METABOLIC AND GENETIC PROFILING FOR PRIMARY AND SECONDARY METABOLITES IN TOMATO

A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in Molecular Biology and Genetics

by
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January 2017
İzmir

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ACKNOWLEDGMENTS

First of all, I would like to thank my supervisor Prof. Dr. Sami Doğanlar and committee member Prof. Dr. Anne Frary for their support, advice, concern, patience and most importantly their trust in me and my decisions during my doctorate education and thesis.

Special thanks to Specialist Neslihan Tek for helping me during metabolic characterization, Asst. Prof. Ali Uncu for helping to interpret GBS data, Res. Asst. İbrahim Çelik for helping with QTL analysis, and lastly thanks to Multi Tarım Company for growing the tomato population.

Thanks to my friends in IYTE, especially those in my laboratory for their help, patience, and understanding.

Most importantly, I would like to thank my family for their endless support, understanding and love all these years.

ABSTRACT

METABOLIC AND GENETIC PROFILING FOR PRIMARY AND SECONDARY METABOLITES IN TOMATO

Understanding the metabolic content of plants and its genetic basis is important to determine the most appropriate breeding strategies for traits such as yield, fruit quality, nutritional content, tolerance to disease factors, adaptation to various environmental conditions, and tolerance to abiotic and biotic stresses. Recently, post-genomic studies such as metabolomics, proteomics and transcriptomics are attracting attention and being used in conjunction with genomic studies. These studies help to speed research progress with crops as well as model plants. Tomato (*Solanum lycopersicum)* is an important crop which is cultivated widely in the world and in our country, and is also important for our daily diet. Although there is growing attention to studies on tomato day by day, there are limited studies that identify the quantitative trait locus (QTL) regions responsible for important agronomic, economic, nutritional and health related traits by correlation of metabolomics and genomics data. In this study, an interspecific IBL (inbred backcross line) population derived from the cross *S. lycopersicum* cv. Tueza x *S. pimpinellifolium* (LA1589) was both genotyped via genotyping by sequencing (GBS) and quantified for primary and secondary metabolites affecting yield, quality and nutritional value of the fruit to determine QTL regions for the targeted metabolites. In total, 187 QTLs were identified for 143 important traits.

The data obtained from this study will help to shed light on the genetic control of plant metabolism and to develop high yield and nutrient-rich cultivars with improved agronomic traits by breeding strategies.

ÖZET

DOMATESTE BİRİNCİL VE İKİNCİL METABOLİTLER İÇİN METABOLİK VE GENETİK PROFİLLEME

Bitkilerin metabolik içeriklerinin ve genetik esaslarının anlaşılması verim, meyve kalitesi, besinsel içerik, hastalık etmenlerine dayanıklılık, farklı çevre koşullarına adaptasyon, abiyotik ve biyotik stres töleransı gibi karakterlerin ıslahı için en uygun ıslah stratejilerinin geliştirilmesinde önemlidir. Son zamanlarda genetik çalışmalar ile birlikte metabolomik, proteomik ve transkriptomik gibi post-genomik çalışmalar da ilgi çekmektedir. Bu çalışmalar model bitkilerde olduğu kadar tarla bitkilerinde de yapılan araştırmalara hız kazandırmıştır. Domates (*Solanum lycopersicum)* dünyada ve ülkemizde geniş ölçüde tarımı yapılan ve günlük diyetimizde önemli bir yer tutan tarım ürünlerinden birisidir. Günden güne domateste yapılan çalışmalara ilgi artmasına rağmen, önemli agranomik, ekonomik, besinsel ve sağlıkla ilgili karakterlerden sorumlu kantitatif karakter lokus bölgelerini belirleyen sınırlı sayıda çalışma vardır. Çalışmamızda hedef metebolitlerden sorumlu QTL bölgelerinin belirlenmesi için *S. lycopersicum* cv. Tueza x *S. pimpinellifolium* (LA1589) çaprazlanması ile ilde edilen interspesifik IBL (inbred geri çaprazlanmış hat) populasyonu hem sekans ile genotiplemeyle (GBS) genotiplenmiş hem de meyvenin verimini, kalitesini ve besinsel içeriğini etkileyen birincil ve ikincil metabolitler ölçülmüştür. Toplamda 143 önemli karakter için 187 QTL tanımlanmıştır.

Bu çalışmadan elde edilen veriler bitki metabolizmasının genetik kontrolünü aydınlatmaya ve geliştirilmiş agranomik karakterler ile verimi ve besinsel içeriği yüksek olan kültürlerin geliştirilmesi için ıslah çalışmalarına yardımcı olacaktır.

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INTRODUCTION

1.1. Plant Domestication and Breeding

The world population reached 7 billion people in 2015 and it is estimated that it will grow 30 % over the next 35 years and reach 9 billion by the year 2050 (Fita et al. 2015). As a result of its huge population, hunger and malnutrition are realities of today's world. More than 800 million people suffer from hunger with 15 million people dying of this cause each year (Esquinas-Alcázar 2005). Agricultural production should increase at least 60 to 70% to cope with the current problem and to meet increasing food demands from the growing population (Alexandratos and Bruinsma 2012; Godfray et al. 2010). Thus, plant breeding and the development of new cultivars are essential.

The origins of plant breeding lie in plant domestication which was a key factor for the transition from the hunter-gather lifestyle to agriculture. Domestication is the process of gradually changing a species for human benefit by selection, mating and growth under agriculture (Gepts 2014). Morphological, physiological, and genetic comparisons between wild crop relatives and existing crops suggest that farming and domestication is less than 10.000 years old (Vaughan, Balazs, and Heslop-Harrison 2007).

Plant domestication and breeding are crucial for improving the yield and quality of crops in order to meet human food demands (Heslop-Harrison and Schwarzacher 2011). Domesticated crops differ from their wild progenitors because selection and growth under agriculture have led to morphological and physiological changes such as size of harvested part, yield, yield stability and product quality (Gepts 2014; Vaughan, Balazs, and Heslop-Harrison 2007). As a result, most domesticated plants need human intervention for survival and reproduction.

Improved genomic techniques allow more efficient and effective selection of plants for domestication and breeding. Selection of appropriate individuals based on genotype was a big step forward for agriculture (McClure et al. 2014). Future agricultural production depends on the wise use and conservation of agricultural biodiversity and

genetic resources. The Industrial Revolution caused rapid changes in population size, ecological degradation, agricultural mechanization and globalization which resulted in reduction of crop genetic diversity (Esquinas-Alcázar 2005). The Green Revolution also dramatically affected genetic diversity. Between the 1940s and 1970s new scientific and technical approaches in agriculture led to dramatically improved crop yields. This era in agriculture is called the Green Revolution (GR) (Borlaug and Dowswell 2005). Major innovations in the cultivation of wheat were made by the development of high yielding, disease-resistant and semi-dwarf cultivars. These innovations spread to other crops. Unfortunately, the increases in yield obtained during the GR required monoculture practices, massive use of agrochemicals including pesticides and chemical fertilizers, mechanization of labor, and increased irrigation of crop land. The development of high yielding and standard, homogeneous varieties by public and private plant breeders had a high price: the loss of local species and innumerable heterogeneous traditional farmers' varieties. Thus, the GR was accompanied by a loss of crop genetic diversity which is also known as genetic erosion. Genetic erosion has reduced the amount of diversity in the gene pool that is available for both natural and artificial fertilization and selection. As a result, today's agricultural crops are more vulnerable to changes in climate and the appearance of new pests and diseases (Esquinas-Alcázar 2005).

In other words, although GR brought unquestionably positive effects, it also caused serious side effects that may hamper future progress in plant breeding. One aspect of this problem is the fact that the high-input agricultural systems used during the GR may not be sustainable. These systems rely on greenhouses for continuous production of certain crops throughout the year, lack of crop rotation, the massive and uncontrolled use of chemical fertilizers and pesticides, and the cultivation of crops with high water requirements in semiarid regions. These negative aspects may cause serious problems today and in the near future as new pathogens/diseases arise and as climate change leads to depletion, contamination, and/or salinization of soil and ground water (Dehaan and Taylor 2002; Mcdonald and Linde 2002).

The GR also had direct effects on the human diet. The total amount of protein and energy available to people increased in staple crops but not the nutritional value of the the crop. In addition, the variety of products consumed by people, especially poor people, was reduced, causing malnutrition or "hidden hunger" (Welch and Graham 2002).

1.1.1. Domestication Syndrome

The term "domestication syndrome (DS)" describes traits that were selected for during the domestication process and which make domesticated crops distinctive from their wild relatives. These traits are phenotypic characteristics which are associated with seed retention and germination, growth habit, size, color, and edible parts of the crop. These characteristics overcome problems with germination/planting, cultivation, and poor harvest (Heslop-Harrison and Schwarzacher 2011; Gur and Zamir 2004; Meyer, Duval, and Jensen 2012; Sakuma and Komatsuda 2011). Genetic and genomic research done to date prove that the allelic diversity present in domesticated species is less than found in their wild relatives/ancestors (Heslop-Harrison and Schwarzacher 2011). This loss of genetic diversity in crops is known as a "genetic bottleneck" (Doebley 2004; Doebley, Gaut, and Smith 2006; Xu et al. 2011). Such bottlenecks arise during domestication and breeding because only favorable genotypes are retained during selection (Shi and Lai 2015). The loss of genetic diversity varies from crop to crop depending on mating system and amount of selection/breeding. For example, while the reduction in variation is less than one fold in rice and maize, it is about threefold in tomato and cucumber (Huang et al. 2012).

1.2. Tomato

1.2.1. Botanical Description of Tomato

Tomato is an economically important plant belonging to the *Solanaceae* family which contains more than 106,300 species including potato, eggplant, pepper, petunia, physalis and tobacco. The largest genus of the *Solanaceae* family is Solanum which contains 1250 to 1700 species. *Solanum* plants can be grown on all temperate and tropical continents and show a wide range of morphological and ecological diversity. The genus contains species producing medicinal compounds and economically important crops (Weese and Bohs 2007). Tomato was identified as *Solanum pomiferum* when it was introduced to Europe in the 16th century. Although Linnaeus classified tomato under the specific name *Solanum lycopersicum* in 1753, the genus assignment of tomato was controversial for a long time (Foolad 2007; Peralta and Spooner 2007). In 1940, Müller

regrouped and identified six tomato species separated into two sections. The first subgenus was eriopersicon including *Lycopersicon peruvianum, L. cheesmaniae, L. hirsutum* and *L. glandulosum*. The second subgenus was eulycopersicon, including *L. esculentum* (cultivated tomato) and *L. pimpinellifolium.* In the 1960s and 70s, Rick proposed a classification for tomato based on the abilty of the wild speices to cross with cultivated ones. He classifed wild tomato species into two groups based on their crossability: the Esculentum section and the Peruvianum section. Although the Peruvianum section displays extreme diversity and potential for crop improvement, usage of this group is restricted by difficulties in hybridization with cultivated tomato and the need for special techniques such as embryo rescue for producing hybrids (Foolad 2007). The Esculentum section consisted of *L. esculentum, L. pimpinellifolium, L. cheesmaniae, L. pennellii, L. hirsutum, L. chmielewskii* and *L.parviflorum*, and the Peruvianum section consisted of *L. chilense* and *L. peruvianum* (Bergougnoux 2014).

In the 2000s it was understood that the classification of tomato is more complex. A phylogenetic study based on the sequence of the granule-bound starch synthase gene placed tomato in the Solanum section Lycopersicon and divided it into three groups: series Lycopersicon, series Eriopersicon and series Neolycopersicon (Peralta and Spooner 2001) (Figure 1). Current names and features of the wild tomato species are summarized in Table 1.

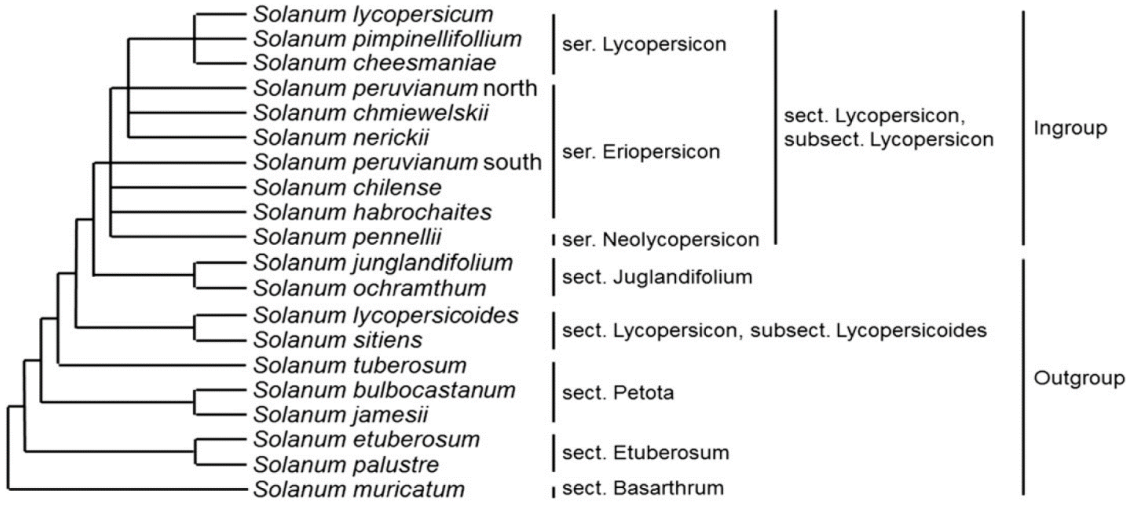

Figure 1. General phylogenetic tree based on the analysis of GBSSI gene sequences (Source: Peralta and Spooner 2001).

Table 1. Comparision of wild tomato species (Solanum L. section *Lycopersicon* subsection *Lycopersicon*) (Source: Peralta, Knapp, and Spooner 2005; Spooner, Peralta, and Knapp 2005). SC: self-compatible, SI: self-incompatible, At: autogamous, Al: allogamous

Species (*Solanum* name)		***Lycopersicon* equivalent**	**Fruit color**	**Reproductive system**	**Importance for breeding purposes**
S. cheesmaniae		*L. cheesmaniae*	Yellow, orange	SC, exclusively At	Salt tolerance; Lepidoptera and virus resistance
S. galapagense		*L. cheesmaniae var. minor*	Yellow, orange	SC, exclusively At	Salt tolerance; Lepidoptera and virus resistance
S. lycopersicum		*L. esculentum*	Red	SC, facultative Al	Moisture tolerance, resistance to wilt, root-rotting, and leaf-spotting fungi
S. pimpinellifolium		*L. pimpinellifolium*	Red	SC, At, facultative Al	Color and fruit quality; resistance to insect, nematode and disease
S. chilense		*L. chilense*	Green, purple stripes	SC, Al	Drought resistance
S. chmielewskii		*L. chmielewskii*	Green	SC, facultative Al	High sugar content
S. habrochaites		*L. hirsutum*	Green	SI	Cold and frost tolerance; resistance to insects due to their glandular hairs
S. pennellii		*L. pennellii*	Green	SI	Drought resistance; resistance to insects
S. neorickii		*L. parviflorum*	Pale green	SC, At	
S. peruvianum north	*S. arcanum*	*L. peruvianum var. hirsutum*	Green	Typically SI, Al, rare population	Resistance to virus, bacteria, fungi, aphid and nematode
	S. huaylasense	*L. peruvianum*	Green	SC, At with a trend to reduce variability in Northern races	
S. peruvianum south	*S. peruvianum*	*L. peruvianum*	Green		
	S. corneliomuelleri	*L. peruvianum var. glandulosum*	Green		

1.2.2. Habitat and Diversity of Tomato

Wild tomato species grow in a wide range of habitats in arid to rainy climates. They are native to western South America including coastal and high Andes regions ranging from central Ecuador, through Peru, to northern Chile, and the Galapagos Islands. In general wild species are adapted to a particular climate and soil type and it is thought that both different ecological habitats and climates have contributed to wild tomato diversity. As seen in Table 1, morphological, physiological and sexual characteristics contribute to wild tomato's diversity (Peralta, Knapp, and Spooner 2005; Spooner, Peralta, and Knapp 2005).

1.2.3. As a Model Plant

Tomato is an excellent model crop because it: (1) has a relative short life cycle, (2) can be grown in different conditions and has adaptability to abiotic stress conditions, (3) has high self-fertility and homozygosity and is easily hybridized, (4) is photoperiod insensitive, (5) can be propagated asexually by grafting, (6) can be regenerated from different parts, (7) has a simple and relatively small genome, (8) lacks extensive gene duplication, and (9) has a sequenced genome. The genomes of cultivated tomato and its closest relative *S. pimpinellifolium* were published in 2012 (Tomato Genome Consortium) while the *S. pennellii* genome was published in 2014 (Bolger et al. 2014). To date, partial sequence data is available for approximately 360 accessions (Aflitos et al. 2014; Lin et al. 2014). As a model species, tomato has been the subject of many scientific studies in both theoretical and applied science. For example, tomato has been used to study fleshy fruit development (Klee and Giovannoni 2011), gene regulation (Karlova et al. 2014; Rohrmann et al. 2011; Seymour et al. 2013) and metabolic shifts during fruit development and ripening (Carrari and Fernie 2006; Tohge, Alseekh, and Fernie 2014).

1.2.4. Economic Importance

Tomato is part of the daily diet in most of the world and is widely used both fresh and also processed in products such as paste, soup, juice, powder and concentrate. Worldwide tomato production was 160,443,775 tons in 2013 and it is one of the most important crops after maize, rice, wheat, potatoes, soybeans and cassava (FAOSTAT). During the last two decades worldwide tomato production and production area heve nearly doubled (Fig. 2).

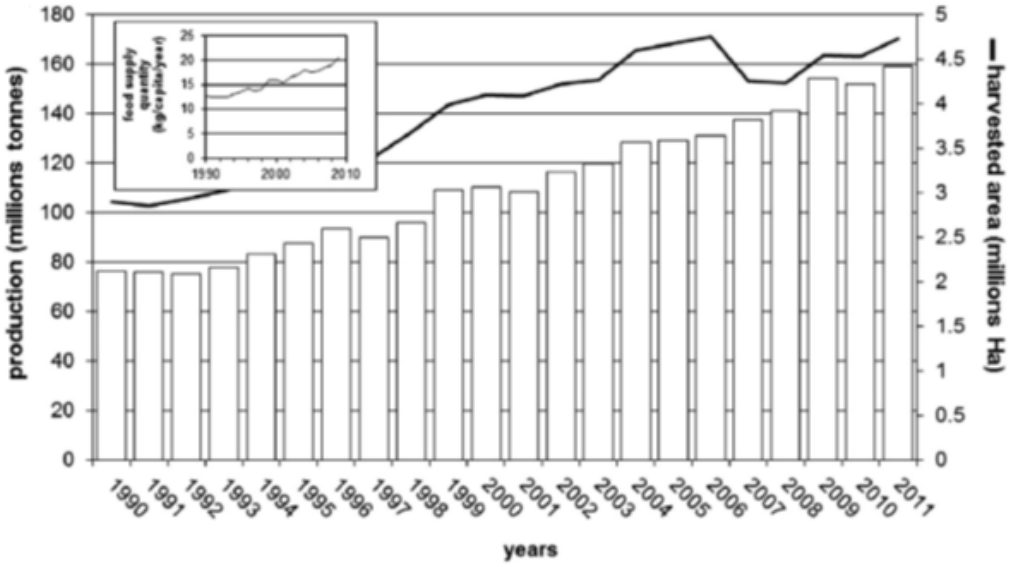

Figure 2. Changes in worldwide tomato production and harvested area based on years (Source: Bergougnoux 2014).

In the 1990s the United States dominated the tomato market, today Asia is the most imporatant producer. While China was the top producer in 2013, Turkey ranked fourth after India and the USA with 11,820,000 tonnes. (Fig. 3) (FAOSTAT). Thus, tomato is important for Turkey's economy. Our country exported 585,478 tonnes fresh tomato and was fifth among tomato exporters worldwide. Production has increased slowly over the past 15 years (Fig. 4) (TUIK 2016).

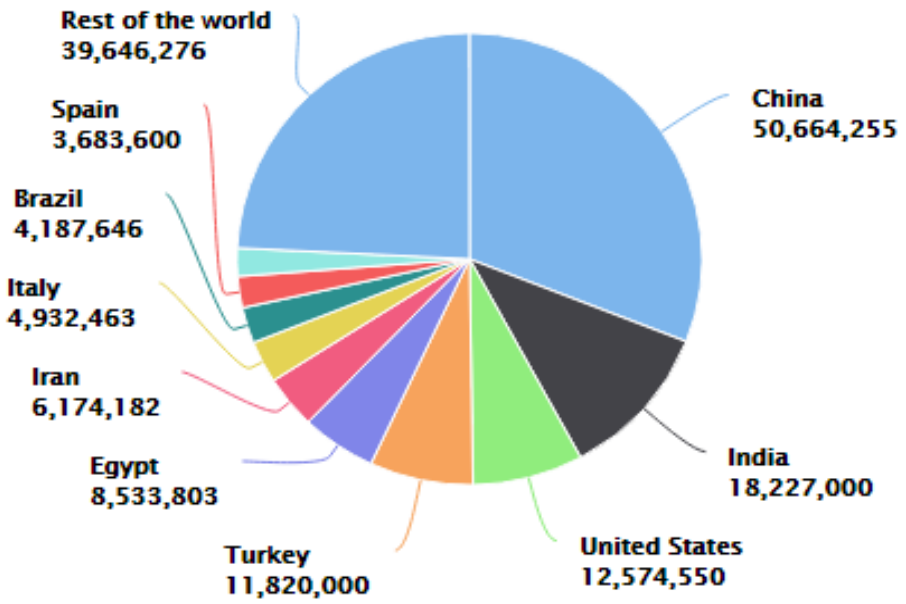

Figure 3. Top tomato producers and production in quantities (tonnes) in 2013 (Source: FAOSTAT).

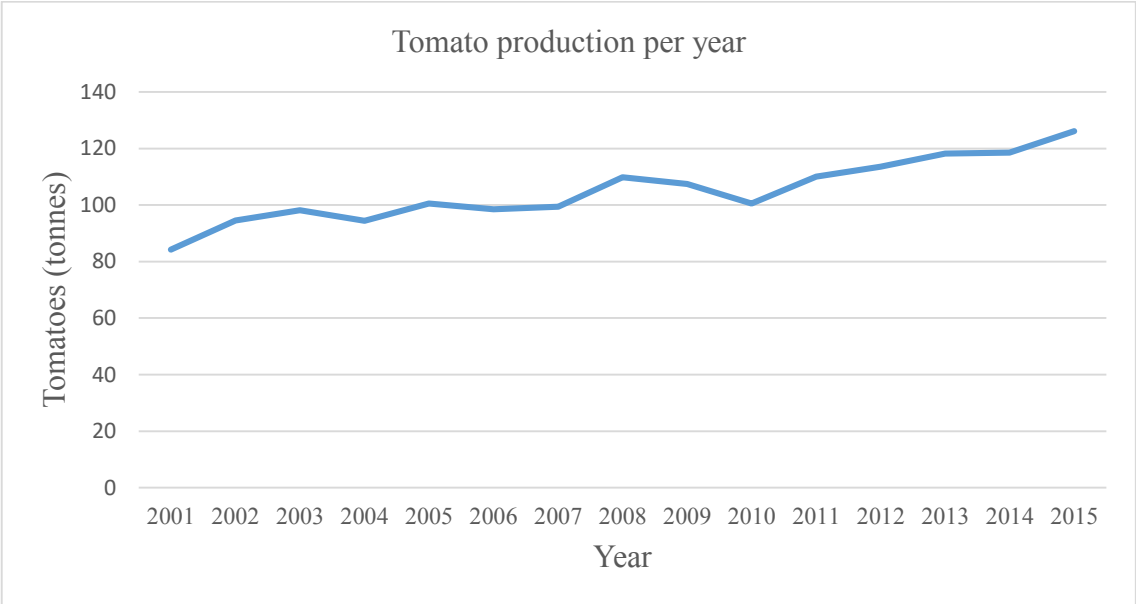

Figure 4. Tomato production in Turkey over the past 15 years (Modified from TUİK).

1.3. History Of Tomato Breeding

1.3.1. Domestication of Tomato

The origin of tomato domestication is still under debate with two hypotheses under consideration: Peruvian vs. Mexican origin. The Peruvian hypothesis was first described by De Candolle in 1882 who claimed that initial domestication was achieved in Peru and then tomato was transported from Peru to Europe. According to this hypothesis, cultivated tomato originated from the wild cherry tomato which grew in coastal Peru, and distribution of cultivated tomato occurred by garden escapes from Peru (Peralta and Spooner 2007). The Mexican hypothesis was first described by Jenkins in 1948. His hypothesis was based on the fact that there was no early evidence of tomato cultivation in South America whereas good evidence was available in Mexico. He also claimed that the word "tomato" comes from Mexican "tomatl" which means "plants bearing globous and juicy fruit" (Bauchet and Causse 2012). In a recent study based on single nucleotide polymorphism, it was confirmed that *S. lycopersicum var. cerasiforme* is the ancestor of cultivated tomato, and that domestication of tomato began in the Andean region and was completed in Mesoamerica. These findings support both the Peruvian and Mexican hypotheses as both areas had important roles in the crop's domestication. (Blanca et al. 2012).

1.3.2. Tomato Breeding

At the end of the 19th century, numerous cultivars of tomato were available which were products of domestication and early breeding and considered as landraces. At the begining of the 20th century, public institutes and private companies became more involved in tomato breeding and breeding shifted from open pollinated cultivars to hybrids. Hybrids carry desired characters from both parents and this provides an advantage over inbred lines. On the other hand, hybrid varieties are more expensive and discourage seed propagation by farmers. The first hybrid tomato cultivar, "Single Cross," was released to the market in 1946. Today nearly all tomato cultivars for both fresh market and processing are hybrids (Bai and Lindhout 2007).

Fresh tomatoes are available in a wide range of shape, size and color; from small cherry to large beef and from pink to black tomatoes. Breeders improve their breeding lines using naturally occurring recombination events to produce cultivars with favorable traits by new crosses within their own material or using their competitors' cultivars. Although wild relatives represent high genetic diversity, new traits are rarely introduced from wild germplasm because it takes years to remove deleterious genes by backcrossing and selection (Bai and Lindhout 2007).

Despite the difficulties associated with using wild species, their genetic variation has been examined to find new resources for tomato improvement (e.g. Walter 1967; Rick and Chetelat 1995; Larry and Joanne 2007). Cultivated tomato has reduced variability compared to its wild relatives. It is estimated that cultivars contain only 5% of the genetic variation of their wild relatives (Miller and Tanksley, 1990). Although sensitive molecular markers were used, very few polymorphisms could be identified in the cultivated tomato gene pool (García-Martínez et al. 2006; Park, West, and Clair 2004; Tam et al. 2005; van Der Beek et al. 1992; Villand et al. 1998). This decreased diversity is because selection of tomato during domestication and early breeding was done on a single plant basis with few plants in the germplasm. In addition, genetic variation tends to decrease in inbreeding species even without selection (Bai and Lindhout 2007). Breeders have tried to reintroduce some favorable traits from the wild relatives of tomato. These traits include biotic and abiotic stress tolerance (Zamir 2001, Takeda and Matsuoka 2008, Frary et al. 2010) and fruit size and shape variation (Tanksley 2004). This approach pre-dates the use of transgenic approaches (Tieman et al. 2010) and metabolic engineering (Fridman et al. 2004; Mutschler et al. 1996; Perez-Fons et al. 2014; Schauer et al. 2006; Schilmiller et al. 2010). Agronomic traits of interest available from wild tomato species are summarized in Table 2.

Table 2. Non-exhaustive list of agronomic traits of interest available from wild tomato species (Source: Bergougnoux 2014).

Phenotype	**Germplasm source (*Lycopersicum* name)**
Biotic stress	
Resistance to bacteria	*L. pimpinellifolium, L. hirsutum, L. pennellii, L. peruvianum*
Resistance to fungi	*L. cheesmaniae, L. esculentum, L. pimpinellifolium, L. chilense, L. hirsutum, L. pennellii, L. parviflorum, L. peruvianum*
Resistance to virus	*L. esculentum, L. pimpinellifolium, L. chilense, L. hirsutum, L. peruvianum*
Resistance to insects	*L. pimpinellifolium, L. peruvianum*
Abiotic stress	
Cold (low temperature)	*L. pimpinellifolium, L. hirsutum*
Drought	*L. pimpinellifolium, L. pennellii*
Salt	*L. pimpinellifolium, L. pennellii*
Plant characteristics	
Branch number	*L. cheesmanii*
Male sterility	*L. pimpinellifolium*
Growth habit	*L. peruvianum, L. pimpinellifolium*
Height	*L. pennelli, L. pimpinellifolium, L. hirsutum, L. cheesmanii*
Self-pruning	*L. chmielewskii, L. pimpinellifolium*
Fruit characteristics	
Antioxidant capacity	*L. pennellii*
Ascorbic acid	*L. pennellii*
Citric acid	*L. pennelli*
Color	*L. pimpinellifolium, L. peruvianum, L. hirsutum, L. parviflorum, L. chmielewski, L. pennellii*
β-carotene	*L. cheesmanii, L. hirsutum, L. pennellii, L. parviflorum*
Lycopene	*L. pimpinellifolium, L. parviflorum, L. pennellii*
Orange	*L. pennellii*
Yellow	*L. parviflorum*
Cracking	*L. pennellii, L. pimpinellifolium*
Diameter	*L. pimpinellifolium*
Shape	*L. pimpinellifolium, L. peruvianum, L. hirsutum, L. parviflorum, L. pennellii*
Firmness	*L. pimpinellifolium, L. peruvianum, L. hirsutum, L. parviflorum, L. chmielewskii, L. pennellii*
Sugars	*L. pennellii, L. hirsutum*

(cont. on next page)

Table 2. **(cont.)**

Phenotype	**Germplasm source (*Lycopersicum* name)**
Length	*L. pimpinellifolium*
Locule number	*L. pimpinellifolium*
Maturity	*L. pimpinellifolium, L. peruvianum, L. hirsutum, L. parviflorum*
Ripening	*L. pennellii, L. pimpinellifolium, L. peruvianum, L. cheesmanii*
Soluble solids	*L. chmielewskii, L. cheesmanii, L. pennelli, L. pimpinellifolium, L. Hirsutum*
Viscosity	*L. pimpinellifolium, L. peruvianum, L. hirsutum, L. parviflorum, L. pennellii*
Weight	*L. pennellii, L. chmielewskii, L. cheesmanii, L. pimpinellifolium, L. peruvianum, L. hirsutum*
Yield	*L. chmielewskii, L. pennelli, L. pimpinellifolium, L. peruvianum, L. hirsutum, L. parviflorum*
Jointless	*L. cheesmanii*

1.3.3. Traits of Modern Tomato Cultivars

Although the goals of tomato breeding programmes depend on location, use and resources, generally breeding goals have gone through four stages: (1) in the 1970s breeding to improve yield, (2) in the 1980s breeding to improve shelf-life, (3) in the 1990s breeding to improve taste and flavor, and (4) current breeding to improve nutritional quality. Considered from an economic perspective, breeding goals have not changed: low production costs with maximum yield of high quality fruit (Bai and Lindhout 2007). Thus many characters should be taken into account in breeding programmes.

1.3.3.1. Yield

Yield represents not only fruit number but also fruit weight. A cultivar with improved agronomic or nutritional traits will not be considered in breeding programmes if it does not have high yield. Yield is a complex trait and is affected directly or indirectly by many genetic and environmental factors. For example, temperature influences plant growth and plant growth influences yield. In one study, researchers were able to increase

yield under hot and humid conditions by breeding tomatoes for resistance to high temperature (Scott, Bryan, and Ramos 1997).

1.3.3.2. Heterosis

Heterosis occurs when a hybrid is more vital, adaptive and productive compared to its parents. The genetic basis of heterosis is still unclear but it is thought that dominancy and additive effects induce heterosis (Birchler, Yao, and Chudalayandi 2006; Semel et al. 2006). Besides the advantage of heterosis, breeders develop F1 hybrids for their uniformity and to protect against illegal production.

1.3.3.3. Resistance to Stress

Cultivated tomatoes are exposed to a wide variety of pests and pathogens, more than 200, which cause significant losses. These pests and pathogens are controlled with chemical agents, but these chemicals bring about many disadvantages: (1) limited effectiveness, (2) high costs, (3) potential risk for growers, consumers and the environment, (4) compliance wth chemical-use laws, and (5) development of resistance to chemicals and the need to develop new chemicals (Bai and Lindhout 2007, Bergougnoux 2014). Thus, resistance to biotic stresses is a major breeding objective. Wild species have a great wealth of resistances. Many of the characters related to resistance are simply inherited, so transferring disease resistance genes to tomato has been quite successful. The first example was achieved in 1934 by transferring resistance to *Cladosporium fulvum*, the fungus responsible for leaf mold, from *S. pimpinellifolium* (Bai and Lindhout 2007).

Since tomato is grown in a wide variety of areas all over the world, it is exposed to various environmental conditions such as excessive water or drought, soil salinity or alkalinity, and high or low temperature. Genetic variation for such abiotic stresses is high in wild tomato germplasm and useful for breeding programmes (e.g. Rick and Chetelat, 1995; Venema et al. 2005; Wang, Vinocur, and Altman 2003).

1.3.3.4. Fruit Quality

Fruit quality is a complex trait and explained by the combination of both visual features such as size, shape and color and sensory stimuli such as sugar, acidity and taste. Tomato cultivars have wide variation in fruit size, from cherry tomato which is less than 20 g to beef tomato which can reach 500 g in weight. They also display wide variation in shape which can be round, oblate, pear-shaped, torpedo-shaped or bell-shaped (Bergougnoux 2014). The ripening process (or fruit maturation) and the determination of soluble solid content, which is affected by ripening, dominate research on fruit quality (Rick and Chetelat 1995). Biochemical reactions during the ripening process cause both beneficial changes for the fruit such as acquisition of color, accumulation of sugars and volatile compounds, and unfavorable changes such as loss of fruit firmness and reduction in shelf-life (Lelievre et al. 1997).

Total soluble solids content is important for processing tomatoes. Soluble solids consist of mainly sugars, and the relative concentrations of sugars and organic acids contribute to flavor. Also pH of the final product is determined by organic acid content. Acidity of tomato fruit is important for flavor and also because microorganisms can develop above pH 4.5 which causes spoiling of the final product. On the other hand, insoluble solids which are composed of the cell wall and proteins determine the firmness of the fruit and viscosity of the final processed product such as ketchup, soup, tomato paste and juice (Bergougnoux 2014).

Tomato color includes the color of the skin and the flesh. Changes in color, which is the most obvious trait of fruit ripening, depend on the quantity of carotenoid pigments which consist of mostly lycopene and, to a lesser degree, β-carotene (Ruiz-Sola and Rodríguez-Concepción 2012).

Flavour mainly depends on sugars, acids and volatile compounds (Tieman et al. 2006). Therefore, flavour is a complex trait and determined by several factors. It has been shown that increases of sugar and acid content resulted in significant improvement in tomato flavour (Jones and Scott 1983). Although many QTLs have been identified for volatile and non-volatile compounds important to flavour, intensive breeding for these compounds has not yet been performed (Tikunov et al. 2005; Tieman et al. 2006).

1.3.3.5. Nutritional Value

Nutritional value is not considered to be only protein, lipid and sugar content but also the amount of compounds which are important to human health such as antioxidants like lycopene, β-carotene and ascorbic acid. Tomato is the main source of lycopene is the human diet and lycopene is important to protect against cancer or cardiovascular disease (Rao and Agarwal 2009). The nutritional value of tomato is summarized in Table 3.

Table 3. Nutritional value of 100 g of red fresh tomato (Source: USDA).

Proximates	**Unit**	**Quantity**
Water	g	94.52
Energy	kcal	18
Protein	g	0.88
Total lipid	g	0.2
Fibers	g	1.2
Sugars	g	2.63
Minerals		
Calcium	mg	10
Magnesium	mg	11
Phosphorus	mg	24
Potassium	mg	237
Sodium	mg	5
Fluoride	µg	2.3
Vitamins		
Vitamin C	mg	13.7
Choline	mg	6.7
Vitamin A	µg	42
α-Carotene	µg	449
β-Carotene	µg	101
Lycopene	µg	2573
Lutein + zeaxanthin	µg	123
Vitamin K	µg	7.9

1.4. Genomics

The main objective of breeding programmes is to develop plant cultivars which have resistance to pathogens, biotic and abiotic stresses, and improved yields and nutritional value. Control and inheritance of these traits are complex. Genomics is the analysis and sequencing of an organism's entire genetic make-up and provides molecular insights to understand the structure, function, and inheritance of genes and to improve complex traits (Omboki et al. 2015; Siva et al. 2012).

1.4.1. Comparative, Structural And Functional Genomics & Plant Breeding

Comparative genomics allows identification of regions of agronomic importance which can be improved in crops by comparing co-evolved genomes. In addition comparative genetics help to discover new regions important to breeding objectives (Omboki et al. 2015; Saintenac, Jiang, and Akhunov 2011). Identification of coding sequences is a more complex issue, but the advantage is that coding sequences are generally conserved among related species. This allows researchers to create gene models which help in the design of algorithms that help in identification of coding sequences (Flicek et al. 2013). Moreover, although it is difficult to identify regulatory elements in a genome, comparative genomics allows such research (Jessica and Kerstin 2013). Comparative genomics can also be used for improving crop biodiversity analysis, identification of better varieties, marker assisted selection, and gene cloning (Nelson, Naylor, and Jahn 2004)

Structural genomics helps to identify the structure of biomolecules coded for by genes using experimental and computational methods. In structural genomics studies, the main focus is linking the structure of the biomolecule to function (Structural genomics, 2006). Today there is an increasing number of sequenced genomes; thus, there is a need to study proteins that are coded for by the genes for a better understanding of plant physiology and chemistry (Helene and Jacque 2004). Structural genomics can help us to understand how transcription and translation occur, and also how plants respond to abiotic and biotic stresses (Vanderschuren et al. 2013). Moreover, studying proteins allows us to better understand flower development, cell division, cell differentiation, plant organs,

development of seeds and seed germination which can help to improve crop cultivars (Takac, Pechan, and Jozef 2011).

Functional genomics studies gene function, gene expression profile, regulation, interaction between the genes, and the responses they exhibit in phenotype (Saraswathy and Ramalingam 2011). Moreover, information from full length cDNA can be used to identify introns, exons, regulatory elements and other regions of agronomic interest that can be helpful in breeding programmes (Omboki et al. 2015). Functional genomics not only studies data that are generated from the genome by molecular biology techniques but also involves metabolomics and phenomics (Saraswathy and Ramalingam 2011). In addition plant functional genomics allows improvements in related fields, most importantly in mapping of quantitative trait loci (QTL). QTLs are used to identify the location of genes, determine if they are major or minor alleles, and to investigate their effects in crops. Hundreds of genes have been mapped and their function identified in crops using QTL analysis (Salvi and Tuberosa 2015).

1.4.2. Genetic Mapping

Developments in genetics since the 1950s including identifying DNA as a genetic material in 1953, using isozymes in 1959, and discovery of other molecular markers constitute milestones in genetics. These milestones help us to understand genetics from the molecular level to phenotype, to improve screening methods for selection of superior genotypes, and to develop decision-making processes in breeding strategies. Genetic mapping (linkage mapping/meiotic mapping) is one of the most useful applications of molecular markers. Genetic mapping reveals the relative positions of markers/genes on a chromosome or plasmid and the distance between them (Collard et al. 2005; Paterson 1996).

The first step of using molecular markers in plant breeding was the construction of detailed genetic maps with high levels of genome coverage (Tanksley et al. 1989). After that many genetic maps were developed to: (1) allow detailed genetic analysis of qualitative and quantitative traits and to localize genes or QTL (Doerge 2002; Mohan et al. 1997; Yim et al. 2002), (2) facilitate marker assisted selection, (3) allow comparative mapping and to estimate similarity between gene order and function between species (Ahn and Tanksley 1993; Paterson et al. 2000), (4) provide a framework for anchoring

physical maps based on DNA sequence or chromosome translocations (Yim et al. 2002), and (5) allow map-based cloning of genes responsible for economically important traits (Mohan et al. 1997; Vuysteke et al. 1999).

1.4.2.1. Principles of Genetic Mapping

Genetic maps are constructed based on analysis of genes (markers or loci) in segregating progeny which carry chromosome recombination events (Paterson 1996). During recombination, which is defined as the exchange of chromosome sections during homologous chromosome pairing at the begining of meiosis, DNA molecules interact with one another to create rearrangements of the genetic information. Thus, new allelic combinations, differing from both parents, appear in the progeny. Sometimes crossing over does not occur and so parental gametes are produced (Semagn, Bjørnstad, and Ndjiondjop 2006). Linked genes have a recombination frequency less than 50% while unlinked genes have a recombination frequency more than 50% (Harlt 1988). The distance between two genes and the chance of a crossover producing recombination between that genes are directly related. Therefore, recombination frequency increases when the distance between two markers on a chromosome increases and determination of recombination frequency can be used to calculate the distance between genes/markers (Semagn, Bjørnstad, and Ndjiondjop 2006).

Construction of a genetic linkage map includes: (1) developing an appropriate population with the appropriate number of individuals, (2) choosing the marker system, (3) screening parents and the population for marker polymorphism, and (4) performing linkage analysis using mapping programmes (Semagn, Bjørnstad, and Ndjiondjop 2006).

1.4.2.2. Types of Genetic Maps

A genetic map represents the specific order of genetic markers located on a chromosome and the distances between them. There are three types of maps: (i) linkage maps, (ii) cytogenetic maps, and (iii) physical maps (Dixit et al. 2014; Singh and Singh 2015).

1.4.2.2.1. Linkage Maps

A linkage map represents the relative locations of genetic markers on the chromosome by using recombination frequency data for pairs of markers in a population derived from biparental cross (Dixit et al. 2014). Genetic markers are grouped into linkage groups based on genetic distance, and their relative order in the linkage group is represented on the linkage map with distances expressed in centiMorgans. Conventional linkage maps are constructed using the target traits as genetic markers, and describe the genes responsible for different phenotypic traits. On the other hand, functional maps, which are a different type of linkage map, also describe the genes responsible for different phenotypic traits, but are constructed using molecular markers located within the gene sequence. Genes responsible for a specific trait, genes with known function, and QTLs can be mapped on a functional map (Singh and Singh 2015).

1.4.2.2.2. Cytogenetic Maps

A cytogenetic map represents the locations of genes on the chromosome based on specific, microscopically observable landmarks. Each chromosome has a characteristic banding pattern. The banding pattern becomes observable with specific staining protocols such as Giemsa. Occasionally the banding pattern can be present naturally as in polytene chromosomes of Drosophila. Cytogenetic maps can be constructed using the heritable heterochromatic regions of identifiable shape and also morphological landmarks such as centromeres, nucleolus organizing regions or knobs. Cytogenetic maps are useful in determining the direction of the linkage groups in relation to the morphology of the respective chromosome and association of linkage groups with specific chromosomes (Dixit et al. 2014; Singh and Singh 2015).

1.4.2.2.3. Physical Maps

A physical map represents the order of genes or molecular markers on the chromosome. The distance between the genes or molecular markers is expressed in base pairs, which is a physical distance and determined by probe hybridization or sequence alignment to a reference genome (Singh and Singh 2015). Molecular biology techniques

are needed for the construction of physical maps. High density genetic maps and large insert genomic libraries are important tools in construction of physical maps. Physical maps represent the entire genome of an organism as a set of overlapping cloned DNA fragments that are ordered with sequence alignment to a reference genome (Dixit et al. 2014).

1.4.2.3. Quantitative Trait Loci

A quantitative trait locus (QTL) is a region of the genome which contains a gene or genes that lead to variation in a quantitative trait. Mapping of QTLs that are responsible for natural variation in quantitative or complex traits by examining the relationship between polymorphic loci and Mendelian segregation has been done since the beginning of the 20th century (Sax 1923). But until the 1980s, only a few model organisms were studied, because of a lack of polymorphic markers (Shrimpton and Robertson 1988). During the 1980s, the discovery of abundant molecular markers and improvements in genotyping and statistical methods methods provided rapid advances in QTL mapping (Mackay, Stone, and Ayroles 2009).

QTL mapping is a powerful technique to study complex agronomical traits, and the only requirement for QTL mapping is the presence of polymorphic alleles in the mapping population (Paterson 2002). Although large phenotypic effects can be identified easily by QTL mapping, the main disadvantage of the technique is that QTLs are highly affected by the environment and parental lines and such studies are time and labor intensive (Nogue et al. 2016). Despite these challenges, QTL mapping is commonly used in many crops including tomato and is an important first step toward marker assisted selection.

1.4.2.4. Marker Assisted Selection

Marker assisted selection (MAS) is a powerful technique in breeding programmes which involves DNA marker detection and selection. In the past two decades, numerous studies were performed on a wide range of crop species to identify molecular markers and the QTLs responsible for important agronomic traits

In theory, all the QTLs responsible for the desired trait should be taken into account in MAS, because quantitative characters are controlled by more than one QTL. However, selecting all QTLs simultaneously is usually impossible and unnecessary. The relative efficiency of MAS is tightly correlated to the number of QTLs involved (Moreau et al. 1998). This means if a quantitative character is controlled by many genes, the efficiency of MAS will decrease. Although five QTLs were used to improve fruit quality in tomato (Lecomte et al. 2004), generally three QTLs are considered to be the maximum for feasible and efficient MAS (Ribaut and Betran 1999). On the other hand, with developing technology, especially gentoyping technologies and SNP markers, more QTLs can be used effectively in MAS (Kumpatla et al. 2012).

Moreover, the number of markers used is also important in MAS. The success in selecting a QTL will be greater if more markers associated with the QTL are used. On the other hand, in practice when resources and facilities are considered, use of two tightly linked markers is suggested for selection of a single QTL. The markers should be close enough to the QTL, maximum 5 cM away, to be sure that only a minor proportion of selected individuals will have recombination between the QTL and marker(s) (Collard and Mackill 2008).

Other concerns in MAS are the number of generations and population size. Generally from two to four consecutive generations in a segregating population are screened for the markers. If the markers are in close proximity to the QTL, fewer markers can be used and fewer generations will be enough for marker selection (Bonnett, Rebetzke, and Spielmeyer 2005).

Although MAS is a powerful technique, there are limitations such as interactions between QTLs, recombination between genes present on the same chromosome, low reproducibility of QTLs in different genetic backgrounds, and requirements for time and money. The closer proximity and association between the marker and QTL generally overcome these limitations (Nogue et al. 2016; Semagn, Bjørnstad, and Xu 2010).

1.4.2.4.1. MAS for Qualitative and Quantitative Traits

Many economically important characters such as male sterility, self incompatibility, resistance to pest and disease, color, and shape are controlled by QTLs in crop plants. MAS for improvement for agronomic characters is difficult because in

addition to being polygenic, QTLs are also affected by environment and epistasis which should be take into account. Each gene may have a small effect on the phenotype and its expression may be strongly affected by environmental factors. Thus identifying marker-phenotype association in agronomic traits is difficult and reduces the efficiency of MAS. Therefore, repeated field tests are needed to evaluate the effects of QTLs and the environment (Bauchet and Causse 2012).

1.4.2.5. DNA Markers In Plant Breeding

A DNA marker is a fragment of DNA and can be used to identify polymorphism in a particular sequence of DNA in a population or different genotypes. DNA markers are categorized as molecular markers and have major applications in plant breeding. In efficient MAS applications, DNA markers should be: highly polymorphic, distributed in the whole genome, codominant, single copy, easy to use and maintain, and cost efficient. Moreover they should not have pleiotropic or detrimental effects on phenotype. Although there are many types of DNA markers, only single nucleotide polymorphism (SNP) markers will be described here (Bauchet and Causse 2012).

SNP markers represent differences in single nucleotide bases among individuals or two DNA sequences. SNPs can be categorized as transitions (C/T, G/A) and transversions (C/G, A/T, C/A, T/G). SNPs are very common in both animals and plants. The frequency of SNP presence is one SNP for every 100-300 bp in plants (Edwards et al. 2007; Xu 2010). SNPs can be present at different frequencies both in noncoding and coding regions of genes and in intergenic regions. There are several SNP genotyping assays: primer extention, allele specific hybridization, ligation and invasive cleavage (Sobrino, Brion, and Carracedo 2005) There are also different detection methods: mass spectrophotometry, chromatography, gel electrophoresis, arrays, chips or fluorescence polarization (Gupta, Roy, and Prasad 2001).

SNPs have drawn attention in genetic and breeding studies because they are: linked to genes, present in the simplest form of polymorphism, codominant markers, and suitable for automation. However, SNPs still have limitations in breeding programs, because start up and marker development prices are expensive, high quality DNA is required, and expensive equipment is needed (Bauchet and Causse 2012).

1.4.2.6. Mapping Populations

Mapping populations can be developed by crossing individuals of one species or related species, which differ in the traits that will be studied. When choosing a mapping population, two parameters are important: (i) polymorphism between the parental lines for the trait to be studied and (ii) reproductive mode (self-compatibility or self-incompatibility) of the plant. It is a problem to obtain homozygous lines in self-incompatible plants while self-compatible lines allow development of pure homozygous lines (Meksem and Kahl 2005). F2 plants, recombinant inbred lines (RIL), backcross (BC) populations, introgression lines assembled in exotic libraries, and doubled haploid lines (DH) can be used as mapping populations (Meksem and Kahl 2005). Only backcross populations will be described here.

Backcross populations are useful to analyze specific DNA fragments derived from parent A, the donor. An F1 plant is backcrossed to parent B, the recipient. Unlinked donor fragments from parent A are separated by the segregation process and also linked fragments are reduced by recombination with the recurrent parent. To minimize number and size of donor fragments, backcrossing with the recurrent parent is repeated (Figure 5). In each backcross generation, the individual with the highest value for the trait is selected and backcrossed to the recurrent parent (Bauchet and Causse 2012). Backcrossing can also be followed by self-pollination to achieve homozygosity. Using backcross lines in breeding is an important tool if a single trait, such as resistance, has to be introduced into a cultivar that already contains other desirable traits (Schneider 2005).

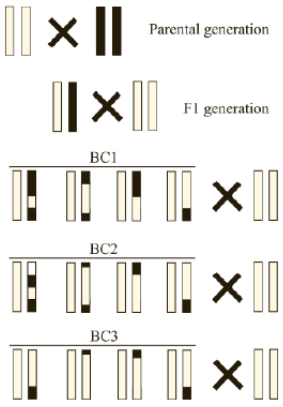

Figure 5. Generation of backcross lines (Source: Schneider 2005).

1.5. Metabolomics And Metabolic Profiling

Besides genomics studies, metabolomics, transcriptomics, phenomics and phylogenetics are widely used technologies for breeding purposes, however, only metabolomics will be described here. The entire set of molecules found in an organism is described as the metabolome. Profiling of metabolites is useful to understand compositional quality of plant products. Many techniques including chromatography, mass spectrophotometry, and nuclear magnetic resonance can be used in metabolite profiling depending on the purpose and facilities. Metabolite profiling provides information about metabolic networks and their regulation which can be used in breeding of plants with favorable compositional quality (Fernie and Schauer 2008; Khakimov, Bak, and Engelsen 2014). Plants produce a wide variety of metabolites. Primary metabolites directly participate in essential reactions in growth and development while secondary metabolites are not directly related to growth and development but are mostly involved in plant-environment interactions. Sensory quality of fruits is primarily defined by metabolite composition. Sugars, organic acids, volatile compounds and free amino acids are all components of taste. An alteration in content and composition of these compounds leads to altered sensory and nutritional quality. Changes in the levels of pigments, sugars, acids and aroma volatiles during the ripening process define the taste and aroma of the ripe fruit. Most physiological changes occur under the control of plant hormones (Kusano et al. 2015; Oms-Oliu et al. 2011).

In plants, natural genetic variation leads to quantitative variation in metabolite content and qualitative variation in chemical composition (Kroymann 2011). The metabolome of a cell, unlike the genome, is highly dynamic and highly affected by the environment and developmental stage of the plant. Moreover, genetic modifications, and responses to abiotic and biotic stresses can cause changes in the synthesis or degradation of metabolites (Allwood, Ellis, and Goodacre 2008). Thus, the metabolome is complex and includes many different chemical groups such as lipid soluble metabolites, aqueous polar metabolites, stable and unstable metabolites and acidic and basic metabolites. Biochemical diversity is predicted to include approximately 200.000 distinct metabolites with a range of 4000 to 20.000 for a single plant species (Dixon and Strack 2003). Although thousands of metabolites can be quantified, detecting the entire metabolome is not possible because of its dynamic nature and differences in physicochemical properties.

There are two approaches in metabolic profiling: (i) targeted metabolite profiling focuses on a specific group of metabolites, while (ii) untargeted metabolic profiling allows quantitative evaluation of unknown metabolites (Wienkoop et al. 2008, 2010).

Metabolomic approaches toward identifying variations in metabolite production coupled with quantitative genomics to understand their underlying molecular basis have gained much attention and significance in the last ten years (Carreno-Quintero et al. 2012). Robust, fast and sensitive analytical techniques are available for metabolic profiling studies. Because it is possible to detect and measure a wide range of plant metabolites, QTL mapping of these metabolic traits and further utilization in breeding programs is now feasible. In addition to identifying QTL, previously unknown enzymatic steps in metabolic pathways can be identified (Rowe et al. 2008). A QTL associated with metabolic and biochemical pathways is referred to as metabolic QTL (mQTL). In addition to the improvement of agronomic characteristics, identification of mQTL is promising for the improvement of pharmaceutical and nutritional characters (Saito and Matsuda 2010). As well as being used for marker assisted breeding, QTL maps of biochemical traits enable the further identification and cloning of genes controlling the traits. Until now, not many genes have been identified by mQTL analysis however, development of multi-parallel approaches that combine gene expression, metabolic and protein profiling analysis have potential for the identification of genes that regulate metabolic pathways. mQTL analysis of diverse populations and different tissue types allows interspecific or intraspecific comparison of metabolic profiles and their underlying genetic basis. Comparative analysis of metabolic traits provides information on the structure and topology of complex metabolic pathways (Toubiana et al. 2012). Metabolic QTL research is also important for evolutionary studies and understanding epistatic relationships (Kliebenstein 2009). Integration of metabolomics with genomic analysis is not limited to breeding studies but also proved successful in evaluating genetically modified organisms (Carreno-Quintero et al. 2012).

1.6. Tomato Metabolomics

Three techniques are used in tomato metabolomics: gas chromatography-mass spectrophotometry (GC-MS), liquid chromatography-mass spectrophotometry (LC-MS), and nuclear magnetic resonance (NMR) (Tohge and Fernie 2015). Numerous metabolite

profiling studies were achieved in tomato including analysis of both primary and secondary metabolites.

1.6.1. Metabolite Profiling of Primary Metabolites in Tomato

Metabolic shifts, changes in primary metabolism and metabolic flux during fruit ripening have been studied by scientists. Carrari et al. (2006) and Mounet et al. (2009) studied gene metabolite networks during the early to late developmental stages of tomato fruit via metabolic and transcriptomic profiling. Correlation analysis showed that sugar phosphates, pigments and intermediates of the tricarboxylic acid (TCA) cycle were highly correlated, but the rest of the metabolites did not show statistically significant correlation. The importance of TCA cycle intermediates, especially malate, was demonstrated in other work (Centeno et al. 2011; Osorio et al. 2013), in which changes in malate content caused reciprocal alterations in the content of transitory starch and soluble sugars, and also post-harvest properties of tomato fruits. Similar studies identified 37 direct gene to metabolite correlations which involved regulatory genes such as bZIP and MYB transcription factors (Mounet et al. 2009). The role of metabolic shifts in development of tomato fruit was also demonstrated (Carrari and Fernie 2006). Studies on changes in primary metabolism during fruit development and ripening were consistent with other studies (Alba et al. 2005; Enfissi et al. 2010; Karlova et al. 2011). In another study, transcriptomic and proteomic data were evaluated together to understand the regulation of metabolism during ripening. They found that (i) post-transcriptional regulatory mechanisms play important roles in the regulation of metabolism during ripening, (ii) strong correlation was found between ripening-associated transcripts and organic acids, sugars and cell wall-related metabolites (Tohge and Fernie 2015).

Metabolite content of the plant is affected by the environment and much variability between cultivars and their wild type relatives, and even among individuals in a population can be seen. The primary metabolite content of fruits and leaves of cultivated tomato and five of its wild relatives, namely *S. pimpinellifolium, S. neorickii, S. chmielewskii, S. habrochaites* and *S. pennellii* were studied (Schauer, Zamir, and Fernie 2005). They showed that there are significant changes in metabolite content and composition, especially in hexoses and proline content, which may reflect adaptation to environmental stress. There were also differences in the content of essential amimo acids,

and vitamins, which are of nutritional importance. In another study of an IL population derived from *S. lycopersicum* and *S. pennellii,* 889 QTLs were found to be responsible for the accumulation of 74 metabolites, which were generally accompanied by a yield penalty (Schauer et al. 2006). The scientists further investigated the heritability and mode of inheritance of these QTLs in lines that were heterozygous for the introgression (ILHs) derived from *S. pennellii* (Schauer et al. 2008). This comparative study of the ILs and ILHs proved that metabolic QTLs could be both dominantly inherited and display additive or recessive modes of action, and that few of the QTLs had overdominant inheritance. Also the mode of inheritance was variable between different groups of metabolites, but similar mode of action was observed for metabolites sharing the same pathway. In addtion, a brix QTL on chromosome 9 (Friedman et al. 2004; Schauer et al. 2008), QTLs for vitamin E, and branched chain amino acids were identified in tomato and consistent with previous studies (Maloney et al. 2010; Schauer et al. 2008; Quadrana et al. 2013). In a recent study, which is the first description of genome-wide association metabolite mapping in tomato, loci controlling variation in compounds including amino acids, sugars and vitamin C were mapped in a core collection of 163 tomato accessions derived from of *S. lycopersicum, S. lycopersicum cv. cerasiforme* and *S. pimpinellifolium* (Sauvage et al. 2014). Researchers quantified the metabolite content in the accessions which were genotyped with 6.000 SNP markers. This analysis allowed identification of 44 loci which were strongly associated with 19 traits including sucrose, vitamin C, malate and citrate contents. This study provided powerful information and candidate genes for improvement of crop composition.

