CHARACTERIZATION AND GENETIC MAPPING OF HEALTH RELATED TRAITS IN TOMATO

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ABSTRACT

Tomato is consumed in the daily diet and has a diverse nutritional content including antioxidants, lycopene, vitamin C and phenolics. However, the content of these compounds in tomato cultivars could be higher. There are two ways of increasing the nutritional quality of tomato. One of them is a transgenic way which is still questioned and is not approved in many countries. The other way is molecular breeding which is based on introgression of wild genes into cultivated tomatoes via the combination of classical breeding with molecular marker analysis. This method lets us obtain nontransgenic tomatoes with increased nutritional quality. In this project a population that was obtained from a cross between wild type *L.pimpinellifolium* and cultivated tomato *L.esculentum* was used. This population includes 145 inbred backcross lines (IBLs). It was expected that some individuals of this population had increased nutritional quality derived from the wild parent. In order to test this hypothesis, antioxidant activity of each line was measured. The method used to measure antioxidant activity was based on spectrophotometric measurement which gave μmol Trolox equivalent antioxidant activity. It was observed that the IBLs were phenotypically close to *L.esculentum* and that some individuals did have higher antioxidant activity than the cultivated parent. The population was also mapped with molecular markers and 31 possible QTLs which control antioxidants in tomato were found. The source of alleles associated with increased antioxidant characters was usually *L.pimpinellifolium*. This result confirmed that wild type tomatoes can be a resource for increasing nutritional quality of tomatoes.

ÖZET

Temel sebze olarak domates günlük diyetimizde tüketilmektedir. Domatesin besin içeriği antioksidantlar, likopen, C vitamini ve fenolik bileşikler bakımından çeşitlilik göstermektedir. Bu nedenle, domatesin besin kalitesini yükseltmek moleküler biyologlar tarafından ilgi çekici bir konu haline gelmiştir. Domatesin besin kalitesini yükseltmenin iki yolu vardır. Bunlardan biri transgenik yoldur ki hala sorgulanmakta ve birçok ülke tarafından onaylanmamaktadır. Diğer bir yol ise yabani tip domates genlerinden kültür domatesine gen aktarımını baz alan ve bunu klasik ıslah ve moleküler işaretleyici analizi yoluyla yapan moleküler ıslahtır. Bu ikinci metod bize besin kalitesi yüksek ve transgenik olmayan domates sağlamaktadır. Bu projede kullanılan populasyon yabani bir tür olan *L. pimpinellifolium* ile kültür domatesi *L.* esculentum'un melezlenmesi sonucu elde edilmiştir. Populasyonda 145 adet geri çaprazlanmış kendilenmiş hat (Inbred Backcross Line) bulunmaktadır. Populasyondaki bazı bitkilerin yabani türden kaynaklı yüksek besin kalitesine sahip olması beklenmektedir. Bu hipotezi test etmek için her bir hattın antioksidant aktivitesi hidrofilik ve lipofilik fraksiyonlarda ölçülmüştür. Antioksidant aktiviteyi ölçmek için kullanılan metod spektrofotometrik ölçüme dayalıdır ve μmol Trolox eşdeğeri antioksidant aktiviteyi vermiştir. Ölçümler sonucu bu domates hatlarının fenotipik olarak *L. esculentum* anaca daha yakın oldukları ve bazı hatların bu ebevenyden daha yüksek antioksidant aktiviteye sahip olduğu gözlenmiştir. Populasyon ayrıca moleküler işaretleyiciler ile haritalanmış ve domateste antioksidantları kontrol eden lokuslar (QTL) belirlenmiştir. Yüksek antioksidant karakteri ile ilişkili allellerin genelde *L. pimpinellifolium* anaca ait olduğu saptanmıştır. Bu sonuçlar, yabani tür domateslerin, domatesin besin kalitesini yükseltmek için yüksek besin içeriği kaynağı olabileceğini doğrulamıştır.

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CHAPTER 1

INTRODUCTION

1.1. Free Radicals

Free radicals are atoms or molecules that contain one or more unpaired electrons. Energetically, electrons have the tendency to occur in pairs. Sometimes one of the pairs can be lost or a single electron can be gained by a nonradical. Free radicals are neutral, structurally unstable and bonding deficient. They tend to react with other components in order to restore normal bonding. This is why free radicals are highly reactive.

Free radicals include reactive oxygen species (ROS). ROS is a term which covers all highly reactive, oxygen-containing molecules. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, the nitric oxide radical, the hypochlorite radical and various lipid peroxides. ROS are generated by a number of pathways. (Percival et al. 1998).

One pathway is through normal aerobic metabolism (mitochondrial electron transport system). Because oxygen has a bi-radical nature, it is susceptible to conversion to reactive forms such as superoxide (O_2•), hydrogen peroxide (H_2O_2), hydroxyl (OH), peroxyl (ROO), alkoxy (RO) and nitric oxide. These conversions are a series of reduction reactions and continue until oxygen is reduced to water. In the electron transport system (ETS) four electrons are transferred and molecular oxygen is reduced to water. During this process, radicals are generated. Most of the superoxide radicals are generated in the ETS of mitochondria. Thus production of ROS is observed during normal aerobic metabolism and increases under pathological conditions.

Another pathway that generates ROS is the cell-mediated immuno defensive process, i.e., oxidative burst from phagocytes. Phagocytosis involves polymorphonuclear leukocytes (e.g. neutrophils, eosinophils, basophils) monocytes and lymphocytes that kill bacterial pathogens via production of superoxide and hydroxyl radicals and hydrogen peroxide (Madhavi et al. 1996).

The xenobiotic metabolism of drugs and chemicals also produces ROS. Xenobiotics are synthetic chemicals that affect living systems. Some xenobiotics are themselves free radicals. Others are not themselves free radicals but cause free radical

formation. For example carbon tetrachloride is a xenobiotic which causes hepatoxicity in humans. Carbon tetrachloride is a colorless gas. When air, drinking water or soil is contaminated with it, carbon tetrachloride can damage the liver, kidneys and nervous system. This xenobiotic was formerly used in the production of refrigeration fluid, as a pesticide and in fire extinguishers. Because of its harmful effects, it is banned except in some industrial applications. (WEB_1). Smoking, alcohol abuse and air pollution are involved in the toxicity of xenobiotics as well.

ROS are also generated by exposure to ionizing radiation. UV rays can excite an electron of a molecule and transfer that electron to oxygen and sometimes excite oxygen to produce singlet oxygen. Oxygen radicals are involved in many biological processes such as enzymatic reactions in the mitochondria, detoxifying reactions of the cytochrome P450 system and cytopathological reactions triggered by exposure to ionizing radiation. (Vichnevetskaia and Roy 1999).

Enzymatic and nonenzymatic catalysis of bond homolysis also produces ROS. Lipid peroxidation reactions belong to this pathway of ROS production.

Oxygen dependent organisms are always at risk from free radicals, because oxygen and its derivatives contribute to free radical formation. Once an oxygen molecule is reduced with an electron and a superoxide radical is formed ($O_2^{\bullet -}$), the chain reaction continues until the molecular oxygen is reduced to water. During these reactions, many free radicals are formed as shown below.

$$O_2 \xrightarrow{e^- \text{ reduction}} O_2^{\bullet -} \quad \text{(superoxide radical)}$$

$$O_2 \xrightarrow{2e^- \text{ reduction}} O_2^{2-} \quad \text{(peroxide anion)}$$

$$O_2^{2-} + 2H^+ \longrightarrow H_2O_2 \quad \text{(protonated form of peroxide)}$$

$$O_2 + 4H^+ \longrightarrow 2\,H_2O \quad \text{(protonated form of } O_2^{\bullet -}\text{)}$$

Among these metabolites $O_2^{\bullet -}$ and H_2O_2 may be catalyzed by iron and copper as follows.

$$O_2^{\bullet -} + Fe^{3+} \longrightarrow O_2 + Fe^{2+}$$

$$H_2O_2 + Fe^{2+} \longrightarrow OH^- + HO^{\bullet} + Fe^{3+}$$

A hydroxyl radical ($HO^{\bullet}$) is formed at the end of these reactions. The hydroxyl radical is known as the most reactive radical in living cells. Hydroxide ions in water

(OH -) can be ionized, losing an electron, to give •OH (hydroxyl free radicals). Hydroxyl radicals can react with alcohols and phospholipids and the aromatic rings of purine and pyrimidine bases in DNA and RNA. Moreover this radical can transfer an electron to organic and inorganic compounds. In addition $^1O_2$ (singlet oxygen) is a very reactive oxygen form. Superoxide anion radicals ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) are less reactive than HO• and $^1O_2$. However superoxide is very common in cells with as much as 2% of the oxygen consumed by mitochondrial respiration converted to this ROS. Superoxide itself does not directly cause damage but leads to the production of reactive oxygen species which are much more reactive. The superoxide radical cannot diffuse across lipid membranes because it has a negative charge and charged species generally have much lower solubility in lipids than uncharged molecules. Unfortunately when two superoxide radicals react with two protons, hydrogen peroxide, which is more dangerous than superoxide, is produced.

NO is another type of free radical and may react with a superoxide radical resulting in highly toxic peroxynitrite (ONOO):

$$O_2^{\bullet -} + NO\bullet \leftrightarrow {}^{-}OONO \text{ (peroxynitrite)}$$

If peroxynitrite is formed at physiological pH, it turns to peroxynitrous acid (HOONO)

$$HOONO \longrightarrow H^{+} + NO_3^{-}$$

or

$$HOONO \leftrightarrow HO\bullet + \bullet ONO \text{ (nitrogen dioxide)}$$

Beside the production of HO•, peroxynitrous acid is a powerful oxidant. It can initiate lipid peroxidation.

The reaction between a lipid molecule and a molecule of oxygen is called autoxidation. Autoxidation, as a free radical chain reaction, can be investigated in three steps: initiation, propagation and termination.

Initiation of autoxidation occurs when oxygen comes in contact with an unsaturated lipid. Formation of a lipid radical (L•) from a lipid is the first step of the chain reaction (Vichnevetskaia and Roy 1999). Once free radicals are formed at the initiation step, they are converted into other radicals. This process is called propagation. When a lipid radical is oxidated, this reaction yields new free radical species such as peroxy radicals (ROO·) (reaction1). Lipid peroxy radicals may react with other molecules and form lipid hydroperoxides plus lipid free radicals (reaction2).

$$R\cdot + {}^3O_2 \longrightarrow ROO\cdot \quad (1)$$

$$ROO\cdot + RH \longrightarrow ROOH + R\cdot \quad (2)$$

Reaction 1 is a very fast reaction and requires almost no activation energy. If reaction 2 occurs repeatedly, hydroperoxides accumulate. The more unsaturated the lipid or fatty acid is, the more accumulation will occur. This accumulation of hydroperoxides causes further propagation. The action of lipoxygenase can also form lipid hydroperoxides. Thus lipid hydroperoxides are the primary products of autoxidation.

Termination of autoxidation occurs by dimerization of two radicals types. For example:

$$R\cdot + R\cdot \text{ ------> } R\text{-}R$$

$$R\cdot + ROO\cdot \text{ -----> } ROOR$$

$$ROO\cdot + ROO\cdot \text{ --------> } ROOR + O_2$$

This dimerization reaction has a very low energy of activation. Termination is controlled by radical concentration and by stereochemistry, which causes radicals to find each other in the correct orientation.

Unsaturated fatty acid content is relatively higher in biomembranes than in other parts of the cell. Thus the autoxidation rate is high in biomembranes. Free radical production by the plasma membrane is derived from arachidonic acid metabolism. Arachidonic acid is stored within the cell membrane and esterified to glycerol in phospholipids. This esterification reaction is a receptor dependent reaction which initiates phospholipids hydrolysis and releases the fatty acid into the intracellular medium. The enzymatic oxidation of arachidonic acid results in carbon and oxygen-centered free radicals which are capable of reacting with other cell components.

In living systems, lipid peroxidation is a very complicated process. Free radicals, oxygen, metal ions and a number of enzymes are involved in autoxidation. For example in microsomes from liver, microsomal NADPH-cytocrome P450 reductase and iron are responsible for lipid peroxidation. Enzymes which are responsible for lipid peroxidation are present mostly in biomembranes. These enzymes induce the oxidative stress which leads to the formation of ROS. Phospholipids of the cellular membrane play an important role in lipid peroxidation. Phospholipids are composed of polyunsaturated fatty acids and they are defined by Vladimirov et al. (1981) as the most unstable molecules of the organic world. The presence of both unsaturated fatty acids and active catalysts of oxidation (hemoproteins and non-heme iron and copper and

manganese complexes) in membranes, promote lipid peroxidation in cellular membranes. Peroxidation can have many effects on the membrane. For example fluidity and permeability are altered, enzymes may be inactivated and cell division may be negatively affected. However cell membranes can exist under such conditions because during evolution, cells have developed protection mechanisms against oxidation.

