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QTL MAPPING OF EARLY BLIGHT RESISTANCE AND FRUIT QUALITY RELATED TRAITS IN A *Lycopersicon esculentum* × *L. pimpinellifolium* RIL POPULATION OF TOMATO

A Thesis in

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by

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Abstract

Early blight (EB) resistance and high fruit quality are among the most important and challenging characteristics in tomato breeding. This is due to complexity of these traits, which are also influenced by pleiotrophic and confounding effects of other characteristics. These challenges have undermined breeding efforts to improve tomatoes with genetic resistance to EB and higher fruit quality at the same time. The confounding and pleiotrophic effects of these traits with other horticulturally important traits can be detected by application of molecular breeding tools, such as genetic linkage maps, graphical genotyping, statistics and biometrical procedures and QTL analysis. The objectives of the current thesis research were to find the relationships and to identify QTLs for EB resistance and fruit quality traits.

A *Lycopersicon pimpinellifolium* accession (PSLP125) was determined to be resistant to EB with exceptionally high lycopene and soluble solid content. In order to exploit the genetic recourses of this accession in breeding cultivated tomato, an $F_{2:7}$ derived RIL population (n=172) of *L. esculentum* × *L. pimpinellifolium* cross was developed. The population was evaluated for EB resistance under field conditions and for fruit quality characteristics in the field and laboratory for three years and generations (2004-F_7, 2005-F_8 and 2006-F_9). A genetic linkage map was constructed based on 275 RFLP, ESTs (mainly candidate resistant genes), CAPS and SSR markers to perform QTL analysis. The map spanned 1061 cM of the tomato genome with an average of 3.8 cM between markers. Skewed segregation was observed for ~29.5% of the markers on all chromosomes except chromosomes 11 and 12. The average heterozygosity in the population was 6.8%, which was 4.7 times greater than what was expected (1.5%) for a RIL population at F_7 generation.

In order to identify QTLs for EB resistance, the population was evaluated for EB resistance for three years in the field. Simple, composite and multiple interval mapping procedures were employed and QTL analysis was carried out. In total 10 QTLs (LOD $\geq$ 2.4, $P \leq 0.001$) for resistance to EB were identified with individual effects ranging from 3.0% to 16%. Two QTLs on chromosomes 5 and 6 were highly consistent across the years/generations. Co-localizations of QTLs with several candidate genes such as *Mi-1*, ethylene response factor-5, lipoxygenase B,

wound-induced protein-1 were observed, suggesting potential involvement of these genes in EB resistance. Thus, it is speculated that the candidate-gene approach is an effective way of identifying and mapping new disease resistance genes in tomato.

The RIL population was also evaluated for three years in the field and laboratory for four fruit quality characteristics, including Fruit Weight (FW), Soluble Solids Concentration (SSC), pH and colorimetric parameters of purée. QTL analysis was employed to identify the QTLs for these fruit quality related traits. Several QTLs for FW on chromosomes 1, 2, 3, 4, 7 and 11, pH on chromosomes 1, 2, 6, 8, 10 and 12, SSC on chromosomes 1, 3, 6, 8, 9 and 10 lycopene on chromosomes 2, 4, 7, 11 and 12 were identified. The QTL analysis of lycopene using HPLC, spectrophotometer, statistical model derived data in previous generations and fruit color evolutions in the field, led to the identification of two major QTLs for lycopene on tomato chromosomes 7 and 12 and three minor QTLs on chromosomes 2, 4, and 11. Because of the consistency of the QTLs on chromosomes 7 and 12, they seem to be promising for developing QTL-NILs, as well as for improving tomato lycopene content by marker-assisted breeding.

In addition to QTL analysis, in 2006 (F_9), HPLC and spectrophotometric assays were employed to measure the amount of lycopene in a sample (n=127) of RILs. The results indicate that the spectrophotometric assay preserves the accuracy of the measurement using HPLC but eliminates the cost and degradation issues inherent with HPLC-based assays. Lycopene estimates obtained from this assay were highly correlated (r = 0.94) with estimates obtained from the HPLC method, suggesting the utility of this method as a substitute for HPLC assay. Furthermore, based on the HPLC assay a simple regression model was developed to estimate the amount of lycopene in previous generations/years using colorimetric values as independent variables.

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CHAPTER 1. General Introduction

Human and the Tomato

It is believed that tomato, *Lycopersicon esculentum* Mill. (syn. *Solanum lycopersicum*) was domesticated around 5000 BC (Stark 1981). Since then, it has been improved either consciously or unconsciously by humans. One aspect of the human-tomato connection is that the route to the tomato's domestication and cultivation has always been continuous and is still ongoing. As a result, new varieties of the cultivated species have integrated into different human societies, cultures and economies (Harvey et al. 2002).

The Tomato in America

The tomato plant originated in the Andes, a high mountain range along the west coast of South America (Smith 2000). It is not known whether indigenous people consumed tomatoes before the Spanish conquest. There exists evidence suggesting that tomato was domesticated in Mexico; however, it is not clear how wild tomatoes were moved from the Andes to Central America in pre-Columbian era (Smith 2000).

Spanish came across tomato for the first time between 1519 and 1521 after the occupation of the new world (Smith 2000). In 1529, a Franciscan, Bernardino de Sahagún, immigrated from Spain to Mexico and started learning the language of Aztec tribe (Nahuatl). He was the first known European who used the word "tomato" in his writings. Shortly after the Hernan Cortés's conquest of Mexico, the tomato was introduced to Spain and Italy, and from there throughout the Europe (Smith 2000).

In the United States, because tomato was long thought to be poisonous like its close wild relative nightshades, no one dared to eat it. Robert Gibbon Johnson is believed to have been the first American who ate tomatoes in September 1820 in Salem, New Jersey (Smith 2000). He had imported some tomato seeds from South America and planted them in his garden. After his tomatoes set fruits and ripened, he decided to eat them. On the steps of the Salem's courthouse,

the onlookers were waiting for him to eat tomato and die. “What are you afraid of? Being poisoned?” He asked the crowd. “Well I am not, and I’ll show you fools that these things are good to eat” (Smith 2000). Of course, Johnson did not die, and later established a huge tomato industry.

Who did introduce tomato to America? When was it first used in American cuisine? Where was it first cultivated? These, as well as many other similar questions, have been asked by Americans for more than 150 years. However, most of them remain unanswered. Andrew Smith has a comprehensive review of the history of tomato in America in his book “*The Tomato in America*” (Smith 2000).

Biosystematics of Tomato

According to the USDA Natural Resources Conservation Services (USDA 2006) the nightshade family (*Solanaceae*) belongs to Order “*Solanales*”, Subclass “*Asteridae*”, Class “*Magnoliopsida”* / “Dicotyledons”, Division “*Magnoliophyta*”/ “Flowering plants”, superdivision “*Tracheobionta*” / “Vascular plants” and Kingdom “*Plantae*” / “Plants”. According to relatively old references *Solanaceae* family consists of 91 genera (D'Arcy 1979) and ~3500 species (D'Arcy 1986). Based on on-line resources the *Solanaceae* family contains 41 genera and 285 accepted taxa overall (USDA 2006). Distribution of many species is still unknown and how to assign individual species into genera is far from being agreed on by botanists, systematists and plant breeders.

Many important crop plants belong to this family, playing an important role in human life, including being in peoples’ daily diet, having pharmaceutical purposes, serving as ornamentals, and many other applications. It is not an exaggeration if one says “I have at least one of the members of this plant family in my daily diet in one form or another”. They are either fruit-bearing plants such as tomato, pepper or eggplant or tuber-bearing plants like potato. Tomato, eggplant and potato are members of the genus *Solanum*, which is the biggest genus of the *Solanaceae* family.

Lycopersicon or Solanum?

The *Solanum* genus has recently become more important by incorporating the tomato genus (*Lycopersicon*) as a member, which also led to changing the scientific name of tomato from *L. esculentum* to *S. lycopersicum* (Marshall et al. 2001; Peralta et al. 2005; Spooner et al. 1993). The tomato was originally considered to be a member of *Solanum* genus as named by Linnaeus in 1753. Later, Philip Miller (Miller 1754, 1768) used *L. esculentum* in the *Gardeners Dictionary. L. esculentum* was an accepted name for tomato for more than two and a half centuries. Recently, there has been debate among botanists and plant breeders with respect to the classification and nomenclature of tomato. To be consistent with previously published papers and books and to prevent any confusion I will use the Miller's classification (*Lycopersicon*) hereafter.

Composition of the genus *Lycopersicon*

The genus *Lycopersicon* contains the cultivated tomato, *L. esculentum,* and eight closely related wild species (Rick 1976). The genus was originally divided into two groups (Muller 1940):

(a) Color-fruited species
(b) Green-fruited species.

This classification, however, does not necessarily reflect the fundamental differences between the species. Rick (1976) classified and defined sub-generic species of *Lycopersicon* based on their crossability with the cultivated tomato and recognized the *esculentum*-complex and *peruvianum*-complex.

Species in the Esculentum-complex

This is the largest complex in the *Lycopersicon* genus. It consists of seven species, including *L. esculentum*, *L. pimpinellifolium,* and *L. cheesmanii* as the colored (red-fruited) species, and *L. parviflorum, L. chmielewskii*, *L. hirsutum,* and *L. pennellii* as the green-fruited species.

Species in the Peruvianum-complex

L. chilense Dun. and *L. peruvianum* (L.) Mill are the two known species in this complex. Due to the presence of critical hybridization barriers, making interspecific crosses between these species and *L. esculentum* are not possible without employing techniques such as embryo rescue or pollen mixture (Smith 1944). This was the main reason for considering them as a separate complex (Rick 1976).

Other closely related Solanum Species

The *Solanum* is a vast genus in the plant kingdom. It is often hard to distinguish closely related species within the *Solanum* genus from one another. There are a number of *Solanaceae* genera that are very similar to tomato. These species belong to a section of *Petota* and sub-section *Potatoe* (D'Arcy 1972; Taylor 1986). One of these sub-sections (series *Juglandifolia*) contains species that are highly similar to the genus *Lycopersicon* (Table 1-1). The series *Juglandifolia* consists of four species. Two of them, *S. lycopersicoides* and *S. rickii*, morphologically resemble each other and to the tomato. However, there are two others, *S. ochranthum* and *S. juglandifolium*, that are far from the other two and from tomato but are very close to each other. For instance, *S. pennellii* used to be considered as a member of *Juglandifolia* but Correll (1962) removed it from this classification and considered it as a member *Lycopersicon* species (Correll 1962). According to this classification, S. *lycopersicoides* and *S. rickii* can be considered as new members of *Lycopersicon*. Then series *Juglandifolia* would have been more homogeneous than it is now. There are still debates regarding classification of tomato and closely wild relatives among taxonomists and plant breeders. To update the current changes in tomato systematics the new findings based cpDNA and nuclear DNA markers will be discussed below.

New classification and nomenclature of tomato

As described above, the late Charles Rick (1915-2002), the former curator of Tomato Genetic Resources Center (TGRC), Department of Vegetable Crops, UC Davis, recognized two species complexes based on crossing relationship. The *esculentum*-complex comprises seven species that are inter-crossable. The *peruvianum*-complex includes two species, *L. chilense* and *L. peruvianum*. More recent studies have provided molecular evidence for justifying the inclusion

of tomato in *Solanum* species and no longer considering *Lycopersicon* as a separate genus within *Solanaceae* (Peralta et al. 2005). In the most recent studies recognition of species has been based on morphological, molecular and ecological data rather than crossability of the collected specimen. Morphological data has been used for identification of many specimens within the TGRC. The new classification, names and equivalent former names have been revised by Knapp and Peralta (Knapp 2006; Peralta et al. 2005). A prominent feature of the new nomenclature is that tomato complexes have kept intact but the name of *Solanum* is being used instead of *Lycopersicon* for the genus and for cultivated tomato *S. lycopersicum* is adopted instead of *L. esculentum*.

Botany

Perennial or annual?

The tomato is a short-lived perennial plant that is planted as an annual crop in most area (Rick 1976). It is highly sensitive to fall or winter frosts; however it can be planted in the greenhouse all year round.

Flower Structure

The tomato has perfect, hypogynous and regular flowers (Taylor 1986). The number of floral organs varies from species to species, but in cultivated tomato there are five or more sepals and five or more petals. Stamens are normally alternate with petals and there are two or more joined carpels. In the early stages of the growth of a flower bud, the petals are enclosed within the calyx. When the sepals separate, petals get exposed, elongate and extend beyond the calyx. In a fully opened flower, the petals curl backward which in turn expose the anther cone. The anther cone normally surrounds the style and stigma. Once pollination occurs and fruit sets, petals, stigma and style will abscise, while the pedicle, calyx and ovary continue to grow.

Pollination and Fertilization

Most modern tomato cultivars are self-pollinated (Ho and J.D.Hewitt 1986). The length of style is under genetic control and is affected by growing conditions. It has been reported that low light or high temperature conditions may cause disproportionate exertion of the style (Levy et al.

1978). A mature pollen grain is ready for pollination right after the time of anthesis, while the stigma is receptive two days before anthesis and remains receptive for 4 days or more (Smith 1935). Within an hour after pollen grains reach the stigma, pollen tubes start to grow. They reach to micropyle in ~12 hours at 25°C. Most of the ovules are fertilized within 30 hours at 20°C (Ho and J.D.Hewitt 1986). As mentioned above, the cultivated and most of the wild tomato species are self-compatible, though a few wild species within the genus are self-incompatible.

Morphology of fruit and ripening Process

Botanically, tomato is a fruit because it develops from an ovary. However, it is often consumed as a vegetable in cooking. The tomato fruit is a typical example of a berry type fruit, containing seeds that are located in a locule cavity and supported by a fleshy pericarp developed from an ovary. Pericarp walls and skin (exocarp), including placenta, locular tissue and seeds make up the tomato fruit content. The ovary wall makes the pericarp, which consists of an exocarp or skin, mesocarp and a single-celled layer of endocarp. The fleshy tissue of the fruit is nothing more than pericarp and placenta. Tomatoes can have either a bilocular or multilocular structure, though a majority of cultivated tomatoes are multilocular except cherry tomatoes.

The ripening of the fruit is a complex process in which a series of synthetic and degradative reactions occur (Grierson and Kader 1986). Dramatic changes in color, soluble solids, pH, aroma, flavor and texture of the fruit occur during the transition from mature green to fully ripe stage. According to USDA classification there are six distinct stages of fruit maturity, including green, turning, breaker, pink, light red and red. To test whether or not a fruit is mature, one needs to cut a fruit in half. If the knife cuts the seeds the fruit is still immature, otherwise the fruit is in one of the other ripening stages. In the breaker to pink stage, α- and β-carotene concentrations reach to maximum. During this stage a half ripe tomato appears to have an orange color. As the ripening proceeds in red-fruited tomatoes, a rapid accumulation of lycopene gives a tomato its red color.

Growth habit

In terms of growth habit, tomatoes are classified as “determinate” or indeterminate” (Atherton and Harris 1986). In some cases, a third semi-determinate type can be seen. The indeterminate

plants can grow indefinitely in optimal conditions. This gives the plants a prostrate or scandent look.

A young four-week-old tomato plant produces 5 to 7 leaves. Then it produces flower clusters. Each is so called a “hand”. In indeterminate plants, the main shoot continues to grow upward. It terminates with a flower cluster followed by a new leaf emergence. Right before flower cluster formation a new leaf bud emerges and occupies its own position for later growth. It continues to grow until the next leaves and flower cluster form. Usually three leaves form with a flower cluster between the second and third leaves. In the mean time lateral buds grow and produce the side branches. The main branch and the side branches continue to grow in the same way, giving the plant a prostrate, upright prostrate or scandent appearance.

In determinate tomato plants the axis of the last formed leaf of the main branch may change into an inflorescence and terminate there without further leaf initiation. However, the sympodial bud continues to grow to form a leaf above the inflorescence. Since no new leaves initiate after this leaf, extension of the shoot axis is terminated with the formation of the second inflorescence. Primary shoot axis then extend by addition of further lateral shoots to the sympodium until the last growing point fails to initiate formation of new leaves and ends up with only an inflorescence (Atherton and Harris 1986).

Color

In addition to flavor, firmness, shelf life and other desirable characters, color is one the most important traits of tomato fruit in a breeding program. Tomato fruit can be yellow, orange, pink or red. Red is the most prevalent and desirable color. The red color of tomato is preliminary due to a member of carotenoids called lycopene. *β*-carotene is another major carotenoid found in tomato fruit that determines the orange color of tomato. The amount of *β*-carotene also determines the amount of vitamin A activity in the fruit (Stevens and Rick 1986). Carotenoids are natural fat-soluble pigments mainly found in plants, algae and photosynthetic bacteria. More than 600 carotenoids occurring in the nature (Ong and Tee 1992). In non-photosynthetic organisms like yeast or molds, carotenoids have a protective role. Animals are unable to synthesize carotenoids; however they incorporate and use them in their diet as an antioxidant

agent and a source of vitamin A (Mera Pharmaceuticals Inc 2002). Carotenoids characteristics are discussed in more detail in Chapter 4.

Flavor

Flavor is perceived by the composition of sugar and acid content of the fruit in addition to many volatiles. It is believed that the amount of volatiles such as 2-isobutylthiazole, methyl salicylate and eugenol is a major factor in perception of flavor (Stevens 1970). The sugars found in tomato fruit are mainly glucose and sucrose (Baxter et al. 2005). Citrate and malate are the two majors acids found in tomato fruit (Stevens and Rick 1986). The best tomato flavor is characterized by high sugar/acid content. High-sugar/low-acid tomatoes are bland, low-sugar/high-acid tomatoes are tart and low-sugar/low-acid ones are tasteless (Peet 2006).

Fresh market and processing tomato

There are major differences between the tomato bought in a market (fresh) and processing tomatoes. Fresh market tomatoes are normally picked in the mature-green or turning stage and promoted to ripen using the plant hormone 'ethylene', whereas processing tomatoes are picked naturally ripe and red. Processing tomatoes usually are machine-harvested and they have to be transported to the cannery by an uncovered truck within few hours. Processing tomatoes are characterized by having oblong (elongated) or square- round shape, more like plum or pear, while fresh market tomatoes are usually round flattened in shape. Processing tomatoes have thicker walls, more soluble solids and are firmer than fresh market tomatoes. Therefore they hold their shape when cooked. Fruit firmness is quantitatively controlled and has resulted from many generations of selection. Processing tomatoes can be used to make paste, ketchup, sauce, canned tomatoes and many other products. Fresh market tomatoes are juicier with higher water content and about 5% solids. The fruit solids consist of water-soluble substances like sugars and acids and water-insoluble (alcohol-soluble) materials such as cell walls and other cell components. In breeding processing tomatoes, a high ratio of insoluble solids to total solids is desirable (Peet 2006).

Crop production

Recent trends in production/consumption in the U.S and the world

The cultivated tomato has become the second most important vegetable crop in dollar value in the U.S. since 1900 and is highly popular elsewhere (Rick 1980b; University of Georgia 2006). In the past 10 years, world production has increased by ~42% (ERS-USDA 2006b). Production increased from 95.6 million metric tons (MT) in 1995 to 136.4 in 2005. The average of 10 years of production in the leading countries shows that China is the leading country in tomato production with 24.6 million MT followed by the United States with 12.6 million MT (Figure 1-2) The total harvested acreage of tomato has decreased since 2000, however, the total production has increased (Figure 1-2). This is mainly due to better cultural practices and the use of high yield genotypes or better cultivars. There has been a 40% increase in fresh-market tomato consumption per capita in the U.S. from 1979 to 2006, reaching to nearly 20.7 pounds in 2006. The average retail price has also risen from $1.29/lb in 1997 to $1.61 in 2005. However the trend for monthly average retail price has been in a steady state from year to year (2000-2005).

Trade

Fresh market tomatoes

International trade has been an important component of the fresh-market tomato in the U.S. Currently, approximately one-third of the fresh tomatoes consumed in the U.S. are imported, as compared to one-fifth during 1980s (ERS-USDA 2006a). The amount of exported tomatoes has remained relatively constant (~7%) since 1980. Over the past decade, Canada's hothouse tomato market has become so successful that it has taken the control of the market from Mexico. For the winter and early spring, Florida and Mexico have been competing historically for the U.S. market. The imports from Mexico peak in winter, while early spring tomatoes are mostly provided by the Florida tomato industry as Mexico production declines. Florida tomato production eventually controls the market during the entire spring. However, Mexico is still the major exporter of fresh-market tomato to the U.S. after hothouse tomatoes from Canada. Canada is also a main importer of U.S. tomatoes with Mexico and Japan ranking in the second and third.

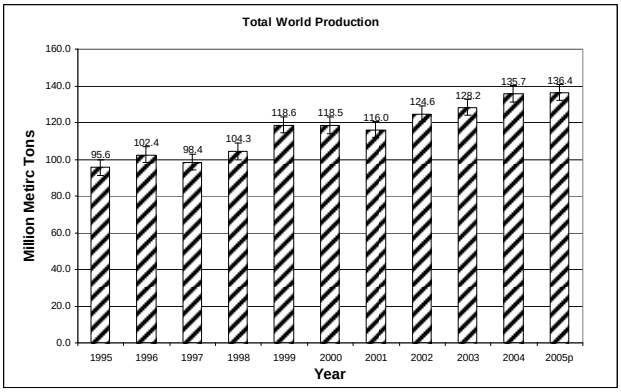

Figure 1-1 World Production of Tomato, 1995-2005 (ERS-USDA 2006b)

**The error bars represent the standard error of data.* p = preliminary data

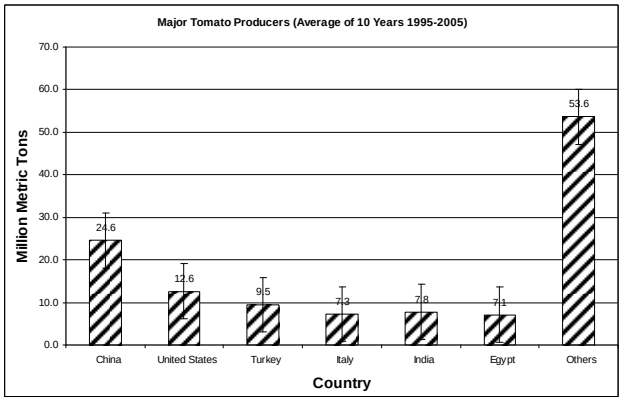

Figure 1-2 Major Tomato Producers of Tomato, 1995-2005 (ERS-USDA 2006b)

**The error bars represent the standard error of data.*

Processing tomatoes

More than 50% of the U.S. produced fruits and vegetables are being processed (ERS-USDA 2006a) Among vegetables, the two topmost are tomato and potato. Tomato accounts for 85% and potato accounts for 68% of the sales. The U.S. has been a major exporter of processed tomatoes since 1990s. On average, about 6% of processed tomatoes are exported (ERS-USDA 2006a). The major importers of the U.S. products are Canada, Mexico, Japan and South Korea. Canada alone accounts for 50% of the U.S. exports. Exported products include tomato sauce, followed by paste, ketchup and whole canned fruits. In terms of imported processed tomatoes, about 6% of

tomato products consumed in the U.S. are imported. This is mainly ketchup. Canada is the largest exporter of processed tomato to the U.S. followed by Chile, Mexico, Italy and Israel.

History of tomato breeding

In Europe, Italians were the first tomato breeders. They began breeding about 200 years ago (Boswell 1937; Taylor 1986). They started breeding tomatoes with a wide range of fruit color and shape probably through selection within heterogeneous populations. In the U.S. tomato breeding started a little more than a century ago. Before that, most of the cultivars grown in the U.S. were imported from England and a few were imported from France (Stevens and Rick 1986). Livingston (1821-98) is known to be the first tomato breeder in the U.S. In 1870 he introduced his first variety, called "The Paragon Tomato". It was the first perfectly and uniformly smooth tomato in the U.S. He claimed it was the first such tomato in the world. (Livingston 1998). He later introduced more cultivars such as Acme, Perfection, Golden Queen and Large Rose Peach. He did not believe in hybridizing tomatoes after failed attempts to make crosses. Most of today's cultivars are bred through hybridization and selection among the segregating progeny. In 1921, the first disease resistant cultivar in the U.S. (Louisiana Wilt Resistance) was introduced by Essary and Edgeton (Stevens and Rick 1986). By the mid 1950s, most of the efforts were focused on breeding for multipurpose tomatoes that could meet the needs of both the fresh market and processing industries. However, the progresses were made in both industries and created the option for developing tomatoes that could serve the needs of both industries. There are some desirable traits that are common between the two industries, such as yield, disease, insect or drought resistance, earliness, resistance to cracks caused by rain, good flavor and many other fruit quality related characteristics.

