected, most of the *L.hirsutum* alleles (approximately 61%) that were identified for antioxidant traits were responsible for improvement of these antioxidant traits. However, for agronomic traits such as fruit color, fruit weight, etc. *L.hirsutum* is expected to negatively influence quality of the elite line. A total of 56% of the identified *L.hirsutum* alleles negatively affected the agronomically important traits. However, in some cases *L.hirsutum* alleles were associated with increased value of some antioxidant and agronomically important traits even when the parental line was inferior for these traits such as lycopene content, internal and external fruit color. For example, *lyc10.1, inc9.1* and *exc9.1* alleles from *L.hirsutum* positively affected these traits by 46, 41 and 30%, respectively. This is because of transgressive segregation of alleles in the

population. Thus, formation of different combinations of alleles from the parents can lead to generation of progeny that can exceed both parental lines. As a result, the phenotype of the wild species does not always reflect its genetic potential. Thus, the use of molecular marker-based techniques can reveal the real potential of this exotic germplasm. By analysis of the genetic potential of wild species, may new and useful genes or alleles can be identified for improvement of existing cultivar types.

The presence of associations between molecular markers and genes of interest indicates the potential usefulness of Marker Assisted Selection (MAS) for improvement of these traits. If a marker is tightly linked with a desired trait, the possibility that the marker and locus will be transmitted together is very high due to low recombination frequency. Therefore, screening of the population with a marker linked to the desired trait makes it feasible to select individuals that have the desired trait or traits without phenotypic characterization. In addition, MAS can also be used for negative selection which means that undesired traits can be eliminated in the population. MAS also does not require completely mature plants, thereby selection can be done at seedling stage with a higher efficiency of selection. By doing this, requirements for time, space and labour are greatly reduced. In this study, for improvement of health related traits, marker TG566 linked with *lyc10.1* (46% allelic effect $P = 0.001$) and At2g06530 linked with *waox12.1* (12% allelic effect $P = 0.0002$), *vitc12.1* (9% effect $P = 0.02$) and *phe12.1* (9% effect $P = 0.02$) may be candidates for use in MAS. The region where *lyc10.1* was located was previously identified to contain the *nor* locus (Tanksley, et al. 1992). *vitc12.1* was also identified in a previous study (Rousseaux, et al. 2005, Stevens, et al. 2007). For agronomic traits, the most significant markers are At3g09925 linked with both *exc9.1* (41% allelic effect $P = 0.004$) and *inc9.1* (30% effect $P = 0.008$) and At1g47830 which was associated with both *exc4.2* and *inc4.1*. MAS also can be used for negative selection; for example At4g33985 is linked with a *fw2.1* QTL that negatively affected fruit weight (approximately 30% reduction in weight $P = 0.0002$). It was also identified and cloned by Frary et al. (2000). So, progeny that possess the *L.hirsutum* allele for this marker could be eliminated through MAS. MAS decreases the time needed for trait improvement approximately 3 or 4 years.

These identified QTLs can be cloned by using map based cloning techniques. After isolation of the sequences for the desired antioxidant or agronomic trait genes, these genes can be transferred into other crops with transgenic approaches. Also

indentification of gene sequence gives an opportunity to determine gene products and their roles in formation of phenotypic expression.

To increase antioxidant capacity of tomato will not only positively affect human health but it will also impact the plant health. Because antioxidant compounds have important roles in plant defence systems, production of high amounts of antioxidants makes plants more vigorous against both biotic and abiotic stress conditions. As a result, producers can obtain higher quality and better yielding crops.