Oxidative stress is an imbalance between protective oxidants and damaging free radicals (Vichnevetskaia and Roy 1999). When there are relatively more free radicals than protective oxidants, free radicals in membranes destroy membrane structure and cause the loss of function of cell organelles. Receptors of the membrane are also inactivated. These effects can be considered as the primary effects of lipid peroxidation. Secondary effects of lipid peroxidation are initiation of the free radical reactions and diffusion within the cell or transport into the bloodstream. After diffusion within the cell, free radicals bind covalently to nucleic acids. Nucleic acids, especially the amino groups of DNA, are cross linked to the lipid peroxidation products. As a result of such reactions mutation or cancer may occur in organisms (Vichnevetskaia and Roy 1999).

In the cells, biomembranes are not the only source of reactive oxygen species. Soluble components of the cytosol such as thiols, hydroquinons, catecholamins, and flavins are able to undergo oxidation–reduction reactions. As a result of these reactions, intracellular free radicals, especially superoxide radicals, are produced. In addition to soluble components, many enzymes (various oxidases and reductases) generate free radicals during their catalytic activity. Endoplasmic reticulum and nuclear membranes have special cytochromes which can oxidize unsaturated fatty acids and xenobiotics, and reduce oxygen, so that they form superoxide radical or H_2O_2.

Consequently, free radicals are present in healthy tissues because they are generated as a result of normal metabolism; however, production of ROS increases under pathological conditions. ROS are able to attack lipids, proteins/enzymes, carbohydrates, and DNA in cells and tissues, causing membrane damage, protein modification, and DNA damage and cell death. To overcome such attacks of free radicals, cells have special protective compounds which are called antioxidants.

1.2. Antioxidants

Antioxidants are an organism's most important compounds of defense against free radicals and are critical for maintaining health and well being. They can be

classified according to many criteria. The first criterion for classification is solvent medium which divides antioxidants into two classes; lipophilic and hydrophilic. For example vitamin C is a hydrophilic antioxidant. A second criterion for classification is their origin. Antioxidants can be classified as exogenous or endogenous antioxidants. Exogenous ones come into the organism from outside. Endogenous ones are synthesized in the organism and transported by systems to the sites where they will be used. A third criterion is the mode of action of the antioxidant: chain breaking and preventive. Chain breaking antioxidants prevent the oxidation of lipids by scavenging peroxy radicals. On the other hand, preventive antioxidants diminish the formation of lipid radicals. Last of all antioxidants can be divided into two groups, natural and synthetic antioxidants. In this report, only natural antioxidants will be discussed. Natural antioxidants occur in and are extracted from plant and animal tissues (Vichnevetskaia and Roy 1999). Some naturally occurring antioxidants include various vitamins, carotenoids, amino acids and phytonutrients (Madhavi et al. 1996).

The most important vitamin with antioxidant activity is vitamin C, ascorbic acid. Vitamin C is synthesized from glucose in the liver of most mammalian species except humans, other primates and guinea pigs (Padayatty et al. 2003). These species lack a special enzyme that is essential for the vitamin C synthesis pathway. That is why humans must consume vitamin C to survive. Vitamin C is an antioxidant because it is an electron donor. In fact, vitamin C is the most important water soluble antioxidant. It can neutralize reactive oxygen species in aqueous phase before lipid peroxidation is initiated (Percival 1998). When vitamin C donates electrons, it becomes a free radical. Fortunately the ascorbyl radical is relatively stable and is fairly unreactive. This feature explains why ascorbate may be a preferred antioxidant. Reduction of reactive free radicals by the formation of a less reactive compound is called free radical scavenging or quenching. Once an ascorbyl radical is formed, it can be reduced back to ascorbic acid. In humans the ascorbyl radical is partially reduced but not recovered. If the ascorbyl radical is not reduced back, it is metabolized into oxalate. Oxalate may result in kidney stones in some people. Ascorbate can also prevent protein or amino acid oxidation and radical propagation. Proteins are one of the building blocks of all organisms. In addition to this main function, proteins undergo oxidation by several mechanisms (Padayatty et al. 2003). A peptide chain can be cleaved by oxidants, or specific amino acids can be oxidized (e.g. methionine and cysteine). Ascorbate can prevent this from occurring.

Another vitamin which has antioxidant activity is vitamin E (α tocopherol) (Singh et al. 2004). It is the major lipid-soluble antioxidant. In addition, it is known as the most efficient chain breaking antioxidant. It protects fatty acids of the cell membrane from lipid peroxidation. Whole grains and high quality vegetable oils are major sources of vitamin E. α tocopherol interacts with low density lipoproteins (LDL) (Padayatty et al. 2003). At the end of this reaction the tocopheroxyl radical is produced. This radical can be reduced by ascorbate back to α tocopherol.

In phototrophs, carotenoids are accessory pigments in addition to chlorophyll. Carotenoids with conjugated double bonds quench reactive oxygen species and act as antioxidants at low oxygen pressure (Simonne et al. 1997). They are in the class of lipophilic antioxidants. Carotenoids have a protective role in lipid-rich tissues. They protect tissues against free radical damage and peroxidation. The most well known carotenoid is β-carotene which is a precursor of vitamin A. β-carotene not only turns into vitamin A in the body but is also itself a quencher of singlet oxygen. So it is essential for proper functioning for the immune system. β-carotene works synergistically with vitamin E. Lycopene is another important dietary carotenoid with an acyclic structure and 11 conjugated double bonds all in the trans configuration (Yaping et al. 2002). It is found in fruits such as fresh ripe tomato, watermelon and grapefruit. It is also the primary carotenoid component found in human tissues and fluids in cis isomers after absorption. Lycopene has the ability to scavenge free radicals, thus protecting cell components against oxidative damage. It has been reported that lycopene can quench singlet oxygen and scavenge free radicals of nitrogen dioxide (Yaping et al. 2002). The location of lycopene in the fruit changes depending on fruit type. For example lycopene is mostly located in the outer pericarp and skin of tomato (Dewanto et al. 2002). Thus lycopene is the major membrane bound antioxidant which is a highly effective singlet oxygen quencher (Andrews et al. 2004).

Glutathione is a water soluble antioxidant and is synthesized from the amino acids cysteine, glycine and glutamate (Percival 1998). Glutathione plays an important role in xenobiotic metabolism. Moreover it can quench reactive oxygen species. It is reported that glutathione works interactively with vitamin C to quench free radicals.

In addition to vitamins and amino acids, plants contain phytonutrients or phytochemicals which may have antioxidant properties. It has been proposed that these phytochemicals are the major contributors to the antioxidant capacity of fruits. For example, phenolic compounds are important natural antioxidants (Chu et al. 2002). The

hydroxyl groups of these compounds contain mobile hydrogen atoms which react easily with peroxide radicals and break oxidation chain reactions.(Ermilova et al. 2000) Phenolics can be classified as free (soluble) phenolics and bound phenolics. When bound phenolics are extracted with ethyl acetate two fractions are obtained, a water soluble and an ethanol soluble fraction. Among phenolics, flavonoids are the most studied. They are found in glucosylated forms in leaves, flowers, fruits and seeds of plants. They occur in plants as metabolic intermediates and may accumulate in the vacuoles (Chu et al. 2002). In plants, flavonoids serve as protectors against environmental stress. In humans, flavonoids are "biological response modifiers" which means they have anti-inflammatory, anti-allergenic, anti-viral, anti-aging and anti-carcinogenic activity (Percival et al. 1998). Fresh fruits and vegetables are rich in phenolics. Increased consumption of fruits and vegetables containing high levels of phytochemicals has been associated with preventing or reducing oxidative stress in the human body (Chu et al. 2002). Therefore it is recommended that fresh fruits and vegetables are consumed in the daily diet.

When plants are subjected to environmental stress, the production of reactive oxygen species occurs. In order to avoid the harmful effects of these reactive molecules, plants have evolved an effective scavenging system composed of antioxidant molecules and antioxidant enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11), and glutathione reductase (GR; EC 1.8.5.1) (Karabal et al. 2003). Production of reactive oxygen species is one of the earliest and most effective defense reactions of plants. ROS play a role in many defense processes including direct antimicrobial action, lignin formation, and the hypersensitivity response. H_2O_2 plays a central role in plant defense response (Karabal et al. 2003).

Oxidative stress occurs when either the antioxidant capacity of the cell decreases or the amount of reactive oxygen species increases. When the balance between oxidants and antioxidants is disturbed by overproduction of free radicals, it will lead to oxidative stress and DNA damage. ROS produced via oxidative stress may also affect host cells. Fortunately, plant tissues are under control of an antioxidant defense system which contains enzymes in addition to low molecular weight antioxidants such as α-tocopherol, flavonoids, ascorbate and glutathione. Not only plants but also other biological systems have the same defense mechanism against free radicals (Chu et al. 2002). These enzymes can metabolize ROS. The antioxidant enzymes require cofactors for their optimum catalytic activity (Percival 1998). For example, selenium is required for glutathione peroxidase, iron for catalase and copper, zinc and manganese for SOD

activity. These antioxidant enzymes can be classified as primary and secondary enzymes. SOD, catalase and glutathione peroxidase, as primary enzymes, eliminate ROS directly. SOD and CAT remove H_2O_2 from the plant and peroxidases regulate the level of H_2O_2 in plant tissues (Abushita et al. 2000). Their modes of actions are schematized in Figure 1.1. On the other hand, glutathione reductase, glucose-6-phosphate dehydrogenase and cytosolic GST, as secondary enzymes, help primary enzymes to detoxify the ROS by decreasing peroxide levels or by supplying metabolic intermediates for optimum activity of primary enzymes.

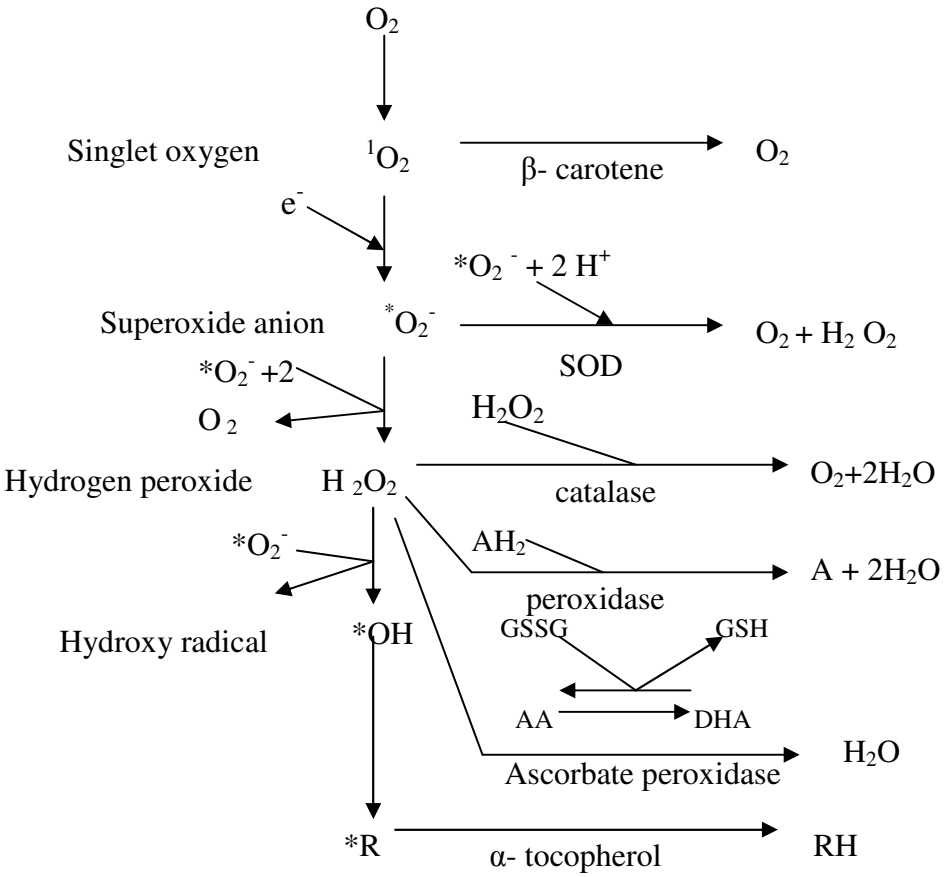

Figure 1.1. Formation of reactive oxygen species and interactions of ROS with antioxidants. AA, ascorbic acid; DHA, dehydroascorbate; GSH, glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase. (Slater et al. 2003)

As previously described, free radicals have harmful effects on organisms and are responsible for many diseases. Antioxidants have preventive and protective roles against free radicals. Inhibition of free radical oxidation causes alleviation of clinical symptoms of diseases which are related to free radical formation. Some examples of such diseases are atherosclerosis, hypertension, neurological degeneration, cancer, and cataracts (Abdullin et al. 2002). Previous research on the effects of antioxidants shows that there is a positive correlation between foods with rich antioxidant content and health. It is reported that optimum intake of vitamin C and vitamin E play an important role in minimizing the risk of cancer and heart diseases. In addition, carotenoids inhibit singlet oxygen-mediated oxidation of low density lipoprotein in plasma, thereby reducing the risk of cardiovascular diseases (Vichnevetskaia and Roy 1999). To understand the mechanism and relationship between free radicals, antioxidants and disease, it is first necessary to explain some of the diseases and their causes. Cancer is an abnormal mass of tissue. Diet and life style are two factors that affect the incidence of cancer. Transformation of a normal cell into a carcinogenic cell involves permanent alteration of the genetic information in the cell. Both diet and environment are able to generate oxygen radicals. Free radical reactions are involved in carcinogenesis which is the subject of much epidemiological research. Cardiovascular diseases are a serious health problem and are the most common cause of death around the world (WEB_2). Atherosclerosis or hardening of blood vessels is initiated when the vascular endothelium is damaged through oxidative injury. As a result of injury, monocytes and macrophages are activated and they secrete $O_2^{\bullet -}$, and H_2O_2 which injure neighboring cells. Dietary antioxidants have been recommended for lowering plasma cholesterol levels and lowering the risk of atherosclerosis. Cataract is opacity of the eye lens. The primary reason for cataracts is changes in lens proteins. Other potential reasons are oxidative damage of lipids and DNA in the eye lens. The human lens has a defense mechanism including a high concentration of antioxidant enzymes and vitamin C to protect itself from free radical attacks. According to epidemiological studies, vitamin C, vitamin E, and β-carotene appear to be associated with lowering the risk of cataracts.