Desirable traits for fresh market tomatoes

There are several specific traits that are more desirable in the fresh tomato industry than the processing industry. These traits include long shelf life, large fruit size, round fruit shape, smooth skin, uniform redness in all layers, smaller stem joint point wound, free from physiological disorders, firmness of pericarp tissue, good taste and flavor (Stevens and Rick 1986).

Desirable traits for processing tomatoes

The needs of the processing industry can be divided into two subcategories. One is plant type related traits and the other is fruit quality related ones. With respect to plant type, it should be determinate, compact and jointless to make it amenable to machine harvest. With respect to fruit type, firmer fruit with thicker walls and reduced locular tissue, higher solids and viscosity of juice with higher yield potential (which is very difficult to breed for) and adequate pH (Stevens and Rick 1986).

Tomato genetics

Tomato is an excellent model organism for genetic studies. It has a short life span, enabling plant geneticists to have 2-3 generations in a year in the greenhouse or in the field in warm areas such as California and Florida. It also has a relatively small genome size (950 Mb), which has packed in 12 chromosomes in its haploid form (NRCPB 2007; SGN 2007). Most of the tomato genome comprises of heterochromatin (75%) which to our knowledge, does not have any genes (SGN 2007). The majority of the genes found in distal part of chromosomal arms in highly dense euchromatic regions (SGN 2007).

All cytogenetics studies have confirmed that tomato is a diploid plant and chromosomal unbalances are not very tolerable. The maximum number of extra chromosomal segments that are tolerable by the genome is three and no euchromatic deficiency of any size has been found to be tolerable (Stevens and Rick 1986).The first linkage studies on tomato started in as early as 1917 by Jones (Jones 1917; Stevens and Rick 1986). Like other organisms the progress was very slow at first, but it was expedited by applying cytogenetics techniques. Since then nearly 1070 monogenic mutants (in 622 loci) have become available to provide materials for basic research and to find phenotypic markers (Chetelat 2007; Emmanuel and Levy 2002 ; Stevens and Rick 1986; Stubbe 1972a, b; TGRC 2007). Aneuploids were used to map markers to chromosomes and even to chromosomal arms (Stevens and Rick 1986). Once the framework maps were constructed, other markers were added to the map for linkage analysis. Construction of the first genetic linkage map of tomato was reported in late 1960s (Butler 1968). This map contained all 12 tomato chromosomes with ~153 morphological and physiological markers. This genetic map has grown more saturated until the mid 1970s and early 1980s when it had a total of 324 makers,

of which 89 were only assigned to chromosomes but their positions were not conclusively determined (Rick 1975; Rick 1980a). Before the advent of DNA markers in early 1980s isozymes markers became very popular in tomato genetic mapping, largely due to several advantages over phenotypic markers such as being co-dominant with no epitasis effect. The disadvantages of isozymes markers include their limited number (~35 isozymes have been mapped in tomato) and lack of polymorphism in closely related species. The introduction of DNA markers (Bostein et al. 1980) started a new era in the construction of tomato genetic maps. They had several advantages over phenotypic and isozyme markers, including being abundant in number, free of pleiotropic effects, and some being co-dominant such as RFLP (Restriction Fragment Length Polymorphism) and SSR (Simple Sequence Repeats) markers. These and other advantages led to the development of new genetic maps based on DNA markers in the mid 1980s. The first tomato molecular genetic map based on DNA markers was developed in 1986 (Bernatzky and Tanksley 1986), consisting of 18 isozyme and 94 RFLP markers with the probes derived from cDNA clones. The first saturated molecular linkage map of tomato was published in 1992 (Tanksley et al. 1992). This map was originally developed using 67 segregating individuals of an F_2 population of a cross between *L. esculentum* cv VF36-*tm2a* and *L. pennellii* LA716. The map consisted of 1030 markers, including morphological, isozyme and RFLP makers, with a mean marker interval of 1.2 map units (cM). By 1996 the map was more saturated with a mean marker interval of ~1.0 cM (Pillen et al. 1996). With the use of new genetic markers such as AFLP, SSR, CAPS, EST and COS, new genetic maps have been developed. For example, another high density map based on an F_2 population of a cross between *L. esculentum* LA925 and *L. pennellii* LA716 has been developed that consists of 2302 markers (1342 RFLPs, 785 CAPS, 19 SNPs and 156 SSRs) (Fulton et al. 2002). The number of sequenced ESTs is 213,947 as of 02/18/2007 corresponding to 41,425 unique sequences in the most recent EST assembly (Computational Biology and Functional Genomics Laboratory 2007). The linkage between mutants and DNA sequences is known only for a few dozen tomato genes (Emmanuel and Levy 2002). A rough estimate for the number of tomato genes ranges from 30,000 to 40,000 (Emmanuel and Levy 2002). Tomato sequencing project is far behind many other plant genome sequencing projects. The tomato sequencing project is coordinated among 10 different countries around the globe with each country being responsible for sequencing one chromosome except that the Unite States is sequencing chromosomes 1, 10 and 11 (SGN 2007).

Why are new and species-specific genetic linkage maps needed?

Considering that there already exist highly saturated maps available, why do we need to develop new genetic maps? The answer goes back to the nature of the population these maps founded on. The haploid genome size of tomato is 950 Mb. Considering 2302 markers on a map and assuming that they are uniformly distributed (which is not the case), it means that each centiMorgan (cM) in the map is equal to ~410 kb. Therefore, theoretically it should be possible to find any gene of interest within a few cM vicinity of a genetic marker. However, there are reasons that necessitate construction of new genetic linkage maps. First of all, not all genes of interest are segregating in the population used to construct the high-density molecular linkage map of tomato, including genes responsible for horticulturally-important traits. Second, not all markers in the high-density map are polymorphic and useful in other populations; in particular populations derived from crosses between *L. esculentum* and closely-related wild species *L. pimpinellifolium*. Due to this limitation, most of the newly developed maps are medium density and are not saturated. For example in a BC_1 population developed from *L. esculentum* × *L. pimpinellifolium* cross only 30% of the makers from the high density map were polymorphic between the two parents (Chen and Foolad 1999). To overcome this problem and to exploit the genetic potential of close relatives of tomato it is necessary to develop new markers that can detect polymorphism in populations derived from crosses of *L. esculentum* and closely related wild relatives. Such attempts are underway to develop new PCR based markers such as SSR, SNP and EST makers that can be used in many labs around the world (Frary et al. 2005; Labate and Baldo 2005).

Populations used in genetic linkage map construction

In order to construct any genetic linkage map a segregating population is needed. In self-pollinated plants a segregating population is often generated from a cross between two inbred lines. The F_1 progeny is self-pollinated to produce an F_2 population. F_2 populations have maximum linkage disequilibrium and segregation of genes and markers, and thus can serve as mapping populations. In some cases, researchers use backcross populations as mapping populations by backcrossing the F_1 to one of the parents. Backcross Inbred Lines (BILs) (Paran et al. 1995) and Recombinant Inbred Lines (RILs) (Goldman et al. 1995; Paran et al. 1995;

Saliba-Colombani et al. 2000) are two other useful populations that are being used in genetic linkage mapping. The procedure to generate RILs will be discussed in detail in Chapter 2.

L. esculentum × *L. pimpinellifolium maps*

Tomato has a very narrow germplasm base, largely due to two genetic bottlenecks that occurred in the past. Therefore finding polymorphic markers in crosses between *L. esculentum* × *L. esculentum* is a tedious task. Because of this, researchers have often developed populations using cultivated tomato as one parent and a wild relative as the other parent to assure presence of marker polymorphism. However, the degree to which polymorphism is detected is not always same in all interspecific crosses varying from species to species and even within species.

To date there have been several tomato linkage maps developed based on crosses between the cultivated tomato and its close wild species *L. pimpinellifolium* (Table 1-1). In most cases, except the map in the current study (F_7-RIL) and previously developed F_2 map in our lab, the *pimpinellifolium* accession used was either LA1589 or LA722. In the current study a *L. pimpinellifolium* accession (PSLP125), which is high in lycopene and exhibits resistance to early blight was used to develop the genetic linkage map in crosses with a *L. esculentum* superior breeding line (NCEBR-1) developed in the North Carolina State University tomato breeding program PSLP125 was first selected from among ~250 wild tomato accessions that were evaluated in the Penn State tomato-breeding program in the field and greenhouse for early blight resistance and other horticulturally important traits. Additional descriptions of the parents of the mapping population are provided in later chapters.

Table 1-1 Genetic linkage maps of tomato developed based on *L. esculentum* × *L. pimpinellifolium* crosses

L. esculentum × *L. pimpinellifolium*	Population type	Pop. size	No. of markers	Type of makers	Reference
M82-1-7 × LA1589	BC_1	257	120	RFLP,RAPD, Morph	(Grandillo and Tanksley 1996a)
NC84173× LA722	BC_1	119	151	RFLP	(Chen and Foolad 1999)
Giant Heirloom × LA1589	F_2	200	90	RFLP, CAPS	(Lippman and Tanksley 2001)
E6203 × LA1589	BC_2-F_6-BIL	196	127	RFLP	(Doganlar et al. 2002)
NC84173× LA722	F_{10} - RIL	119	191	RFLP, RGA	Foolad et al. (unpublished)
NCEBR1× PSLP125	F_2	172	256	RFLP, EST, RGA	Foolad et al. (unpublished)
NCEBR1× PSLP125	F_7 -RIL	172	275	RFLP, EST, CAPS, SSR	Foolad et al. (Current study)

QTL mapping methods

Quantitative traits are usually influenced by multiple genes and various environmental factors. Detecting and mapping individual genes underlying such complex traits is a difficult task. In 1923, Sax reported the association of seed coat color (a qualitative trait) in bean with seed size (a quantitative trait). He interpreted his finding as the linkage of a single gene controlling seed color with polygenes controlling seed size (Sax 1923). Thoday (1961) first proposed the idea of using two markers to bracket a chromosomal region to map the QTLs (Thoday 1961). With the advent of genetic markers, finding association between quantitative traits and chromosomal regions was no longer a limitation. Detection of QTLs was first preformed by Single Marker Analysis (SMA) using a simple *t*-test (Luo and Kearsey 1989; Weller 1986). A disadvantage of SMA is that the power of the analysis decreases with the increase in the distance between the marker locus and a given QTL. Precision of the method also depends on having a dense molecular linkage map. With the development of DNA markers and molecular maps it has become possible to scan the genome of an organism to locate QTLs for various quantitative traits. An improved method, called Interval Mapping (IM) was proposed by Lander and Botstein (Lander and Botstein 1989) to map the position of QTLs more precisely. In IM method, a marker interval is tested against the phenotype of a trait. At each cM position in a given marker interval the likelihood ratio (LR) is calculated. The entire genome of an organism can be scanned in this way as long as a complete genetic linkage map is available. An LR profile can be produced along the chromosomes to

identify the position of the significantly largest LR as an estimate of the QTL position. This approach takes into account one QTL at a time, and therefore, suffers from being biased to identify QTLs (Haley and Knott 1992; Kao et al. 1999; Lander and Botstein 1989; Zeng 1994). To overcome this problem, a combination of IM and a multiple regression method called "Composite Interval Mapping" (CIM) was independently developed by Zeng (1993; 1994) and Jansen (1993). In this approach while the presence of QTLs is being tested in a given marker interval, other markers are being used as covariates to reduce the residual variance in a way to minimize the bias in QTL position estimate (Jansen 1993; Zeng 1993). Although the CIM method is more precise than IM in identification of QTLs, however, methods that are more accurate were needed to estimate the combined effects of each individual QTLs. Subsequently, Multiple Interval Mapping (MIM) was proposed by Kao et al. (1999), which is more powerful in detecting QTLs and can easily search for and analyze epistatic interaction among the identified QTLs (Kao et al. 1999). By using MIM, one can estimate breeding values, heritability, genetic and phenotypic variances of the quantitative trait.

QTL mapping in tomato

In tomato QTL mapping started with the early work of Paterson et al. (1988). They conducted a comprehensive study using DNA markers to find QTLs for fruit size, SSC and pH. Since then more and more quantitative traits have been mapped using different genetic linkage maps and populations (Chen et al. 1999; deVicente and Tanksley 1993; Eshed and Zamir 1994; Foolad 1999a, b; Foolad et al. 1998; Foolad et al. 2003; Grandillo and Tanksley 1996b; Leonards-Schippers et al. 1994; Nienhuis et al. 1987; Robert et al. 2001). The final goal in all of these efforts is to have a better understanding of the nature of complex traits. Some of the QTL mapping efforts have led to cloning of underlying genes, including cloning of a QTL for fruit weight in tomato (Frary et al. 2000). This demonstrates the power of mapping and statistics to reveal the underlying genetic mechanisms of complex traits. With the advances in molecular cloning and new technologies cloning QTLs for more agronomically important traits such as yield and resistance to complex biotic and abiotic stresses in plants should not be difficult.

Breeding strategies

The majority of tomato species are self-fertilized with a low percentage of cross-pollination in exerted stigma genotypes or species with self incompatibility such as *L. peruvianum*. For a conventional plant breeding, most of the common protocols of breeding self-pollinated plants are applicable to tomato. These methods include pedigree, bulk, and backcross breeding methods, which have been used in the past and are still used in breeding programs. They have advantages and disadvantages. A plant breeder usually decides to choose one or a combination of these methods based on his/her own breeding program. In certain cases the original protocol may not be applicable for specific needs or goals of the plant breeder; therefore a modified form of the protocol can be used, such as a modified pedigree or backcross method.

Conventional plant breeding protocols have been the key methods of tomato improvement in the past and in present, and their contributions toward introduction of new cultivars and varieties are not expected to disappear. However, they are not the only tools available for genetic improvement of tomatoes. The traditional breeding protocols are often lengthy and time consuming. Furthermore, the pedigree of breeding populations, need to be well documented and managed. The average time needed to introduce a new cultivar using conventional breeding methods is about 10-15 years. New methodologies such as DNA marker techniques can speed up the process of cultivar development and need to be integrated into traditional breeding programs.

Due to its narrow germplasm, improving cultivated tomato may require interspecific hybridizations or introduction of exotic genes to its genome by genetic engineering techniques. However, in the U.S., genetically modified plants are subject to per-market "approval" by three federal agencies, including the USDA (United State Department of Agriculture), The EPA (Environmental Protection Agency) and the FDA (Food and Drug Administration). The FDA is usually not involved in the safety determination of new varieties of vegetables such as tomato; however, it has the authority to remove a product from the market if there is enough evidence to believe that a product is unsafe. Therefore private companies or governmental plant breeding institutes should be prepared for addressing concerns by the FDA (Lindemann 1993).

Using traditional, new or a combination of both methods still remains as a crucial means to cope with the growing demand for food in the world. The world population is currently ~6.6 billion

and is expected to reach ~9.6 billion in 2050 (U.S.CensusBureau 2006). The world population has doubled since 1950, but the food production has tripled (Ehrlich 1994). This increase in food production is due mainly to the green revolution in early 1960s, better agricultural practices/managements such as crop rotation, mass production, the use of petroleum based fertilizers and chemical pesticides, better irrigation systems to exploit more arid and semi-arid lands and the use of superior genetically improved cultivars. It is estimated that at least a half of this increase has been due to the use of genetically improved varieties (Eric et al. 2004). To prevent future food crises in the world, it is estimated that the food production should be doubled or preferably tripled by 2050 (Eric et al. 2004; Herrera-Estrella 2000). Many developing countries have used most of their arable land and there is no more room to expand cultivated areas. Therefore, there are two ways to increase the crop yield in such regions. The first is through better agricultural practices and the second is using genetically superior and improved cultivars. Have we used the maximum potential and power of plant breeding? If the answer to this question is "No," we have to use better and more efficient plant breeding methods.

Goals and objectives of this thesis research

The purpose of this thesis research was to construct a genetic linkage map based on an F_7-RIL population derived from a cross between *L. esculentum* and *L. pimpinellifolium* and to use the map to identify the position of QTLs for EB resistance and fruit quality characteristics. Through this research, I aimed to accomplish the following specific objectives:

1- To complete development of a Recombinant Inbred Line (RIL) population of tomato originated from a cross between the cultivated tomato *L. esculentum* cv. NCEBR-1 breeding line and wild tomato *L. pimpinellifolium* PSLP125 (chapter 2).

2- To construct a genetic linkage map of this population based on RFLP, candidate resistance genes and other molecular markers (chapter 2).

3- To evaluate the RIL population in different generations/years for early blight (EB) resistance under field conditions and to identify QTLs controlling EB resistance (chapter 3).

4- To evaluate the population for fruit quality traits such as fruit weight, soluble solid content (SSC), pH and lycopene content in different generations/years and identify QTLs for such traits (chapter 4)

5- To examine association between mapped ESTs and QTLs conferring EB resistance in order to identify potential candidate genes for EB resistance (chapter 3).

6- To compare and verify stability of QTLs for EB resistance and fruit quality traits across generations and years (chapters 3 and 4).

8- To develop a statistical model to estimate the amount of lycopene from chromaticity values (LAB color system) in tomato (chapter 4).

Hypotheses

In this thesis research, the following hypotheses have been tested:

1) EB resistance in tomato is genetically controlled, and novel genetic factors (QTLs) can be identified and mapped;
2) At least a proportion of the QTLs affecting variation for EB resistance correspond to candidate genes associated with disease resistance;
3) Candidate-gene approach can be used to identify and map new *R* genes for EB resistance in this population;
4) Lycopene is quantitatively controlled and QTLs for lycopene can be identified.

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CHAPTER 2. A molecular linkage map of tomato based on an F_7-RIL population of a cross between *L. esculentum* and *L. pimpinellifolium* displaying the position of several candidate resistance gene markers

Abstract

The cultivated tomato, *Lycopersicon esculentum* Mill., has a narrow germplasm base, restricting crop genetic improvement using intra-specific variation. The related wild species of tomato, however, are a rich source of useful genes for crop improvement and basic genetic studies. Utilization of wild germplasm in plant breeding programs can be facilitated by development and use of molecular linkage maps. A *L. pimpinellifolium* accession (PSLP125) from the red-fruited wild species of tomato has been identified in the tomato breeding program at the Pennylvania State University. This accession has highly desirable horticultural characteristics, including early blight resistance and high fruit lycopene content. Because of the close genetic relationship between *L. pimpinellifolium* and *L. esculentum* and the limited marker polymorphism between the two, the previously developed high-density map of tomato based on a *L. esculentum* × *L. pennellii* cross can not be readily utilized for genetic mapping and exploitation of useful genes in *L. pimpinellifolium*. To overcome this problem, a genetic linkage map was developed based on an F_7-RIL population (N = 172) of a cross between PSLP125 (staminate parent) and an advanced tomato breeding line (NCEBR-1) and 275 molecular markers, including RFLP, EST, CAPS and SSR markers. The map spanned 1066.1 cM of tomato genome with an average marker distance of 3.8 cM. Significant deviation from expected 1:1 ratio between the two homozygous classes was found in 29.4% of the markers. In about half of these, *L. esculentum* alleles were present in greater frequency than the *L. pimpinellifolium* alleles. The distinct feature of this map is the inclusion of more than 130 Expressed Sequence Tag (EST) markers, selected based on their sequence similarities (DNA OR AA) with known resistance (*R* genes) or defense-response genes, signal-transduction genes, transcriptional regulatory factors, genes encoding pathogenesis-related proteins and lycopene synthesis. This map will be useful for marker-

assisted exploitation of desirable characteristics of PSLP125 and the genetic variation that exists within *L. pimpinellifolium* or *L. esculentum*, map-based cloning and QTL mapping of various traits in the population.

Introduction

Based on molecular evidence, the amount of genetic variation in the cultivated species of tomato, *Lycopersicon esculentum* Mill.*,* accounts for ~4.5% of the total variation in the genus *Lycopersicon* (Miller and Tanksley 1990). The limited variation in the cultivated tomato is believed to be due to major genetic bottlenecks imposed by selection and inbreeding during its domestication and cultivation (Rick 1976). One of the consequences of reduced genetic variation is inbreeding depression that may increase the chance of extinction of vulnerable plants (Swart et al. 1996). As a result plants become more vulnerable to newly emerged races of diseases and insects and jeopardize a sustained genetic improvement. While modern cultivars of tomato have the lowest percentage of total variation, wild type accessions especially green fruited species such as *L. peruvianum* (33.3%), *L. pennellii* (24.6%) and *L. hirsutum* (16.8%) have the highest percentages of total variation (Miller and Tanksley 1990). One way to increase the extent of variation in the cultivated tomato is introgression of genes from its related wild species via sexual hybridization. Thus, during the past several decades, wild type tomatoes have received much more attention than before as they bear a wealth of genetic variation for many agriculturally important characteristics. For instance, sources of desirable traits such as high lycopene, increased soluble solid content (SSC), resistance to biotic and abiotic stresses have been found mainly within tomato wild species. However, one of the major obstacles to use wild species in tomato breeding is genetic linkage drag. This is especially more challenging when distantly-related green-fruited wild species of tomato are being used in tomato breeding. Elimination of undesirable traits before starting the breeding program is referred to as pre-breeding, which in turn is a time consuming and an expensive process. However, by the advent of molecular linkage maps, overcoming these limitations may no longer be insurmountable.

The advent of molecular markers in 1980s revolutionized plant and animal breeding methods. For instance in the field of plant sciences, molecular markers have found a wide range of applications in cultivar identification (Moisan-Thiéry et al. 2001), phylogenetic studies (Spooner

et al. 2005), and genetic map construction (Bernatzky and Tanksley 1986b; Tanksley 1983). Perhaps, development of genetic linkage maps has been one of the most powerful and versatile applications of molecular markers in the past few decades. Molecular linkage maps have been developed for many crop species including tomato and have found applications in QTL (quantitative trait locus) mapping (Grandillo and Tanksley 1996b; Paterson et al. 1988), marker assisted selection (MAS) and map-based cloning (Brommenschenkel and Tanksley 1997; Chunwongse et al. 2002; Ling et al. 1999; Martin et al. 1993a).

Among crop species, tomato has the most saturated genetic linkage map with more than a thousand of DNA markers (Pillen et al. 1996; Tanksley et al. 1992). Construction of the high-density linkage map of tomato (Tanksley et al. 1992) provided the foundation for subsequent development of a number of other molecular linkage maps (Bernacchi and Tanksley 1997; Chen and Foolad 1999; Eshed and Zamir 1995; Fulton et al. 1997a; Fulton et al. 2000; Paran et al. 1995; Zhang et al. 2002). In this respect, however, developing molecular linkage maps based on either intraspecific crosses within the cultivated species or crosses between a cultivated tomato and a closely-related wild species are highly desirable in exploitation of genetic variation of these genetic resources. This is because of elimination or reduction of pre-breeding efforts. Among the eight identified wild species in the genus *Lycopersicon*, the red-fruited species *L. pimpinellifolium* is the closest relative of the cultivated tomato (Miller and Tanksley 1990), for which natural introgression in the cultivated tomato has taken place (Chen and Foolad 1999). Furthermore, genetic introgression from this species to the cultivated tomato has been made through plant breeding (Grandillo and Tanksley 1996b; Palmer and Zamir 1982; Rick 1982). In addition, *L. pimpinellifolium* accessions readily and bidirectionally can be crossed with the cultivated tomato, make them more amenable to tomato genetic studies. Moreover, there are also less undesirable horticultural and agronomical traits within *L. pimpinellifolium* accessions, which make them more useful for tomato breeding purposes compared to the more distantly related wild accessions such as *L. pennellii* and *L. hirsutum* (Chen and Foolad 1999). These and other important considerations, make *L. pimpinellifolium* accessions more appealing to tomato breeding programs.