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APPENDIX

RAW DATA FOR PHENOTYPIC CHARACTERIZATION

Table A-1. Raw data for antioxidant traits

Ped.#	AOX µmol Trolox/kg	Phenolic (mg/kg)	Flavonoid (mg/kg)	Vitamin C mg/kg	Lycopene mg/kg
7S0001	3925,1	303,1	83,3	160,7	3,9
7S0002	2574,9	207,3	54,6	165,1	89,4
7S0003	3801,2	255,2	172,2	237,6	83,4
7S0006	3601,4	215,9	69,9	177,4	153,9
7S0008	5092,8	454,6	126,8	314,9	76,7
7S0009	3512,3	226,6	168,9	194,8	64,4
7S0012	4402,3	333,8	58,8	230,7	81,1
7S0013	4209,9	273,1	76,8	254,3	107,0
7S0015	3557,6	196,6	55,1	182,9	71,8
7S0030	2746,3	204,4	76,4	171,3	21,0
7S0031	3672,6	253,8	151,3	268,1	17,7
7S0032	3332,0	240,2	215,2	177,4	83,1
7S0037	2804,9	275,9	88,4	178,7	91,7
7S0091	4586,1	307,4	77,3	228,9	129,1
7S0095	3429,6	252,3	72,2	173,3	95,8
7S0105	3264,7	169,4	75,9	169,4	43,6
7S0108	3695,0	284,5	137,4	224,9	137,5
7S0113	3513,9	220,2	60,6	190,8	84,9
7S0114	3713,2	266,6	54,6	243,0	101,4

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Table A-1. (Cont.) Raw data for antioxidant traits

Ped.#	AOX µmol Trolox/kg	Phenolic (mg/kg)	Flavonoid (mg/kg)	Vitamin C mg/kg	Lycopene mg/kg
7S0115	2894,1	228,7	82,4	174,8	51,2
7S0116	3482,9	221,6	57,4	167,6	36,8
7S0123	3741,5	194,4	55,5	238,8	49,0
7S0124	2614,2	290,9	61,5	165,9	72,8
7S0126	3918,2	225,2	58,3	109,8	33,5
7S0131	3670,7	233,0	84,7	163,4	36,7
7S0132	4438,7	271,6	103,2	180,1	48,1
7S0139	3684,1	269,5	59,7	319,1	69,4
7S0143	4300,3	273,8	57,8	207,5	65,5
7S0146	2926,8	180,8	62,9	171,8	60,0
7S0148	3604,1	222,3	63,4	312,9	45,0
7S0151	2734,1	273,8	75,9	322,8	61,6
7S0153	3565,6	318,8	158,7	163,4	83,8
7S0165	3437,3	343,8	72,2	176,3	66,0
7S0171	3907,7	220,2	57,4	222,1	95,8
7S0174	3139,5	187,3	66,6	125,3	62,7
7S0177	2996,2	254,5	88,4	243,4	64,4
7S0181	2918,0	188,0	74,0	167,6	41,0
7S0195	3348,2	175,1	81,0	158,1	64,8
7S0196	2510,6	218,7	172,2	113,0	53,5
7S0203	2439,5	182,3	83,3	174,8	97,7
7S0208	2988,7	166,6	62,9	235,0	94,5
7S0210	2066,6	225,9	58,3	124,2	105,6
7S0225	3529,6	181,6	70,3	196,3	70,0

(cont. on next page)