1.6.2. Metabolite Profiling of Nonvolatile Secondary Metabolites in Tomato

Tomato produce a wide variety of secondary metabolites, most importantly polyphenols, carotenoids and alkaloids. To date, approximately 250 metabolites have been documented in tomato fruit (Tohge and Fernie 2015). So far, 49 glycoalkaloids, 122 flavonoids, 56 hydroxycinnamates and 36 acyl-sugars have been documented in tomato. It was shown that phenylpropanoids and flavonoids are synthesized in nearly all of the tomato plant including in seeds, organs and glandular trichomes, and that these compounds show diversity both in structure and quantity within and between plant

species and accessions (Kim et al. 2014; Saito et al. 2013; Schmidt et al. 2011, 2012; Tohge et al. 2013). Although metabolic profiling studies on flavonoids have been done, profiling of flavonoid derivatives using a wide variety of tomato accessions has not yet been extensively reported. Thus diversity and metabolic profiling studies of flavonoids will be attractive in future studies (Tohge and Fernie 2015).

Carotenoids in tomato have been subjected to many scientific studies and the genetic background underlying tomato carotenoids has been examined using mutants (Bird et al. 1991; Galpaz et al. 2008; Isaacson et al. 2002; Kachanovsky et al. 2012; Ronen et al. 2000; Thompson et al. 1999; Wilkinson et al. 1995) or IL populations (Fantini et al. 2013; Fraser et al. 2007; Lee et al. 2012; Liu et al. 2003; Pan et al. 2012). Fraser et al. (2007) identified QTLs for the carotenoids canthaxanthin and lycopene in an IL population derived from *S. pennellii*. A similar study in the same population also identified many QTLs, but also provided information that there were some factors, outside of the structural genes, influencing carotenoid metabolism (Liu et al. 2003). This information led researchers to identify other factors in carotenoid metabolism, and they found that a transcription factor, SIERF6, was involved in carotenoid biosynthesis (Lee et al 2012). In a similar study, researchers analyzed transcription factors during tomato ripening and maturation, and one of the transcription factors was homologous to the *Arabidopsis thaliana*, Arabidopsis Response Regulator2-Like gene (APRR2-Like). This gene is upregulated in wild type tomato fruits which results in increasing plastid number, area and pigment content, and carotenoid content in red ripe fruits (Pan et al 2012). In addition, Fantini et al (2013) studied the effects of silencing of nine structural genes for 45 carotenoid isomers using the VIGS technique.

Acyl sugars are important to investigate the cross-talk between primary and secondary metabolism. Acyl sugars are found at low quantities in *S. lycopersicum* and *S. pennellii* (Alseekh et al. 2015). They are found at higher levels in glandular trichomes of wild tomato species, but not in domesticated tomato cultivars. Natural variation of acylsugars has been reported and it was found that *S. habrochaites* (Ghosh, Westbrook, and Jones 2013; Kim et al. 2012) and *S. pennellii* (Shapiro, Steffens, and Mutschler 1994) have different acyl sugar profiles. Acyl sugars have biological activity against aphids (Goffreda and Mutschler 1989; Rodriguez, Tingey, and Mutschler 1993), leafminer (Hawthorne et al. 1992), whitefly (Kisha 1981; Liedl et al. 1995) and worms, including fruit worm (Dias et al. 2013; Juvik et al. 1994; Williams et al. 1980). QTL studies identified several key genes responsible for acyl sugar synthesis which encode BAHD

acyl-transferases (Schilmiller et al. 2012) and 3-ketoacyl-acyl carrier protein synthase (Slocombe et al. 2008). Metabolic engineering and breeding strategies for acylsugars are complex because sugar metabolism and acyl-CoA metabolism which is synthesized from fatty acid metabolism and branched amino acid (BCAA) catabolism play roles in acyl sugar synthesis (Schilmiller, Charbonneau, and Last 2012; Slocombe et al. 2008).

A recent study in tomato evaluated a wide range secondary metabolites including flavonols, phenylpropanoids, glycoalkaloids and acyl sugars in an IL population derived from *S. pennellii* (Alseekh et al. 2015). Totally 679 mQTL were identified and it was shown that the majority of mQTLs revealed dominant or additive modes of inheritance.

AIM OF THE PROJECT

The main goal of the project is to elucidate the genetic control of important metabolic traits affecting the quality (taste, flavor, and color) and nutritional value of tomato fruit. Carotenoids, water and fat soluble vitamins, glutathione, fatty acids, organic acids, sugars, phenolic acids and volatile compounds were measured. In addition, brix, pH, dry matter, fruit weight, fruit number, fruit shape, firmness, stem scar, locule number, wall thickness, internal and external color were measured and scored as morphological characters in this study. Genetic data were obtained with the aid of the next generation sequencing technique, genotyping by sequencing (GBS). A QTL mapping approach was carried out to associate phenotypic and biochemical data collected from metabolic analysis with molecular marker data. Thus, QTLs that are responsible for the control of yield and fruit quality associated traits were identified. Identification of QTLs that regulate accumulation of primary and secondary metabolites will allow a better understanding of those metabolic pathways and their regulation. Data produced in our project are available for molecular breeding studies focused on improved resistance, yield and nutritional attributes as well as comparative genome analysis of *Solanaceae* species. Realization of such studies would also enable development of lines tailored for agronomic, industrial and pharmaceutical purposes.

CHAPTER 2

GENOTYPING BY SEQUENCING (GBS)

2.1. Introduction

Two major strategies; classical breeding and molecular breeding, are followed in plant breeding. Classical breeding involves interbreeding of individuals selected based on phenotype to develop new cultivars. Selection, evaluation and crossing of useful genotypes require long periods of time and several generations (Tester and Langridge 2010). On the other hand, molecular breeding uses molecular biology and/or biotechnology techniques to develop new cultivars. DNA polymorphism is used to select useful genotypes, which reduces the selection period and number of generations evaluated. Molecular breeding includes two major approaches: marker assisted selection (MAS) and genetic transformation or genetic engineering (Moose and Mumm 2008). Genetic engineering applications in plant breeding are very limited because of food safety and environmental concerns (Nicolia et al. 2014). However, MAS has been widely applied in plant breeding. MAS is an effective method which uses molecular markers for indirect selection of traits such as crop yield, tolerance to abiotic and biotic stresses and crop quality. Recent advances in next generation sequencing (NGS), especially genotyping-by-sequencing (GBS), provide powerful MAS tools for plant breeding and crop improvment (He et al. 2014).

In recent years, GBS has become feasible for highly diverse and large-genome species as a result of reductions in the cost of DNA sequencing due to advances in NGS technologies (Elshire et al. 2011). GBS is a simple multiplexed system to construct reduced representation libraries (RRL) and generates whole genome sequence data useful for SNP (single nucleotide polymorphism) genotyping and other genetic analyses (Beissinger et al. 2013). Advantages of GBS are: (i) low cost, (ii) easy to scale up, (iii) reduction PCR and purification steps, (iv) no need for size fractionation, (v) efficient barcoding, (vi) reduced sample handling, (vii) no reference sequence limits, (viii) no requirement for prior genomic knowledge and (ix) ability to simultaneously perform SNP discovery and genotyping (Davey et al. 2011; Kim et al. 2016). On the other hand,

genotyping errors can occur as a result of low coverage of NGS reads. Moreover, the technology is still challenging in polyploids and outcrossing species (Kim et al. 2016). Despite the disadvantages of GBS, the advances and advantages of NGS technologies make GBS an ideal platform in genetic and genomic studies, especially in plant breeding.

2.2. Materials And Methods

2.2.1. Plant Material

An interspecific IBL (inbred backcross line) population derived from the cross *S. lycopersicum* cv. Tueza x *S. pimpinellifolium* (LA1589) was used as plant material in the study. Tueza is a cultivated fresh market tomato line with large (150 – 160 g), red, slightly flattened round fruits. LA1589 is a wild type tomato with small, red, round fruits. The IBL population and parents were grown by Multi Tohum seed company (Antalya, Turkey). A total of 10 plants per genotype were grown in double rows with 140 cm between wide rows and 50 cm between narrow rows. Within rows, plants were spaced at 40 cm intervals. For basal fertilization, 500 kg 15:15:15 (N:P:K) fertilizer and 50 t of composted manure were applied per ha. Drip irrigation was used with fertigation (1.4 dS m^{-1} EC value) at each irrigation using 1-2-1 fertilizer until first fruit set, 2-1-1 fertilizer until first fruit ripening and 1-1-2 fertilizer after first fruit ripening. Total genomic DNA was isolated from the leaf tissue of the parental accessions and 93 individuals of the IBL population using a CTAB method (Doyle and Doyle 1990). Genomic DNA was quantified using *Qubit*™ quantitation assay (Life Technologies). DNA integrity was checked on a 1% agarose gel.

2.2.2. DNA Extraction, Library Preparation, and Sequencing

DNA was extracted from leaves from each parent and progeny tomato using miniprep isolation method and quantified using Quant-iT™ PicoGreen ® dsDNA Kit (Invitrogen) at the University of Wisconsin (UW) center. Whole genome amplification was performed using 10 ng of DNA and the Illustra™ GenomiPhi™ V2 DNA Amplification Kit (GE Healthcare) in at the UW center. Amplified DNA (1.0 µg) was plated and dried using a vacuum centrifuge. Dried DNA was resuspended and digested at

75 °C for 2 h using a 10 µl mix containing 4 units of *ApeKI* restriction endonuclease (New England Biolabs, Ipswich, MA, USA) and 1 µl of 10× NEBuffer 3, then cooled on ice. A total of 96 unique barcode adapters were used to track individual DNA samples (Elshire et al. 2011). Dried barcode adapters were resuspended by pipeting 40 µl of a ligation mix containing 4 units of T4 DNA Ligase (Promega) in 2× rapid ligation buffer (Promega). Resuspended barcode adapters were mixed with cooled digested DNA. Ligation was performed at room temperature for 60 min, followed by incubation at 65 °C for 30 min to inactivate the enzyme and then cooling on ice until the next step. Ligation products were purified using 90 µl of Agencourt AMPure (Beckman Coulter) beads per the manufacturer's instructions and eluted in 35 µl of EB Buffer (Qiagen). Single-end sequencing of the library was done with a Genome Analyzer II device in a single flowcell channel (Illumina Inc., San Diego, CA). Library preparation and sequencing were carried out at the University of Wisconsin-Madison Biotechnology Center.

2.2.3. SNP Calling, Localization and Distribution in the Reference Genome

The raw sequence data were processed into SNP genotype files in HapMap format using the TASSEL 3.0 GBS pipeline (Glaubitz et al. 2012). First, all of the 100 bp reads that contained a known barcode along with the expected *Ape*KI cut-site remnant were converted into 64 base sequence tags (where, barring sequencing errors, each tag represented an allele) by trimming off the barcode along with excess 3′ nucleotides. Reads containing N's within the first 64 bases after the barcode were rejected. Reads that contained either the beginning of the common (non-barcoded) adapter (from short restriction fragments) or a full *Ape*KI site (from incomplete digest or chimera formation) within the first 64 bases after the barcode were truncated accordingly. A master tag list was constructed comprising all tags that were observed at least 10 times across all of the samples. These tags were then aligned to the tomato reference genome (The Tomato Genome Consortium 2012). Tags located at the same, unique position on the tomato reference genome and containing no more than two SNPs relative to the reference were then aligned against each other, which, along with information from the barcodes indicating which samples contained each tag, allowed SNP genotypes to be called.

2.3. Results

A total of 120,983,088 reads were generated by sequencing of the 95-plex library. From these reads, 448,539 sequence tags were generated. A majority of the sequence tags (84.4%, 378,659) were uniquely aligned to the tomato genome. The remaining tags were either aligned to multiple positions (13.8%, 61,793) or could not be located (1.8%, 8,087) to the tomato genome assembly. The 378,659 sequence tags uniquely aligned to the genome were used for genome- wide high-throughput SNP discovery.

Tag alignment to the reference genome revealed 23,677 unique SNP loci (merged SNPs) between the *S. lycopersicum* and *S. pimpinellifolium* genomes. The SNP loci were found on all 12 chromosomes of tomato (T1-T12). The physical map constructed with the identified SNP loci had high coverage and contained SNPs that were evenly distributed along the chromosomes (Figure 6, 7 and 8). After filtration based on parameters such as minimum Taxon Coverage (mnTCov: 0.01), minimum Site Coverage (mnSCov: 0.2), linkage disequilibrium with neighboring SNPs (hLD: TRUE), minimum R^2 value for the LD filter [-mnR2]: 0.2, and minimum Bonferroni-corrected p-value for the LD filter [-mnBonP]: 0.005, a total of 3,125 SNP loci were retained (Figure 10). While the average distance between adjacent loci was 33.8 kb for the merged SNPs, frequency was reduced to one SNP per 256.4 kb after filtering (Table 4). Chromosome T6 had the highest frequency of filtered SNPs with an average distance of 129.7 kb between adjacent markers.

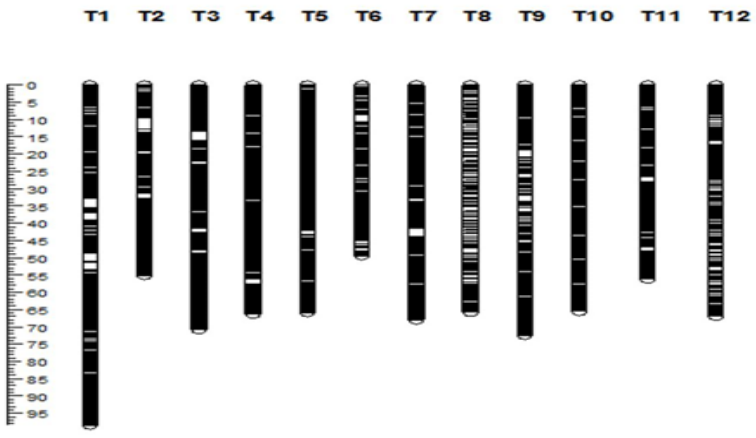

Figure 6. Physical map of the 23,677 SNPs identified in the tomato genome.

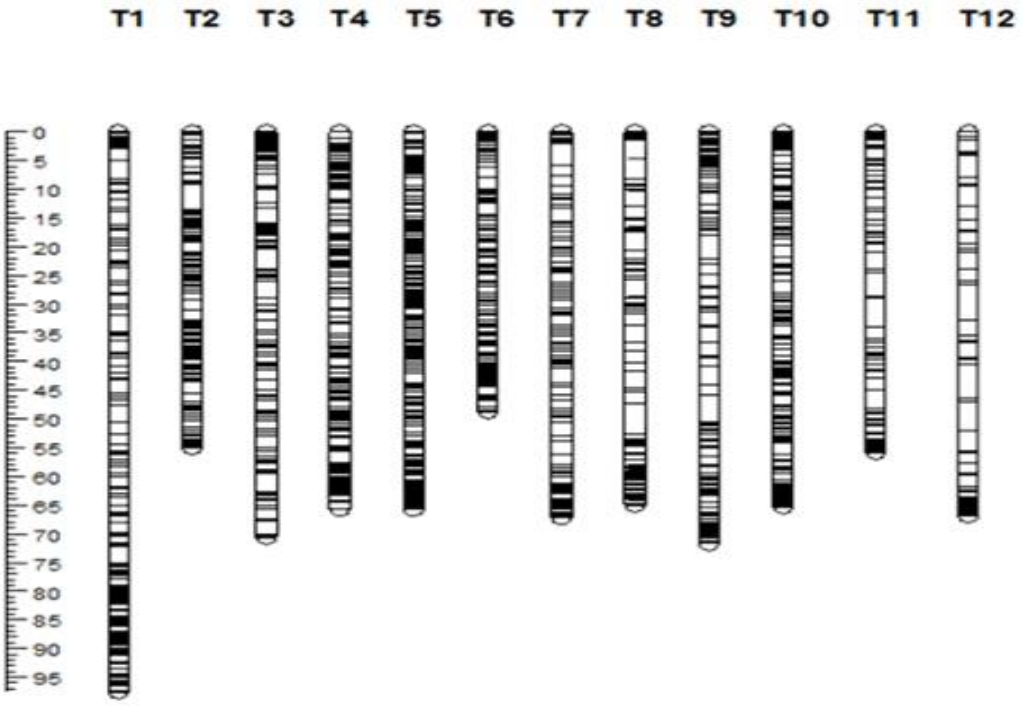

Figure 7. Physical map of the 3,125 SNPs retained after the filtering process.

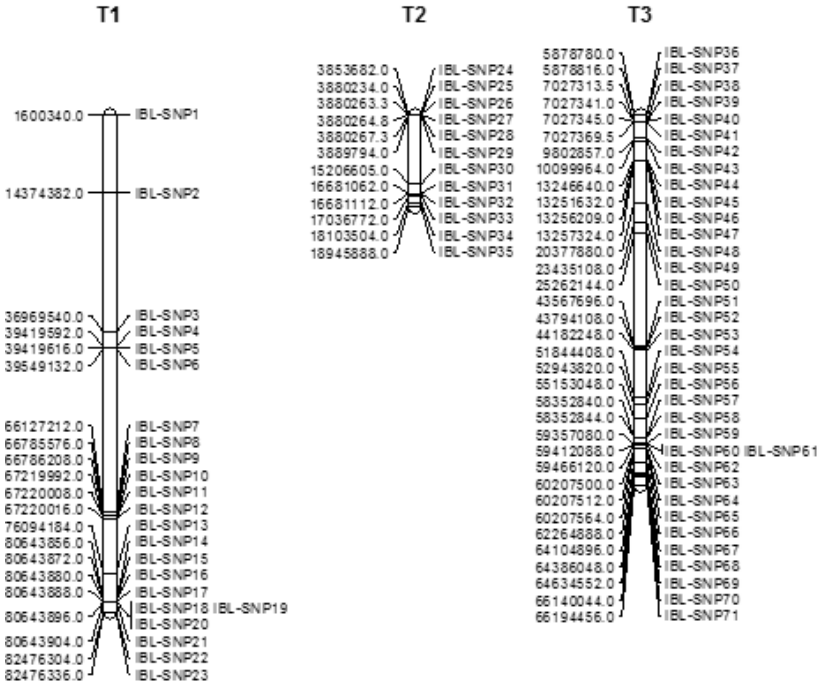

Figure 8. Physical locations of SNP markers on tomato chromosomes.

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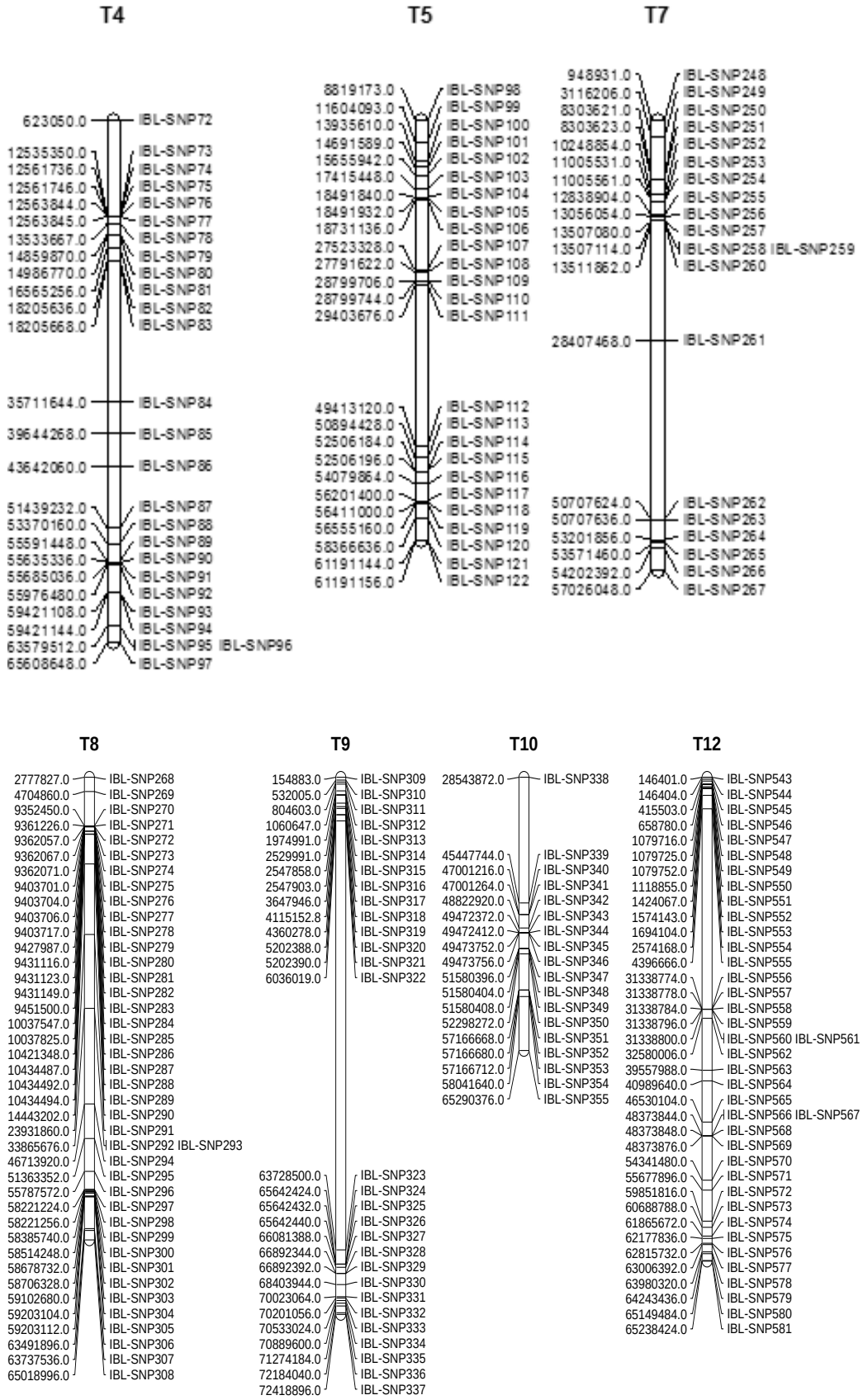

Figure 8. **(cont.)**

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Figure 8. **(cont.)**

Table 4. Numbers and frequencies of merged and filtered SNP loci in tomato *S. lycopersicum* and *S. pimpinellifolium* genomes.

		Number of SNPs		Frequency (kb) of SNPs	
Chromosome	**Size of LG (Mb)**	**Merged***	**Filtered ****	**Merged***	**Filtered****
T1	98.4	2193	293	44.9	335.9
T2	55.2	1773	297	31.2	186.0
T3	70.8	1979	200	35.8	353.8
T4	66.4	1816	271	36.6	245.1
T5	65.9	2002	442	32.9	149.0
T6	49.5	3521	382	14.1	129.7
T7	68.0	1551	213	43.9	319.4
T8	65.8	1360	176	48.4	373.9
T9	72.4	2664	299	27.2	242.2
T10	65.5	1503	299	43.6	219.1
T11	56.2	2430	165	23.1	340.8
T12	67.1	885	88	75.8	762.1
Total	801.3	23677	3125	33.8	256.4

* Number and frequency of SNPs physically mapped in tomato genome.
** Number and frequency of SNPs retained after the filtration process.

The majority of the SNPs (56.2%) identified in this study were transition mutations (A/G or C/T) as expected (Table 5). The most frequently observed substitution types, A/G and C/T transitions, had similar frequencies: 28.2 and 28.0%, respectively. C/G transversion was the least common substitution type (7.9 %). The observed transition/transversion ratio was 1.28.

Table 5. Types of substitutions represented by the identified SNP loci.

Transition/ Transversion	Number of SNPs	Frequency (%)
Transition		
C/T	6619	28.0
A/G	6679	28.2
Total	13298	56.2
Transversion		
C/G	1862	7.9
A/C	2892	12.2
G/T	2858	12.1
A/T	2767	11.7
Total	10379	43.8

2.4. Discussion

The analysis of GBS data in conjunction with a well-established reference genome is a relatively straightforward route for SNP calling and marker ordering along chromosomes (Poland and Rife 2012). In this work, the proportion of sequence tags (84.4%) that uniquely aligned to the tomato reference genome was much higher than the values obtained in GBS analyses of *Miscanthus sinensis* (23%) (Ma et al. 2012), whereas the result was comparable to that obtained with oil palm (88%) (Pootakham et al. 2015). These comparisons emphasize the importance of reference genomes in GBS analyses, since working with species with complete genome assemblies such as tomato and oil palm results in high proportions of tags mapped to their physical locations.

In the present study, a very high number of SNP markers was discovered in the tomato genome (23,677 SNPs) and 3,125 SNP loci were validated in the IBL population. The TraitGenetics EXPIMP2012 linkage map had the highest number of interspecific SNP markers (4,491 SNPs) (Sim et al. 2012). Thus, this study increased the number of polymorphic SNP loci between *S. pimpinellifolium* and *S. lycopersicum* by 69.6%. The Solanaceae Genomics Network is a database that contains 9,226 tomato-specific SNP loci identified by sequencing of a few individuals in tomato genomic studies (Sim et al. 2012). The number of SNP loci identified in the present study corresponds to 33.9% of the total currently in the database. The present research is the first report of high-throughput SNP discovery using GBS in tomato and demonstrated that this approach was efficient for SNP

identification in tomato.

The average frequency of SNPs identified in this study was 1 SNP per 33.8 kb, much higher than reported for the *S. lycopersicum* × *S. pimpinellifolium* linkage maps developed by Salinas et al. (2013) (1 SNP per 8,482 kb) and Capel et al. (2015) (1 SNP per 4,077 kb). The SNPs identified in this study also had 6.2-fold higher resolution than the EXPIMP2012 map (1 SNP per 211 kb). Despite the sufficient SNP marker resolution obtained in this work, there are reports of significantly higher SNP frequencies obtained through GBS. One example is in oil palm (Pootakham et al. 2015), where the average interval between adjacent markers is as low as 0.66 kb. The relatively low frequency of SNPs in the tomato genome compared to oil palm is due to the higher genetic diversity in trees than annual plants. These comparisons show that the number and frequency of SNP loci identified by GBS depend on the diversity level of the plant species under study rather than GBS efficiency. The GBS-based SNP map developed in this work will be useful for both gene mapping and MAS in *S. lycopersicum* × *S. pimpinellifolium*-derived populations.

CHAPTER 3

MORPHOLOGICAL AND AGRONOMIC CHARACTERS

3.1. Introduction

Although agricultural product quality can be defined in many ways (Arana, Jaren, and Arazuri 2004; Jarén and García 2002), a general definition can be described as characteristics/traits that consumers wish to find in the product. Quality parameters are related to flavor, texture, and suitability for harvesting and transport (Arazuri et al. 2007). Thus, general quality parameters for tomato are firmness, internal and external color, total soluble solid content, size, shape, pH and titratable acidity.

Firmness is an important quality parameter for breeders and the tomato industry, especially in the canning industry. The trait is related to fruit ripeness and is important in determining the tomato's susceptibility to damage during harvesting, post harvest handling such as storage or transportation, and processing. Ripe fruits become soft and this decreased firmness causes easier breakage. The canning industry can utilize soft fruits, however, they are sold at a lower price than whole tomatoes (Arazuri et al. 2007). Increased firmness also allows easy mechanical harvest of the fruit (Gould 1992).

Another agronomically important trait is fruit color, with both external and internal color being significant. Tomato color is also related with ripeness. Therefore, tomato color determines the harvesting time of the fruit. Red color is favored by both consumers and the tomato industry. The intense red color of some tomatoes attracts consumers. Moreover the CieLab system, which uses two coordinates (a and b) and the ratio a to b to define color, is used to determine tomato color in the canning industry. A positive value of a represents red color, while a negative value represents green color. A positive value of b represents yellow color, while a negative value represents blue color (Renquist and Reid 1998).

In processing tomato, another important quality parameter is total soluble solids content (or Brix). Total soluble solids content is mainly composed of hexose sugars, glucose and fructose. The amounts and ratios of these sugars along with parameters including organic acids, mainly citric and malic acid, and lipids determine the

organoleptic quality of tomato (Arazuri et al. 2007). Total soluble solids content determines the viscosity of final processed products, such as ketchup, soups, tomato paste and juice (Bergougnoux 2014). Moreover, sugars contribute to tomato flavor while organic acids contribute to pH and titratable acidity, which are also important parameters in processing tomato. Organic acids are the main determinants of pH and acidity in tomato, and acidity also affects tomato flavor (Anthon, Lestrange, and Barrett 2011). In addition, acidity is important for food safety because it prevents spoilage. Desirable pH for food safety is 4.4 and optimum pH should be 4.25.

Fruit size and shape have huge variation in tomato and all types have usage areas, especially in canning industry. Tomato varieties can be classified as peeled and concentrated varieties based on their usage (Arazuri et al. 2007). Peeled tomatoes have long shape and can be canned whole. Thus these tomatoes should be high quality and resistant to mechanical damages. On the other hand, tomatoes used for concentrate are spherical and may be sold as crushed. Thus the shape of the tomato may not be not important and mechanical damage can be ignored because the fruit will be pureed, diced or crushed.

3.2. Materials And Methods

3.2.1. Plant material

The IBL population described in Chapter 2 was used for metabolic analysis.

3.2.2. Scoring and Measuring Traits

Tomato fruits at the normal market stage were evaluated for 11 qualitative fruit traits: fruit weight, dry matter weight, external color, internal color, locule number, wall thickness, firmness, fruit shape, stem scar, total soluble solids content and pH. Fruit weight (FW) was determined by bulking the fruit from 10 plants and calculating the mean weight of 10 representative tomato fruits. Fruits from 10 plants per genotype were bulked and characterized for external and internal color, fruit firmness, shape, stem scar, locule number, wall thickness and total soluble solids content. External (EXC) and internal fruit colour (INC) were visually determined for each individual using a scale from 1 to 5 (1 =

yellow or orange, 5 = most intense red). A total of 100 g tomato fruits were dried and weighed to calculate fruit dry matter. Fruit firmness (FIRM) was determined by hand squeezing using a scale of 1 to 5 (1 = soft, 5 = very firm). Ratio of fruit length to fruit width represented fruit shape (FS) with a scale from 1 to 5 (1 = round, 5 = elongated). Stem scar diameter (1 = small, 5 = very large) represented stem scar size (SCAR). Locule number (LN) was counted in transversely-cut fruits. Fruit wall thickness (pericarp thickness) (WALL) was visually determined using a scale from 1 to 5 (1 = thin, 5 = very thick) (Tanksley and Nelson 1996). Total soluble solids content of the tomato fruits was measured using a refractometer. The pH of the fruits was measured with a pH meter.

3.2.3. QTL Mapping

QGene version 4.0 (Joehanes and Nelson, 2008) was used for QTL analysis. The CIM (*Composite Interval Mapping)* QTL analysis method uses both interval mapping and multiple regression analysis and was performed with automatic forward cofactor selection and a scan interval of 0.2 Mb. LOD threshold ≥ 3 was used.

3.3. Results

3.3.1. Phenotypic variation

A total of 94 IBL individuals and parental accessions were characterized for 11 fruit quality traits including fruit weight, dry matter weight, external and internal color, locule number, wall thickness, firmness, fruit shape, stem scar, soluble solids content and pH. The parents of the IBL population had extreme phenotypes for fruit weight, wall thickness, stem scar and soluble solids content traits (Table 6). All of the traits segregated in the IBL population (Figure 9). Fruit weight, wall thickness, firmness and stem scar traits displayed the highest variation in the population with coefficients of variation (CV) ranging from 33.5 to 46.4%. With the exception of soluble solids content and pH, the remaining traits (dry weight, external color, internal color, locule number and fruit shape) had considerable variation in the population (CVs ranging from 21.2 to 27.5%). The pH and soluble solids contents displayed the lowest variation with 7 and 9.4% CV, respectively. All traits except external color, locule number and fruit shape displayed

normal, continuous distributions.

Table 6. Statistics for fruit quality traits measured in IBL population and parents; *S. lycopersicum* cv. Tueza and *S. pimpinellifolium* cv. LA1589.

	Parents		IBL population		
Fruit traits	**Tueza**	**LA1589**	**Mean**	**Range**	**CV%**
Fruit weight (g)	118.4	0.8	65.5 ± 3.1	10.4 - 190.2	46.4
Dry matter weight (g)	4.6	5.2	5.1 ± 0.1	1.3 - 8.4	21.2
External color (1-5)	3	5	4.0 ± 0.1	1 - 5	21.6
Internal color (1-5)	4	3	3.3 ± 0.1	1 - 5	27.5
Locule number (1-5)	3	2	3.2 ± 0.1	1 - 4	24.6
Wall thickness (1-5)	3.5	1	2.8 ± 0.1	1 - 5	42.6
Firmness (1-5)	3	3.5	3.1 ± 0.1	1 - 5	33.9
Fruit shape (1-5)	1	1	1.0 ± 0	1 - 2	22.5
Stem scar (1-5)	4	1	3.1 ± 0.1	1 - 5	33.5
Soluble solids content	4.4	8.2	5.2 ± 0.05	4 - 6.8	9.4
pH	4	4	4.0 ± 0	3.7 - 6	7

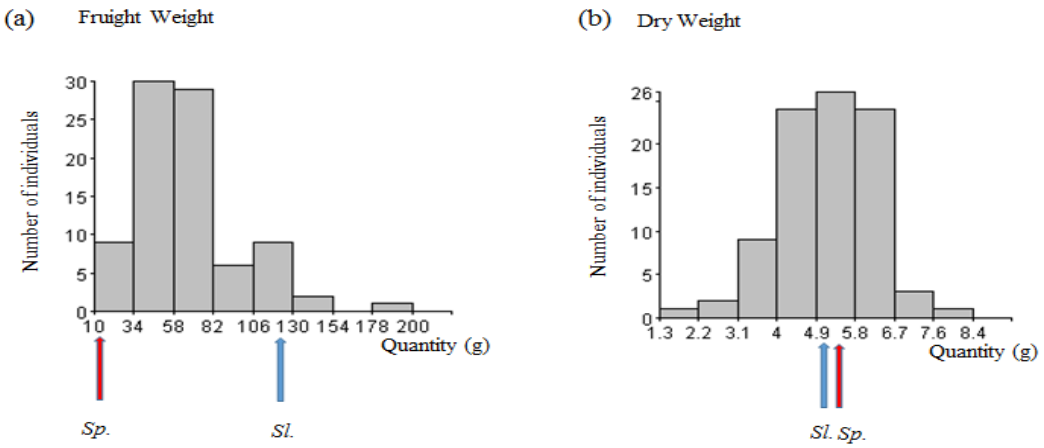

Figure 9. Distribution of traits in IBL population. (a) Distribution of fruit weight in IBL population, (b) Distribution of dry weight in IBL population. Arrows indicate means for Sl.: *S. Lycopersicum* cv. Tueza and Sp.: *S. pimpinellifolium* cv.

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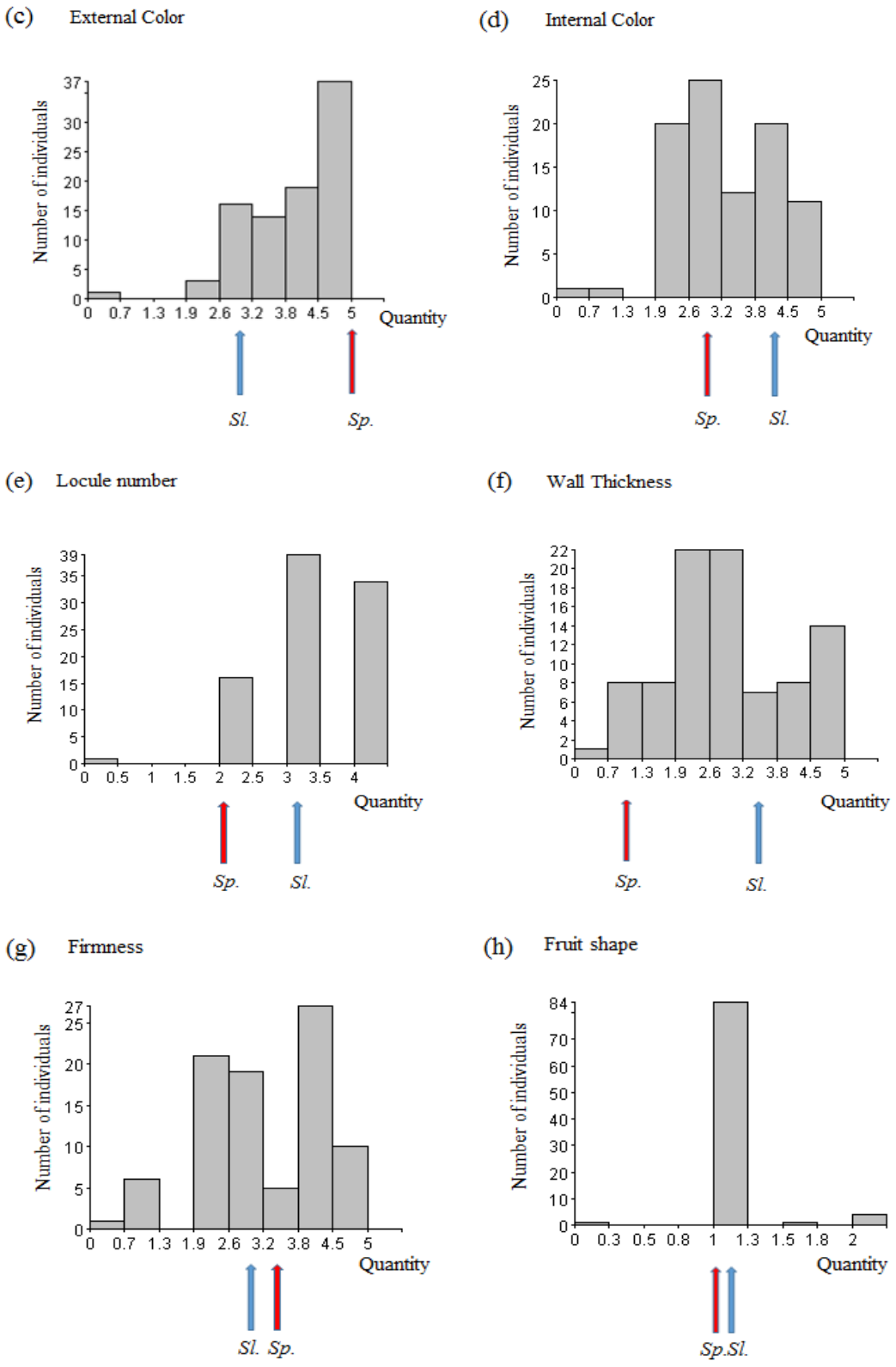

Figure 9. **(cont.)** (c) Distribution of external color in IBL population, (d) Distribution of internal color in IBL population, (e) Distribution of locule number in IBL population, (f) Distribution of wall thickness in IBL population. (g) Distribution of firmness in IBL population, (h) Distribution of fruit shape in IBL population.

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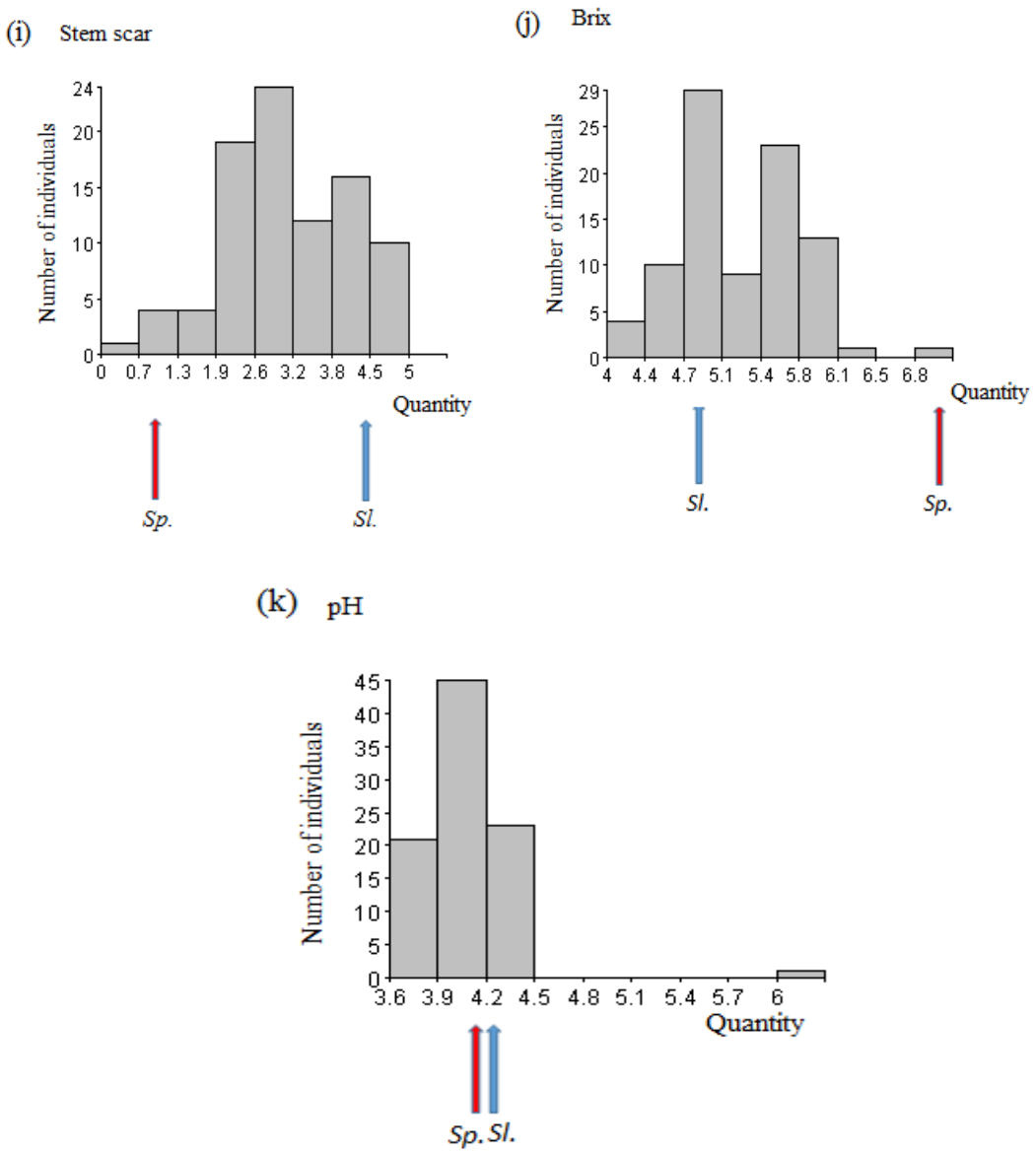

Figure 9. **(cont.)** (i) Distribution of stem scar in IBL population. (j) Distribution of brix in IBL population, (k) Distribution of pH in IBL population.

3.3.2. QTL Mapping

The 3,125 genome-wide SNP loci that were retained after filtering (see Chapter 2) were used in QTL mapping of the fruit quality traits. Composite interval mapping (CIM) analysis was performed and a logarithm of odds (LOD) threshold ($p<0.05$) generated by 1,000 permutations was used to identify QTLs for each trait. For fruit weight, the LOD threshold was 3.1 and three QTLs (*fw2.1*, *fw4.1* and *fw6.1*) were

identified on chromosomes T2, T4 and T6. The percentage of phenotypic variation (PVE) explained by the QTLs varied from 15 to 26%. The QTL on chromosome T4 (*fw4.1*) had the highest PVE, 26%. Three QTLs were identified for dry matter weight on chromosome T7 based on a LOD threshold of 3.3. The PVEs of these loci were 19, 15 and 14% for *dw7.1*, *dw7.2* and *dw7.3*, respectively (Table 7).

A total of 11 QTLs were identified for external and internal color in tomato based on LOD thresholds of 3.1 and 3, respectively. For the external color, two QTLs were identified on chromosomes T1 and T2 with a total PVE of 21%. A total of nine QTLs were identified for internal color on chromosomes T2, T4, T6, T7, T8, T10, and T12. The percentage of phenotypic variation (PVE) explained by the loci varied from 14 to 24% (Table 7).

Two QTLs were identified for locule number on chromosomes T2 and T4 with a LOD threshold of 3.6. PVEs of the QTLs were 30% for *ln2.1* and 13% for *ln4.1*. LOD threshold for wall thickness was 3 and two QTLs were identified on chromosomes T10 and T12. PVEs of the QTLs were 15 and 13%, for *wall10.1* and *wall12.1*, respectively.

A total of four QTLs with LOD scores higher than the threshold (3.2) were identified for firmness on chromosomes T1, T4 and T10. PVEs of the QTLs varied from 14 to 24. For fruit shape, four QTLs were detected on chromosomes T4, T10 and T12 (with LOD scores greater than the threshold, 5). PVEs of the QTLs for the trait ranged from 14 to 26%. The QTL on chromosome T4 had the highest PVE (26%). Two QTLs were determined for stem scar (LOD greater than 3) on chromosomes T7 and T10. PVEs of the QTLs were 16 and 3% for *sc7.1* and *sc10.1*, respectively (Table 7).

LOD thresholds for soluble solids content and pH traits were 3.1 and 6.2, respectively. For soluble solids content, four QTLs were identified on chromosomes T1, T2, T8 and T10. PVEs of the QTLs varied from 17 to 34%. The QTL on chromosome T2 had the highest PVE (34%). For pH, two QTLs were identified on chromosomes T1 and T8. The QTL on chromosome T1 had a major allelic effect with a PVE of 47%. The PVE of the QTL on chromosome T8 was 14% (Table 7). Colocalization of the QTLs indicates that a given QTL has an effect on more than one trait and this pleiotropism is not uncommon in eukaryotic genomes. In the present study, a few colocalized QTLs were detected. QTLs on chromosomes T2 and T4 for locule number colocalized with QTLs for fruit weight and fruit shape, respectively. QTLs on chromosomes T10 and T12 for wall thickness colocalized with QTLs for soluble solids content and fruit shape, respectively. QTLs for pH and external color colocalized on chromosome T1 (Table 8).

Table 7. QTLs identified for 11 fruit quality traits.

Trait	QTL	Chr.	Position (Mb)*	Marker interval	LOD	PVE**	Additive effect***
Fruit weight	fw2.1	T2	51.6 - 52	SpimpSNP_chr2_51653038 - SpimpSNP_chr2_52236461	3.1	15	*S. pimpinellifolium*
Fruit weight	fw4.1	T4	22.5 - 22.9	SpimpSNP_chr4_21588199 - SpimpSNP_chr4_23188806	5.6	26	*S. lycopersicum*
Fruit weight	fw6.1	T6	24.2 - 19.8	SpimpSNP_chr6_23671779 - SpimpSNP_chr6_24889074	3.6	17	*S. lycopersicum*
Dry matter weight	dw7.1	T7	6.07.2008	SpimpSNP_chr7_2225863 - SpimpSNP_chr7_9627011	4.1	19	*S. lycopersicum*
Dry matter weight	dw7.2	T7	27.8 - 29.4	SpimpSNP_chr7_26481282 - SpimpSNP_chr7_28434174	3.2	15	*S. lycopersicum*
Dry matter weight	dw7.3	T7	39.6 - 44.8	SpimpSNP_chr7_39834929 - SpimpSNP_chr7_44678356	3	14	*S. lycopersicum*
External color	exc1.1	T1	67.6 - 67.8	SpimpSNP_chr3_67613866 - SpimpSNP_chr3_67813317	3	11	*S. pimpinellifolium*
External color	exc2.1	T2	62.3 - 62.5	SpimpSNP_chr4_62352850 - SpimpSNP_chr4_62544061	3	10	*S. pimpinellifolium*
Internal color	inc2.1	T2	23.6 - 23.8	SpimpSNP_chr2_23655570 - SpimpSNP_chr2_24268112	4.9	23	*S. pimpinellifolium*
Internal color	inc2.2	T2	34.8	SpimpSNP_chr2_34886535 - SpimpSNP_chr2_35242658	4.5	20	*S. pimpinellifolium*
Internal color	inc2.3	T2	2.03.2008	SpimpSNP_chr2_640497 - SpimpSNP_chr2_3894978	5	20	*S. pimpinellifolium*
Internal color	inc4.1	T4	16.5 - 16.7	SpimpSNP_chr4_16565256 - SpimpSNP_chr4_18177074	3.4	15	*S. pimpinellifolium*
Internal color	inc6.1	T6	30.4	SpimpSNP_chr6_30399172 - SpimpSNP_chr6_31009885	3.1	14	*S. lycopersicum*
Internal color	inc7.1	T7	34.3 - 34.4	SpimpSNP_chr7_33799287 - SpimpSNP_chr7_34463608	4.2	16	*S. lycopersicum*
Internal color	inc8.1	T8	10.04.2011	SpimpSNP_chr8_10421348 - SpimpSNP_chr8_15081462	3.8	17	*S. pimpinellifolium*
Internal color	inc10.1	T10	51.4	SpimpSNP_chr10_51446730 - SpimpSNP_chr10_53466408	5.5	24	*S. pimpinellifolium*
Internal color	inc12.1	T12	23.7 - 24.1	SpimpSNP_chr12_21186959 - SpimpSNP_chr12_24152718	5.2	23	*S. lycopersicum*
Locule number	ln2.1	T2	47.2 -51.4	SpimpSNP_chr2_47074933 -SpimpSNP_chr2_51653038	7	30	*S. lycopersicum*
Locule number	ln4.1	T4	5.7	SpimpSNP_chr4_5137285 - SpimpSNP_chr4_6526895	3.8	13	*S. lycopersicum*

* Peak position of QTL.
** Percentage of phenotypic variation explained by identified QTL.
*** Parental allele associated with increased trait value.

(cont. on next page)

Table 7. **(cont.)**

Trait	QTL	Chr.	Position (Mb)*	Marker interval	LOD	PVE**	Additive effect***
Wall thickness	wall10.1	T10	21.6 - 22.6	SpimpSNP_chr10_19888032 - SpimpSNP_chr10_23051275	3.4	15	*S. lycopersicum*
Wall thickness	wall12.1	T12	62.5	SpimpSNP_chr12_52326486 - SpimpSNP_chr12_63747215	3	13	*S. lycopersicum*
Firmness	firm1.1	T1	2.08.2003	SpimpSNP_chr1_2881522 - SpimpSNP_chr1_8892676	3.5	14	*S. pimpinellifolium*
Firmness	firm1.2	T1	80.2 - 80.4	SpimpSNP_chr1_79834277 - SpimpSNP_chr1_80687203	3.8	18	*S. pimpinellifolium*
Firmness	firm4.1	T4	2.5	SpimpSNP_chr4_1705692 - SpimpSNP_chr4_2593932	4	19	*S. pimpinellifolium*
Firmness	firm10.1	T10		SpimpSNP_chr10_1689980 - SpimpSNP_chr10_2014825	5.3	24	*S. pimpinellifolium*
Fruit shape	fs4.1	T4	5.7	SpimpSNP_chr4_5717067 - SpimpSNP_chr4_6526895	6,1	26	*S. lycopersicum*
Fruit shape	fs10.1	T10	11.2 - 11.8	SpimpSNP_chr10_10418801 -SpimpSNP_chr10_12085921	5.2	14	*S. lycopersicum*
Fruit shape	fs12.1	T12	33.1 - 35.5	SpimpSNP_chr12_33126847 - SpimpSNP_chr12_36718114	5.5	20	*S. lycopersicum*
Fruit shape	fs12.2	T12	59.7 -62.7	SpimpSNP_chr12_52326486 - SpimpSNP_chr12_63747215	5.2	23	*S. lycopersicum*
Stem scar	sc7.1	T7	64.4	SpimpSNP_chr7_64272106 - SpimpSNP_chr7_64876647	3.6	16	*S. lycopersicum*
Stem scar	sc10.1	T10	29.8 - 35.6	SpimpSNP_chr10_36881278 - SpimpSNP_chr10_29632232	3.3	3	*S. pimpinellifolium*
Soluble solids content	ssc1.1	T1	26.6 - 27.2	SpimpSNP_chr1_24085783 - SpimpSNP_chr1_27278048	5.3	23	*S. pimpinellifolium*
Soluble solids content	ssc2.1	T2	36.6 -38.6	SpimpSNP_chr2_36642750 - SpimpSNP_chr2_38643210	8.2	34	*S. pimpinellifolium*
Soluble solids content	ssc8.1	T8	60.2 - 61.2	SpimpSNP_chr8_60424301 - SpimpSNP_chr8_61250753	3.7	17	*S. lycopersicum*
Soluble solids content	ssc10.1	T10	22.8	SpimpSNP_chr10_23051275 - SpimpSNP_chr10_19888032	3.6	17	*S. lycopersicum*
pH	ph1.1	T1	66.8	SpimpSNP_chr1_65817896 - SpimpSNP_chr1_67006382	12	47	*S. lycopersicum*
pH	ph8.1	T8	63.8	SpimpSNP_chr8_63683606 - SpimpSNP_chr9_755740	6.4	14	*S. lycopersicum*

Table 8. Fruit quality QTLs that colocalized.

Trait	QTL	Chr.	Position*
pH	ph1.1	T1	66.8
External color	exc1.1	T1	67.6 - 67.8
Locule number	ln2.1	T2	47.2 -51.4
Fruit weight	fw2.1	T2	51.6 - 52
Locule number	ln4.1	T4	5.7
Fruit shape	fs4.1	T4	5.7
Soluble solids content	ssc10.1	T10	22.8
Wall thickness	wall10.1	T10	21.6 - 22.6
Wall thickness	wall12.1	T12	62.5
Fruit shape	fs12.2	T12	59.7 -62.7

Correlations between fruit weight and all traits except fruit shape, internal color, dry matter weight and pH demonstrated that fruit weight was associated with fruit quality traits such as locule number, wall thickness, firmness and stem scar. Fruit weight had a high positive correlation with locule number. Negative correlations were observed for fruit weight with external color and soluble solids content (Table 9).

Table 9. Significant ($P < 0.05$) correlations between tomato fruit traits. Correlations with P value > 0.05 were considered to be non-significant (NS). FW = Fruit weight, DW = Dry matter weight, EXC = External color, INC = Internal color, LN = Locule number, WALL = Wall thickness, FIRM = Firmness, FS = Fruit shape, SCAR = Stem scar, SSC = Soluble solids content.

Traits	FW	DW	EXC	INC	LN	WALL	FIRM	FS	SCAR	SSC	pH
FW	1	NS	-.27	NS	.40	.50	.31	NS	.32	-.26	NS
DW		1	.33	.30	NS	NS	NS	NS	NS	.467	NS
EXC			1	.38	.25	NS	NS	NS	NS	NS	NS
INC				1	.26	.24	NS	NS	.37	NS	NS
LN					1	.25	NS	NS	.55	NS	NS
WALL						1	.20	.30	.33	NS	NS
FIRM							1	.15	NS	NS	NS
FS								1	NS	NS	NS
SCAR									1	NS	NS
SSC										1	NS
pH											1

3.4. Discussion

The IBL population and parental genotypes were evaluated for 11 fruit quality traits in order to identify associated QTLs. The efficiency of QTL mapping to reveal the genetic basis of a trait depends on segregation of the trait in the mapping population and phenotypic differences between parents (Collard et al. 2005). Sizable variation for all traits except soluble solids content and pH, and normal continuous distribution of all but three traits (external color, locule number and fruit shape) were observed in the IBL population. Thus, all traits segregated in the IBL population. External color and locule number tended to skew toward more intense red color and higher locule numbers due to the unbalanced nature of the IBL population which favors the recurrent parent genotype. The parents of the IBL population had extreme alleles for fruit weight, wall thickness, stem scar and soluble solids content traits. Although the parental alleles for soluble solids content were extreme, low variation was observed in the IBL population for the trait. This finding implies an unbalanced introgression of *S. pimpinellifolium* alleles for soluble solids content into the *S. lycopersicum* genome.

The present study demonstrated correlations between fruit quality traits, however, most of the significant correlations were weak. Correlations between fruit weight and all traits except fruit shape, internal color, dry matter weight and pH demonstrated that fruit weight was associated with fruit quality traits such as locule number, wall thickness, firmness and stem scar. Fruit weight had a high positive correlation with locule number. This is expected because increased locule number has a direct effect on fruit size and weight. Negative correlations of fruit weight with external color and soluble solids content indicate that intensity of external color decreases with increased fruit size due to decreased lycopene concentration. The results also suggest that sucrose content is negatively correlated with fruit volume. This negative correlation was also reported by Chen et al. (1999); Doganlar et al. (2002); Sun et al. (2012); Fulton et al.(1997). Correlation results between fruit weight and fruit quality traits were consistent with the results of Lippman and Tanksley (2001), Ökmen et al. (2011) and Fulton et al. (1997). A direct effect of soluble solids content on dry matter weight was observed in the IBL population. The positive correlation between internal color and external color was expected and consistent with previous reports (Fulton et al. 1997, 2000; Ökmen et al. 2011). These correlations can also be attributed to the fact that some of the traits are

directly related to each other (e.g., color traits) and/or the pleiotropic effects of genes on different fruit quality traits.

Fruit quality parameters are important agronomic traits that increase the market value of both fresh market and processing tomatoes. Thus, there are many reports on QTL identification for fruit quality traits. All previous QTL mapping studies were performed using low density linkage maps constructed with primers and RFLP probes. Various parental lines and mapping populations such as BC_2F_2, IBL and RIL were used in these previous studies. This is the first study in which QTLs for fruit quality traits were identified by constructing a high density SNP-based physical map using a recently developed IBL population that carries introgressions from the *S. pimpinellifolium* genome. The physical map of SNP markers was useful for QTL mapping as IBLs are unbalanced populations which are not suitable for linkage map construction.