It is obvious that the full genetic potential of *L. pimpinellifolium* accessions has not been exploited yet. This can be inferred from the limited genetic linkage maps (Chen and Foolad

1999; Graham et al. 2004; Lippman and Tanksley 2001; Villalta et al. 2005) that have been developed based on interspecific crosses between *L. esculentum* and *L. pimpinellifolium*. In addition to the usefulness of *L. pimpinellifolium* accessions in tomato breeding programs, developing genetic linkage maps based on permanent segregating populations such as recombinant inbred lines (RIL) is also important for basic as well as applied research. For example, using RILs in molecular mapping has several advantages. First, a RIL population is a permanent population that can be propagated without any further segregation. It is unlike F_2 or backcross populations that DNA becomes exhausted after one generation. Thus, the same mapping population can be used by several laboratories and evaluated for several traits in different years and environments. This characteristic is useful for analysis of quantitative traits because it allows minimize the amount of environmental variance (Paran et al. 1995). Second, RILs undergo multiple rounds of meiosis and recombination before homozygosity is achieved. As a result, linked genes have a grater probability of shuffling (Haldane and Waddington 1931). Because in a RIL population the basic linkage associations have been established, detection of non-allelism becomes easier and map distance can be estimated more accurately (Burr et al. 1988). This is because confidence intervals are a function of the percentage of observed recombination – with the additional round of meiosis, there is a greater chance of detecting recombination between two linked markers (Burr and Burr, 1991). On average, the amount of observed recombination in a RIL population produced by selfing is twice that of other populations derived from a single meiosis (Haldane and Waddington 1931). Third, the increase in the number of homozygous loci will be expected to increase the phenotypic differences between the lines and make mapping of dominant markers more precise (Sandal et al. 2006). Forth, the genetic map developed from a RIL population can be used to detect loci controlling quantitative traits (QTLs). In QTL mapping, the use of homozygous lines that can be replicated in different locations or several years gives an additive bonus for more accurate measurements of the quantitative traits. This in turn compensates for the reduced linkage disequilibrium (LD) in the RIL population (Paran et al. 1995; Tanksley 1993). Because of these important considerations, RIL populations are often preferred populations for genetic mapping.

In tomato, previously RIL populations were used to construct a number of genetic linkage maps, including in populations developed from crosses between *L. esculentum* and *L. cheesmanii*

(Paran et al. 1995), *L. esculentum* and *L. esculentum* var. *cerasiforme* (Saliba-Colombani et al. 2000), *L. esculentum* var. cerasiforme and *L. pimpinellifolium* or *L. cheesmanii* (two populations) (Villalta et al. 2005), and two populations of *L. esculentum* and *L. pimpinellifolium* (Graham et al. 2004) (Foolad et al. unpublished data). In all of these studies the authors have recognized the importance of using closely-related wild species in exploitation of genetic variation within these genetic resources, to reduce or eliminate the pre-breeding efforts.

The advantages of utilizing *L. pimpinellifolium* accessions in developing segregating populations combined with the use of a RIL population in genetic mapping will have an additional benefit in studying complex and quantitatively inherited characteristics, especially when the *L. pimpinellifolium* is exceptional in horticulturally important traits. In this respect the choice of molecular markers is also crucial to take the most advantage of using RIL population and *L. pimpinellifolium* accessions. For example, the use of random genomic DNA sequences as markers in developing the genetic maps is not as informative as the use of expressed sequence tags (ESTs). The EST markers can be selected based on their role in disease resistance, defense response, carotenoid biosynthesis, ripening, soluble solid contents and many other horticulturally and agronomically important traits depending on the breeding program goals (see below). As more and more ESTs become available in data bases, this mapping approach also known as candidate gene mapping become more promising in identification of the genes monogenic or quantitative trait loci (Wang et al. 2001).

Recently, a *L. pimpinellifolium* accession (PSLP125) was identified through an extensive screening experiment in the tomato genetic and breeding program at Penn Stat University. This accession was determined to be superior in resistance to early blight (*Alternaria solani*), fruit color and soluble solid contents (Hyman 2001; M R Foolad, unpublished data). To aid the characterization and exploitation of the full genetic potential of this accession, a genetic linkage map from a cross between this accession and a fresh-market tomato breeding line has been constructed, as described in this chapter. This map will provide a basis for the identification and characterization of useful genes and QTLs in *L. pimpinellifolium* accession PSLP125, and also for the introgression of useful traits into the cultivated tomato. Previously, several molecular linkage maps of tomato based on different crosses between *L. esculentum* and *L. pimpinellifolium* were reported by Grandillo and Tanksley (1996b), Graham (2004), Lippman

(2001) and Chen (1999). The present map, however, is different, but complementary to the previous maps, as it contains a decent number of ESTs along with RFLP anchor markers to facilitate basic and applied investigations of complex traits such a early blight resistance and lycopene content of the tomato fruit. More than 224 ESTs from a pool of ESTs available in tomato genome index (Computational Biology and Functional Genomics Laboratory 2007) were selected based on two criteria, those that were involved in events associated with the recognition of, and interaction with, elicitor molecules from pathogen and those involved in plant defense responses. The ESTs markers were selected in a classified manner to cover all four main groups of cloned resistance genes, including genes encoding serine/threonine kinases, genes coded for proteins with nucleotide binding site (NBS) and leucine-rich repeats (LRR), genes whose product contain LRR motif and genes encoding receptor like protein kinases (Hammond-Kosack and Jones 1997). A total of 12 ESTs that were part of structural genes for carotenoids synthesis (especially for lycopene synthesis pathway) were also selected for mapping purposes. Furthermore, comparisons were made between *L. esculentum* × *L. pimpinellifolium* maps with other molecular maps of tomato with a discussion of similarities and differences in relation to phylogenetic relationships between parents of the various mapping populations

Materials and methods

Plant materials

NCEBR-1 (*L. esculentum* Mill.) breeding line, a horticulturally superior, multiple-disease resistant, fresh-market tomato breeding line and PSLP125 (*L. pimpinellifolium* Jusl.), a self compatible, inbred wild accession, which easily hybridizes with *L. esculentum*, were used as parental lines to generate the RIL population as follows. In 1998, PSLP125 was identified at Penn State as a rich source of genes for a number of desirable horticultural and agronomic characters (M R Foolad, unpublished data). In 1999, NCEBR-1 breeding line and PSLP125 were hybridized and F_1 progeny were produced. A single F_1 hybrid plant was self-fertilized to produce F_2 seeds. In 2000, a total of 900 F_2 plants along with the parental lines were grown under field conditions. Plants with undesirable characteristics were eliminated, and 172 individuals were grown to maturity, self-pollinated and produced seed of F_3 progeny (MR Foolad, unpubl. data). In 2001, plants of the 172 F_3 families were grown under field conditions. One individual plant from each family was chosen, self-fertilized, and produced seed of F_4 progeny. In summer of 2002, F_4 progeny were grown to maturity under field conditions, and F_5 seeds were collected from one individual plant per each of 172 F_4 families (MR Foolad, unpublished. data). Upon my arrival at Penn State in the Fall of 2002, I took over the F_5 seed and continued advancing the population via single-seed descent method. Using greenhouse and field facilities, the population was advanced to F_7 generation by 2004. During the summer of 2004, the 172 F_7-RILs were grown under field conditions and screened for various traits, as described in chapters 3 and 4. However, to develop a linkage map of this population, in 2004 one plant of each of the 172 F_7 lines was selected from which leaf tissue was collected for DNA extraction and molecular marker analyses, as described below. Subsequently, the F_7 population was advanced to F_8 and F_9 generations, as described in chapters 3 and 4. In this chapter I describe the molecular map developed based on the F_7 generation.

Tissue collection and DNA extraction

Leaf tissue of 172 F_7 individuals (a single plant representative of each line) was collected in 50 mL Falcon tubes. Immediately after collection in the field, the tissues were freeze-dried by dipping the tube in liquid nitrogen for 1-2 minutes and kept on ice until transferred to -70 °C

freezer in the laboratory. CTAB method of DNA extraction (Bernatzky and Tanksley 1986a; Foolad et al. 1993) was used to extract the genomic DNA from 10-15 grams frozen leaf tissues. The extracted DNA was treated with 1 µL *RNase A* (10 mg/mL, Sigma) per 100 µL DNA for an hour at room temperature. DNA concentration was then measured by spectrophotometer at 260 nm.

DNA Markers

Different molecular markers such as Restriction Fragment Length Polymorphism (RFLP), Expressed sequence tags (ESTs), Cleaved Amplified Polymorphic Sequences (CAPS), Conserved Ortholog Set (COS) and Microsatellites or Simple Sequence Repeats (SSR) were used to develop a genetic linkage map, as described below.

Sources of RFLP Probes

The DNA probes included 124 random tomato genomic (TG) or cDNA (CD or CT) clones obtained from S.D. Tanksley, Cornell University, Ithaca, N.Y., as well as 5 germination cDNA clones (denoted on the genetic map as C and CEL) obtained from Dr. Kent Bradford, University of California, Davis, CA., and 1 cDNA clone of potassium-transport related genes from tomato or potato obtained from Dr. Leon Kochain, Cornell University, Ithaca, N.Y. The tomato genomic and cDNA clones were chosen based on the high-density molecular map of tomato (Pillen et al. 1996; Tanksley et al. 1992). On average ~10 RFLP (anchor) makers evenly distributed on each chromosome were selected from the high-density molecular map of tomato to provide a uniform coverage of the genome. Based on an independent study in our laboratory, graduate student M. Kinkade found a RAPD marker putatively linked to late blight resistance in another *L. esculentum* × *L. pimpinellifolium* population (Foolad *et al.,* unpubl. data). The plausible linked fragment was gel purified using QIAquick gel purification kit (QIAGEN). It was then re-amplified with the same RAPD primer; purified with QIAquick PCR cleaning kit (QIAGEN) and used as a probe (designated as OP16EBR1) to map the position of the polymorphic copy of this fragment in the current population.

Sources of EST probes

The EST clones were supplied from either Clemson University or the Boyce Thompson Institute for Plant Research, Cornell University Ithaca, NY (Table 2-1). Upon the receipt of the clones, 10 µL of bacterial cells were streaked on LB-agar plates containing 50 µg/mL carbenicillin for an overnight growth. One single colony from each plate was picked to inoculate 1.0 mL LB media containing 50 µg/mL carbenicillin for an overnight growth. Plasmid DNA was extracted from 1 mL of the LB-grown bacteria using QIAperp Spin Miniprep Kit (German town, MD USA). The fragment was PCR-amplified using T3 and T7 universal forward and reverse primers. All PCR reactions were performed in 25-µL volume using the Promega PCR core kit (Madison, WI) and a Bio-Rad PCT-100 Thermocycler (formerly MJ Research PCT100). The thermocycler was programmed as an initial 95 °C for 30 seconds, followed by 35 cycles of 94 °C for 1 min DNA denaturation, 50 °C primer annealing for 1.0 minute, 72 °C extension for 2 or 2.5 minutes, followed by a final 72 °C for 5 minutes. A 3.0 µL of the amplified product was run on 1.2% agarose gel (Sigma Co.) to verify only one single fragment was amplified. The PCR amplified probes were purified using *QIAquick PCR purification* kit from QIAGEN according to manufacturer instruction (QIAGEN 2002). A 3.0 µL of the purified fragment was run on a 1.2% agarose gel. The fragment concentration was determined by comparing the purified band with the corresponding band of DNA ladder I (GENECHOICE 2002). In order to verify the nucleotide sequence of each EST clone with the one published in gene bank, 10 µL of purified fragments (40 ng/µl) were sent to University of Wisconsin-Madison sequencing facility for sequencing. RFLP procedure using the EST clone was carried out using 20 ng of each probe for parental survey or 50 ng for the whole population hybridization.

RFLP analysis

The extracted DNAs were diluted to 1.0 µg/µL concentration working solution, 15 µg DNA was digested overnight at 37 °C with eight restriction enzymes, *DraI*, *EcoRI*, *EcoRV*, *HaeIII*, *HindIII*, *RsaI*, *ScaI* and *XbaI*, according to the manufacturer's instructions (Promega, Madison WI, USA). To 50 µL digestion cocktail, 8.3 µL of 6X loading dye containing Xylene Cyanol and Bromophenol Blue was added and loaded into a 0.8% Agarose gel. Electrophoresis carried out at constant voltage of 35 V for an overnight. The DNA was alkaline transferred to N+ or XL nylon membranes (GE Healthcare Bio-Sciences Corp, NJ. formerly Amersham Biosciences) using

Southern blotting overnight. After Southern transfer, blots were baked for 2 hrs at 80 °C, washed in 2X SSC solution for 2 minutes, semi dried with blotting papers, preserved in protective sheets and kept in refrigerator for future RFLP procedure. Radioactive labeling of the RFLP or EST probes and hybridization procedure was carried out as described elsewhere (Bernatsky and Tanksley 1986; Feinberg and Vogelstein 1983; Foolad et al. 1997).

Sources of CAPS markers

A total of 32 CAPS or COS markers were surveyed for their polymorphism between the parents of the RIL population (Table 2-2). The CAPS markers were selected from a previously published genetic linkage maps of tomato based on *L. esculentum* LA925 × *L. pennellii* LA716 cross (Frary et al. 2005; Fulton et al. 2002; SGN 2007) and from Solanaceae genome network web site (SGN 2007). One of the CAPS markers on chromosome 11 happened to be a Known Functional Gene (KFG) marker linked to jointless locus (KFG-J1). Moreover, one CAPS marker (TG328) which is putatively known to be linked to *ph3* gene (located on tomato chromosome 9) conferring resistance to several races of *Phytophthora infestans* was kindly provided by Dr. Martha Mutchler, Cornell University, Ithaca, NY. Forward and reverse primers for all PCR based markers were synthesized by Integrated DNA Technologies, Inc. (IDT 2007).

CAPS/COS procedure

DNAs of the two parents were amplified using 32 CAPS/COS primer pairs (Appendix A) using Green GoTaq DNA polymerase (Promega, Madison, WI). PCR was preformed in 100 µL reaction volume using 50 µL of 2X GoTaq Green Master Mix ® Reaction Buffer containing: 400 µM each of dATP, dGTP, dCTP and dTTP, 3 mM $MgCl_2$, 4 µL of each of the downstream and upstream primers (10 µM) to obtain the final concentration of 0.4 µM, 0.5 µL GoTaq® DNA Polymerase (5U/µL), 15 µL template DNA (10 ng/µL) and 26.5 µL Nuclease Free Water. All PCR reactions were performed using a Bio-Rad PCT 100 Thermocycler (formerly MJ Research PCT100). The thermocycler was programmed as: 1 Cycle of 94° - 2 minutes, 40 cycles of 94° - 30 seconds, 55°C - 45 seconds , 72°C - 2 minutes, and 1 Cycle of 72°C – 5 minutes, followed by storage at 4°C. To verify amplification, 5 µL of the PCR products were resolved on a 2.0% agarose gel at constant voltage of 100 V for 2-3 hours. The gel was pre stained by ethidium bromide (1 µL of 10 mg/mL solution for 100 mL of agarose gel). The amplified PCR products

were digested with the enzyme suggested by Frary *et al.* (2005). All but one (TG320) marker were monomorphic with the suggested enzymes. Therefore, more restriction enzymes were tried to seek polymorphic CAPS markers. The restriction enzymes were supplied by either Promega (Madison, WI) or New England Biolabs, aka NEB (Ipswich, MA) and digestions were carried out according to manufacturers' instructions.

Source of SSR markers

Five SSR markers for chromosome 11 and one for chromosome 12 were selected from a previously published genetic linkage map of tomato based on *L. esculentum* LA925 × *L. pennellii* LA716 cross (Fulton et al. 2002; SGN 2007).

SSR procedure

Forward and reverse primers were synthesized via IDT (IDT 2007) (Table 2-2) and were used for DNA amplification. In order to find polymorphic makers, DNAs of the two parents were amplified using 6 primer pairs using Flexi GoTaq® DNA polymerase (Promega, Madison, WI). PCR reactions were preformed in 12.5 µL volume. Each PCR reaction contained 2.5 µL of 5X colorless Flexi GoTaq® reaction buffer, 1.0 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 0.4 µM forward and reverse primers, 0.25 U GoTaq® DNA polymerase (Prmega, Madison, MI) and 50 ng of template DNA. All PCR reactions were performed using a Bio-Rad PCT 100 Thermocycler, programmed for 1 cycle at 94°C for 5 min, 35 cycles of 30 seconds at 94°C, 45 seconds at 50, 55 or 65 °C (depends on annealing temperature of the primer used), 45 seconds at 72°C, and followed by 1 cycle of 5 min at 72°C for final extension and storage at 4°C). Separation of PCR products was as described elsewhere (Creste et al. 2001), but briefly equal volume of denaturing loading-dye (10 mM NaOH; 0.05% xylenecyanol [w/v]; 0.05% bromophenol blue [w/v]; 20 mM formamide) was added to the amplification product and 2 µL of each sample was separated on a urea:acryrlamide (6% acrylamide:7 M Urea) denaturing sequencing gel at ~1800-2000 V for ~2.5 hours at 50-55°C, using a S3S Owl separation system (Thermo Fisher Scientific Inc. Portsmouth, NH). The gel was silver stained using Promega silver stain kit (Promega, Madison, WI). To make a permanent record of the gel, Promega Automatic Processor Compatible (APC) films (Promega, Madison, WI) were used to take picture of the gel. Polymorphic markers were identified and used to survey the whole population. Because of the

large size of the population (n=172) and limited wells for sequencing gels (~70 wells), two sequencing gels were used to run the entire population with two times sample loading on each gel.

Mapping procedure

MAPMAKER/EXP v. 3.0 (Lander et al. 1987) was used to construct the genetic map. The group command with the LOD (Log of Odds) score of 3.0 and maximum marker distance of 26 cM (centiMorgan) was used to group the markers. Markers in each linkage group were assigned to the corresponding chromosome based on the location of anchor markers. The ORDER and RIPPLE commands in MAPMAKER and RECORD Software (Hans Van et al. 2005) were used to find the best and most likely order of the markers.

To construct the genetic map, a back bone map was first developed based on only random genomic and cDNA RFLP clones derived from the high density map of tomato (Pillen et al. 1996; Tanksley et al. 1992). *TRY* command was used to add EST, SSR and SNP markers to their corresponding chromosome. For example, to add a new marker to the map, the markers were first grouped. After finding the linkage group of markers using the *TRY* command, the *ORDER* and *RIPPLE* commands were used to find the most likely order/position of the markers. In cases where the marker belonged to groups consisting of two chromosomes, it was tried on both chromosomes to find the most likely position of the marker using the maximum likelihood values obtained by the software. In most cases the results of *TRY, ORDER* and *RIPPLE* commands were the same and comparable. After locating all markers, the markers on each chromosome were sequenced using the *SEQUENCE* command. Then the *MAP* command was used to map the markers to a particular chromosome. *MAKE CHROM* and *ASSIGN* commands were used to assign the markers to the corresponding chromosomes. In some cases after assigning markers to chromosomes, the user may receive the UNASSIGNED error message. In such cases, those markers needed to be re-sequenced, and attached to the corresponding chromosome using the "*ATTACH CHROM #*" command. After attaching the marker(s) to the chromosome, the markers on each particular chromosome needed to be sequenced again and reassigned. At the end, the *FRAMEWORK* command was used to make the framework of the map and to generate the MAP file containing the map information. This file then was imported

into QTL Cartographer software for QTL mapping. The Map file was also modified according to MapChart software manual (Voorrips 2002) to draw the map. MapChart package was also used to make a joint comparison between F_7-RIL and F_2 maps (Appendix A).

Statistical analysis

Pair-wise comparisons of maps were made based on common marker intervals. In order to carry out this comparison, the distances of all common maker intervals were identified and subjected to correlation analysis. The SPSS v. 14.0 package was used to perform all correlation analyses (SPSS 2005). Microsoft Excel was used to calculate χ^2 values for deviation from 1:1 ratio at each marker locus and also to calculate allele frequencies for RILs to generate the corresponding graphs.

Results

Marker polymorphism between *L. esculentum* (NCEBR-1) and *L. pimpinellifolium* (PSLP125)

RFLPs

A majority of the RFLP probes that were mapped in this population were also polymorphic in other *L. esculentum* × *L. pimpinellifolium* populations as follows. A total of 109 RFLP markers (82.5%) were common between the F_7–RIL map (denoted E×PM-1) and F_2 (denoted E×PM-2) map of the same cross (Foolad et al. unpublished data). About 82 RFLP markers (62%) were previously mapped in our laboratory in a *L. esculentum* (NC84173) × *L. pimpinellifolium* (LA722) BC_1 population (denoted E×PM-3) (Chen and Foolad 1999) and 54 (41%) were common with a previously published *L. esculentum* (M82-1-7) × *L. pimpinellifolium* (LA1589) population (denoted E×PM-4 map) (Grandillo and Tanksley 1996a). From among the 151 RFLP markers that were used in the E×PM-3 map, 11 (7%) were monomorphic between NCEBR-1 breeding line and PSLP125 (this study). Furthermore, of the 115 markers that were mapped in the E×PM-4 map, 7 were monomorphic between NCEBR-1 breeding line and PSLP125. Sixty-nine RFLP probes from the E×PM-3 and 54 from the E×PM-4 maps were not surveyed for polymorphism in the course of developing the current map or the E × PM-2 map. From among 120 RFLP probes that were directly selected from the high-density map of tomato, ~40 (32%) were polymorphic between NCEBR-1 breeding line and PSLP125. This level of polymorphism was similar to that previously reported by Chen and Foolad (1999) for construction of a *L. esculentum* (NC84173) × *L. pimpinellifolium* (LA722) BC_1 population (E×PM-3).

ESTs

The degree of polymorphism for EST markers was higher than that for the RFLP makers. In order to add EST makers to the F_2 map (E×PM-2), another colleague (Arun Sharma) previously surveyed 140 EST probes, of which 96 (68.5%) were polymorphic between NCEBR-1 breeding line and PSLP125. To meet the objectives of this thesis project, more ESTs had to be added to the F_7-RIL map (E×PM-1). Therefore, another set of 96 EST probes were surveyed, from which 68 (71.0%) were polymorphic between the two parents. Collectively, from among 236 EST probes 164 (~69.5%) were polymorphic between NCERBR-1and PSLP125. Mapped ESTs were

not uniformly distributed throughout the 12 tomato chromosomes: chromosome 1 had the highest number of ESTs (20 out of 128) and chromosome 11 the minimum number (4). EST clusters in the genome were found at several chromosomal locations. For example seven ESTs were clustered on the short arm of chromosome 2 within a 0.6 cM region. Similarly, nine ESTs at the middle of chromosome 4 within a 15.5 cM region and eight EST on long arm of chromosome 10 close to centromer within a 7.0 cM region were clustered. Similar EST clusters (with at least 4 ESTs within 10 cM) were also observed on all other chromosomes except 6, 9 and 11 (Figure 2-1). Because ESTs were mapped in all chromosomal regions, consequently they were co-localized with characterized or defined resistance loci. Moreover, the ESTs that were part of characterized genes were mapped in their correct positions. For example, two copies of an EST clone (cLEC34L10a and b) of the *Cf-9* gene family were mapped to distal part of chromosome 1 where *Cf-9* gene and other *Cf* gene family members (*Cf2.1*, *Cf2.2* and *Cf4*) were previously located. The EST marker cTOF26E9 of *Prf* gene was also mapped to chromosome 5 where the *Prf* gene locus is located. Co-localization of ESTs with known resistance/defense response genes or QTLs for early blight is discussed in chapter 3.

PCR based markers

A total of 12 CAPS/COS/KFG markers out of 34 (35%) detected polymorphism between the two parents (Table 2-3). The percentage of polymorphism was similar to that reported by Frary et al. (2005). The only KFG-J1 marker used in this study was also polymorphic; however, the jointless trait (with which KFG-J1 is associated) was not segregating phenotypically in the RIL population. From among 5 SSRs that were subjected to parental survey, 2 markers (SSR80 and SSR345) were polymorphic between the two parents. SSR345 and SSR80 were mapped to chromosomes 11 and 12, respectively.