To date, much research has been conducted on food stuff in order to understand the antioxidant content and nutritional quality of foods. For example Matsukawa et al. (1997) have compared antioxidant activities in seaweeds. Chu et al. (2002) have worked on antioxidant activities of some vegetables (broccoli, cabbage, carrot, spinach etc.). It is generally understood that fresh fruits and vegetables have high antioxidant activity.

Some examples of antioxidant activity of fresh fruits and vegetables are shown in Table 1.1.

Table 1.1. Total antioxidant concentrations of some vegetables and fruits. The values in mmol/100 g are mean values of three different replicates of the same sample. The total antioxidant values were determined by FRAP assay (Chu et al. 2002).

Vegetable or fruit	Species name	Antioxidant level (mmol/100 g)
Chili pepper	*Capsicum annuum*	2,46
Kale/curly kale	*Brassica oleracea var acephala*	2,34
Red cabbage	*Brassica oleracea var capitata*	1,88
Grape	*Vinis vinifera*	1,45
Orange	*Citrus sinensis*	1,14
Plum	*Prunus domestica*	1,06
Lemon	*Citrus limon*	1,02
Spinach	*Spinacia oleracea*	0,98
Kiwi fruit	*Actinida chinensis*	0,91
Lime	*Citrus aurantifolia*	0,73
Fig	*Ficus carica*	0,73
Broccoli	*Brassica oleracea*	0,58
Apricot	*Prunus armeniaca*	0,52
Tomato	*Lycopersicon esculentum*	0,31
Garlic	*Allium sativum*	0,21
Eggplant	*Solanum melongena*	0,17

Among these vegetables and fruits, tomato has an important place. According to FAOSTAT data (WEB_3), 116 million metric tons of tomato were produced around the world in 2004 (Table 1.2.). Turkey is in the third place behind China and U.S. with 8 million metric tons. In Turkey, 220 hectares of tomatoes were harvested in 2004 with 363,636 Kg/Ha yield. Tomato is a main source of lycopene in the daily diet. In addition, it contains different phenolic compounds (e.g. chlorogenic acid, quercetin, keampferol

and naringenin) and vitamin C. It has been reported that consumption of tomatoes and tomato products improved the antioxidant activity of plasma and reduced LDL oxidation in human. Moreover, epidemiological studies show that diets rich in lycopene decrease the risk of many chronic diseases such as cancers and hearth diseases (Podsedek et al. 2003).

1.3. Functional Foods

Food quality directly affects human health in terms of maintaining healthiness. Low quality nutrition may cause various diseases. In order to maintain healthiness and decrease the risk of various diseases, functional foods are needed. Functional foods are food products that provide health benefits by their nutritional quality. Compared to some exceptions with high antioxidant activity, staple foods such as eggplant and tomato contain relatively low levels of antioxidant activity (Figure 1.1.). This situation is a disadvantage in terms of nutritional quality, because the most consumed food stuffs are not very nutritious. In order to increase the nutritional quality of staple foods, scientists have focused on ways of increasing food quality. There are two approaches of increasing antioxidant level of foods. One of them is transgenic approach and the other is nontransgenic approach which is also called conventional breeding.

The "Golden Rice" project (Ye et al. 2000) is a good example of the transgenic approach. Rice endosperm lacks provitamin A. Three genes in the β-carotene pathway were introduced to rice endosperm. Consequently this research resulted in transgenic rice with carotenoid. Another example of the transgenic approach is about increasing vitamin C level in strawberries. In this work, D-galactronic acid reductase, which is an enzyme in the vitamin C pathway, was overexpressed in strawberries by genetic engineering in order to increase vitamin C levels (Agius et al. 2003). In addition Verhoeyen et al. (2002) induced flavonoid production in tomato through transgenic approach. The transgenic tomato lines had 78 fold more total flavonols. Additionally chalcone synthase and flavonol synthase transgenes were found to act synergistically to increase flavonol synthesis in tomato flesh by Verhoeyen et al (2002).

Table 1.2. Worldwide tomato production in 2004. First 10 countries are given in this table (WEB_3).

Production Mt		Area harvested(Ha)		Yield (Hg/Ha)	
China	30,142,040	**China**	1,255,103	**Netherlands**	4,538,462
United States of America	12,400,000	**Belgium**	900	**Sweden**	3,507,692
Turkey	8,000,000	**Czech Republic**	850	**Norway**	3,000,000
India	7,600,000	**El Salvador**	850	**Finland**	2,868,853
Egypt	6,780,000	**Congo, Republic of**	666	**Belgium**	2,777,778
Italy	6,500,000	**New Zealand**	630	**Iceland**	2,500,000
Spain	3,900,000	**Latvia**	600	**Denmark**	2,200,000
Brazil	3,394,677	**India**	540	**United Kingdom**	1,777,778
Iran, Islamic Rep of	3,150,000	**Kuwait**	540	**Austria**	1,764,706
Mexico	2,148,130	**Panama**	520	**Germany**	1,454,546

Traits related with antioxidant activity are polygenic, so it is hard to introgress these genes into cultivars by conventional breeding. However scientists have succeeded in conventional breeding to produce more nutritious crops (Verhoeyen et al. 2002). Willits et al. (2005) crossed *L. esculentum* and *L. pennellii v. puberulum* and F1 hybrids showed increased flavonoid accumulation. Because fruits were seedless, it was not possible to breed the next generation. In this research, however, the potential of genetic resources of wild species to create a nontransgenic high flavonoid tomato were demonstrated. Jones et al. (2003) introgressed anthocyanin fruit phenotype into *L. esculentum* from *L. chilense*. Introduction of the anthocyanin fruit characteristic into tomatoes provides benefit to develop new cultivars rich in antioxidants.

Tomato is one of the most studied crops in work aimed at improving nutritional quality. In addition to the fact that tomato is a staple vegetable and the main source of lycopene, there is one more important reason for this focus on tomato which is the fact that tomato is a model genetic system. Regardless of which approach (transgenic or breeding) is used to produce functional foods, information about genetic control of antioxidant content needs to be known. Tomato is a model system for such work because it has been the subject of much past research. The first high density DNA-based molecular map was constructed by Tanksley et al. in 1992. RFLP markers were used in this map to identify genes controlling qualitative traits. This is very helpful for breeders in order to develop better cultivars via marker assisted selection (MAS) techniques. In addition, qualitative trait loci (QTL) map of whole tomato genome was first done by Paterson et al. (1988). Further research has been conducted on genetically fixed lines. The first plant disease resistance gene was cloned (Martin et al. 1993) and also the first plant QTL was isolated (Frary et al. 2000) in tomato.

The above research lights the way to develop more nutritive tomato cultivars. Studies on measuring total antioxidant activity and antioxidative compounds like vitamin C, lycopene and phenolics of some tomato lines were conducted. For example Scalfi et al. (2000) worked on Corbarini small tomatoes and their antioxidant activities. Leonardi et al. (2000) surveyed antioxidant activity in addition to carotenoid and tomatine contents in different types of tomatoes. Raffo et al. (2002) researched the antioxidant activity, phenolic compounds and carotenoid content at different ripening stages. George et al. (2004) worked on different genotypes and their antioxidant activities which is resulted in finding cherry varieties with high levels of antioxidant for germplasm improvement programme. Besides these studies there is very little on

measuring and mapping of these traits. Rousseaux et al. (2005) conducted the first such research. Nutritional and antioxidant content of introgression lines (ILs) derived from *L.pennelli* and *L.esculentum* were detected and they were used to identify QTL for nutritional and antioxidant content. Twenty QTL were identified.

The purposes of the research presented in this thesis were to measure the total antioxidant activity of inbred backcross lines (IBLs) and reveal the correlations between antioxidant activity and the content of vitamin C, lycopene and phenolic compounds. In addition to this phenotypic characterization, these health related traits were genetically characterized with microsatellites and CAPs markers. This characterization revealed the number of QTL controlling each trait, their chromosomal locations, allelic effects and possible correspondence with known genes and the QTL identified by Rousseaux et al.(2005).

CHAPTER 2

MATERIALS AND METHODS

2.1. Plant Materials

The plant materials used in this project are inbred backcross lines (IBLs). These lines were derived from an *L.esculentum* by *L.pimpinellifolium* interspecific cross. (Doğanlar et al. 2002). *L.esculentum* cv.E6203 was used as the recurrent parent and pollinated with *L.pimpinellifolium* (LA1589). The F1 generation obtained from this cross was backcrossed with the recurrent parent in order to generate a BC1F1 population. BC1F1 lines were backcrossed once more to fix the *L.esculentum* traits in the population. The BC2F1s were then selfed for six generations to increase homozygosity. Seeds of the 145 different BC2F6 lines were sown in March 2004 in the greenhouse and transferred to the field of Ege Tarımsal Araştırma Enstitüsü as three replicates in May 2004. They were harvested at the end of August 2004 when fruits were fully ripe. Tomatoes of each line were bulked, weighed and separated into four plastic bags. Bags of tomatoes were stored at -20 ^{o}C as it has been reported that tomatoes can be stored frozen for up to six months in order to prevent tomatoes from losing their antioxidant activities (Lisiewska and Kmiecik 2000)

2.2. Preliminary Experiments

Preliminary experiments were done to determine the conditions for measuring antioxidant activity. During this process homogenization conditions, different concentrations of tomato juice and different solvents were used. For example, the blending operations were conducted at different sample/water ratios (1/2, 2/1) and homogenization operations following blending were conducted by using 5 or 10 g sample and 10 or 15 ml water or solvent. The solvents tested were different concentrations of ethanol (25, 50, 75 and 100%), acetone (25, 50, 75 and 100%) and methanol (100%). After many measurements, conditions for measuring highest hydrophilic or lipophilic antioxidant activities were determined.

Antioxidant activity of tomatoes was measured according to Re et al. (1999). This method is based on kinetic measurement of radical cation decolorization. ABTS (2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) is a chemical which is decolorized in the presence of antioxidants. More specifically the ABTS radical cation (ABTS ˙+) which is blue-green in color is decolorized. ABTS ˙+ was produced by mixing ABTS stock solution with potassium persulfate (7mM ABTS and 2.45 mM potassium persulfate). The ABTS radical cation solution was kept at room temperature and in the dark for 12-16 hours before it was used for measurements.

Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) was used as an antioxidant standard in this experiment. The stock solution of trolox was prepared as 2,5 mM in ethanol (100 ml). Four different concentrations of trolox solution were used in order to prepare the standard curve. The final trolox concentrations in reaction mixture were 0,0045/0,03,/0,015 and 0, 0075 μmol. The measurements were conducted at 734 nm using a spectrophotometer (SHIMADZU 1700 UV-Visible Spectrophotometer) equipped with with a constant temperature cell holder at 30 °C.