Fruit weight is the focus of many studies because increased fruit weight has direct effects on tomato yield (Capel et al. 2015; Chen et al. 1999; Doganlar et al. 2002; Lippman and Tanksley 2001; Saliba-Colombani et al. 2001; Prudent et al. 2009). Fruit size is also an important trait that directs consumer preferences. Medium and large tomatoes are usually preferred by consumers (Oltman, Jervis and Drake 2014). In this work, three QTLs were identified on chromosomes T2, T4 and T6 for fruit weight. Previous studies identified three major and two minor QTLs on chromosomes T1, T2, T3, T7 and T11. Although QTL locations varied among these studies, all studies identified a QTL with major effect on chromosome T2 corresponding to a cloned gene that controls fruit weight (*fw2.2*) (Frary et al 2000). In the present study, the fruit weight QTL on chromosome T2 explained 15% of varioation for the trait, a value which is relatively low when compared with the same QTL in other studies (PVEs ranged between 15 and 40%). Differences in QTL magnitudes of effect are most likely due to differences in population type used in the studies. The present work is most similar to the work of Doganlar et al. (2002) which also studied an IBL population but used a processing tomato as the recurrent parent. The PVE of the QTL on chromosome T2 was the same as that reported by Doganlar et al. (2002) (15%) due to the similarity of the genetic structures of the populations used in the two studies. Identification of previously undetected QTLs on chromosomes T4 and T6 in the present work can be attributed to variation in the genetic backgrounds of the two mapping populations which is due to the use of different recurrent parents.

Because dried tomatoes have a high economic value, fruit dry matter weight can be as important as fruit weight. A previous QTL mapping study performed by Saliba-Colombani et al. (2001) identified dry matter QTLs (with PVEs ranging from 9 to 25%) on chromosomes T2, T4 and T9 in a RIL population developed from the cross between a cherry tomato cultivar and *S. lycopersicum*. In other work, QTLs were identified on chromosomes T8, T10, T11 and T12 using 20 introgression lines carrying *S. chmielewski* introgressions in a *S. lycopersicum* genetic background (Prudent et al. 2009). In the present study, none of the above mentioned QTLs were detected. This result can be due to insufficient variation for dry matter weight between the parents and the moderate coefficient of variation detected for the trait in the mapping population. PVEs of identified QTLs ranged from 14 to 19%, suggesting that in contrast to fruit weight, dry matter weight is not controlled by major effect QTLs in our population.

In the present study, while a total of nine QTLs were identified for internal color, only two loci were identified for external color. The low number of QTLs identified for external color might be due to the unbalanced segregation of the trait in the IBL population. Previous work detected QTLs for external color on chromosomes T1, T3, T4, T7, T8, T9, T11 and T12 (Monforte et al. 2001; Ökmen et al. 2011). Although a QTL was also identified on chromosome T1 in this work, the physical position of the closest marker (C2_At5g13030: 1.1 Mb) to the locus on the same chromosome by Ökmen et al. (2011) reveals that the two QTLs are not identical. For internal color, previous studies identified QTLs on chromosomes T1, T3, T4, T7, T8, T9 and T12 with PVEs that ranged between 5 to 30% (Bernacchi et al. 1998; Ökmen et al. 2011). In the present work, QTLs for internal color were identified on chromosomes T4, T7 and T8. The physical positions of the markers (65.4 Mb, 55 Mb and 58.1 Mb for At1g47830, T0671 and TG307, respectively) linked to the three QTLs indicated that they do not overlap with the QTLs identified in previous work.

Previous studies showed that locule number is controlled by six QTLs on chromosomes T2, T3, T4, T7, T10 and T12 (Monforte et al. 2001; Ökmen et al. 2011). In addition, a major gene for locule number was mapped at the 48.1 Mb position on chromosome T2 (Muños et al. 2011). The major QTL (*ln2.1*) containing this single gene (*lc*) was also identified in the present study (PVE of 30%). In addition to this major QTL, a new QTL with minor effect was identified on chromosome T4.

Wall thickness and firmness are important fruit quality traits that define the shelf life of tomatoes. QTLs with minor effects on wall thickness were reported on

chromosomes T6, T8, T11 and T12 (Ökmen et al. 2011), however, these loci do not overlap with those reported in the present work. Previously, QTLs for firmness were identified on chromosomes T1, T2, T3, T4, T5, T8 and T10 (Doganlar et al. 2002; Ökmen et al. 2011). In addition to these previously identified QTLs, four new QTLs were identified for firmness trait in this work.

Fruit shape and stem scar are appearance traits analysed in this study. Globular fruits with small stem scar are favoured in the market. More than 10 QTLs for fruit shape were identified in previous studies (Doganlar et al. 2002; Frary et al. 2000; Ökmen et al. 2011). In addition to these QTLs, four new QTLs were identified in this work with minor effects on fruit shape. For stem scar, seven QTLs were previously identified in tomato (Doganlar et al. 2002; Lippman and Tanksley 2001; Ökmen et al. 2011). One of the two QTLs identified in this study for the stem scar was previously reported at the 65.5 Mb position on chromosome T7 with a low PVE of 8% (Doganlar et al. 2002).

Soluble solids content and pH are important traits for fresh market tomatoes as they help define flavor (Stevens, Kader and Albright 1979). A total of five QTLs were detected on chromosomes T1, T6, T8 and T9 in previous studies for soluble solids content (Capel et al. 2015; Chen et al. 1999; Doganlar et al. 2002; Saliba-Colombni et al. 2001). The present report demonstrated that different QTLs (chromosome T1, T2, T8 and T10) control soluble solids content in fresh market tomatoes. For pH, a total of six QTLs were identified in tomato on chromosomes T1, T2, T4, T5, T9 and T12 in previous studies (Capel et al. 2015; Chen et al. 1999; Saliba-Colombni et al. 2001). While the position of the previously identified QTL on chromosome T1 (Chen et al. 1999) was at 86 Mb, the major effect QTL (47%) identified on the same chromosome in this work was positioned at 66.8 Mb. Thus, the QTL identified in this study is close to the QTL previously identified by Chen et al. (1999). These two QTLs might actually overlap because the SNP based map of the present study has much higher resolution than the linkage map of Chen et al. (1999).

In the present study, expected colocalized QTLs were detected for fruit quality traits in the tomato genome. QTLs for locule number colocalized with fruit weight and fruit shape because increased locule number leads to larger tomatoes. However, colocalization of a QTL for pH with one for external color and colocalization of loci for wall thickness and soluble solids content were unexpected. These unexpected colocalizations might be due to linkage of the genes that control the traits (He and Zhang 2006).

The present study confirmed the high breeding potential of *S. pimpinellifolium* by detecting useful alleles for breeding of fruit quality traits such as fruit weight, external and internal color, firmness, soluble solids content and stem scar. The findings were expected for external color and soluble solids content because *S. pimpinellifolium* had higher values than cultivated tomato. In contrast, although *S. pimpinellifolium* had lower values than *S. lycopersicum* for fruit weight, internal color and stem scar, favorable *S. pimpinellifolium* alleles were detected for these traits. This result was consistent with the work of Top et al. (2014). In that study, although *S. pimpinellifolium* had lower values than cultivated tomato for fruit weight and firmness, some individuals from an IBL (BC_2F_9) population derived from the cross *S. lycopersicum* and *S. pimpinellifolium* (LA1589) showed higher values than *S. lycopersicum* (TA209). Overall, knowledge of the locations and magnitudes of effect of fruit quality QTLs will be very useful in MAS for these traits.

CHAPTER 4

TOMATO FLAVOR CHARACTERS

4.1. Introduction

Many consumers complain about the flavor of fresh market tomatoes (*S. lycopersicum*). Flavor is a complex trait which is determined by both taste and olfaction (aroma compounds) and their interactions. In tomato, taste is mainly determined by sugars and organic acids and volatile compounds determine olfaction (Baldwin et al. 2000; Tieman et al. 2012, 2017). Metabolites contributing to flavor are greatly affected by environmental factors, and the study of flavor is problematic due to a lack of high throughput assays. Because of these difficulties, until recently breeders have focused on yield and resistance to biotic and abiotic stresses instead of tomato flavor (Tieman et al. 2017). Moreover, previous breeding objectives resulted in the loss of tomato flavor (Maul et al. 2000).

The types and quantities of sugars, and their ratios to each other determine sweetness which is the main determinant of quality and marketability of tomato fruits. Sugar content and variation in sugar types are highly genotype-dependent characters, and also related to total soluble solids content, pH, titratable acidity and fruit size (Georgelis 2002). In addition to genotype, plant nutrition, climate, soil, and storage conditions also affect sugar accumulation in the fruit (Whiting 1970). Moreover, sugar content changes during ripening and maturation (Nookaraju et al. 2010) and sugar accumulation differs in different tissues such as mesocarp and locular tissues of tomato (Mounet et al. 2009). The major sugars present in tomato are hexose sugars: glucose and fructose. The only exceptions are a few wild species such as *S. chmielewskii* and *S. habrochaites* which contain sucrose as the major sugar (Nookaraju et al. 2010). Sucrose level decreases and the levels of hexose sugars increase during maturation due to changes in enzyme activity. During maturation, the activities of sucrose synthase, glucokinase and fructokinase decrease. At the same time, the activity of invertase, the enzyme that catalyzes conversion of sucrose to glucose and fructose, increases in both cultivated tomato and most wild species (Carrari et al. 2006; Steinhauser et al. 2010; Yelle et al. 1991).

In addition to contributions to flavor, sugars also have different biological functions. Sugars play roles in carbon and energy metabolism and polymer biosynthesis such as the synthesis of starch. Low starch synthesis is related to high glucose levels while high starch levels are related to high sucrose levels and also nuclear expansion (Tsai, Salamini, and Nelson 1970; Weber et al. 1998). Moreover, hexose sugars control developmental processes and metabolism and sucrose regulates differentiation and storage in developing seeds (Rolland et al. 2006; Weber, Borisjuk, and Wobus 1997; Wobus and Weber 1999). Sugars are also reported to have hormone-like roles as primary messengers in signal transduction (Koch 1996; Lalonde et al. 1999).

Organic acids affect fruit flavor by changing acidity. They regulate basic cellular processes such as modification of cellular pH and redox state (Drincovich, Voll, and Maurino 2016). Citric acid and malic acid are found in high concentrations in tomato and their contribution to tomato flavor is significant (Anthon et al. 2011). In addition, organic acids have diverse functions in plants such as playing roles in transportation of molecules across biological membranes (de Angeli et al. 2013; Hedrich and Marten 1993), acting as messengers in signalling (Finkemeier et al. 2013), responding to abiotic stress (Dyson et al. 2016) and modification of proteins (Weinert et al. 2013; Zhang et al. 2011).

The most important compound of flavor is volatile compounds, which are the major determinant of aroma and, therefore, flavor. Aroma depends on the composition and concentration of individual volatile compounds and the interactions between them (Baldwin et al. 2008; Tucker 1993). Although ripe tomato fruit synthesize more than 400 volatile compounds, only 29 of them are present in relatively high concentration (Table 10). Of these, 15 to 20 have positive major effects on tomato flavor: cis-3-hexenal, hexanal, 3-methylbutanal, trans-2-hexenal, trans-2-heptenal, 2-phenylacetaldehyde, β-ionone, 1-penten-3-one, β- damascenone, 6-methyl-5-hepten-2-one, cis-3-hexenol, 2-phenylethanol, 3-methylbutanol, 1-nitro-2-phenylethane, 2-isobutylthiazole, and methyl salicylate (Baldwin et al. 2000; Buttery 1993; Wang, Baldwin, and Bai 2016).

Table 10. Volatiles present in fresh tomato fruit (Source: Wang et al. 2016).

Volatile compound	Precursor	Concentration (ng L^{-1})	Odor threshold (ng L^{-1})	Log odor unit	Odor description
Aldehydes					
cis − 3-Hexenal	Fatty acids	12.000	0.25	3.7	Tomato, green
Hexanal	Fatty acids	3100	4.5	2.8	Green, grassy
3-Methylbutanal	Branched chain amino acids	27	0.2	2.1	Musty
trans − 2-Hexenal	Fatty acids	270	17	1.2	Green
trans − 2-Heptenal	Fatty acids	60	13	0.7	Green
2-Phenylacetaldehyde	Phenylalanine	15	4	0.6	Floral, alcohol
β-Cyclocitral	Carotenoids	3	5	-0.2	Mint
Geranial	Carotenoids	12	32	-0.4	Citrus
trans − 2-Pentenal	Fatty acids	140	1500	-1	Strawberry, fruity, tomato
Neral	Carotenoids	2	30	-1.2	Lemon
Pseudoionone	Carotenoids	10	800	-1.9	Balsamic
Ketones					
β-Ionone	Carotenoids	4	0.007	2.8	Fruity, floral
1-Penten-3-one	Fatty acids	520	1	2.7	Fruity, floral, green
β-Damascenone	Carotenoids	1	0.002	2.7	Fruity
6-Methyl-5-Hepten-2-one	Carotenoids	130	50	0.4	Fruity, floral
Geranylacetone	Carotenoids	57	60	-0.02	Sweet, floral, estery
Epoxy-β-ionone	Carotenoids	1	100	-2	Fruity, sweet, wood

(cont. on next page)

Table 10. **(cont.)**

Volatile compound	Precursor	Concentration (ng L^{-1})	Odor threshold (ng L^{-1})	Log odor unit	Odor description
Alcohols					
cis − 3-Hexenol	Fatty acids	150	70	0.3	Green
2-Phenylethanol	Phenylalanine	1900	1000	0.3	Nutty, fruity
3-Methylbutanol	Branched chain amino acids	380	250	0.2	Earthy, musty
Linalool	Geranyl pyrophosphate	2	6	-0.5	Citrus, fruity, sweet
1-Penten-3-ol	Fatty acids	110	400	-0.6	Sweet, fruity, grassy
Pentanol	Fatty acids	120	4000	-1.5	Balsamic
Hexanol	Fatty acids	7	500	-1.9	Resin, flower, green
Nitrogen- and oxygen-containing compounds					
1-Nitro-2-phenylethane	Phenylalanine	17	2	0.9	Musty, earthy
1-Nitro-3-methylbutane	-	59	150	-0.4	-
Esters					
Methyl salicylate	Phenylalanine	48	40	0.008	Wintergreen
Sulfur- and nitrogen-containing heterocyclic compounds					
2-Isobutylthiazole	Branched chain amino acids	36	3.5	1	Tomato vine, green
Nitrogen compounds					
3-Methylbutanenitrile	Branched chain amino acids	13	1000	-1.9	Pungent

The concentrations of volatile compounds increase during ripening, especially in later ripening stages. Amino acids, fatty acids, and carotenoids are precursors, and ethylene also plays a role in the synthesis of volatile compounds. Fatty acid-derived volatile compounds are C5 and C6 aldehydes and their alcohols, and are synthesized through the oxylipin pathway. Carotenoid-derived volatile compounds are apocarotenoid volatiles and are synthesized through carotenoid cleavage dioxygenases. Amino acid-derived volatile compounds are phenolic volatiles and are synthesized directly via amino acid metabolism. Moreover, composition and concentration of volatile compounds are highly dependent on genetic background, environmental factors, and pre-and post harvest conditions (Wang, Baldwin, and Bai 2016).

4.2. Materials And Methods

4.2.1. Plant material

The IBL population described in Chapter 2 was used for metabolic analysis.

4.2.2. Metabolic Profiling

Currently there is no single method for extraction and detection of metabolites because the metabolome is complex and consists of a wide varity of metabolites including lipid soluble metabolites, aqueous polar metabolites, stable and nonstable metabolites, and acidic and basic metabolites. Despite this challenge, different chromatographic techniques with different extraction methods based on the structure of the metabolite are powerful techniques to study metabolomics. Thus, two different extraction methods were applied to samples to extract polar and nonpolar metabolites. Also the chromatographic techniques for the target metabolites were optimized.

4.2.3. Sample Preparation

Samples (100 g) of tomato fruit from the 94 individuals and 2 parents of the IBL population were lyophilized. Fine powder was obtained from dried samples by grinding with a knife mill grinder.

A total of 1 g of each dried tomato sample was extracted in 5 ml hexane:dichloromethane (1:1, v/v) on an orbital shaker at 400 rpm and 18 °C overnight. Samples were centrifuged at 4 °C, 4000 rpm for 20 minutes. Supernatants were saved and pellets were subjected to extraction with the same solvent overnight once more. Samples were centrifuged at 4 °C and 4000 rpm for 20 minutes. Supernatants were combined, aliquoted and kept at -80 °C until the analysis. Pellets were subjected to another extraction with chloroform:methanol:water (1:3:1, v/v/v) on an orbital shaker at 400 rpm and 18 °C overnight. The procedure was the same as the hexane:dichloromethane extraction. Supernatants were combined, aliquoted and kept at -80 °C until the analysis.

4.2.4. Quantification of Metabolites

An expanded literature survey was done, promising methods were chosen and some modifications were applied for the optimization of the analytic methods.

Glucose, fructose, and sucrose were analysed with a isocritic method of HPLC-RI with a modified method (Petkova et al. 2013). The chloroform: methanol: water (1:3:1, v:v:v) extract of tomato was used. Sugars were analyzed on amino column (NH_2, 5 µm – 25 x 4,6 mm) at 40 °C using water: acetonitrile (10:90, v:v) as the mobile phase with a flow rate 1 ml/min. Sample injection was 20 µl and standard solutions were prepared in water. Detection was done on positive mode at 40 °C with RI detector.

Summary of method parameters are below:

Column: NH_2 (5 µm – 25 x 4,6 mm)

Column Temperature: 40 °C

RI: 40 °C, positive

Flow rate: 1 ml/min

Injection volume: 20 µl

Solvent: Water

Mobil phase: Water: Acetonitrile (10:90, v:v)

The organic acids including citric acid, malic acid, tartaric acid, succinic acid, lactic acid, fumaric acid, butyric acid, shikimic acid were analyzed by derivitization with methoxamine hydrochloride and N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) on a thermogradient method of GC-FID with a combined and modified method (Namgung et al. 2010; Roessner et al. 2000). The only exception was acetic acid which

was analyzed together with volatile compounds by GC-MS (as described below). The chloroform: methanol: water (1:3:1:, v:v:v) extract of tomato was used. Samples (100 µl) of extract were evaporated at 30 °C in a vacuum evaporator. Then 40 µl of methoxamine hydrochloride (20 mg/ml in pyridine, and freshly prepared before use) was added to dried sample. The sample was dissolved in methoxamine hydrochloride in ultrasonic bath for 5 minutes. Derivatization was performed by holding samples at 37 °C for 90 minutes. Second derivatization was done with MSTFA. Thus, 60 µl of MSTFA was added to sample which was held at 37 °C for 30 minutes. Derivatized sample was centrifuged at 14.000 rpm for 5 minutes. Supernatant was injected to GC-FID.

Organic acids were analyzed on Rtx 5DA (0,25 mm x 0,25 mm, 30 m) column with a thermogradient program. The column temperature was programmed from 100 °C (1 min held) to 150 °C at a rate of 5 °C/min, from 150 °C (1 min held) to 280 °C at a rate of 5 °C/min, 2 minutes held at the final temperature was applied. Injection port temperature was held at 250 °C while detector temperature was held at 300 °C. Carrier gas was nitrogen (N_2) and split ratio was 1/25. Detection was done by FID.

Summary of method parameters is below:

Column: Rtx 5DA (0,25 mm x 0,25 mm, 30 m)

Column Temperature: 100 °C to 280 °C

Column Gas Flow: 1,52 ml/min

Carrier Gas: N2

Injection Volume: 1 µl

Split Ratio: 1/25

Injection Port Temperature: 250 °C

Detector Temperature: 300 °C

Column temperature programme:

°C / min	°C	min
_	100	1
5	150	1
5	280	2

Volatile compounds were analysed using GC/MS with solid phase microextraction (SPME) with a modified method (Maggi et al. 2011). Samples were incubated 50 °C for 15 minutes for absorption on fiber in SPME. Volatile compounds

were analyzed on Rxi 5Sil MS (0,25 mm x 0,25 mm, 30 m) column with a thermogradient program. The column temperature was programmed from 40 °C (3 min held) to 230 °C at a rate of 4 °C/min, 50 minutes held at the final temperature was applied. Injection port temperature was held at 250 °C. Carrier gas was helium (He) and split ratio was 1/10. Detection was done by mass spectrometry with electrospray ionization. Temperature of ionization source was set to 200 °C. Detection was performed in scan mode (m/z 35 – 450).

Summary of method parameters:

Column: Rxi 5Sil MS (0,25 mm x 0,25 mm, 30 m)

Column Temperature: 40 °C -230 °C

Column Gas Flow: 90 kPA

Carrier Gas: He

Split Ratio: 1/10

Injection Port Temperature: 250 °C

Column temperature Programme:

°C / min	°C	min
_	40	3
4	230	50

4.2.5. QTL mapping

The method for QTL mapping was explained in chapter 3 using the data obtained from GBS (chapter 2) to identify metabolic QTLs.

4.3. Results

4.3.1. Metabolite Variation

A total of 94 IBL individuals and parental accessions were characterized for sugars including glucose, fructose and sucrose; for organic acids including citric acid, malic acid, tartaric acid, succinic acid, lactic acid, fumaric acid, butyric acid, salicylic

acid shikimic acid and acetic acid; for volatile compounds including β-ionone, 2-methyl-1-butanol, 3-methyl-1-butanol, β-cyclocitral, 1-nitro-3-methylbutane, 1-octen-3-ol, 1-pentanol, 1-penten-3-one, 2,3-butanediol, 2,3-butanedione, 3-hydroxy-2-butanone, 2-heptanone, 2-hexenal, 2-ısobutylthiazole, 2-nonanone, 2-pentanone, 3-methyl-2-pentanone, 2-pentenal, 3-ısopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane, 3-pentanone, geranyl acetone, 6-methyl-5-hepten-2-ol, 6-methyl-3,5-heptadien-2-one, 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, acetaldehyde, acetic acid, ethyl ester, acetic acid, methyl ester, isovaleric acid, cyclohexasiloxane dodecamethyl, cyclopentane, cyclopentasiloxane decamethyl, perillen, methyl capronate, methyl isovalerate, neryl acetone, 1-nitro-pentane, 1-nitro-propane, 2-nitro-propane, and sulcatol.

4.3.1.1. Sugars

The parents of the IBL population had high glucose and fructose content (Table 11). All of the traits segregated in the IBL population. Glucose displayed variation in the population with a coefficient of variation (CV) of 24.0 % while fructose had CV of 28.0 %. Both traits displayed normal and continuous distributions within the population (Figure 10). On the other hand, sucrose was not detected in both parents and the population.

Table 11. Statistics for sugar content measured in IBL population and parents: *S. Lycopersicum* cv. Tueza and *S. pimpinellifolium* cv. LA1589. Quanitities of metabolites are given as mg/100g DW.

	Parents		**IBL Population**		
	Tueza	**LA1589**	**Mean**	**Range**	**CV%**
Glucose	8738.04	4153.29	6596.45	0 - 9897.50	24.0
Fructose	8401.38	3967.70	5839.52	0 - 9457.71	28.0

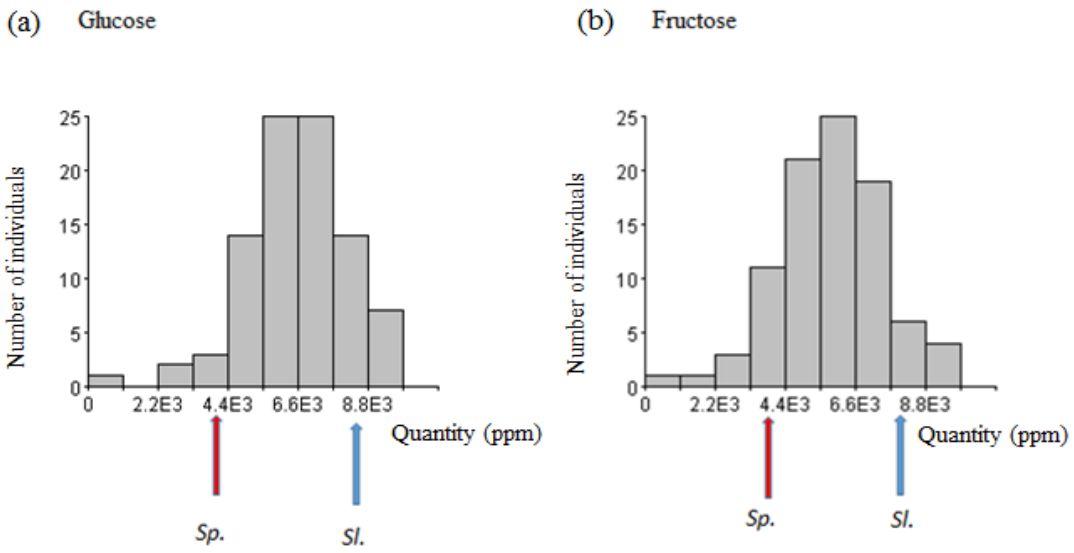

Figure 10. Distribution of sugar content in IBL population. (a) Distribution of glucose content (ppm) in IBL population, (b) Distribution of fructose content (ppm) in IBL population. Arrows indicate means for Sl.: *S. Lycopersicum* cv. Tueza and Sp.: *S. pimpinellifolium* cv. LA1589.

4.3.1.2. Organic Acids

Tartaric acid, fumaric acid and butyric acid could not be detected in parents or in the IBL population. The parents of the IBL population had high citric acid content. Tueza had especially high malic acid content while *S. pimpinellifolium* had high acetic acid content. The parents did not have high amounts of other organic acids (Table 12). Organic acids displayed variation in the population with CV ranging from 61.9 % to 286.8 %. Organic acids did not display continuous distribution within the population except shikimic acid and acetic acid (Figure 11).

Table 12. Statistics for organic acid contents measured in IBL population and parents; *S. Lycopersicum* cv. Tueza and *S. pimpinellifolium* cv. LA1589. Quanitities of metabolites were explained as mg/100g DW.

	Parents		IBL Population		
	Tueza	**LA1589**	**Mean**	**Range**	**CV%**
Succinic acid	0.01	0.02	0.03	0-0.50	286.8
Lactic acid	0.31	0.04	0.18	0-1.35	98.0
Malic acid	6.19	0.86	3.58	0-27.0	100.7
Shikimic Acid	0.94	0.00	1.17	0-3.78	89.8
Citric acid	10.40	8.51	7.59	0-20.60	61.9
Acetic acid	9.0	82.51	38.24	0-59.24	99.7

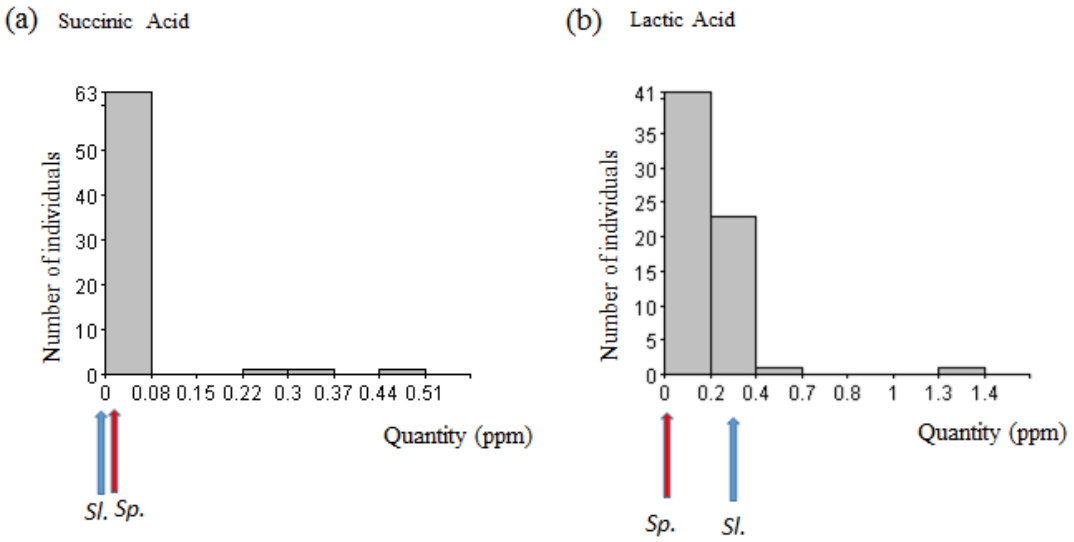

Figure 11. Distribution of organic acid contents in IBL population. (a) Distribution of succinic acid content (ppm) in IBL population, (b) Distribution of lactic acid content (ppm) in IBL population. Arrows indicate means for Sl.: *S. Lycopersicum* cv. Tueza and Sp.: *S. pimpinellifolium* cv. LA1589.

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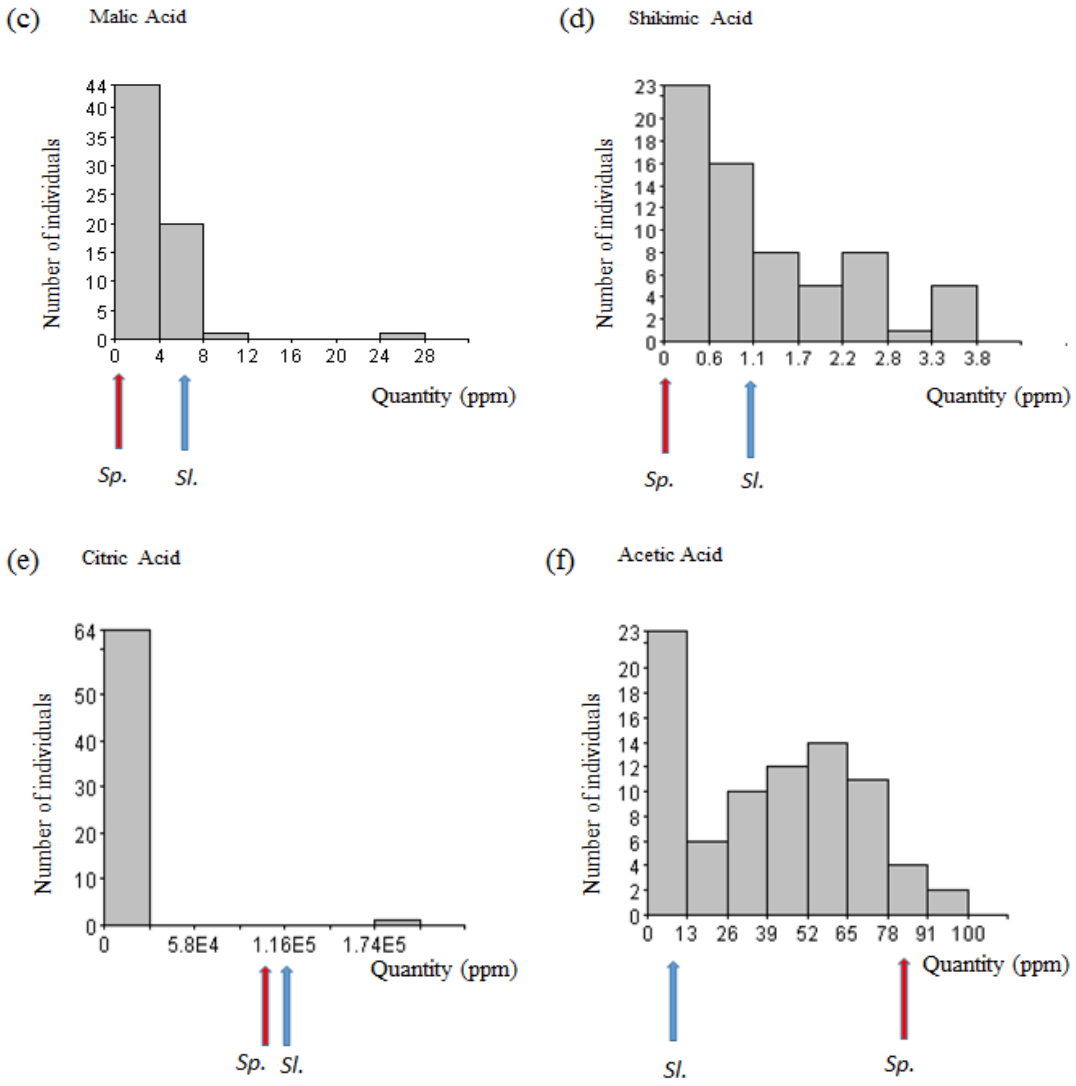

Figure 11. **(cont.)** (c) Distribution of malic acid content (ppm) in IBL population, (d) Distribution of shikimic acid content (ppm) in IBL population, (e) Distribution of citric acid content (ppm) in IBL population, (f) Distribution of acetic acid content (ppm) in IBL population.

4.3.1.3. Volatile Compounds

The parents of the IBL population had high β-cyclocitral, 3-hydroxy-2-butanone, 6-methyl-3,5-heptadien-2-one, 6-methyl-5-hepten-2-one, acetic acid and its ethyl ester, cyclohexasiloxane dodecamethyl and cyclopentane content. Besides these, the parent Tueza had high 1-penten-3-one, 2-heptanone, 2-isobutylthiazole, 3-methyl-2-pentanone, 3-ısopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane, 3-pentanon, geranyl acetone, methyl isovalerate, 1-nitro pentane, 1-nitro propan and 2-

nitro-propane content. While the *S. pimpinellifolium* parent had high 2,3-butanediol, 6-methyl-5-hepten-2-one, acetaldehyde, acetic acid methyl ester, hexanoic acid methyl ester, neryl acetone and 1- nitro-pentan content (Table 13). All of the volatile compounds segregated in the IBL population, except 3-methyl-1-butanol, 1-nitro-3-methylbutane, 1-octen-3-ol, 1-pentanol, 2-hexenal, 2-pentenal, acetic acid methyl ester, methyl isovalerate, 1-nitro-pentane, 2-nitro propane, and sulcatol. Volatile compounds displayed variation in the population with CV ranging from 28.6 % to 92841.9 %. Volatile compounds did not display continuous distributions within the population except β-cyclocitral, 2-heptanone, 2-isobutylthiazole, 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tri(trimethylsiloxy) tetrasiloxane, 3-pentanone, geranylacetone, 6-methyl-3,5-heptadien-2-one, 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, 6-methyl-5-hepten-2-oneB, acetic acid ethyl ester, cyclopentane, furan-3,4-methyl-3-pentenyl and methyl capronate (Figure 12).

Table 13. Statistics for volatile compounds measured in IBL population and parents: *S. lycopersicum* cv. Tueza and *S. pimpinellifolium* cv. LA1589. Quantities of metabolites were explained as mg/100g DW.

	Parents		**IBL Population**		
	Tueza	**LA1589**	**Mean**	**Range**	**CV%**
β-Ionone	0.00	0.00	0.03	0-67327	92841.9
2-Methyl-1-butanol	0.00	0.00	0.18	0-420248	22440.7
3-Methyl-1-butanol	0.00	0.00	3.58	0-4454954	932.9
β-cyclocitral	39.62	47.99	1.17	0-146529	1759.7
1-nitro-3-methylbutane	0.00	5.35	7.59	0-538795	458.9
1-Octen-3-ol	0.00	0.00	5.84	0-77618	20.7
1-Pentanol	0.00	0.00	4.65	0-1751231	165.1
1-Penten-3-one	2.17	0.00	10.02	0-2475705	91.2
2,3-Butanediol	0.00	100.00	6.83	0-1746592	96.4
2,3-Butanedione	0.00	61.34	9.75	0-1014101	39.7
3-Hydroxy-2-butanone,	14.98	21.57	16.81	0-29801873	118.9
2-Heptanone	47.09	0.00	26.09	0-259754	54.6
trans 2-Hexenal	0.00	0.00	3.37	0-94949	28.6
2-Isobutylthiazole	67.58	0.00	16.37	0-352805	48.6
2-Nonanone	0.00	0.00	10.79	0-178623	59.5
2-Pentanone	0.00	0.00	6.60	0-1159916	65.9
3-Methyl-2-pentanone	50.22	0.00	8.82	0-1085668	39.1

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Table 13. **(cont.)**

	Parents		IBL Population		
	Tueza	**LA1589**	**Mean**	**Range**	**CV%**
2-Pentenal	0.00	0.00	2.27	0-912220	104.7
3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	16.34	0.00	7.80	0-272518	76.2
3-Pentanone	85.92	0.00	29.10	0-1394255	51.1
Geranyl acetone	31.69	0.00	13.50	0-780681	73.7
6-methyl-5-Hepten-2-ol	0.00	0.00	4.70	0-3170863	101.3
6-Methyl-3,5-heptadien-2-one	29.24	100.00	19.87	0-356326	65.3
6-Methyl-5-hepten-2-one	0.00	77.53	20.59	0-26984952	67.3
6-Methyl-5-hepten-2-ol	0.00	0.00	8.66	0-5695040	95.6
Acetaldehyde	0.00	33.65	10.03	0-405296	79.9
Acetic acid, ethyl ester	4.58	12.62	15.24	0-25751787	99.7
Acetic acid, methyl ester	0.00	68.10	21.56	0-3291477	69.2
3-methyl- butanoic acid	0.00	0.00	7.40	0-174084	87.4
Cyclohexasiloxane dodecamethyl	31.45	32.50	23.02	0-353438	43.2
Cyclopentane	54.13	15.11	25.41	0-832947	65.5
Cyclopentasiloxane,decamethyl	27.02	18.66	18.94	0-303093	55.6
Perillen	0.00	0.00	11.75	0-137323	61.2
Methyl capronate	0.00	16.43	12.56	0-177522	64.7
Methyl isovalerate	93.88	0.00	4.94	0-445305	129.2
Neryl acetone	0.00	23.28	11.57	0-637776	89.4
1-Nitro-pentane	10.16	0.00	3.27	0-2108109	229.4
1-Nitro-propane	65.77	0.00	6.62	0-2448930	52.1
2-Nitro- Propane	0.00	97.39	4.05	0-1163451	79.0
Sulcatol	0.00	3.32	2.61	0-4900429	107.8

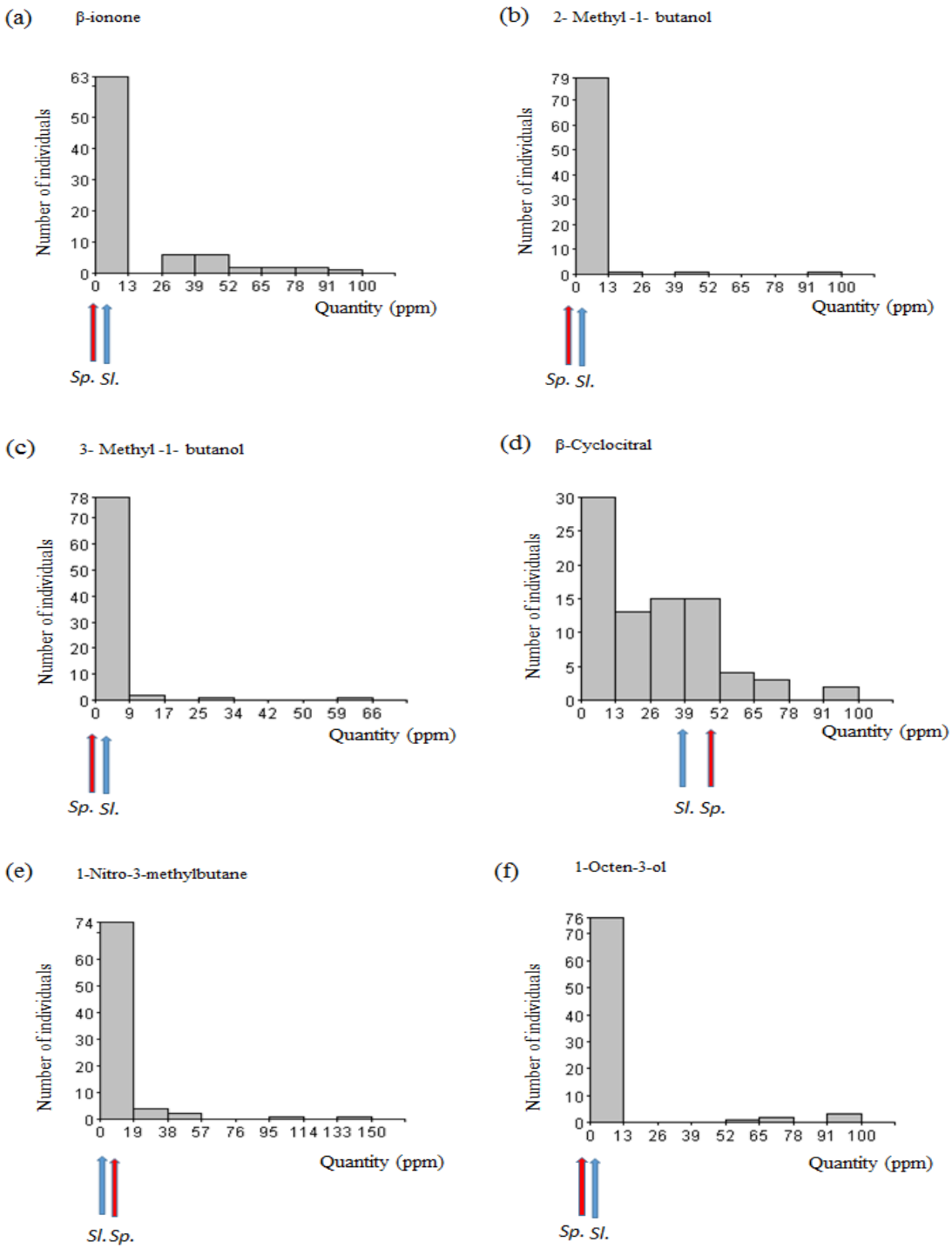

Figure 12. Distribution of volatile compounds in IBL population. (a) Distribution of β-ionone content (ppm) in IBL population, (b) Distribution of 2-methyl-1-butanol content (ppm) in IBL population, (c) Distribution of 3-methyl-1-butanol content (ppm) in IBL population, (d) Distribution of β-cyclocitral content (ppm) in IBL population, (e) Distribution of 1-nitro-3-methyl butane content (ppm) in IBL population, (f) Distribution of 1-octen-3-ol content (ppm) in IBL population. Arrows indicate means for Sl.: *S. Lycopersicum* cv. Tueza and Sp.: *S. pimpinellifolium* cv. LA1589.

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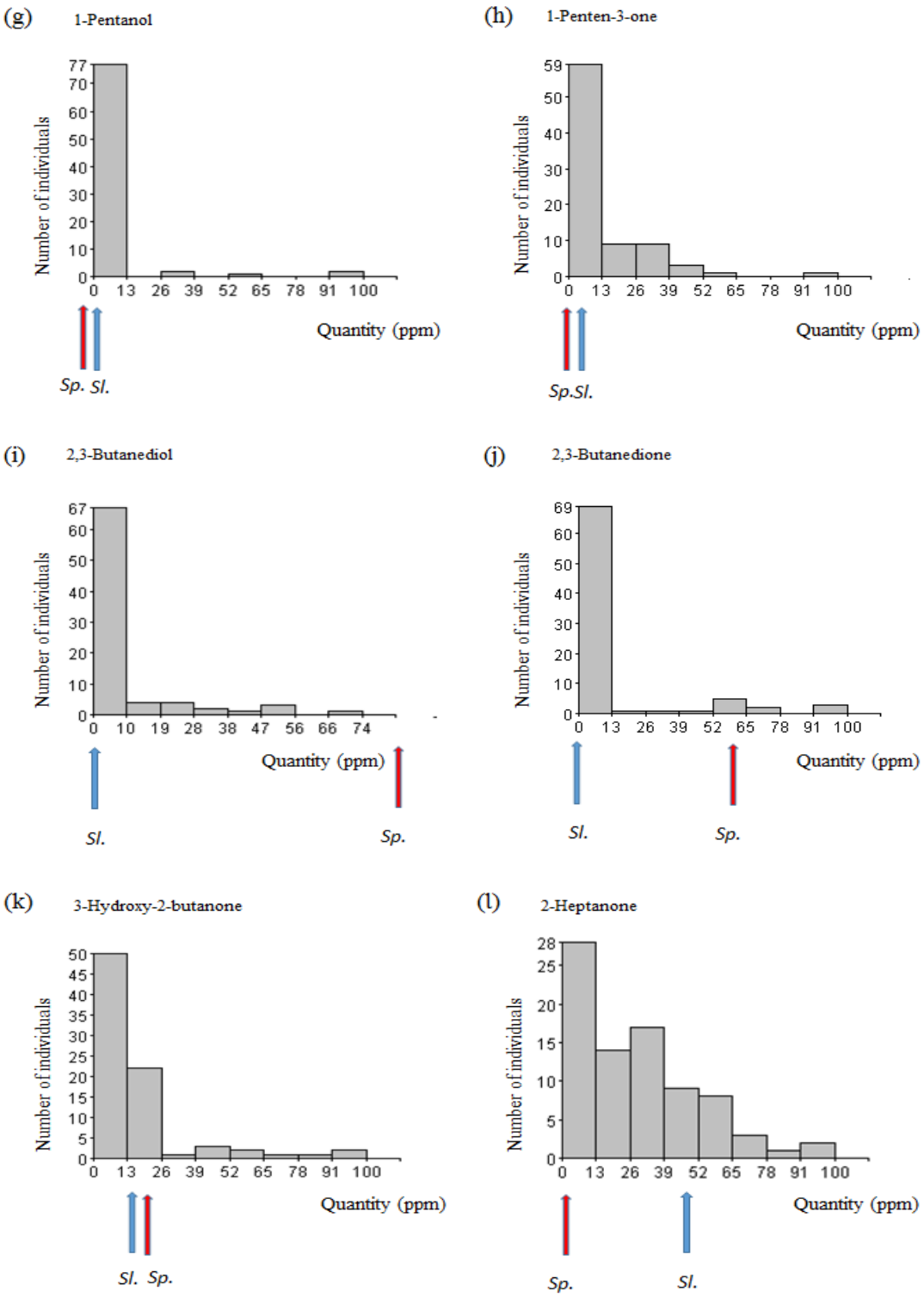

Figure 12. **(cont.)** (g) Distribution of 1-pentanol content (ppm) in IBL population, (h) Distribution of 1-penten-3-one content (ppm) in IBL population, (i) Distribution of 2,3-butanediol content (ppm) in IBL population, (j) Distribution of 2,3-butanedion content (ppm) in IBL population, (k) Distribution of 3-hydroxy-2-butanone content (ppm) in IBL population, (l) Distribution of 2-heptanone content (ppm) in IBL population.

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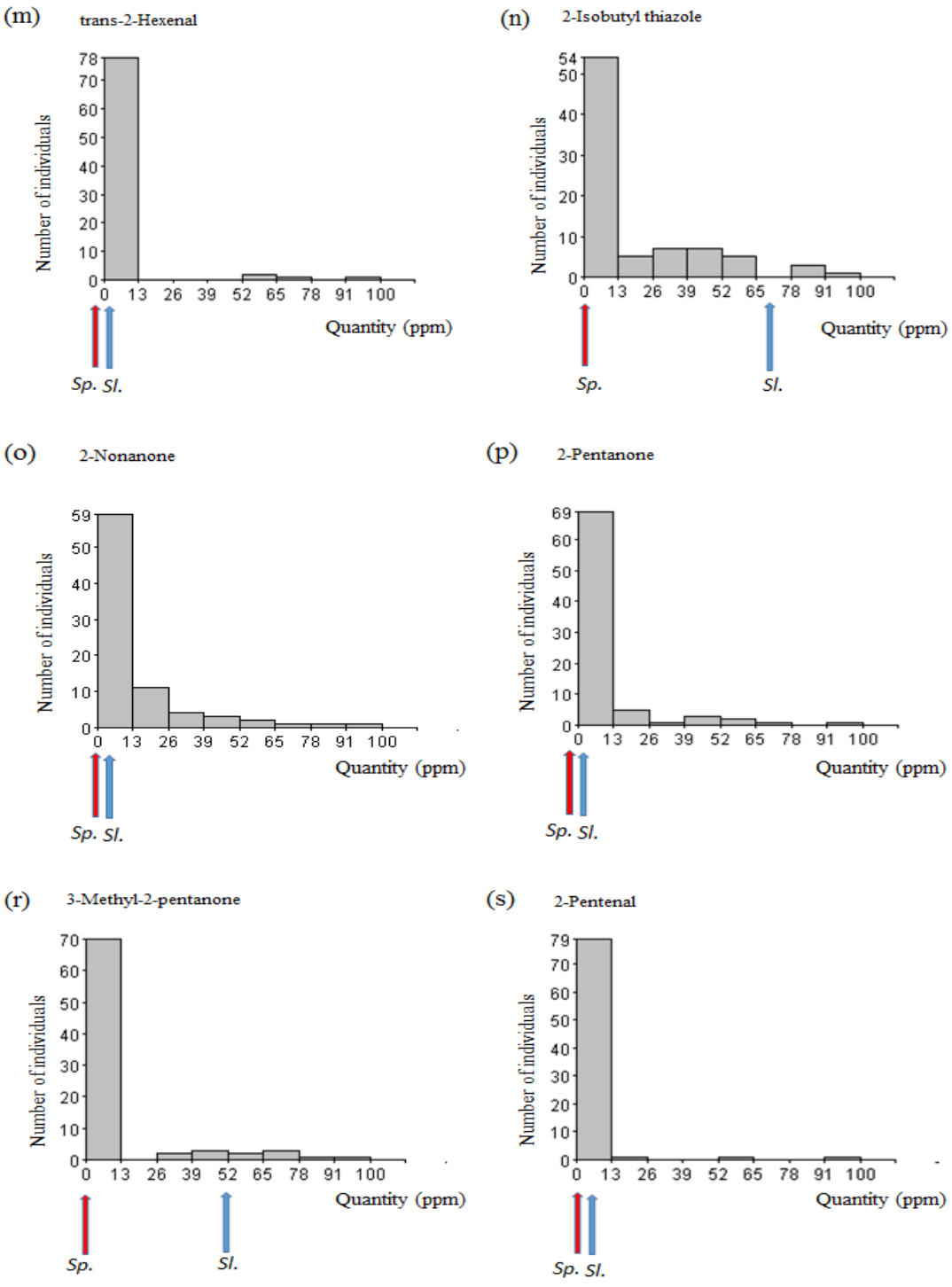

Figure 12. **(cont.)** (m) Distribution of trans-2-hexanal content (ppm) in IBL population, (n) Distribution of 2-isobutyl thiazole content (ppm) in IBL population, (o) Distribution of 2-nonanone content (ppm) in IBL population, (p) Distribution of 2-pentanone content (ppm) in IBL population, (r) Distribution of 3-methyl-2-pentanone content (ppm) in IBL population, (s) Distribution of 2-pentanal content (ppm) in IBL population.

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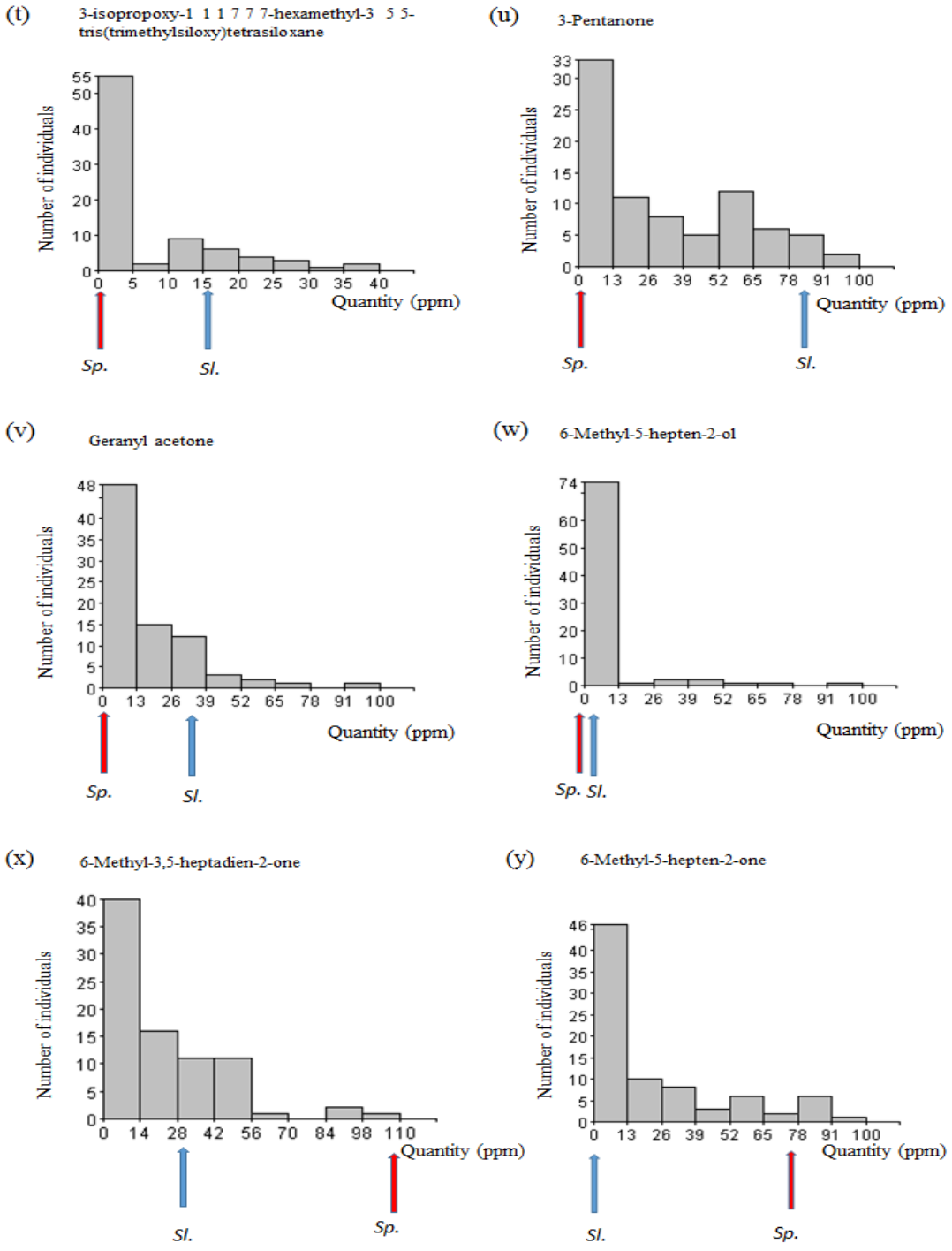

Figure 12. **(cont.)** (t) Distribution of 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane content (ppm) in IBL population, (u) Distribution of 3-pentanone content (ppm) in IBL population, (v) Distribution of geranyl acetone content (ppm) in IBL population, (w) Distribution of 6-methyl-5-hepten-2-ol content (ppm) in IBL population, (x) Distribution of 6-methyl-3,5-heptadien-2-one content (ppm) in IBL population, (y) Distribution of 6-methyl-5-hepten-2-on content (ppm) in IBL population.

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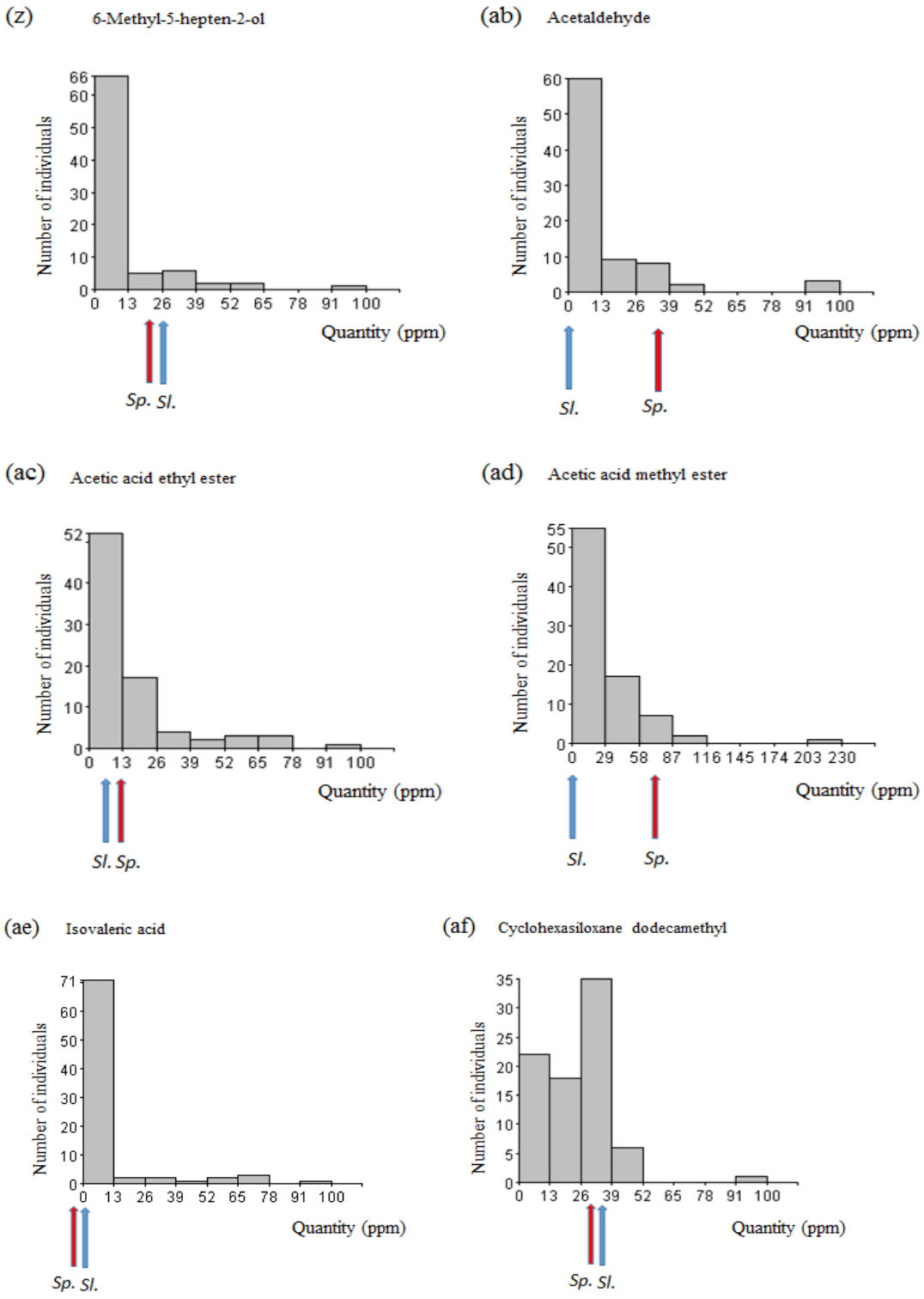

Figure 12. **(cont.)** (z) Distribution of 6-methyl-5-hepten-2-ol content (ppm) in IBL population, (ab) Distribution of acetaldehyde content (ppm) in IBL population, (ac) Distribution of acetic acid ethyl ester content (ppm) in IBL population, (ad) Distribution of acetic acid methyl ester content (ppm) in IBL population, (ae) Distribution of isovaleric acid content (ppm) in IBL population, (af) Distribution of cyclohexasiloxane dodecamethyl content (ppm) in IBL population.