Marker coverage and map density

A medium-density genetic linkage map was constructed using the F_7-RIL population (n = 172) with 275 DNA markers, including 130 RFLPs, 132 ESTs, 6 CAPS, 4 COS, 1 Known Functional Gene (KFG) and 2 SSR markers (Figure 2-1 and Figure 2-2). The map spanned 1066.1 cM of the tomato genome with an average marker distance of 3.88 cM. The length of the chromosomes in the map varied from 65 cM for chromosome 6 to 125 cM for chromosome 1, with an average of

88.8 cM. On average ~10 RFLP (anchor) makers are evenly distributed throughout each chromosome, providing a uniform coverage of the tomato genome. ESTs had the highest level of polymorphism, followed by RFLP and CAPS markers.

Skewed segregation

A total of 82 markers (29.8% of 275 markers) were skewed (Figure 2-1and Figure 2-3), of which 40 markers (14.9%) distorted in favor of *L. pimpinellifolium* alleles (*PM* alleles) and 42 (15.2%) in favor of *L. esculentum* alleles (*E* alleles). Chromosome 6 had the maximum number of skewed markers while markers on chromosomes 11 and 12 segregated normally (Table 2-4). The most extreme skewed segregation toward PM alleles was observed on chromosome 3 for EST marker cLEX12O16b (Ethylene response factor 5) which had the minimum number of *E* alleles (n = 58 from a total of 302 alleles) and the most extreme in the other direction was RFLP marker TG279 on chromosome 6 with the maximum number of *E* alleles (n = 326 from a total of 340 alleles).

Genome composition of the F_7-RIL population

Genome composition of the 172 RILs (F_7) was determined based on 275 co-dominant markers using Microsoft Excel and the GG32 packages (Ralph van Berloo 1999). The percent residual heterozygosity in the population was 6.8% which was 5.3% higher than the expected 1.5% for the RIL population at F_7 generation. Genome composition of the RILs ranged from 26.7% to 90.7% from the E parent (Figure 2-4) with an average of 50.2%. As it can be inferred from the distribution there were lines in the population with > 90% of the genome from *L. esculentum* and possibly desirable traits from the wild parent including high fruit lycopene content. Such lines can be highly useful for breeding purposes (chapter 3 and 4).

Comparison of chromosome size in the current map with other E × PM maps

The size of the chromosomes of the E×PM-1 map was compared with other *L. pimpinellifolium* maps, as well as with the high density map of tomato (E×P). The total length of the E×PM-1 map was 97.5 cM (~10% increase) longer than the E×PM-2 map. Similar comparisons were made for all combinations of E×PM-1 map with other E×PM maps. Overall the length of the E×PM-1 map was shorter than other maps (Table 2-5).

Comparison of common marker intervals of the current map with other E × PM maps

A paired comparison was made between E×PM-1 map with three other *L. esculentum* × *L. pimpinellifolium* genetic maps as well as the high density genetic linkage map of tomato (Table 2-6). Similar comparisons were made elsewhere (Chen and Foolad 1999; Grandillo and Tanksley 1996a), however, adding two maps (F_7-RIL and F_2) to the previously published maps justified conducting the current comparisons. In total 78 common paired marker intervals were identified between the E×PM1 and E×P. In 9 (11%) intervals, the distances between markers in the E×PM1 map were at least two-fold that in the high-density map of tomato. The intervals were located on chromosomes 1, 6, 7, 10, 11 and 12. The maximum number of marker interval expansion was on chromosome 7, where 3 intervals were expanded at least by two-fold. A number of consensus marker interval expansions among almost all maps were also observed. For example, marker interval of TG73-TG283 on chromosome 1 was at least doubled in the E×PM-1, E×PM-2 and E×PM-4 compared to the high density map. The marker intervals TG356-TG365 on chromosome 6 and CT234-CT11 on chromosome 10 were doubled in all E×PM-1, E×PM-2, E×PM-3 and E×PM-4 maps relative to the high density map but were consistent when they were compared between each other. The comparison of marker interval TG183-TG128 on chromosome 7 on E×PM-1 and E×PM-2 maps showed that this interval was expanded at least two-fold not only in the high density map but also in the E×PM-3 and E×PM-4 maps. This interval was consistent in length between the E×PM-1 and E×PM-2 maps (Table 2-6).

Correlation between maps based on common marker intervals

Pair-wise comparisons of different maps were made based on common marker intervals (Table 2-7). In order to carry out this type of comparison, the distances of all common maker intervals were identified and subjected to correlation analysis. The maximum number of identified common intervals was between E×PM-1 (F_7-RIL) and E×P maps (n=61). This comparison showed the smallest correlation coefficient (r = 0.59), but it was significant ($P < 0.01$). The highest correlation was observed between the E×PM-1 and E×PM-4 (r = 0.83 $P < 0.01$) or E×PM-2 (r = 0.82, $P < 0.01$) maps. The same degree of resemblance was observed between the E×PM-3 and E×P (r = 0.82, $P < 0.01$) and between the E×PM-4 and E×P (r = 0.70, $P < 0.01$) maps.

Discussion

A genetic linkage map was developed using an F_7-RIL population of a cross between *L. esculentum* breeding line NCEBR-1 and *L. pimpinellifolium* accession PSLP125. This map is complementary to the other genetic maps that were previously developed in our program at Penn State as well as in other laboratories. A genetic map associated with a RIL population provides a permanent germplasm resource for basic and applied tomato genetics and breeding research. In particular, this map has applications in mapping of genes and QTLs for disease resistance and high fruit quality, marker-assisted selection (MAS) and breeding, and map based gene/QTL cloning.

The current genetic map is different in many aspects from other molecular maps of tomato. First, the wild parent used in the construction of this map is a desirable accession selected from among 270 wild tomato accessions based on several years of field and greenhouse evaluations for early blight resistance, drought tolerance, high lycopene content, high soluble solids content and many other important traits. Second, this map is based on a permanent population, which can be shared with other laboratories for basic and applied research. Third, the population size is relatively large, which allows a more accurate estimate of marker intervals as well as trait segregation. Forth, mapping 128 ESTs for disease resistance or defense response genes provides a valuable source for dissecting the genetic nature of disease resistance including resistance to early blight resistance. To my knowledge, most of these ESTs were not previously mapped in other tomato populations. Fifth, a total of 4 ESTs involved in carotenoid synthesis have also been mapped in the RIL population providing another resource for further investigation of the nature of high lycopene characteristic in this population. Sixth, having markers from the high density linkage map of tomato at the distal parts of all chromosomes in the current map indicates that the genetic map does not extend beyond these markers. Thus, any QTL identified at the distal parts of chromosomes in this population may not be false QTLs. This matter has been reviewed by Tanksley (1992) and is discussed in chapter 4 of this thesis.

The survey of the parental lines of this population indicated that there was an adequate amount of marker polymorphism between the two parents for map construction. However, the level of polymorphism was less than what was observed in crosses between *L. esculentum* and distantly

related wild species such as *L. hirsutum* and *L. pennellii.* However, the use of EST fragments as RFLP probes resulted in identification of more polymorphism than the random genomic DNA fragments. This observation is contradictory with the general belief that ESTs are more conserved sequences among species and are expected to be less polymorphic. This observation needs further investigation using other populations.

For mapping purposes it is ideal to have markers segregating in Mendelian fashion. In the current study, however, skewed segregation (SKS) was observed for ~29% of the marker loci. Skewed segregation for marker loci is a common phenomenon in populations derived from interspecific crosses of tomato (Bernacchi and Tanksley 1997; Foolad 1996; Fulton et al. 1997b; Grandillo and Tanksley 1996a; Haanstra et al. 1999b; Paran et al. 1995; Paterson et al. 1988). Skewed segregation in interspecific crosses of tomato has been attributed to various causes, including self-incompatibility (SI), unilateral incongruity, gametophytic selection and viability selection of segregating plants (Foolad 1996; Trognitz and Schmiediche 1993). Segregation distortion has also been reported in interspecific crosses of other crop species, including peanut (Halward et al. 1993), rice (Fukuta et al. 2000; Guiderdoni et al. 1989; Harushima et al. 1996), alfalfa (Kaló et al. 2000), pearl millet(Liu et al. 1994) and soybean (Kopisch-Obuch and Diers 2006). It should be noted that SKS does not affect genetic distance calculation (Ott 1999) or recombination fraction (Liu 1998). However, in genome mapping projects it is crucial to interpret the linkage hypothesis carefully when marker data is distorted. To date, all mapping packages cannot take into account distortion models to have an unbiased estimate of the map distances (Liu 1998).

The degree to which makers show SKS varies from population to population. For instance, 83% of the markers that Paran et al. (1995) used in constructing their *L. esculentum* × *L. cheesmanii* RIL genetic map were skewed toward one or the other parent. Hannstra et al. (1999b) reported skewed segregation in two F_2 populations. The first population was developed from a cross between *L. esculentum* var.VF-36-*tm2a* and *L. pennellii* acc. LA716 and SKS was observed on chromosomes 2, 4, 7, 8, 10, 11 and 12. The second population was developed from a cross between *L. esculentum* cv. Allround and *L. pennellii* acc. LA716 and SKS was observed on chromosomes 9, 10 and 12. The most distorted region was reported on chromosome 10 for both populations for RFLP maker TG230. They attributed the observed SKS to selection against pollen of *L. esculentum* than zygote abortion. A rather large percentage of SKS (62%) was

observed in a backcross population of a cross between *L. esculentum* (NC84173) and *L. hirsutum* (PI126445), with makers on chromosome 1, 5 and 6 severely skewed. In a selective genotyping study of a backcross population of the same cross, 62 out of 179 makers (34.6%) exhibited skewed segregation toward one of the parents (Zhang et al. 2003). Generally, SKS is higher in filial generations (average 70%) than in backcross populations (average 40%) (Bernacchi and Tanksley 1997). However, in the current filial population, only 29% of the markers exhibited skewed segregation. This percentage is much lower than the average of SKS for filial generations possibly because *L. pimpinellifolium* is much closer to the cultivated tomato when compared to distantly related wild species of tomato that Bernacchi and Tanksley (1997) used. In the F_7-RIL map, severed skewed segregation was observed on chromosomes 3, 5 and 6. Markers on all other chromosomes but 11 and 12 also showed SKS, however, these skewed segregations were only at 3-7 markers per region and were clustering together. In another F_2 population of the same cross, Foolad et al. (unpublished) observed distorted segregation for co-dominant (1:2:1) and dominant (3:1) RFLP, EST and RGA makers on chromosomes 1, 3, 4, 5 and 6.

In the current study, the observed skewness on chromosome 3 was toward *L. pimpinellifolium*, starting from TG114 and ending at marker CT85 that spanned ~70 cM of the chromosome length. The EST marker cLEX12O16b (ethylene response factor 5) on this chromosome had the minimum number of *E* alleles (n = 58 out of 302). In the skewed region a large QTL for fruit weight was observed with an additive effect from the *L. esculentum* parent (Chapter 4). However, it does not seem that there is any relationship between this QTL and skewed segregation in this region. To the best of my knowledge any of the phenotypic traits that have been mapped to chromosome 3 such as aureate (*aut)*, baby lea syndrome (*bls)*, cana (*cn)*, divaricata (*div)*, fulgens-3 (*ful-3)*, Lanata *(Ln),* (maculonecrotic) *mcn*, (male-sterile-9) *ms-9*, pudica *pdc*, and yellow flesh (*r)* (Stevens and Rick 1986; TGRC 2007) are not segregating in this population.

The SKS of the markers on chromosome 6 can be attributed to the self-pruning gene (*sp*) family which is tightly linked to marker TG279 (Carmel-Goren et al. 2003; Pnueli et al. 1998). Most commercial cultivars and breeding lines are determinate and homozygous for the gene (*sp*/*sp*) vs. wild type genotypes that are mostly indeterminate. To date two mutant alleles have been identified for the *sp* gene. Almost all commercial tomato cultivars including the breeding line

used in this study carry *sp*[1] identified by Yeager (Yeager 1927) and MacArthur (MacArthur 1932); *sp*[2] was identified by Rick and Butler in VFNT cherry tomatoes (Rick and Butler 1956). In the current study, although no conscious selection against indeterminacy was made during population development, a majority of RILs turned out to be either semi-determinate or determinate. Phenotypic mapping of this trait (in 2006) placed the gene for self-pruning in a 2 cM distance from TG279 (data not shown). This result was consistent with previously reported skewed segregation on this chromosome (Foolad et al. 2002). Markers on chromosome 5 were also skewed toward the cultivated parent breeding line (NCEBR-1). The observed SKS on this chromosome can be attributed to QTLs for resistance to early blight, which its additive effect is inherited from *L. esculentum* (NCEBR-1) parent (chapter 3). Markers on chromosome 5 have been also severely skewed in another study but toward wild type *L. hirsutum* (PI126445) parent (Foolad et al. 2002).

The percent residual heterozygosity in the population was 6.8%, which is higher than the expected heterozygosity for the RIL population at an F_7 generation (1.56%). Excess heterozygosity has been reported in interspecific crosses of tomato (Paran et al. 1995), peanut (Halward et al. 1993), rice (Fukuta et al. 2000; Guiderdoni et al. 1989; Harushima et al. 1996), Alfalfa (Kaló et al. 2000), pearl millet (Liu et al. 1994) and soybean (Kopisch-Obuch and Diers 2006). Excess heterozygosity has also been attributed to map expansion in both F_2 and RIL populations (Knox and Ellis 2002). In tomato, excess heterozygosity has been reported as a result of unintentional selection against plants with low fertility or cross-pollination of the plants during population development (Paran et al. 1995). Theoretically, each generation of inbreeding following the F_1 generation should reduce the percentage of heterozygosity by 50%. However, heterozygosity may be maintained steady in each meiosis throughout the inbreeding generations. In *Arabidopsis*, heterozygosity of 0.42% at an F_8 generation showed that in this species there was no heterozygous advantage to maintain heterozygosity (Lister and Dean 1993).

The length of the current map is ~140 cM shorter than the average length of the tomato genetic linkage maps previously published. There are many factors that affect genome coverage and genetic map length, such as population type (i.e. F_2, BC, DH, BIL or RIL), population size, number of markers, type of markers (dominant or co-dominant), distribution of markers, errors in genotype scoring and data entry, frequency of double recombination, mapping function and the

software used. Population size has a significant effect on accuracy of the obtained genetic distances (Ferreira et al. 2006). While the most accurate genetic distances are obtained by population sizes of 500 to 1000 individuals, F_2 and RIL populations with co-dominant markers are among the best populations for mapping. In our studies, in both F_2 and F_7-RIL mapping projects the size of the populations was close to the optimum population size (n=200) to provide mapping accuracy (Ferreira et al. 2006). Although the map length is important but the order of the markers is a more important factor. Therefore, the order of the markers on each chromosome was also verified by comparing the result of MapMaker/EXP (MM) with another software called RECORD (Hans Van et al. 2005) that has solely designed for this purpose. The results obtained by RECORD were exactly the same as MM, indicating that the algorithm that MM and RECORD use are very similar and that the order presented in the map in this study is most likely the best order.

The effect of the algorithm used in mapping packages was also investigated by comparing the mapping results obtained from MM and JoinMap 3.0 package (JM) (Van-Ooijen and Voorrips 2001). In order for MM to be able to load the data set, all heterozygote notations in the data file should be removed and considered as missing data, while JM can take them into account. In spite of this, when JM was used to construct the genetic map (using the same data set and considering the heterozygote alleles), the total map length decreased significantly. In the mean time when JM was used to determine the best order of the markers on each linkage group, for a few regions of linkage groups the marker order was somewhat different from the order determined by MM (data not shown). To further investigate this matter, the data of F_2 population were examined where similar results were obtained (data not shown); it appeared that JM calculated less recombination than MM did with applying the same map function. The only explanation for this discrepancy is that JM and MM use different algorithms to make calculations for map distances. This issue needs further investigation by using more map data from other labs. In order to be consistent with other maps that have already constructed in tomato including the high-density map of tomato, in the present work MM was used to construct the map. As a result, the order of anchor markers in 99.6% of the cases was consistent with most genetic maps of tomato that were constructed previously using the same program (Chen and Foolad 1999; Fulton et al. 2001; Grandillo and Tanksley 1996a; Tanksley et al. 1992).

Comparison of the current map with the previously published maps of tomato showed longer map distances for certain areas of all chromosomes. This is not unexpected since recombinant inbred lines undergo several generations of recombination leading to map expansion. The concept of map expansion has been discussed in detail by (Martin and Hospital 2006). The fraction of recombination in a RIL population is increased because of the simple fact that there are several meioses before the population reaches rather complete homozygosity and used for map construction. Many authors tend to use the term "map expansion". However, this term is misleading because there is only one map and using the recombination fraction observed in the mapping population (*R*) instead of recombination rate per meiosis (*r*) does not lead to generation of a new map. The fact is that RIL population provides a map with a higher resolution (Martin and Hospital 2006).

Pair-wise comparison of common marker intervals of E×PM1 map and other *L. esculentum* × *L. pimpinellifolium* maps showed substantial differences between these intervals. The differences are expected because the population types, sizes and the parents that were used in construction of these maps were different. However, this comparison was useful in finding marker intervals that were at least doubled in E×PM1 compared to other maps. The odds that this has happened by chance are remote. For example, an interval on chromosome 7 (TG183-TG128) has increased at least three-fold in E×PM1 compared to all four maps. When comparing the current map with the high-density map of tomato, the maximum increase observed in three marker intervals on chromosome 7 and two on chromosome 1. The remaining maker interval increases were sporadic and were not chromosome specific. Marker interval contractions were also observed in these two maps but they were not chromosomal region specific or clustered together. The common aspect of E×PM1 and E×PM2 maps was that in 95% of the cases the same maker interval was increased compared to E×P map. Correlation analysis indicated that there were more similarities between E×PM1 map with any of E×PM2, E×PM3 and E×PM4 maps than with the E×P map. This can be due to the fact that the former maps are developed based on crosses of *L. esculentum* and *L. pimpinellifolium* species while E×P map is based on far more distantly related species (*L. esculentum* and *L. pennellii*).

Comparison of E×PM1 and E×PM2 maps was more useful in terms of finding hot spots for recombination. Because the two maps share common parents and both populations have the same

size. It seemed that the increase in marker intervals in these two maps was not chromosomal region specific. In all chromosomes but 2, 9 and 11 there was at least one chromosomal interval showing an increase in map distances. On chromosome 1, four marker intervals showed increases by at least two fold. Five marker intervals (> 5.0 cM) on E×PM1 map showed contractions relative to E×PM2 map (Appendix A). This can be attributed to random error in mapping. Generally, a population needs to be large enough (N > 1000) to accurately determine the real distances between markers. As mentioned before, the order of the markers is more important than the distances between them, therefore these few discrepancies that were also observed in a another study (Chen and Foolad 1998) should not affect further QTL analyses using both E×PM1 and E×PM2 maps.

The map presented here will be useful in studies of QTL identification for different traits in different locations or years, and in MAS breeding. Because the population is permanent, the constructed map can be shared with other laboratories and even more markers can be added to the map in order to make it more specific for other candidate genes. This map is also useful for chromosome landing (Tanksley et al. 1995) and ultimately cloning major genes or QTLs controlling important traits in *L. pimpinellifolium* accession PSLP125.

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Tables

Table 2-1 List of ESTs mapped in the *Lycopersicon esculentum* × *L. pimpinellifolium* F_7- RIL population, their putative function and chromosomal location

EST Clone Name	Putative function	Chromosome No.
cTOF3A14	Cytosolic Cu, Zn Superoxide dismutase, *L. Esculentum*	1
cTOE7J7a	Endo-1,4-beta-glucanase, *L. esculentum*	1
cLED27E12	Cold acclimation protein WCOR413-like protein form, *O. sativa*	1
cTOE6F10	Lipoxygenase, *L. esculentum*	1
cLEG9N2	Subunit A of ferredoxin-thioredoxin reductase, *S. tuberosum*	1
cLES9N20	ASC1 (Alternaria stem canker resistance protein), *L. esculentum*	1
cLEC6O2	Polyamine oxidase, *A. thaliana*	1
cTOF20P4	Carotenoid cleavage dioxygenase 1-2, *Lycopersicon esculentum*	1
cLEZ11K12	Snakin2 precursor, *L. esculentum*	1
cLEC345L10a	*Lycopersicon pimpinellifolium* Cf-9 resistance gene cluster	1
cLEC345L10b	*Lycopersicon pimpinellifolium* Cf-9 resistance gene cluster	1
cTOS16I16	Glucan endo-1,3-beta-glucosidase A precursor (Acidic beta-1.3-glucanase)	1
cLEC71F17	Glucan endo-1,3-beta-glucosidase B precursor (Basic beta-1.3-glucanase)	1
cLEC72D1	Endo-1,4-beta-glucanase	1
cLEI6L6	Ribosomal protein S6 family protein (RFC3), [Annotation Temporary]	1
cLEG61B21	Lipoxygenase	1
cLEG37I4	Lipoxygenase	1
cTOF13C14	Stress enhanced protein 1 (SEP1). *Arabidopsis thaliana*	1
cLEC66G13b	Xyloglucan-specific fungal endoglucanase inhibitor protein	2
cTOA9C11	Similar to WRKY transcription factor Nt-SubD48, *N. tabacum*	2
cLET10E15	Acidic 26kDa endochitinase precursor, *L. esculentum*	2
cTOF19J9	Phosphoribosylanthranilate isomerase, *A. thaliana*	2
cLEY1K9	Pathogen-inducible alpha-dioxygenase, *N. attenuata*	2
cLEI6D5	Protein kinase	2
cLEC73K6	Phytoene synthase	2
cLPP13J1	Receptor-like protein kinase	2
cLEC66G13a	Xyloglucan-specific fungal endoglucanase inhibitor protein	2
cLEC67B16	Jasmonic acid 2	2
cTOA29P9	Ethylene responsive element binding factor	2
cLEC73I19	Shikimate kinase, chloroplast precursor	2
cTOD16E7	Acidic 26kDa endochitinase precursor	2
cLEC72P14	Acidic 27kDa endochitinase precursor	2
cTOF16A9	Calmodulin 3 protein, *L. esculentum*	3
cLER17H16	Elicitor-inducible cytochrome P450, *N. tabacum*	3
cTOF18P1	Serine palmitoyltransferase, *S. tuberosum*	3
cLEC40M5	Hypothetical protein HMA8/weakly similar to putative disease related protein 2	3
cTOF5N18	Ribosomal protein L6 family protein	3
cTOF9P13	Serine/threonine protein kinase pk23	3
cLEI4N5	Serine/threonine protein kinase Fen	3
cLEX12O16	Ethylene response factor 5, *L. esculentum*	3
cTOF14B17	Anthocyanin 5-O-glucosyltransferase, *S. sogarandinum*	4
cLED15E5	Shikimate kinase chloroplast precursor, *L. esculentum*	4
cLEN13D5	Chorismate synthase 1 precursor, *L. esculentum*	4
cTOS21D12	Similar to heat shock factor, *N. tabacum*	4
cTOF10N11	Myo-inositol-1-phosphate synthase, *L. esculentum*	4
cLEC78C22	Late blight resistance protein, *Solanum demissum*	4
cLEY17F12	Tropionone reductase II	4
cLEX4M3	Protein kinase	4
cLEY12N4	Acetyl Co-A acetyltransferase	4
cLEF42D20	Stress enhanced protein 2(SEP2). *Arabidopsis thaliana*	4
cLEG50P8	Abscisic stress ripening protein 1	4
cLEN8H11	Lipoxygenase, Lycopersicon esculentum	4
cLEN7N12	MYC transcription factor / (Jasmonic acid 3, *Lycopersicon esculentum*	4
cLER5E19	Phospholipase PLDb1, *L. esculentum*	5
cTOC2J14	Weakly similar to disease resistance gene homolog Mi-copy1, *L. esculentum*	5
cTOF26E9	Prf, *L. pimpinellifolium*	5

Table 2.1 (Contd.)