Table A-1. (Cont.) Raw data for antioxidant traits

Ped.#	AOX µmol Trolox/kg	Phenolic (mg/kg)	Flavonoid (mg/kg)	Vitamin C mg/kg	Lycopene mg/kg
7S0226	3941,3	265,9	193,4	261,9	96,3
7S0231	3189,8	281,6	104,6	233,5	48,0
7S0233	3442,8	308,8	66,6	246,6	89,7
7S0237	4007,4	249,5	58,8	177,8	127,4
7S0239	2612,8	186,6	77,3	118,4	117,9
7S0240	4043,3	235,9	227,7	245,3	42,3
7S0250	2997,1	220,2	84,7	218,7	58,0
7S0252	3066,6	185,1	59,7	171,8	30,8
7S0267	4272,2	328,8	74,5	237,0	87,7
7S0276	3652,5	304,5	99,5	266,6	40,1
7S0287	3618,2	207,3	62,9	155,1	83,6
7S0290	2941,2	205,9	80,5	224,9	34,5
7S0294	4483,8	199,2	56,5	235,8	92,6
7S0306	3355,8	245,9	74,0	130,2	44,1
7S0313	3297,7	181,6	70,3	188,7	72,9
7S0314	3288,5	304,5	75,9	201,6	105,0
7S0319	3405,1	201,6	56,5	184,6	29,6
7S0322	3319,6	311,7	119,4	190,7	56,3
7S0325	3673,2	253,8	176,8	145,2	28,6
7S0326	4012,1	245,9	72,7	245,7	28,8
7S0328	2705,8	213,7	149,0	82,6	69,1
7S0329	3819,5	371,0	79,1	217,0	43,7
7S0331	2919,0	170,1	77,3	168,1	76,1
7S0333	2669,5	217,3	61,5	273,8	54,2

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Table A-1. (Cont.) Raw data for antioxidant traits

Ped.#	AOX µmol Trolox/kg	Phenolic (mg/kg)	Flavonoid (mg/kg)	Vitamin C mg/kg	Lycopene mg/kg
7S0338	5048,7	273,8	139,3	252,1	64,6
7S0342	3729,6	317,4	217,0	145,0	31,1
7S0347	4111,8	230,9	59,2	216,1	52,5
7S0360	3341,7	258,0	79,1	206,4	94,5
7S0392	3638,6	254,5	68,0	246,6	102,3
7S0410	3724,9	291,6	62,0	222,6	48,1
7S0417	3116,4	261,6	69,9	204,3	68,2
7S0435	3013,9	211,6	215,7	161,7	38,9
7S0439	2651,5	243,0	69,0	167,0	86,0
7S0460	3694,5	323,1	78,2	138,9	66,8
7S0461	3262,6	204,4	81,4	199,9	53,2
7S0467	3247,6	318,8	88,4	255,1	67,4
7S0470	4146,5	318,8	64,3	238,5	43,2
7S0471	3780,3	326,0	62,9	227,9	73,1
7S0476	4174,0	226,6	59,2	240,7	31,4
7S0492	3614,8	312,4	61,1	216,9	53,1
7S0499	3775,8	277,3	96,7	204,8	117,7
7S0502	3931,7	226,6	115,7	253,6	78,4
7S0510	4318,4	376,7	98,6	195,4	48,2
7S0511	3325,7	176,6	90,2	132,4	43,5
7S0524	2615,1	217,3	68,0	146,2	139,8
7S0534	2576,4	180,1	87,5	189,6	70,3
7S0547	3544,4	199,4	67,6	119,8	171,8
7S0548	2796,6	169,4	64,8	190,7	111,7

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Table A-1. (Cont.) Raw data for antioxidant traits

Ped.#	AOX µmol Trolox/kg	Phenolic (mg/kg)	Flavonoid (mg/kg)	Vitamin C mg/kg	Lycopene mg/kg
7S0552	2583,2	161,5	58,8	199,0	60,1
7S0555	2733,7	203,7	74,5	148,6	78,6
7S0559	3019,3	229,5	162,9	192,8	66,5
7S0561	2825,0	256,6	59,2	190,8	105,5
7S0563	3697,6	278,8	80,5	196,9	119,1
7S0571	3245,4	202,3	83,8	215,6	56,9
7S0572	3715,8	322,4	93,5	190,3	78,9
7S0575	2242,4	185,9	68,5	190,0	82,4
7S0579	2974,4	259,5	85,6	204,6	58,3
7S0580	2887,5	228,7	74,0	163,4	118,0
7S0581	2160,1	212,3	46,7	161,7	84,8
7S0583	3295,7	222,3	69,0	161,3	57,6
7S0584	3532,8	220,2	54,6	196,9	46,1
7S0586	3110,1	193,7	69,9	209,3	75,1
7S0593	2960,1	269,5	61,1	240,0	68,6
7S0596	2764,0	168,0	241,1	155,6	117,9
7S0597	3264,2	336,0	59,7	102,8	83,9
7S0598	2876,1	230,2	249,9	174,5	31,4
7S0599	2557,5	209,4	166,6	215,1	55,9
7S0601	3127,5	206,6	67,6	218,7	136,2
7S0602	1618,1	168,0	77,3	118,6	76,6
7S0604	3161,7	296,6	190,7	224,0	137,7
7S0606	2288,2	183,0	59,7	192,7	115,4
7S0608	3339,1	140,8	59,7	159,2	83,2