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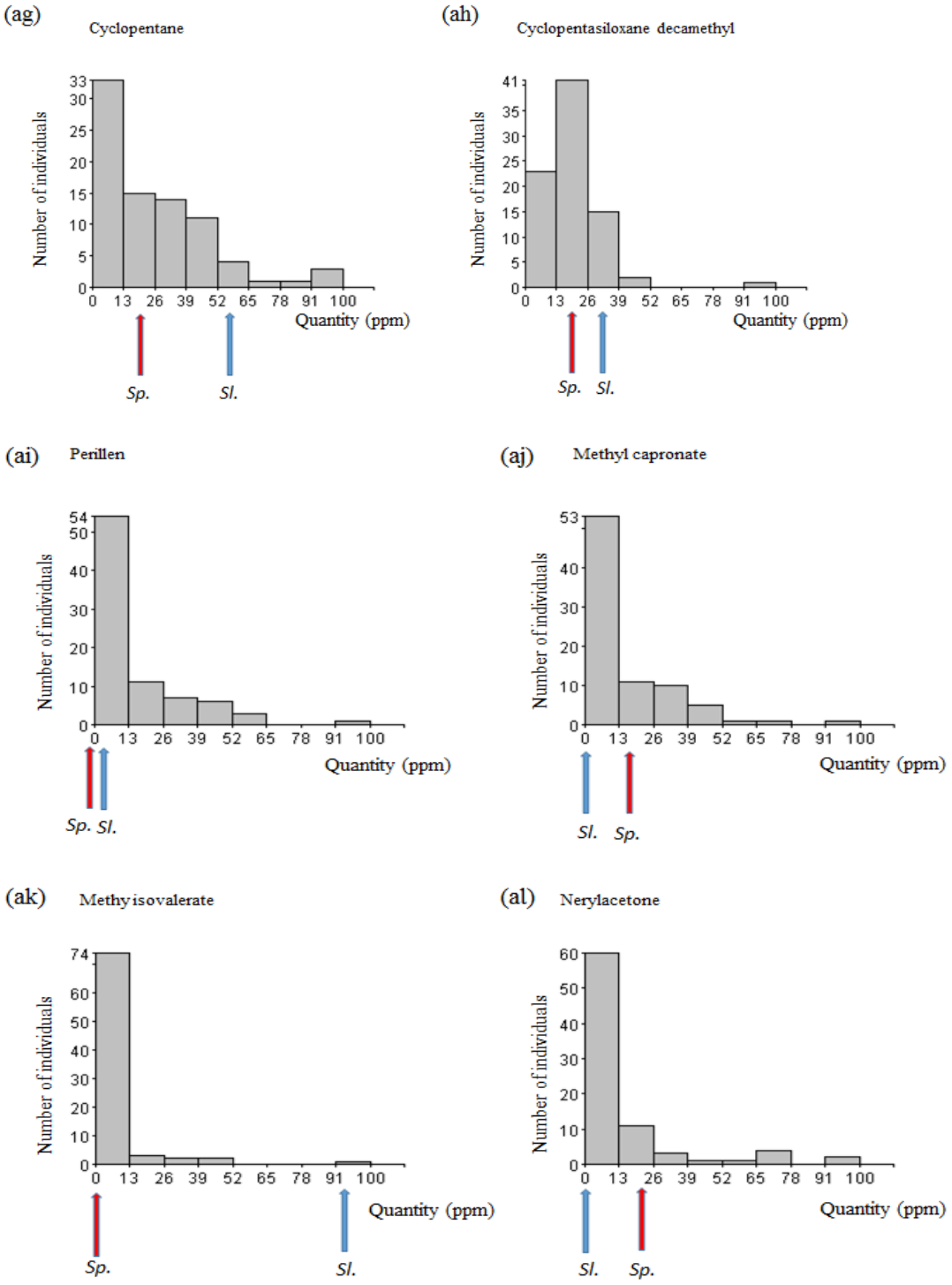

Figure 12. **(cont.)** (ag) Distribution of cyclopentane content (ppm) in IBL population, (ah) Distribution of cyclopentasiloxane decamethyl content (ppm) in IBL population, (ai) Distribution of perillen content (ppm) in IBL population, (aj) Distribution of methyl capronate content (ppm) in IBL population, (ak) Distribution of methyl isovalerate content (ppm) in IBL population, (al) Distribution of nerylacetone content (ppm) in IBL population.

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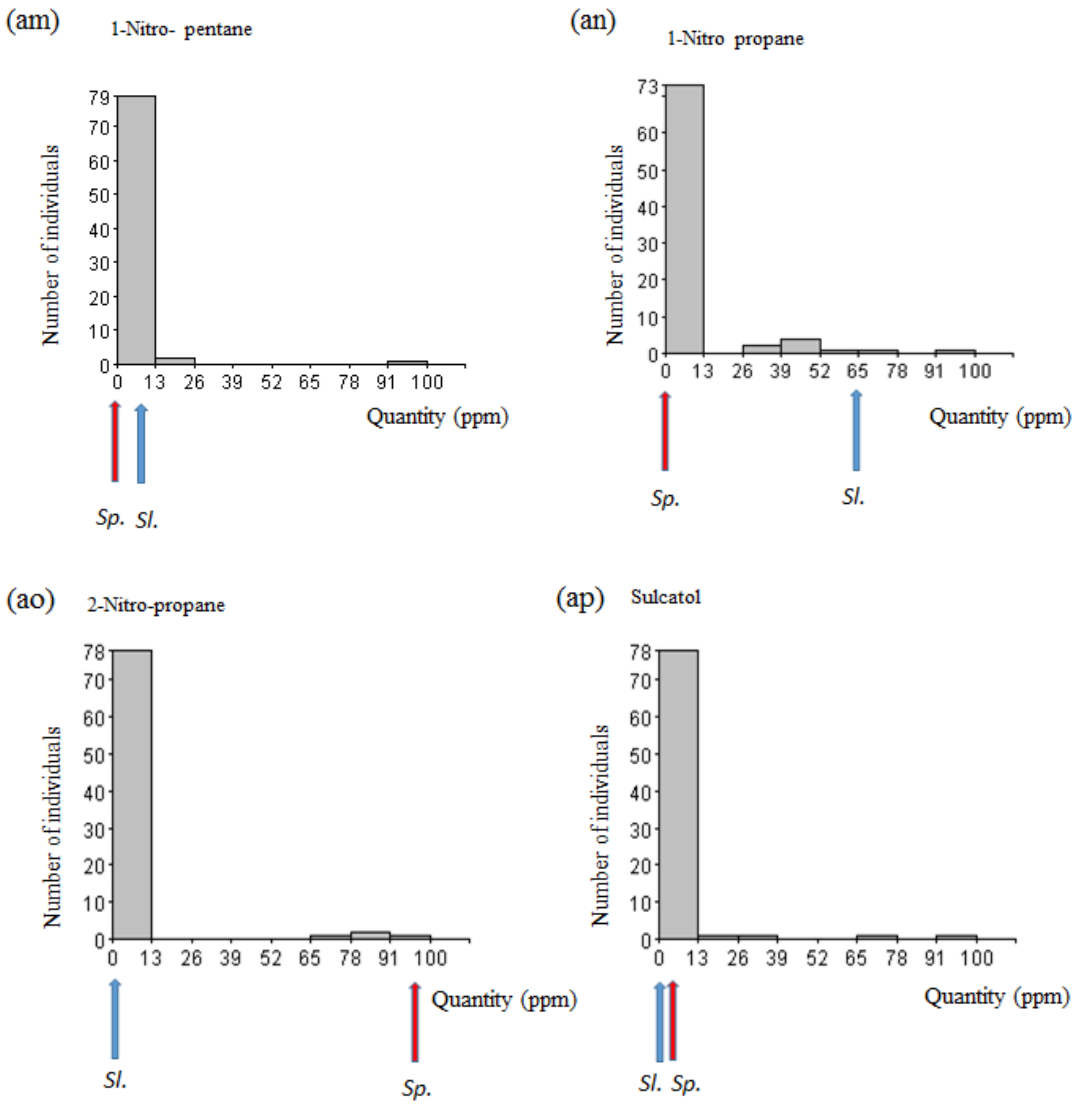

Figure 12. **(cont.)** (am) Distribution of 1-nitro-3-pentane content (ppm) in IBL population, (an) Distribution of 1-nitro propane content (ppm) in IBL population, (ao) Distribution of 2-nitro-propane content (ppm) in IBL population, (ap) Distribution of sulcatol content (ppm) in IBL population.

4.3.2. QTL Mapping

4.3.2.1. Sugars

Although sugars in tomato showed continous distribution and variation, no QTL were detected for sugar content.

4.3.2.2. Organic Acids

A total of 17 QTLs were identified for organic acids based on LOD thresholds of 3. For succinic acid, 6 QTLs were identified on chromosomes T5 and T7 with the percentage of phenotypic variation explained (PVE) varying between 19% and 45%. The identified QTLs had moderate effect with the exception of QTL (sa7.2) on chromosome 7 with the highest PVE, 45%. Thus, sa7.2 can be considered a major QTL. For shikimic acid only one QTL was identified on chromosome T12 with a PVE of 20%, a moderate effect. For citric acid, 10 QTLs were identified on chromosomes T1, T3, T7, T10 and T12 with PVE varying between 20% - and 62%. Of these, four QTLs were considered to be major: ca 1.2 with PVE 62 % and ca 1.3 with PVE 30 %, both on chromosome 1; ca 3.1 with PVE 50 % on chromosome 3; and ca 10.1 with PVE 58 % on chromosome 10 (Table 14).

Table 14**.** QTLs identified for organic acids.

Trait	QTL	Chr	Position (Mb) *	Marker Interval	LOD	PVE **	Additive Effect***
Succinic acid	*sa5.1*	T5	4.1	S5_141731 - S5_8117157	5.8	0.30	*S. pimpinellifolium*
Succinic acid	*sa7.1*	T7	12.2	S7_2225863 - S7_32224294	4	0.24	*S. pimpinellifolium*
			16.2	S7_2225863 - S7_32224294	3.2	0.19	
Succinic acid	*sa7.2*	T7	24.2	S7_2225863 - S7_32224294	5.1	0.30	*S. pimpinellifolium*
			26.2	S7_2225863 - S7_32224294	8.4	0.45	
Succinic acid	*sa7.3*	T7	4.2	S7_2225863 - S7_32224294	4.1	0.25	*S. pimpinellifolium*
			6.2	S7_2225863 - S7_32224294	3.9	0.24	
Succinic acid	*sa7.3*	T7	36.2	S7_32224294 - S7_58280573	3	0.19	*S. pimpinellifolium*
			38.2	S7_32224294 - S7_58280573	3.9	0.23	
			40.2	S7_32224294 - S7_58280573	3.3	0.2	
			42.2	S7_32224294 - S7_58280573	4.1	0.25	
			44.2	S7_32224294 - S7_58280573	3.9	0.24	
			46.2	S7_32224294 - S7_58280573	3.9	0.24	
Shikimic Acid	*sh12.1*	T12	38.1	S12_26140976	3.3	0.2	*S. pimpinellifolium*
Citric acid	*ca1.1*	T1	20.6	S1_663411 - S1_28625691	10.8	0.53	*S. pimpinellifolium*
			22.6	S1_663411 - S1_28625691	13.9	0.62	*S. pimpinellifolium*

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Table 14. **(cont.)**

Trait	QTL	Chr	Position (Mb) *	Marker Interval	LOD	PVE **	Additive Effect***
Citric acid	*ca1.2*	T1	64.6	S1_56607342 - S1_70630742	3.9	0.24	*S. pimpinellifolium*
			66.6	S1_56607342 - S1_70630742	3.9	0.24	*S. pimpinellifolium*
			68.6	S1_56607342 - S1_70630742	5.9	0.32	*S. pimpinellifolium*
Citric acid	*ca1.3*	T1	48.6	S1_32640248 - S1_56607342	5.5,	0.3	*S. pimpinellifolium*
			50.6	S1_32640248 - S1_56607342	3.5	0.21	*S. pimpinellifolium*
Citric acid	*ca3.1*	T3	52	S3_46076617 - S3_64075209	9.8	0.5	*S. pimpinellifolium*
Citric acid	*ca3.2*	T3	12	S3_2015299 - S3_18037470	9.5	0.49	*S. pimpinellifolium*
Citric acid	*ca7.1*	T7	2.2	S7_2225863 - S7_32224294	3.4	0.21	*S. pimpinellifolium*
Citric acid	*ca10.1*	T10	30	S10_18026339 - S10_32088336	5.5	0.32	*S. pimpinellifolium*
			34	S10_32088336 - S10_42019169	12.1	0.58	*S. pimpinellifolium*
Citric acid	*ca10.2*	T10	58	S10_54018912 - S10_62061848	5	0.29	*S. pimpinellifolium*
			60	S10_54018912 - S10_62061848	3.8	0.24	*S. pimpinellifolium*
Citric acid	*ca10.3*	T10	8	S10_4007994 - S10_10077455	3.2	0.2	*S. pimpinellifolium*
Citric acid	*ca12.1*	T12	32.1	S12_26140976	5.2	0.3	*S. lycopersicum*

* Peak position of QTL.
** Percentage of phenotypic variation explained by identified QTL.
*** Parental allele associated with increased trait value.

4.3.2.3. Volatile Compounds

A total of 66 QTLs were identified for volatile compounds based on LOD thresholds of 3. For β-ionone, 4 QTLs were identified on chromosomes T3 and T9 with the percentage of PVE varying between 16% and 31%. A major QTL (βi3.1) was identified with PVE 30% on chromosome 3, and another (βi3.3) with moderate effect with PVE 30% was also identified on chromosome 3. The other two QTLs had minor effects with PVE less than 20%. For 2-methyl-1-butanol, only one QTL (2mb9.1) was identified on chromosome T9 with PVE 22%, a moderate effect. For 3-methyl-1-butanol, only one QTL (3mb9.1) was identified on chromosome T11 with minor effect (PVE 18%). For β-cyclocitral, 3 QTLs with minor effects were identified on chromosomes T3 and T11 with the PVE varying between 16% and 17%. For 1-nitro-3-methyl butane, 2

QTLs with minor effects were identified on chromosomes T4 and T9 with the PVE varying between 16% and 17%. For 1-pentanol, 5 minor QTLs were identified on chromosome T8 with the PVE varying between 17% and 19%. For 2,3-butanedione, 4 QTLs were identified on chromosomes T2, T3 and T10 with the PVE varying between 18% and 32%. A major QTL (bon2.1) was identified on chromosome 2 with PVE 32 % while the QTL (bon10.1) on chromosome 10 had a moderate effect (PVE 22 %). The other two QTLs had minor effects with PVE less than 20%. For isobutylthiazole, only one QTL (ibt5.1) was identified on chromosome T5 with PVE 20%. For 2-pentanone, 2 QTLs with moderate effects were identified on chromosomes T3 and T5 with the PVE varying between 22% and 24%. For 2-pentanone-3-methyl, 3 QTLs with moderate effects were identified on chromosomes T3 and T6 with PVE varying between 20% and 24%. For 2-pentanal, only one minor QTL was identified on chromosome T11 with PVE 17%. For 6-methyl-5-hepten-2-ol, only one minor QTL was identified on chromosome T4 with PVE 16%. For sulcatol, 11 QTLs were identified on chromosomes T1, T3, T5, T7 and T12 with PVE varying between 16% and 31%. Three major QTLs were identified for sulcatol: (s3.1) and (s3.2) on chromosome 3 with PVE 30% for both, and (s7.3) on chromosome 7 with PVE 31%. One QTL (s5.2) on chromosome 5 had moderate effect while the other QTLs had minor effects. For acetic acid methyl ester, 5 QTLs, with minor effects were identified on chromosome T8 with the PVE varying between 16% and 19%. For cyclopentane, 2 QTLs were identified on chromosomes T3 and T7. The QTL on chromosome 3 had moderate effect with PVE 25% while the QTL on chromosome 7 had minor effect with PVE 17%. For cyclopentansiloxane decamethyl, 5 QTLs were identified on chromosome T8 with PVE varying between 16% and 30%. A major QTL (cpm8.5) on chromosome 8 with PVE 30% was identified while the effects of the other identified QTLs were moderate. For methyl capronate, 3 QTLs were identified on chromosomes T1, T10 and T11 with PVE varying between 15% and 24%. One of the QTL (mca1.1) on chromosome 1 had moderate effect with PVE 24 % while the other two QTLs had minor effects. For 1-nitro pentane, 11 QTLs were identified on chromosomes T1, T2, T3, T10 and T12 with the PVE varying between 15% and 47%. Four major QTLs were identified for this compound: npan1.1 on chromosome 1 with PVE 34 %, npan2.1 on chromosome 2 with PVE 33 %, npan3.2 on chromosome 3 with PVE 41, and npan10.1 on chromosome 10 with PVE 47. One QTL (npan10.3) had minor effect with PVE 18 %. The other identified QTLs had moderate effects with PVE ranging from 22 % to 28%. For 2-nitro propane, only one minor QTL was identified on chromosome T11 with PVE

16% (Table 15).

Table 15**.** QTLs identified for volatile compounds.

Trait	QTL	Chr	Position (Mb) *	Marker Interval	LOD	PVE **	Additive Effect***
β-ionone	*βi3.1*	T3	20	S3_18037470 - S3_46076617	6.8	0.31	*S. pimpinellifolium*
			22	S3_18037470 - S3_46076617	3	0.16	
			24	S3_18037470 - S3_46076617	3.5	0.18	
β-ionone	*βi3.2*	T3	36	S3_18037470 - S3_46076617	4	0.2	*S. pimpinellifolium*
			38	S3_18037470 - S3_46076617	3.6	0.18	
β-ionone	*βi3.3*	T3	52	S3_46076617 - S3_64075209	5.9	0.28	*S. pimpinellifolium*
β-ionone	*βi9.1*	T9	68.7	S9_68734489 - S9_70745825	3.1	0.16	*S. pimpinellifolium*
2-Methyl-1-butanol	*mb9.1*	T9	0.7	S9_755740 - S9_44727247	4.4	0.22	*S. pimpinellifolium*
3-Methyl-1-butanol	*mb11.1*	T11	4.3	S11_356158 - S12_179179	3.7	0.18	*S. pimpinellifolium*
β-cyclocitral	*βc3.1*	T3	20	S3_18037470 - S3_46076617	3.2	0.16	*S. lycopersicum*
β-cyclocitral	*βc3.2*	T3	38	S3_18037470 - S3_46076617	3.3	0.17	*S. lycopersicum*
β-cyclocitral	*βc11.1*	T11	36.3	S11_356158 - S12_179179	3.4	0.17	*S. lycopersicum*
1-Nitro-3-methyl butane	*nmb4.1*	T4	20.5	S4_16565256 - S4_32558453	3.5	0.17	*S. lycopersicum*
1-Nitro-3-methyl butane	*nmb9.1*	T9	68.7	S9_68734489 - S9_70745825	3.1	0.16	*S. lycopersicum*
1-Pentanol	*pol8.1*	T8	20	S8_10004731 - S8_30052255	3.7	0.19	*S. pimpinellifolium*
1-Pentanol	*pol8.2*	T8	40	S8_30052255 - S8_60041028	3.7	0.19	*S. pimpinellifolium*
			42	S8_30052255 - S8_60041028	3.6	0.18	
			44	S8_30052255 - S8_60041028	3.7	0.19	
			46	S8_30052255 - S8_60041028	3.4	0.17	
			48	S8_30052255 - S8_60041028	3.7	0.19	
			50	S8_30052255 - S8_60041028	3.7	0.19	
			52	S8_30052255 - S8_60041028	3.6	0.18	
1-Pentanol	*pol8.3*	T8	4	S8_18476 - S8_10004731	3.3	0.17	*S. pimpinellifolium*
			6	S8_18476 - S8_10004731	3.7	0.19	
			8	S8_18476 - S8_10004731	3.7	0.19	
1-Pentanol	*pol8.4*	T8	16	S8_10004731 - S8_30052255	3.7	0.19	*S. lycopersicum*
			18	S8_10004731 - S8_30052255	3.4	0.17	
			20	S8_10004731 - S8_30052255	3.7	0.19	
			22	S8_10004731 - S8_30052255	3.6	0.18	
1-Pentanol	*pol8.5*	T8	28	S8_10004731 - S8_30052255	3.7	0.19	*S. lycopersicum*
			30	S8_30052255 - S8_60041028	3.7	0.19	
			32	S8_30052255 - S8_60041028	3.7	0.19	
			34	S8_30052255 - S8_60041028	3.7	0,18	
			36	S8_30052255 - S8_60041028	3.7	0.19	

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Table 15. **(cont.)**

Trait	QTL	Chr	Position (Mb) *	Marker Interval	LOD	PVE **	Additive Effect***
			38	S8_30052255 - S8_60041028	3.1	0.17	
2,3-Butanedione	bon2.1	T2	18.2	S2_18214735 - S2_22281850	6.9	0.32	S. lycopersicum
2,3-Butanedione	bon3.1	T3	46	S3_46076617 - S3_64075209	3.5	0.18	S. lycopersicum
2,3-Butanedione	bon10.1	T10	30	S10_1802633 - S10_32088336	4.5	0.22	S. pimpinellifolium
2,3-Butanedione	bon10.2	T10	16	S10_1208592 - S10_18026339	3.8	0.19	S. pimpinellifolium
2-Isobutyl thiazole	*ibt5.1*	T5	4.1	S5_141731 - S5_8117157	3.9	0.2	*S. lycopersicum*
2-Pentanone	*pon3.1*	T3	6	S3_2015299 - S3_18037470	4.5	0.22	*S. pimpinellifolium*
2-Pentanone	*pon5.1*	T5	46.1	S5_38149609 - S5_50171107	4.9	0.24	*S. pimpinellifolium*
2-Pentanone-3-methyl	*polm3.1*	T3	60	S3_46076617 - S3_64075209	3.9	0.2	*S. lycopersicum*
			62	S3_46076617 - S3_64075209	4.2	0.21	
2-Pentanone-3-methyl	*polm6.1*	T6	30.8	S6_24889074 - S6_42876082	4.8	0.24	*S. lycopersicum*
2-Pentanal	*pan11.1*	T11	4.3	S11_356158 - S12_179179	3.2	0.17	*S. lycopersicum*
6-Methyl-5-hepten-2-ol	*mhol4.1*	T4	24.5	S4_16565256 - S4_32558453	3.2	0.16	*S. lycopersicum*
Sulcatol	*s1.1*	T1	4.6	S1_663411 - S1_28625691	3.5	0.18	*S. lycopersicum*
Sulcatol	*s3.1*	T3	30	S3_18037470 - S3_46076617	6.4	0.3	*S. lycopersicum*
			32	S3_18037470 - S3_46076617	3	0.16	
Sulcatol	*s3.2*	T3	40	S3_18037470 - S3_46076617	6.5	0.3	*S. lycopersicum*
Sulcatol	*s3.3*	T3	48	S3_46076617 - S3_64075209	3.3	0.17	*S. lycopersicum*
Sulcatol	*s5.1*	T5	40.1	S5_38149609 - S5_50171107	3.6	0.18	*S. lycopersicum*
Sulcatol	*s5.2*	T5	58.1	S5_50171107	4	0.2	*S. pimpinellifolium*
Sulcatol	*s7.1*	T7	20.2	S7_2225863 - S7_32224294	3	0.16	*S. pimpinellifolium*
			22.2	S7_2225863 - S7_32224294	4	0.2	
Sulcatol	*s7.2*	T7	38.2	S7_32224294 - S7_58280573	3.5	0.18	*S. lycopersicum*
Sulcatol	*s7.3*	T7	42.2	S7_32224294 - S7_58280573	6.6	0.31	*S. lycopersicum*
Sulcatol	*s12.1*	T12	20.1	S12_179179 - S12_24152718	3.2	0.17	*S. lycopersicum*
Sulcatol	*s12.2*	T12	40.1	S12_26140976	3.4	0.17	*S. lycopersicum*
Acetic acid methyl ester	*aam8.1*	T8	8	S8_18476 - S8_10004731	3.7	0.18	*S. lycopersicum*
			6	S8_18476 - S8_10004731	3.2	0.16	
Acetic acid methyl ester	*aam8.2*	T8	14	S8_10004731 - S8_30052255	3.6	0.18	*S. pimpinellifolium*
			16	S8_10004731 - S8_30052255	3.8	0.19	
			20	S8_10004731 - S8_30052255	3.5	0.18	
Acetic acid methyl ester	*aam8.3*	T8	24	S8_10004731 - S8_30052255	3.6	0.18	*S. pimpinellifolium*
			26	S8_10004731 - S8_30052255	3.8	0.19	
			30	S8_30052255 - S8_60041028	3.7	0.19	
			32	S8_30052255 - S8_60041028	3.2	0.16	
			36	S8_30052255 - S8_60041028	3.1	0.16	

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Table 15. **(cont.)**

Trait	QTL	Chr	Position (Mb) *	Marker Interval	LOD	PVE **	Additive Effect***
Acetic acid methyl ester	*aam8.4*	T8	38	S8_30052255 - S8_60041028	3.4	0.17	*S. lycopersicum*
			40	S8_30052255 - S8_60041028	3.7	0.19	
			42	S8_30052255 - S8_60041028	3.8	0.19	
			44	S8_30052255 - S8_60041028	3.1	0.16	
Acetic acid methyl ester	*aam8.5*	T8	46	S8_30052255 - S8_60041028	3.7	0.19	*S. lycopersicum*
			48	S8_30052255 - S8_60041028	3.6	0.18	
			50	S8_30052255 - S8_60041028	3.3	0.17	
			52	S8_30052255 - S8_60041028	3.2	0.17	
Cyclopentane	*cpan3.1*	T3	68	S3_64075209	5	0.25	*S. pimpinellifolium*
			70	S3_64075209	3.2	0.16	
Cyclopentane	*cpan7.1*	T7	36.2	S7_32224294 - S7_58280573	3.3	0.17	*S. lycopersicum*
Cyclopentasiloxane decamethyl	*cpm8.1*	T8	4	S8_18476 - S8_10004731	4.8	0.23	*S. pimpinellifolium*
			6	S8_18476 - S8_10004731	4.8	0.26	
			8	S8_18476 - S8_10004731	5.4	0.26	
Cyclopentasiloxane decamethyl	*cpm8.2*	T8	14	S8_10004731 - S8_30052255	5.5	0,26	*S. pimpinellifolium*
			18	S8_10004731 - S8_30052255	5.8	0.28	
			20	S8_10004731 - S8_30052255	5.6	0,27	
			22	S8_10004731 - S8_30052255	5.6	0,27	
Cyclopentasiloxane decamethyl	*cpm8.3*	T8	24	S8_10004731 - S8_30052255	5.5	0.26	*S. lycopersicum*
			26	S8_10004731 - S8_30052255	5.3	0.26	
			30	S8_30052255 - S8_60041028	5.5	0.26	
			32	S8_30052255 - S8_60041028	5.2	0.25	
			34	S8_30052255 - S8_60041028	5.4	0.26	
Cyclopentasiloxane decamethyl	*cpm8.4*	T8	36	S8_30052255 - S8_60041028	5.1	0.25	*S. pimpinellifolium*
			38	S8_30052255 - S8_60041028	5.1	0.25	
			40	S8_30052255 - S8_60041028	5.5	0.26	
			42	S8_30052255 - S8_60041028	5.5	0.26	
			44	S8_30052255 - S8_60041028	5.2	0.25	
Cyclopentasiloxane decamethyl	*cpm8.5*	T8	46	S8_30052255 - S8_60041028	4.5	0,22	*S. lycopersicum*
			48	S8_30052255 - S8_60041028	6.4	0,30	
			50	S8_30052255 - S8_60041028	6.3	0,29	
			52	S8_30052255 - S8_60041028	6.4	0.3	
Methyl capronate	*mca1.1*	T1	0.6	S1_663411 - S1_28625691	5	0.24	*S. pimpinellifolium*

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Table 15. **(cont.)**

Trait	QTL	Chr	Position (Mb) *	Marker Interval	LOD	PVE **	Additive Effect***
Methyl capronate	mca10.1	T10	4	S10_4007994 - S10_10077455	3	0.15	S. lycopersicum
Methyl capronate	*mca10.2*	T10	28	S10_18026339 - S10_32088336	3.2	0.16	*S. lycopersicum*
Methyl isovalerate	*mca11.1*	T11	36.3	S10_32088336 - S10_42019169	3.2	0.16	*S. pimpinellifolium*
1-Nitro-pentane	*npan1.1*	T1	16.6	S1_663411 - S1_28625691	4	0.2	*S. pimpinellifolium*
			20.6	S1_663411 - S1_28625691	4	0.2	
			22.6	S1_663411 - S1_28625691	7.4	0.34	
1-Nitro-pentane	*npan1.2*	T1	48.6	S1_32640248 - S1_56607342	4.8	0.24	*S. lycopersicum*
1-Nitro-pentane	*npan1.3*	T1	64.6	S1_56607342 - S1_70630742	3.9	0.2	*S. lycopersicum*
			68.6	S1_56607342 - S1_70630742	5.8	0.28	
1-Nitro-pentane	*npan2.1*	T2	18.2	S2_18214735 - S2_22281850	7.3	0.33	*S. pimpinellifolium*
1-Nitro-pentane	*npan3.1*	T3	12	S3_2015299 - S3_18037470	4.5	0.22	*S. lycopersicum*
1-Nitro-pentane	*npan3.2*	T3	46	S3_46076617 - S3_64075209	9.6	0.41	*S. lycopersicum*
1-Nitro-pentane	*npan10.1*	T10	16	S10_12085921 - S10_18026339	11.4	0.47	*S. pimpinellifolium*
1-Nitro-pentane	*npan10.2*	T10	26	S10_18026339 - S10_32088336	3.6	0.18	*S. lycopersicum*
			30	S10_18026339 - S10_32088336	4.6	0.22	
			34	S10_32088336 - S10_42019169	4.4	0.22	
1-Nitro-pentane	*npan10.3*	T10	58	S10_54018912 - S10_62061848	3.6	0.18	*S. pimpinellifolium*
1-Nitro-propane	*npr10.1*	T10	62	S10_54018912 - S10_62061848	3.5	0.18	*S. pimpinellifolium*
1-Nitro-pentane	*npan12.1*	T12	32.1	S12_26140976	3	0.15	*S. lycopersicum*
2-Nitro-propane	*npan11.1*	T11	24.3	S11_356158 - S12_179179	3.1	0.16	*S. lycopersicum*

4.3.3. Correlated and Colocalized Traits

Correlation analysis of metabolic characters demonstrated that some compounds had significant correlation. Most of the correlations were positive. Glucose and fructose are positively strongly correlated ($r^2 = 0.91$). 3-methyl-1-butanol and 2,3-butanediol ($r^2 = 0.455$), 2-heptanone and cyclohexasiloxane dodecamethyl ($r^2 = 0.402$), 2-hexanol and acetaldehyde ($r^2 = 0.526$), 2-pentanal and acetaldehyde ($r^2 = 0.565$), 3-isopropoxy-1 1 1 7 7 7-hexamethyl-3 5 5-tris(trimethylsiloxy)tetrasiloxane and 6-methyl-5-heptene-2-one ($r^2 = 0.409$), 6-methyl-5-heptadiene-2-one and neryl acetone ($r^2 = 0.442$) showed moderate correlation. Also for many metabolites weak correlations between some

metabolites, especially between volatile compounds, were determined (Table 16).

Colocalized QTLs were detected for flavor and aroma components. 1-nitro pentane and citric acid QTL were colocalized at the 48.6 Mb and 68.6 Mb position on chromosome 1, at 12 Mb position on chromosome 3, and at 58 Mb position on chromosome 10. 1-nitro pentane also colocalized with 2,3-butanedione at 18.2 Mb position on chromosome 2, at 46 Mb position on chromosome 3, at 16 Mb position on chromosome 10, and colocalized with both 2,3-butanedione and citric acid on 30 Mb position on chromosome 10. β-ionone and β-cyclocitral were colocalized at 20 and 38 Mb position on chromosome 3. β-ionone also colocalized with 1-nitro-3-methyl butane at 68.7 Mb positionn on chromosome 9. 2-isobutyl thiazole and salicylic acid colocalized at 4.1 Mb position on chromosome 5. Succinic acid QTL were also colocalized with cyclopentane at 36.2 Mb position on chromosome 7. 1-pentanol, acetic acid methyl ester and cyclopentanesiloxane decamethyl were colocalized at 6, 8, 20, 30, 32, 36, 38, 46 and 48 Mb position on chromosome 8. 2-pentanal and 3-methyl-1-butanol were colocalized at 4.3 Mb position on chromosome 11 (Table 17).

Table 16. Correlation between flavor traits. Significant ($P < 0.4$) correlations between tomato fruit traits. Correlations with P value > 0.4 were considered to be non-significant (NS). Glc = Glucose, Frc = Fructose, 3m1b = 3-methyl-1-butanol, 2,3b = 2,3-butanediol, 2hn = 2-heptanone, Chd = cyclohexasiloxane dodecamethyl, 2hl = 2-hexanol, Aa = Acetaldehyde, 2p = 2-pentanal, 6m5h2o = 6-methyl-5-heptene-2-one, 6m5hn2o = 6-methyl-5-heptadiene-2-one, 3ihtts = 3-isopropoxy-1 1 1 7 7 7-hexamethyl-3 5 5-tris(trimethylsiloxy)tetrasiloxane, Na = neryl acetone.

Traits	**Glc**	**Frc**	**3m1b**	**2,3b**	**2hn**	**Chd**	**2hl**	**Aa**	**2p**	**6m5h2o**	**6m5hn2o**	**3ihtts**	**Na**
Glc	1	0.91	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Frc		1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
3m1b			1	0.455	NS	NS	NS	NS	NS	NS	NS	NS	NS
2,3b				1	NS	NS	NS	NS	NS	NS	NS	NS	NS
2hn					1	0.402	NS	NS	NS	NS	NS	NS	NS
Chd						1	NS	NS	NS	NS	NS	NS	NS
2hl							1	0.526	NS	NS	NS	NS	NS
Aa								1	0.565	NS	NS	NS	NS
2p									1	NS	NS	NS	NS
6m5h2o										1	NS	0.409	NS
6m5hn2o											1	NS	0.442
3ihtts												1	NS
Na													1

Table 17. QTLs for fruit metabolites that colocalized.

Trait	QTL	Chr.	Position (Mb)
1- Nitro pentane	*npan1.2*	T1	48.6
Citric Acid	*ca1.3*	T1	48.6, 50.6
1- Nitro pentane	*npn1.3*	T1	64.6, 68.6
Citric Acid	*ca1.2*	T1	64.6, 66.6, 68.6
2,3 - Butanedione	*bon2.1*	T2	18.2
1- Nitro pentane	*npan2.1*	T2	18.2
β-Ionone	*βi.3.1*	T3	20, 22, 24
β- cyclocitral	*βc.3.1*	T3	20
β-Ionone	*βi.3.2*	T3	36,38
β- cyclocitral	*βc.3.2*	T3	38
2,3 - Butanedione	*bon3.1*	T3	46
1- Nitro pentane	*npan3.2*	T3	46
1- Nitro pentane	*npan3.1*	T3	12
Citric Acid	*ca3.2*	T3	12
2-Isobutyl thiazole	*ibt5.1*	T5	4.1
Succinic Acid	*sa5.1*	T5	4.1
Cyclopentane	*cpan3.1*	T7	36.2
Succinic Acid	*sa7.3*	T7	36.2, 38.2, 40.2, 42.2, 44.2, 46.2
Sulcatol	*s7.2*	T7	38.2, 42.2
Succinic Acid	*sa7.3*	T7	36.2, 38.2, 40.2, 42.2, 44.2, 46.2
1-Pentanol	*pol8.4*	T8	16, 18, 20, 22
Acetic Acid Methyl Ester	*aam8.2*	T8	14, 16, 20
Cyclopentasiloxane decamethyl	*cpm8.2*	T8	14, 18, 20, 22
1-Pentanol	*pol8.2*	T8	40, 42, 44, 46, 48, 50, 52
Acetic Acid Methyl Ester	*aam8.4*	T8	46, 48, 50, 52
Cyclopentasiloxane decamethyl	*cpm8.5*	T8	46, 48, 50, 52
1-Pentanol	*pol8.3*	T8	4, 6, 8
Acetic Acid Methyl Ester	*aam8.1*	T8	6, 8
Cyclopentasiloxane decamethyl	*cpm8.1*	T8	4, 6, 8
1-Pentanol	*pol8.5*	T8	28, 30, 32, 34, 36, 38
Acetic Acid Methyl Ester	*aam8.3*	T8	24, 26, 30, 32, 36, 38
Cyclopentasiloxane decamethyl	*cpm8.3*	T8	24, 26, 30, 32, 34
Cyclopentasiloxane decamethyl	*cpm8.4*	T8	36, 38, 40, 42, 44
β-Ionone	*βi.3.1*	T9	68.7
1-Nitro-3-Methyl Butane	*nmb9.1*	T9	68.7
2,3-Butanedione	*bon10.1*	T10	30
1-Nitro-pentane	*npan10.2*	T10	26, 30, 34
Citric Acid	*ca10.1*	T10	30
2,3-Butanedione		T10	16
1-Nitro-pentane	*npan10.1*	T10	16
1-Nitro-pentane	*npan10.3*	T10	58
Citric Acid	*ca10.2*	T10	58, 60
3-Methyl-1-butanol	*3mb11.1*	T11	4.3
2-Pentanal	*pan11.1*	T11	4.3

4.4. DISCUSSION

Tomato breeding objectives have shifted somewhat from high yielding, resistant cultivars to nutritional and flavor-rich cultivars as a result of consumer preferences (Tieman et al. 2017). Despite advances in molecular techniques and markers, breeding studies for fruit flavor are difficult because these traits are polygenic and strongly affected by environmental factors (Bertin et al. 2009). Flavor is a complex trait influenced by taste and aroma. Taste is determined by sweetness and sourness which are influenced by soluble sugars and acids, respectively (Kader et al. 1977; Malundo, Shewfelt, and Scott 1995; Stevens et al. 1977a, 1977b), and aroma is determined by volatile compounds (Rambla et al. 2016; Tieman et al. 2007). Besides being essential to fruit flavor, sugars and organic acids constitute over 60% of dry matter and contribute to soluble solids content (SSC) (Baldwin, Goodner, and Plotto 2008; Bastias et al. 2011; Davies and Hobson 1981; Goff and Klee 2006; Kader 2008). Moreover, soluble sugars, acids and potassium salts determine osmotic pressure, which induces turgor pressure resulting in cell expansion in tomato fruit. Thus sugar and acid content play roles in regulation of fruit size by controlling osmotic pressure (Ho, Grange, and Picken 1987). Bertin et al. (2009) identified QTLs for SSC, dry matter, sugar content and titratable acidity. They concluded that the region at the top of chromosome 9 may control fruit size in tomato with combined QTL effects on cell division, cell wall synthesis, carbon import and carbon metabolism.

High sugar is a favorable trait in tomato breeding because of its contributions to fruit sweetness (Beauvoit et al. 2014, Osorio, Ruan, and Fernie 2014; Patrick, Botha, and Birch 2013). However, up to now, sugar content was evaluated by SSC instead of individual sugar content in breeding studies. Moreover, organic acid content was usually evaluated by titratable acids (Ruggieri et al. 2014; Saliba-Colombani et al. 2001; Sauvage et al. 2014; Xu et al. 2013; Zhang et al. 2015). Thus, QTL mapping studies focusing on individual sugar and organic acid content are limited in the tomato literature (Fulton et al. 2002; Ruggieri et al. 2014; Schauer et al. 2006, 2008; Sauvage et al. 2014). In addition, studies focused on individual sugars and acids are also limited to a few main metabolites such as glucose, fructose, sucrose, citric acid, malic acid and ascorbic acid (Fulton et al. 2002; Osvald, Petrovic, and Demsar 2001; Ruggieri et al. 2014; Schauer et al. 2005).

In the present work, the IBL population and parental genotypes were evaluated for three simple sugars; glucose, fructose and sucrose in order to identify associated

QTLs. Both glucose and fructose segregated in the IBL population and had normal continuous distributions. Parental alleles for glucose and fructose content were extreme and Tueza contained, about two-fold more glucose and fructose that the *S. pimpinellifolium* parent (Table 11 and Fig. 10).

Sucrose was not detected in the parents or in the population. This result was expected because it was reported that *S. lycopersicum* and *S. pimpinellifolium* accumulate hexose sugars (Yelle et al. 1988) instead of sucrose due to high activity of the sucrose hydrolyzing enzyme, acid invertase, in these species (Miron and Schaffer 1991; Stommel 1992; Yelle et al 1991). Sucrose that accumulates at the early stages of development is converted to hexose sugars, glucose and fructose by invertase(s) at late stages of development (Beauvoit et al. 2014; Osorio et al. 2014; Sagor et al. 2016). Interestingly, *S. peruvianum* and *S. habrochaites* were reported in 1966 as sucrose accumulator wild species (Davies 1966), thus, they accumulate sucrose instead of hexose sugars. In addition, *S. chmielewskii* is reported as being a sucrose accumulator (Chetelat, De Verna, and Bennett 1995; Yelle et al. 1988).

The efficiency of QTL mapping depends on segregation of the trait in the mapping population and quantitative differences between parents (Collard et al. 2005). Sugars, both glucose and fructose, displayed a continuous distribution in IBL population. However, despite the segregation of sugar content in the population, QTLs for sugar synthesis could not be detected. This could arise from repulsion phase linkage. QTL effects are strongly affected by linkage. When they are in repulsion phase linkage, it is possible that no QTLs are detected (Kearsey 2002; Liu 1998; Sofi and Rather 2007).

There are limited studies in the literature to identify QTLs for flavor-related traits, sugars, organic acids and volatile compounds, and also individual determination or correlation of these traits. In most of these studies, *S. pennellii*, *S. chmielewskii* and *S. lycopersicum var. ceasiforme* (Cervil) were used as the donor parents and IL populations derived from wild species and cultivated tomato were investigated.

Ikeda et al. (2013) investigated QTLs related to brix content, because soluble sugars (glucose, fructose, sucrose) determine brix. They mapped a 300 kb region of chromosome 8 for high Brix content. In addition, changes in sugar content during fruit development were studied using an IL8-3 population derived from *S. lycopersicum* M82 and *S. pennellii*. Significant differences in glucose and fructose content were found between IL8-3 and M82 at late stages of fruit development. Sugar content also showed differences between developmental stages. Sucrose content was high but glucose and

fructose content was low at the early stage of fruit development. Later, sucrose was degraded into glucose and fructose resulting in high concentrations of glucose and fructose and low concentration of sucrose in ripe fruit.

Prudent et al. (2009) detected 12 QTLs for total sugar content on chromosomes 1, 3, 4, 6, 7, 8, 9, 10 and 12 using an IL population derived from *S. lycopersicum* and *S. chmielewskii* using SSR markers. Saliba-Colombani et al. (2000) detected 5 QTLs for total sugar content on chromosomes 2, 3, 10 and 11 using an RIL population derived from Cervil and Levovil with RFLP markers. Causse et al. (2007) found 4 QTLs for total soluble sugar content on chromosomes 2, 10 and 11 using NIL populations derived from single introgressions from Cervil into the Levovil genetic background, and cumulating isogenic lines (CIL) population derived from five introgressions from Cervil into the Levovil genetic background with SSR and RFLP markers. They also demonstrated that Cervil alleles provided increased sugar content. In other work done by Causse et al. (2002), 4 QTLs were identified for total soluble sugars on chromosomes 2, 3 and 10 using a RIL population derived from Cervil and Levovil with RFLP, RAPD and AFLP markers. Work done by Prudent et al. (2011) evaluated physiological processes including assimilate supply, transformation of sugars into other compounds, and dilution of sugars by water uptake underlying QTLs for fruit sugar concentration. They identified 14 QTLs for fruit load, 11 QTLs for sugar supply, 15 QTLs for metabolic transformation of sugars, 12 QTLs for dilution of sugars using 20 IL populations carrying single or multiple introgressions of *S. chmielewskii* in *S. lycopersicum* (Moneyberg) genetic background. They also demonstrated that *S. chmielewskii* alleles provided increases in sugar content.

Causse et al. (2004) detected 13 QTLs for sugars (glucose and fructose) on chromosomes 1, 2, 4, 5, 7, 8, 9, 10, 11 and 12 using an IL population derived from *S. lycopersicum* M82 and *S. pennellii* using RFLP markers. Sugar content was found to be increased by *S. pennellii* alleles. They observed continuous distribution for sugars and found a positive correlation between SSC and glucose. Zhao et al. (2016) performed association mapping with SSR markers among 174 tomato accessions composed of 123 *S. lycopersicum var. ceasiforme* (Cervil) accessions and 51 *S. lycopersicum* (Levovil) accessions. They detected significant associations including 4 marker-trait associations for glucose on chromosomes 5, 6 and 9; 6 marker-trait associations for fructose on chromosomes 1, 5, 6, 7 and 9; 10 marker-trait associations for sucrose on chromosomes 1, 3, 6, 7, 8, 9. Schauer et al. (2006) identified 889 fruit metabolite loci including 16 QTLs for glucose on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 10, 11 and 12, 16 QTLs for fructose on

chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 10, 11 and 12, 9 QTLs for sucrose on chromosomes 1, 2, 3, 4, 5, 6, 7, 9 using an IL population derived from *S. lycopersicum* M82 and *S. pennellii.* In an another work done by Schauer et al. (2008), 39 QTLs for 12 sugars were identified in heterozygous IL populations containing introgressions from *S. pennellii* in the genetic background of M82. Capel et al. (2015) identified QTLs for fruit quality traits including sugars using a RIL population derived from *S. lycopersicum cv*. Moneymaker and *S. pimpinellifolium* with SNP, SSR and indel markers. In that study, 2 QTLs for glucose on chromosomes 2 and 7, 1 QTL for fructose on chromosome 2 were identified. Fulton et al. (2002) identifed QTLs affecting flavor in four advanced backcross populations of tomato. *S. lycopersicum* E6203 was used as recurrent parent and four wild species (*S. pimpinellifolium, S. habrochaites, S. neorickii* and *S. peruvianum*) were used as donor parents to develop four different populations. Totally 17 QTLs for citric acid on chromosomes 1, 2, 3, 4, 5, 8, 9, 11 and 12, 21 QTLs for malic acid on chromosomes 1, 2, 3, 5, 7, 8, 9, 10 and 11 were identified.

Findings from the studies mentioned above demonstrated that (i) sugar contents change during different developmental stages, (ii) Cervil and *S. chmielewskii* alleles provide increased sugar content, and (iii) QTLs are common on chromosome 2, 3, and 10 for total soluble sugar content.

Besides sugars, the IBL population and parental genotypes were evaluated for organic acids as another flavor component. Citric acid, malic acid, lactic acid, shikimic acid, succinic acid and acetic acid were quantified in order to identfy associated QTLs. Organic acids, with the exceptions of shikimic acid and acetic acid, did not have normal continuous distributions in the IBL population. Parents of the IBL population did not show significant variation in organic acid content, except for malic acid and acetic acid (Table 12, Fig. 11). Although the parental alleles for malic acid content were extreme, low variation was observed in the IBL population for the trait. These results suggests an unbalanced introgression of *S. pimpinellifolium* alleles for malic acid into *the S. lycopersicum* genome. Malic acid and lactic acid tended to skew toward low malic acid and lactic acid content due to the unbalanced nature of the IBL population which favors the recurrent parent genotype.

In our study although QTLs for sugar content could not be identified, 16 QTLs were identified including those for citric acid which is the organic acid that contributes most to tomato flavor. We also identified QTLs for salicylic acid and shikimic acid which are new for the literature. Salicylic acid is known as a stress hormone and its synthesis is

induced by pathogen attack (Klee 2010; Assman and Albert 2006). Shikimic acid is an important molecule which plays a role in the synthesis of flavonoids (Dorais, Ehret, and Papadopoulos 2008; Wilson and Roberts 2014), isoquinoline alkaloids, anthocyanines, terpenoids (Wilson and Roberts 2014), and aromatic amino acids (Kaushik et al. 2015). Shikimic acid is a key intermediate for drugs such as Tamiflu (Lim and Bowles 2012). The identified QTLs for salicylic acid were mostly localized on chromosome 7, while QTL for shikimic acid were on chromosome 12.

There are a few studies focused on individual organic acids in the literature. Toubiana et al. (2012) identified 8 QTLs for malic acid on chromosomes 1, 4, 5, 6 and 10, and 7 QTLs for citric acid on chromosomes 1, 2, 4, 6, 8, 10 and 11 using an IL population derived from *S. lycopersicum* and *S. pennellii* using SNP markers. Causse et al. (2004) detected 7 QTLs for citric acid on chromosomes 4, 5, 7, 8, 9 and 10; and 5 QTLs for malic acid on chromosomes 3, 4, 8 and 12 using an IL population derived from *S. lycopersicum* M82 and *S. pennellii* using RFLP markers. Citric acid content was found to be increased by *S. pennellii* alleles but not for malic acid. They observed continuous distribution for organic acids and found positive correlation between citric acid and malic acid, but negative correlation between SSC and malic acid. Also titratable acidity was found to be positively correlated with both citric and malic acid. Zhao et al. (2016) performed association mapping with SSR markers among 174 tomato accessions composed of 123 *S. lycopersicum var. ceasiforme* (Cervil) accessions and 51 *S. lycopersicum* (Levovil) accessions. They detected significant associations including 18 marker-trait associations for citric acid on chromosomes 1, 2, 4, 5, 6, 7, 8, 9 and 12; 1 marker-trait association for malic acid on chromosome 9. Schauer et al. (2006) identified 889 fruit metabolite loci including 9 QTLs for citric acid on chromosomes 2, 4, 5, 7, 9 and 10, and 14 QTLs for malic acid on chromosomes 1, 2, 3, 5, 7 and 10 using an IL population derived from *S. lycopersicum* M82 and *S. pennellii.* In other work done by Schauer et al. (2008), 102 QTLs for 22 organic acids were identified in heterozygous IL populations containing introgressions from *S. pennellii* in the genetic background of M82. Capel et al. (2015) identified QTLs for fruit quality traits including organic acids using a RIL population derived from *S. lycopersicum cv*. Moneymaker and *S. pimpinellifolium* with SNP, SSR and indel markers. In that study, 5 QTLs for citric acid on chromosomes 6, 10 and 11, and 1 QTL for malic acid on chromosome 1 were identified. Fulton et al. (2002) identifed QTLs affecting flavor in four advanced backcross populations of tomato. *S. lycopersicum* E6203 was used as recurrent parent and four wild species (*S.*

pimpinellifolium, S. habrochaites, S. neorickii and *S. peruvianum*) were used as donor parents to develop four different populations. Totally 23 QTLs for fructose on chromosomes 1, 2, 3, 4, 6, 7, 8, 9, 10 and 12, 18 QTLs for glucose on chromosomes 1, 2, 3, 4, 6, 7, 8, 9, 10,11 and 12, 10 QTLs for sucrose on chromosomes 2, 4, 6, 8, 9 and 10 were identified.

Findings from the studies mentioned above and our study together demonstrated that wild species of tomato can be used to improve both sugar and organic acid content in cultivated tomato. QTLs were identified for glucose, fructose, citric acid and malic acid among all 12 tomato chromosome in different studies. We identified QTLs for citric acid on the same chromosomes with the other works which are chromosome 1 (Capel et al. 2015, Fulton et al. 2002, Toubiana et al. 2012, Zhao et al. 2016), 3 (Capel et al. 2015, Fulton et al. 2002), 7 (Causse et al. 2004; Schauer et al. 2006), 10 (Capel et al. 2015; Causse et al. 2004; Schauer et al. 2006; Toubiana et al. 2012) and 12 (Capel et al. 2015; Zhao et al. 2016).

It is known that wild species can be used to increase the genetic variability of cultivated tomato resulting in improved agronomic value. Thus, quantification and determination of important agronomic traits and metabolites in wild species is important to develop introgression lines carrying wild species alleles. In the peresent study, we found that introgressions from *S. pimpinellifolium* increased the trait values of organic acids in the *S. lycopersicum* genetic background.Totally 15 *S. pimpinellifolium* alleles, including 5 for succinic acid, 1 for shikimic acid, and 9 for citric acid increased the trait value. The work done by Fulton et al. (2002) supported that wild type alleles could be used to improve flavor in tomato. *S. lycopersicum* E6203 was used as recurrent parent and four wild species including *S. pimpinellifolium* were used as donor parents to develop advanced backcross lines. Specifically for the population carrying *S. pimpinellifolium* alleles they identified 1 QTL for glucose on chromosome 1, no QTLs for fructose or sucrose, 3 QTLs for citricacid on chromosome 1, 3 and 8, and 6 QTLs for malic acid on chromosome 1, 3, 5, 7 and 11.

Other studies also showed that alleles of other wild species improve agronomic value of the cultivated tomato. Overy et al. (2005) developed six IL populations (IL1-4, IL3-2, IL4-3, IL4-4, IL5-4 and IL7-3) derived from *S. lycopersicum* M82 and *S. pennellii*. They showed that *S. pennellii* contains much more glucose, fructose, citric acid and malic acid than cultivated tomato M82. They also found that IL populations with different introgressions showed variation in the mentioned traits, and that IL3-2 showed especially

significant differences from cultivated tomato. Indeed, there are succesful commercial tomato hybrids carrying introgressions from wild species such as cultivars containing introgression from *S. pennellii* with increased fruit SSC (Friedman, Pleban, and Zamir 2000) and cultivars improved in β-carotene levels (Ronen et al. 2000).

In other work, Schauer et al. (2005) evaluated natural genetic diversity of five wild species of tomato including *S. pimpinellifolium*, *S. neorickii*, *S. chmielewskii*, *S. habrochaites* and *S. pennellii* and compared them to cultivated tomato *S. lycopersicum* for metabolite content and breeding potential. They observed that some compounds had high concentrations in wild species as compared to cultivated tomato, while others had low concentrations in wild species. Specifically, *S. pimpinellifolium* contained high levels of fruit metabolites from the group of organic acids such as aconitic acid, citramalate, threonic acid, citric acid, dehydroascorbic acid, chlorogenic acid, galactrunic acid, isocitric acid, malic acid and nicotinitic acid. This wild species contained low levels of gluconic acid, succinic acid, shikimic acid as compared to cultivated tomato. *S. pimpinellifolium* also contained high levels of sugars and sugar alcohols such as fructose, glucose, raffinose, galactose, glycerol, rhamnose, isomaltose and mannose, but low levels of fucose, inositol, maltose and xylose as compared to cultivated tomato. In contrast, in our study we found that cultivated tomato "Tueza" contained high level of sugars for both glucose and fructose than *S. pimpinellifolium*. Moreover, the mean value of sugars measured in the IBL population was higher than *S. pimpinellifolium.* On the other hand, *S. pimpinellifolium* had higher levels of succinic acid and acetic acid, but not other organic acids.

The other metabolite group that strongly influences tomato flavor is volatile compounds. It has been stated that although more than 400 volatile compounds are synthesized in tomato (Buttery and Ling, 1993), only 15 to 20 of these compounds have major effects on tomato flavor (Baldwin et al. 2000). Very few genes or QTLs were identified in the tomato flavor volatiles synthesis pathway because of the complexity of the trait. Indeed, volatile compounds are strongly affected by environmental conditions, and can be present in nanomolar concentrations which makes quantification of these compounds extremely difficult (Tieman et al. 2017).

The IBL population and parental genotypes were evaluated for 40 volatile compounds including β-ionone, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-nitro-3-methylbutane, 1-pentanol, 1-penten-3-one, 2-hexanal, 2-isobutylthiazole, 2-pentanal, geranylacetone, 6-methyl-5-hepten-2-one which are known major contributors to tomato

flavor (Baldwin et al. 2000). Volatile compounds, except 14 of them which are β-cyclocitral, 2-heptanone, 2-isobutylthiazole, 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tri(trimethylsiloxy) tetrasiloxane, 3-pentanone, geranylacetone, 6-methyl-3,5-heptadien-2-one, 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, 6-methyl-5-hepten-2-oneB, acetic acid ethyl ester, cyclopentane, furan-3,4-methyl-3-pentenyl and methyl capronate, did not have normal continuous distribution in the IBL population. Parents of the IBL population did not show significant variation in all volatile compound content, however, variation was seen for 2,3-butanediol, 2,3-butanedion, 2-heptanone, 2-isobutylthiazole, 2-pentanone-3-methyl, 3-isopropoxy - 1,1,1,7,7,7 - hexamethyl - 3,5,5 tri (trimethylsiloxy) tetrasiloxane, 3-pentanone, geranylacetone, 6-methyl-3,5-heptadien-2-one, 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-oneB, acetaldehyde and acetic acid methyl ester (Table 15, Fig. 15). Although the parental alleles for 2,3-butanediol, 2,3-butanedion, 2-pentanone-3-methyl, acetaldehyde and acetic acid methyl ester content were extreme, low variation was observed in the IBL populatiom for the trait. These findings suggest an unbalanced introgression of *S. pimpinellifolium* alleles for these compounds into the *S. lycopersicum* genome.