EST Clone Name	Putative function	Chromosome No.
cTOE1K1	Spermidine synthase, *L. esculentum*	5
cTOC20D5	Serine/threonine protein kinase Pto (Pto kinase)	5
cLEC76E11	Ethylene receptor 1 (LeETR1)	5
cLEX12O16a	Ethylene response factor 5	5
cLEY18H8	Coronatine-insensitive 1	5
cTOA24J24	Ribosomal protein L6 (YL16-like)	5
cTOC20J21	60S Ribosomal protein L6 (YL16-like)	5
cLEG32E10	Lipoxygenase B, *L. esculentum*	6
cTOF8F19	Ascorbate peroxidase, *L. esculentum*	6
cLEZ16H16	Contains similarity to disease resistance response protein, *Pisum sativum*	6
cLEC76A13	Wound-induced protein 1	6
cLEG49O24	Phosphoenolpyruvate carboxylase kinase 2	6
cLEN10H12	Alcohol dehyrogenase 2	6
cLEC75A1	Band 7 family protein similar to hypersensitive-induced response protein *Zea mays*	6
cLER16A16	Wound-inducible carboxypeptidase	6
cTOF34C13	Peroxiredoxin Q-like protein, *A. thaliana*	7
cLEN14F9	Sucrose-phosphate synthase, *L. esculentum*	7
cTOF21F12	Dehydroquinate dehydratase/shikimate,NADP oxidoreductase, *L. esculentum*	7
cLEN13G22	1-aminocyclopropane-1-carboxylate oxidase, *L. esculentum*	7
cTOE15M9	MYB-related transcription factor VlMYBB1-1, *Vitis labrusca* × *V. vinifera*	7
cLEY22L20	Peroxidase precursor, *L. esculentum*	7
cTOS19O5	Stress responsive protein homolog, *Arabidopsis thaliana*	7
cTOF18O1	Diacylglycerol kinase	7
cLEC71D23	AtNAC2, Arabidopsis thaliana	7
cLEG57M16	Ethylene responsive element binding factor	7
cLEN14C8	PR-related protein, PR P23 (salt-induced protein), *L. esculentum*	8
cTOF9D16	Pathogenesis-related protein 5-1, *L. esculentum*	8
cTOF28D12	Polyphenol oxidase E, chloroplast precursor, *L. esculentum*	8
cLEN10H3	Heat shock factor protein HSF8 (Heat shock transcription factor 8), *L. esculentum*	8
cLEI16E21	Cold-induced glucosyl transferase, *L. esculentum*	8
cTOF2N15	Osmotin-like protein OSML13 precursor (PA13), *L. esculentum*	8
cTOE23J12	Monodehydroascorbate reductase, *L. esculentum*	8
cLES6H6	UDP-glucose:salicylic acid glucosyltransferase, *Nicotiana tabacum*	8
cLEC71I10	UDP-glucose:salicylic acid glucosyltransferase, *Nicotiana tabacum*	8
cLEC35F1	UDP-glucose:salicylic acid glucosyltransferase, *Nicotiana tabacum*	8
cLEC73B1	PR5-like protein	8
cLEX11E19	Putative NADH-ubiquinone oxireductase, *A. thaliana*	9
cTOE10J18	PR protein sth-2, *S. tuberosum*	9
cLEC13E21	P14 (PR-Protein), *L. esculentum*	9
cLEX10N16a	PR Protein	9
cLEC79A23	Lipoxygenase	9
cLED4N20	Wound-induced proteinase inhibitor I precursor	9
cLEZ6E21	Ubiquitin, *L. esculentum*	10
cLED18G6	Similar to WRKY-like drought-induced protein, *Retama raetam*	10
cTOD4I20	Tyrosine aminotransferase, *A. thaliana*	10
cLHT11J12	Diacylglycerol kinase, *L. esculentum*	10
cLER4F5a	Ferredoxin-I chloroplast precursor *L. esculentum*	10
cLER4F5b	Ferredoxin-I chloroplast precursor *L. esculentum*	10
cTOF30K21	Chloroplast ferredoxin I,, *L. esculentum*	10
cTOF22M16	NADH-ubiquinone oxidoreductase 23 kDa subunit, L. esculentum	10
cLEN14K6	Multi resistance protein homolog, A. thaliana	10
cLEX10N16b	PR protein, L. esculentum	10
cLEC18O1	Basic 30kDa endochitinase precursor, L. esculentum	10
cTOA21D22	Apoptosis inhinitory 5 (API5) family protein contains Pfam domain	10
cLPP2M12	Fructokinase	10
cLED13I7	Resistance complex protein I2C-1, *L. esculentum*	11

Table 2.1 (Contd.)

EST Clone Name	Putative function	Chromosome No.
cTOF28I23	Resistance complex protein I2C-5, *L. pimpinellifolium*	11
cTOF17F8	60S Ribosomal protein L6 (YL16-like)	11
cTOF22B15	Pto-responsive gene 1 protein	11
cTOF29F6	10-hydroxygeraniol oxidoreductase, - *L. esculentum*	11
cTOS21D14	WRKY transcription factor IId-2, *L. esculentum*	12
cLPT1G11	S-adenosyl-l-homocysteine hydrolase, *L. esculentum*	12
cLEZ15E8	Extensin class I, *L. esculentum*	12
cTOF12F19	Lycopene epsilon cyclase, chloroplast precursor	12
cLEG53C20	Similar to jasmonic acid regulatory protein-like, *Arabidopsis thaliana*	12
cLEC80G6	Disease resistance protein RPP8, *Arabidopsis thaliana*	12

The information on putative function of each EST has been obtained from http://www.tigr.org/tigr-scripts/tgi/T_reports.cgi?species=tomato or (Computational Biology and Functional Genomics Laboratory 2007)

Table 2-2 Primer sequences of PCR based markers used for parental survey of the *Lycopersicon esculentum* × *L. pimpinellifolium* F_7-RIL population

Marker	Chr.	Type	Forward Primer (5′ → 3′)	Reverse Primer (5′ → 3′)
T1480	2	CAPS	ACCACCTTGGATGAATACCG	TGCAACAGCTTTTCCCTCTC
T1621	3	CAPS	GACTGGTGAGGACGATGATG	CGGCAATCTCTTCGTCAAA
TG585	3	CAPS	TGGAAAGCCAGACACACAGA	CAGGGGTATCAGTAGGCAGTG
TG23	5	CAPS	GGTGGTTCAAATCCTTATTGG	AAAGTGTTGGGGTAACGCAC
TG279	6	CAPS	GTAGAATCCGCTGTCGCTTC	TATGTCCACGAAGTCGGTGA
T892	6	CAPS	TGGCTCTTCGGACTTTAGTGA	AGCACCTTCTGCGTTCATCT
TG174	7	CAPS	TTCCAAGATCTTTTAGCGTCTC	CTGTTGCGGATGTGATCATT
TG302	8	CAPS	CTCTCCGGGTGGCTATTACA	TCTTGGGACTCCTCCTTTTCT
T1190	9	CAPS	GCGTTCTCGTTACTGGTGCT	GTTGCATGGTTGACATCAGG
TG254	9	CAPS	GACTTCGGGGCAATTATCTG	AAACGAGCACTGCATTCATG
TG328	9	CAPS	GGTGACTGCTTGACTTGGG	AAGGTCTAAAGAAGGCTGGTGC
TG303	10	CAPS	CGTAAAGGGTTGTTCTTGTGC	TGTTTTCGAGTGGGGTTCAT
TG400	11	CAPS	TCCAAATCCACCACCTATCC	AGCATTGCTCCCTGCTAAAG
TG497	11	CAPS	CGGAGAGTGAAGATGCATTG	AAGTTCCAGAGGGAGCACAA
T302	11	CAPS	TGGCTCATCCTGAAGCTGATAGCGC	AGTGTACATCCTTGCCATTGACT
C2_At3g52220	11	COS	TGCTCGGGTGGATGGTCTTGG	TGATGGTGAACTTGGTTCTTCCC
C2_At2g28600	11	COS	AGCGATGATTCCATTCAGAGAAGG	TCCCCATGTATGGATGAAGAAAGGC
C2_At3g60830	11	COS	ATGCTGGTTCTAAATTTCTCAAAGC	ATATGCGTCCAACTGCATAAAGCG
C2_At5g16710	11	COS	ACTTGATGAGCTGACAGCTTTCAATG	AGCTTTGGTCCAAGCGACAAATC
C2_At2g22570	11	COS	ACTGAAGAGTGAGATTCCGGTGGAG	TCTGTTCCAGTGATACAATGAGGAGG
C2_At4g22260	11	COS	TCCTCTAACGGTCTAGAGAAATGGG	AGGAACTCTTGCAATTGTTTCCAGAAC
C2_At3g02870	11	COS	TGAAGCTGCTAAAAAAGCTGGAGAG	ACAAAAGGGAACCCGTGCACAAAG
C2_At1g21690	11	COS	ATGCAGAGCTCTCAGCCATGGG	ACCTACAGCAACAGCAGCAAAGTTC
C2_At1g44446	11	COS	AGATCTTGCACACGCACCTTTCAC	TCCTTGCAGACCAGATGCAGGAGTC
C2_At1g44790	11	COS	TCGGTTTTATCAAAGGCTATCGTC	TGTTACTGTTCTACCTGGGAATTCTGG
C2_At2g14260	11	COS	AGGATCTATACCCCTCTATAGAGCC	TTATTGGGTGAAGTCCCACCTCC
C2_At5g12200	11	COS	TCCTGACTTCCATTTTGCAGCAAG	TGCTCTGATTGGTGGGCTCATGAC
C2_At4g10050	11	COS	ATCACCCTTCTGCCTTTTCTTC	ATCTGGGATCTGAATGTCATCCTC
C2_At5g04590	11	COS	ATCACCACAGTCCTTGCACAGGG	AGGACAAAGTGGAAAAGCTGGG
COSOH57	11	COS	TGCCCAAAAGCACAGTACAA	CGCCTCCTATCTTCCAAACTT
KFG J1	11	KFG	CATCCACCGCTATGTACGTG	CACCACTCACCCATCTTGTG
SSR46	11	SSR	CCGAGGCGAATCTTGAATAC	GCACCATCTCTTGTGCCTCT
SSR67	11	SSR	GCACGAGACCAAGCAGATTA	GGGCCTTTCCTCCAGTAGAC
SSR76	11	SSR	ACGGGTCGTCTTTGAAACAA	CCACCGGATTCTTCTTCGTA
SSR80	11	SSR	GGCAAATGTCAAAGGATTGG	AGGGTCATGTTCTTGATTGTCA
SSR136	11	SSR	GAAACCGCCTCTTTCACTTG	CAGCAATGATTCCAGCGATA
SSR345	12	SSR	AAGCCAAGCTCGAACCTGTA	ATCCATGCTGTCGCTTTCAT
cLET8K4	12	CAPS	CACTTTGTGGCAATCGACAT	TGCCTTATGCCAAACAGAAA
TG68	12	CAPS	TCCACCTAGGATGAGTTTGGA	CATGTCAAGGGGATTGAACA
TG180	12	CAPS	TCTCAGTGGACTAAGGGGTCA	TCACAGCAGACATGTCGGAC

Table 2-3 Description of CAPS/COS/KFG markers on the genetic map of *Lycopersicon esculentum* × *L. pimpinellifolium* F_7 RIL population

Marker	Chr.	TM °C	Band Size (bp)	Polymorphic band size between NCEBR1/PSLP125	Enzyme
T1480	2	50	1300	Monomorphic	*N/A*
T1621	3	55	1300	Monomorphic	*N/A*
TG585	3	55	491	180/100+80	*Dpn II*
TG23	5	55	1700	Monomorphic	*N/A*
TG279	6	55	750	Monomorphic	*N/A*
T892	6	55	1400	1300+100/1400	*Scrf I*
TG174	7	55	1500	Monomorphic	*N/A*
TG302	8	50	750	320/500	*Alu I*
T1190	9	50	2000	750+100/850	*Rsa I/Dpn II*
TG254	9	55	1700	700/650+50	*Alu I*
TG328	9	55	482	480/240+240	*Bst NI/Scrf I*
TG303	10	55	400	400/300+100	*Dpn II*
TG400	11	55	404	Monomorphic	*N/A*
TG497	11	55	800	Monomorphic	*N/A*
T302	11	55	950/850	Monomorphic	*N/A*
C2_At3g52220	11	55	300	200+50/200+100	*HinfI*
C2_At2g28600	11	55	1400	Monomorphic	*N/A*
C2_At3g60830	11	55	2800	1000+900/2800	*HaeIII*
C2_At5g16710	11	55	1600	Monomorphic	*N/A*
C2_At2g22570	11	55	1250	Monomorphic	*N/A*
C2_At4g22260	11	55	700	Monomorphic	*N/A*
C2_At3g02870	11	55	300	150/120†	*HindIII*
C2_At1g21690	11	55	1600	150/180†	*BstNI*
C2_At1g44446	11	55	300	Monomorphic	*N/A*
C2_At1g44790	11	55	280	300/180+120	*AluI*
C2_At2g14260	11	55	1500	Monomorphic	*N/A*
C2_At5g12200	11	55	120	Monomorphic	*N/A*
C2_At4g10050	11	55	650	400+250/650*	*BstNI,TaqI,CfoI*
C2_At5g04590	11	55	1100	Monomorphic	*N/A*
COSOH57	11	55	250	250/150+100	*Tth111I*
KFG J1	11	55	1000	850/750	*BstUI*
cLET8K4	12	55	1100	Monomorphic	*N/A*
TG68	12	50	440	Monomorphic	*N/A*
TG180	12	50	1000	1000/450+550	*Taq I*

Table 2-4 Significant deviations from the expected 1:1 ratios in the *Lycopersicon esculentum* × *L. pimpinellifolium* F_7 RIL population (E = *esculentum* allele, PM = *pimpinellifolium* allele)

		Genotype			
Locus	**Chromosome**	**E/E**	**PM/PM**	**E/PM**	**χ^2 Probability**
cLEC34L10b	1	57	94	13	0.0026
cLEC71F17	1	55	100	14	0.0003
cLEC72D1	1	53	105	14	0.0000
TG125	1	53	105	12	0.0000
cLEI16L6	1	50	105	14	0.0000
cTOF3A14	1	56	102	10	0.0003
cTOD16E7	2	61	97	14	0.0042
cLEC72P14	2	62	97	13	0.0055
cLET10E15	2	62	97	13	0.0055
TG463	2	62	100	10	0.0028
CG21	2	62	99	8	0.0035
TG645	2	61	96	10	0.0052
cLEY1K9	2	60	96	14	0.0039
TG114	3	60	99	13	0.0020
TG132	3	60	99	13	0.0020
TG585CAPS	3	57	97	11	0.0013
TG66	3	50	106	14	0.0000
cLEC73K6a	3	46	109	15	0.0000
cTOF5N18	3	41	98	11	0.0000
TG387	3	40	110	20	0.0000
cTOF9P13	3	37	112	19	0.0000
cLEX12O16b	3	29	122	16	0.0000
cTOF18P1	3	39	118	15	0.0000
cTOF16A9	3	35	119	17	0.0000
cTG515	3	41	109	14	0.0000
cLEI4N5	3	48	98	12	0.0000
CT178	4	61	94	15	0.0080
TG272	4	62	95	13	0.0084
C25	4	57	100	13	0.0006
CT167	5	99	55	11	0.0004
CT93	5	103	51	16	0.0000
TG503	5	104	50	16	0.0000
cLER5E19	5	111	48	11	0.0000
cTOF26E9	5	107	50	11	0.0000
cTOC20D5	5	105	51	15	0.0000
cLEC76E11	5	106	43	8	0.0000
cLEX12O16a	5	116	42	2	0.0000
cLEY18H8	5	106	46	13	0.0000

Table 2-4 (Contd.)

Locus	Chromosome	Genotype			χ^2 Probability
		E/E	PM/PM	E/PM	
TG351	5	111	46	12	0.0000
TOC2J14	5	117	43	10	0.0000
cTOC20J21	5	119	42	6	0.0000
cTG185	5	115	42	14	0.0000
cLEC76A13	6	99	59	10	0.0015
TG590	6	98	63	9	0.0058
cLEG49O24	6	100	63	5	0.0038
CT285	6	94	60	2	0.0061
cLEN10H12	6	114	53	4	0.0000
TG356	6	130	37	4	0.0000
TG365	6	144	22	5	0.0000
TG253	6	151	17	4	0.0000
cLEC75A1	6	151	17	4	0.0000
C54	6	162	7	2	0.0000
TG279	6	163	7	2	0.0000
cLEZ16H16	6	154	15	2	0.0000
TG477	6	152	15	5	0.0000
cLER16A16	6	138	26	7	0.0000
cLEC71D23	7	96	57	14	0.0016
TG438	7	91	59	18	0.0090
cTOA29P9b	7	96	58	17	0.0022
cLEG57M16	7	93	57	15	0.0033
TG499	7	93	61	18	0.0099
C21	8	97	63	10	0.0072
CT92	8	100	58	12	0.0008
TG349	8	96	55	12	0.0008
cTOF28D12	8	97	63	10	0.0072
CT265	8	97	64	11	0.0093
cLEC73B1	8	99	58	14	0.0011
cTOF2N15	8	98	58	16	0.0014
cLEN14C8	8	97	59	15	0.0023
cTOF9D16	8	99	60	13	0.0020
cTOE23J12	8	95	60	11	0.0049
CT279	9	56	94	11	0.0019
cLEC79A23	9	60	98	13	0.0025
TG348	9	61	98	12	0.0033
TG303CAPS	10	64	97	11	0.0068
cLHT11J12	10	61	101	11	0.0093

Table 2-4 (Contd.)

Locus	Chromosome	Genotype			χ^2 Probability
		E/E	PM/PM	E/PM	
cLER4F5a	10	61	100	10	0.0017
cTOF22M16	10	58	102	10	0.0021
cLEX10N16b	10	58	102	11	0.0005
cLEN14K6	10	59	101	8	0.0005
cLPP2M12	10	63	99	12	0.0009
cLEC18O1	10	64	97	8	0.0047

Table 2-5 Pair-wise comparison of the present map (E × PM-1) with other maps of tomato for individual chromosome lengths

	Chromosome length (cM)													
Linkage Map[a]	1	2	3	4	5	6	7	8	9	10	11	12	Average	Total
E × PM-1 (F_7-RIL)	125.8	92.4	97.4	85.1	92.9	65.2	82.5	77.5	82.2	78.8	107.9	78.4	88.8	1066.1
E × PM-2 (F_2)	97.2	92.6	84.5	71.0	68.3	72.5	69.8	82.5	93.6	79.0	81.8	75.8	80.7	968.6
E × PM-1/E × PM-2	1.3	1.0	1.5	1.2	1.8	0.9	1.8	0.9	1.3	1.0	1.5	1.0	1.26	
E × PM-3 (BC1)	129.7	121.9	133.8	108.0	94.1	82.8	91.3	64.4	104.8	84.9	78.2	92.6	98.9	1186.5
E × PM-1/E × PM-3	1.0	0.8	0.7	0.8	1.0	0.8	0.9	1.2	0.8	0.9	1.4	0.8	0.93	
E × PM-2/E × PM-3	0.7	0.8	0.6	0.7	0.7	0.9	0.8	1.3	0.9	0.9	1.0	0.8	0.84	
E × PM-4	149.6	98.2	116.6	97.2	108.2	85.2	116.4	86.1	104.2	101.5	107.0	105.2	106.3	1275.4
E × PM-1/E × PM-4	0.8	0.9	0.8	0.9	0.9	0.8	0.7	0.9	0.8	0.8	1.0	0.7	0.76	
E × PM-2/E × PM-4	0.6	0.9	0.7	0.7	0.6	0.9	0.6	1.0	0.9	0.8	0.8	0.7	0.83	
E × P (BC_1)	133.5	124.2	126.1	124.8	97.4	101.9	91.6	94.9	111.0	90.1	88.0	93.1	106.4	1276.6
E × PM-1/E × P	0.9	0.7	0.8	0.7	1.0	0.6	0.9	0.8	0.7	0.9	1.2	0.8	0.83	
E × PM-2/E × P	0.7	0.7	0.7	0.6	0.7	0.7	0.8	0.9	0.8	0.9	0.9	0.8	0.76	

E × PM-1, *L. esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) map (RIL-F_7); E × PM-2 , *L. esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) map (selected F_2 population map); E × PM-3, *L. esculentum* (NC 84173) × *L. pimpinellifolium* (LA722) map (Chen and Foolad 1999); E × PM-4, *L. esculentum* (M82-1-7) × *L. pimpinellifolium* (LA1589) map (Grandillo and Tanksley et al. 1992); E × P, *L. esculentum* (VF36-*Tm*2) × *L. pennellii* (LA716) map (Tanksley et al. 1992).

Table 2-6 Comparison of map distances based on common marker intervals between four molecular linkage maps of tomato[†]

Interval	Chr.	Marker interval map distance (cM)												
		E×PM-1[a]	E×PM-2[b]	E×PM-3[c]	E×PM-4[d]	E × PM-1 (E × PM-3	E × PM-1 E × PM-4	E × PM-2 E × PM-3	E × PM-2 E × PM-4	E×P[d]	E × PM-1 E × P	E × PM-2 E × P	E × PM-3 E × P	E × PM-4 E × P
TG301-TG125	1	27.0	-	27.2	41.0	1.0	0.7	-	-	13.0	2.1*	-	2.09*	3.15*
TG125-TG70	1	12.6	8.1	7.0	12.5	1.8	1.0	1.2	0.6	13.0	1.0	0.6	0.54	0.96
TG70-TG273	1	22.2	24.2	-	34.7	-	0.6	-	0.7	8.9	2.5*	2.7*	-	3.90*
TG273-TG59	1	7.1	8.7	8.8	4.1	0.8	1.7	1.0	2.1	15.7	0.5	0.6	0.56	0.26
TG59-CT191	1	8.9	10.0	13.2	9.7	0.7	0.9	0.8	1.0	17.4	0.5	0.6	0.76	0.56
CT137-TG580	1	8.3	6.4	8.6	-	1.0	-	0.7	-	16.0	0.5	0.4	0.54	-
TG608-CT205	2	11.5	-	7.8	14.0	1.5	0.8	-	-	16.2	0.7	-	0.48	0.86
CT205-CT176	2	6.8	-	6.2	6.1	1.1	1.1	-	-	6.3	1.1	-	0.98	0.97
CT176-TG453	2	17.2	20.0	29.5	-	0.6	-	0.7	-	14.4	1.2	1.4	2.05*	-
TG453-CT103	2	10.4	10.7	13.3	-	0.8	-	0.8	-	15.0	0.7	0.7	0.89	-
TG554-TG453	2	6.8	6.6	-	-	-	-	-	-	0.0	-	-	-	-
TG453-TG145	2	0.0	0.4	-	-	-	-	-	-	6.8	0.0	0.1	-	-
TG145-CT103	2	10.4	10.3	-	-	-	-	-	-	10.1	1.0	1.0	-	-
CT103-TG151	2	34.1	-	38.2	-	0.9	-	-	-	52.8	0.6	-	0.72	-
TG151-TG620	2	4.4	-	2.5	-	1.8	-	-	-	11.2	0.4	-	0.22	-
CT176-TG582	2	-	5.6	18.9	-	-	-	0.3	-	5.4	-	1.0	3.50*	-
CT59-TG620	2	-	2.6	-	-	-	-	-	-	3.5	-	0.7	-	-
TG114-TG132	3	0.0	0.0	8.9	-	0.0	-	0.0	-	13.0	0.0	0.0	0.68	-
TG132-CT22	3	10.6	-	13.4	-	0.8	-	-	-	14.0	0.8	-	0.96	-
CT22-TG66	3	16.5	-	20.0	-	0.8	-	-	-	9.0	1.8	-	2.22*	-
TG66-CT225B	3	-	6.6	-	-	-	-	-	-	0.7	-	9.4*	-	-
TG66-CT85	3	44.2	40.4	58.9	-	0.8	-	0.7	-	33.0	1.3	1.2	1.78	-
CT85-TG214	3	18.7	20.7	32.6	19.8	0.6	0.9	0.6	1.0	23.5	0.8	0.9	1.39	0.84
CT225B-CT82	3	-	9.0	-	-	-	-	-	-	7.5	-	1.2	-	-
CT82-TG515	3	-	12.7	-	-	-	-	-	-	4.3	-	3.0*	-	-
TG123-TG182	4	13.5	12.5	-	-	-	-	-	-	11.9	1.1	1.1	-	-
TG182-TG609	4	5.9	5.5	-	-	-	-	-	-	5.1	1.2	1.1	-	-
TG609-CT178	4	12.0	11.9	-	-	-	-	-	-	12.0	1.0	1.0	-	-
TG123-TG272	4	32.8	-	37.2	-	0.9	-	-	-	29.0	1.1	-	1.28	-
CLE5-CT101	5	5.3	4.0	6.1	-	0.9	-	0.7	-	-	-	-	-	-
CT101-CT242	5	-	6.9	6.4	-	-	-	1.1	-	9.0	-	0.8	0.71	-
CT167-CT93	5	15.2	19.3	8.9	16.4	1.7	0.9	2.2*	1.2	13.9	1.1	1.4	0.64	1.18
CT93-TG351	5	33.0	17.7	52.1	48.3	0.6	0.7	0.3	0.4	41.6	0.8	0.4	1.25	1.16
TG351-TG185	5	19.2	10.7	14.2	12.7	1.4	1.5	0.8	0.8	23.5	0.8	0.5	0.60	0.54
TG503-TG96	5	-	2.5	-	-	-	-	-	-	3.2	-	0.8	-	-

Table 2-6 (Contd.)