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Table A-1. (Cont.) Raw data for antioxidant traits

Ped.#	AOX µmol Trolox/kg	Phenolic (mg/kg)	Flavonoid (mg/kg)	Vitamin C mg/kg	Lycopene mg/kg
7S0610	2209,2	206,6	67,6	198,6	79,6
7S0615	3464,1	260,9	69,9	174,5	50,5
7S0616	3959,0	309,5	78,7	251,1	61,8
7S0617	3614,9	193,7	56,0	237,1	58,5
7S0618	4319,6	248,8	215,7	249,1	71,0
7S0619	4509,0	255,9	62,9	200,5	33,6
7S0627	3942,5	208,7	140,7	183,2	47,7
7S0633	3902,7	265,2	112,5	213,7	57,1
7S0634	4257,4	290,9	79,6	324,2	21,2
7S0635	3309,5	248,8	95,3	125,9	126,2
7S0637	4024,8	240,2	64,3	230,4	19,6
7S0638	3356,3	295,2	78,2	186,9	26,7
7S0639	4755,2	240,9	70,8	259,2	28,8
7S0641	4048,7	308,1	57,4	219,8	22,1
7S0642	2402,2	278,8	66,6	181,2	131,0
7S0643	3283,8	172,3	73,6	150,8	58,8
7S0644	3299,1	280,9	59,7	247,2	27,4
7S0651	3281,5	209,4	64,8	207,3	46,6
7S0663	3416,6	298,8	82,8	243,0	40,6
7S0664	2991,3	213,0	77,7	192,7	54,7
7S0673	3973,5	233,7	74,0	226,7	51,6
7S0679	3063,3	209,4	128,2	200,6	38,0
7S0680	3181,1	230,2	218,4	178,3	85,4
7S0682	3439,6	269,5	60,2	198,8	96,5

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Table A-1. (Cont.) Raw data for antioxidant traits

Ped.#	AOX µmol Trolox/kg	Phenolic (mg/kg)	Flavonoid (mg/kg)	Vitamin C mg/kg	Lycopene mg/kg
7S0683	3705,4	189,4	54,6	224,0	53,5
7S0684	4126,0	288,8	69,9	247,8	46,4
7S0685	3279,0	215,9	161,0	206,8	61,0
7S0686	3359,9	194,4	57,4	165,9	83,6
7S0687	3154,3	196,6	142,5	181,2	30,8
7S0689	3147,8	299,5	89,3	221,6	87,1
7S0691	3538,7	183,7	74,0	158,1	89,9
7S0692	3054,1	168,7	70,3	174,6	54,7
7S0693	3341,6	213,0	78,7	258,5	46,4
7S0699	2502,2	198,0	92,6	194,8	41,5
7S0700	2791,7	208,7	77,3	155,0	49,1
7S0701	3343,6	311,7	87,9	265,5	84,3
7S0707	2521,0	215,9	72,7	168,7	80,4