In this study, we identified 65 QTLs for 40 volatile compounds located on all 12 tomato chromosomes. The identified QTLs mostly localized on chromosomes 3 (14 QTLs), 8 (15 QTLs), and 10 (8 QTLs). Specifically, among the volatiles which are major contributors to tomato flavor: 4 QTLs for β-ionone on chromosomes 3 and 9, 1 QTL for 2-methyl-1-butanol on chromosome 9, 2 QTLs for 1-nitro-3-methyl butane on chromosomes 4 and 9, 5 QTLs for 1-pentanol on chromosome 8, 1 QTL for 2-isobutylthiazole on chromosome 5, and 1 QTL for 2-pentanal on chromosome 11 were identified. For most of the volatile compounds, introgressions from *S. pimpinellifolium* increased the trait value of the volatile compounds in the genetic background of *S. lycopersicum.*

Although there are limited studies to find QTLs for volatile compounds, existing studies identified QTLs on all 12 tomato chromosome for different volatile compounds. Mathieu et al. (2009) identified 30 QTLs for 27 of volatile compounds on chromosomes 2, 3, 4, 5, 6, 7, 10, 11 and 12 using an IL population derived from *S. lycopersicum* and *S. habrochaites*. Saliba-Colombani et al. (2000) detected 29 QTLs for 18 aroma related volatile compounds on chromosomes 1, 2, 3, 4, 6, 8, 9 and 12 using a RIL population derived from Cervil and Levovil with RFLP markers. Causse et al. (2002) detected 23 QTLs for 12 volatile compounds on chromosomes 1, 2, 4, 6, 7, 8, 9 and 12 using the RIL

population derived from Cervil and Levovil with RFLP, RAPD and AFLP markers. Zhang et al. (2015) performed association mapping and identified 82 marker-trait associations for 28 volatile compounds on all chromosomes using 123 accessions of *S. lycopersicum* var. cerasiforme and 51 accessions of *S. lycopersicum* with SSR markers. Perez-Fons et al. (2014) identified 42 QTLs for volatile compounds on all chromosomes except chromosome 7 using an IL population derived from *S. lycopersicum* M82 and *S. pennellii*. Rambla et al. (2016) found 102 QTLs for 39 volatile compounds using 12 RIL populations containing single introgression from *S. pimpinellifolium* in the genetic background of *S. lycopersicum*. The identified QTLs mostly localized on chromosome 1. They also showed that *S. pimpinellifolium* alleles provide higher levels of volatiles for 42 loci, especially for synthesis of carotenoid-derived volatiles, while reduced levels were associated with the other 60 loci.

Liu et al. (2016) investigated ILs containing different chromosomal segments of *S. pennellii* in the genetic background of *S. lycopersicum* cv M82. They observed that IL 4-4 was significantly altered in fruit flavor and nutritional quality (including 21 volatile compounds) compared to M82. Moreover fine mapping of the loci affecting metabolic traits in IL4-4 showed that fatty acid-derived volatile compounds (C5 volatiles) localized within a small segment on chromosome 4 introgressed from *S. pennellii*.

Tieman et al. (2006) identified 25 loci on all 12 chromosomes of tomato for 23 volatile compounds in an IL population derived from *S. lycopersicum* cv M82 and *S. pennellii.* In a recent study performed by Tieman et al. (2017), flavor deficiency in modern tomato cultivars was investigated in 398 modern, heirloom and wild species accessions using whole genome sequencing and genome wide association study to identify loci affecting flavor-related compounds, including sugars, organic acids and volatile compounds with 8K SolCap Illumina Infinium SNP tomato array. A total of 398 accessions grown in Florida, an F2 population (derived from Maglia Rose cherry and modern inbred line FLA 8059), and a second overlapping population grown in Israel had significant common associations for volatile compounds including geranylacetone on chromosome 3, 6- methyl-5-heptene-2-ol on chromosome 3 and 9, and guaiacol on chromosome 9. Two metabolically linked volatiles, guaiacol and methylsalicylate contributed negatively to consumer liking, and were found localized on E8 area on chromosome 9 by associated SNPs. In addition, alleles of two carotenoid-derived volatile compounds, geranylacetone and MHO, were found to be lost during human selection. For these compounds they identified 1 locus for MHO and 4 loci for geranylacetone and noted

strong associations on chromosome 3 for both compounds. Moreover, they identified two loci for glucose and fructose content on chromosome 9 and 11. Both of these loci are located on the same region with the introgressed region for fruit weight in domesticated tomato cultivars. Further examination in the F2 population for sugar content showed that the locus on chromosome 9 for sugar content colocalized with extracellular invertase, *Lin5*. These findings indicate that selection for fruit weight during domestication caused low sugar content. Ballester et al. (2016) worked on IL populations derived from *S. chmielewski* introgression in the *S. lycopersicum* (Moneyberg) genetic background using SNP markers, and showed that 9D region on chromosome 9 provided volatile compound accumulation due to the presence of the IL 9D introgression.

It is not always the case that introgressions from wild type species increase tomato flavor in a favorable way. Tadmor et al. (2002) investigated IL lines (IL 8-1, IL 8-2, IL 8-3) containing *S. pennellii* introgressions in the *S. lycopersicum* cv M82 genetic background. They found that high levels of 2-phenylethanol and 2-phenyl acetaldeyhde coming from *S. pennellii* chromosomal segments of chromosome 8 caused malodorous tomato odor and flavor.

The findings from the studies mentioned above and our study together demonstrated that wild species of tomato can be used to improve volatile compounds content in cultivated tomato. Indeed *S. pimpinellifolium* has good potential in terms of breeding for cultivated tomato flavor as also indicated in other studies (Rambla et al. 2016; Vogel et al. 2010; Tieman et al. 2012).

In addition, colocalized QTLs were detected for flavor and aroma components. 1-nitro pentane and citric acid QTLs were colocalized at 48.6 Mb and 68.6 Mb position on chromosome 1, at 12 Mb position on chromosome 3, and at 58 Mb position on chromosome 10. 1-nitro pentane also colocalized with 2,3-butanedione at 18.2 position on chromosome 2, at 46 position on chromosome 3, at 16 Mb position on chromosome 10, and colocalized with both 2,3-butanedione and citric acid on 30 Mb position on chromosome 10. β-ionone and β-cyclocitral were colocalized at 20 and 38 Mb position on chromosome 3. β-ionone also colocalized with 1-nitro-3-methyl butane at 68.7 Mb position on chromosome 9. 2-isobutyl thiazole and salicylic acid were colocalized at 4.1 Mb position on chromosome 5. Salicylic acid was also colocalized with cyclopentane at 36.2 Mb position on chromosome 7. 1-pentanol, acetic acid methyl ester and cyclopentanesiloxane decamethyl were colocalized at 6, 8, 20, 30, 32, 36, 38, 46 and 48 Mb position on chromosome 8. 2-pentanal and 3-methyl-1-butanol were colocalized at

4.3 Mb position on chromosome 11. Several volatile compounds were colocalized together on different regions of different chromosomes. These colocalizations might be due to sharing common biosynthesis pathways and enzymes which catalyze common pathways might be on the same chromosomal regions. Besides colocalization of volatile compounds, organic acids also colocalized with volatile compounds. These colocalizations might be due to linkage of the genes that control the traits. Moreover, correlation analysis between flavor-related traits demonstrated significant positive correlation between glucose and fructose (r^2 = 0.91). This correlation was expected because sucrose is made from glucose and fructose units by condensation reaction. During ripening, sucrose is hydrolyzed into glucose and fructose (Miron and Schaffer 1991). Other correlations, both positive and negative, between sugars, organic acids and volatile compounds are weak. Many weak correlations were observed between volatile compounds as expected. This was expected because volatile compounds are synthesized from common pathways.

Overall, the QTLs reported in populations such as ILs, NILs, and RILs derived from different wild species such as *S. pimpinellifolium*, *S. neorickii*, *S. chmielewskii*, *S. habrochaites* and *S. pennellii*, were overlapping with only a minor portion of our QTLs. This discrepancy could arise from: the different methods used to quantify flavor related metabolites, the different markers used, the different populations structures and the different wild species used as donor parents. Taken all together, these results suggest that there is huge genetic variation already available in wild species for breeding studies and development of new cultivars with improved fruit flavor by modulating flavor-related traits.

CHAPTER 5

TOMATO NUTRITIONAL CHARACTERS

5.1. Introduction

Hunger, starvation, malnutrition and poor health are the world's biggest problems. Today about 800 million people suffer from hunger and malnutrition (Baldermann et al. 2016), and about 3.1 million children die because of poor nutrition every year (World Food Program). Moreover, malnutrition prevents normal physical and mental development in children and also causes weakened health and productivity in adults (Triantaphylides and Havaux 2009). Malnutrition and disease are closely linked. The incidence of many diseases, such as cancer, cardiovascular diseases and diabetes, increases as a result of malnutrition and unbalanced daily diets (Baldermann et al. 2016). Thus vegetables are important as a source of vitamins and essential compounds for human health (Almeida et al. 2011). Fats, proteins, carbohydrates, vitamins and minerals define the nutritional value of vegetables (OECD, 2015).

Phytosterols are plant sterols and have similar biological functions as sterols found in animals, mainly cholesterol, which is cell membrane stabilization (Moreau et al. 2002). Phytosterols can be found in both free and conjugated forms in plants (Wojciechowski 1991). Phytosterol content and concentration in plants depends on genetic background and environmental factors (Piironen et al. 2003). The most abundant phytosterols in plants are β-sitosterol, campesterol, stigmasterol and avenasterol (Nes 1987). Phytosterols play roles in maintaining membrane fluidity, transportation of molecules across the membrane, modulating the activities of membrane-associated proteins, and are precursors of other bioactive molecules (Dyas et al. 1993, Hartmann 1998, Clouse 2000, Wojciechowski 1991). Plant sterols are also benefical to human health. Dieatary phytosterols help to reduce LDL cholesterol in serum (Hallikainen and Uusitupa 1999; Hendriks et al. 1999; Jones et al. 1999; Miettinen et al. 1995) and may have protective effects against different types of cancer such as stomach, rectal or colon cancer (Awad and Fink 2000).

Fatty acids are important molecules with diverse functions such as being the main components of cell membranes, preventing organ fusion, limiting water loss because they are constituents of the surface layers, playing roles in cell signalling, and being carbon and energy storage compounds (Dyer and Taylor 2008; Troncoso-Ponce et al. 2016). Moreover, fatty acids contribute to tomato flavor because they are precursors of some volatile compounds (Stone, Hall, and Kazeniac 1975; Galliard et al. 1977; Vick and Zimmerman 1986). Besides structural and metabolic functions, fatty acids are important to human health. Palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and alphalinolenic acid (18:3) are the most important fatty acids for human health (Dyer et al 2008). Moreover, epidemiological studies showed that essential molecules such as amino acids and fatty acids as well as particular elements and minerals are necessary to maintain human health and protection again specific diseases (Boeing et al. 2012).

Another group of health-related metabolites is vitamins. They are essential and should be taken with the diet, because humans cannot synthesize vitamins (Asensi-Fabado and Munne 2010). Vitamin deficiency causes severe diseases such as blindness in vitamin A deficiency, beriberi in vitamin B1 deficiency, pellagra in vitamin B3 deficiency, anemia in vitamin B6 deficiency, neural tube defects in fetus during pregnancy in vitamin B9 deficiency, scurvy in vitamin C deficiency, and rickets in vitamin D deficiency. Such deficiencies can be lethal in some cases. For example vitamin A deficiency increases the risk of mortality and morbidity from infectious diseases in children (Asensi-Fabado and Munne 2010, Kraemer et al. 2008; Wright et al. 2007).

Health-related metabolites have increasingly become the focus of breeding programmes, because consumers are paying more attention to the health and nutritional aspects of agricultural products.

5.2. MATERIALS AND METHODS

5.2.1. Plant material

The IBL population described in Chapter 2 was used for metabolic analysis.

5.2.2. Metabolic Profiling and Sample Preparation

Samples were prepared as mentioned in Chapter 4.

5.2.3. Quantification of Metabolites

Phytosterols, desmosterol, ergosterol, fucosterol, cholesterol, campestrol, stigmasterol, and β-sitosterol, were analyzed with a thermocritic method of GC-FID which modified from the method mentioned in Xu et al. (2012). The chloroform: methanol: water (1:3:1:, v:v:v) extract of tomato was used. Phytosterols were analyzed on Rtx 5DA (0,25 mm x 0,25 mm, 30 m) column with a thermocritic program. The column temperature was set at 300 °C and column gas flow was 0.52 ml/min. Injection port temperature was held at 310 °C while detector temperature was held at 315 °C. Carrier gas was nitrogen (N_2) and split ratio was 1/50. Detection was done by FID.

Summary of method parameters is below:

Column: Rtx 5DA (0,25 mm x 0,25 mm, 30 m)

Column Temperature: 300 °C

Column Gas Flow: 0.52 ml/min

Carrier Gas: N2

Injection Volume: 1 µl

Split Ratio: 1 / 50

Injection Port Temperature: 310 °C

Detector Temperature: 315 °C

Free fatty acids were analyzed with derivatization by converting methyl esters. C6, C8, C10, C11,C12, C13, C14, C14-9, C15, C16, C16-1, C17, C18, C18-1, t-C18-1, C18-2, C20, C20-11, C20-11,14, C21, C22, C22-1, C23 fatty acids were analyzed with a thermogradient method of GC-FID which modified from the method mentioned in Lissitsyna et al. (2012). Samples (500 µl) of dichloromethane:hexane (1:1, v/v) extracts of tomato were taken and solvent was evaporated in a vacuum evaporator at 30 °C. Then 2 ml of 10% sulfuric acid in methanol was added to dried samples, and incubated at 57 °C for 40 minutes while shaking at 140 rpm. After samples were cooled to room

temperature, 1 ml of 2% sodium bicarbonate was added. Then 1 ml of hexane was added to the solution and mixed well by vortexing. Samples were kept at room temperature for phase separation. The upper phase, which was hexane phase, was taken for GC analysis.

Fatty acid methyl esters were analyzed on stabilwax DA (0,25 mm x 0,25 mm, 60 m) column with a thermogradient program. The column temperature was programmed from 100 °C (4 min held) to 245 °C at a rate of 20 °C/min (40 min held), from 245 °C to 250 °C at a rate of 100 °C/min, 5 minutes held at the final temperature was applied. Injection port temperature was held at 250 °C while detector temperature was held at 280 °C. Carrier gas was nitrogen (N_2) and split ratio was 1/25. Detection was done by FID.

Summary of method parameters is below:

Column: Stabilwax DA (0,25 mm x 0,25 mm, 60 m)

Column Temperature: 100 °C to 250 °C

Column Gas Flow: 1,5 ml/min

Carrier Gas: N2

Injection Volume: 1 µl

Split Ratio: 1 / 25

Injection Port Temperature: 250 °C

Detector Temperature: 280 °C

Column temperature Programme:

°C / min	°C	min
_	100	4
20	245	40
100	250	15

Vitamin A, vitamin D3, and vitamin K3 were analysed with an isocritic method of HPLC- PDA/FLD which was combined and modified from the methods mentioned in Bakre et al. (2015) and Turner and Burri (2012). Dichloromethane:hexane (1:1, v/v) extracts of tomato were used. Fat soluble vitamins were analyzed on reverse phase (RP C18, 5 µm – 25 x 4,6 mm) column at 40 °C using acetonitrile:methanol (75:25, v:v) as the mobile phase with a flow rate 1.5 ml/min. Sample injection was 20 µl and standard solutions were prepared in methanol (vitamin A) and acetonitrile: methanol (80:20, v:v for vitamin D3 and vitamin K3). Detection was done at 280, 265 and 325 nm for vitamin

A, vitamin D3 and vitamin K3, respectively, with PDA detector.

Summary of method parameters is below:

HPLC- PDA/FLD method:

Column: RP-C18 (5 µm – 25 x 4,6 mm) Column Temperature: 40 °C

Flow rate: 1,5 ml/min

Injection volume: 20 µl

PDA wavelength: 280, 265 and 325 nm

Solvent: Methanol (vitamin A), acetonitrile: methanol – 80:20- v:v (vitamin D3, vitamin K3)

Mobile phase: Acetonitrile:Methanol – 75:25, v:v

Pantothenic acid, niacin, folic acid, vitamin B12 and riboflavin were analysed with a isocritic method of HPLC- PDA/FLD which combined and modified from the methods mentioned in Li and Chen (2001a, 2001b). Choloroform: methanol: water (1:3:1, v:v:v) extracts of tomato were used. Pantothenic acid, niacin, folic acid were analyzed on reverse phase (RP C18, 5 µm – 25 x 4,6 mm) column at 40 °C using methanol: potassium dihydrogenphosphate buffer (KH_2PO_4, 0.1M, pH=7) (10:90, v:v) as the mobile phase with a flow rate 1 ml/min. Sample injection was 20 µl and standard solutions were prepared in water with the exception of folic acid which was prepared in 10 mM KH_2PO_4. Detection was done at 204, 261, 265 and 282 nm with PDA detector.

Summary of method parameters is shown:

HPLC- PDA method:

Column: RP-C18 (5 µm – 10 x 2,1 mm) Column Temperature: 40 °C

Flow rate: 1 ml/min

Injection volume: 20 µl

PDA wavelength: 204, 261, 265 and 282 nm

Solvent: 10 mM KH_2PO_4, pH = 7,5 (folic acid); the other vitamins dissolved in water.

Mobile phase: Methanol : 0.1 M KH_2PO_4, pH=7 – 10:90, v:v

Vitamin B12 and riboflavin were analyzed on reverse phase (RP C18, 3 µm – 10 x 2,1 mm) column at 40 °C using methanol: potassium dihydrogenphosphate buffer (KH_2PO_4, 0.1M, pH=7) (45:55, v:v) as the mobile phase with a flow rate 0.3 ml/min. Sample injection was 20 µl and standard solutions were prepared in water. Detection was

done at 204 and 361 nm with PDA detector, and fluorescence detector was set at 450 nm for excitation and 510 nm for emission.

Summary of method parameters is shown:

For vitamin B12 and riboflavin:

HPLC- PDA/FLD method:

Column: RP-C18 (3 µm – 10 x 2,1 mm)

Column Temperature: 40 °C

Flow rate: 0,3 ml/min

Injection volume: 20 µl

PDA wavelength: 204 and 361 nm

FLD: excitation – 450 nm, emission – 510 nm

Solvent: Water

Mobile phase: Metanol : 0,1M KH_2PO_4, pH=7 – 45:55, v:v

5.2.4. QTL mapping

QTL mapping was performed as described in Chapter 3 using data obtained from GBS as explained in Chapter 2.

5.3. Results

5.3.1. Metabolite Variation

5.3.1.1. Phytosterols

None of the phytosterols could be detected in the parents and IBL population.

5.3.1.2. Fatty acids

The parents of the IBL population had high fatty acid content with the only exception being the myristic acid (C14) content of Tueza (Table 18). All of the traits segregated in the IBL population except palmitoleic acid (C16-1). Fatty acids displayed

variation in the population with coefficients of variation (CV) ranging from 26.6 % to 172.6 %. Normal continuous distribution was observed for fatty acids except palimitoleic acid (C16-1) (Figure 13).

Table 18. Statistics for fatty acid content measured in IBL population and parents; *S. lycopersicum* cv. Tueza and *S. pimpinellifolium* cv. LA1589. Quanitities of metabolites are given as mg/100g DW.

	Parents		IBL Population		
	Tueza	**LA1589**	**Mean**	**Range**	**CV%**
C14	0.00	22.22	22.42	0-35.68026	40.8
C16	275.86	88.39	153.12	0-383.30	36.9
C16:1	48.30	17.35	22.76	11.45-389.61	172.6
C18	307.04	103.24	187.23	92.64-333.44	26.6
C18:1	29.69	33.32	46.00	3.90-210.30	81.8
C18:2	157.59	43.26	107.28	14.40-515.86	77.6

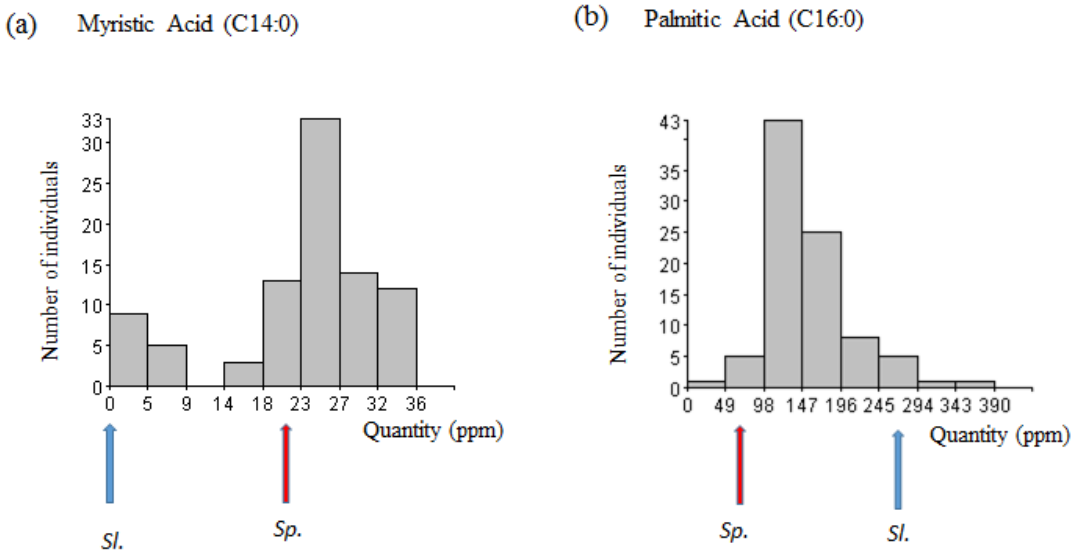

Figure 13. Distribution of fatty acid content in IBL population. (a) Distribution of myristic acid content (ppm) in IBL population, (b) Distribution of palmitic acid content (ppm) in IBL population. Arrows indicate means for Sl.: *S. Lycopersicum* cv. Tueza and Sp.: *S. pimpinellifolium* cv. LA1589.

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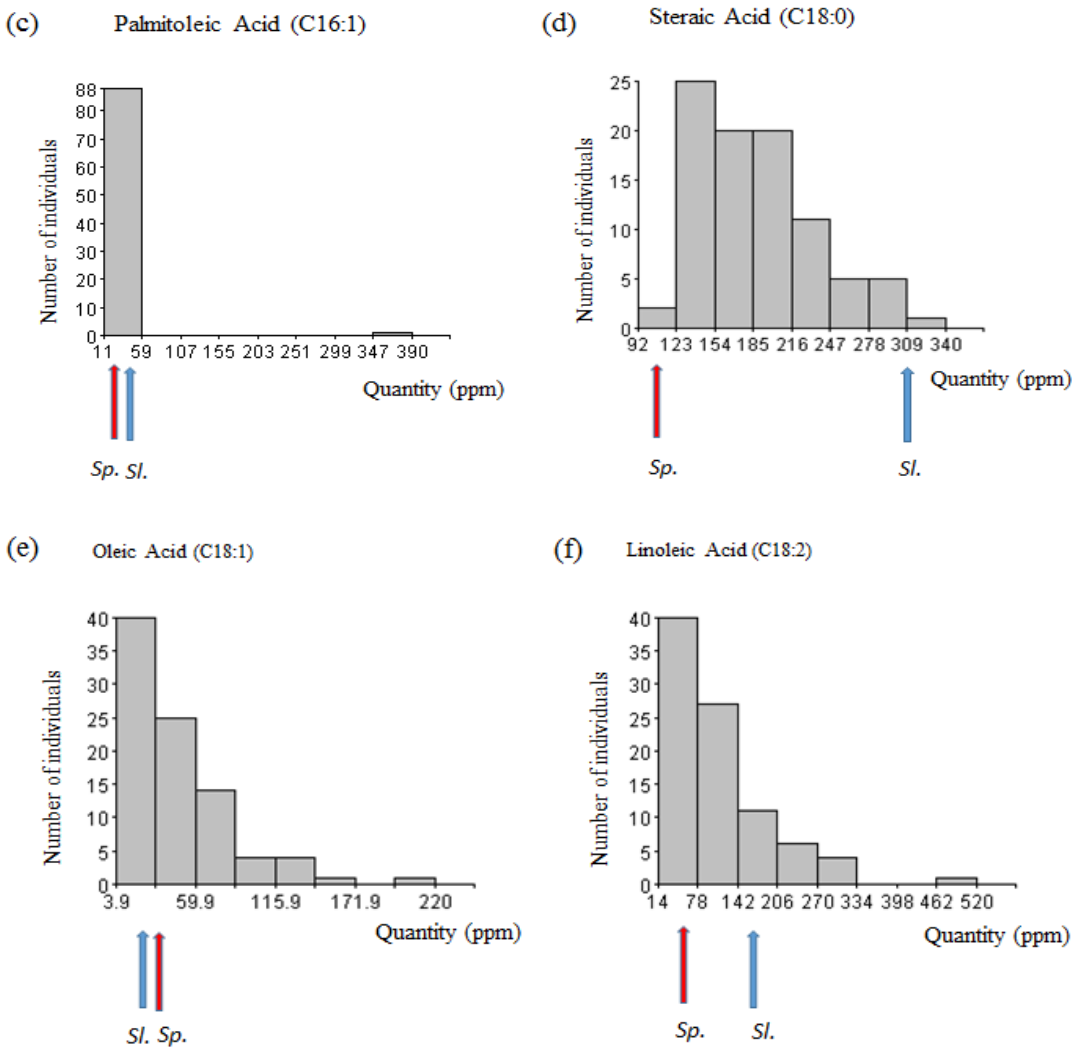

Figure 13. **(cont.)** (c) Distribution of palmitoleic acid content (ppm) in IBL population, (d) Distribution of stearic acid content (ppm) in IBL population, (e) Distribution of oleic acid content (ppm) in IBL population, (f) Distribution of linoleic acid content (ppm) in IBL population.

5.3.1.3. Vitamins

The parents of the IBL population did not have high fat soluble vitamin content (Table 19). Vitamin A and vitamin D3 segregated in the IBL population. Fat soluble vitamins displayed variation in the population with coefficients of variation (CV) ranging from 113.8 % to 798.5% Only vitamins A and D3 displayed continuous distributions within the population (Figure 14).

Table 19. Statistics for fat soluble vitamin contents measured in IBL population and parents; *lycopersicum* cv. Tueza and *S. pimpinellifolium* cv. LA1589.

	Parents		IBL Population		
	Tueza	**LA1589**	**Mean**	**Range**	**CV%**
Vit A	0.06	0.07	0.21	0-36617	140.3
Vit D3	0.00	0.09	0.56	0-3.20	113.8
Vit K3	5.04	2.46	840.57	0-62924.77	798.5

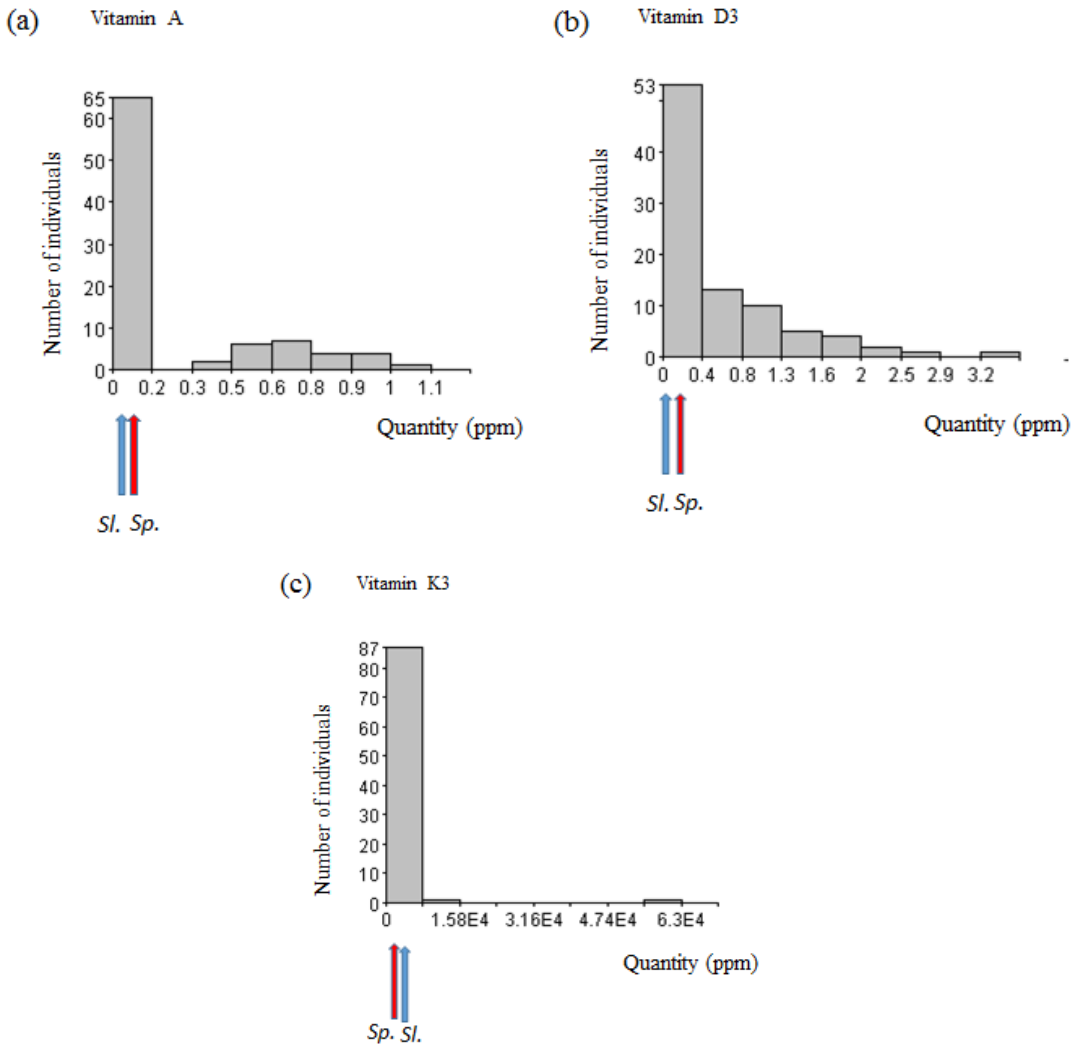

Figure 14. Distribution of fat soluble vitamin content in IBL population. (a) Distribution of vitamin A content (ppm) in IBL population, (b) Distribution of vitamin D3 content (ppm) in IBL population, (c) Distribution of vitamin K3 content (ppm) in IBL population. Arrows indicate means for Sl.: *S. lycopersicum* cv. Tueza and Sp.: *S. pimpinellifolium* cv. LA1589.

Vitamin B12 and riboflavin could not be detected in both parents and IBL population.

The parents of the IBL population had pantothenic acid and niacin content but low folic acid content (Table 20). All of the traits segregated in the IBL population. Water soluble vitamins displayed variation in the population with CV ranging from 68.5 % to 160.2 % (Figure 15).

Table 20. Statistics for water soluble vitamin contents measured in IBL population and parents; *S. lycopersicum* cv. Tueza and *S. pimpinellifolium* cv. LA1589.

	Parents		**IBL Population**		
	Tueza	**LA1589**	**Mean**	**Range**	**CV%**
Panthatonic acid	105.18	159,56	11.99	0-126.45	160.2
Folic acid	6.65	3.60	2.38	0-7.48	83.3
Niacin	110.58	44.96	5.60	0-24.45	68.5

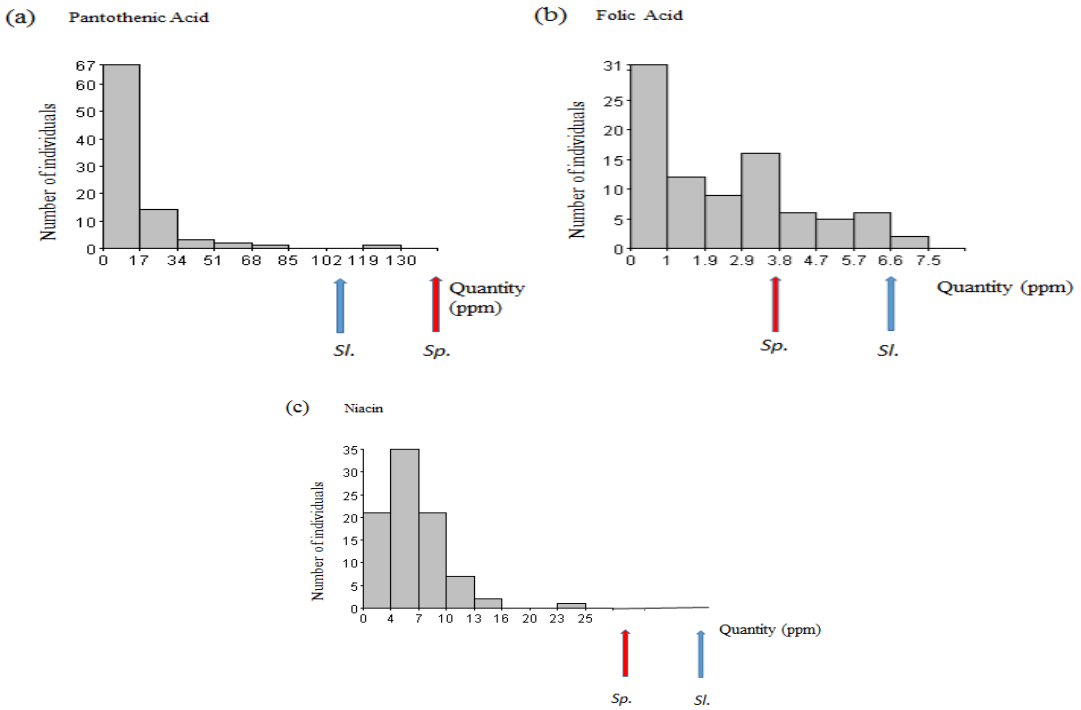

Figure 18. Distribution of water soluble vitamin content in IBL population. (a) Distribution of Pantothenic acid content (ppm) in IBL population, (b) Distribution of Niacin content (ppm) in IBL population, (c) Distribution of Niacin content (ppm) in IBL population. Arrows indicate means for Sl.: *S. lycopersicum* cv. Tueza and Sp.: *S. pimpinellifolium* cv. LA1589.

5.3.2. QTL Mapping

5.3.2.1. Fatty Acids

A total of 16 QTLs were identified for fatty acids based on LOD thresholds of 3. A minor QTL was identified for myristic acid on chromosome T11 with PVE of 15%. For palmitoleic acid, 11 QTLs were identified on chromosomes 1, 2, 3, 10 and 12 with the PVE varying between 16% and 55%. Four of the QTLs (*pa1.1, pa2.1, pa3.1, pa10.1*) were major for palmitoleic acid on chromosomes 1, 2, 3, and 10 with PVE 38, 40, 48 and 55%, respectively. Four of the QTLs (*pa1.2, pa3.2, pa10.2, pa10.3*) on chromosomes 2, 3 and 10 had moderate effects with 20 % < PVE < 30 %. The remaining four QTLs had minor effects. For oleic acid, 2 minor QTLs were identified on chromosomes 5 and 10 with PVE between 15% and 18%. For linoleic acid, a minor QTL was identified on chromosome 5 with PVE < 20 % (Table 21).

Table 21**.** QTLs identified for fatty acids.

Trait	QTL	Chr	Position (Mb)	Marker Interval	LOD*	PVE **	Additive Effect***
Myristic Acid (C:14)	*ma11.1*	T11	10.3	S11_356158	3.6	0.15	*S. lycopersicum*
Palmitoleic Acid (C16:1)	*pa1.1*	T1	20.6	S1_663411 - S1_28625691	4.8	0.22	*S. lycopersicum*
			22.6	S1_663411 - S1_28625691	9.2	0.38	
Palmitoleic Acid (C16:1)	*pa1.2*	T1	48.6	S1_32640248 - S1_56607342	4.7	0.22	*S. lycopersicum*
Palmitoleic Acid (C16:1)	*pa1.3*	T1	64.6	S1_56607342 - S1_70630742	3.9	0.18	*S. lycopersicum*
Palmitoleic Acid (C16:1)	*pa2.1*	T2	18.2	S2_18214735 - S2_22281850	9.7	0.4	*S. lycopersicum*
Palmitoleic Acid (C16:1)	*pa3.1*	T3	46	S3_46076617 - S3_64075209	12.8	0.48	*S. lycopersicum*
			52	S3_46076617 - S3_64075209	5.7	0.25	
Palmitoleic Acid (C16:1)	*pa3.2*	T3	12	S3_2015299 - S3_18037470	6.3	0.28	*S. lycopersicum*
Palmitoleic Acid (C16:1)	*pa10.1*	T10	16	S10_12085921 - S10_18026339	15.2	0.55	*S. lycopersicum*

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Table 21. **(cont.)**

Trait	QTL	Chr	Position (Mb)	Marker Interval	LOD *	PVE **	Additive Effect***
Palmitoleic Acid (C16:1)	pa10.2	T10	30	S10_18026339 - S10_32088336	5.7	0.26	S. lycopersicum
			34	S10_32088336 - S10_42019169	6.5	0.28	
Palmitoleic Acid (C16:1)	*pa10.3*	T10	58	S10_54018912 - S10_62061848	5.7	0.25	*S. lycopersicum*
Palmitoleic Acid (C16:1)	*pa10.4*	T10	8	S10_4007994 - S10_10077455	3.6	0.17	*S. lycopersicum*
Palmitoleic Acid (C16:1)	*pa12.1*	T12	32.1	S12_26140976	4.1	0.19	*S. lycopersicum*
Palmitoleic Acid (C16:1)	*pa12.2*	T12	40.1	S12_26140976	3.3	0.16	*S. lycopersicum*
Oleic Acid (C18:1)	*oa5.1*	T5	2.1	S5_141731 - S5_8117157	3.8	0.18	*S. lycopersicum*
			4.1	S5_141731 - S5_8117157	3.9	0.18	*S. lycopersicum*
			6.1	S5_141731 - S5_8117157	3.7	0.17	*S. lycopersicum*
Oleic Acid (C18:1)	*oa10.1*	T10	56	S10_54018912 - S10_62061848	3.1	0.15	*S. lycopersicum*
Linoleic Acid (C18:2)	*la5.1*	T5	2.1	S5_141731 - S5_8117157	4.2	0.19	*S. lycopersicum*
			4.1	S5_141731 - S5_8117157	4.1	0.19	*S. lycopersicum*
			6.1	S5_141731 - S5_8117157	3.2	0.15	*S. lycopersicum*

* Peak position of QTL.
** Percentage of phenotypic variation explained by identified QTL.
*** Parental allele associated with increased trait value.

5.3.2.2. Vitamins

A total of 3 QTLs were identified for fat soluble vitamins based on LOD thresholds of 3. For vitamin A content, only one minor QTL was identified on chromosomes 9 with PVE of 10%. For vitamin K3, a major QTL on chromosome 12 with (PVE 33%) and a minor QTL on chromosome 4 were identified (Tab1e 22).

Table 22. QTLs identified for fat soluble vitamins.

Trait	QTL	Chr	Position (Mb)	Marker Interval	LOD*	PVE**	Additive Effect***
Vitamin A	*va9.1*	T9	52.7	S9_44727247 - S9_58720803	3.2	0.1	*S. pimpinellifolium*
Vitamin K3	*va12.1*	T12	2.1	S12_179179 - S12_24152718	7.8	0.33	*S. lycopersicum*
Vitamin K3	*va4.1*	T4	10.5	S4_10551519 - S4_12563845	3.2	0.15	*S. lycopersicum*

* Peak position of QTL.
** Percentage of phenotypic variation explained by identified QTL.
*** Parental allele associated with increased trait value.

A total of 18 QTLs were identified for water soluble vitamins based on LOD thresholds of 3. For pantothenic acid, two minor QTLs were identified on chromosomes 3 and 6 with PVE between 17% and 18%. For niacin, 16 QTLs were identified on chromosomes 1, 2, 3, 6, 8, 9 and 10 with PVE varying between 15% and 29%. All of the QTLs identified for niacin had moderate effects with 20 % < PVE < 30 % except 5 QTLs (ni1.4, ni2.2, ni3.2, ni 8.1, ni10.1) on chromosomes 1, 2, 3, 8 and 10 which had minor effects with PVE < 20 % (Table 23).

Table 23. QTLs identified for water soluble vitamins.

Trait	QTL	Chr	Position (Mb)	Marker Interval	LOD*	PVE**	Additive Effect***
Panthatonic Acid	*pa3.1*	T3	68	S3_64075209	3.5	0.17	*S. pimpinellifolium*
Panthatonic Acid	*pa6.1*	T6	42.8	S6_42876082 - S6_46815418	3.8	0.18	*S. lycopersicum*
Niacin	*ni1.1*	T1	40.6	S1_32640248 - S1_56607342	4.9	0.23	*S. pimpinellifolium*
Niacin	*ni1.2*	T1	48.6	S1_32640248 - S1_56607342	4.8	0.2	*S. pimpinellifolium*
Niacin	*ni1.3*	T1	58.6	S1_56607342 - S1_70630742	4.7	0.22	*S. pimpinellifolium*
Niacin	*ni1.4*	T1	68.6	S1_56607342 - S1_70630742	3.4	0.16	*S. pimpinellifolium*
Niacin	*ni1.5*	T1	74.6	S1_72610586 - S1_80687203	5	0.23	*S. pimpinellifolium*
Niacin	*ni2.1*	T2	12.2	S2_6233827 - S2_14253555	5.4	0.25	*S. pimpinellifolium*

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Table 23. **(cont.)**

Trait	QTL	Chr	Position (Mb)	Marker Interval	LOD*	PVE**	Additive Effect***
			14.2	S2_14253555 - S2_18214735	3.7	0.18	*S. pimpinellifolium*
Niacin	*ni2.2*	T2	28.2	S2_28240228 - S2_34211204	3.3	0.16	*S. lycopersicum*
			30.2	S2_28240228 - S2_34211204	3	0.15	
			32.2	S2_28240228 - S2_34211204	4.9	0.23	
Niacin	*ni2.3*	T2	20.2	S2_18214735 - S2_22281850	4.5	0.21	*S. pimpinellifolium*
Niacin	*ni3.1*	T3	4	S3_2015299 - S3_18037470	4.3	0.2	*S. lycopersicum*
Niacin	*ni3.2*	T3	34	S3_18037470 - S3_46076617	3.8	0.18	*S. pimpinellifolium*
			38	S3_18037470 - S3_46076617	4.7	0.22	
Niacin	*ni8.1*	T8	62	S8_60041028	3.5	0.17	*S. pimpinellifolium*
Niacin	*ni9.1*	T9	58.7	S9_58720803 - S9_68734489	4.6	0.2	*S. lycopersicum*
Niacin	*ni9.2*	T9	66.7	S9_58720803 - S9_68734489	4	0.2	*S. lycopersicum*
Niacin	*ni10.1*	T10	28	S10_18026339 - S10_32088336	3.5	0.17	*S. pimpinellifolium*
Niacin	*ni10.2*	T10	32	S10_32088336 - S10_42019169	6	0.27	*S. pimpinellifolium*
Niacin	*ni10.3*	T10	40	S10_32088336 - S10_42019169	6.4	0.29	*S. pimpinellifolium*

* Peak position of QTL.
** Percentage of phenotypic variation explained by identified QTL.
*** Parental allele associated with increased trait value.

5.3.3. Correlated and Colocalized Traits

Correlation analysis of metabolic characters demonstrated that there was only one very weak but significant correlation between pantothenic acid and niacin ($r^2 = 0.105$). Other correlations between nutritional traits were non-significant (Table 24).

Table 24. Correlation between flavor traits. Significant ($P < 0.05$) correlations between tomato fruit traits. Correlations with P value > 0.05 were considered to be non-significant (NS). MA= Myristic acid, PA= Palmitic acid, PoA= Palmitoleic acid, SA= Stearic acid, OA= Oleic acid, LA= Linoleic acid, VitA= Vitamin A, VitD3= Vitamin D3, VitK3= Vitamin K3, PnA= Pantothenic acid, FA= Folic acid.

Traits	MA	PA	PoA	SA	OA	LA	VitA	VitD3	VitK3	PnA	FA	Niacin
MA	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PA		1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PoA			1	NS	NS	NS	NS	NS	NS	NS	NS	NS
SA				1	NS	NS	NS	NS	NS	NS	NS	NS
OA					1	NS	NS	NS	NS	NS	NS	NS
LA						1	NS	NS	NS	NS	NS	NS
VitA							1	NS	NS	NS	NS	NS
VitD3								1	NS	NS	NS	NS
VitK3									1	NS	NS	NS
PnA										1	NS	0.105
FA											1	NS
Niacin												1

Only one colocalized QTL for nutritional traits was identified for oleic acid (*oa5.1*) and linoleic acid (*la5.1*) on chromosome 5 at 2.1, 4.1 and 6.1 position (Table 25).

Table 25. Nutritional traits QTLs that colocalized.

Trait	**QTL**	**Chr.**	**Position**
Oleic Acid	*oa5.1*	T5	2.1, 4.1, 6.1
Linoleic Acid	*la5.1*	T5	2.1, 4.1, 6.1

5.4. DISCUSSION

Diet has always been an important factor for human health. Malnutrition, especially vitamin and mineral deficiency, causes many diseases. For example, vitamin C deficiency is associated with scurvy and niacin (vitamin B3) deficiency is associated with pellagra. (Jukes 1989). Tomato consumption is reported to decrease cancer development due to its bioactive metabolites such as vitamins, dietary fiber, phenolics

and phytosterols (Giovannucci et al. 2002;Pinela et al. 2012). The nutritional value of a crop is defined by fats, proteins, carbohydrates, vitamins and minerals. Nowadays crops are bred for higher nutritional value or improved health benefits and this is also a growing trend in tomato breeding (OECD 2015). In this study, we focused on phytosterol, fatty acid and vitamin content. Most of these are ignored in tomato breeding with the exceptions of vitamin C and vitamin E.

It was reported that phytosterol uptake is a more efficient strategy to reduce the risk of heart diseases than restricted uptake of saturated fats (Moreau et al. 2002). In addition, phytosterols may have anti-tumor activity, especially in colon cancer (Oomah and Mazza 1999). Thus, the IBL population and parental genotypes were evaluated for seven phytosterols: cholesterol, fucosterol, ergosterol, desmosterol, campesterol, stigmasterol and β-sitosterol. The analytical method for phytosterols was optimized and the analytical standards of phytosterols were detected and quantified. In addition, different extraction methods, including nonpolar solvents such as hexane and dichloromethane which are reported to be capable of extracting >95 % of free phytosterols (Moreau, Powell, and Hicks 1996) were tested. Despite this optimization, we could not quantify phytosterols in the IBL population or in the parents. This could arise if the phytosterols are in a conjugated form instead of a free form. Phytosterols can be present in conjugated forms with fatty acids, phenolic acids and glycosides and conjugated forms compose 85 to 90 % of total phytosterols (Moreau et al. 2002; Piironen et al. 2003). There are a few reports on tomato phytosterol content in the literature (Cetkovic et al. 2012; Ramos-Bueno et al. 2017). It is reported that tomato contains lower levels of phytosterols than in other fruits and vegetables and that the main sterols in tomato are campesterol, stigmasterol and β-sitosterol (Moreau et al. 2002).

Triacylglycerols including saturated and unsaturated fatty acids, and sterols such as cholesterol and phytosterols are dietary lipids (Fenyvesi, Vikmon, and Szente 2015). Phytosterol content in tomato fruit is variable and this variability is attributed to maturity stage. Ramos-Bueno et al. (2017) worked on four tomato varieties: Cherry, Racimo, Raf and Pera which were at the moderate ripening stage (not fully ripe). They found that β-sitosterol had the highest quantity while stigmasterol was lowest. Total phytosterol content ranged between 918 mg/kg dry weight (Racimo) and 1570 mg/kg dry weight (Cherry).

The other group of triacylglycerols, fatty acids, participitate in plant cell membrane structure (Millar, Smith, and Kunst 2000), are a source of energy (Kachroo

and Kachroo 2009), play roles in plant defence by conjugating with other molecules like sugars to form acylsugars (Blauth et al. 1999; Leckie et al. 2014), act as signalling molecules in plant defence and can be directly involved in plant defence (especially 16 and 18 carbon fatty acids) (Kachroo and Kachroo 2009). Moreover, fatty acids contribute to tomato flavor because they are precursors of volatile compounds which determine tomato flavor (Domiguez et al. 2010; Wang et al. 1996, 2001). Besides having a pivotal role in plants, fatty acids also contribute to human nutrition and health. Limited intake of polyunsaturated fatty acids (PUFAs) has cardiovascular benefits, but excess amounts of PUFAs have potential adverse effects. In addition, monosaturated fatty acids (MUFAs) modulate insulin sensitivity, mediate glycemic control and blood pressure, and promote healty blood lipid profiles (Gillingham, Harris-Janz, and Jones 2011). Moreover, some fatty acids including linoleic acid, α-linolenic acid eicosapentaenoic acid, docosahexanoic acid, and arachidonic acid, are essential and cannot be synthesized by humans. Therefore these fatty acids should be obtained in the daily diet. Essential fatty acids play roles in the prevention of hypertension, inflammatory and autoimmune diseases, coronary artery disease, arthritis and cancer (Simopoulos 1999).

Tomato has low fatty acid content compared to other vegetables (Kalogeropoulos et al. 2012; OECD 2015; Ramos-Bueno et al. 2017). In our work, the IBL population and the parents were evaluated for 23 fatty acids, 6 of them were quantified in the parents and IBL population. Total fatty acid content was 8.2 g /kg dry weight for *S. pimpinellifolium*, 3.1 g /kg dry weight for cultivated tomato, Tueza, and the mean total fatty acids for the IBL population was 5.4 g /kg dry weight. *S. pimpinellifolium* contained high levels of palmitic acid, palmitoleic acid, stearic acid, and linoleic acid, but low level of myristic acid and oleic acid as compared toTueza (Table 18). The quantified fatty acids, except palmitoleic acid, had normal continuous distribution in the IBL population. Parents of the IBL population showed extreme variation in fatty acid content, except for oleic acid. Although the parental alleles for palmitoleic acid content were extreme, low variation was observed in the IBL population for the trait. The finding implies an unbalanced introgression of *S. pimpinellifolium* alleles for palmitoleic acid into the *S. lycopersicum* genome.

Ramos-Bueno et al. (2017) identified fatty acid profiles in commercial tomato varieties. They found that the dominant fatty acid in quantity was linoleic acid followed by palmitic acid, oleic acid, linoleic acid, stearic acid, vaccanic acid and palmitoleic acid, respectively. These results were compatible with those of Guil-Guerrero and Rebolloso-

Fuentes (2009) for the same cultivars but unlike those we found in our study. Both the IBL population and its parents contained higher concentrations of stearic acid, palmitic acid, linoleic acid than the other fatty acids. This suggests that fatty acid profiles differ between cultivars or varieties.

In the present study, we identified 16 QTLs for fatty acids: 1 QTL for myristic acid (C14:0) on chromosome 11; 12 QTLs for palmitoleic acid (C16:1) on chromosomes 1, 2, 3, 10 and 12; 2 QTLs for oleic acid (C18:1) on chromosomes 5 and 10; and 1 QTL for linoleic acid (C18:2) on chromosome 5 which was the same chromosome found in the study performed by Schauer et al. (2006). Although the parental alleles for palmitic acid (C16:0) and stearic acid (C18:0) content were extreme and normal continuous distribution was observed in the IBL population for the trait, no QTLs were detected. The finding implies an unbalanced introgression of *S. pimpinellifolium* alleles for palmitic acid and stearic acid into the *S. lycopersicum* genome. Moreover, all alleles for increased fatty acid content were coming from the cultivar Tueza instead of *S. pimppinellifolium.* This is most likely due to the unbalanced nature of the IBL population which favors the recurrent parent genotype.

There are a few studies in the literature that examined the genetic control of fatty acids in tomato. Short chain fatty acids conjugate sugars to form acylsugars that are important for insect resistance. Thus, a few study identified QTLs for fatty acids in tomato leaf and demonstrated the contribution of alleles of wild tomato species to insect resistance due to increasing leaf fatty acid content (Blauth et al. 1999; Leckie et al. 2014).

In other work, Toubiana et al. (2012) detected 15 QTLs for pelargonic acid (C9:0) on chromosomes 2, 5, 8, 10, 11 and 12, and 6 QTLs for stearic acid (C18:0) on chromosomes 2, 3 and 4 using an IL population derived from *S. lycopersicum* and *S. pennellii* using SNP markers in tomato fruit. Schauer et al. (2006) identified 889 fruit metabolite loci including 16 QTLs for palmitic acid (C16:0) on chromosomes 2, 4, 5, 6, 7, 8, 9 and 10, 16 QTLs for steraic acid (C18:0) on chromosomes 2, 3, 4, 5, 6, 7, 8 and 10, and 6 QTLs for linoleic acid (C18:2) on chromosomes 5, 7 and 9 using an IL population derived from *S. lycopersicum* M82 and *S. pennellii.*

Another group of molecules important to our daily diet is vitamins. Vitamins are low molecular weight, organic molecules that are essential for human health to maintain homeostasis and diverse metabolic functions such as catalyzing biochemical reactions (Karazniewicz-Lada and Głowka, 2015). Vitamin deficiency and excess amounts of vitamins both cause health problems including anemia, kidney stones, cardiovascular

disease and even depression (Curhan et al. 1999; Fletcher and Fairfield 2002; Judd and Tangpricha 2008). Ascorbic acid (vitamin C), and B group vitamins, which are thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), pyridoxal (vitamin B6), folic acid (vitamin B9) and cyanocobalamin (vitamin B12) are water soluble. Retinol (vitamin A), tocopherol (vitamin E), radiostol (vitamin D) and antihemorrhagic vitamins (vitamin K) are fat soluble vitamins (Karazniewicz-Lada and Głowka, 2015). Moreover, humans cannot synthesize most vitamins, thus both fat soluble and water soluble vitamins must be obtained from our daily diet (Fitzpatricket et al. 2012).

Unfortunately there are limited studies that have examined the diversity of vitamin content and QTLs responsible for vitamins in tomato. Those that have been published focused on vitamin E and vitamim C (Almedia et al. 2011; Capel et al. 2015; Hanson et al. 2014; Ökmen et al. 2010; Schauer et al. 2016; Stevens et al. 2007). Here, we focused on both fat and water soluble vitamins. Vitamin E and vitamin C will be discussed in the next chapter as antioxidants. The IBL population and the parents were evaluated for 4 fat soluble vitamins (vitamin A, D3, E and K3), 6 water soluble vitamins (vitamin C, pantothenic acid, niacin, folic acid, vitamin B12 and riboflavin). Vitamin B12 and riboflavin were not detected in the parents or in the IBL population. The parents of the IBL population had low vitamin A and vitamin D3 content, but high vitamin K3, pantothenic acid, niacin and folic acid content. Most of the individuals of the IBL population had low vitamin A and vitamin D3 content; $\leq$ 2 mg/kg DW vitamin A and <4 mg/kg DW vitamin D3 Vitamin K3 content of the parents was higher than both vitamin A and D3, however, the IBL population had low vitamin K3 content which was not continuously distributed in the population.

The parents had extreme alleles for pantothenic acid, niacin and folic acid and the difference was about two fold between the parents for the traits. However, the IBL population had lower content of these vitamins. Niacin and folic acid had normal continuous distributions and segregated in the IBL population but pantothenic acid did not have a continuous distribution. This finding implies an unbalanced introgression of *S. pimpinellifolium* alleles for pantothenic acid content into the *S. lycopersicum* genome.

QTL studies for tomato vitamin content, except vitamin C and E, are not encountered in the literature. In this study, we identifed 21 QTLs including 3 for fat soluble vitamins and the rest for water soluble vitamins. Specifically, we identifed one QTL for vitamin A on chromosome 9 and two QTLs for vitamin K3 on chromosomes 4 and 12. Although Tueza did not contain vitamin D3 and *S. pimpinellifolium* had a low

amount (0.9 mg/kg DW), IBL population contained vitamin D3 up to 32 mg/kg DW. Although introgressions from *S. pimpinellifolium* increased the trait value of vitamin D3 in the genetic background of *S. lycopersicum*, no QTLs could be detected for vitamin D3.

Two QTLs for pantothenic acid were detected on chromosomes 3 and 6, and 16 QTLs for niacin on chromosomes 1, 2, 3, 8, 9 and 10. Although the parents had high allelic variation for folic acid and continuous distribution was observed in the IBL population, no QTL were detected for the trait.

Correlation analysis demonstrated that there was only one weak but significant positive correlation between nutritional compounds, pantothenic acid and niacin (r^2 = 0.105). This correlation may arise from a metabolic relationship between B group vitamins. Because metabolic and catabolic reactions both require interaction of B groups vitamins with each other. Specifically, niacin is synthesized from the essential amino acid L-tryptophan, and pyridoxal phosphate, which is the metabolic active form of pantothenic acid, acts as a coenzyme for the enzymes needed in tryptophan metabolism. Thus it is expected that while niacin concentration increases, pantothenic acid concentration should also increase because of being an intermediate metabolite in the niacin synthesis pathway (Egashira et al. 1996; Sauberlich 1980).