2.3. Preparations of Extracts

To prepare extracts for spectrophotometric analysis of antioxidant activity, tomatoes were blended in a Waring blender for two minutes at +4 °C. The blending process was done with 200 g tomato and 100 ml distilled water. Resulting tomato puree was kept in an ice bath to prevent loss of antioxidant activity. Ten grams of the tomato puree was then taken and mixed with ten ml distilled water or HPLC grade absolute ethanol according to type of fraction. Thus the hydrophilic fraction was prepared by mixing the puree with distilled water and the lipophilic fraction was prepared by mixing the puree with ethanol. The resulting mixtures were homogenized for one minute by using a Yellowline homogenizer disperser (IKA yellow line DI 18). Homogenate was filtered through four layers of nylon cloth into two 15 ml falcon tubes and tubes were centrifuged at 3000 X G for 10 minutes with all steps performed at +4 °C. After centrifugation, supernatant was collected as clear phase into a tube and was kept in an ice bath in the dark. In preliminary experiments, the pellet was also dissolved and antioxidant activity was measured. Because it was found that pellet contained almost no activity, only the antioxidant activity of the supernatant was measured in subsequent

samples.

2.4. Determination of Antioxidant Activity

In order to measure the antioxidant activity of the hydrophilic fraction, ABTS stock solution was diluted with 5mM phosphate buffer saline (PBS) containing 150 mM NaCl (pH 7,4) until the solution gave absorbance of 0,680-0,720 at 734 nm. For measurement of antioxidant activity of lipophilic fractions, this ABTS stock was diluted in HPLC grade ethanol instead of PBS. Once the diluted ABTS solution was at proper absorbance, the reaction mixture was formed by mixing 2 ml potassium persulfate oxidized ABTS solution in PBS and 2,5 / 5 or 7,5 µl of extract. The mixture was shaken two times quickly and then the cuvet was placed into the spectrophotometer. The kinetic measurement was then started in order to observe decolorization of ABTS radical cation. Total time of the measurement was six minutes. After six minutes, absorbance values at the first, third and sixth minutes were recorded. Each measurement was repeated three times for each concentration of sample. The results were calculated as area under the curve (AUC) and were expressed as µmol Trolox/kg fresh weight tomatoes. To calculate the AUC, the percent inhibition/concentration values for the extracts and Trolox were plotted separately against test periods (1, 3, 6 min) (Demirbüker et al. 2005). Figure 2.1. shows the plot of *L. esculentum* parent (pedigree number 105) and Figure 2.2. shows the plot of Trolox. The ratio of the areas of curves for extracts and Trolox was used to calculate the AUC value.

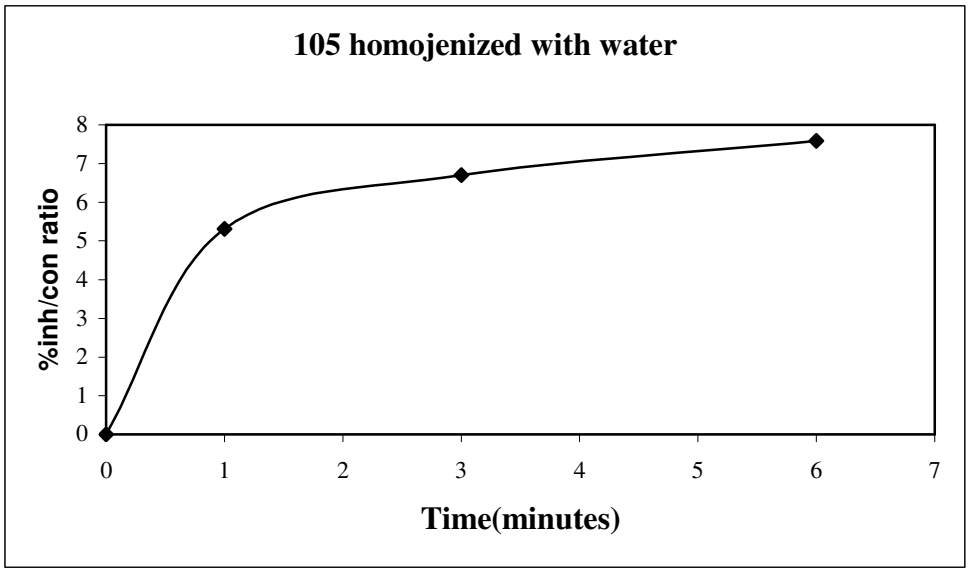

Figure 2.1. Percent inhibition vs. concentration plot of *L.esculentum* at 1st, 3 rd and 6 th min used to measure the area under curve (AUC).

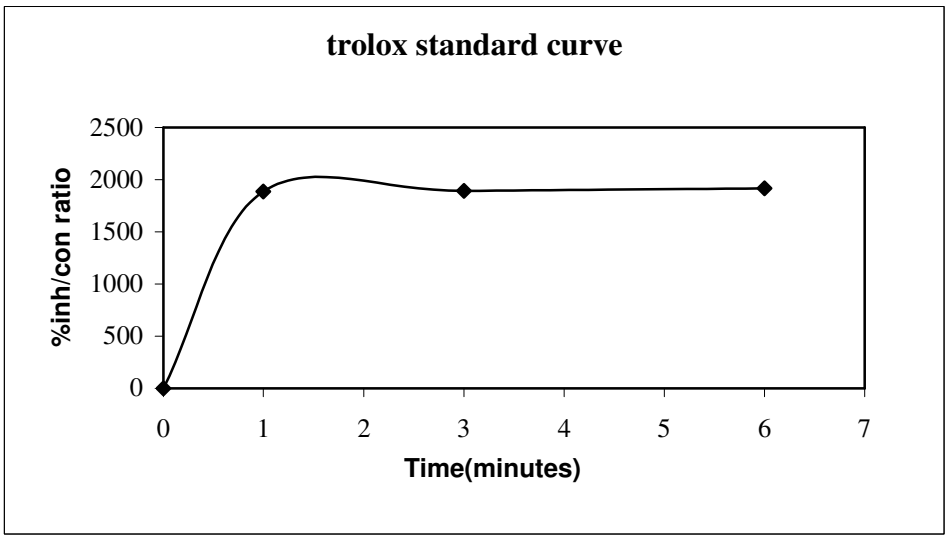

Figure 2.2. Percent inhibition vs. concentration plot of Trolox standard at 1st, 3rd and 6 th min used to measure the AUC.

2.5. Genotyping and Statistical Analysis

In addition to the antioxidant activity data generated for each line in this work, other antioxidant-related traits were also studied in this experiment. The data for these traits (vitamin C, lycopene and phenolic compounds contents) were generated in separate work. Correlation coefficients between traits were calculated by QGENE (Nelson 1997). QTL mapping of antioxidant traits was also performed by QGENE using simple linear regression. Plants were previously genotyped by Doğanlar et al. (2002). These genotypes for 127 markers were used for QTL analysis. A significance threshold of $P < 0,01$ was used for QTL declaration. Estimates of magnitudes of effect (R^2 from QGENE) and trait means were determined for the most significant marker for each QTL.

CHAPTER 3

RESULTS and DISCUSSION

Figure 3.1. *Lycopersicon pimpinellifolium* Wild type tomato with small fruit.

Figure 3.2. *Lycopersicon esculentum* Cultivated tomato.

3.1. Trait Means and Distributions

Antioxidant activities of the hydrophilic and lipophilic fractions of the *L.pimpinellifolium* (Figure 3.1.) and *L.esculentum* (Figure 3.2.) parents and inbred backcross lines are given in Table 3.1. Mean values and standard errors for these and the other antioxidant traits are summarized in Table3.2. Mean AUC value in the hydrophilic fraction of the *L.esculentum* parent was 6197 µmol Trolox/ kg fresh tomato. The same trait for the *L.pimpinellifolium* parent was 1,5 fold more than *L. esculentum*. Mean values of antioxidant activities in the lipophilic fraction were lower than those for the hydrophilic fraction. Thus, values were 50% lower for the lipophilic fraction of *L. esculentum* and 64% lower for the same fraction of *L. pimpinellifolium*. In addition, the wild type parent had a 1,8 fold higher value for lipophilic antioxidant activity than the cultivated type. In the IBLs, the difference between lowest and highest values of antioxidant activities in both fractions was around 3000µmol Trolox/kg. The mean

values of IBLs were mostly close to the *L. esculentum* mean for both fractions of antioxidant activity. According to the distribution histogram of the hydrophilic fraction of antioxidant activity, the distribution of values in the population appeared to be normal but was skewed to the left relative to the *L. esculentum* mean (Figure 3.3.). Thus, although it was expected that the population mean would be similar to the *L. esculentum* mean, it was found that the *L. esculentum* mean was higher than expected. The reason of such distribution could be that the hydrophilic antioxidants were probably dissolved in the cytoplasm quickly and the hydrophilic antioxidants were first used. The distribution of antioxidant activity in the lipophilic fraction of the IBL plants was normal and the *L. esculentum* value fell within the expected range (Figure 3.4.).

For vitamin C content, the *L.pimpinellifolium* mean was 1,6 fold more than the *L.esculentum* mean. The lowest and the highest values of IBLs for vitamin C content were 165 mg/kg fresh weight and 504 mg/kg fresh weight, respectively. Their mean value (290 mg/kg) was nearly identical to the *L.esculentum* parent mean (288 mg/kg). The vitamin C content values of the IBLs had a normal distribution (Figure 3.5.). Similar to vitamin C content, it was found that the *L.pimpinellifolium* parent had 2,5 times more phenolic compound content than the *L.esculentum* parent. The IBL values for this trait ranged from 3540 mg/kg to 11748 mg/kg and were normally distributed (Figure 3.6.). The mean value of inbred backcross lines was 4901mg/kg which was close to the *L. esculentum* parent mean. The lycopene content of the *L.esculentum* parent was 214 mg/kg which is 1,8 fold less than that in the *L.pimpinellifolium* parent. The mean lycopene content of the IBLs was slightly lower than that of *L. esculentum*. As with the other traits, the lycopene values were normally distributed (Figure 3.7.).

Table 3.1. AUC values of the IBLs.

IBL numbers	Genotype	AUC(μmol Trolox/kg) Hydrophilic	AUC(μmol Trolox/kg) Lipophilic
E6203	**T 104**	**6416,8**	**2848,57**
LA 1589	**T 105**	**9337,3**	**5824,58**
TA 2136	T 10	4407,79	3007,75
TA2138	T 11	4302,85	2228,91
TA 2143	T 12	5169,62	2758,62
TA 2149	T 13	4917,54	2554,38
TA 2151	T 14	4382,02	2470,3
TA 2153	T 15	4092,95	2367,5
TA 2155	T 16	4302,85	2433,96
TA 2157	T 17	5037,48	2755,27
TA 2158	T 18	4722,64	2586,99
TA 2159	T 19	5697,15	2932,1
TA 2160	T 20	6296,85	2945,74
TA 2163	T 21	5997	3221,87
TA 2165	T 22	5083,2	3118,44
TA 2166	T 23	5097,45	2806,59
TA 2167	T 24	3898,05	2281,2
TA 2168	T 25	5982	3135,05
TA 2171	T 26	5352,32	2785,49
TA 2172	T 27	4943,03	3186,65
TA 2175	T 28	4722,63	2876,55
TA 2178	T 29	4797,6	2668,6
TA 2181	T 30	5697,15	2743,79
TA 2182	T 31	4636,78	2950,52
TA 2183	T 32	5224,88	2470,5
TA 2184	T 33	5412,3	2998,5
TA 2185	T 34	5352,32	3679,24
TA 2186	T 35	5479,76	3062,5
TA 2188	T 36	5397,3	3817,62
TA 2191	T 37	4512,74	2890,36
TA 2195	T 38	4512,74	2423,62
TA 2196	T 39	4407,8	2488,75
TA 2197	T 40	6454,27	3921,57

(cont. on next page)

Table 3.1. (cont.)

IBL numbers	Genotype	AUC(µmol Trolox/kg) Hydrophilic	AUC(µmol Trolox/kg) Lipophilic
TA 2200	T 41	4947,53	2700,16
TA 2201	T 42	5037,48	2767,94
TA 2203	T 43	4797,6	3133,4
TA 2206	T 44	5479,8	5209,7
TA 2209	T 45	4197,9	2533,33
TA 2210	T 46	5982	3605,54
TA 2219	T 47	5097,4	2723,15
TA 2224	T 48	4917,54	2690,4
TA 2227	T 50	4722,64	3008,07
TA 2229	T 51	5352,32	2638,89
TA 2232	T 52	4610,19	2563,72
TA 2233	T 53	4917,54	3015,87
TA 2238	T 54	4722,64	3133,43
TA 2240	T 55	5667,16	2968,51
TA 2242	T 56	4722,64	2801,84
TA 2247	T 58	4797,6	2949,92
TA 2248	T 59	4610,19	3050,97
TA 2251	T 60	3778,11	2749,19
TA 2253	T 61	4722,64	2820,09
TA 2259	T 63	4917,54	2526,55
TA 2260	T 64	5224,88	2998,5
TA 2262	T 65	5667,17	3133,43
TA 2263	T 66	4617,69	2813,11
TA 2264	T 67	5839,58	3133,43
TA 2265	T 68	5224,89	3158,68
TA 2269	T 69	5397,3	3157,09
TA 2270	T 70	4197,9	2720,59
TA 2273	T 71	5157,42	3190,84
TA 2277	T 72	6936,94	3423,42
TA 2282	T 73	4857,57	3199,31
TA 2291	T 74	3988	2624,36
TA 2292	T 75	4183,67	2572,71
TA 2293	T 76	3898,05	2644

(cont. on next page)

Table 3.1. (cont.)