Table A-2. Raw data for agronomic traits

Ped.#	Internal Color	External Color	Weight	Firmness	Fruit Shape	Stem scar	Locule Number	Wall
7S0001	1	1	6,5	2	1	1	3	1
7S0002	3	3	261,9	3,5	1	5	6	4,5
7S0003	3,5	4	123,5	4	1	3,5	6	2
7S0006	3,5	4	55,8	4,5	4,5	2	2	5
7S0008	1	1	103,4	2	1	4,5	4	1,5
7S0009	2	3	198,4	2,5	1	5	6	3,5
7S0012	-	-	-	-	-	-	-	-
7S0013	4	4	75,5	5	1	3	3	4,5
7S0015	3	3	125,3	3,5	1	4,5	4	2
7S0030	1	1	67	4,5	1,5	4,5	4	1,5
7S0031	1	1	79,7	2	1	1,5	4	1,5
7S0032	3	3,5	65	3	1	4	4	2,5
7S0037	3	2,5	112,7	2,5	1,5	5	5	3
7S0091	2	2,5	86,2	4,5	1,5	3,5	3	4
7S0095	4,5	5	70,9	5	2	2	3	4
7S0105	1,5	1,5	149,6	3	1	4	5	3,5
7S0108	2	2,5	57,4	4	1,5	2,5	3	1,5
7S0113	2	3	76,8	4	1,5	4	5	2,5
7S0114	4	4,5	78	4	2,5	3	4	4
7S0115	1	1	143,6	3,5	1,5	4	5	2,5
7S0116	1,5	1,5	125,3	4	1	4,5	5	2
7S0123	1,5	1,5	111,8	4	1	3,5	4	2,5
7S0124	3	3	143	4	1	5	5	4,5
7S0126	1	1	84,5	4	1,5	4,5	5	3,5

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Table A-2. (Cont.) Raw data for agronomic traits

Ped.#	Internal Color	External Color	Weight	Firmness	Fruit Shape	Stem scar	Locule Number	Wall
7S0131	1	1	121,6	4	1	4	5	2,5
7S0132	2	2	126,4	2	1,5	3,5	5	1,5
7S0139	1	2	128	5	1,5	4	4	2,5
7S0143	1	1	136,7	2	1	5	5	3,5
7S0146	2,5	2,5	126,3	3,5	1	4,5	5	2,5
7S0148	1,5	2	136,4	2	1	5	5	3
7S0151	3	3	71	4	1,5	2,5	4	1,5
7S0153	1,5	2	96,8	2	1,5	3,5	4	3,5
7S0165	1,5	1,5	85,3	3,5	1	4	4	3,5
7S0171	1,5	2	71,5	2	1	2,5	3	3
7S0174	1,5	1,5	73,5	5	1	3	4	2,5
7S0177	2	1,5	36,9	2,5	2	2	3	2
7S0181	1,5	1,5	138	2	1	5	6	4
7S0195	4,5	4	102,7	5	1	5	5	3
7S0196	1	1,5	99,2	3	1	4	6	1,5
7S0203	1,5	2,5	87	2,5	1	4	5	3
7S0208	1,5	3,5	101,2	1,5	1,5	5	4	3
7S0210	1,5	2	133,9	3	1	5	5	2
7S0225	1,5	1,5	118	3	1	3	5	2,5
7S0226	2	2	97,4	1,5	1	5	4	2,5
7S0231	1,5	1,5	106,5	4	1,5	4,5	4	1,5
7S0233	2	2	87,1	2,5	2,5	3,5	3	3,5
7S0237	4	4	40,3	4,5	2	1	3	4
7S0239	3	4	88,2	3,5	1	3,5	5	3
7S0240	2,5	2	96,5	3,5	2,5	4,5	4	3,5

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Table A-2. (Cont.) Raw data for agronomic traits