Colocalized QTLs were detected for nutritional compounds. Palmitoleic acid and niacin were colocalized at 48.6 Mb on chromosome 1. Oleic acid and linoleic acid QTLs were colocalized at 2.1, 4.1 and 6.1 Mb positions on chromosome 5. Colocalization of nutritional components was rare which demonstrated that genes controlling these traits probably are not linked.

Alleles for improvement of vitamin A, pantothenic acid and niacin alleles were provided by *S. pimpinellifolium*. Our study demonstrated that wild species of tomato can be used to improve nutritional value of the cultivated tomato for both fat and water soluble vitamins. Thus understanding the genetic control underlying these nutritional components is crucial to improve the dietary value of tomato.

CHAPTER 6

ANTIOXIDANT CHARACTERS IN TOMATO

6.1. INTRODUCTION

Metabolic processes are a necessity for the continuity of life. However, through these processes, molecules called reactive oxygen species (ROS) are produced. A primary source of ROS is incompletely processed oxygen or electrons at ETS in mitochondria (Elekofehinti et al. 2013). ROS are harmful to the cell since they are highly reactive due to their free radical groups. They disrupt the chemical bonds of any molecule close to them. So, if not neutralized, proteins, lipids and DNA molecules can be damaged by ROS which can cause various diseases such as cancer, neurodegenerative or cardiovascular diseases (Valko et al. 2006; Djordjevic 2004; Willcox et al. 2004). Also, because the liver is a recycling center for ROS, liver diseases are thought to be initiated primarily due to high ROS concentrations (Morisco et al. 2008). For this reason, ROS are neutralized or recycled immediately after they are produced. This function is generally performed by antioxidants, molecules that are capable of neutralizing the harmful effects of ROS (Akanitapichat et al. 2010). This neutralization involves a delicate balance between ROS and antioxidant molecules.

Glutathione, vitamin C and E, carotenoids and phenolic acids are antioxidant molecules (Devasagaym et al. 2004, Raiola et al. 2014; Sacco et al. 2013). Tomato is a good source for antioxidant molecules, especially for carotenoids. Regular consumption of tomato can reduce the risk of chronic disease, cardiovascular disease, different types of cancer, and inflammation due to interaction of tomato phytochemicals with metabolic pathways which are related to the inflammatory response and oxidative stress (Raiola et al. 2014).

Understanding the genetic basis of antioxidants in tomato provides powerful tools for breeders to develop new cultivars rich in antioxidant molecules. Moreover, antioxidant-based drugs or formulations can be developed, and antioxidant rich varieties can be used as a source of phytochemicals for drugs. Clinical trials are also performed with antioxidant-based drugs, such as deprenyl and tocopherol, for therapy of Parkinson's

disease (Devasagaym et al. 2004). Therefore, antioxidant molecules are important for both prevention and treatment of diseases.

6.2. MATERIALS AND METHODS

6.2.1. Plant material

The IBL population described in Chapter 2 was used for metabolic analysis.

6.2.2. Metabolic Profiling and Sample Preparation

Samples were prepared as mentioned in Chapter 4.

6.2.3. Quantification of Metabolites

Reduced and oxidized glutthione were analysed with an isocritic method of HPLC-PDA which modified from the method mentioned in Khan et al. (2011). Choloroform: methanol: water (1:3:1, v:v:v) extracts of tomato was used. Glutathiones were analyzed on reverse phase (RP C18, 3 µm – 10 x 2,1 mm) column at 35 °C using trifluoro acetic acid (aq): methanol (97:3, v:v) as the mobile phase with a flow rate 0.2 ml/min. Sample injection was 20 µl and standard solutions were prepared in 0,05 % trifluoro acetic acid (aq). Detection was done at 208 nm by PDA detector.

HPLC- PDA/FLD method:

Column: RP-C18 (3 µm – 10 x 2,1 mm) Column Temperature: 35 °C

Flow rate: 0,2 ml/min

Injection volume: 20 µl

PDA wavelength: 208 nm

Solvent: 0,05 % trifluoro acetic acid(aq)

Mobile phase: 97 % trifluoro acetic acid(aq) – 3 % Methanol

Vitamin C was analysed with a isocritic method of HPLC-PDA which combined and modified from the methods mentioned in Li and Chen (2001a, 2001b). Choloroform: methanol: water (1:3:1, v:v:v) extracts of tomato were used. Vitamin C was analyzed on

reverse phase (RP C18, 5 µm – 25 x 4,6 mm) column at 40 °C using methanol:potassium dihydrogenphosphate buffer (KH_2PO_4, 0.1M, pH=7) (10:90, v:v) as the mobile phase with a flow rate 1 ml/min. Sample injection was 20 µl and standard solutions were prepared in water. Detection was done at 265 nm with PDA detector.

Summary of method parameters is shown:

HPLC- PDA method:

Column: RP-C18 (5 µm – 10 x 2,1 mm) Column Temperature: 40 °C

Flow rate: 1 ml/min

Injection volume: 20 µl

PDA wavelength: 265 nm

Solvent: Water

Mobile phase: Methanol : 0,1 M KH_2PO_4, pH=7 – 10:90, v:v

Vitamin E was analysed with a isocritic method of HPLC-FLD which combined and modified the methods mentioned in Bakre et al. (2015) and Turner and Burner (2012). Dichloromethane:hexane (1:1, v/v) extracts of tomato were used. Vitamin E was analyzed on reverse phase (RP C18, 5 µm – 25 x 4,6 mm) column at 40 °C using acetonitrile:methanol (75:25, v:v) as the mobile phase with a flow rate 1.5 ml/min. Sample injection was 20 µl and standard solutions were prepared in acetonitrile: methanol. Vitamin E was detected on fluorescence detector at 300 nm excitation and 360 nm emission.

Summary of method parameters is below:

HPLC- PDA/FLD method:

Column: RP-C18 (5 µm – 25 x 4,6 mm) Column Temperature: 40 °C

Flow rate: 1,5 ml/min

Injection volume: 20 µl

FLD: excitation – 300 nm, emission – 360 nm

Solvent: Acetonitril: methanol (80:20, v:v)

Mobile phase: Acetonitrile:Methanol – 75:25, v:v

Lycopene and β-carotene were analysed with an isocritic method of HPLC-PDA which was combined and modified from the methods mentioned in Ishida, Ma and Chan (2001) and Serino et al. (2009). Dichloromethane:hexane (1:1, v/v) extracts of tomato were used. Carotenoids were analyzed on reverse phase (RP C18, 5 µm – 25 x 4,6 mm)

column at 30 °C using methanol:ethyl acetate:acetonitrile (50:40:10, v:v:v, and 0,05 % triethylamine was added to ethyl acetate and acetonitrile) as the mobile phase with a flow rate 1.5 ml/min. Sample injection was 20 µl and standard solutions of lycopene were prepared in methanol:acetone (1:1, v:v) and standard solutions of β-carotene were prepared in dichloromethane. Detection was done at 450 and 469 nm by PDA detector.

Summary of method parameters is below:

HPLC- PDA method:

Column: RP-C18 (5 µm – 25 x 4,6 mm) Column Temperature: 30 °C

Flow rate: 1,5 ml/min

Injeciton volume: 20 µl

PDA wavelength: 450 and 469 nm

Solvent: β-carotene was dissolved in dichloromethane, lycopene was dissolved in methanol:acetone (1:1, v:v).

Mobile phase: Methanol :Ethyl Acetate:Acetonitrile – 50:40:10, v:v:v – 0,05 triethylamine was added to ethyl acetate and acetonitrile.

Lutein and zeaxanthin were analysed with an isocritic method of HPLC-PDA which was combined and modified from the methods mentioned in Ishida, Ma and Chan (2001) and Serino et al. (2009). Dichloromethane:hexane (1:1, v/v) extracts of tomato were used. Carotenoids were analyzed on reverse phase (RP C18, 5 µm – 25 x 4,6 mm) column at 30 °C using acetonitrile:methanol (10:90, v:v and 0,05 % triethylamine was added to acetonitrile) as the mobile phase with a flow rate 1 ml/min. Sample injection was 20 µl and standard solutions of carotenoids were prepared in dichloromethane containing 0,01% BHT. Detection was done at 475 nm by PDA detector.

Summary of method parameters is below:

HPLC- PDA method:

Column: RP-C18 (5 µm – 25 x 4,6 mm)

Column Temperature: 30 °C

Flow rate: 1 ml/min

Injection volume: 20 µl

PDA wavelength: 475 nm

Solvent: Dichloromethane containing 0,01% BHT

Mobile phase: Acetonitrile:Methanol (10:90, v:v, and 0.05% triethylamine was added to acetonitrile)

Phenolic acids were analyzed with a gradient method of HPLC- PDA which was modified from the method mentioned in Gomez-Alonso and Hermosin-Gutierrez (2007). Choloroform: methanol: water (1:3:1, v:v:v) extracts of tomato were used. Phenolic acids were analyzed on reverse phase (RP C18, 5 µm – 25 x 4,6 mm) column at 35 °C using (A) ammonium dihydrogen phosphate buffer ($NH_4H_2PO_4$, 50 mm, pH=2.6), (B) %20 mobile phase A and %80 acetonitrile, (C) 200 mM phosphoric acid as the mobile phase with a flow rate 1 ml/min. Sample injection was 20 µl and standard solutions were prepared in methanol. Detection was done at 280, 320, 360, 520 nm with PDA detector.

Summary of method parameters is below:

HPLC- PDA method:

Column: RP-C18 (5 µm – 25 x 4,6 mm) Column Temperature: 35 °C

Injection volume: 20 µl

PDA wavelength: 280, 320, 360, 520 nm

Solvent: Methanol

Mobile phase: (A) 50 mM $NH_4H_2PO_4$, PH=2.6

(B) %20 mobile phase A and %80 Acetonitrile

(C) 200 mM H_3PO_4

Gradient Programme:

Time (min)	**Flow rate (ml/dk)**	**Mobile Phase A %**	**Mobile Phase B %**	**Mobile Phase C %**
0	1	100	0	0
2	1	100	0	0
5	1	92	8	0
17	1	0	14	86
22	1	0	18	82
29.5	1	0	21	70
55	1	0	33	67
70	1	0	50	50
75	1	0	50	50
78	1	20	80	0
81	1	20	80	0
86	1	100	0	0

6.2.4. QTL mapping

QTL mapping was performed as described in Chapter 3 using data obtained from GBS as explained in Chapter 2.

6.3. Results

6.3.1. Metabolite Variation

6.3.1.1. Gutathione

The parents of the IBL population had high reduced gutathione content (especially the parent Tueza) and low oxidized gutathione content (Table 26). Both traits segregated in the IBL population. Glutathiones displayed variation in the population with coefficients of variation (CV) ranging from 113.4 % to 205.9 % Glutathiones did not display continuous distributions within the population (Figure 16).

Table 26. Statistics for glutathione contents measured in IBL population and parents; *S. lycopersicum* cv. Tueza and *S. pimpinellifolium* cv. LA1589. Quanitities of metabolites are given as mg/100g DW.

	Parents		**IBL Population**		
	Tueza	**LA1589**	**Mean**	**Range**	**CV%**
Reduced Glutathione	17.75	10.79	52.17	7.57-322.38	113.4
Oxidized Glutathione	5.18	0.09	71.43	0-807.41	205.9

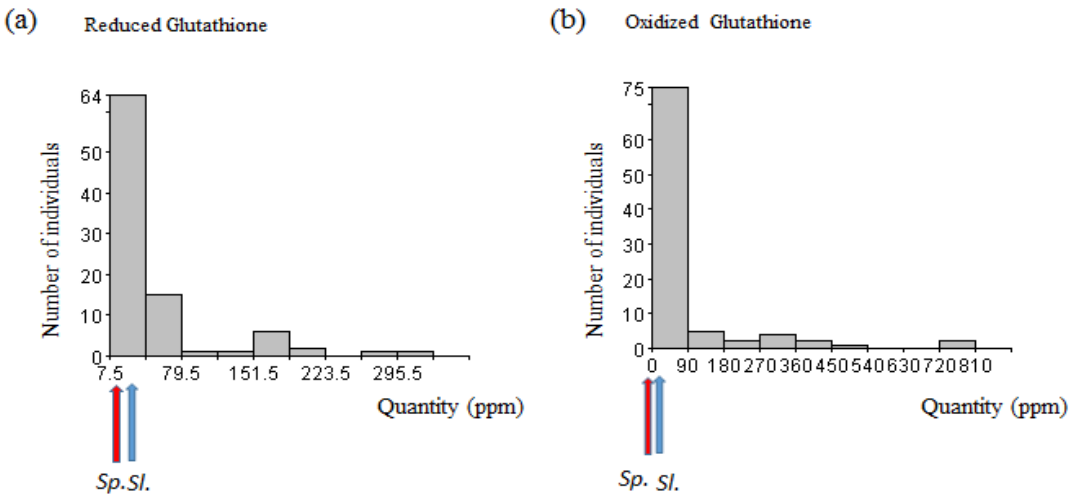

Figure 16. Distribution of glutathione content in IBL population. (a) Distribution of Reduced glutathione content (ppm) in IBL population, (b) Distribution of Oxidized glutathione content (ppm) in IBL population. Arrows indicate means for Sl.: *S. Lycopersicum* cv. Tueza and Sp.: *S. pimpinellifolium* cv. LA1589.

6.3.1.2. Vitamins

The parents of the IBL population had high vitamin C and vitamin E content (Table 27). Both of the traits segregated in the IBL population with CV of 65.2 % for vitamin C and 91.2 % for vitamin E. Traits had continuous distributions within the population (Figure 17).

Table 27. Statistics for vitamin C and vitamin E contents measured in IBL population and parents; *S. lycopersicum* cv. Tueza and *S. pimpinellifolium* cv. LA1589. Quantities of metabolites were explained as mg/100g DW.

	Parents		**IBL Population**		
	Tueza	**LA1589**	**Mean**	**Range**	**CV%**
Vitamin C	19.11	20.17	19.06	0-91.17	65.2
Vitamin E	3.61	20.28	21.53	0-123.87	91.2

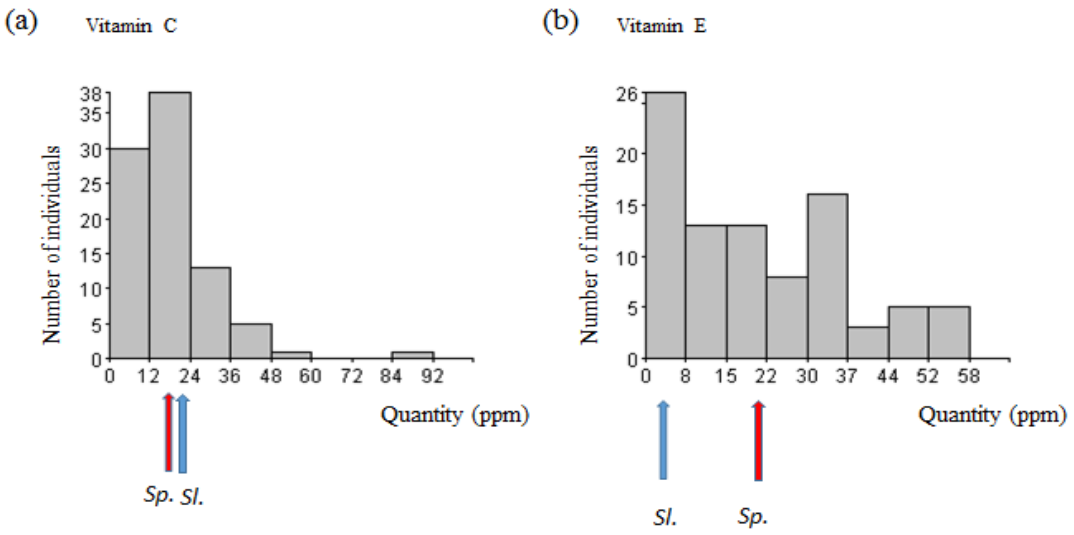

Figure 17. Distribution of vitamin C and vitamin E content in IBL population. (a) Distribution of Vitamin C content (ppm) in IBL population, (b) Distribution of Vitamin E content (ppm) in IBL population. Arrows indicate means for Sl.: *S. Lycopersicum* cv. Tueza and Sp.: *S. pimpinellifolium* cv. LA1589.

6.3.1.3. Carotenoids

The parents of the IBL population had high lycopene and β-carotene, but not lutein and zeaxanthin (Table 28). All of the traits segregated in the IBL population, except lycopene. Carotenoids displayed variation in the population with CV ranging from 15.0 % to 45.8 % Carotenoids displayed good continuous distributions within the population except lycopene (Figure 18). Lycopene content was high in many of the individuals of the IBL population.

Table 28. Statistics for carotenoid contents measured in IBL population and parents; *S. lycopersicum* cv. Tueza and *S. pimpinellifolium* cv. LA1589. Quanitities of metabolites are given as mg/100g DW.

	Parents		**IBL Population**		
	Tueza	**LA1589**	**Mean**	**Range**	**CV%**
Lutein	3.25	5.06	3.83	0.04-7.95	32.4
Zeaxanthin	3.26	2.78	3.60	0.47-9.83	45.8
Lycopene	16141.58	26733.95	16605.08	1474.30-18518.74	15.0
β-Carotene	56.62	36.06	45.98	3.86-88.52	38.8

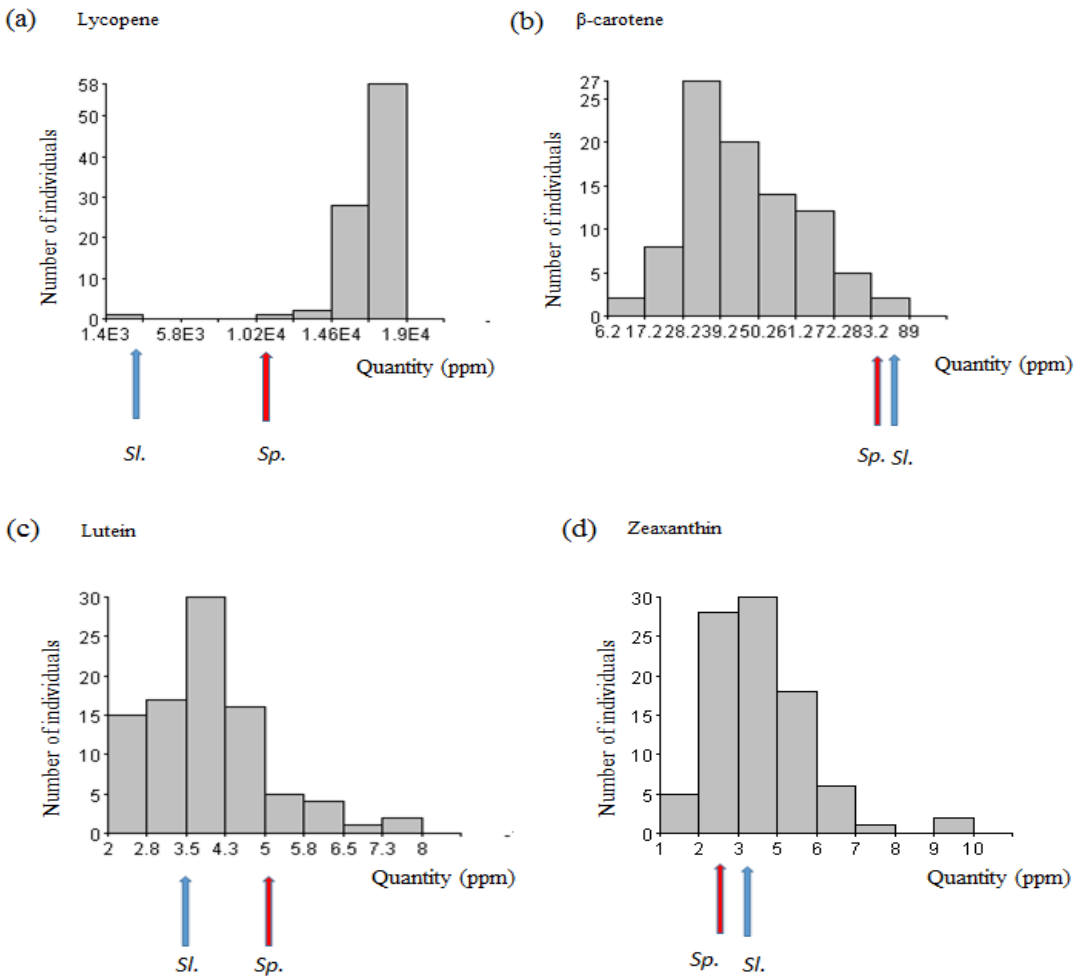

Figure 18. Distribution of carotenoid content in IBL population. (a) Distribution of Lycopene content (ppm) in IBL population, (b) Distribution of β-carotene content (ppm) in IBL population, (c) Distribution of Lutein content (ppm) in IBL population, (d) Distribution of Zeaxanthin content (ppm) in IBL population. Arrows indicate means for Sl.: *S. Lycopersicum* cv. Tueza and Sp.: *S. pimpinellifolium* cv. LA1589.

6.3.1.4. Phenolic Acids

The IBL population and the parents were evaluated for 33 phenolic acids, including flavanols (quercetin, myricetin, syringetin, kaempferol, isorhamnetin), flavones (apigenin, luteolin, chrysin), flavanones (naringenin, taxifolin), flavonol-3-ol (catechin, epicatechin, epigallocatechin), anthocyanins and anthocyanidines (cyanidine, delphinidine, malvidin, pelargonidin, peonidin), hydroxybenzoic acids (3-hydroxy benzoic acid (3-OHBA), 4-hydroxy benzoic acid (4-OHBA), gallic acid, vanillic acid,

syringic acid, salicylic acid), hydroxycinnamic acids (cinnamic acid, coumaric acid, ferulic acid, caffeic acid, sinapic acid, cafteric acid, chlorogenic acid) and stilbenes (resveratrol, pterostilbene). Syringetin, kaempferol, isorhamnetin, naringenin, taxifolin, cyanidine, delphinidine, pelargonidin, peonidin, cafteric acid, resveratrol, pterostilbene could not be detected both in parents and IBL population.

Tueza had high vanillic acid, 4-OHBA, gallic acid, chlorogenic acid, and caffeic acid content while LA1589 had high syringic acid, epicatechin, chrysin, 4-OHBA, myricetin, coumaric acis, and ferulic acid content (Table 29). All of the traits segregated in the IBL population except syringic acid, quercetin and luteolin (Figure 19). Phenolic acids displayed variation in the population with CV ranging from 0.1 % to 29.7 %. Traits did not display normal continuous variation, with the exceptions of gallic acid and chlorogenic acid.

Table 29. Statistics for phenolic acid contents measured in IBL population and parents; *S. lycopersicum* cv. Tueza and *S. pimpinellifolium* cv. LA1589. Quanitities of metabolites are given as mg/100g DW.

	Parents		**IBL Population**		
	Tueza	**LA1589**	**Mean**	**Range**	**CV%**
Hydroxy benzoic acid	6.6	1.37	46.80	0 - 275.41	4.8
Catechin	0.37	0.59	26.53	0 - 249.5	0.6
Vanillic acid	12.8	2.05	72.52	0 - 1132.58	6.2
Syringic acid	3.96	20.4	282.70	0 - 8408.33	0.2
Epigallocatechin	1.92	2.19	2.61	0 - 24.74	0.9
Epicatechin	0.24	5.89	2.45	0 - 44.02	0.04
Chrysin	0.42	2.07	80.12	0 - 888.34	0.2
4-OHBA	8.14	19.3	22.70	0 - 217.91	0.4
Myricitin	10	40	3.37	0 - 32.62	0.2
Quercetin	1.88	1.67	1.25	0 - 22.6	1.1
Gallic acid	31.46	1.06	5.07	0 - 26.33	29.7
Cinnamic acid	0.06	0.11	0.98	0 - 8.2	0.6
Chlorogenic acid	19.2	0.83	0.73	0 - 18.52	23.1
Caffeic acid	9.54	3.32	4.55	0.1 - 56.9	2.9
Coumaric acid	0.24	2.13	1.87	0 - 21.8	0.1
Ferulic acid	2.08	31	3.29	0 - 28.16	0.1
Sinapic acid	1.47	1.29	1.94	0 - 20.42	1.1
Malvidin	0.76	0.75	3.48	0 - 40.56	1.0
Salicylic acid	1.65	2.75	4.63	0 - 53.95	0.6
Apigenin	2.18	1.24	13.04	0.26 - 72.1	1.8
Luteolin	0.62	0.2	0.83	0 - 11.71	3.1

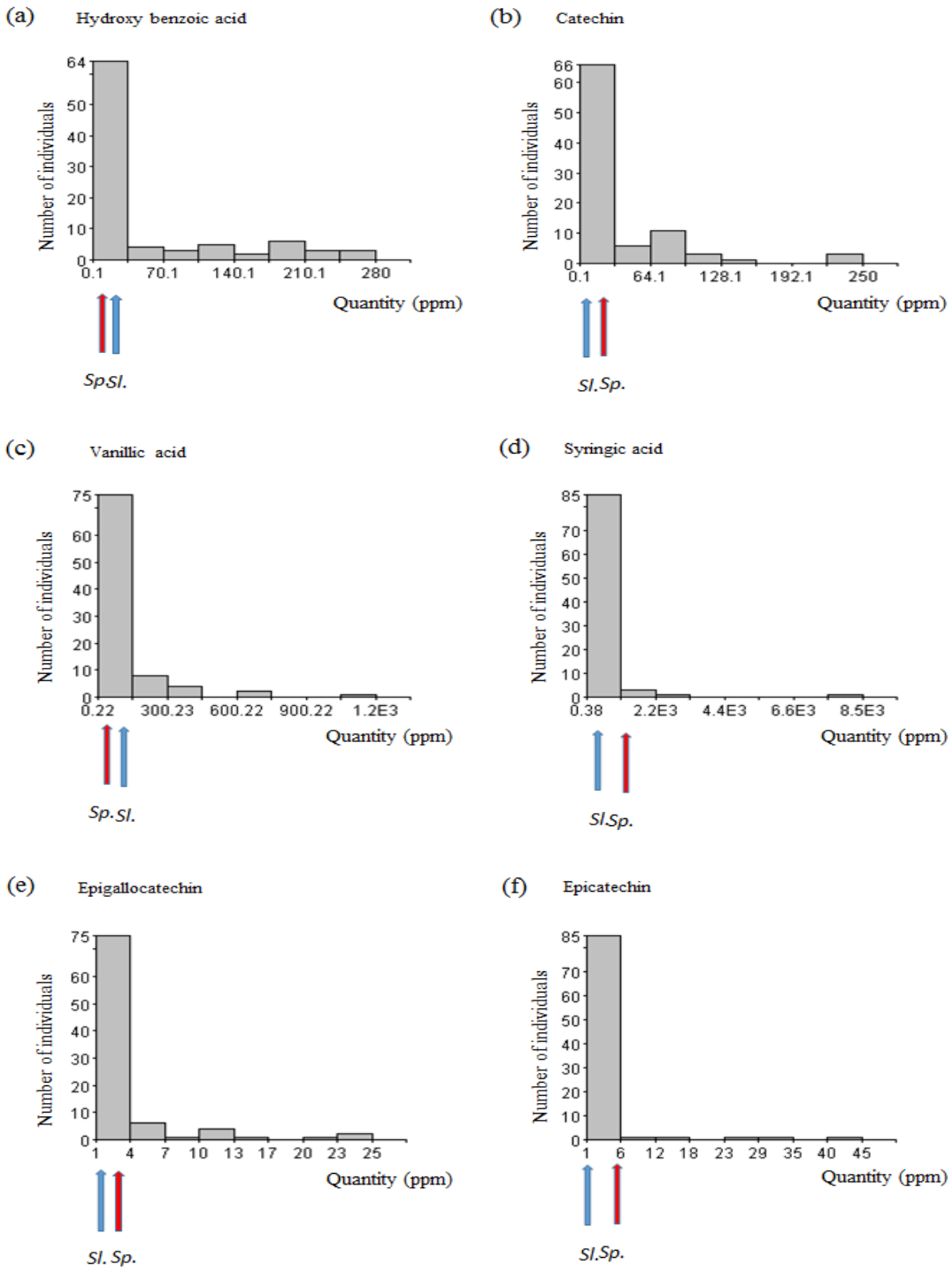

Figure 19. Distribution of phenolic acid content in IBL population. (a) Distribution of Hydroxy benzoic acid content (ppm) in IBL population, (b) Distribution of Catechin content (ppm) in IBL population, (c) Distribution of Vanillic acid content (ppm) in IBL population, (d) Distribution of Syringic acid content (ppm) in IBL population, (e) Distribution of Epigallocatechin content (ppm) in IBL population, (f) Distribution of Epicatechin content (ppm) in IBL population. Arrows indicate means for Sl.: *S. Lycopersicum* cv. Tueza and Sp.: *S. pimpinellifolium* cv. LA1589.

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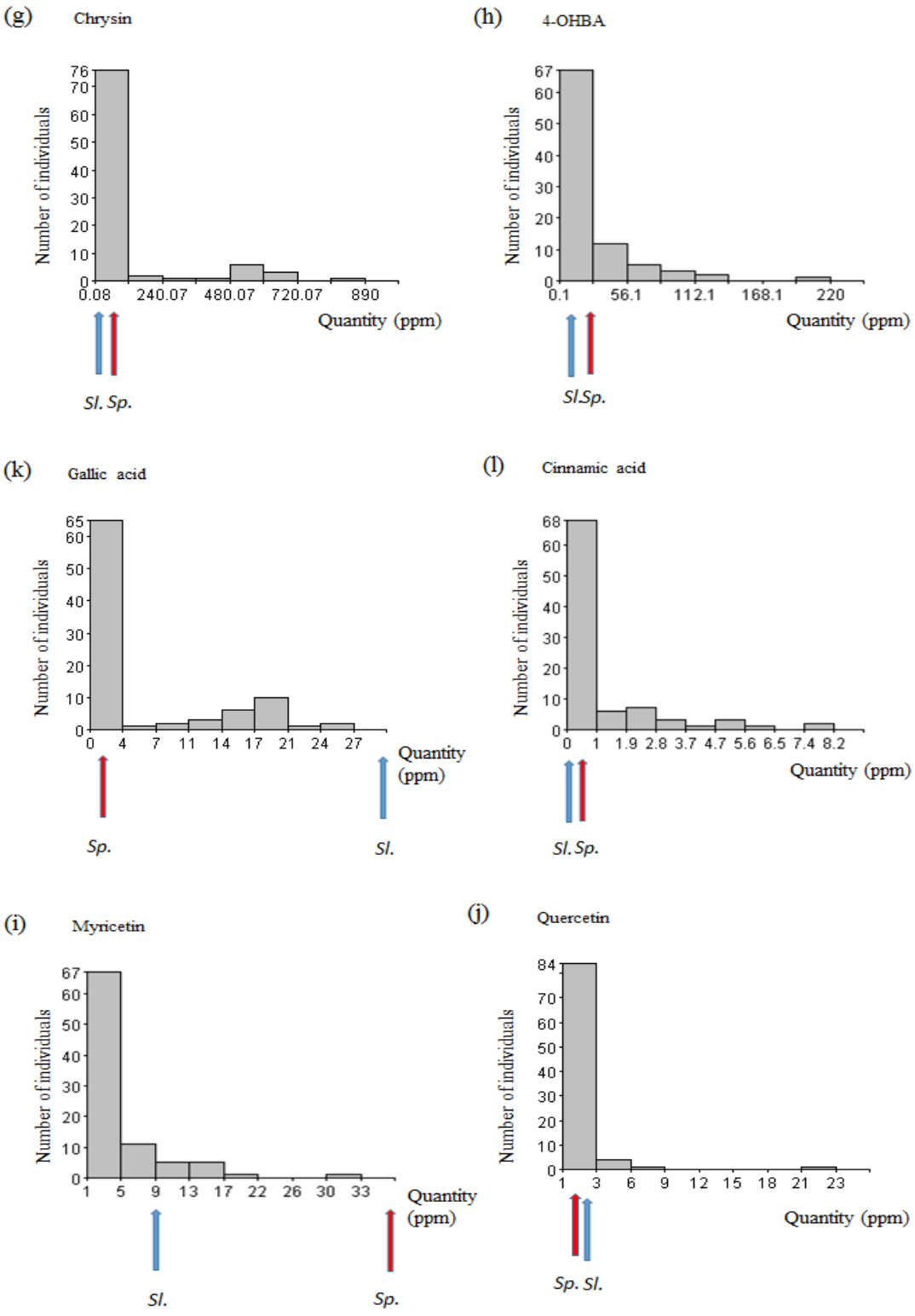

Figure 19. **(cont.)** (g) Distribution of Chrysin content (ppm) in IBL population, (h) Distribution of 4-OHBA content (ppm) in IBL population, (i) Distribution of Myricetin content (ppm) in IBL population, (j) Distribution of Quercetin content (ppm) in IBL population, (k) Distribution of Gallic acid content (ppm) in IBL population, (l) Distribution of Cinnamic acid content (ppm) in IBL population.

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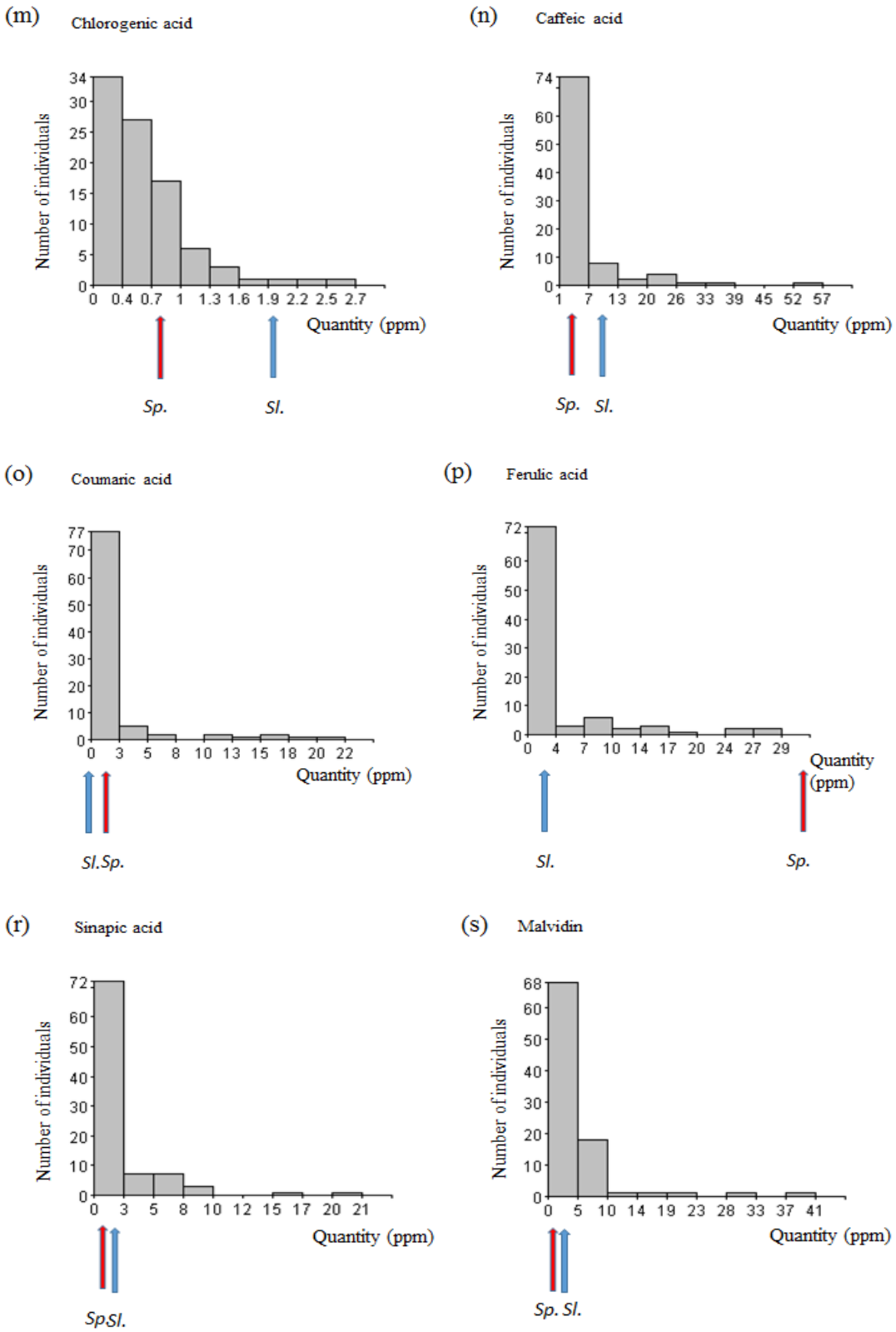

Figure 19. **(cont.)** (m) Distribution of Chlorogenic acid content (ppm) in IBL population, (n) Distribution of Caffeic acid content (ppm) in IBL population, (o) Distribution of Coumaric acid content (ppm) in IBL population, (p) Distribution of Ferulic acid content (ppm) in IBL population, (r) Distribution of Sinapic acid content (ppm) in IBL population, (s) Distribution of Malvidin content (ppm) in IBL population.

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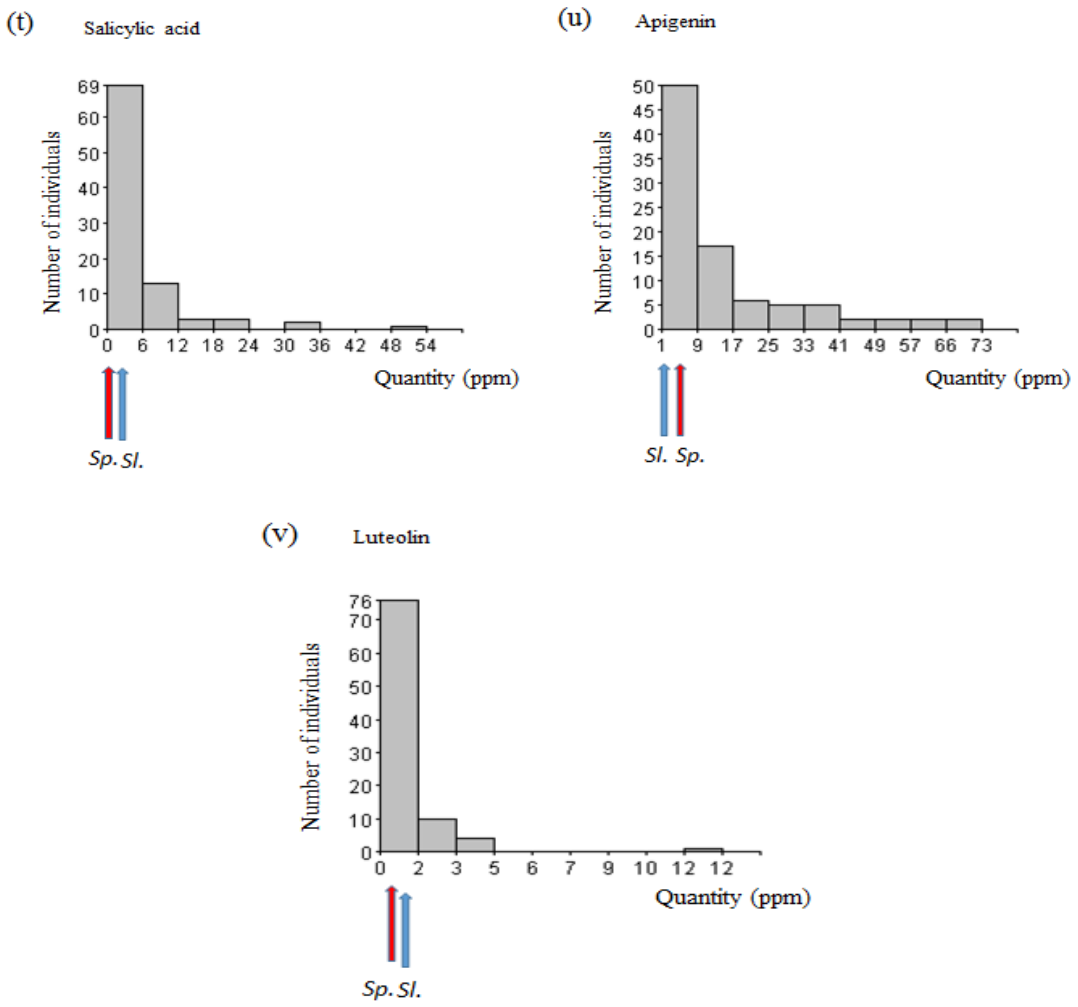

Figure 19. **(cont.)** (t) Distribution of Salicyclic acid content (ppm) in IBL population, (u) Distribution of Apigenin content (ppm) in IBL population, (v) Distribution of Luteolin content (ppm) in IBL population.

6.3.2. QTL Mapping

6.3.2.1. Glutathione

A total of 3 QTLs were identified for glutathione based on LOD thresholds of 3. For the oxidized glutathione content, only one minor QTL was identified on chromosome 6 with PVE of 15%. For reduced glutathione, one QTL was identified on chromosome 6 with moderate effect with PVE of 21%, and one minor QTL was identified on chromosome 7 with the percentage of PVE 16% (Table 30).

Table 30**.** QTLs identified for glutathione.

Trait	QTL	Chr	Position (Mb)	Marker Interval	LOD*	PVE**	Additive Effect***
Reduced Glutathione	*rg6.1*	T6	36.8	S6_24889074 - S6_42876082	4.7	0.21	*S. lycopersicum*
Reduced Glutathione	*rg7.1*	T7	0.2	S7_250810 - S7_2225863	3.4	0.16	*S. lycopersicum*
Oxidized Glutathione	*og6.1*	T6	38.8	S6_24889074 - S6_42876082	3.2	0.15	*S. lycopersicum*

* Peak position of QTL.
** Percentage of phenotypic variation explained by identified QTL.
*** Parental allele associated with increased trait value.

6.3.2.2. Vitamins

For vitamin C content, only one minor QTL was identified on chromosome 10 with the PVE 17% (Table 31). For vitamin E, no QTL were detected.

Table 31. QTL identified for vitamin C.

Trait	QTL	Chr	Position (Mb)	Marker Interval	LOD*	PVE**	Additive Effect***
Vitamin C	*vc10.1*	T10	54	S10_54018912 - S10_62061848	3.5	0.17	*S. pimpinellifolium*

* Peak position of QTL.
** Percentage of phenotypic variation explained by identified QTL.
*** Parental allele associated with increased trait value.

6.3.2.3. Carotenoids

A total of 5 QTLs were identified for carotenoids based on LOD thresholds of 3. For lycopene content, one major QTL was identified on chromosomes 8 with PVE 37%. Another QTL was identified on chromosome 8 with moderate effect with PVE 20%, and one minor QTL were identified on chromosomes 2 with PVE 17%. For β-carotene, 2 minor QTL were identified on chromosome T1 with PVE 14% (Table 32).

Table 32. QTLs identified for carotenoids.

Trait	QTL	Chr	Position (Mb)	Marker Interval	LOD*	PVE**	Additive Effect***
Lycopene	*ly2.1*	T2	42.2	S2_42269471 - S2_48218829	3.7	0.17	*S. lycopersicum*
Lycopene	*ly8.1*	T8	60	S8_60041028	8.9	0.37	*S. lycopersicum*
Lycopene	*ly8.2*		62	S8_60041028	4.5	0.2	*S. pimpinellifolium*
β-Carotene	*βc1.1*	T1	70.6	S1_70630742 - S1_72610586	3.08	0.14	*S. lycopersicum*
β-Carotene	*βc1.2*	T1	82.6	S1_80687203 - S1_86630982	3.05	0.14	*S. lycopersicum*

* Peak position of QTL.
** Percentage of phenotypic variation explained by identified QTL.
*** Parental allele associated with increased trait value.

6.3.2.4. Phenolic Acids

A total of 22 QTLs were identified for phenolic acids based on LOD thresholds of 3. Two minor QTL were detected on chromosomes 11 and 12 for vanillic acid with PVE 14%. Two major QTLs on chromosome 6 with PVE 36% and 63%, and two minor QTLs were detected for syringic acid. Four minor QTLs were detected on chromosome 7 for epicatechin with PVE ranging from 14% to 17%. For quercetin, two minor QTLs were detected on chromosome 12. One QTL with moderate effect on chromosome 6 with PVE 23%, and one minor QTL on chromosome 4 were detected for cinnamic acid. For coumaric acid, two QTLs with moderate effects were found on chromosomes 4 and 12 with PVE 21% and 20%, respectively. In addition, 3 minor QTLs were found on chromosomes 1 and 12 with PVE ranging from 14% to 18%. One minor QTL was detected on chromosome 6 for sinapic acid. Malvidin content was associated with two QTLs: a QTL with moderate effect on chromosome 11 and a major QTL on chromosome 6 with PVE 34% (Table 33).

Table 33**.** QTLs identified for phenolic acids.

Traits	QTL	Chr	Position (Mb)	Marker Interval	LOD *	PVE**	Additive Effect***
Vanillic Acid	*va11.1*	11	34.3	S11_356158	3.108	0.14	*S. lycopersicum*
	va12.1	12	14.1	S12_179179 - S12_24152718	3.09	0.14	*S. lycopersicum*
Syringic Acid	*sya6.1*	6	0.8	6819852 - S6_12894552	3.603	0.16	*S. lycopersicum*
	sya6.2	6	8.8	S6_819852 - S6_12894552	3.599	0.16	*S. lycopersicum*
			10.8		3.482	0.63	
			12.8		3.596	0.16	
	sya6.3	6	18.8	S6_18869825 - S&_24889074	3.506	0.16	*S. lycopersicum*
	sya6.4	6	28.8	S6_24889074 - S6_ 42876082	8.837	0.36	*S. lycopersicum*
			34.8		6.314	0.27	
Epicatechin	*ep7.1*	7	2.2	S7_2225863 - 32224294	3.391	0.15	*S. lycopersicum*
			8.2		3.075	0.14	
	ep7.2	7	26.2	S7_2225863 - 32224294	3.099	0.14	*S. lycopersicum*
			34.2	S7_32224294 - S7_58280573	3.639	0.17	
			36.2		3.030	0.14	
	ep7.3	7	52.2	S7_32224294 - S7_58280573	3.291	0.15	*S. lycopersicum*
	ep7.4	7	60.2	S7_58280573 - S7_62210562	3.140	0.14	*S. lycopersicum*
Quercetin	*qn12.1*	12	38.1	S12_26140976	3.222	0.15	*S. lycopersicum*
	qn12.2	12	58.1	S12_26140976	3.667	0.17	*S. lycopersicum*
Cinnamic Acid	*cna4.1*	4	44.5	S4_32558453 - S4_46515927	3.063	0.14	*S. lycopersicum*
	cna6.1	6	44.8	S6_42876082 - S6_ 46815418	5.352	0.23	*S. lycopersicum*
Coumaric Acid	*coa1.1*	1	26.6	S1_663411 - S1_28625641	3.389	0.15	*S. lycopersicum*
	coa1.2	1	40.6	S1_32640248 - S1 56607342	3.125	0.14	*S. lycopersicum*
			42.6		3.394	0.15	
			46.6		3.130	0.14	
	coa12.1	12	4.1	S12_179179 - S12_24152718	3.320	0.15	*S. lycopersicum*
			8.1		4.486	0.20	
	coa12.2	12	18.1	S12_179179 - S12_24152718	3.007	0.14	*S. lycopersicum*
			20.1		4.085	0.18	
	coa4.1	4	58.5	S4_58546318 - S4_62544061	4.696	0.21	*S. lycopersicum*
Sinapic Acid	*spa6.1*	6	38.8	S6_24889074 - S6_ 42876082	3.886	0.18	*S. lycopersicum*

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Table 33. **(cont.)**

Traits	QTL	Chr	Position (Mb)	Marker Interval	LOD *	PVE**	Additive Effect***
Malvidin	*mn11.1*	11	2.3	S11_356158	4.813	0.21	*S. lycopersicum*
	mn6.1	6	40.8	S6_24889074 - S6_42876082	3.500	0.16	*S. lycopersicum*
			42.8	S6_24889074 - S6_42876082	3.169	0.14	*S. lycopersicum*
			44.8		8.436	0.34	*S. lycopersicum*

* Peak position of QTL.
** Percentage of phenotypic variation explained by identified QTL.
*** Parental allele associated with increased trait value.

6.3.3. Correlated and Colocalized Traits

There were a few significant correlations between the antioxidant traits, many of the correlations were weak. There were significant positive correlation between oxidized glutathione and vitamin C (r^2 = 0.109), but a negative correlation between oxidized glutathione and vitamin E (r^2 = -0.026). Vitamin C and vitamin E were positively correlated (r^2 = 0.047). Moreover, vitamin E and β-carotene were positively correlated (r^2 = 0.015) (Table 34).

Table 34. Significant ($P < 0.05$) correlations between tomato fruit traits. Correlations with P value > 0.05 were considered to be non-significant (NS). OXG = Oxidized glutathione, RDG = Reduced glutathione, VitC = Vitamin C, VitE = Vitamin E, LYC = Lycopene, β-CR = β-carotene, LUT =Lutein, ZXN = Zeaxanthin

Traits	OXG	RDG	VitC	VitE	LYC	β-CR	LUT	ZXN
OXG	1	NS	0.109	-0.026	NS	NS	NS	NS
RDG		1	NS	NS	NS	NS	NS	NS
VitC			1	0.047	NS	NS	NS	NS
VitE				1	NS	0.015	NS	NS
LYC					1	NS	NS	NS
β-CR						1	NS	NS
LUT							1	NS
ZXN								1

Phenolic acids were not included into the correlation table, because no significant correlations were detected between phenolic acids or between phenolic acids and other antioxidant molecules.

Colocalized QTLs were detected for antioxidant molecules. Cinnamic acid and malvidin were colocalized at 44.8 Mb position on chromosome 6. Sinapic acid and oxidized glutathione were colocalized at 38.8 Mb position on chromosome 6 (Table 35).

Table 35. Fruit quality QTLs that colocalized.

Trait	QTL	Chr.	Position
Cinnamic acid		T6	44.8
Malvidin		T6	40.8,42,8,44.8
Sinapic Acid		T6	38.8
Oxidized Glutathione		T6	38.8

6.4. DISCUSSION

Fruits and vegetables are important for the human diet because they are good sources of antioxidants and minerals. Antioxidants can reduce the risk of developing an illness or prevent diseases like cancer or cardiovascular disease. Vegetable crop breeding strategies have begun to select and develop higher nutritional quality vegetables for their health benefits (Raigo´n et al. 2008). Tomato is an important contributor to human health due to its antioxidant content (Almeida et al. 2011). Vitamin C (ascorbic acid), vitamin E (tocopherol), carotenoids and phenolics are the main antioxidant molecules in tomato (Abushita et al. 1997, Frusciante et al. 2007). Glutathione is also a powerful antioxidant molecule but is an ignored molecule in breeding studies in tomato. Vitamin C, vitamin E and glutathione are linked by oxidation-reduction reactions (Lovat et al. 2016; Winkler, Olrselli, and Rex 1994).

Unfortunatly, there are limited studies on antioxidant capacity and antioxidant-related traits in tomato and these have focused on vitamin C, vitamin E, carotenoids and phenolic acids but not glutathione. The existing studies evalutated changes in glutathione content under stress conditions, especially salinity stress (Khan et al. 2017; Sang et al. 2016; Sun et al. 2010; Yan et al. 2016; Zhang et al. 2016; Zhu et al. 2016). In the present study, the IBL population and parental genotypes were evaluated for both reduced and

oxidized glutathione in order to identify related QTLs. *S. lycopersium* had higher levels of both oxidized and reduce glutathione than the other parent *S. pimpinellifolium*. Also most of the individuals had lower levels of reduced and oxidized glutathione, and normal continuous distribution was not observed in the IBL population. Two QTLs for reduced glutathione on chromosomes 6 and 7 and 1 QTL for oxidized glutathione on chromosome 6 were identified with alleles from *S. lycopersicum* associated with increased glutathione content.

Another group of important antioxidants is vitamins, especially vitamin C and vitamin E. The IBL population and parents were evaluted for these two vitamins. We focused on the α isoform of vitamin E, because this isoform is used to estimate the current Recommended Dietary Allowance (RDA) for vitamin E (IOM 2000). Parents of the IBL population had extreme alleles for vitamin E but not for vitamin C. On the other hand, normal continuous distribution in the IBL population was observed for both vitamins. One QTL was detected on chromosome 10 for vitamin C and the allele from *S. pimpinellifolium* was associated with increased vitamin content. This is a new QTL compared to the literature. No QTLs were detected for vitamin E content.

There are a few studies to identify QTLs responsible for vitamin C and E. In these studies mostly IL populations derived from *S. pennellii* introgressions into *S. lycopersicum* genome were used. Rousseaux et al. (2005) identified 1 QTL for vitamin C on chromosome 12 using an IL population. Capel et al. (2015) identified 4 QTLs on chromosomes 1, 3, 7 and 8 for vitamin C using a RIL population derived from *S. lycopersicum cv*. Moneymaker and *S. pimpinellifolium* with SNP, SSR and indel markers. Stevens et al. (2007) used three populations: (i) an IL population derived from *S. lycopersicum* M82 and *S. pennellii*, (ii) an advanced backcross population derived from *S. lycopersicum* and *S. habrochaites*, and (iii) a RIL population derived from Cervil and Levovil to define QTLs for vitamin C using RFLP markers. Several QTLs were identified in their work, but the most important common QTLs for the three populations were identified on bins 2-K, 8-B and 9-J. Moreover positive alleles on bin 2-K were provided by *S. lycopersicum* in two populations. This result was consistent with the finding in work done by Ökmen et al. (2011). They identified 5 QTLs for vitamin C on chromosomes 1, 2, 6 and 12 using an advanced backcross population derived from *S. lycopersicum* and *S. habrochaites* with CAPs and SSR markers. Their findings showed that *S. lycopersicum* alleles were responsible for higher vitamin C content for the chromosome 2 QTLs.

Rigano et al. (2014) studied two chromosomal region, 7.3 and 12.4, using an IL population derived from *S. pennellii* introgressions into the *S. lycopersicum* M82 genome. Both regions are important for vitamin C synthesis. Their work was consistent wth other studies. Matteo et al. (2010) also studied an IL population derived from *S. pennellii* introgressions into the *S. lycopersicum* M82 genome and found that region 12.4 is important for vitamin C synthesis. Sacco et al. (2013) used the same plant material and found that regions 7.3 and 12.4 are important for vitamin C synthesis.

Schauer et al. (2006) identified 889 fruit metabolite loci including 3 QTLs for vitamin C on chromosomes 4, 5, and 12; and 7 QTLs for vitamin E on chromosomes 1, 3, 5, 6, and 9 using an IL population derived from *S. lycopersicum* M82 and *S. pennellii.* Two of the loci for vitamin E on chromosomes 6 and 9 explained variation in the α isoform of vitamin E fruit content. This finding was supported in other work done by Almedia et al. (2011). They studied variation in vitamin E isoforms (α, β, γ and δ) in ILs (IL 6-1, 6-2, 7-4, 7-4-1, 7-5, 8-2, 8-2-1, 9-1 and 9-2-1) derived from *S. lycopersicum* M82 and *S. pennellii* using RFLP and CAPs markers. They found QTLs for α isoform of vitamin E on chromosomes 6 and 9. They also identified additional QTLs on chromosomes 6, 7, 8 and 9 for different isoforms of vitamin E and total vitamin E. Perez-Fons et al. (2014) identified 4 QTLs for the δ isoform of vitamin E on chromosomes 2, 8, 9 and 10, and 2 QTLs for the α isoform of vitamin E on chromosome 8 using the IL population

In the present study, we found that *S. pimpinellifolium* had higher vitamin E content than the other parent Tueza: *S. pimpinellifolium* contained 202.8 mg/kg DW and Tueza contained 36.1 mg/kg DW. Thus, the parents had extreme alleles for the trait. Although vitamin E contents were significant in both parents and segregated in the population, QTLs were not detected for vitamin E.

Another group of antioxidants molecules is carotenoids. Carotenoids content provides the highest contribution to antioxidant capacity of tomato and also the main nutritional importance of tomato consumption is carotenoid intake (Frusciante et al. 2007). The main carotenoid in tomato is lycopene followed by β-carotene. Lutein and zeaxanthin are also found in tomato at low concentrations (Raiola et al. 2014; Liu et al. 2012).

In the present study, the IBL population and the parents were evaluated for 4 carotenoids: lycopene, β-carotene, lutein and zeaxanthin. *S. pimpinellifolium* had higher content of all carotenoids except β-carotene than *S. lycopersicum*. Sizable variation and

normal continuous variation were observed in the IBLs for all carotenoids except lycopene. Parents of the IBL population showed extreme alleles for lycopene and many individuals of the IBL population had higher lycopene content than the parents. Although the traits segregated in the IBL population, QTL could not be detected for lutein and zeaxanthin. This could arise from unbalanced introgression of *S. pimpinellifolium* alleles for the traits into the *S. lycopersicum* genome. On the other hand, 2 QTLs for β-carotene on chromosome 1 were identified while 3 QTLs for lycopene on chromosomes 2 and 8 were identified. *S. lycopersicum* alleles increased the trait value for β-carotene content, and both *S. lycopersicum* and *S. pimpinellifolium* increased the trait value for lycopene content, although parents had extreme alleles for lycopene, and *S. pimpinellifolium* had higher lycopene content. Another study showed that *S. pimpinellifolium* alleles were a good source for antioxidant traits. Hanson et al. (2004) investigated antioxidant traits in 50 *S. lycopersicum* and three *S. pimpinellifolium* Mill entries to evaluate variation and source of the antioxidant traits lycopene, β-carotene, vitamin C, total phenolics and soluble solid content. They found *S. pimpinellifolium* Mill entries contained significantly higher content of lycopene, vitamin C, total phenolics and soluble solid content than *S. lycopersicum* cultivars.