Interval	Chr.	Marker interval map distance (cM)												
		E×PM-1[a]	E×PM-2[b]	E×PM-3[c]	E×PM-4[d]	E×PM-1 / E×PM-3	E×PM-1 / E×PM-4	E×PM-2 / E×PM-3	E×PM-2 / E×PM-4	E×P[d]	E × PM-1 / E × P	E × PM-2 / E × P	E × PM-3 / E × P	E × PM-4 / E × P
CT216-CT285	6	21.5	21.3	17.6	-	1.2	-	1.2	-	17.0	1.3	1.3	1.04	-
CT285-TG356	6	16.8	14.7	19.5	-	0.9	-	0.8	-	10.8	1.6	1.4	1.81	-
TG356-TG365	6	7.8	13.0	11.9	11.8	0.7	0.7	1.1	1.1	4.1	1.9*	3.2*	2.90*	2.88*
TG365-TG253	6	2.5	4.1	5.9	-	0.4	-	0.7	-	10.8	0.2	0.4	0.55	-
TG253-C54	6	3.5	7.4	6.8	-	0.5	-	1.1	-	-	-	-	-	-
C54-TG279	6	3.5	4.0	10.2	-	0.3	-	0.4	-	-	-	-	-	-
TG279-TG477	6	3.4	6.3	6.4	-	0.5	-	1.0	-	2.4	1.4	2.6*	2.67*	-
TG274-TG590	6	-	3.4	-	-	-	-	-	-	10.4	-	0.3	-	-
TG279-TG365	6	9.5	15.5	22.9	13.5	0.4	0.7	0.7	1.1	27.6	0.3	0.6	0.83	0.49
TG113-TG156	7	16.2	19.4	29.0	-	0.6	-	0.7	-	19.7	0.8	1.0	1.47	-
TG156-CT135	7	0.0	-	1.1	-	0.0	-	-	-	0.0	-	-	-	-
CT135-TG174	7	11.9	-	12.3	-	1.0	-	-	-	19.3	0.6	-	0.64	-
TG174-TG128	7	14.1	19.3	13.6	-	1.0	-	1.4	-	3.4	4.1*	5.7*	4.00*	-
TG183-TG128	7	12.7	15.7	3.5	-	3.6*	-	4.5*	-	2.3	5.5*	6.8*	1.52	-
TG128-CT226	7	4.2	3.1	3.5	-	1.2	-	0.9	-	1.6	2.6*	1.9*	2.19*	-
CT226-TG499	7	24.8	16.7	31.8	-	0.8	-	0.5	-	38.5	0.6	0.4	0.83	-
TG499-TG128	7	29.0	19.8	35.3	27.4	0.8	1.1	0.6	0.7	41.9	0.7	0.5	0.84	0.65
TG128-CD57	7	-	-	27.0	31.0	-	-	-	-	25.71	-	-	1.05	1.21
TG176-CD40	8	6.5	8.5	-	19.6	-	0.3	-	0.4	6.0	1.1	1.4	-	3.27*
CD40-CT92	8	8.8	6.2	10.5	3.2	0.8	2.8	0.6	1.9	10.9	0.8	0.6	0.96	0.29
CT92-TG349	8	5.6	7.7	8.5	13.2	0.7	0.4	0.9	0.6	13.4	0.4	0.6	0.63	0.99
TG349-TG302	8	6.5	6.7	8.2	11.0	0.8	0.6	0.8	0.6	6.1	1.1	1.1	1.34	1.80
TG302-CT265	8	27.2	24.8	27.6	30.2	1.0	0.9	0.9	0.8	42.7	0.6	0.6	0.65	0.71
CT265-TG294	8	12.1	12.8	9.6	-	1.3	-	1.3	-	13.1	0.9	1.0	0.73	-
CT143-CT279	9	28.3	33.0			-	-	-	-		-	-		
CT143-TG486	9	-	19.8	-	-	-	-	-	-	32.4	-	0.6	-	-
TG486-CT279	9	-	13.2	-	-	-	-	-	-	9.4	-	1.4	-	-
CT279-TG348	9	8.3	10.2	-	-	-	-	-	-	6.1	1.4	1.7	-	-
TG348-TG421	9	15.6	14.9	-	-	-	-	-	-	17.6	0.9	0.8	-	-
TG421-CT96	9	10.9	6.3	-	-	-	-	-	-	13.3	0.8	0.5	-	-
TG421-TG328	9	14.8	-	22.4	11.9	0.7	1.2	-	-	18.1	0.8	-	1.24	0.66
CT96-TG328	9	3.9	-	-	-	-	-	-	-	4.8	0.8	-	-	-
TG486-CD3	9	-	1.7	–	-	-	-	-	-	1.3	-	1.3	-	-
CD3-CT279	9	-	11.5	–	-	-	-	-	-	5.6	-	2.1*	-	-
CT279-TG35	9	-	1.9	–	-	-	-	-	-	0.0	-	-	-	-
CT16-CT234	10	11.4	12.0	13.8	23.1	0.8	0.5	0.9	0.5	20.8	0.5	0.6	0.66	1.11
CT234-CT11	10	16.3	16.1	12.0	28.4	1.4	0.6	1.3	0.6	4.7	3.5*	3.4*	2.55*	6.04*
CT11-CD34	10	5.2	9.6	10.3	-	0.5	-	0.9	-	18.5	0.3	0.5	0.56	-

Table 2-6 (Contd.)

Interval	Chr.	Marker interval map distance (cM)												
		E×PM-1[a]	E×PM-2[b]	E×PM-3[c]	E×PM-4[d]	E × PM-1 / E × PM-3	E × PM-1 / E × PM-4	E × PM-2 / E × PM-3	E × PM-2 / E × PM-4	E × P[d]	E × PM-1 / E × P	E × PM-2 / E × P	E × PM-3 / E × P	E × PM-4 / E × P
CD34-KJB3	10	17.4	13.8	13.0	-	1.3	-	1.1	-	-	-	-	-	-
KJB3-CC33	10	1.1	0.0	1.7	-	0.6	-	0.0	-	-	-	-	-	-
CC33-TG403	10	15.3	16.0	22.8	-	0.7	-	0.7	-	-	-	-	-	-
TG403-TG63	10	9.0	6.6	11.3	7.9	0.8	1.1	0.6	0.8	16.5	0.5	0.4	0.68	0.48
TG408-CD34 (b)	10	3.1	4.9	-	-	-	-	-	-	14.7	0.2	0.3	-	-
CD34 (b)-TG403	10	33.8	19.8	27.5	-	1.2	-	0.7	-	10.0	3.4*	2.0*	2.75*	-
TG629-TG497	11	0.0	0.0	-	-	-	-	-	-	0.0	-	-	-	-
CT107-TG546	11	13.1	17.7	10.1	31.3	1.3	0.4	1.8	0.6	18.2	0.7	1.0	0.55	1.72
TG546-TG36	11	4.7	6.2	10.8	6.8	0.4	0.7	0.6	0.9	10.3	0.5	0.6	1.05	0.66
TG147-CT55	11	2.8	2.8	-	-	-	-	-	-	8.0	0.4	0.4	-	-
TG400-TG546	11	12.8	16.7	-	-	-	-	-	-	15.3	0.8	1.1	-	-
TG36-TG30	11	2.1	2.6	-	-	-	-	-	-	8.1	0.3	0.3	-	-
TG30-CT65	11	8.2	17.7	-	-	-	-	-	-	1.8	4.6*	9.8*	-	-
CT65-TG26	11	0.0	3.4	-	-	-	-	-	-	0.0	-	-	-	-
TG180-TG68 (b)	12	2.3	-	7.7	5.8	0.3	0.4	-	-	13.8	0.2	-	0.56	0.42
TG68-CT79	12	7.8	6.9	6.7	-	1.2	-	1.0	-	14.4	0.5	0.5	0.47	-
CT79-CT100	12	9.6	15.5	17.0	-	0.6	-	0.9	-	9.8	1.0	1.6	1.73	-
CT100-TG565	12	13.9	30.9	12.0	-	1.2	-	2.6	-	17.0	0.8	1.8	0.71	-
TG565-TG111	12	4.0	7.9	3.4	5.3	1.2	0.8	2.3	1.5	7.4	0.5	1.1	0.46	0.72
TG111-CT80(b)	12	39.4	-	32.9	-	1.2	-	-	-	27.4	1.4	-	1.20	-
CT80(b)-TG473	12	1.3	-	9.4	-	0.1	-	-	-	16.8	0.1	-	0.56	-
CT99-TG618	12	5.5	5.6	-	-	-	-	-	-	0.8	6.9*	7.0*	-	-
TG618-TG111	12	8.0	13.7	-	-	-	-	-	-	6.6	1.2	2.1*	-	-
TG111-TG156	12	40.4	34.9	-	35.1	-	1.2	-	1.0	25.9	1.6	1.3	-	1.36
CT156-TG473	12	14.0	5.8	-	-	-	-	-	-	19.1	0.7	0.3	-	-
TG473-CD2	12	0.3	0.0	-	-	-	-	-	-	1.8	0.2	0.0	-	-

†Only common marker intervals that were different in length by at least two-fold between E × PM-1, E × PM-2, E × PM-3 or E × P linkage maps are shown.

[a] E × PM-1 = *Lycopersicon esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) map (RIL-F_7 map).

[b] E × PM-2 = *Lycopersicon esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) map (F_2 map) (Foolad et al. unpublished)

[c] E × PM-3 = *L. esculentum* (NC84173) × *L. pimpinellifolium* (LA722) map (Chen and Foolad 1999)

[d] E × PM-4 = *L. esculentum* (M82-1-7) × *L. pimpinellifolium* (LA1589) map (Grandillo and Tanksley 1996a)

[d] E × P = *L. esculentum* (VF36-*Tm*2) × *L. pennellii* (LA716) map (Tanksley et al. 1992).

*Different in interval length by at least two-fold. Dashes (−) indicate on common interval for comparison.

Table 2-7 Correlation coefficients between different maps based on common marker intervals[a]

	Marker interval lengths			
	E×PM-1[a]	**E×PM-2**	**E×PM-3**	**E×PM-4**
E×PM-2	0.86** (70)			
E×PM-3	0.88** (60)	0.71** (48)		
E×PM-4	0.83** (28)	0.76** (23)	0.75** (26)	
E×P	0.70** (78)	0.57** (76)	0.74**(57)	0.50**(29)

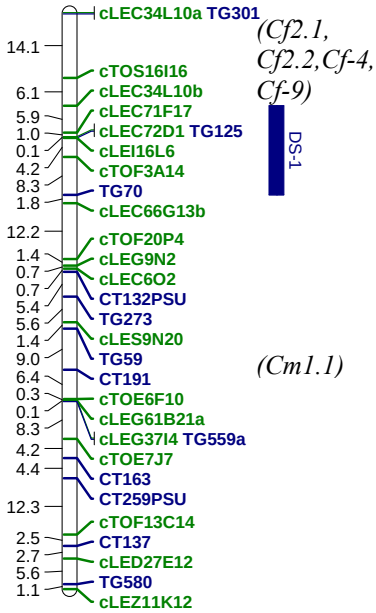
[a] see text for description of various linkage maps

* $P < 0.05$; ** $P < 0.01$, ns= none significant correlation

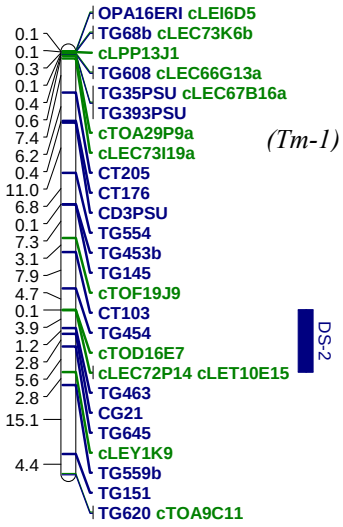
The numbers in parentheses are the number of paired common intervals

Figures

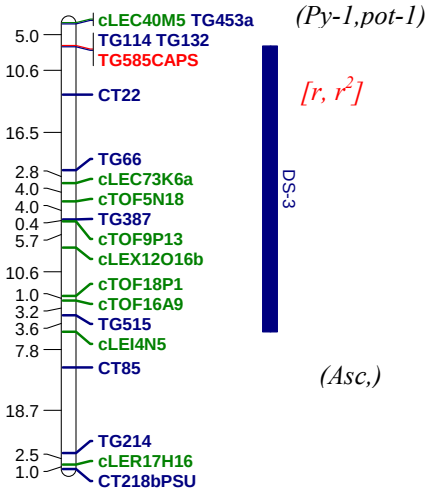
Chromosome 1



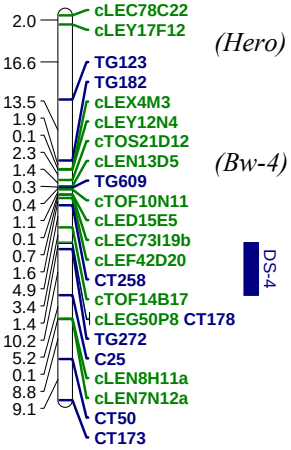
Chromosome 2



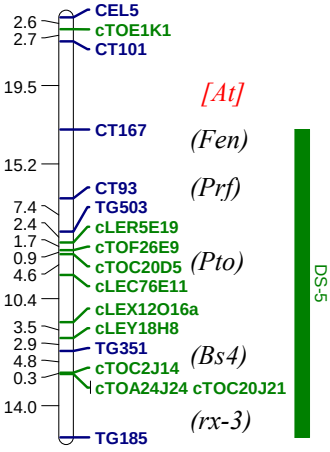
Chromosome 3



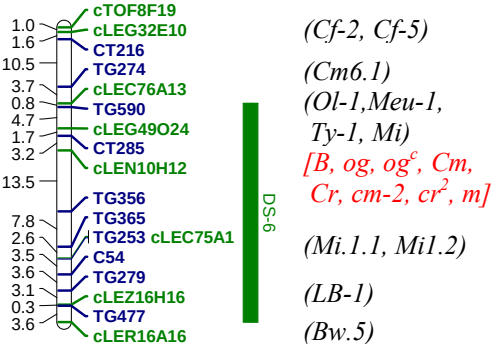
Chromosome 4



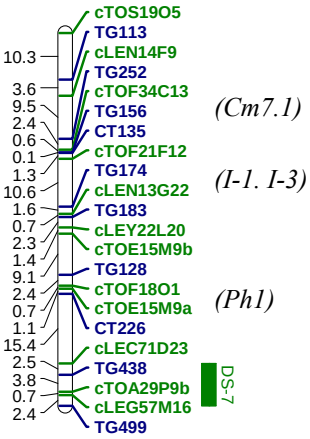
Chromosome 5



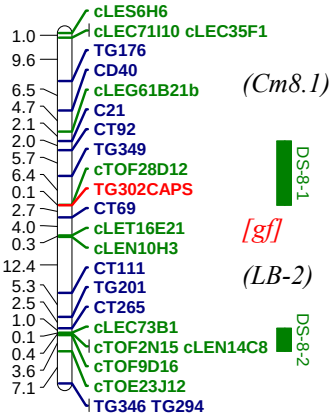
Chromosome 6



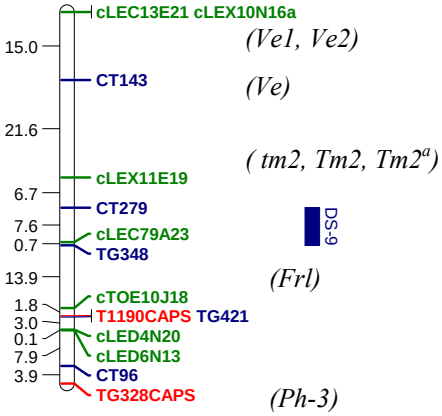
Chromosome 7



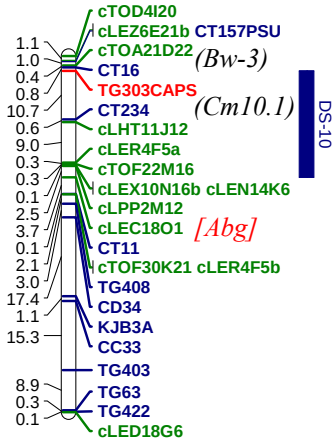
Chromosome 8



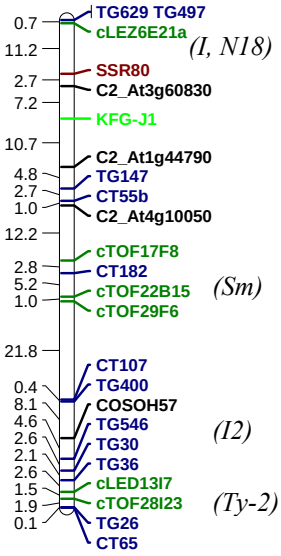
Chromosome 9



Chromosome 10



Chromosome 11



Chromosome 12

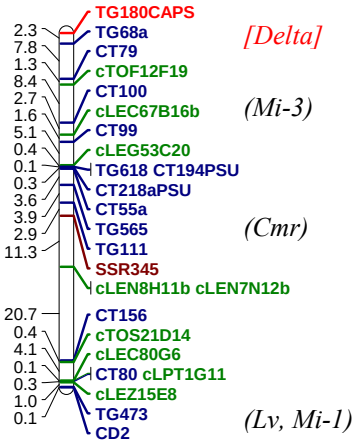


Figure 2-1 The genetic linkage map of RIL-F_7 population based on 269 RFLP, EST and CAPS markers

RFLP markers are depicted in **blue**, ESTs in **green**, CAPS in **red**, COS markers in **black**, Known Functional Genes marker in **florescent green** and SSRs in **brown** color. Markers with distorted segregation (DS) are marked by **blue** (DS toward PM alleles) or **green** (DS toward E alleles) bars. The approximate locations of disease-resistance genes (R genes), quantitative resistance loci (QRLs), and fruit color related genes (depicted in red) as inferred from other published research, are shown in parentheses to the right of chromosomes.

The descriptions of the R genes and QRLs are as follow: *Asc*, resistance to stem canker (*Alternaria alternata*) (van-der-Biezen et al. 1995); *Bw* (*Bw-1, Bw-3, Bw-4, Bw-5)*, QRLs for resistance to bacterial wilt (*Ralstonia solanacearum*)(Mangin et al. 1999); *Cf* (*Cf-1, Cf-2, Cf-4, Cf-5, Cf-9, Cf-ECP2*), resistance to leaf mould (*Cladosporium fulvum*)(Haanstra et al. 1999a; Thomas et al. 1998); *Cm* (*Cm1.1, Cm6.1, Cm7.1, Cm8.1, Cm9.1, Cm10.1*), QRLs for resistance to bacterial canker (*Clavibacter michiganensis*) (Sandbrink et al. 1995); *Fen*, sensitivity to fenthion (Martin et al. 1994); *Fr1*, resistance to Fusarium wilt (*Fusarium oxysporum* f.sp. *radicis-lycopersici*) (Vakalounakis et al. 1997); *Hero*, resistance to potato cyst namatode (*Globodera rostochiensis*) (Ganal et al. 1995); *I* (*I-1, I-2, I-3*), resistance to different races of Fusarium wild (*Fusarium oxysporum* f.sp. *lycopersici*) (Ori et al. 1997); *LB-1* and *LB-2*, QRLs for resistance to tomato late blight (*Phytophthora infestans*) (Frary et al. 1998); *Lv*, resistance to powdery mildew (*Leveuillula taurica*) (Chunwongse et al. 1994); *Mi* and *Mi-3*, resistance to root knot nematodes (*Meloidogyne* spp.) (Veremis et al. 1999; Yaghoobi et al. 1995); *N18*, resistance to tobacco mosaic virus (Whitham et al. 1994); *OI-1*, resistance to powdery mildew (*Oidium lycopersicum*) (van-der-Beek et al. 1994); *Ph* (*Ph-1, Ph-2, Ph-3*), resistance to late blight (*Phytophthora infestans*) in tomato (Chunwongse et al. 1998); *Pto* and *Prf*, resistance to bacterial speck (*Pseudomonase syringae* pv. *tomato*) (Martin et al. 1993b; Salmeron et al. 1996); *Py-1*, resistance to corky root rot (*Pyrenochaeta lycopersici*) (Doganlar et al. 1998); *rx* (*rx-1, rx-2, rx-3*), resistance to bacterial spot (*Xanthomonas campestris*) (Yu et al. 1995); *Sm*, resistance to *Stemphilium* (Behare et al. 1991); *Sw-5*, resistance to tomato spotted wilt virus (Brommenschenkel and Tanksley 1997); *Tm-1* and *Tm-2a*, resistance to tobacco mosaic virus (Young and Tanksley 1989); *Ty-1* and *Ty-2*, resistance to yellow leaf curl virus (Hanson et al. 2000; Zamir et al. 1994); *Ve*, resistance to *Verticillium dahliae* (Diwan et al. 1999). Fruit color genes are designated in red and the description of them are as follows: *Og, Og^c, cr* or old gold crimson (Ronen et al. 2000); *B*, high β-carotene (Ronen et al. 2000); *Abg* or aubergine gene for fruit color, flavor and ripening modification (Chetelat and Meglic 2000); *Delta*, orange mature fruit color due to inhibition of lycopene and increase of δ-carotene (Ronen et al. 1999); *At* or apricot gene confers yellow-pink color of fruit flesh (Mackinney et al. 1956)

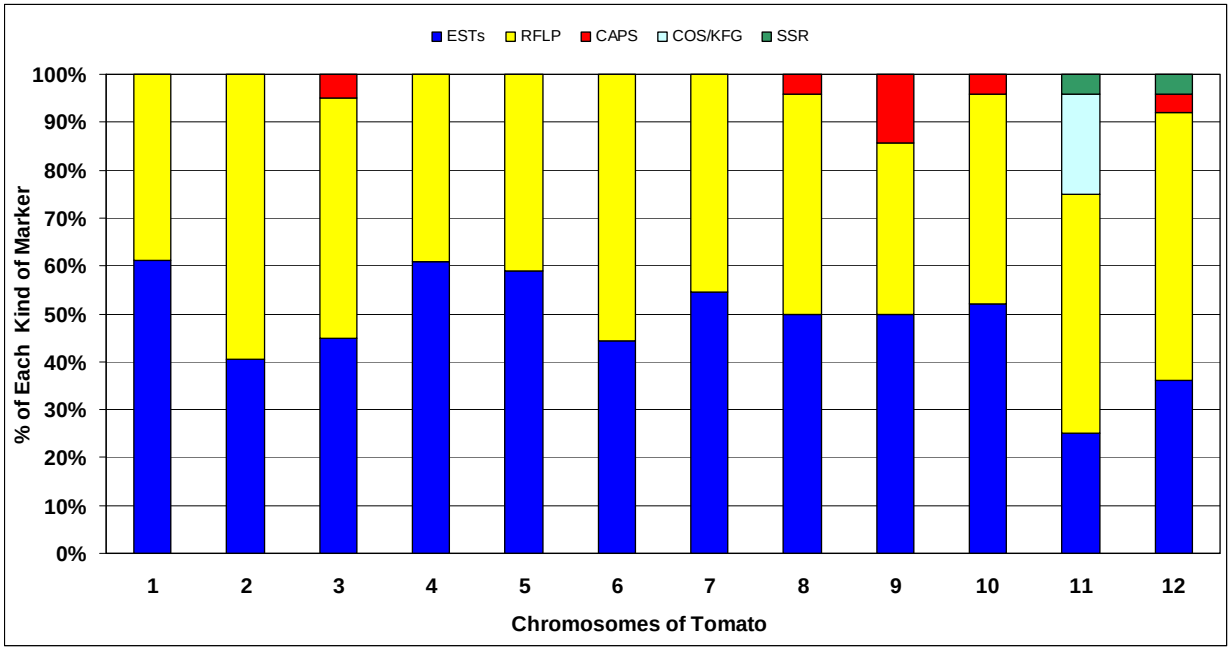


Figure 2-2 Number of EST (blue), RFLP (yellow), CAPS (red), COS/KFG (light blue) and SSR (green) markers mapped to F_7-RIL map. The Y axis is in percentage scale showing the proportion of each marker type on each chromosome.