Ped.#	Internal Color	External Color	Weight	Firmness	Fruit Shape	Stem scar	Locule Number	Wall
7S0250	1	1	59,7	1,5	1	1,5	3	1,5
7S0252	1	1	73,2	3	1	5	4	1,5
7S0267	3,5	4,5	89,7	4	1	4	4	3,5
7S0276	1,5	2,5	65,6	3	1	2	4	2,5
7S0287	1,5	2	100,4	2	1,5	3,5	5	1
7S0290	1	1,5	107,3	3,5	1,5	3,5	4	2
7S0294	4,5	4,5	154,8	2	1	5	5	3,5
7S0306	1,5	2	93,5	2	1	5	5	2
7S0313	1,5	1,5	108,9	1	1	5	6	2
7S0314	3	5	52,5	3	1,5	1,5	3	4
7S0319	1,5	1,5	72	2	1,5	1,5	4	2,5
7S0322	1,5	2	99,5	3	1,5	5	5	3,5
7S0325	1	1	37,1	4,5	1	1,5	4	1
7S0326	1	1	43	3,5	1	1	3	1
7S0328	1,5	2,5	95,5	2,5	2	4	4	3,5
7S0331	2	2	61,2	1,5	1,5	1,5	4	2
7S0333	1	1,5	84,5	4,5	1,5	4,5	4	3,5
7S0338	2	2,5	70,7	1,5	2	3	4	3,5
7S0342	1	1	65	2	1	2	3	3
7S0347	1	1	145,2	2	1	5	6	1,5
7S0360	3	4	89,5	4	1,5	5	4	3,5
7S0392	2	3,5	80	2	1	4	4	2,5
7S0410	1	1,5	46,9	1,5	1	2	4	1,5
7S0417	1,5	1,5	125,9	1,5	1	5	4	2,5
7S0435	2	2,5	119,3	3	1,5	3	5	1,5

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Table A-2. (Cont.) Raw data for agronomic traits

Ped.#	Internal Color	External Color	Weight	Firmness	Fruit Shape	Stem scar	Locule Number	Wall
7S0439	4	4	130,2	1,5	1	5	5	3
7S0460	1	1	78,3	2	1,5	3	4	2,5
7S0461	1,5	2	65	2	1,5	1	3	2,5
7S0467	4,5	4	127,2	3	1	4,5	5	2,5
7S0470	1,5	3	98,2	4	1	4	4	3
7S0471	1,5	3	97,8	4,5	1	2	5	3,5
7S0476	1	1	106,9	3,5	1	4,5	5	2,5
7S0492	3,5	3,5	138,6	3	1	4,5	4	3,5
7S0499	3,5	3,5	76	2	1,5	3	4	2
7S0502	3	3	126,4	3	2	4,5	4	3
7S0510	1,5	1,5	78,9	2	2	2	4	2,5
7S0511	1	1	128,7	5	1	3,5	5	3,5
7S0524	4	4,5	21,7	2,5	1	5	6	5
7S0534	3	3	199,9	3,5	1	5	6	4,5
7S0547	4	4	111,5	4	1,5	5	4	4,5
7S0548	2	2	198	3,5	1	5	5	4,5
7S0552	2	2,5	217	3	1	5	5	4,5
7S0555	2,5	3	52,8	5	4	2	3	4,5
7S0559	1,5	3	63,2	2	1	3	5	1
7S0561	2	2	104,1	2,5	1	4,5	5	1,5
7S0563	1,5	1,5	78,3	2,5	1,5	3	3	3,5
7S0571	2,5	2,5	111,2	4,5	1,5	5	5	2,5
7S0572	1	1,5	58,7	1,5	2	2	3	1
7S0575	2	2,5	63,2	4,5	1	1	4	2,5
7S0579	4	4,5	48	3	2,5	1	4	2,5

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Table A-2. (Cont.) Raw data for agronomic traits