Our QTLs for lycopene on chromosome 2 and 8, and QTLs for β-carotene on chromosome 1, were consistent with some other studies. Ökmen et al. (2011) identified 8 QTLs for lycopene on chromosomes 2, 3, 7, 8, 9, 10, 11 and 12 using advanced backcross population derived from *S. lycopersicum* and *S. habrochaites* with CAPs and SSR markers. The major alleles for increased lycopene were coming from *S. lycopersicum*. Capel et al. (2015) identified 3 QTLs on chromosomes 2, 4 and 6 for lycopene, and 6 QTLs on chromosomes 1, 2, 3, 4, 10 and 12 for β- arotene using RIL population derived from *S. lycopersicum cv*. Moneymaker and *S. pimpinellifolium* with SNP, SSR and indel markers. On the other hand, in other work different QTLs were identified on different chromosomes for β-carotene. Perez-Fons et al. (2013) identified 5 QTLs for β-carotene on chromosomes 2, 4 and 10 using IL population derived from *S. lycopersicum* M82 and *S. pennellii*.

Moreover, Ashrafi (2011) identified 2 major QTLs for lycopene on chromosomes 7 and 12 using RIL population derived from market tomato and *S. pimpinellifolium* with CAPs, EST and RFLP markers. Liu et al. (2003) identified 6 major QTLs for lycopene on chromosomes 2, 3, 5, 6, 11 and 12, and 2 QTLs for β-carotene on chromosomes 10 and 12 using an IL population derived from *S. lycopersicum* M82 and *S. pennellii* with

RFLP markers. Rousseaux et al. (2005) identified 3 QTLs for β-carotene on chromosomes 3 and 6, and 2 QTLs for δ-carotene on chromosome 12, which are provided by *S. pennellii*, using IL populations derived from *S. lycopersicum* M82 and *S. pennellii*. In addition, in another study chromosomal regions 7.3 and 12.4 were validated for carotenoid synthesis (Rigano et al. 2014). They identified a QTL on chromosome 12 (lyc 12.1) for lycopene which was consistent with work done by Kinkade anf Foolad (2013). They used a RIL population derived from *S. pimpinellifolium* introgressions into the *S. lycopersicum* genome and validated the QTL lyc 12.1 using fine mapping.

Candidate genes were also identified for carotenoid synthesis and vitamin C synthesis in tomato. Phytone synthase was located on chromosome 3, lycopene β-cyclase on chromosome 6, lycopene ε-cyclase on chromosome 12 (Perez-Fono et al. 2013), 9-cis-epoxy carotenoid dioxygenase and L-ascorbate oxidase in region 7.3 on chromosome 7 (Calafiore et al. 2016). Moreover, it was shown that the lycopene β-cyclase allele for increased content was provided by wild tomato, *S. pennellii* (Rousseaux et al. 2005).

The largest and most diverse group of antioxidants is phenolic acids. Besides their roles as antioxidants that have important biological activities in plants and health benefits to humans, phenolic acids are taking attention in plant breeding studies. In QTL studies, researchers mainly focused on total antioxidant capacity or total phenolic acid content because of the high positive correlation between antioxidant capacity and phenolic acid content (Stratil, Klejdus, and Kuban 2006).

For example, Rousseaux et al. (2005) identified 4 QTLs for total antioxidant capacity on chromosomes 6, 7, 10 and 12 using an IL population derived from *S. lycopersicum* M82 and *S. pennellii*. They suggested that chromosomes 6, 7 and 12 potentially improve antioxidant capacity, vitamin C content and total phenolic content. Ökmen et al. (2011) identified 6 QTLs for total antioxidant capacity on chromosomes 1, 5, 6, 8 and 12, 5 QTLs for total phenolics on chromosomes 1, 6, 7, 9 and 12, and 4 QTLs for flavonoids on chromosomes 2, 3, 5 and 11 using an advanced backcross population derived from *S. lycopersicum* and *S. habrochaites* with CAPs and SSR markers.

Moreover, specific regions of tomato chromosomes were evaluated in a few studies. Ballester et al. (2016) worked on an IL population derived from *S. chmielewski* introgressions in the *S. lycopersicum* (Moneyberg) genetic background using SNPs, and showed that 17 genes were upregulated in the 5B region on chromosome 5. This upregulation resulted in large quantitative changes in the levels of flavanols, especially kaempferol and quercetin glycosides, and was due to the presence of the IL 5b

introgression. Rigano et al. (2014) studied two chromosomal region, 7.3 and 12.4, using an IL population derived from *S. pennellii* introgressions into the *S. lycopersicum* M82 genome. Both regions are important for phenolic acid synthesis. Their results were consistent with another study. Sacco et al. (2013) studied the same IL population and found that regions 7.3 and 12.4 are important for phenolic acid synthesis.

In the present study we quantified individual phenolic acids instead of total phenolic acid content. We examined individual compounds because phenolic acids are a huge, diverse class and many different phenolic acids are synthesized by different plants, and also different phenolic acids have different biological functions or health benefits (Balasundram, sundram, and Samman 2006; Hermann and Nagel 1989; Matilla and Hellström 2007). The IBL population and its parents were evaluated for 33 phenolic acids, including flavanols (quercetin, myricetin, syringetin, kaempferol, isorhamnetin), flavones (apigenin, luteolin, chrysin), flavanones (naringenin, taxifolin), flavonol-3-ol (catechin, epicatechin, epigallocatechin), anthocyanins and anthocyanidines (cyanidine, delphinidine, malvidin, pelargonidin, peonidin), hydroxybenzoic acids (3-hydroxy benzoic acid (3-OHBA), 4-hydroxy benzoic acid (4-OHBA), gallic acid, vanillic acid, syringic acid, salicylic acid), hydroxycinnamic acids (cinnamic acid, coumaric acid, ferulic acid, caffeic acid, sinapic acid, cafteric acid, chlorogenic acid) and stilbenes (resveratrol, pterostilbene). Among these phenolic acids, 12 of them (syringetin, kaempferol, isorhamnetin naringenin, taxifolin, cyanidine, delphinidine, pelargonidin, peonidin, cafteric acid, resveratrol and pterostilbene) were not detected in parents or in the IBL population. The most abundant phenolic acid in *S. pimpinellifolium* was myricetin (400 mg/kg DW) while gallic acid was the most abundant phenolic acid in *S. lycopersicum*. In addition, some genotypes of the IBL population had extreme quantities of syringic acid (mean value 2.8 g/kg DW), on the other hand caffeic acid and apigenin were detected in all genotypes of IBL population. According to Kaushik et al. (2015), chlorogenic acid (35 mg/kg FW) is the most abundant phenolic acid in tomato. Moreover Rigano et al. (2016) found that chlorogenic acid is the most abundant in *S. lycopersicum* and *S. pennellii*. These findings confirm that individual phenolic content is highly dependent on both genotype and environmental factors.

The parents showed extreme alleles for syringic acid, epicatechin, chrysin, 4-OHBA, myricetin, coumaric acid, and ferulic acid all of which were high in *S. pimpinellifolium*. 3-OHBA, vanillic acid, gallic acid, chlorogenic acid, and caffeic acid were high in *S. lycopersicum.* Despite the presence of extreme alleles in parents, QTLs

could not be detected for chrysin, 4-OHBA, myricetin, ferulic acid, 3-OHBA, gallic acid, chlorogenic acid and caffeic acid. Moreover, phenolic acids did not have normal continuous distributions with the exceptions of 3-OHBA, gallic acid, cinnamic acid, chlorogenic acid and apigenin. In addition, low variation was observed for most of the phenolic acids. This could arise from unbalanced introgression of *S. pimpinellifolium* alleles for the traits into the *S. lycopersicum* genome.

In the present study, we identified 2 QTLs for vanillic acid on chromosomes 11 and 12; 4 QTLs for syringic acid on chromosomes 6; 4 QTLs for epicatechin on chromosome 7; 2 QTLs for quercetin on chromosome 12; 2 QTLs for cinnamic acid on chromosomes 4 and 6; 5 QTLs for coumaric acid on chromosome 1, 4 and 12; 1 QTL for sinapic acid on chromosome 6; and 2 QTLs for malvidin on chromosome 6 and 11. It was also found that *S. lycopersicum* alleles increased the trait value for all the individual phenolic acids. These findings differ from other work done by Perez-Fons et al. (2013). They identified 43 QTLs for phenolic acids including 17 QTLs for coumaric acid on chromosomes 4, 5, 6, 8, 9, 10, 11 and 12; 6 QTLs for ferulic acid on chromosome 1, 2, 4 and 8; 7 QTLs for naringenin on chromosome 1, 6, 8, 9, 11 and 12; 1 QTL for cinnamic acid on chromosome 2; 5 QTLs for caffeic acid on chromosome 2, 3, 8 and 12; 3 QTLs for rutin on chromosome 8, 11 and 12; 1 QTL for kaempferol on chromosome 8; 1 QTL for caffeolic acid on chromosome 11; 1 QTL for lutein on chromosome 12; and 1 QTL for myricetin on chromosome 3 using IL population derived from *S. lycopersicum* M82 and *S. pennellii*. New QTLs were identified for different phenolic acids in our study and Perez-Fons' study. Moreover, most QTL studies in the literature for phenolic acids in tomato were focused on total phenolic content not individual phenolic acids. Thus the identified QTLs in our study were new for the literature and breeding studies.

Moreover, correlation analysis demonstrated that there were weak positive correlations between antioxidant molecules: β-carotene and vitamin E (r^2 = 0.015), vitamin C and vitamin E (r^2 = 0.047), vitamin C and oxidized glutathione (r^2 = 0.109), and negative correlation between vitamin E and oxidized glutathione (r^2 = -0.026). These correlations were expected because there are different antioxidant mechanisms; shikimate pathway, phenylpropanoid pathway, flavonoid pathway, carotenoid pathway, tocochromanol pathway, and ascorbic acid and glutathione redox system. In the ascorbic acid and glutathione redox system vitamin C, vitamin E and glutathione are linked to each other via a series of coupled oxidation-reduction reactions. This relationship provides continuous formation of reduced forms of the molecules from their oxidized forms (Lovat

et al. 2016). Other negative and positive correlations between antioxidant molecules were weak.

Colocalized QTLs were detected for antioxidant molecules. Cinnamic acid and malvidin were colocalized at 44.8 Mb position on chromosome 6. Sinapic acid and oxidized glutathione were colocalized at 38.8 Mb position on chromosome 6. Colocalization of antioxidant components were rare which demonstrated that genes controlling these traits probably are not linked.

Tomato contains high levels of antioxidant compounds. Thus consuming tomato daily is beneficial to human health. Tomato is especially rich in lycopene content which is a powerful antioxidant, and also prevents diseases like cancer and cardiovascular diseases. In this study, we evaluated variation in an IBL population derived from cultivated tomato and identified QTLs for popular compounds such as lycopene, β-carotene, vitamin C and E, and ignored molecules such as lutein, zeaxanthin, oxidized and reduced glutathione. Moreover phenolic acids were individually evaluated instead of total phenolic acid content. Values for nearly all of the antioxidant traits were increased by *S. lycopersicum* (Tueza) instead of *S. pimpinellifolium*. These findings should be useful for breeding studies and to develop new cultivars.

CHAPTER 7

DISCUSSION

GBS represents a powerful, low-cost platform in the field of plant breeding for genomic selection and genotyping breeding populations, to discover molecular markers, to study genomic diversity and for genetic linkage analysis (Poland and Rife 2012). The main advantage of GBS is performing SNP discovery and genotyping simultaneously without requirement for knowledge of the species genome (Narum et al. 2013, Poland and Rife 2012). GBS has begun to be applied to many crop species in plant breeding, such as maize (Romay et al. 2013), soybean (Lam et al. 2010), potato (Uitdewilligen et al. 2013), barley (Fu and Peterson 2011), wheat (Poland et al. 2012a), rice (Heffner et al. 2009; Huang et al. 2009; Jannink et al. 2010), and cassava (Rabbi et al. 2015). The present research is the first report of high-throughput SNP discovery using GBS in tomato and demonstrated that this approach was efficient for SNP identification in tomato.

Genetic determinants of quality parameters of tomato have been studied for a long time. These studies were mostly limited to morphological characters and disease resistance. However, studies have recently begun to focus on single metabolites or groups of metabolites. For example, total sugar content expressed as brix value, or organic acid content measured by acidity have been studied but there are limited QTL mapping or genome wide association studies studying individual sugars or organic acids (Fulton et al. 2002; Schauer et al. 2006, 2008, Ruggier et al. 2014; Sauvage et al. 2014). Therefore, the genetic and moleculer basis of natural variation of tomato metabolites is still far from being clearly understood (Fulton et al. 2002; Schauer et al. 2008; Sauvage et al. 2014). In the present study, a detailed investigation was done for individual metabolites. Eleven agronomic and morphologic traits, 53 flavor-related metabolites including sugars, organic acids and volatile compounds, 38 nutritionally important metabolites including phytosterols, free fatty acids, fat and water soluble vitamins, and 41 health related antioxidant metabolites including glutathiones, vitamin C and E, carotenoids and phenolic acids were screened in the IBL population and its parents. In this way, 37 QTLs for agronomic and morphologic traits, 82 QTLs for flavor-related metabolites, 37 QTLs for nutritionally important metabolites, and 31 QTLs for health-related antioxidant

metabolites were identified among all tomato chromosomes. In total, 187 QTLs were identified for 143 important traits.

An important problem in breeding programmes is reduction in genetic diversity due to both natural and artficial selection. The loss of genetic diversity results in decreasing the chance to fight future challenges such as new diseases or pests, and changing environment (Esquinas-Alcázar 2005). Therefore, it is important to find new resources to increase genetic diversity. Wild species are natural resources with a large genetic diversity (Rick, Chetelat, and DeVenna 1988). Cultivated tomato contains only 5% of the genetic variation of its wild relatives (Miller and Tanskley 1990). Thus exploring the use of wild species alleles to increase genetic diversity in cultivated tomato is important in breeding strategies. In the present study, a wild relative of cultivated tomato, *S. pimpinellifolium*, was used as donor parent. Our study confirmed the high breeding potential of *S. pimpinellifolium* by detecting useful alleles for breeding of fruit quality traits and metabolites. Thus, we demonstrated that *S. pimpinellifolium* alleles can be a good source to increase agronomic and morphological traits such as external and internal color, firmness, and soluble solids content. This species can also be used to improve flavor-related traits such as organic acids and some volatile compounds, as well as nutritionally important compounds such as vitamins and antioxidants.

CHAPTER 8

CONCLUSION

Recent advances in genomics, especially in next generation sequencing (NGS) and post-genomics areas, such as transcriptomics, proteomics and metabolomics provide a chance to apply genomics findings to plant breeding. NGS technology offers cost effective, high throughput DNA sequencing. Avaliability of large genomic data provides wider applications of genomics in the area of agriculture. Many species' genomes including major crops and botanical models have been sequenced (Micheal and Jackson 2013; Paterson 2010). Sequenced crop genomes allow examination of genome organization, evolution, genetic diversity, modifications of specific genes, marker discovery, and discovery of QTLs. These advances help to overcome bottlenecks in breeding programmes (Fiorani and Schurr 2013; Shi and Lai 2015).

In the last two decades, the combination of metabolomics with genomics studies has revealed many new relationships. Understanding the genetic architecture underlying accumulation of metabolites and the relationship between metabolite and phenotype aids crop improvement. Also, together with the other post-genomic approaches, metabolomics enlightens many biological and physiological questions. The application of analytic techniques in post-genomic approaches allow a better understanding of the influence of primary metabolism on plant growth and development as well as evolution of secondary metabolism in plants (Toghe and Fernie 2015). Moreover, environmental adaptations, gene functions, and interaction between gene products can be understood with post-genomic studies which also help to evaluate the agricultural potential of varieties (Zivy et al. 2015).

One of the most popular crops is tomato (*S. lycopersicum*) which was subjected to many studies such as fruit physiology and development (Giovannoni 2001), quantitative genetics and plant breeding (Lippman, Semel, and Zamir 2007; Zamir 2001) with the aid of genomics and post-genomics approaches. Tomato contains many beneficial and essential nutrients in the human diet such as minerals, vitamins and antioxidants (Spencer et al. 2005). These beneficial nutrients are found in fruits and vegetables and are known phytochemicals and bioactive molecules. These molecules are

functional ingredients due to their health promoting potential; thus, they are gaining more importance in human nutrition (Butt et al. 2008). Moreover, FAO and WHO have stressed the role of nutrition in disease prevention and the importance of plant-derived phytochemicals (FAO 2003). Balanced consumption of phytochemicals protects against diseases, especially lifestyle disorders such as cancer, due to altering the body metabolism, and triggering/modulating detoxification mechanisms (Manach et al. 2004). In this regard, tomato is an important source of phytochemicals with beneficial effects. Tomato is consumed every day and is therefore one of the most globally consumed fruits. It is not only consumed fresh, but also consumed as paste, sauce, juice or catchup. Daily consumption of tomato helps to prevent hypertension, diabetes, obesity, cardiovascular diseases, inflammatory processes and digestive tract tumors (Canene-Adams et al. 2005). It is estimated that the health benefits of tomato are mainly related to its antioxidant content, especially phenolic acids, carotenoids, vitamin C and vitamin E (Canene-Adams et al. 2005; Frusciante et al. 2007; Raiola et al. 2014). The chemical composition of tomato is highly affected by genetic background, environmental factors and cultural practices (Periago et al. 2009; Garcia-Valeverde et al. 2013). Tomato has become focus of breeding programmes due to its economic and nutritional importance.

Up to the last decade, plant breeding programmes focused on yield, resistance and tolerance to abiotic and biotic stresses, longer shelf life and varietal diversification. However, consumer awareness and needs are changing due to a geater understanding of the correlation between diet and maintaining health and preventing disease. As a result, functional and nutritional foods have become popular. Therefore, besides yield and resistance, the focus of breeding programmes is shifting toward improving nutritional health-beneficial traits and fruit flavor. However, breeders have faced an important problem: loss of genetic variation in cultivated tomato due to both natural and artificial selection throughout the decades. It is estimated that cultivated tomato has less than 5% of genetic variation compared to its wild species (Miller and Tanksley 2003). The potential of wild species as sources of increasing genetic variation in cultivated tomato has been investigated. Different wild species of tomato such as *Solanum pennelli, Solanum pimpinellifolium, Solanum chmielewskii, Solanum habrochaites, Solanum cheesmanii, Solanum peruvianum* and *Solanum neorickii* were investigated for resistance to abiotic and biotic stresses (Légnani et al. 1996; Frankel et al. 2003), and contents of primary metabolites (Schauer et al. 2005) and secondary metabolites (Alseekh et al. 2015). In these studies, the usefulness of wild species to increase genetic variation was

proven for specific traits. Even so, these studies are very limited, and the potential use of wild species alleles should be further investigated. In the present study, we demonstrated that *S. pimpinellifolium* alleles can be used to improve favorable traits in tomato cultivars. Especially, this wild species can be used to increase trait values for important agronomic quality parameters such as external and internal color, soluble solid content or firmness. Moreover, our study demonstrated that *S. pimpinellifolium* alleles increased both nutritional (such as vitamins) and flavor (such as organic acids and volatile compounds) related traits in the IBL population. Thus, the present study confirmed the high breeding potential of wild relative *S. pimpinellifolium* by detecting useful alleles for breeding of fruit quality traits. In general, the genetic bottlenecks imposed on crops during domestication and through modern breeding practices have greatly reduced the genetic variability that can be used for breeding. For this reason, wild relatives or local landraces are a particularly useful source for allele mining.

In addition, understanding the metabolic constitution of plants and its genetic control promises to facilitate crop improvement strategies. It will help breeders to address potentially more difficult issues such as crop compositional quality including traits such as antioxidant or flavor compounds. The present study combines the latest technologies of genomics and metabolomics to elucidate the metabolic constituents of tomato and their genetic control. The current study will not only aid in improvement of tomato for traditional breeding targets such as yield and disease resistance, it will also provide information needed for quality traits which are becoming more important for consumers. Improvement of quality and health-related traits such as vitamin content, antioxidant or flavor will be added value traits which will result in innovative cultivars representing a new generation of tomato products.

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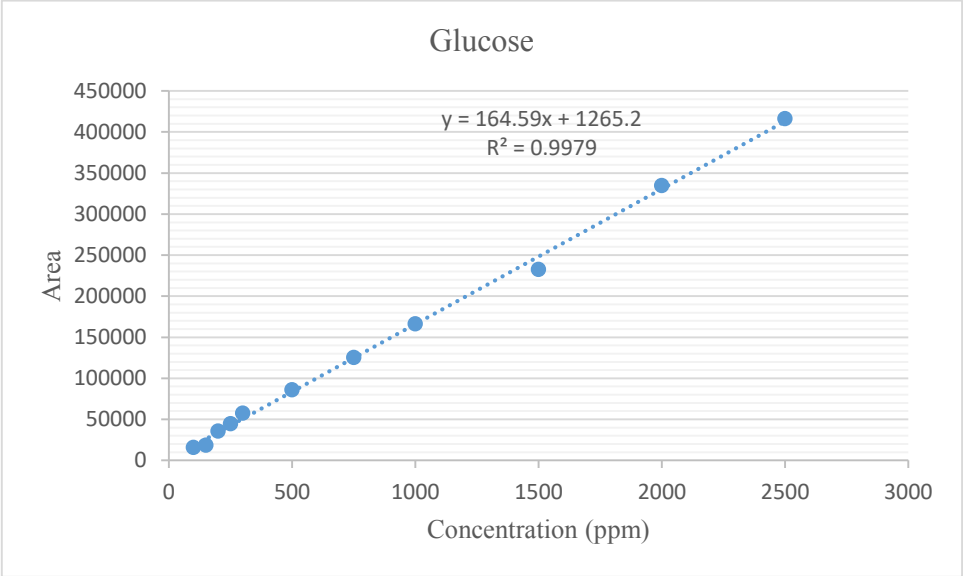
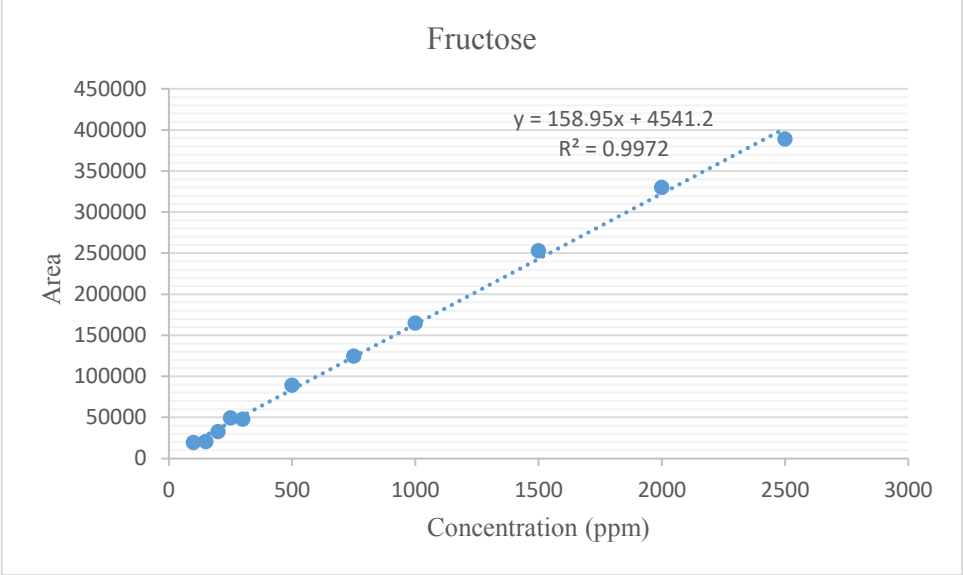
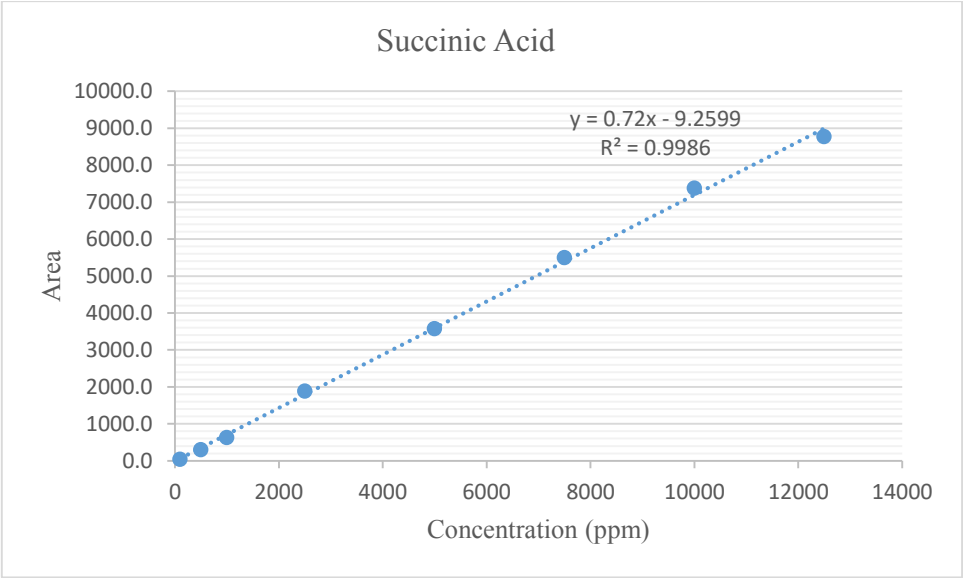
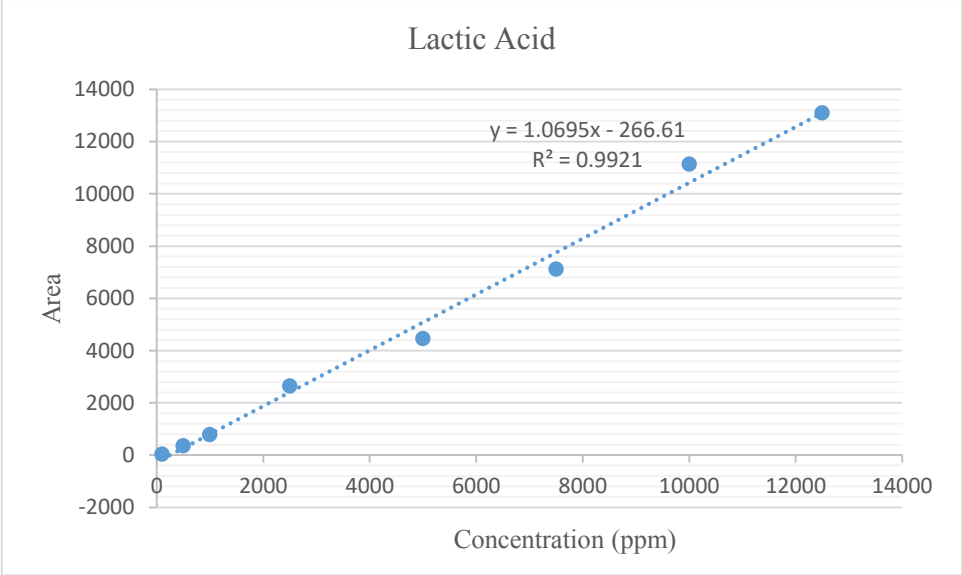
APPENDIX A

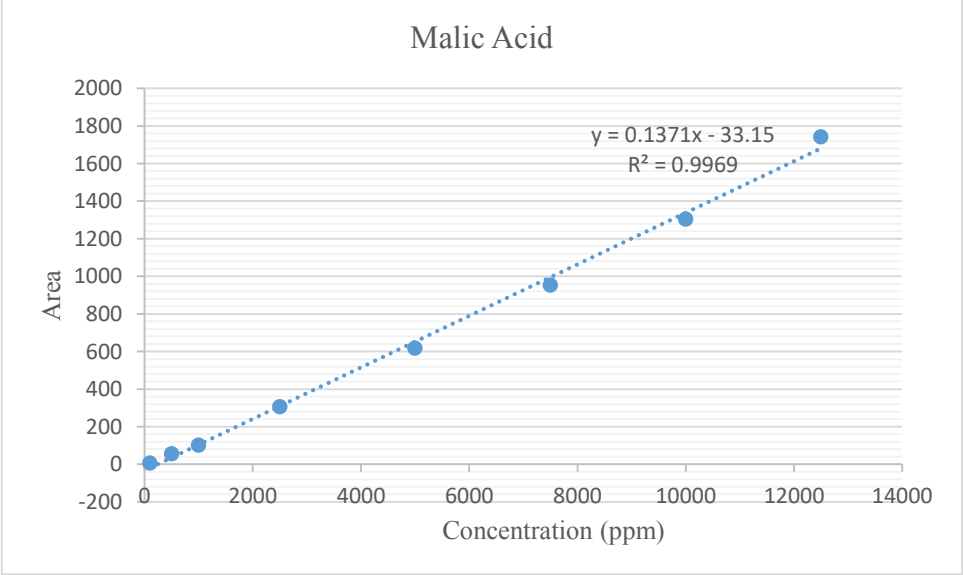
STANDARD GRAPHICS OF METABOLITES

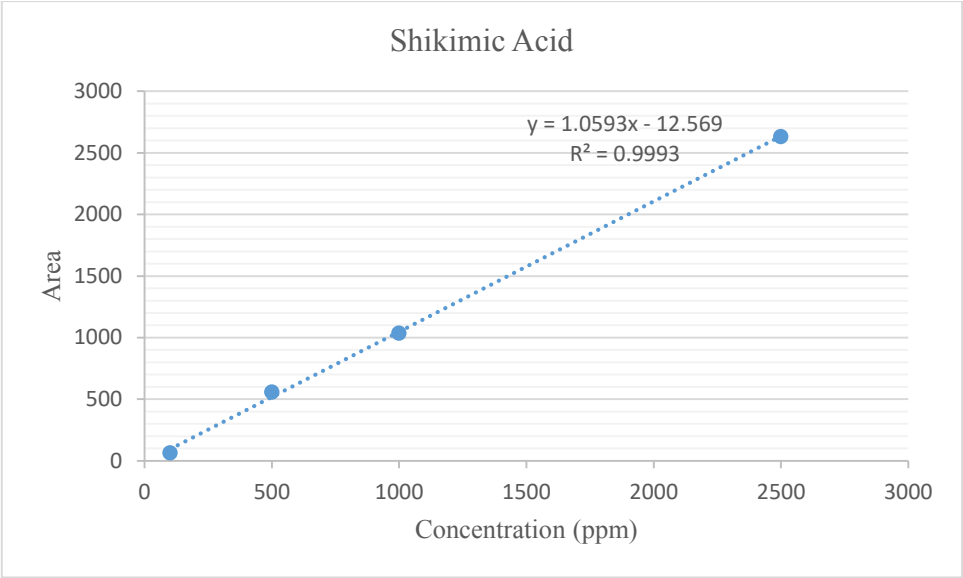


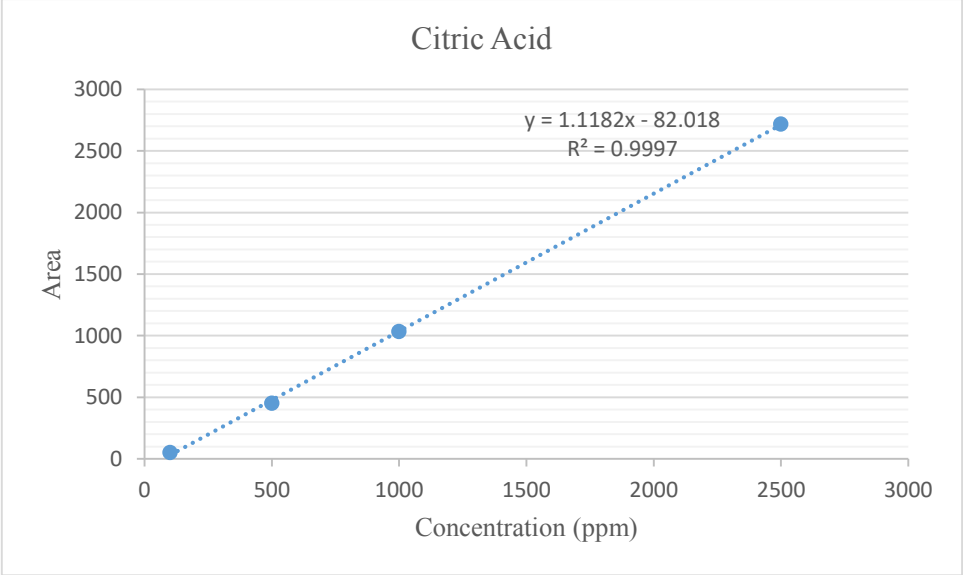


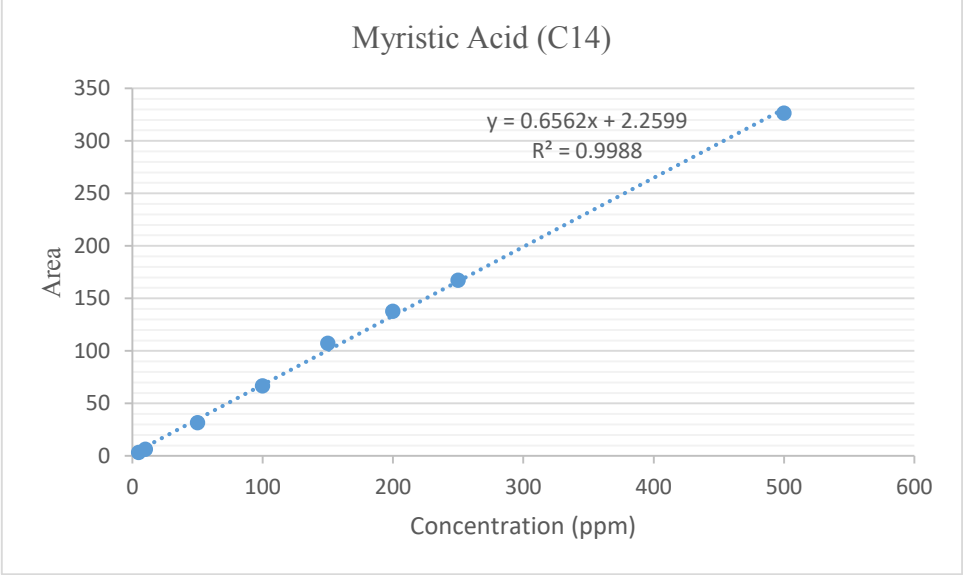

Succinic Acid
y = 0.72x - 9.2599
R² = 0.9986
Area
Concentration (ppm)
10000.0
9000.0
8000.0
7000.0
6000.0
5000.0
4000.0
3000.0
2000.0
1000.0
0.0
0
2000
4000
6000
8000
10000
12000
14000

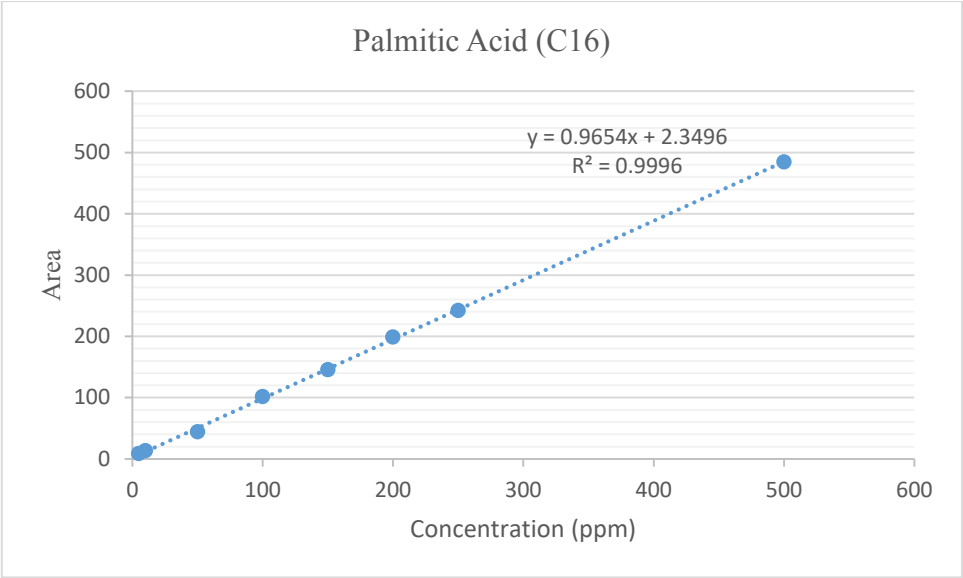

Lactic Acid
y = 1.0695x - 266.61
R² = 0.9921
Area
Concentration (ppm)
14000
12000
10000
8000
6000
4000
2000
0
-2000
0
2000
4000
6000
8000
10000
12000
14000

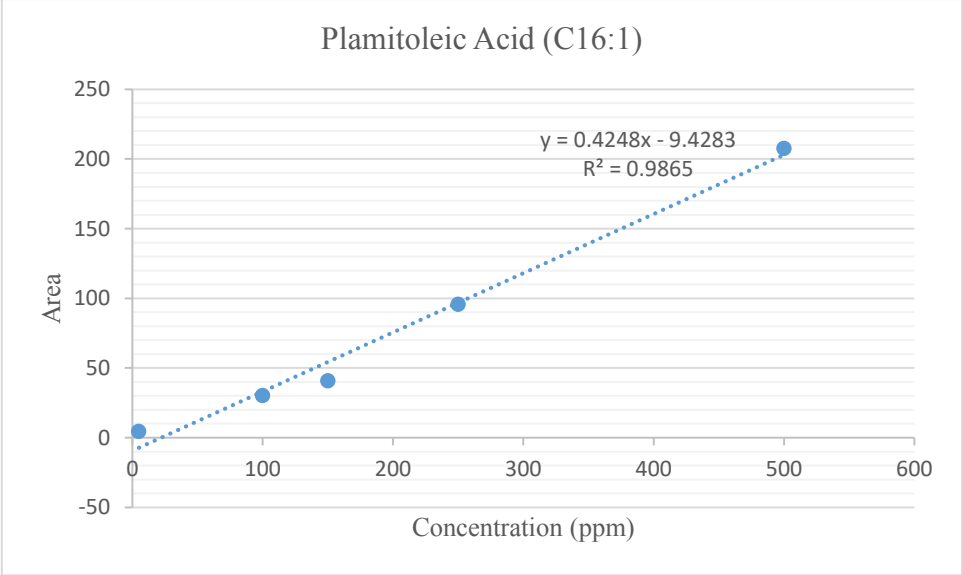

Malic Acid
y = 0.1371x - 33.15
R² = 0.9969
Area
Concentration (ppm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
0
2000
4000
6000
8000
10000
12000
14000

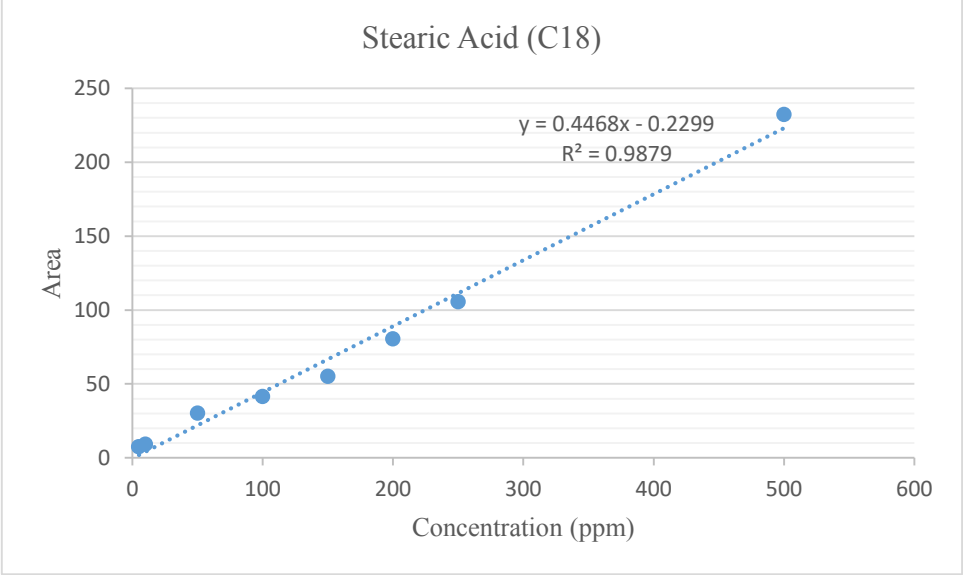

Shikimic Acid
y = 1.0593x - 12.569
R² = 0.9993
Area
Concentration (ppm)
0
500
1000
1500
2000
2500
3000

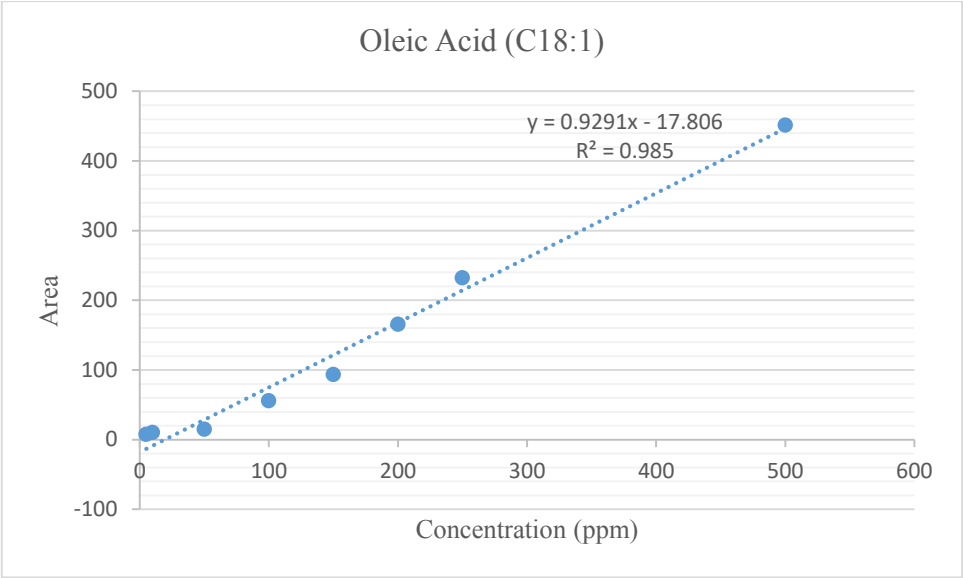

Citric Acid
y = 1.1182x - 82.018
R² = 0.9997
Area
Concentration (ppm)
0
500
1000
1500
2000
2500
3000

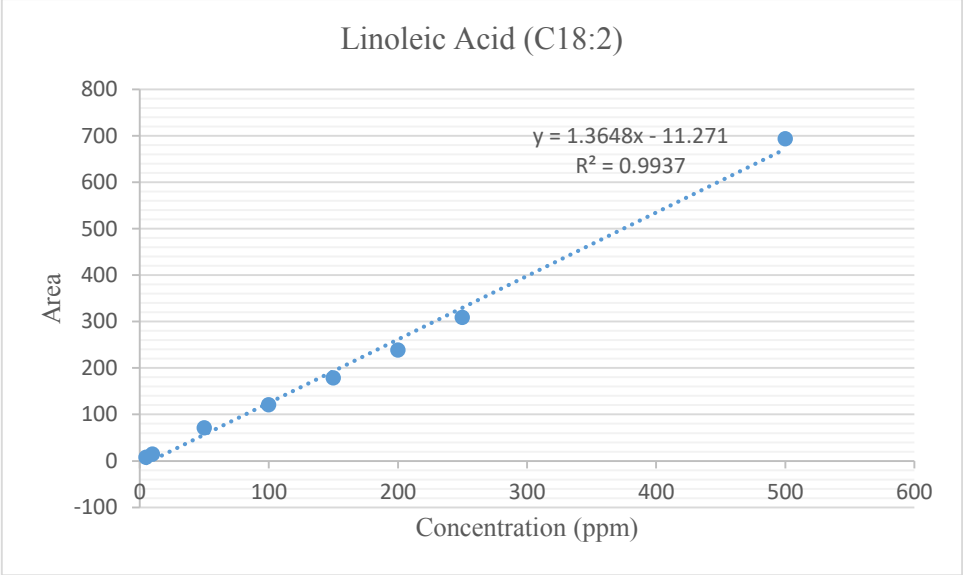

Myristic Acid (C14)
y = 0.6562x + 2.2599
R² = 0.9988
Area
Concentration (ppm)
0
50
100
150
200
250
300
350
400
500
600

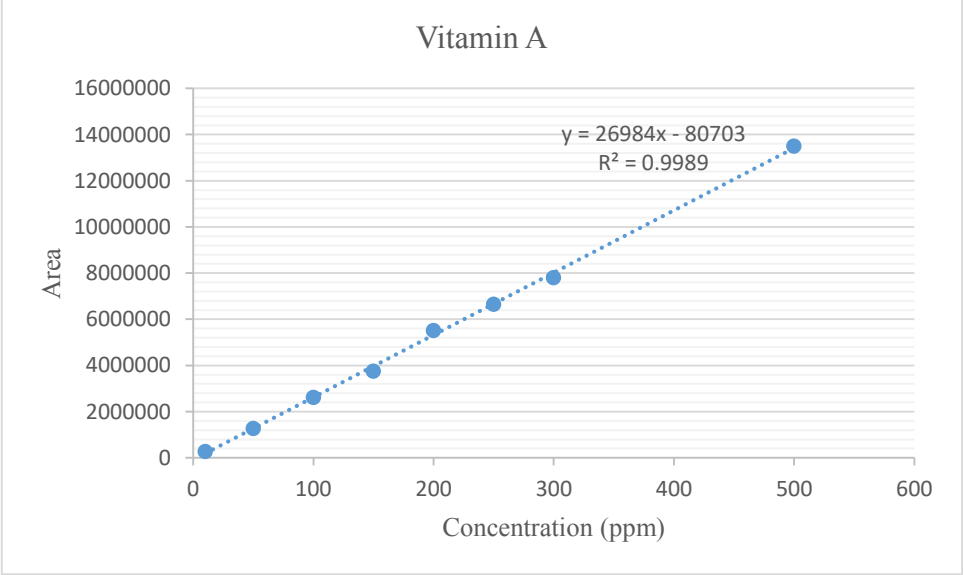

Palmitic Acid (C16)
y = 0.9654x + 2.3496
R² = 0.9996
Area
Concentration (ppm)
0
100
200
300
400
500
600

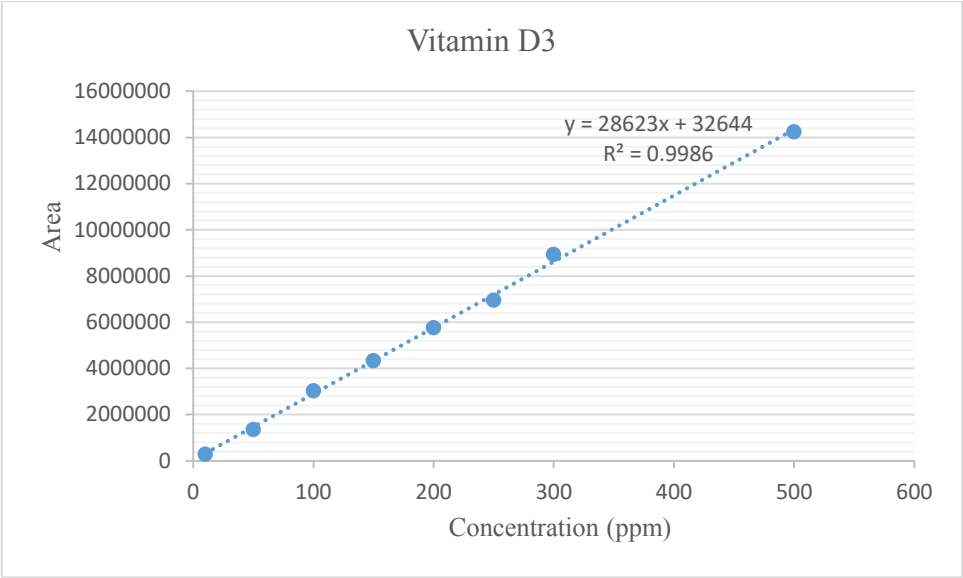

Plamitoleic Acid (C16:1)
y = 0.4248x - 9.4283
R² = 0.9865
Area
Concentration (ppm)
-50
0
50
100
150
200
250
300
400
500
600

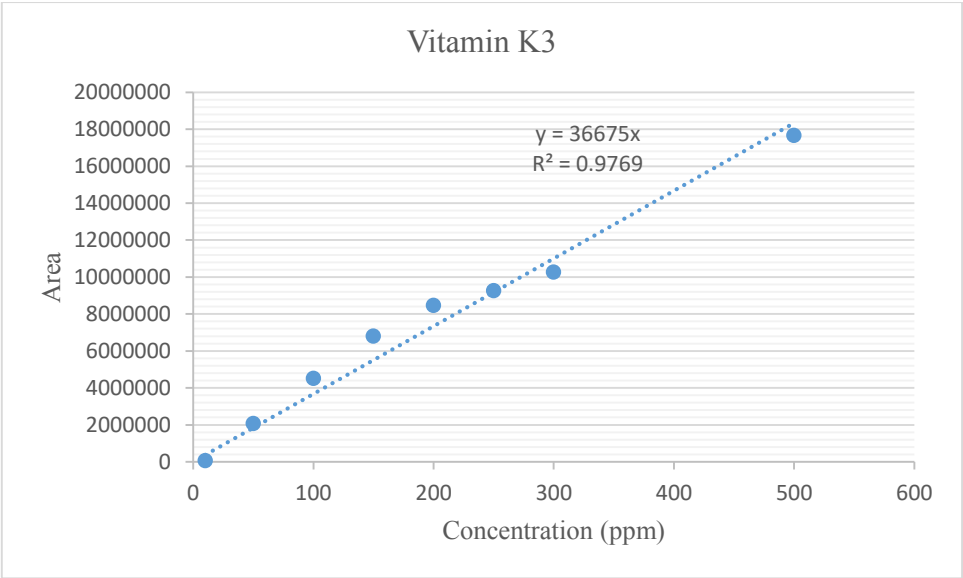

Stearic Acid (C18)
y = 0.4468x - 0.2299
R² = 0.9879
Area
Concentration (ppm)
0
50
100
150
200
250
300
400
500
600

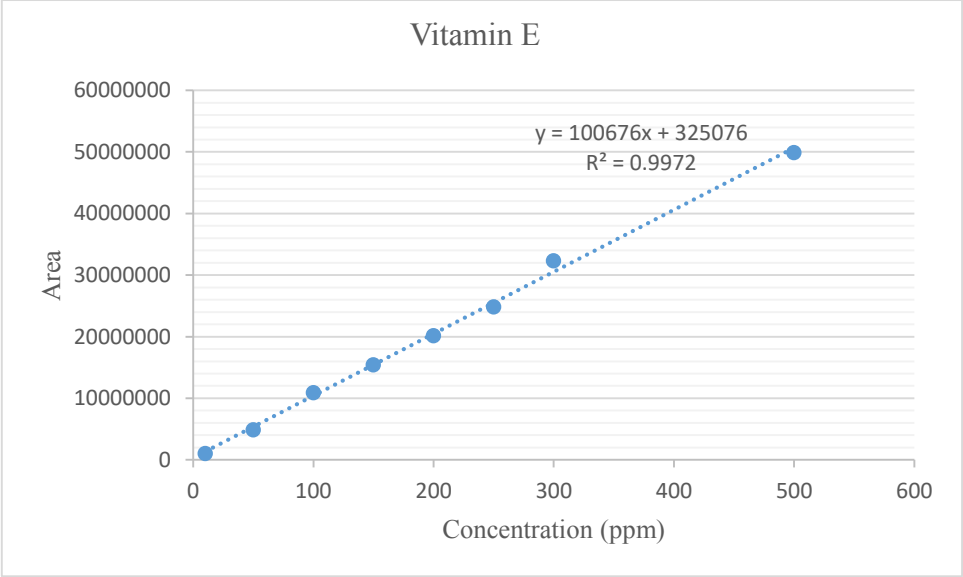

Oleic Acid (C18:1)
y = 0.9291x - 17.806
R² = 0.985
Area
Concentration (ppm)
500
400
300
200
100
0
-100
0
100
200
300
400
500
600

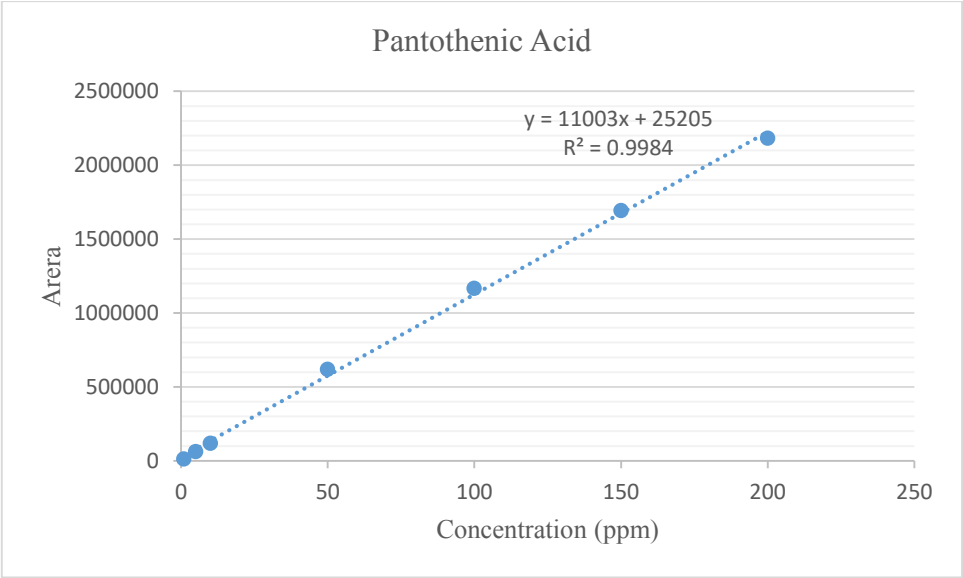

Linoleic Acid (C18:2)
y = 1.3648x - 11.271
R² = 0.9937
Area
Concentration (ppm)
800
700
600
500
400
300
200
100
0
-100
0
100
200
300
400
500
600

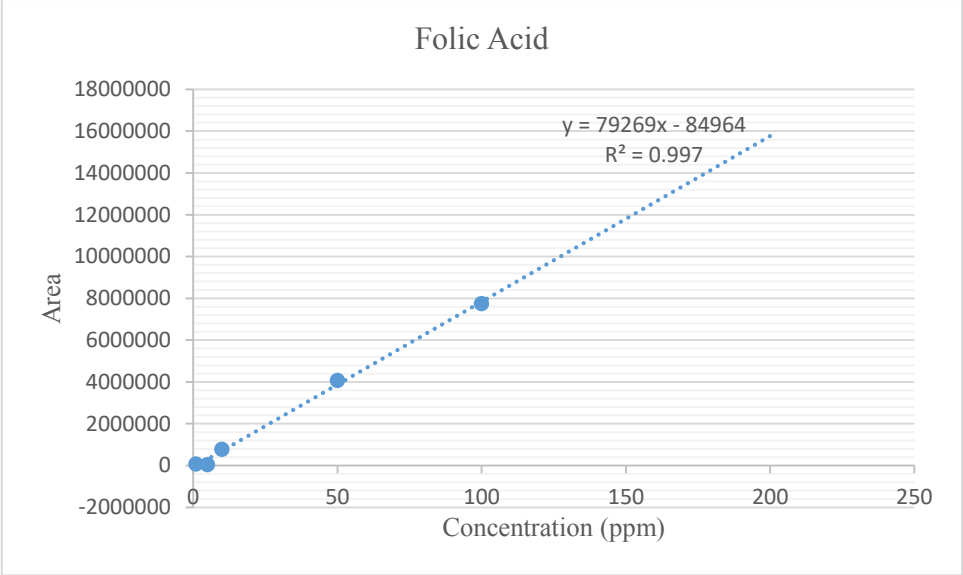

Vitamin A
y = 26984x - 80703
R² = 0.9989
Area
Concentration (ppm)
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
100
200
300
400
500
600

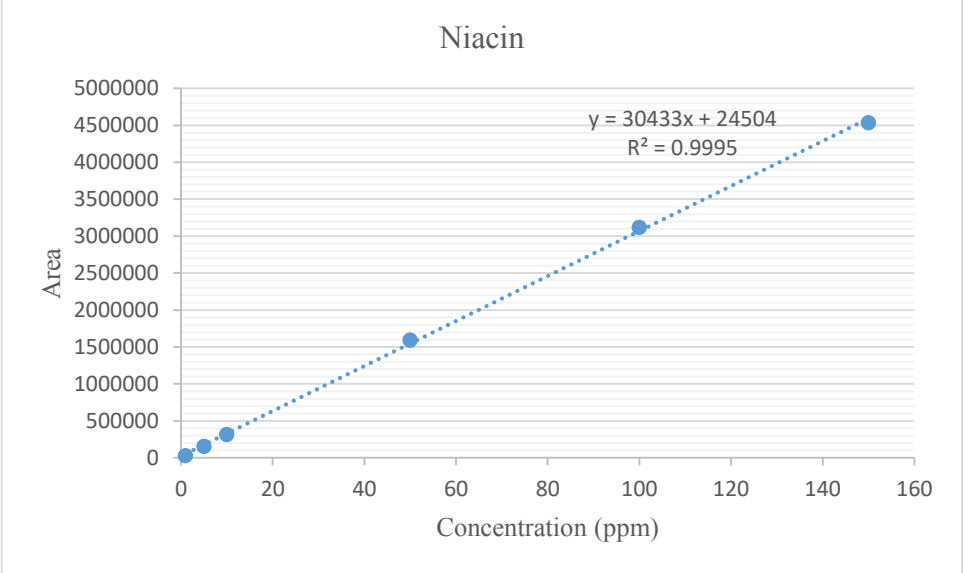

Vitamin D3
y = 28623x + 32644
R² = 0.9986
Area
Concentration (ppm)
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
100
200
300
400
500
600