IBL numbers	Genotype	AUC(µmol Trolox/kg) Hydrophilic	AUC(µmol Trolox/kg) Lipophilic
TA 2294	T 77	5224,88	2972,58
TA 2296	T 78	5547,22	3257,23
TA 2297	T 79	4970,01	2571,65
TA 2300	T 80	5097,45	2963,87
TA 2301	T 81	4722,64	2563,72
TA 2302	T 82	5114,15	3268,36
TA 2304	T 83	4722,64	2515,53
TA 2303	T 84	5262,37	2848,57
TA 2305	T 85	5097,45	2825,09
TA 2308	T 87	6296,85	3301,74
TA 2309	T 88	4917,54	2658,85
TA 2310	T 89	4722,64	3395,06
TA 2311	T 90	5037,48	3264,84
TA 2314	T 91	4407,79	2396,15
TA 2316	T 92	3688,15	2438,08
TA 2318	T 93	4557,72	2905,19
TA 2319	T 94	4917,54	3133,43
TA 2321	T 95	4407,79	2700,83
TA 2322	T 96	4741,93	2496,77
TA 2323	T 97	3898,05	2523,08
TA 2325	T 98	5097,45	3133,43
TA 2327	T 99	6327,33	3555,55
TA 2328	T 100	4797,6	2698,65
TA 2332	T 101	4610,19	3150,8
TA 2335	T 102	4197,9	2835,83
TA 2337	T 103	5697,15	3874,17
TA 2137	T 207	5352,32	3341,83
TA 2139	T 208	4407,79	2360,6
TA 2140	T 209	5397,3	3133,43
TA 2142	T 210	4557,72	2666,1
TA 2144	T 211	4917,54	2594,02
TA 2145	T 212	4917,54	2955,17
TA 2146	T 213	4497,75	2843,95

(cont. on next page)

Table 3.1. (cont.)

IBL numbers	Genotype	AUC(µmol Trolox/kg) Hydrophilic	AUC(µmol Trolox/kg) Lipophilic
TA 2147	T 214	5352,32	2878,79
TA 2148	T 215	3898,05	2982,73
TA 2152	T 217	5097,45	2923,54
TA 2154	T 218	3847,33	2792,23
TA 2162	T 220	5045,04	2342,34
TA 2174	T 222	3778,11	2978,05
TA 2176	T 223	3598,2	2677,66
TA 2177	T 224	5352,32	3059,58
TA 2179	T 225	4407,79	3132,91
TA 2187	T 226	4197,9	2903,22
TA 2189	T 227	4197,9	2848,29
TA 2193	T 228	4354,58	2881,19
TA 2204	T 229	4917,54	2791,6
TA 2205	T 230	4497,75	2731,41
TA 2207	T 231	4722,64	2698,65
TA 2211	T 232	3988	2785
TA 2212	T 233	5697,15	2953,56
TA 2213	T 234	4092,95	2874,2
TA 2214	T 235	3988	2620,69
TA 2215	T 236	4407,79	2782,68
TA 2216	T 237	5697,15	3438,91
TA 2217	T 238	5097,45	2748,09
TA 2221	T 239	4407,79	2515,92
TA 2228	T 240	4197,9	2840,33
TA 2230	T 241	5224,88	2551,72
TA 2239	T 242	5509,7	2245,45
TA 2244	T 243	5127,4	3389,8
TA 2249	T 244	3628,18	2338,83
TA 2257	T 245	5262,37	3148,94
TA 2258	T 246	5532,23	3204,4
TA 2266	T 247	5224,88	2987,42
TA 2271	T 248	4722,64	2987,42
TA 2278	T 249	6306,3	3303,3

(cont. on next page)

Table 3.1. (cont.)

IBL numbers	Genotype	AUC(µmol Trolox/kg) Hydrophilic	AUC(µmol Trolox/kg) Lipophilic
TA 2280	T 250	3898,05	2536,6
TA 2285	T 251	5037,5	2891,7
TA 2286	T 252	4407,79	2848,57
TA 2290	T 253	4272,86	2637,63
TA 2307	T 254	4610,19	3027,95
TA 2313	T 255	5554,72	3675,28
TA 2317	T 256	4497,75	2773,61
TA 2324	T 257	6296,85	4197,9
TA 2331	T 258	4677,66	2909,65
TA 2333	T 259	5697,15	3283,35
TA 2334	T 260	4917,54	2523,24
TA 2336	T 261	6596,7	3725,49
TA 2338	T 262	6454,27	3328,33
	T 107	4197,9	2706,15

Table 3.2. Mean values and standard errors of parents and IBLs for the antioxidant traits. Numbers of lines were given in parentheses. Values followed by a different letter are significantly different at P<0.05 as determined by Student's t-test.

	***L. esc.* parent**	***L. pimp.* parent**	**Inbred backcross lines**	
Trait	**Mean ± SE (n)[1]**	**Mean ± SE (n)[1]**	**Mean ± SE (n)**	**Range**
Hydrophilic AUC (µmol Trolox/kg)	6197 ± 265 (3) a	9265 ± 238 (3) b	4917,4 ± 67 (144) c	3598 to 6937
Lipophilic AUC (µmol Trolox/kg)	3133 ± 0 (3) a	5917 ± 92 (3) b	2919,9 ± 40 (144) a	2229 to 5210
Vit-C(mg/kg)	288,1 ± 0 (3) a	463,9 ± 8.0 (3) b	290,6 ± 5,9 (144) a	164,7 to 503,9
Phenolic compounds(mg/kg)	4685 ± 87 (3) a	11748 ± 38 (3) b	4901,2 ± 55 (144) a	3540,42 to 11748,36
Lycopene(mg/kg)	213,6 ± 0,8 (3) a	398,4 ± 0 (3) b	207,2 ± 4,1 (144) a	110,34 to 398,44

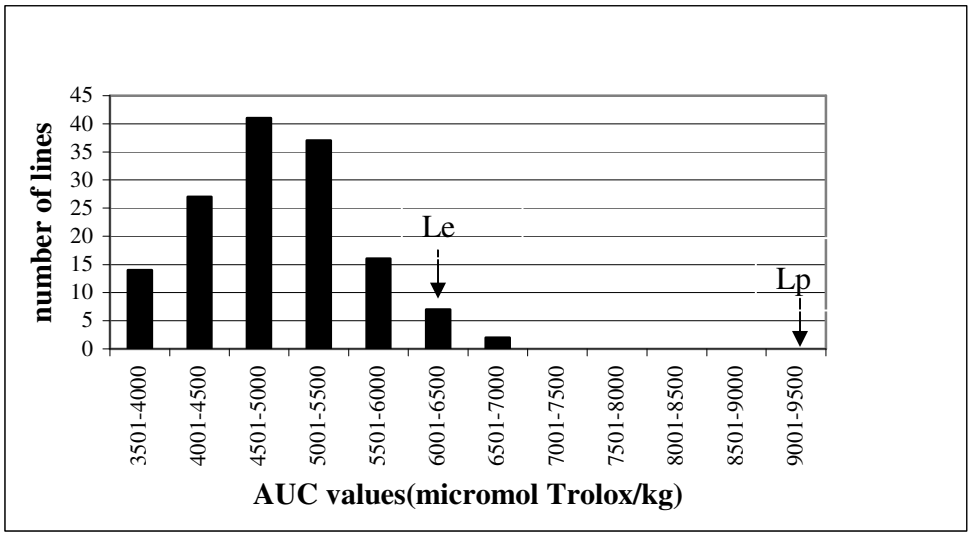

Figure 3.3. Distribution histogram of antioxidant activities in hydrophilic fraction. Le and Lp indicate locations of *L.esculentum* and *L.pimpinellifolium* means, respectively.

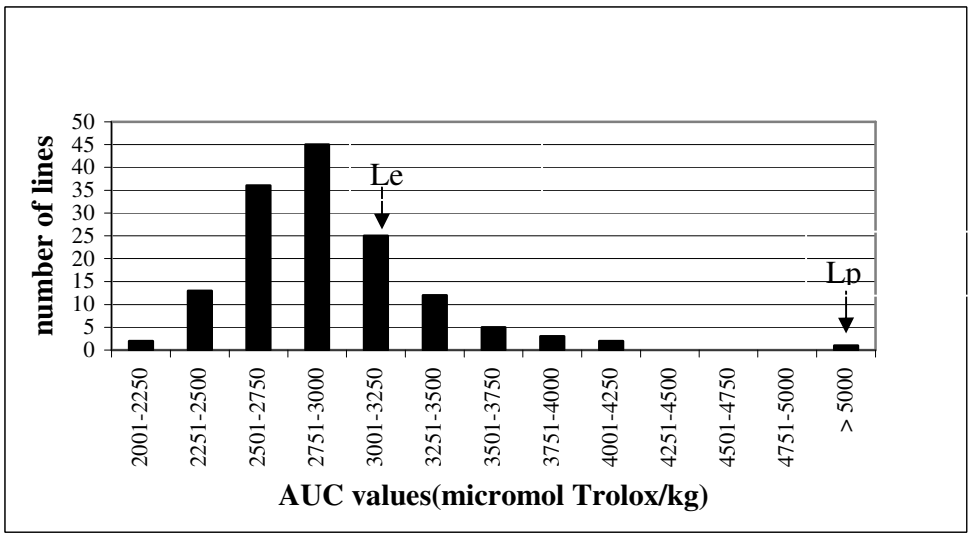

Figure 3.4. Distribution histogram of antioxidant activities in lipophilic fraction Le and Lp indicate locations of *L.esculentum* and *L.pimpinellifolium* means, respectively.

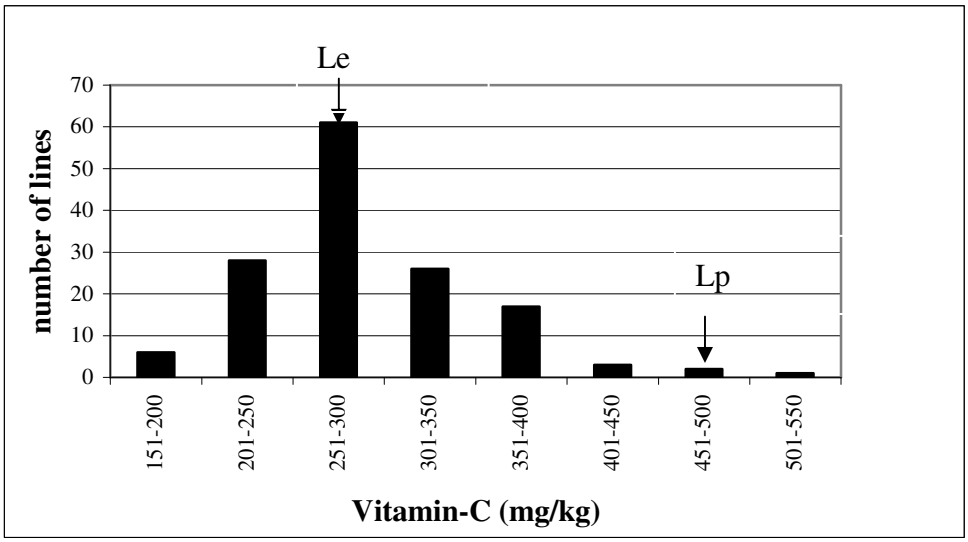

Figure 3.5. Distribution histogram of Vitamin-C content. Le and Lp indicate locations of *L.esculentum* and *L.pimpinellifolium* means, respectively.