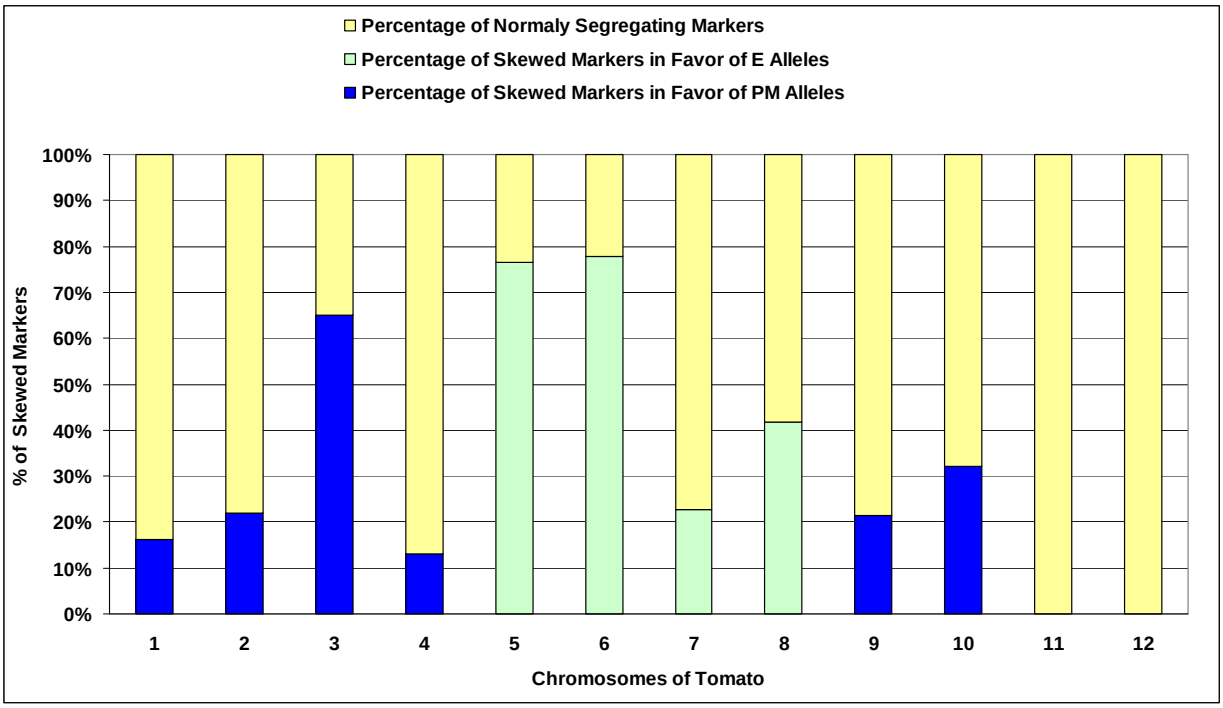


Figure 2-3 Percentage and number of skewed (blue and light green bars) and non-skewed segregating markers (yellow bars)

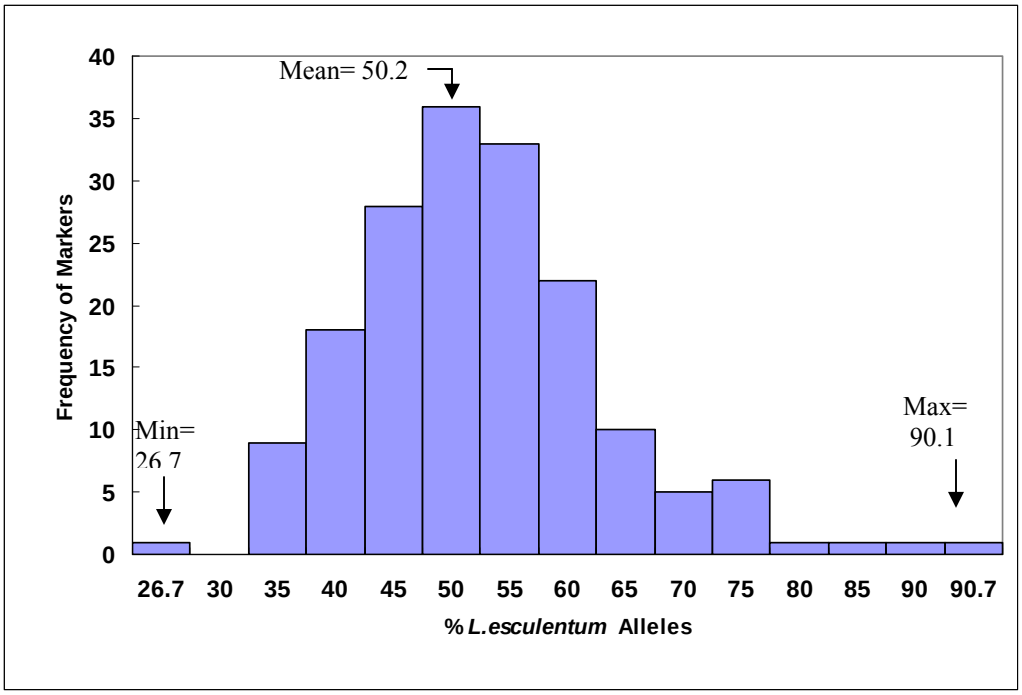


Figure 2-4 Distribution of *L. esculentum* (NCEBR-1) alleles based on 275 DNA markers

CHAPTER 3. Mapping of QTLs for early blight resistance and examination of their co-localization with candidate resistance genes in a RIL population of tomato

Abstract

Most cultivars of tomato, *Lycopersicon esculentum* Mill., are susceptible to early blight (EB), a devastating fungal (*Alternaria solani* Sorauer) disease of tomato in the northern and eastern part of the US and elsewhere in the world. Early blight of tomato is characterized by dark colored leaf spots that expand and coalesce to cause defoliation. Currently, the most common measures to control the disease are sanitation, crop rotation and fungicide spray. Use of resistant cultivars is the most economically acceptable and environmentally safe measure to control the disease. Unfortunately, due to its narrow germplasm base, no genetic source of EB resistant is known within the cultivated species of tomato. However, genetic sources of resistance exist within the related wild species of tomato, including *L. hirsutum, L. peruvianum* and *L. pimpinellifolium*. EB resistance does not follow the gene-for-gene model of host-pathogen interaction. Mapping QTLs conferring horizontal resistance is potentially an effective approach for studying complex resistance traits such as EB. Furthermore, structural similarities among different resistance genes lead to the speculation that common resistance pathways exist in plants, and thus the use of a candidate gene strategy to find new resistance genes. The purpose of this study was to 1) identify quantitative trait loci (QTLs) conferring resistance to EB in an accession (PSLP125) of the tomato wild species *L. pimpinellifolium* and identify ESTs that co-localized with QTLs, 2) measure heritability of EB resistance, and 3) calculate correlations between EB resistance and other horticulturally important characteristics segregating in the population. The experiment initiated with the development of 172 recombinant inbred lines (RILs) from a cross between *L. esculentum* breeding line NCEBR-1 and *L. pimpinellifolium* accession PSLP125. The RILs were grown in replicated field trials in three successive years using F_7, F_8 and F_9 generations. In each year, the population was evaluated three to four times for early blight incidence in ten-day intervals and area under disease progress curve (AUDPC) were determined. Also, in each year, the last evaluation was considered as the final % defoliation (disease severity). As described in

chapter 2, the 172 RILs were genotyped with 275 molecular markers and a genetic linkage map was constructed, spanning 1066.1 cM of the 12 tomato chromosomes. Three QTL mapping approaches, simple interval mapping (SIM), composite interval mapping (CIM) and multiple interval mapping (MIM) were employed to identify QTLs for EB resistance. The SIM and CIM resulted in identification of the same QTLs, though CIM identified QTLs with substantially more precision. In total 10 QTLs (LOD $\geq$ 2.4, $P \leq$ 0.001) were identified for EB resistance with individual phenotypic effects ranging from 3.0% to 16%. The MIM analysis revealed the combined effects of all QTLs to be ~39% of total phenotypic variation. Six QTLs had the positive alleles form the disease-resistance wild parent and four had the positive alleles form the cultivated parent. Two QTLs on chromosomes 5 and 6 were consistent throughout the years and generations. Co-localization of QTLs with candidate ESTs such as *Mi-1*, ethylene response factor-5, lipoxygenase B, wound-induced protein-1, phosphoenolpyruvate carboxylase kinase-2 and others may be due to potential involvement of these genes with EB resistance. The candidate-gene approach is considered an effective approach to identifying and mapping new resistance genes (*R* genes) in tomato. Further investigation may lead to the identification of genes underlying EB resistance in tomato.

Introduction

Background

One of the most destructive diseases of tomato in northeast of the U.S. and elsewhere in the world is early blight (EB), caused by fungus *Alternaria solani* (Ellis & Martin) Sorauer. The disease can lead to yield losses up to 79% in some regions with high humidity and heavy rainfalls, including Canada, India, Nigeria and the U.S. (Basu 1974; Chaerani and Voorrips 2006; Datar and Mayee 1985; Sherf and MacNab 1986). It has been estimated that seedling losses due to collar rot (see below) can reach 20% - 40% (Sherf and MacNab 1986). Currently, the most common measures of controlling the disease are sanitation, crop rotation and fungicide sprays (Foolad et al. 2002a; Foolad et al. 2002b; Madden et al. 1978; Sherf and MacNab 1986). However, in area similar to northeast of the U.S., where humidity is high, during rainy season of tomato cultivation these measures are limited in controlling the disease. Application of fungicides is also costly because ~15 applications per planting season are needed to obtain an

adequate protection against the crop loss (Madden et al. 1978; Sherf and MacNab 1986). In addition, fungicide application is not safe and has hazardous effects on the environment. Availability of cultivars with adequate resistance against the disease would reduce the need for fungicide application. Therefore, there are reasons to believe that, the most economically acceptable and environmentally safe measure to control EB is by the use of resistant cultivars.

Biology of the pathogen

A. solani is a member of imperfect fungi and belongs to the *Dematiaceae* family. *Alternaria* species are either parasites on living plants or saprophytes on organic substrates (Rotem 1994). No known races of *A. solani* have been found to exist, suggesting that the host recognition of the pathogen may occur in a non-specific manner (Lawrence et al. 1996; Lawrence et al. 2000; Rotem 1994). It overwinters as conidia or mycelia on host debris or tubers remained in the field from previous season tomato or potato cultivation. Chlamydospores may serve as a primary source of inoculum early in the season until the secondary disease cycle starts. The chlamydospores have thick walls, which make them resistant to low temperatures. To date, no sexual stage has found for *A. solani*, conversely, it has a high variation both in morphology and genetic composition *in vivo* and *in vitro* (Rotem 1994; Van der Waals et al. 2004; Weir et al. 1998). The possible hypothesis for high variability among isolates of *A. solani* is heterokaryosis (Stall 1958; Stall and Alexander 1957). Heterokaryosis is a phenomenon by which genetically different nuclei enter the same cell. Heterokaryosis is the result of branching out and reconnection of the hyphae, a phenomenon called ‘anastomosis’ in pathology. The heterokaryosis may be maintained or lost during further cell division (Stall 1958).

The fungus is readily cultured on artificial media such as V-8 juice agar where it produces a deeply pigmented gray/black hairy colony (Rotem 1994). The mycelium is haploid and septate, becoming darkly pigmented with age. The asexual conidia are borne singly or in a chain of two on distinct conidiophores. The beaked conidia may possess 9 to11 transverse septa as well as vertical septa (Rotem 1994).

A. solani's symptoms

A. solani affects all above ground parts of the plant and depending on the symptoms three distinct phases in disease incidence can be distinguished: collar rot, leaf blight (early blight) and fruit rot (Barksdale 1969; Foolad et al. 2002b; Sherf and MacNab 1986). The symptom of *A. solani* on tomato seedlings is known as collar rot. When a tomato seedling with collar lesions is transferred to the field, the lesions encircle the stem and damage the vascular system, eventually killing the plant (Horsfall and Huberger 1942). The leaf blight symptom, known as EB, normally appears during the adult phase of the tomato plant, though it can also be seen at earlier stages. It first appears as dark, small and coalescing concentric lesions, usually on lower older leaves and progresses upward as the plant reaches maturity. This phase is the most important phase of the disease, which may lead to defoliation of the whole plant towards the end of the season (Barksdale and Stoner 1977). Fruit rot is another phase of the disease, which occurs in mid to the end of the growing season and contributes to a significant crop loss. Throughout this chapter, I will only refer to EB symptoms of the disease as no specific measurement was made for collar rot or fruit rot during the season.

Environmental conditions for pathogen growth

In nature, the most favorable conditions for *A. solani* are periods of heavy dew, rainfall, and high humidity. In the laboratory or greenhouse, 24 hrs of continuous humidity after inoculation and temperatures between 10 °C to 20 °C are essential for successful infection. The effects of temperature on conidial germination of different *Alternaria* species have been studied in detail, either *in vitro* or *in vivo* elsewhere (Evans et al. 1992; Strandberg 1988). According to these studies, conidial germination of *Alternaria* species starts within 1-3 hrs over a wide range of temperatures (3-35 °C) at 100% relative humidity (RH). In *Alternaria linicola*, which affects linseed, under continues wetness and darkness and at temperatures between 10 °C and 25 °C, germination starts within 2 hrs of inoculation and reaches maximum (100%) germination by 8 – 24 hrs (Vloutoglou et al. 1996). It has been shown that inoculum concentration is important in the final percentage of infection. An increase in inoculum concentration (conidia/mL) from 6.2 to 11.5 leads to an increase in the percentage of leaf area infected, which eventually leads to more defoliation (Vloutoglou and Kalogerakis 2000). For a successful germplasm screening in

the greenhouse, inoculation of tomato plants at 3-4 leaf stage with inoculum concentration of 20,000 spores/mL and keeping plants in mist chamber at 21 – 24 °C before transferring them to the greenhouse where temperature is maintained at 24 ± 3 °C is recommended. Usually, one week after successful inoculation and infection, the symptoms of the disease are visible (Barksdale 1969).

Mechanism of action of A. solani

In spite of the recent advances in molecular biology and pathology, the pathogenicity mechanism of *A. solani* in tomato has not been clearly determined. However, it is known that upon a successful infection, *A. solani* spores germinate on both resistant and susceptible leaves resulting in the production of germ tubes which primarily penetrate into the epidermis (Rotem 1994). The fungus enters epidermis cells during the initial stages of pathogenesis and then grows in the intercellular spaces in a biotrophic manner (Rotem 1994). It produces some phytotoxins, which complicate the investigations of the disease (described below). Two major compounds, *alternaric acid* and *ziniol* have been recovered from *A. solani* culture filtrates (Brian et al. 1949; Cotty et al. 1983; Pound and Stahmann 1951). Alternaric acid produces symptoms characteristics of early blight on tomatoes, with leaf lesions, chlorosis and necrosis (Maiero et al. 1991). Ziniol causes stem wilting and leaf necrosis on zinnias but its effect on tomato is unknown. Two crystalline pigments, 5-methysulphony- methylenealtersolanol-A and tetrahydroaltersolanol-B, have been isolated from the liquid culture of a strain of *A. solani*. The antimicrobial activity of altersolanol-A was examined and showed an inhibitory effect on Gram-positive bacteria, *Micrococcus luteus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as well as cultured cells of *Nicotiana rustica* (Okamura et al. 1996). It acts as a potent stimulator of NADH oxidation in mitochondria isolated from *N. rustica* cells. The simulation was also observed in mitochondria in which the respiratory chain was blocked by electron transport inhibitor (Haraguchi et al. 1996). No relationship between the amount of alternaric acid production in isolates and susceptibility of tomato cultivars has been found, because some highly virulent isolates produce little amount of alternaric acid (Brian et al. 1952). Therefore, toxin susceptibility is not a reliable marker when screening for resistance to this pathogen (Maiero et al. 1991). It has been suggested that resistance to *A. solani* most likely does not depend on detoxification of

alternaric acid. However, there might be a mechanism involved by which prior to and/or upon pathogen infection, the plant creates an antifungal environment.

Molecular basis of resistance

In recent years, the successful cloning of more than 20 disease resistance genes in plants has dramatically advanced our understanding of the molecular basis of disease resistance. Over the past two decades, numerous major resistance genes were cloned from several plant species, including tomato, tobacco, *Arabidopsis*, and flax (Baker et al. 1997; Bent 1996; Martin et al. 1993a). Despite these advances, our knowledge of molecular basis of resistance to *A. solani* is limited to a handful of studies that have been conducted in the recent past. Here, I summarize what we know today of the molecular basis of EB resistance and possible pathways involved.

The role of hydrolytic enzymes in breakdown of fungal cell wall polysaccharides has been well demonstrated (Meins et al. 1992; Simmons 1994). Constitutive production of hydrolytic acid enzymes such as acidic or basic chitinase and β-1,3-glucanse are involved in this process (Lawrence et al. 2000). Western blot analysis demonstrated that upon challenging plants with *A. solani*, the four isozymes of chitinase (26, 27, 30, and 32 kDa) were induced in all genotypes in the experiment, including susceptible and resistant lines such as NCEBR-1 and NCEBR-2 breeding lines (Lawrence *et al.*, 1996). However, resistant lines had significantly greater 30 kDa chitinase activity. In a more recent study, Lawrence et al. (2000) found that during *A. solani* infection a highly-resistant breeding line (NC24-E) rapidly accumulates mRNA transcripts coding for multiple PR genes, including antifungal isozymes of chitinase and β-1,3-glucanase isozymes. Nevertheless, purified tomato chitinase or β-1,3-glucanase isozymes did not show any antifungal activity *in vitro*. On the other hand, the elevated levels of both acidic (extracellular) and basic (intracellular) isozymes of PR proteins have been observed in tomatoes infected by *A. solani*, further suggesting that multiple defense pathways such as salicylic acid (SA), ethylene and/or jasmonate–dependent pathways are involved (Lawrence et al. 2000). This conclusion is based on previous experiments in which the effects of SA and ethylene had been investigated in response to *A. solani*. For instance, application of exogenous SA on tomato roots caused enhanced expression of PR-1 gene (Spletzer and Enyedi 1999) and spraying tomato leaves with arachidonic acid increased the level of a PR-1 like protein (Coquoz et al. 1995). Ethylene is also

known to be produced in response to many biotic and abiotic stresses (Schlagnhaufer et al. 1997). Elevated expression of *ST-ACS4* and *ST-ACS5* genes (members of 1-aminocyclopropane-1-carboxylic acid gene) which are precursors in ethylene synthesis pathway was observed in potato in response to *A. solani* (Schlagnhaufer et al. 1997).

In an attempt to transfer the β-1,3-glucanase gene to tomato, Schaefer et al. (2005) created a series of transgenic tomato lines carrying the M-GLU (a maize glucanase) gene. As a result, enhanced resistance to EB was observed in only one of the transgenic lines compared to the control line. The same group also tried another kind of antimicrobial peptides, which were previously identified in *Mirabilis jalapa* (Cammue et al. 1992). Theses peptides (*Mj*-AMP1 and *Mj*-AMP2) are highly basic with homodimeric structures. Similar to M-GLU gene, from among 5 transgenic tomato lines carrying *Mj*-AMP1 construct, only one showed enhanced resistance to EB. Although these studies are valuable *per se,* comprehensive studies on the mechanism of action of the pathogen and the resistance mechanism by which tomato or potato plants respond to *A. solani* are yet to be done in future. Despite all the efforts that have been made to date, effects of genes underlying EB resistance are still unknown. New strategies are needed for the identification, validation and effective transfer of genes for EB resistance from wild species into the cultivated tomato.

Heritability of EB resistance

Resistance to EB is reportedly a low heritability (h^2) trait, as compared to many resistance traits controlled by major genes. Therefore, progress based on phenotypic evaluations alone is most likely slow (Chaerani and Voorrips 2006). The narrow sense heritability (h^2) estimate of 0.26-0.38 was previously reported by Nash and Gardner (1988a). Based on parent-off spring regression analysis, Foolad et al. (Foolad et al. 2002a; 2002b) reported a heritability of 0.65-0.75 for EB. Parent-off spring method is an applicable procedure to estimate heritability in early generations such as F_2 and F_3. However, in the case of advanced generations such as recombinant inbred lines (RILs), methods such as restricted maximum likelihood combined with mixed models with replications over the environments should be applied to measure the heritability. In the current study, family mean basis and plot mean basis analyses have been applied to estimate the narrow-sense heritability for EB (see Materials and Methods). For such a

low-heritability, complex and polygenic trait, quantitative trait locus (QTL) mapping seems to be promising. QTL mapping is an effective approach for studying complex forms of plant disease resistance. With QTL mapping, the role of specific resistance loci can be described, race-specificity of partial resistance genes can be assessed, and interactions between resistance genes, plant development, and the environment can be analyzed. QTL mapping also provides a framework for marker-assisted selection (MAS) of complex disease resistance characters and positional cloning of partial resistance genes (Young 1996).

Challenges in breeding for EB resistance

There are many hindering factors in developing tomatoes with an adequate level of resistance to EB. These factors are either pathogen related or plant related. For instance, in tomato, resistance to many diseases is inherited in a single gene fashion, but with respect to *A. solani* resistance is believed to be controlled by multiple genes each with a minor effect (Zhang et al. 2002). EB is also associated with physiological maturity and fruit load of the plant (Barksdale and Stoner 1977; Barratt and Richards 1944; Gardner 1988; Nash and Gardner 1988b). Late maturity and/or low yielding plants appear resistant, while they may not possess genetic resistance (Foolad et al. 2002b). On the other hand, indeterminate plants may outgrow the disease and emerge as resistant, while they may not have genes for EB resistance. Evaluation of tomato plants for EB resistant in populations segregating for self-incompatibility, indeterminate growth habit and late maturity would be greatly confounded by the effects of such factors. To date, no genetic source of resistance to EB is known within the cultivated species of tomato (Barksdale and Stoner 1977; Foolad et al. 2002b; Gardner 1988). It appears that a genetic bottleneck exists among commercial varieties of tomato (Miller and Tanksley 1990) as to EB resistance. This is one of the obstacles for breeders to develop tomato cultivars with resistance to EB. Therefore, the search is going on to find resistant genes among close relatives of tomato and to introgress them to the cultivated elite lines. Nevertheless, there are limitations associated with the use of this strategy, including genetic linkage drag, incompatibility, and confounding effects such as late maturity and indeterminacy.