Ped.#	Internal Color	External Color	Weight	Firmness	Fruit Shape	Stem scar	Locule Number	Wall
7S0580	2,5	2,5	70,9	3	1	3,5	5	2,5
7S0581	2	3	89,4	4,5	1,5	3	3	4
7S0583	1,5	3	69,4	4,5	1,5	4	4	3
7S0584	1	1	83,2	2,5	1	2,5	4	1,5
7S0586	1,5	1,5	109,3	3,5	1	4,5	5	2,5
7S0593	1,5	2	110,5	3,5	2,5	2	2	2
7S0596	1,5	1,5	123,5	5	1	5	5	3,5
7S0597	1,5	2	65,7	4	1	4	4	2,5
7S0598	2	2	140	4	1	5	5	2,5
7S0599	1,5	2	111,2	4,5	1	5	5	1,5
7S0601	2	2,5	191,1	1,5	1	5	5	3,5
7S0602	2	2,5	201,8	2	1	5	5	2
7S0604	2,5	2,5	72,4	2	1	5	5	2,5
7S0606	3	2	42,8	3	2	2	3	3
7S0608	1,5	2	83,7	3	1,5	3	5	2,5
7S0610	2	2,5	88,5	5	1	3,5	3	3,5
7S0615	2,5	3,5	52,6	3,5	2	2,5	3	2,5
7S0616	1,5	1	69,7	5	1,5	2,5	4	4,5
7S0617	1,5	2	67	4	1	2,5	5	3,5
7S0618	1	1	75,9	5	1	4	6	2
7S0619	1	1	90,9	4,5	1,5	4,5	5	2,5
7S0627	4,5	4	73	5	1,5	3,5	4	2,5
7S0633	3	4	49,3	1,5	1,5	5	4	3,5
7S0634	1	1	53,7	4,5	1,5	2	3	2
7S0635	2	3	94	3	1	4,5	5	2

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Table A-2. (Cont.) Raw data for agronomic traits

Ped.#	Internal Color	External Color	Weight	Firmness	Fruit Shape	Stem scar	Locule Number	Wall
7S0637	1	1	109,2	2	1	4	5	1,5
7S0638	1	1	99,4	2	1	4	4	2
7S0639	1	1	52,9	4,5	1,5	3	4	2
7S0641	1	1	95	4,5	1	3,5	3	2
7S0642	3,5	3	149,5	2	1	5	5	3
7S0643	2	1,5	77,9	5	1	2	3	4
7S0644	1	1	118,2	4	1	1	4	2
7S0651	1	1	148	1,5	1	5	4	4,5
7S0663	1	1	96,3	2,5	1,5	3,5	4	1,5
7S0664	1	1	98	2,5	1,5	5	4	3,5
7S0673	1,5	1,5	128,3	2	1	4,5	5	2,5
7S0679	2	1,5	94,3	2,5	1	3,5	5	3
7S0680	2	2,5	73,9	3,5	1	3,5	4	3,5
7S0682	4,5	3,5	96,7	2	1	4,5	5	4
7S0683	2	3	104,8	4,5	1	5	5	2
7S0684	1	1,5	90	1,5	1	4,5	6	2,5
7S0685	1	1	85,8	4	1,5	3,5	4	2,5
7S0686	2	2,5	127,3	2,5	1	4,5	4	4
7S0687	1,5	1,5	56,5	3,5	1,5	4,5	4	4
7S0689	2,5	2,5	171,9	4	1	4,5	6	4
7S0691	2,5	2,5	71	2,5	1	4,5	6	2,5
7S0692	1,5	2	41,9	2,5	2,5	3	3	4
7S0693	1,5	1,5	72,2	4	1	4	6	2,5
7S0699	1,5	1,5	56,9	2	1	2	5	2,5
7S0700	1,5	1,5	82,5	5	1	5	5	2,5

(cont. on next page)

Table A-2. (Cont.) Raw data for agronomic traits

Ped.#	Internal Color	External Color	Weight	Firmness	Fruit Shape	Stem scar	Locule Number	Wall
7S0701	1,5	2	46,5	3,5	1	2,5	4	2,5
7S0707	1,5	1,5	51,5	2	1,5	4	3	3,5