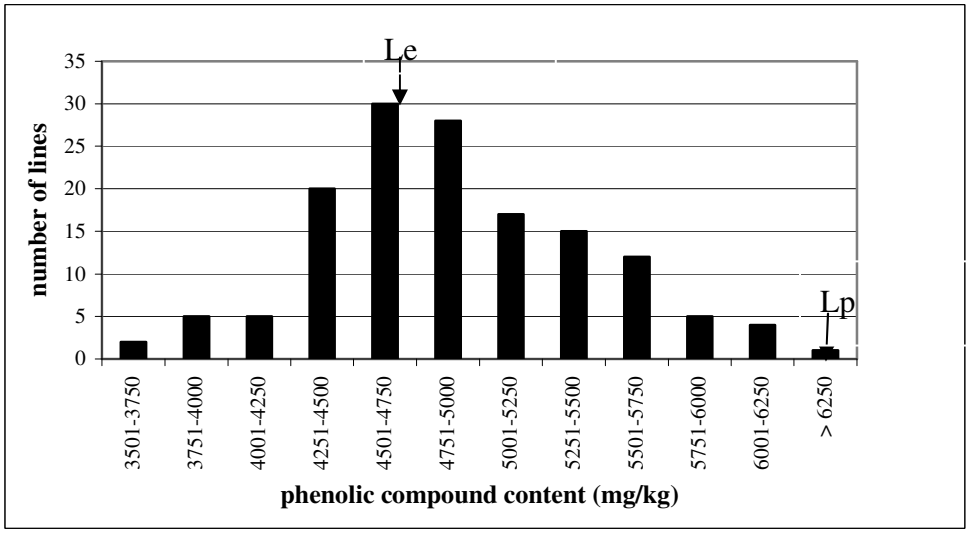

Figure 3.6. Distribution histogram of phenolic compound content. Le and Lp indicate locations of *L.esculentum* and *L.pimpinellifolium* means, respectively.

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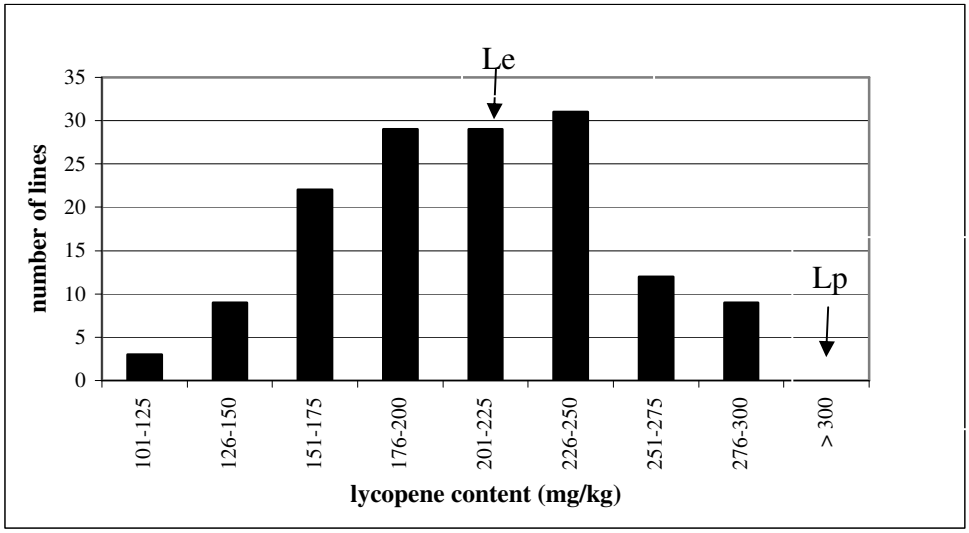

Figure 3.7. Distribution histogram of lycopene content. Le and Lp indicate locations of *L.esculentum* and *L.pimpinellifolium* means, respectively.

3.2. Comparison with Previous Research

3.2.1. Total Antioxidant Activity

Results from this research were compared with previous research. Many different assays have been applied to measure antioxidant activity in previous research. These methods include TRAP (Total Radical Trapping Parameter), ORAC (Oxygen Radical Absorbance Capacity), ARP (Anti-Radical Power), ILP (Inhibition of Lipid Peroxidation) (Hanson et al. 2004). The current research was based on the TEAC (Trolox Equivalent Antioxidant Capacity) assay. All these assays can be divided into two categories (Ou et al. 2002). In the first category of antioxidant activity assays, methods are based on hydrogen atom transfer reaction. ORAC and TRAP assays are in this category. For example, in the ORAC test, the $ROO^{\bullet}$ (peroxyl radical) abstracts a hydrogen atom from the antioxidant. These assays are not total antioxidant activity assays because they only measure antioxidant activity against specific radicals.

The second category is assays based on single electron transfer. This category includes the FRAP and TEAC assays. In these assays, antioxidants are oxidized by oxidants such as Fe(III) or $ABTS^{+\bullet}$. A single electron is transferred from antioxidant molecule to the oxidant. The FRAP assay measures the capacity of a solution to reduce

the ferric ion. In this assay, any compound that has a lower redox potential than the ferric ion can theoretically reduce Fe(III) to Fe(II). As a result, this assay measures many different compounds, not just antioxidants. This method is also too slow to be of any practical use as each assay takes hours. On the other hand, the TEAC assay is rapid, easy and correlates with the biological activity of antioxidants. Despite the problems with the FRAP assay, some scientists have used it to measure antioxidant activity (Halvorsen et al. 2001 and George et al. 2004). In recent work, Hanson et al. (2004) used the ARP and ILP assays to determine antioxidant activity of different tomato lines including fifty *L. esculentum* lines and three *L. pimpinellifolium* accessions. The ARP and ILP assays are similar to the TEAC assay. Based on comparison with this previous work, antioxidant activity of LA1589 was 50% more than other *L.pimpinellifolium* examples. In addition the *L.esculentum* parent of IBLs had almost two fold more antioxidant activity than other *L.esculentum* examples. The reason for the comparatively low antioxidant activity of the tomato lines that were measured by Hanson et al. might be the way the samples were prepared. Samples of the current research were cut into four slices and then were frozen at -20^{o}C. However the samples of the Hanson et al. work were blended and then were frozen at -70^{o}C. The blending step in the sample preparation might have started the antioxidant decreasing process.

Antioxidant activities of different tomato cultivars were determined by Toor and Savage (2005). They used TEAC assay with different fractions of tomato such as peel, pulp and seeds. Antioxidant activity of pulp fraction can be compare with the results of IBL parents. Antioxidant activities were present as μM Trolox.100g^{-1}. Antioxidant activity in hydrophilic fraction was 81,8 μM Trolox.100g^{-1} and in lipophilic fraction was 7,0 μM Trolox.100g^{-1}.

3.2.2. Other Antioxidant Traits

The other health related traits measured in this study such as vitamin C (ascorbic acid), phenolics and lycopene have also been measured by Hanson et al. (2004). In that work, the vitamin C content of *L.pimpinellifolium* examples were 1,3 fold less than that obtained for *L.pimpinellifolium* LA1589, the IBL's parent. In addition, the *L.esculentum* parent of the IBLs had almost 1,6 fold more vitamin C content than the *L.esculentum* examples measured by Hanson et al.(2004). There were also differences

between the results for phenolic compound content. Phenolic compounds in the *L.pimpinellifolium* examples measured in the previous study were 7,6 fold less than LA1589 and the *L.esculentum* examples were 5,4 fold less than the *L.esculentum* parent of the IBLs. It was reported that phenolics made the major contribution to the total antioxidant activity. Finally, the lycopene content of *L.pimpinellifolium* and *L.esculentum* examples used by Hanson et al. were 1,9 and 3,3 fold less than the IBL parents, respectively. In addition to the health related traits, Hanson et al.(2004) also measured some agronomical traits, color, soluble solids and fruit size and correlated these characters with the health related traits.

George et al. (2004) conducted similar research on *L.esculentum*. The same health related traits measured in this research were also determined by George et al. (2004). In this paper, these compounds were called bio-antioxidants and were measured in 12 tomato genotypes. The measurements were done in two different tissues, pulp and peel. The results of the research conducted by George et al. (2004) were presented on both a fresh weight basis and a dry weight basis. The data that were obtained in the current research were compared with the pulp fraction values in fresh weight basis that were determined by George et al. (2004). Vitamin C content of their lines was nearly identical to the vitamin C content of the *L.esculentum* parent of the IBLs. On the other hand, phenolic compound content of the *L.esculentum* parent of the IBLs was 19,5 fold more and lycopene content was 6 fold more than the mean values of these traits of the *L.esculentum* cultivars studied by George et al. (2004). Additionally George et al. (2004) measured the total soluble solids and titratable acidity.

Toor and Savage (2004) measured the health-related traits of three tomato cultivars in different fractions. The fractions were skin, pulp and seeds. Ascorbic acid, phenolic compounds and lycopene contents of the pulp fraction were compared with the *L.esculentum* (E6203) parent of the IBLs. The vitamin C content of the *L.esculentum* parent of IBLs was 3 fold higher than the mean value of the three cultivars that were used by Toor and Savage (2004). Secondly, total phenolics were measured in two fractions: hydrophilic and lipophilic fractions. For this reason their values cannot be directly compared with current values. Last of all the lycopene content of E6203 was almost 10 fold higher than the mean of the three cultivars studied by Toor and Savage (2004). Interestingly, total antioxidant activity, vitamin C, phenolics both in hydrophilic and lipophilic extracts, and lycopene were higher in the skin than the seeds and pulp. When seeds and pulp were compared, except for lycopene, all the traits were higher in

seeds than pulp. Such a separation of tissue types was not necessary for the current research because usually all of these tomato tissues are consumed in the daily diet.

3.3. Correlations

Table 3.3 shows the correlations between the traits that were measured. Correlations were performed between these traits using the QGENE computer program (Table 3.3.). According to the data, some of the traits have strong, positive correlations. The strongest correlations were seen between phenolic compounds content and both hydrophilic and lipophilic antioxidant activities (r = 0.66). In addition, phenolics were also strongly and significantly correlated with vitamin C content. Vitamin C content was also positively correlated with antioxidant activity in the lipophilic fraction (r = 0.51). Antioxidant activities of the hydrophilic and lipophilic fractions were also well correlated. All of these significant positive correlations were expected because vitamin C, lycopene and phenolic compounds have antioxidant activities and all contribute to total antioxidant activity. Phenolic compounds can be both hydrophilic and lipophilic, therefore, they contribute to both fractions of total antioxidant activity. It has been reported that flavonoids, a class of phenolic compound, support the primary ascorbate-dependent scavenging system in plants (Sakihama et al. 2002). Thus, the correlation between vitamin C and phenolic compounds seen in this study was not unexpected and confirms the idea that phenolics work together with ascorbate to neutralize reactive oxygen species (Sakihama et al. 2002). Vitamin C as a water soluble compound has an effect on hydrophilic antioxidant activity which also explains the positive correlation between these two traits (r = 0.28). Lycopene has lipophilic characteristics, so its correlation with lipophilic antioxidants (r = 0.23) was also expected.

Table 3.3. Correlations between antioxidant traits for IBL population. P values are given in parenthesis. Only correlations with P-value <0.05 are considered to be significant.

Trait	VitC	Lycopene	Phenolics	AUCwater
Lycopene	0,02 (0,864)			
Phenolics	0,53 (< 0,0001)	0,24 (0,020)		
AUCwater	0,28 (0,009)	0,1 (0,302)	0,66 (<0,0001)	
AUCEtOH	0,51 (<0,0001)	0,23 (0,029)	0,66 (<0,0001)	0,58 (<0,0001)

The correlations of antioxidant traits for IBL population were compared with correlations that have been made by Hanson et al. (2004). Similar to the IBL population, the strongest correlations were seen between phenolic compounds content and both hydrophilic and lipophilic antioxidant activities (r = 0,90 and r = 0,83 respectively). Phenolics were also strongly and significantly correlated with vitamin C content (r =0,81). Antioxidant activities that were measured by the ARP and ILP methods were also strongly and significantly correlated with each other. Thus, the correlations between antioxidant traits were very similar between this study and the previous study.

3.4. Identification and Mapping of QTL

Complex biochemical traits such as vitamin C content or total antioxidant activity are under the control of more than one gene. In order to identify and map the quantitative trait loci (QTL) controlling the traits examined in this study, QGENE software was used. For this analysis, the phenotypic values for antioxidant activity of the hydrophilic and lipophilic fractions obtained in this study were used. In addition, values for vitamin C, lycopene and phenolic compounds content obtained in separate work were analyzed. Genotypes for the IBLs were previously published (Doganlar et al. 2002). The QGENE program performed single regression analysis for each trait and a significance threshold of $P \leq 0.01$ was used. R-squared values were used to determine the percentage of phenotypic variance explained by each locus. For the five traits analyzed in this work, 31 loci were identified (Table 3.4). Figure 11 shows the most likely position of each QTL on the genetic map.

Table 3.4. QTL identified for antioxidant traits. R square values indicate effect of each QTL to the total phenotype. AA column gives mean value for individuals homozygous for *L.esculentum* alleles at each marker. aa gives mean value for individuals homozygous for *L.pimpinellifolium* alleles at each marker. The last column lists which parental alleles were associated with higher antioxidant activity for each QTL.