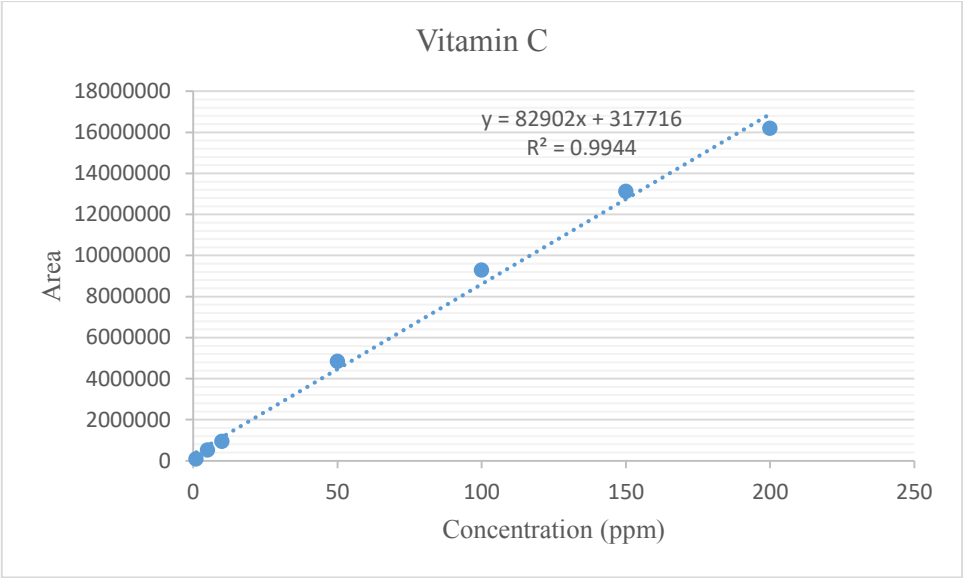

Vitamin K3
y = 36675x
R² = 0.9769
Area
Concentration (ppm)
20000000
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
100
200
300
400
500
600

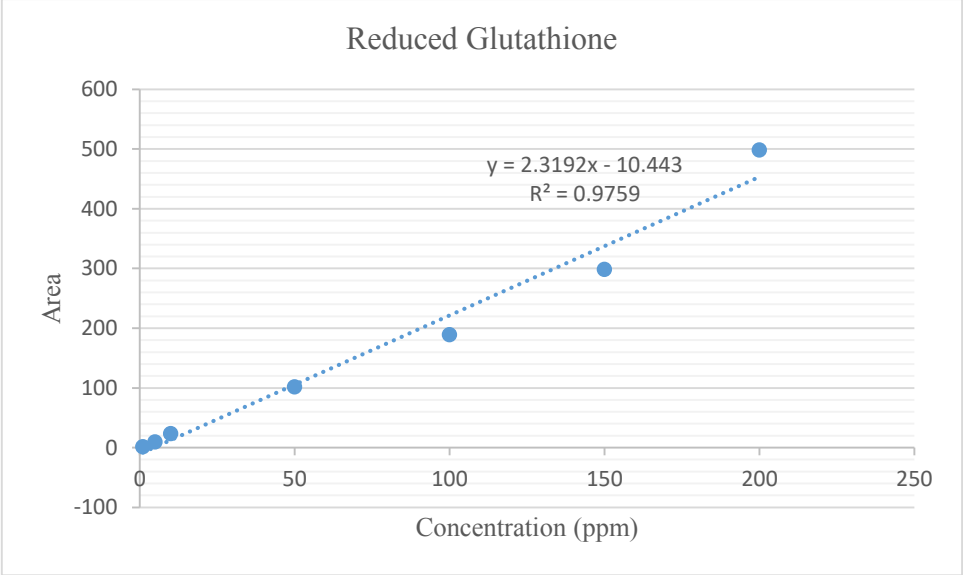

Vitamin E
y = 100676x + 325076
R² = 0.9972
Area
Concentration (ppm)
60000000
50000000
40000000
30000000
20000000
10000000
0
0
100
200
300
400
500
600

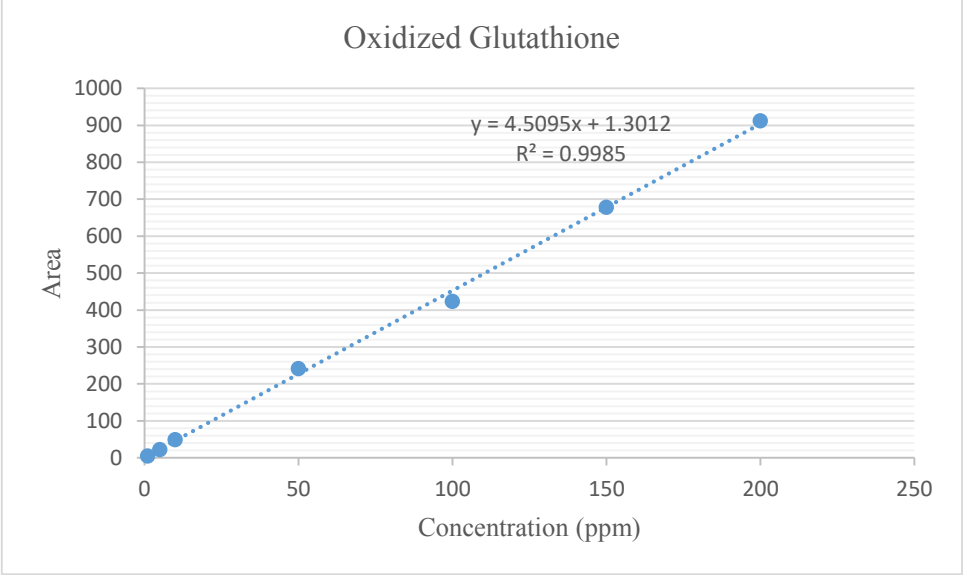

Pantothenic Acid
y = 11003x + 25205
R² = 0.9984
Arera
2500000
2000000
1500000
1000000
500000
0
0
50
100
150
200
250
Concentration (ppm)

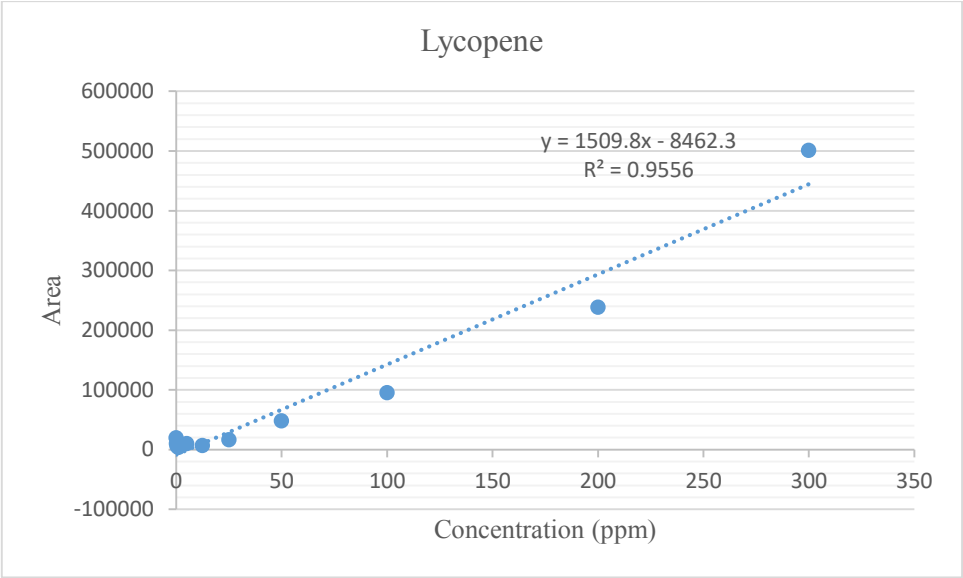

Folic Acid
y = 79269x - 84964
R² = 0.997
Area
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
-2000000
0
50
100
150
200
250
Concentration (ppm)

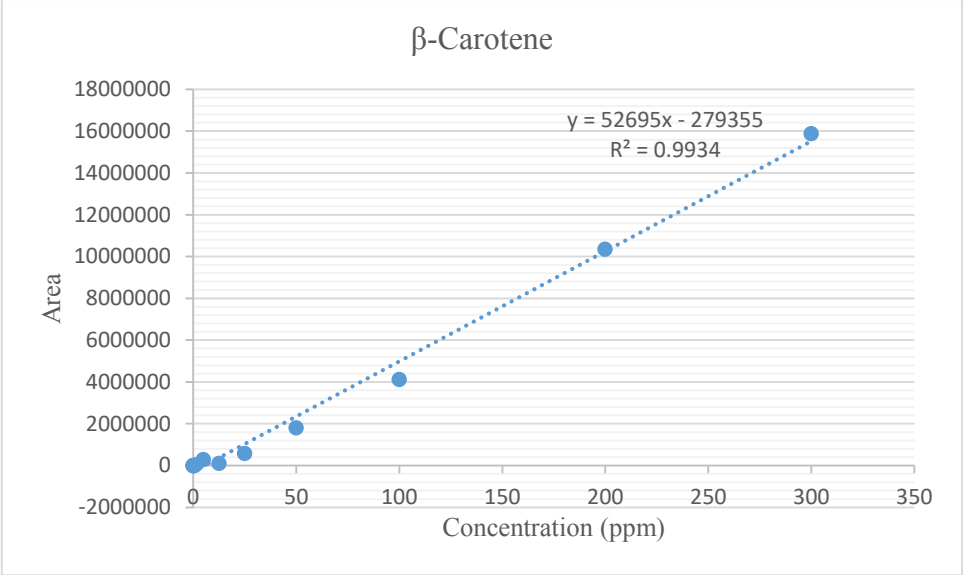

Niacin
y = 30433x + 24504
R² = 0.9995
Area
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
0
20
40
60
80
100
120
140
160
Concentration (ppm)

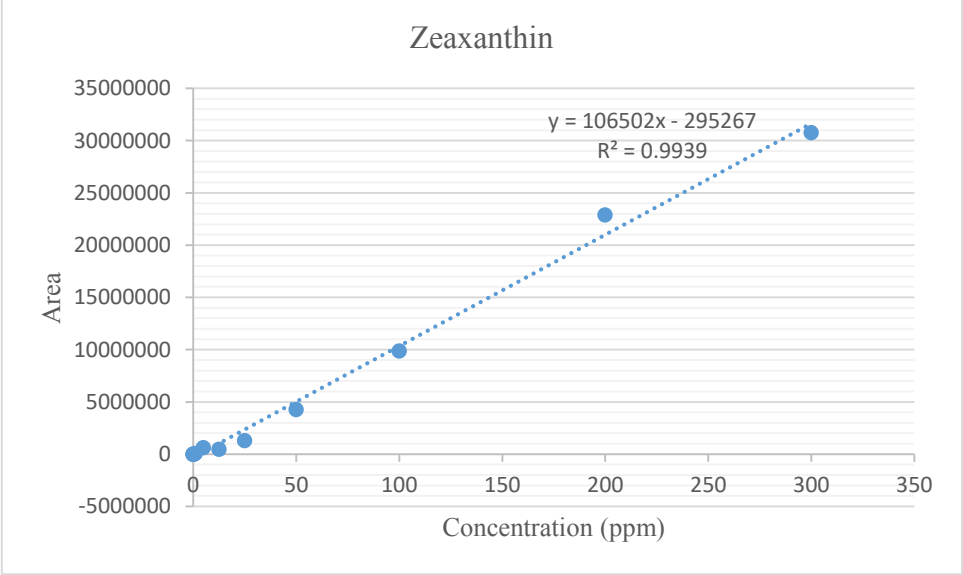

Vitamin C
y = 82902x + 317716
R² = 0.9944
Area
Concentration (ppm)
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
50
100
150
200
250

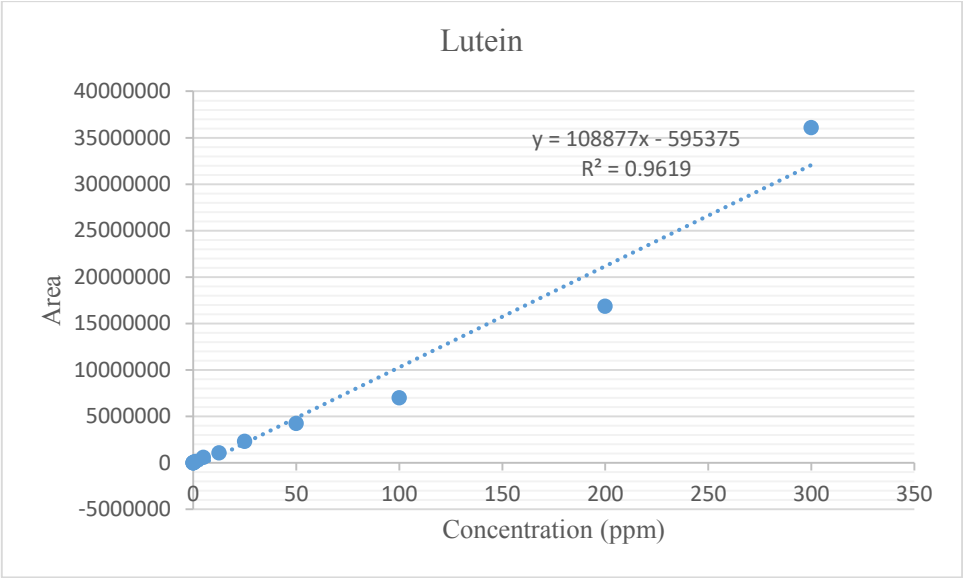

Reduced Glutathione
y = 2.3192x - 10.443
R² = 0.9759
Area
Concentration (ppm)
600
500
400
300
200
100
0
-100
0
50
100
150
200
250

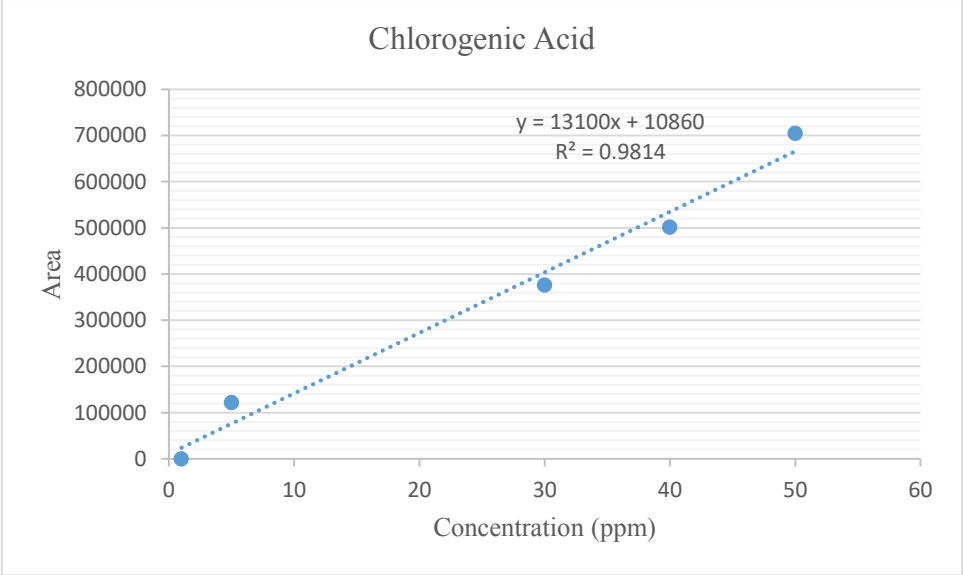

Oxidized Glutathione
y = 4.5095x + 1.3012
R² = 0.9985
Area
Concentration (ppm)
1000
900
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250

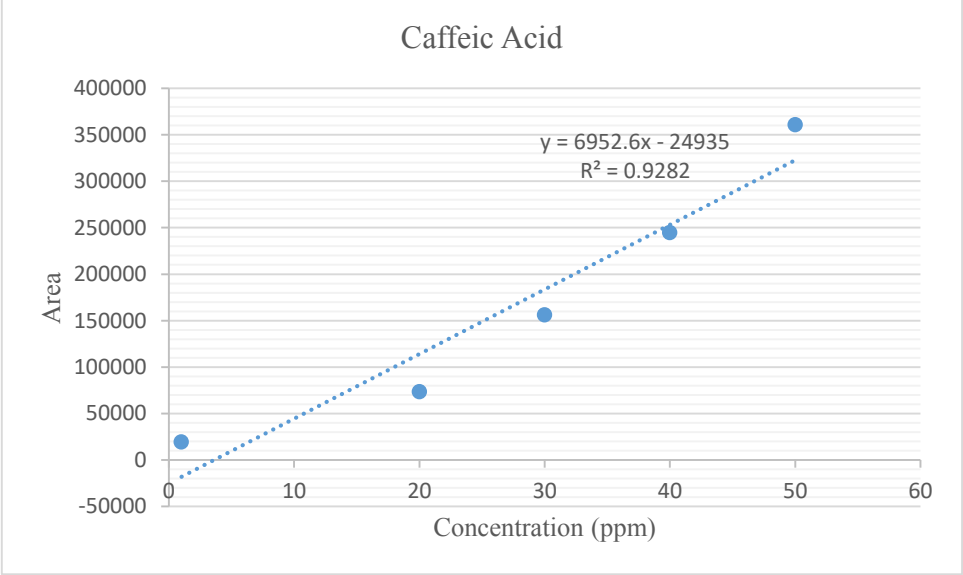

Lycopene
y = 1509.8x - 8462.3
R² = 0.9556
Area
Concentration (ppm)
600000
500000
400000
300000
200000
100000
0
-100000
0
50
100
150
200
250
300
350

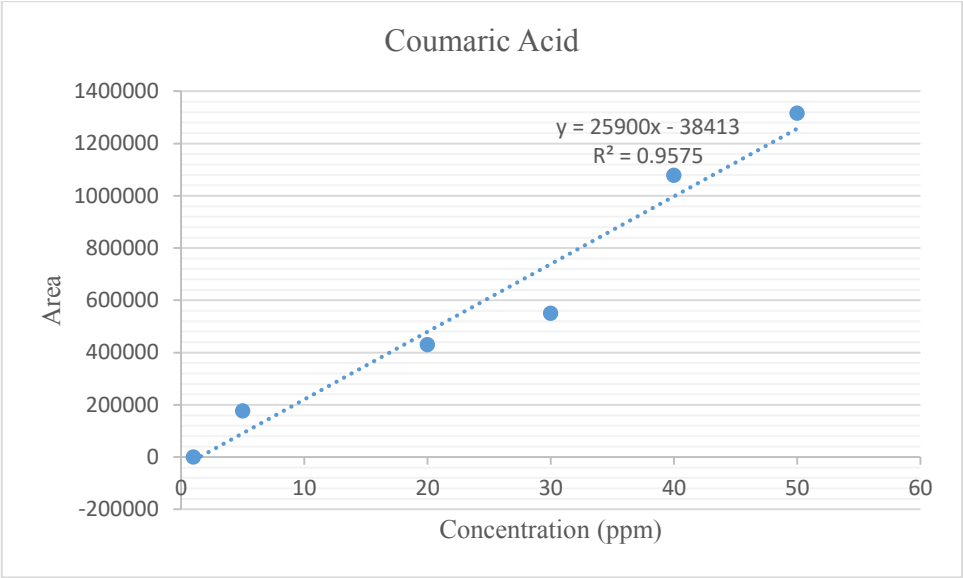

β-Carotene
y = 52695x - 279355
R² = 0.9934
Area
Concentration (ppm)
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
-2000000
0
50
100
150
200
250
300
350

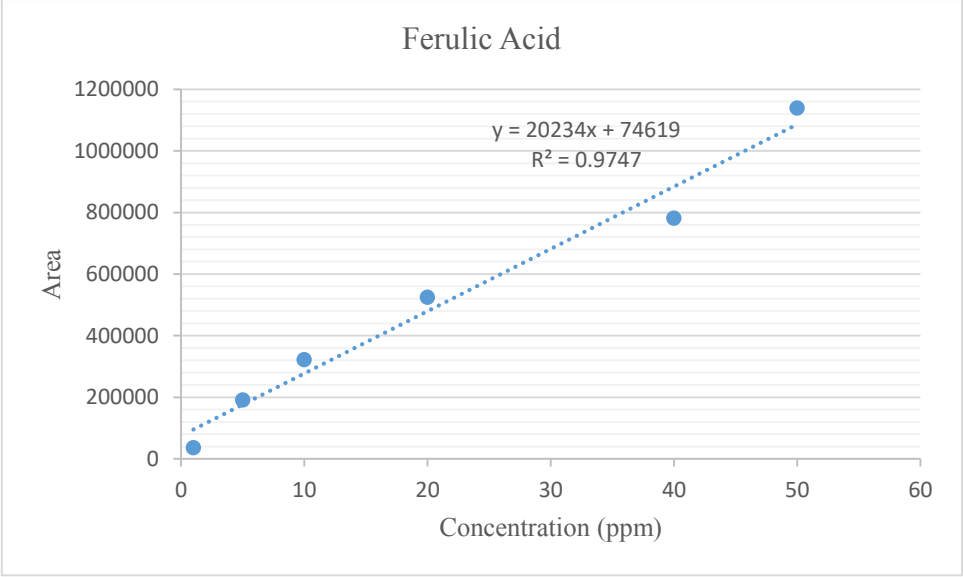

Zeaxanthin
y = 106502x - 295267
R² = 0.9939
Area
Concentration (ppm)
35000000
30000000
25000000
20000000
15000000
10000000
5000000
0
-5000000
0
50
100
150
200
250
300
350

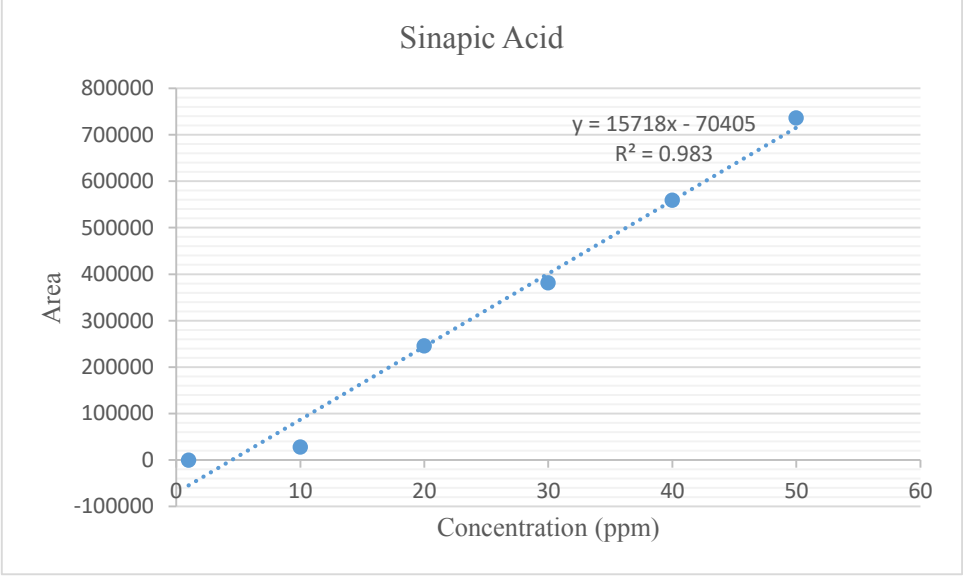

Lutein
y = 108877x - 595375
R² = 0.9619
Area
Concentration (ppm)
40000000
35000000
30000000
25000000
20000000
15000000
10000000
5000000
0
-5000000
0
50
100
150
200
250
300
350

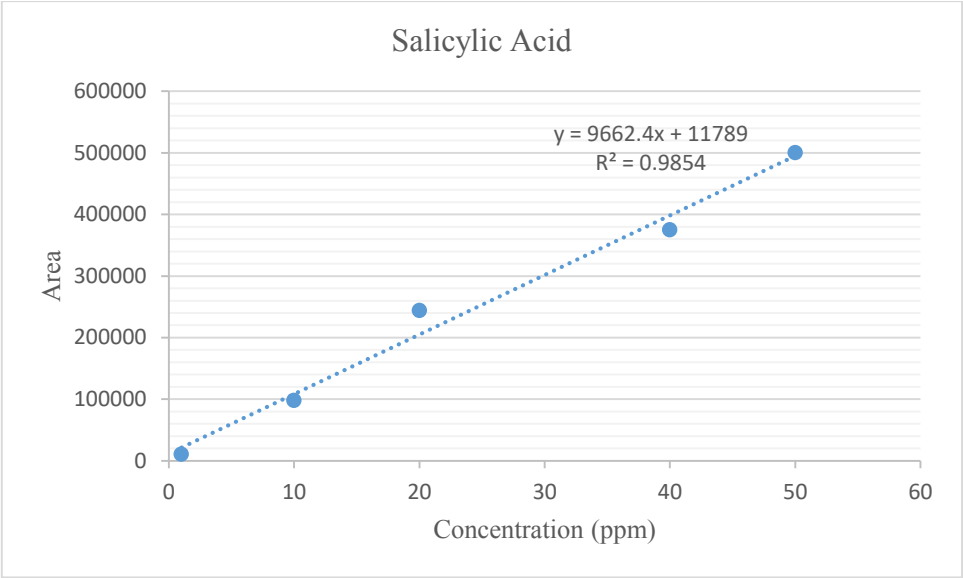

Chlorogenic Acid
y = 13100x + 10860
R² = 0.9814
Area
Concentration (ppm)
800000
700000
600000
500000
400000
300000
200000
100000
0
0
10
20
30
40
50
60

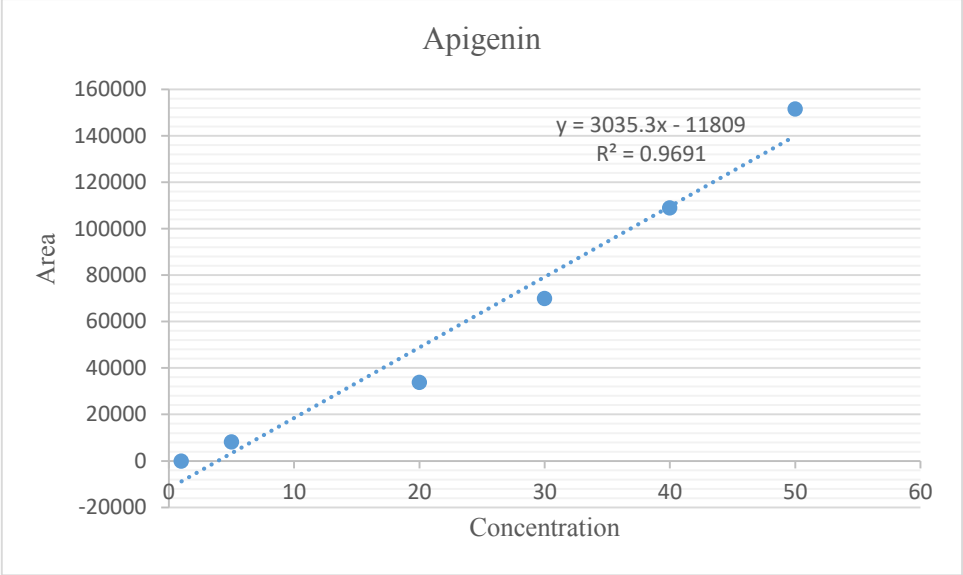

Caffeic Acid
y = 6952.6x - 24935
R² = 0.9282
Area
Concentration (ppm)
400000
350000
300000
250000
200000
150000
100000
50000
0
-50000
0
10
20
30
40
50
60

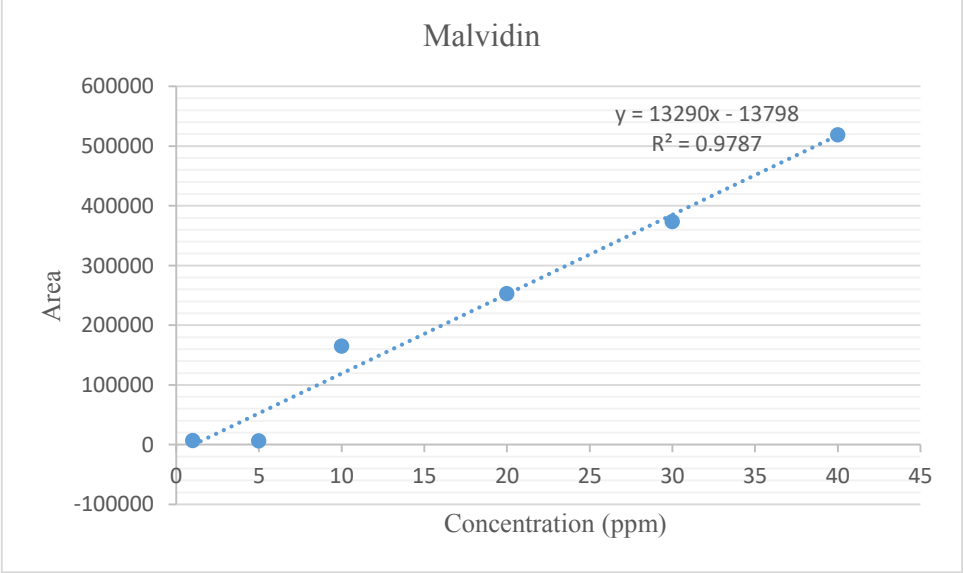

Coumaric Acid
y = 25900x - 38413
R² = 0.9575
Area
Concentration (ppm)
1400000
1200000
1000000
800000
600000
400000
200000
0
-200000
0
10
20
30
40
50
60

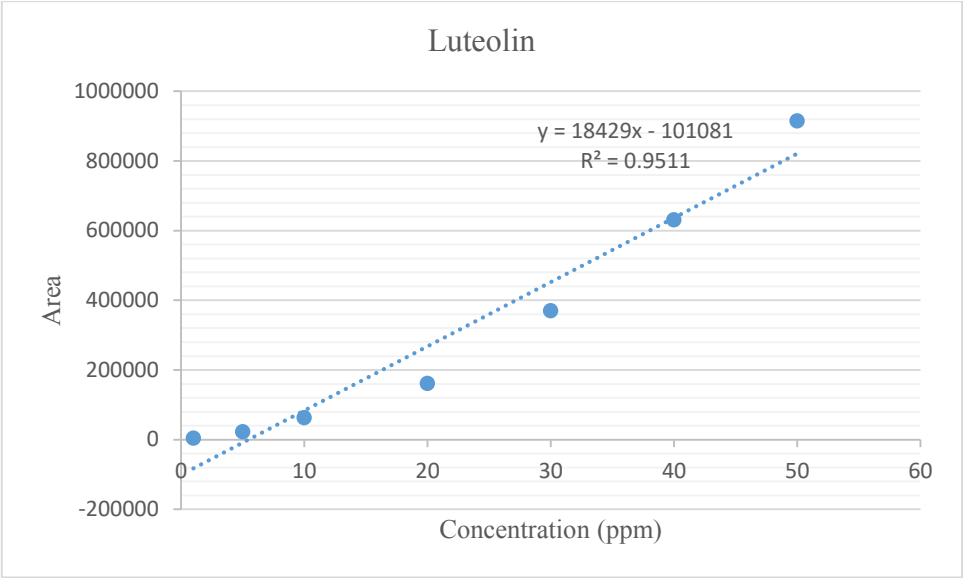

Ferulic Acid
y = 20234x + 74619
R² = 0.9747
Area
Concentration (ppm)
1200000
1000000
800000
600000
400000
200000
0
0
10
20
30
40
50
60

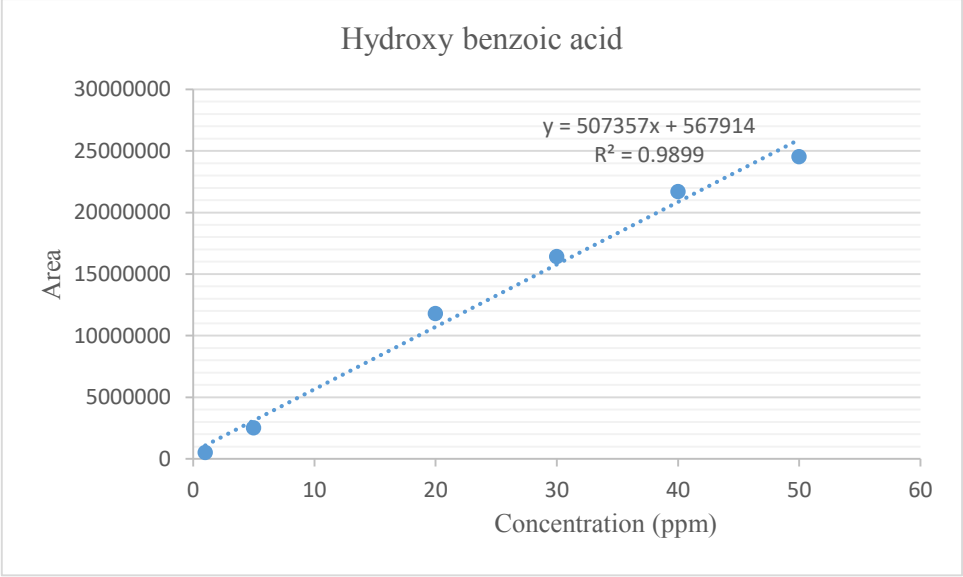

Sinapic Acid
y = 15718x - 70405
R² = 0.983
Area
Concentration (ppm)
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
0
10
20
30
40
50
60

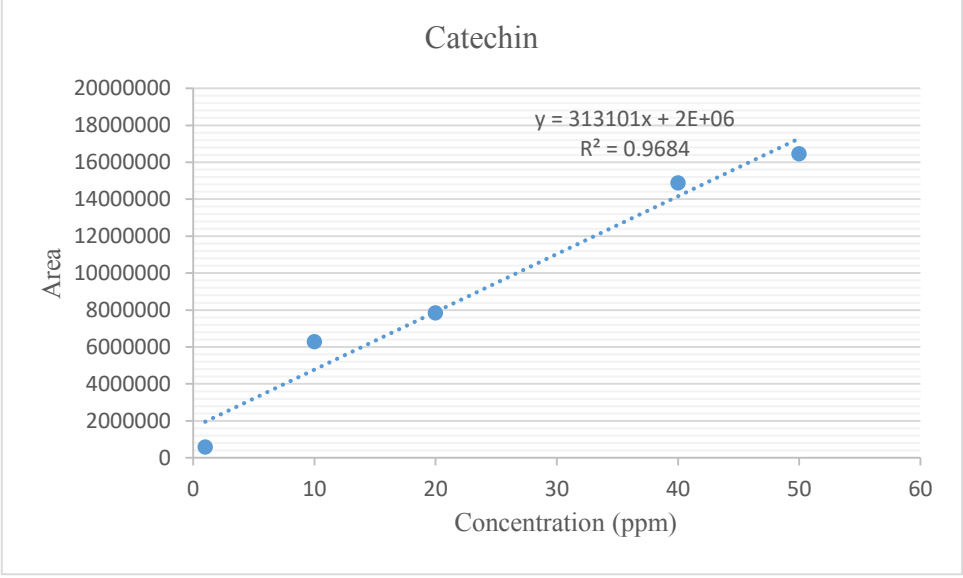

Salicylic Acid
y = 9662.4x + 11789
R² = 0.9854
Area
Concentration (ppm)
600000
500000
400000
300000
200000
100000
0
0
10
20
30
40
50
60

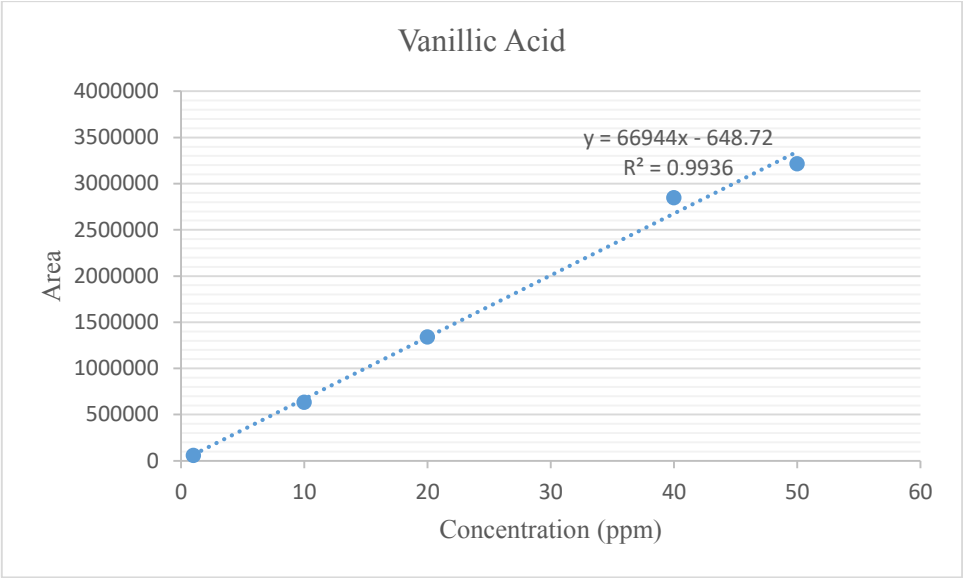

Apigenin
y = 3035.3x - 11809
R² = 0.9691
Area
Concentration
160000
140000
120000
100000
80000
60000
40000
20000
0
-20000
0
10
20
30
40
50
60

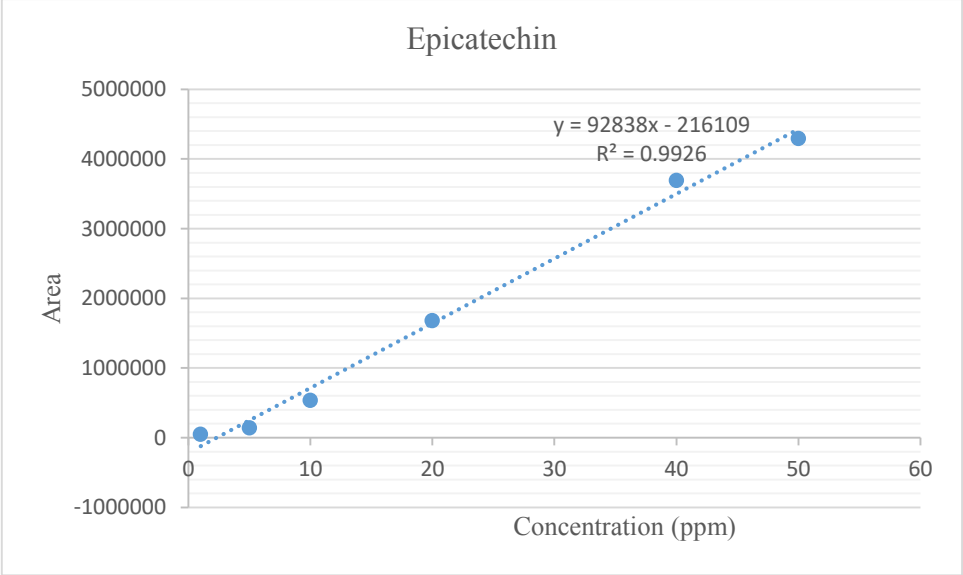

Malvidin
y = 13290x - 13798
R² = 0.9787
Area
Concentration (ppm)
600000
500000
400000
300000
200000
100000
0
-100000
0
5
10
15
20
25
30
35
40
45

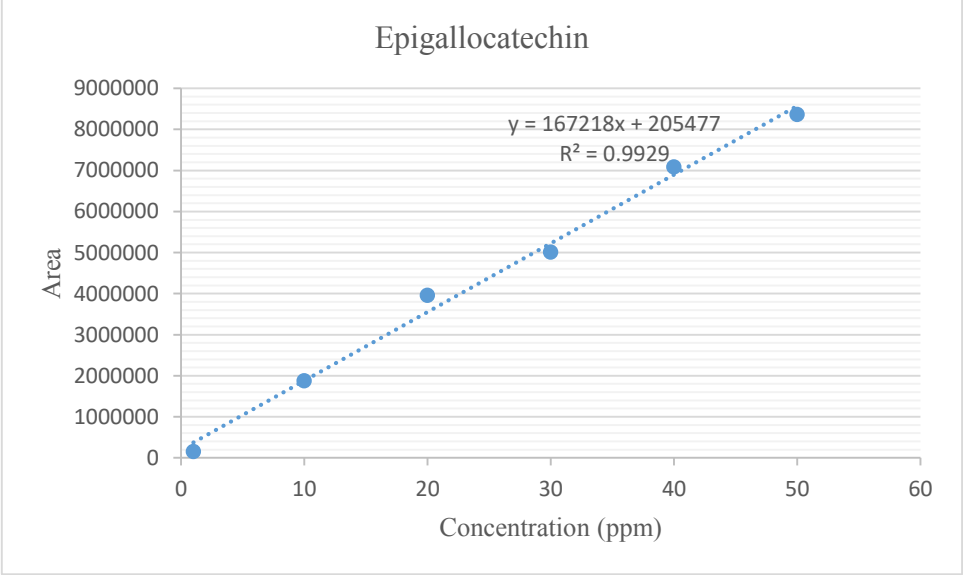

Luteolin
y = 18429x - 101081
R² = 0.9511
Area
Concentration (ppm)
1000000
800000
600000
400000
200000
0
-200000
0
10
20
30
40
50
60

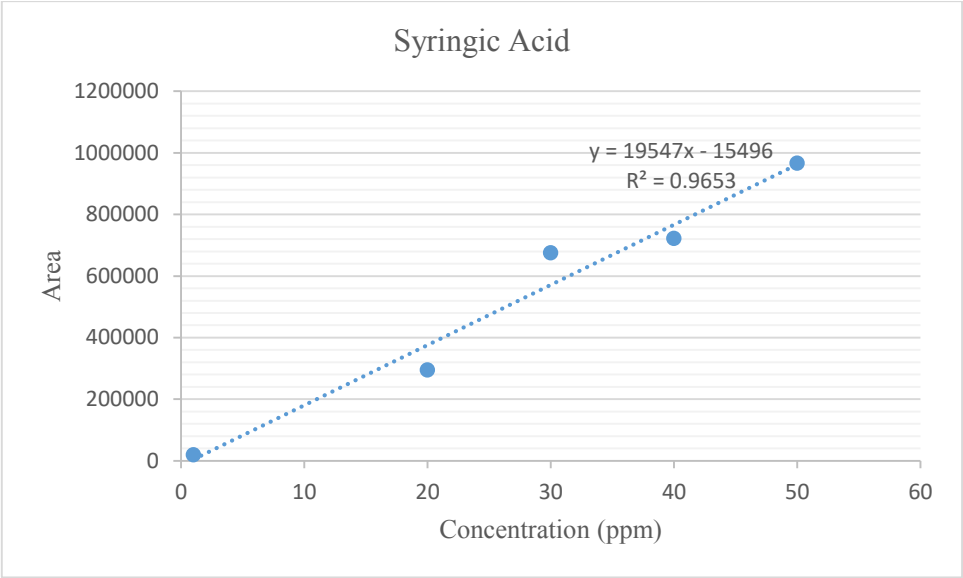

Hydroxy benzoic acid
y = 507357x + 567914
R² = 0.9899
Area
Concentration (ppm)
30000000
25000000
20000000
15000000
10000000
5000000
0
0
10
20
30
40
50
60

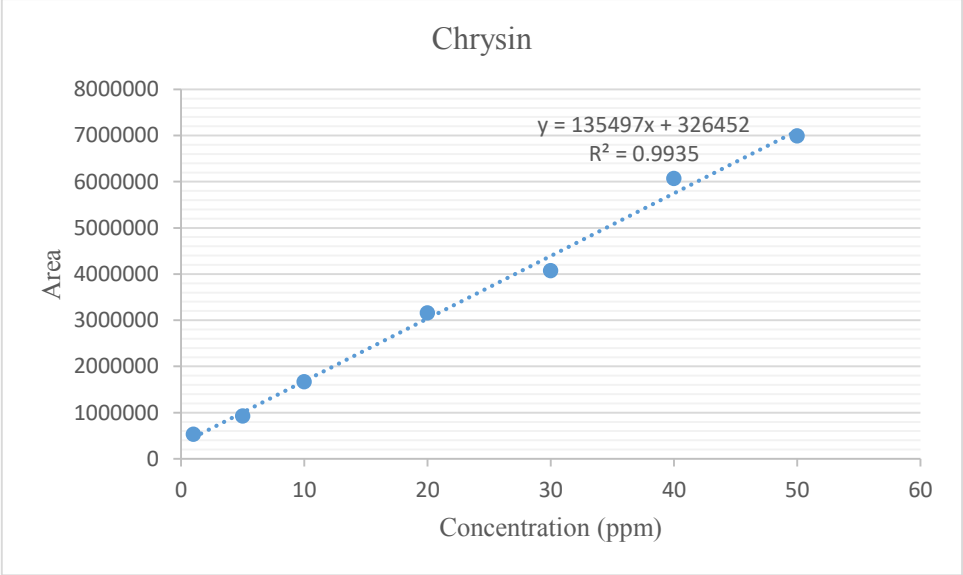

Catechin
y = 313101x + 2E+06
R² = 0.9684
Area
Concentration (ppm)
20000000
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
10
20
30
40
50
60

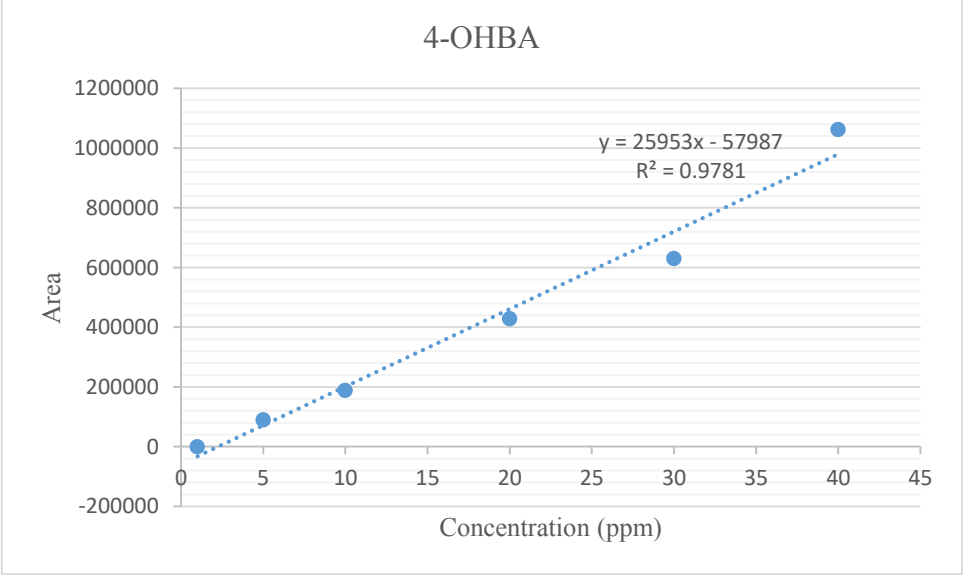

Vanillic Acid
y = 66944x - 648.72
R² = 0.9936
Area
Concentration (ppm)
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
0
10
20
30
40
50
60

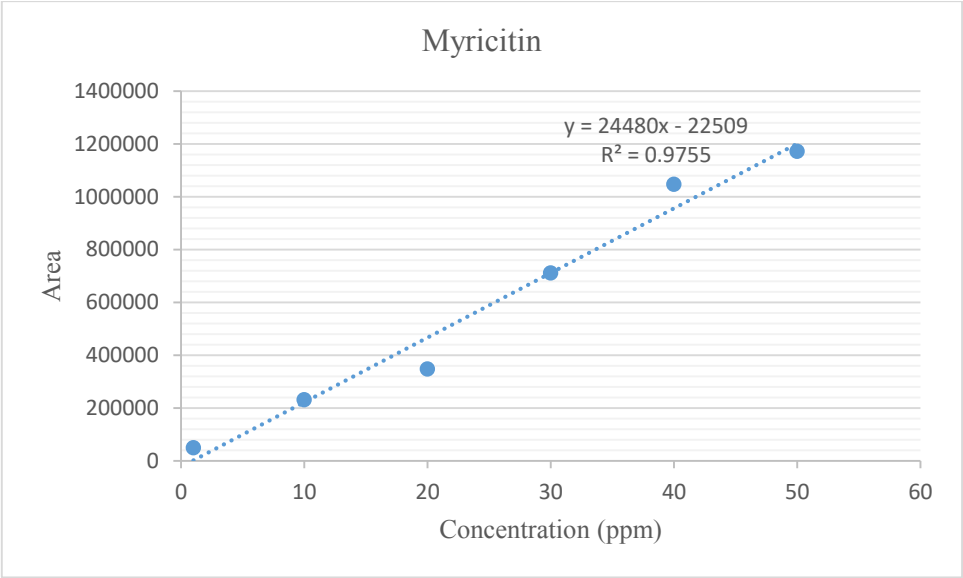

Epicatechin
y = 92838x - 216109
R² = 0.9926
Area
Concentration (ppm)
5000000
4000000
3000000
2000000
1000000
0
-1000000
0
10
20
30
40
50
60

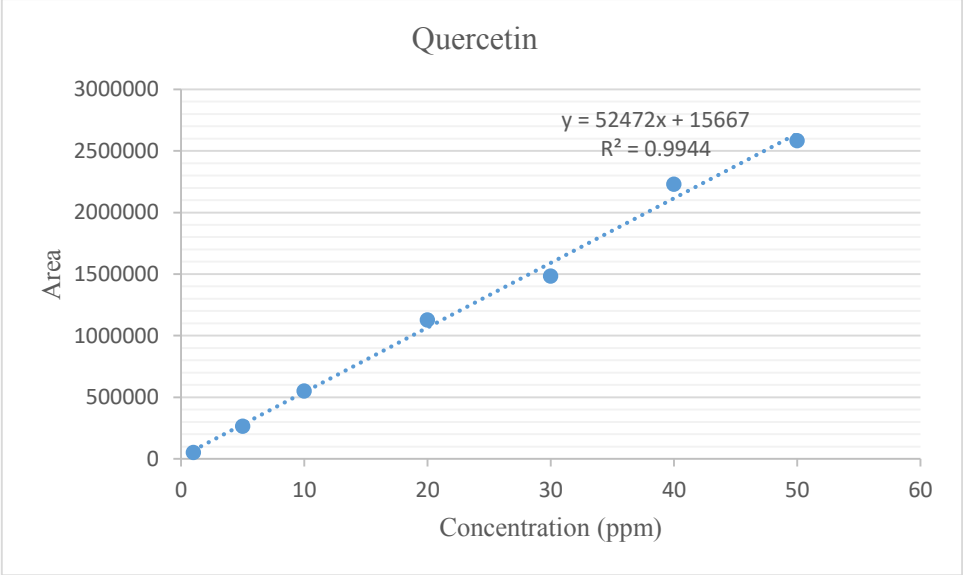

Epigallocatechin
y = 167218x + 205477
R² = 0.9929
Area
Concentration (ppm)
9000000
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
0
10
20
30
40
50
60

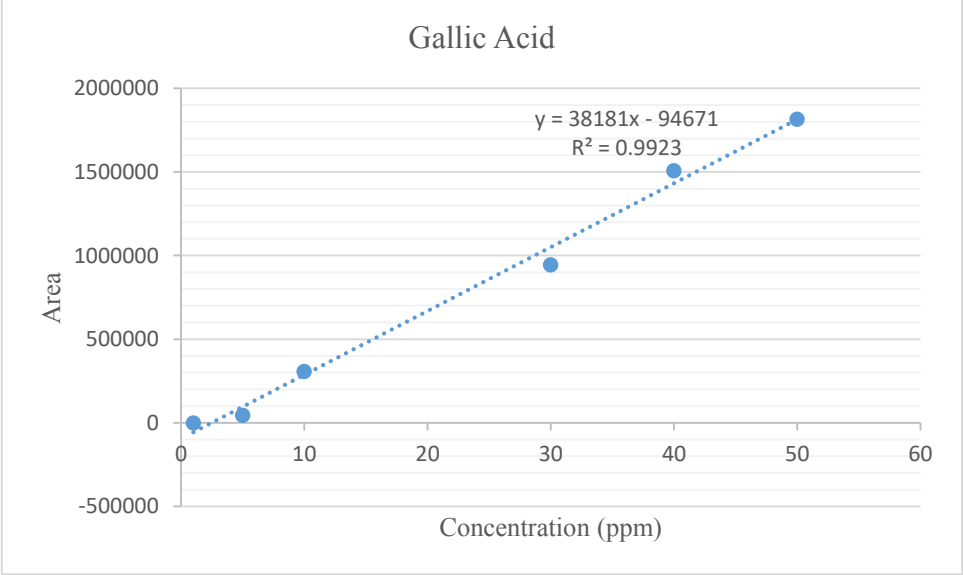

Syringic Acid
y = 19547x - 15496
R² = 0.9653
Area
Concentration (ppm)
1200000
1000000
800000
600000
400000
200000
0
0
10
20
30
40
50
60

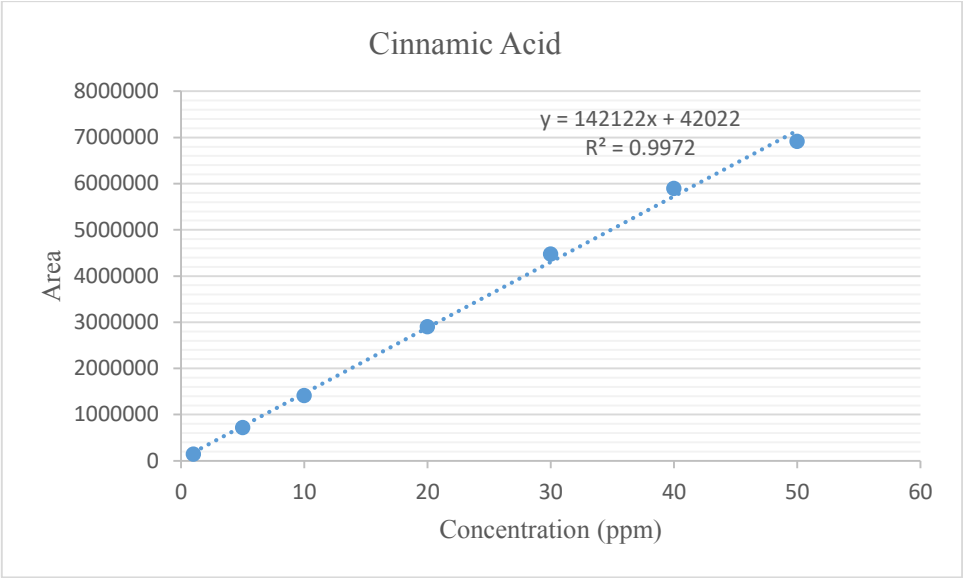

Chrysin
y = 135497x + 326452
R² = 0.9935
Area
Concentration (ppm)
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
0
10
20
30
40
50
60


4-OHBA
y = 25953x - 57987
R² = 0.9781
Area
Concentration (ppm)
1200000
1000000
800000
600000
400000
200000
0
-200000
0
5
10
15
20
25
30
35
40
45


Myricitin
y = 24480x - 22509
R² = 0.9755
Area
Concentration (ppm)
1400000
1200000
1000000
800000
600000
400000
200000
0
0
10
20
30
40
50
60


Quercetin
y = 52472x + 15667
R² = 0.9944
Area
Concentration (ppm)
3000000
2500000
2000000
1500000
1000000
500000
0
0
10
20
30
40
50
60


Gallic Acid
y = 38181x - 94671
R² = 0.9923
Area
Concentration (ppm)
2000000
1500000
1000000
500000
0
-500000
0
10
20
30
40
50
60


Cinnamic Acid
y = 142122x + 42022
R² = 0.9972
Area
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
0
10
20
30
40
50
60
Concentration (ppm)

VITA

Date of Birth: March 19, 1985, İstanbul

Work Experience:

December 2009-Present: Research asistant-İzmir Institute of Technology, Molecular Biyology and Genetics Departmant

Education and Training:

PhD- İzmir Institute of Technology, Departmant of Molecular Biyology and Genetics (2010-2017)

Master- İzmir Institute of Technology, Departmant of Molecular Biyology and Genetics (2007-2010)

Graduate: Ege Universty , Biochemistry Departmant (Biotecnology option) (2003-2007)

Certificates and Traning:

September 2012: RND project market in chemical substances and manufacture

April 2011: Iso9001 Seminar

April 2010: Molecular Dynamics Workshop

Ekim 2007: National Biotecnolgy Congress (poster presentation)

May 2006: HACCP ve İSO 9001 Training

Scientific Papers:

Ökmen, B., Sığva, H.O., Gürbüz, N., Ülger, M., Frary, A., Doğanlar, S. 2011. "Quantitative Trait Loci (QTL) Analysis For Antioxidant and Agronomically Important Traits in Tomato (*Lycopersicon Esculentum*)." Turk J Agric & Forest, 35: 501-514.

Gürbüz, N., Karabey, F., Öztürk, T. K., Kılınç, A., Frary, A., Doğanlar, S. 2015. "Glycoalkaloid Isolation from *Solanum Linnaeanum* Berries." Fruits, 70 (6): 371-375.

Çelik, İ., Gürbüz, N., Uncu, A. T., Frary, A., Doğanlar, S. 2017. "Genome-wide SNP Discovery and QTL Mapping for Fruit Quality Traits in Inbred Backcross lines (IBLs) of *Solanum Pimpinellifolium* Using Genotyping by Sequencing." BMC Genomics, 18 (1).