Trait	QTL symbol	Chromosome	Marker	P value (<0,001)	R Sq value %	AA	aa	High Antioxidant Source
Hydrophilic Antioxidant Activity	*aoxh 1.1*	1	TG301	0,0026	6,3	4882,35	5716,7	LA1589
	aoxh 6.1	6	TG365	0,0022	8,4	4887,21	5888,8	LA1589
	aoxh 6.2	6	TG314	0,001	7,5	4886,46	5992	LA1589
	aoxh 11.1	11	TG393	0,0031	8	4925,53	4736,4	E6203
Lipophilic Antioxidant Activity	*aoxl 1.1*	1	TG301	0,0039	5,8	2897,85	3382,7	LA1589
	aoxl 2.1	2	CT205	<0,0001	18,9	2878,85	2962,2	LA1589
	aoxl 2.2	2	TG48	0,0001	13,6	2870,33	3051,8	LA1589
	aoxl 5.1	5	CT167	<0,0001	10,9	2880,23	2840,4	LA1589
	aoxl 5.2	5	CT118	<0,0001	16,2	2897,98	2808,4	LA1589
	aoxl 6.1	6	TG314	0,0024	6,4	2900,9	3520	LA1589
	aoxl 7.1	7	CT52	0,0063	7	2911,34	2873,1	LA1589
	aoxl 10.1	10	CT234	0,0019	8,6	2906,87	2830,8	E6203
	aoxl 11.1	11	CT182	0,0093	6,5	2882,51	3165,5	LA1589
	aoxl 11.2	11	TG393	<0,0001	27,9	2889,53	2876,4	LA1589
	aoxl 12.1	12	TG473	0,0012	9,3	2907,13	2868	LA1589
Vitamin C	*vitc 1.1*	1	TG301	0,0007	7,8	286,7	370,33	LA1589
	vitc2.1	2	CT205	0,0013	9,2	284,17	339,55	LA1589
	vitc 5.1	5	CT167	0,0094	6,6	288,88	123	E6203
	vitc 6.1	6	TG365	0,0079	6,7	287,15	371,2	LA1589
	vitc 6.2	6	TG314	0,0123	4,4	288,09	364	LA1589
	vitc 7.1	7	CD57	0,0006	10,1	281,9	342,11	LA1589
	vitc 8.1	8	CD40	0,0002	11,8	282,93	342,11	LA1589
	vitc 11.1	11	TG36	0,0105	6,3	285,8	318,23	LA1589

(cont. on next page)

Table 3.4. (cont.)

Trait	QTL symbol	Chromosome	Marker	P value (<0,001)	R Sq value %	AA	aa	High Antioxidant Source
	vitc 12.1	12	CT156	0,0054	7,3	295,09	231,7	E6203
Phenolic	*phe 1.1*	1	TG301	0,0095	4,7	4869,44	5447,3	LA1589
Compounds	*phe 4.1*	4	TG163	0,0063	5,2	4860,21	5338	LA1589
	phe 5.1	5	CT167	0,0006	10,4	4867,97	4629,6	E6203
	phe 6.1	6	TG365	0,0083	6,7	4871,94	5588	LA1589
	phe 11.1	11	CT182	0,0025	8,2	4841,52	5372	LA1589
	phe 12.1	12	TG360	0,005	7,8	4908,08	4694	E6203
Lycopene	*lyc 9.1*	9	CT283	0,013	6,1	211,5	181,61	E6203

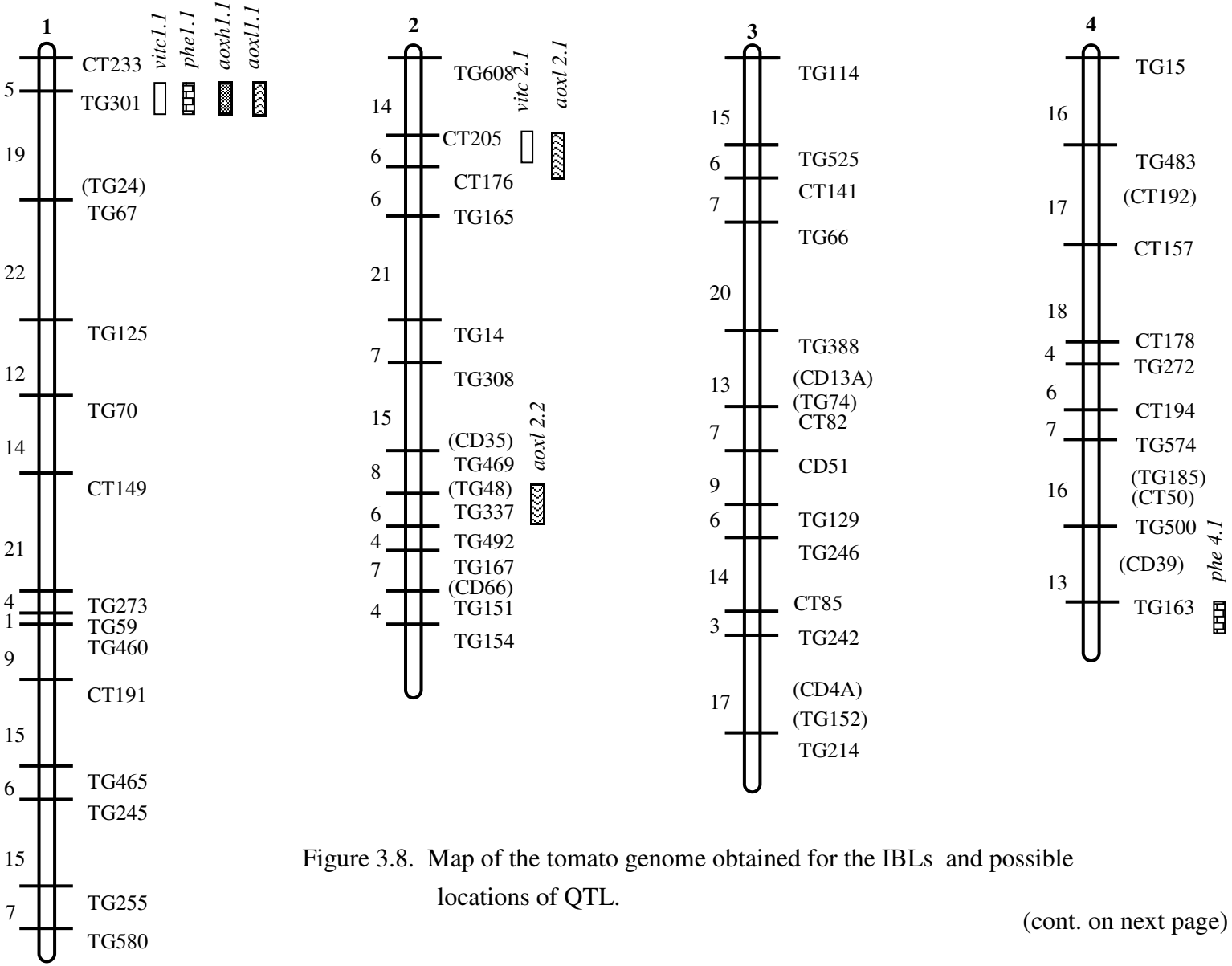

Figure 3.8. Map of the tomato genome obtained for the IBLs and possible locations of QTL.

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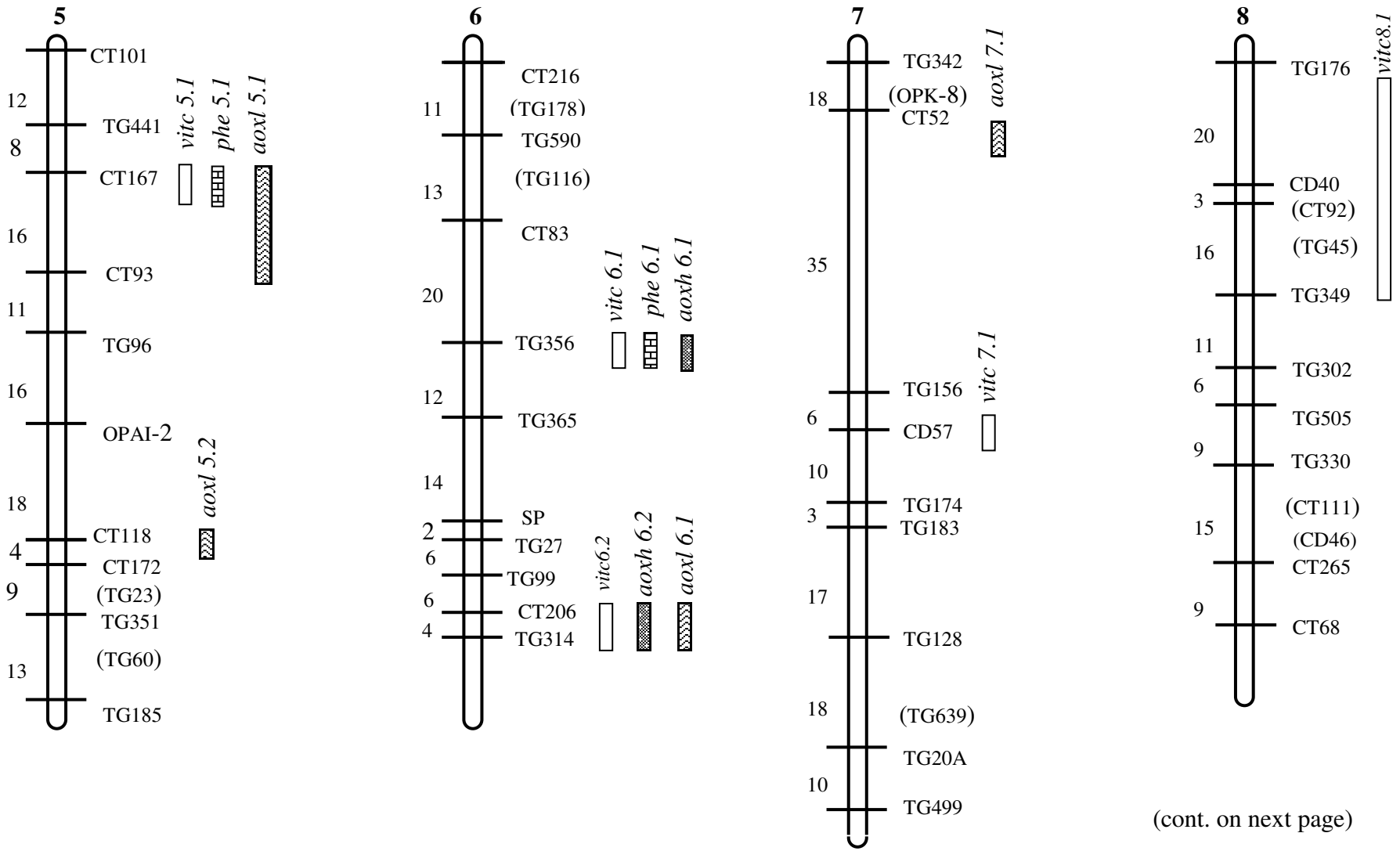

Figure 3.8.(cont.)

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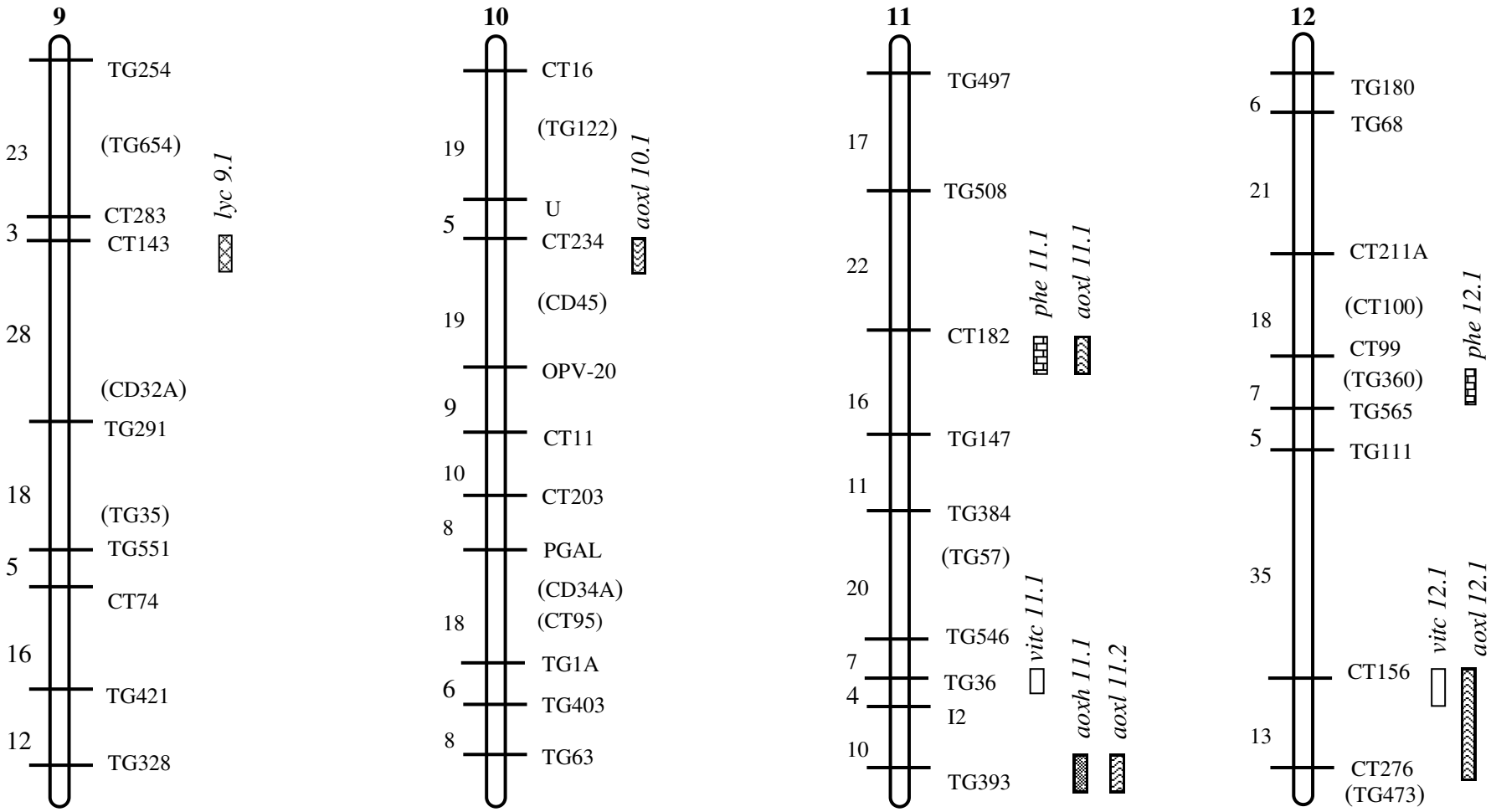

Figure 3.8. (cont.)

3.4.1. Hydrophilic Antioxidant Activity

Four QTL for antioxidant activity in hydrophilic fraction were identified. The most significant QTL, *aohx6.2*, (P = 0,001) was on chromosome 6 and explained 7.5% of the phenotypic variance for this trait. A second locus was also identified on chromosome 6, aoxh6.1, and accounted for 8,4% of the variance. For three of the four QTL, the alleles from the wild parent, *L. pimpinellifolium*, were the source of higher antioxidant activity. However, for *aoxh11.*1, on chromosome 11, the *L. esculentum* allele was associated with increased antioxidant activity.

3.4.2. Lipophilic Antioxidant Activity

Eleven significant (p<0,01) QTL were identified for antioxidant activity in the lipophilic fraction. The most significant QTL is *aoxl 11.2* with a P value less than 0,0001. Its contribution to the phenotype is 27,9 %. The other QTL, which were highly significant, were on chromosome 2 (*aoxl 2.1* and *aoxl 2.2*) and chromosome 5 (*aoxl 5.1* and *5.2*). The source of high antioxidant activity for all of these loci was the wild type allele. The contributions of these loci to phenotypic variance were 18,9%, 13,6%, 10,9% and 16,2%, respectively. Besides the highly significant QTL, another locus was identified on chromosome 10 (P=0,0019). For this QTL, the source of high antioxidant activity was the *L.esculentum* allele.

3.4.3. Vitamin C

Nine QTL for vitamin C were identified. *Vitc 8.1,* marked by CD40, and *vitc 7.1*, marked by CD57, were the most significant QTL (P- values = 0,0002 and 0,0006, respectively) The contribution to the total phenotype (R^2 value) of *vitc8.1* was 11,8%. In addition, R^2 value of *vitc7.1* was 10,1%. Except for two QTL, *L. pimpinellifolium* alleles were associated with higher vitamin C content for all QTL. *L. esculentum* was the source of high vitamin C content for QTL on chromosome 5 and chromosome 12. These QTL, *vitc 5.1* (P= 0,0094) and *vitc 12.1* (P= 0,0054), had 6,6% and 7,3% contributions to the total phenotype, respectively.

3.4.4. Phenolic Compounds Content

Six QTL were identified for phenolic compounds content. They were on chromosomes 1, 4, 5, 6, 11 and 12 with the markers TG301, TG163, CT167, TG365, CT182 and TG360, respectively. The QTL with the highest contribution to the phenotype was on chromosome 5 (R^2= 10,4%) and the L.esculentum allele was the source of high phenolic compounds content. The only other QTL for which the *L. esculentum* allele was the source of high phenolic compound content was *phe 12.1*. The *L .pimpinellifolium* parent was associated with the higher phenolic compounds content for the other QTL.

3.4.5. Lycopene

In this experiment, lycopene content was found to be controlled by one QTL on chromosome 9. This locus was marked by CT283. The contribution of this QTL to the phenotype was 6,1 % with a P-value of 0,013. The source of high lycopene content was the *L.esculentum* allele.

3.5. Colocalization of QTL

The QTL for the five traits and their possible colocalizations were determined. Clusters of these QTL help explain the correlations between these traits and also give an idea about the genes controlling these traits and the interactions between them. Loci for hydrophilic and lipophilic antioxidant activities colocalized on chromosome 1, chromosome 6 and chromosome 11. These colocalizations support their strong and positive correlations. Most likely their pathways or the key points of the pathways are controlled by the same loci. Hydrophilic antioxidant activity colocalized with vitamin C QTL on chromosome 1 and in two locations on chromosome 6. Vitamin C has a hydrophilic characteristic so genes that increase vitamin C content would also be expected to increase overall hydrophilic antioxidant activity.. On the other hand, lipophilic antioxidant activity loci and vitamin C loci were clustered on chromosomes 1, 2, 5, 6 and 12. The reason for such clusters may be strong correlations between

hydrophilic and lipophilic antioxidant activities. While vitamin C was associated with hydrophilic antioxidant activity, the same trait might be associated with lipophilic antioxidant activity. In addition to antioxidant activity loci, vitamin C QTL colocalized with QTL for phenolic compounds content. On chromosomes 1, 5 and 6 this colocalization was observed. Their similar pathways and complementary action against reactive oxygen species support their positive correlation and colocalizations. These same QTL clusters for vitamin C and phenolic compounds content were also associated with antioxidant activity loci. The loci marked by TG301 on chromosome 1 are an example for such an association. In addition, on chromosome 5 and chromosome 11, phenolic compounds loci clustered with loci for antioxidant activity in lipophilic fraction. However on chromosome 6 phenolic compounds and hydrophilic antioxidant activity QTL colocalized. The explanation of these clustering might be the high percent of contribution of phenolic compound contents to the total antioxidant activity.

3.6. Possible conservation of QTL

In this research an IBL population was used to map traits related to antioxidant activity. The locations of the loci controlling these traits were compared with previously mapped QTL and mutant genes. QTL analysis of antioxidants in tomato was also done in *Lycopersicon pennellii* introgression lines (ILs) by Rousseaux et al. (2005). Some QTL in this previous research matched with QTL identified in the current research. In order to declare that two QTL matched, they had to be located within 20 cM of each other. For example, a QTL (*ao6*-2) for hydrophilic antioxidant activity in ILs overlapped with a QTL (*aoxh 6.*1) for the same trait in IBLs. In addition *ao6*-3 matched with *aoxh6.*2. QTL for vitamin C (ascorbic acid) content in ILs, which were *aa12-3* and *aa12-4,* had a possible match with *vitc12.1* in IBLs. Phenolic compound content of ILs had also QTL match with IBLs. The overlapped QTL for the phenolic compounds were *phe6-2* in ILs and *phe6.1* in IBLs. Thus, the locations of several QTL were confirmed by the presence of similar loci in a different population. Moreover, these results suggest that genes controlling antioxidant traits have been conserved during evolution of cultivated tomato and its wild relatives from a common ancestor.

In an attempt to learn more about the QTL identified in this study, their locations were compared with the positions of known color and ripening mutations in tomato. Table 3.5 lists these color mutants and their locations.

Table 3.5. Color and ripening mutants of tomato and their locations

Name of mutant	Symbol	Location	Fruit Color
β-Carotene	*B*	Chromosome 6	Orange
Beta modifier	Mo_B	Chromosome 6	Orange
Tangerine	*t*	Chromosome 10	Orange
Delta	*Del*	Chromosome 12	Reddish orange
High Pigment	*hp*	Chromosome 2	Dark Red
High Pigment	*hp2*	Chromosome 1	Dark Red
Green Stripe	*gs*	Chromosome 7	Red with stripe
Green Flesh	*gf*	Chromosome 8	Purplish brown
Yellow Flesh	*r*	Chromosome 5	Yellow
Never Ripe	*Nr*	Chromosome 9	Green
Ripening Inhibitor	*rin*	Chromosome 5	Green
Non-ripening	*nor*	Chromosome 10	Green
Alcobaca	*alc*	Chromosome 10	Green

Some color mutants were associated with QTL that were identified in this research. Some of these mutant genes were found close to antioxidant QTL (within 20 centimorgan, cM). For example beta locus (*B*) and modified beta locus (Mo_B) on chromosome 6 were marked by $OPAR18_{1100}$ and UBC 792_{830} (Zhang and Stommel 2000). These loci are two of the loci responsible for β-carotene content of tomato at the expense of lycopene and give orange color to tomato. QTL for hydrophilic and lipophilic antioxidant activity were located within 20 cM of B and Mo_B. Thus, part or all of the effect on antioxidant activity displayed by this QTL may, in fact, be caused by the β-carotene gene at this location. The tangerine (*t*) mutant causes trans- lycopene instead of cis- lycopene in tomato (Isaacson et al. 2002). This change of isomers gives rise to orange color. The tangerine locus on chromosome 10 was mapped based cloned by Isaacson et al. (2002). According to markers of tangerine there was no relationship between this gene and the QTL identified in this work. High pigment mutants (*hp* and *hp2*) give dark red color to tomato. It could be estimated that there was a relationship between *hp* locus and the QTL for lipophilic antioxidant activity on chromosome 2 as they were located within 20 cM of each other. On chromosome 1, QTLs for all traits

except lycopene were within 20 cM of the *hp2* mutant. Green stripe (*gs*) mutant on chromosome 7 had a possible match with vitamin C QTL on the same chromosome. The green flesh (*gf*) mutant on chromosome 8 was possibly close to the vitamin C QTL on chromosome 8. Yellow flesh (*r*) locus codes for phytoene synthase, an enzyme which condenses two molecules of geranyl geranyl diphosphate and is the first of four specific enzymes necessary for β-carotene biosynthesis in plants (Burkhardt et al. 1997), and may have possible match with lipophilic antioxidant activity QTL on chromosome 5 (Moore et al. 2002). The ripening inhibitor mutant (*rin*) on chromosome 5 also has a possible match with a lipophilic antioxidant activity QTL. Thus, overlaps between mutant genes and QTL help in understanding the possible gene action and metabolic pathways affected by each QTL.

CHAPTER 4

CONCLUSION

In this research, Inbred Backross Lines (IBLs), derived from an initial cross between *L. esculentum* and *L. pimpinellifolium* LA1589, were grown in the field as three replicates. The tomatoes were harvested when they were fully ripe for biochemical analysis and the resulting data were used for molecular mapping of health related traits. The aims of this research were to survey the antioxidant capacity of the IBLs and to map the loci controlling antioxidant traits with molecular markers. It was found that the antioxidant capacity of the IBLs was similar to the *L. esculentum* parent. This was expected because the population was fixed by backcrossing and was carrying mostly *L. esculentum* alleles. On the other hand, some lines had high antioxidant capacity that showed the effect of *L. pimpinellifolium* alleles in the introgressed regions. Mapping with molecular markers supported the known relationships between antioxidant traits as it was found that QTL for related traits were colocalized. The mapping results were also compared with previous research and it was determined that several QTL were conserved. The knowledge obtained from the current research could be used for different purposes in the future. Breeding high nutrition capacity tomatoes can be one of the purposes. Some lines in the IBL population had high antioxidant activity like *L.pimpinellifolium* parent, while most of IBLs have moderate antioxidant activity like *L.esculentum* parent. The lines with high antioxidant activity could be commercially produced. In order to develop such lines, lines with high antioxidant capacity should be chosen. These lines should be backcrossed to *L. esculentum* with genotypic and phenotypic selection to retain the wild type alleles for high antioxidant capacity and to remove alleles and traits with negative effects. In addition, the QTL for any of the antioxidant traits could be cloned and sequenced. Such information would reveal the protein product of the locus and its mode of action. Thus, sequenced QTL could help explain the mechanisms of many pathways such as β-carotene production and neutralization of reactive oxygen species. Transgenic tomato lines with high antioxidant capacity could also be developed using the information gained from gene isolation.

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