Disease Control

As alluded to before, most commercial cultivars of tomato are susceptible to EB (Foolad et al. 2000; Martin and Hepperly 1987) and at present, sanitation, crop rotation, and application of fungicides are the most common measures to control the disease (Foolad and Lin 2003). Tomato breeding lines with moderate levels of resistance to early blight have been developed at the North Carolina State University (Gardner 1988; Gardner and Shoemaker 1999; Nash and Gardner 1988b). There are also several other NCEBR lines, which are suggested to be moderately to highly resistance to EB. However, most of the resistant lines are low-yielding and late maturing genotypes (Barksdale 1971; Barksdale and Stoner 1977; Gardner 1988). Attempts to develop high yielding varieties with an acceptable level of EB resistance are underway (Foolad et al. 2005; Foolad et al. 2002b; Thirthamallappa et al. 2000). Because of the complexity of the disease and variable environmental conditions, from year to year and location to location it is hard to ascertain that one cultivar has a broad spectrum of resistance in all environmental conditions and locations. For example, while NCBER-2 exhibited moderate resistant to collar rot and early blight, NCEBR-1 was mainly resistant to early blight (Maiero et al. 1991). Furthermore, to date, no single-gene resistance to EB has been identified in the cultivated tomato. This and other limitations have caused breeders to seek for EB resistant in close relatives or wild type tomato. Genetics and breeding of EB resistance in tomato, the number and effects of QTLs in different mapping populations have been reviewed by Foolad et al. (2005). BC_1-derived *L. hirsutum* population has been compared with an F_2 derived population of *L. esculentum* cv. NCEBR-1 and *L. pimpinellifolium* (filial generation of the RIL population used in this thesis research, Foolad et al. unpubl.). Comparative analysis between these two interspecific populations (*L. esculentum* × *L. hirsutum* or *L. esculentum* × *L. pimpinellifolium*) revealed 30% similarity between QTLs on chromosomes 3, 5, and 11 in the two populations. Presence of similar QTLs across species validates the authenticity of these QTLs for further exploitation in breeding for EB resistance. Other uncommon QTLs between the two populations were cross specific indicating that *L. hirsutum* and *L. pimpinellifolium* harbor different QTLs because both mapping populations have one *L. esculentum* parent in common (different accessions of the same species). The results of this study are complementary to our previous knowledge about EB

resistance and are aimed to identify candidate ESTs for EB resistance in a more classified manner.

Candidate gene and QTL mapping strategies

In searching for gene(s) involved in a genetic trait, investigators usually focus on candidate genes. A gene is considered a candidate gene either if its encoded protein represents a logical possibility for being involved in the trait of interest or if the gene is physically located within a region of the genome known to be containing the gene for the trait. Sequence analysis of the predicted proteins reveals that resistance genes of diverse origin and with different pathogen specificity share similar structural motifs, indicating that a common surveillance strategy has been adopted by plant species to detect invading pathogens (Michelmore and Meyers 1998). The second situation is frequently encountered in positional cloning projects, where a resistance gene is identified based on its position within the genome. In a typical positional cloning project a small genomic region is first identified containing the gene(s) of interest with all the genes residing in that region immediately becoming possible candidate genes. If something is known or can be inferred from the encoded protein of a given candidate gene and if a convincing case can be made for how that protein may be involved in the expression of the disease resistance then that gene would likely be considered as a strong candidate gene. The candidate gene approach has successfully led to the identification of new genes in human, mouse and rice (Copeland et al. 1993; Wang et al. 2001). Candidate gene method has been particularly productive for the investigation of pest and disease resistance because many genes involved in these resistance pathways have been characterized (Ramalingam et al. 2003). The application of this strategy in tomato has been limited by the lack of known polymorphic expressed sequence tags (ESTs). As more and more plant ESTs are mapped, the candidate-gene approach emerges as a promising method for identifying genes for monogenic and polygenic characters. For example, the structural similarity among different resistance genes leads us to speculate that a few common resistance pathways may exist in plants. Furthermore, the applications of this strategy are not limited to disease or defense mechanisms. Candidate gene approach also has been used for analyzing organ pigmentation loci in the *Solanaceae* (Thorup et al. 2000).

The candidate-gene approach has emerged as a promising method of merging QTL analysis with the extensive data available on cloning and characterization of genes involved in plant defense. In this strategy, genes potentially involved in biochemical pathways leading to trait expression are employed as molecular markers for QTL analysis (Faris et al. 1999). Many major resistance genes show a high degree of similarity in many plant species. The corresponding genes or partial sequences of the genes are currently available in databases. These sequences can be used in genetic linkage maps. The association and co-localization of the markers with QTLs can be an indication of their involvement in the trait of interest. Previously, the application of this strategy to plant species was limited because of the lack of ESTs available in databases. Currently, however, the abundance of ESTs in databases provides a great opportunity to identify genes underlying monogenic and polygenic traits. Wayne and McIntyre (2002) proposed to combine QTL mapping with microarray data to map candidate genes for which there are not *a priori* information and to narrow down the number of candidate genes for ovariole number (a quantities trait) in *Drosophila melanogaster*. The proposed method was highly efficient to reduce the number of candidate genes from 5286 to 34, suggesting the efficacy of microarray technology combined with QTL mapping.

Multiple genes and various environmental factors usually influence quantitative traits. Detecting and mapping individual genes underlying such complex traits is a difficult task. In 1923, Sax reported the association of seed coat color (a qualitative trait) in bean with seed size (a quantitative trait). He interpreted his finding as the linkage of single gene controlling seed color with polygenes controlling seed size (Sax 1923). Thoday (1961) reported the use of single marker traits to map and characterize individual polygenes controlling quantitative traits. Nowadays with the development of DNA marker maps for most crop species, it is possible to search throughout the genomes to locate QTLs for various quantitative traits. The idea of mapping QTLs is very straightforward. The individuals in any mapping population (e.g. F_2, backcross, and recombinant inbred line) are analyzed for DNA marker genotypes and also the phenotype of the trait(s) of interest. For each DNA maker, the individuals in the population are classified according to their marker genotypes. Mean and variance of parameters are calculated and compared among the classes. A significant difference between classes suggests there is a putative relationship between the DNA marker and the trait of interest (Young 1996).

Since the advent of molecular markers, numerous papers in QTL mapping of tomato have been published. This technique has been used to identify genetic factors for many traits such as disease resistance, fruit quality and tolerance to abiotic stresses (Bernacchi et al. 1998; Chaerani et al. 2007; Chen et al. 1999; Frary et al. 2004; Goldman et al. 1995; Paterson et al. 1988; Rousseaux et al. 2005). The underlying assumption in using marker loci to detect polygenes is that linkage disequilibrium exists between alleles at the marker locus and alleles of the linked polygene(s). Linkage disequilibrium is very high in F_2/F_3 or backcross populations derived from controlled mating. On the other hand, in a RIL population LD is reduced, since this population undergoes several generation of meiosis and recombination during its construction. However, RILs have the advantage of homozygous lines that can be replicated and retested for more accurate measurement of the quantitative trait (Paran et al. 1995; Tanksley 1993) (Chapter 2).

To expedite the breeding efforts for EB resistance in tomato using marker technology, QTL mapping and conventional breeding research projects have been established at Penn State to facilitate determination of the genetic basis of EB resistance, including identification, mapping and characterization of QTLs for resistance in both cultivated and wild species of tomato. As a part of this project, more than 270 accessions of *L. pimpinellifolium* was screened (Foolad 2000) and several accessions exhibited resistance to EB in the filed as well as under greenhouse and laboratory conditions. One wild accession, named "Penn State *L. pimpinellifolium 125*" (PSLP125) was among the best in terms of resistance to EB and other horticulturally important characteristics such as fruit quality. In this chapter I am addressing the use of both candidate gene strategy and QTL mapping to identify QTLs for resistant to EB and find possible co-localization of the QTLs and candidate genes a RIL population of a cross between NCEBR-1 breeding line and PSLP125.

Materials and Methods

Genetic materials

In 1999, breeding line NCEBR-1 (*L. esculentum* Mill.) was hybridized with PSLP125 (*L. pimpinellifolium* Jusl.), a self compatible, inbred accession. One F_1 progeny plant was self-fertilized to produce F_2 seed. A total of 900 F_2 plants along with the parental lines were grown under greenhouse conditions and subsequently transplanted into the field in the summer of 2000. In order to generate a RIL population, 172 F_2 plants were randomly chosen and self pollinated to produce F_3 seed. The F_3 families were grown under field conditions in 2001, and one individual plant representative of each family was self-fertilized to produce F_4 seed. During the summer of 2002, the F_4 families were grown under field conditions and produced F_5 seed. When I joined Penn State in August of 2002, I took over the advancement of the population to develop the RIL population. In 2003, the F_5 families were grown in two replications with 10 plants per family to produce F_6 seed. Plant spacing was 120 cm between plants and 150 cm between rows, covering a total of 8400 m^2 (~2 acres). From 2003 to 2006 (F_9), the population was grown in the field each year in the same manner. In summer 2004 (F_7), a single plant was selected from the first replication of the experiment and all fruit quality measurements were carried out using the fruits of single plants of each RIL. This single plant was the source of DNA for marker analysis and map construction as well as the source of seed for the next generation. In summers of 2005 and 2006, fruits were collected in bulk from each line within each replication and measurements were done on plants/fruits for the entire line within the two replications of the experiment separately.

Genetic map construction

Genomic DNA was extracted from leaf tissue of the 172 individual plants of each RIL, using standard protocols for tomato (Bernatzky and Tanksley 1986; Foolad et al. 1997). A medium density genetic linkage map was developed using 275 molecular markers, including RFLPs, EST (candidate resistance genes), SSR, COS, KFG and CAPS markers, as described in Chapter 2. The map spanned 1066.1 cM of tomato genome with an average 3.8 cM marker distance and used for QTL analysis.

Disease screening and evaluations

Disease screening to choose the parents for making the RIL population was previously conducted (Foolad *et al.,* 2000). In late spring of the years 2004 (F_7-RIL), 2005 (F_8-RIL) and 2006 (F_9-RIL) eight-week-old seedlings were transplanted into the field in two replications. The planting scheme was as described above. No artificial inoculation was made as there was natural occurrence of EB under field conditions in Centre County (PA) every year.

For scoring disease development, the traditional Horsfall and Barrett (Horsfall and Barratt 1945) disease scale was used (see below). Plants were evaluated for the incidence of EB in three to four intervals each year starting with observation of the first symptoms of the disease: in 2004 on Aug 25, Sep 10 and Sep 22, in 2005 on Aug 24, Sep 5, Sep 19 and Oct 1 and in 2006 on Aug 21, Sep 9 and Sep 24.

In each replication, plants (10 plants per plot) were scored based on percent infection/defoliation caused by EB on a scale of 0 to 100, in which 0 indicated no sign of the disease on the whole plot and 100 indicated complete defoliation of all 10 plants. In rare cases, other diseases or disorders were observed. Due to their confounding effects on EB evaluations, necessary adjustments were made visually to partition the defoliation caused by EB or other factors and only EB fraction was recorded. The last evaluation in each year was considered as the final % defoliation. Area Under Disease Progress Curve (AUDPC) was calculated for each RIL in each replication using the following formula.

$$AUDPC = \sum_{i=1}^{n} \frac{(R_{i+1} + R_i)}{2}(t_{i+1} - t_i)$$

where R_i is rating (estimated proportion of defoliation) at the ith observation and t_i = time (days) since previous rating at the ith observation, and n = total number of observations (Tooley and Grau 1984). The AUDPC is usually expressed as *percent-day* or *proportion days* (Campbell and Madden 1990; Torres-Barragán et al. 1996). The AUPDC and the final % defoliation (hereafter referred to as % defoliation) values of the RILs were used for QTL analysis in the F_7, F_8, and F_9 generations.

Evaluation of other traits

In order to investigate the correlation between other plant or fruit characteristics and EB, a number of traits were either visually evaluated in the field or were measured in the lab each year. The traits that were evaluated in the field were as follows:

a) Plant growth habit: Each RI line was scored as determinate (D), semi-determinate (SD) or indeterminate (I). For correlation analysis a dummy variable was defined in which D = 1, SD = 2 and I = 3

b) Plant type: The plants were scored as prostrate type (PR), upright-prostrate (UP/PR) or upright (UP). For correlation analysis a dummy variable was defined in which PR = 1, UP/PR = 2 and UP = 3.

c) Plant size: The plant size scoring varied from 1-5 in which 1 indicated the smallest plant stature and 5 the biggest. The *L. esculentum* parent was scored as 2 and the *L. pimpinellifolium* parent (PSLP125) was scored as 5. The sizes of RILs were measured relative to the parental sizes.

d) Maturity: Plants were scored based on the level of fruit maturity when at least 50% of the fruits were ripened under field conditions. Scoring ranged between 1 (very early maturity) and 5 (very late maturity).

e) Fruit size and fruit weight: Fruit size was first visually evaluated in the field, ranging from 1 (small fruits with diameter ≈ 10 mm, similar to the *L. pimpinellifolium* parent) and 5 (largest fruits with diameter of 60 mm or greater, similar to *L. esculentum* parent). Fruit weight was measured in the lab based on the average weight of 10 – 20 random mature fruit.

f) Fruit yield: The yield of each RI line was visually scored based on its overall fruit load, where 1 indicated the lowest and 5 the highest fruit load.

g) pH: The pH of the purée of each RI line was measured in the lab as described in Chapter 4.

i) Soluble Solid Content (SSC): The SSC of the purée of each RI was measure in the lab as described in Chapter 4.

Statistical analysis

ANOVA and Correlations Analyses

MINITAB v 14 (MINITAB 2003) was used for the analysis of variance (ANOVA) and summary statistics analyses. Both summary statistics analysis and ANOVA were carried out for the data in each year separately and the pooled data over three years and generations. The SPSS v 14 software (SPSS 2005) was used to perform all correlation analyses.

Heritability analysis

Before the introduction of PROC MIXED to SAS (SAS Institute, Inc 2004) plant breeders were relying on calculation of variance components based on traditional least square analysis with PROC GLM or ANOVA in statistical packages such as SAS. Variance components estimates based on MIXED models and GLM are almost identical if the data are balanced. However, in case of unbalanced data, as it is the case most of the times in agricultural research, the variance components would be biased if they were obtained by GLM models. Restricted Maximum Likelihood (REML) models are free from these bias estimations and are more precise in estimation of variance components. However, without computers and appropriate software packages the calculations are very cumbersome. By introduction of PROC MIXED to SAS (Little et al. 1996) and GENSTAT (Payne and Arnold 1998) there is now more interest in measuring variance components by mixed models and REML procedure even when the data are balanced. For these reasons, in this study the heritability was estimated for EB resistance using restricted maximum likelihood (REML) method and PROC MIXED procedure of SAS (Little et al. 1996) according to Holland et al. (2003) and Nyquist (1991). Dr. James Holland (NCSU-ARS) kindly provided the SAS codes, and necessary modifications were made to the program accordingly. In addition to REML analysis, variance components estimation was also carried out based on traditional least square methods (GLM model) and narrow-sense heritability was calculated based on family mean for both methods as follow.

Family heritability on a plot basis, giving the response to selection among plot means within one replication of one environment as measured in independent environment.

$$\widehat{h}_{f1}^2 = \frac{\widehat{\sigma}_F^2}{\widehat{\sigma}_F^2 + \widehat{\sigma}_{FE}^2 + \widehat{\sigma}_{\varepsilon'}^2} = \frac{\widehat{\sigma}_F^2}{\widehat{\sigma}_P^2}$$

Heritability of family means, giving the response to selection among family means averaged across the years as measured in independent environments.

$$\widehat{h}_{f1}^2 = \frac{\widehat{\sigma}_F^2}{\widehat{\sigma}_F^2 + \frac{\widehat{\sigma}_{FE}^2}{e} + \frac{\widehat{\sigma}_{\varepsilon'}^2}{er}} = \frac{\widehat{\sigma}_F^2}{\widehat{\sigma}_P^2}$$

Where:

$\widehat{h}_{f1}^2$ is the estimation of heritability from the first population to the next base population

$\widehat{\sigma}_F^2$ is the family variance component

$\widehat{\sigma}_{FE}^2$ is the family by environment interaction

$\widehat{\sigma}_{\varepsilon'}^2$ is the experimental error variance component

e is the number of environments

r is the number of replications per environment

Calculation of standard error of heritability statistically is complex and has been thoroughly explained elsewhere (Holland et al. 2003).

As an alternative approach h^2 estimate was computed by correlation analysis of F_7, F_8 and F_9 generations as described elsewhere (Falconer and Mackay 1996; Foolad and Lin 2001).

Mapping QTLs

QTL analyses were carried out based on disease evaluation data collected from the field experiments in 2004, 2005 and 2006. Both, final % defoliation and the AUDPC data for each replication and the mean of two replications within each year were used for the QTL analysis. Windows QTL Cartographer v2.5 software (Wang et al. 2006) was used for QTL mapping. Simple interval mapping (SIM) and composite interval mapping (CIM) using the default parameters (model 6) were employed. A stepwise regression was used to perform the CIM analysis to enter or remove background markers from the model. In order to develop a model for MIM analysis the output file from CIM was scanned for putative QTLs with a minimum LOD score of 2.0 and minimum distance of 5 cM between two adjacent markers separated by at least 1.0 LOD score from top to valley of QTL peak. Several MIM models were created for each trait and tested in a stepwise regression procedure to add or remove QTLs from model and to test their significance after each cycle. The model with least Bayesian Information Criterion (BIC) (Piepho and Gauch 2001) was selected as the best model. According to BIC criterion, if no more QTL could be added to the model, each pair of QTL in the model was tested for epistatic interactions. The epistatic effects that decreased the BIC criterion were chosen to be included in the model (Kurti et al. 2006). SIM analysis was also carried out using QGENE package (Nelson 1997) and the results were compared to that of Windows QTL cartographer software (data not shown). A 1000 time permutation was performed in order to obtain the LOD threshold to declare a significant QTL. Windows QTL cartographer generated a relatively higher values (LOD > ~3.0) for the thresholds than QGENE (LOD > ~2.4). Because the LOD threshold values obtained by Windows cartographer are very stringent it is likely that it leads to increase frequency of Type-II error (not detecting valid QTLs), therefore a reduced LOD score of 2.4 ($P = 0.001$) was chosen to guarantee both enough stringency and reducing the chance of Type-I error (false positive).

Results

Responses of the parental and F_1 progeny to EB

The severity of the EB disease in the three years for parental lines and the F_1 progeny is presented in Table 3-1. The mean of the final % defoliation and AUDPC for parental lines showed that NCEBR-1 breeding line is more susceptible than both PSLP125 and F_1 generation. However, in 2005 due to a lower rainfall the severity of disease on NCEBR-1 breeding line parent was lower than any of PLSP125 parent or F_1 progeny.

Response of F_7, F_8 and F_9 RIL generations to EB

Final % Defoliation: The summary statistics for three or four evaluations per year of the leaf defoliation in three generations of the RIL population are presented in Table 3-2. The last % defoliations which are the last evaluation in each year are highlighted in the table. The mean of two replications per year ranged from 42.71±1.90 in F_8 (2005) to 69.32±1.83 and 68.96±2.10 in F_7 (2004) and F_9 (2006), respectively. The overall mean of final % defoliation of the two replications of the experiment per year was calculated, which ranged from 85 to 94 with an standard deviation of 22.92 – 26.95, respectively. As it is depicted in Figure 3-1, in F_7 and F_9 generations, the % defoliation distributions were skewed toward the higher values, while in F_8 (2005) due to less severity of the disease the skewness was positive and toward the lower (less disease) values.

AUPDC: The statistical analysis of the AUDPC was also carried out (Table 3-3). The mean of the AUDPC over the two replications ranged from 957.3 ± 49.1 *percent-day* in F_8 to 1356.0 ± 48.8 and 1288.3 ± 42.9 *percent-day* in F_7 and F_9, respectively. Similar to the final % defoliation, distribution of the AUDPC was skewed toward the lower values in F_8, however, it was close to normal distribution in F_7 and F_9 (Figure 3-1).

Analysis of variance of parental lines and F_1

The analysis of variance was carried out using the data of final % defoliation and AUDPC. Because the results were the same, only the results of final % defoliation are shown here. The ANOVA showed that there was a significant difference ($P < 0.01$) among the parents and F_1 for the severity of the EB in all three years (Table 3-4). Because the years were significantly

different, analysis of mean comparisons of the years was carried out (Appendix B). The results indicated that observed significant difference among the years was due to the year 2005. In order to eliminate the significant effect of the year from ANOVA table, the data was reanalyzed without the data of the year 2005. As it was expected the year 2004 and 2006 were not significantly different ($P > 0.05$) (data not shown). While the years were not statistically different this time, the parents and F_1 were still significantly ($P < 0.01$) different. Tukey's test of mean comparison was carried out and the results indicated the cultivated parent was more EB susceptible than the wild type parent and F_1 hybrid (statistical analysis is shown in Appendix B).

Analysis of variance for the RIL population in three generations

Similar to the parents and F_1, the analysis of variance for the population was also carried out by employing the data of final % defoliation and AUDPC. Because the use of both data types led to the same conclusion the results of final % defoliation are reported here. Based on ANOVA, there was a significant difference ($P < 0.01$) among the RILs in each generation. The results revealed that the effects of the years, nested replications within the years, and the interaction of the years and the RILs were also highly significant ($P < 0.01$) (Table 3-5). Tukey's test of mean comparison of the years showed that year 2005 was different from both 2004 and 2006. This was also inferred from analysis of the parental data as described above. Removing the data of the year 2005 indicated that the effect of year in ANOVA (2004 and 2006) was not significant ($P > 0.05$). However, this did not change the significance difference among other sources of variation in ANOVA table such as the differences among the RILs, nested replications within the years, and the interaction of the years and the RILs.

Correlation analysis

There was significant correlation between the final % defoliation and the AUDPC data in each year. This is not surprising because one of the components of calculation of AUDPC is the final % defoliation. There was a relatively high and significant correlation among different years for % defoliation as well as AUDPC (Appendix B). The correlation of the % defoliation in F_7 and F_8 was the lowest ($r = 0.53$; $P < 0.01$) while that of F_8 and F_9 was the highest ($r = 0.65$; $P < 0.01$) (Table 3-6). The correlation analysis using the AUPDC data resulted in equal correlations among different years ($r = 0.58$; $P < 0.01$) (Appendix B).

The results of correlation analysis of various traits with either final % defoliation or AUDPC are presented in Table 3-7. Correlation analysis in all three years between EB resistance and growth habit resulted in a negative and significant correlation ranging from -0.35 in F_8 to -0.46 in F_9 ($P < 0.01$). A negative and significant correlation ($r = -0.42_{F8}$ to -0.50_{F9}) was observed between the plant type and EB resistance. The same trend was observed for correlation of the plant size and maturity with EB resistance. A positive and highly significant correlation was also observed between the plant yield and either the % defoliation or the AUDPC in all three years. There was almost no significant correlation between the final % defoliation (or AUDPC) and fruit weight in any of the years, though it was very weakly negative. The correlation of pH of the purée and % defoliation varied among the years and generations due to the variability of the trait and no solid conclusion could be made, but overall in most cases it was negative and not significant. The SSC had a negative and significant correlation with final % defoliation in F_8 and F_9 but it was not significant in F_7.

Heritability analysis

Heritability of the EB in three years and generations was estimated based on REML method. Both final % defoliation and AUDPC data were used in separate analyses. Components of variance based on mixed procedure are listed in Appendix B. These components along with compilation of a SAS code generated necessary vectors and matrices to estimate the heritability. Plot basis analysis resulted in a lower heritability than family bases. However, the outcome of both final % defoliation and AUDPC data were the same. Using final % defoliation data the heritability of EB was 0.49 and 0.68 for plot and family based analysis, respectively (Table 3-8).

QTLs identified for early blight

Because the results of SIM and CIM analysis were comparable, only the results of CIM will be reported here (Table 3-9). Two stable QTLs were identified on chromosome 5 and 6 over the three years and generations. The positive effect of one of these QTLs containing several intervals on chromosome 5 was from the *L. esculentum* parent. Depending on the generation and the trait being used (final % defoliation or AUDPC), this QTL explained between 12% and 16% of total variation. The effect of the second QTL on chromosome 6 was inherited from the *L. pimpinellifolium* parent. QTL results were the same for both the final % defoliation and AUDPC.

The maximum LOD score for the QTL on chromosome 5 was 7.4 and that of the QTL on chromosome 6 was 6.38. A QTL on chromosome 1 was identified in F_7 (LOD = 2.1) and F_9 (LOD = 2.4) in cTOS16I16 and cLEC34L10b marker interval, which was repeated in two generations. A number of less congruous QTLs were also identified on chromosomes 2, 3, 9 and 10. One QTL on distal part of chromosome 2 was highly significant (LOD > 4.8) but it was identified only in F_8 generation. The LOD score for this QTL in F_7 and F_9 was < 2 but the contours were following the same trend as in F_8. The additive effect of this QTL was inherited from *L. pimpinellifolium* parent.

Total QTL effects for early blight resistance by MIM

The MIM analysis revealed the total effects of the QTLs identified by the CIM analysis for EB resistance. In F_7, ~39% of the total phenotypic variation explained by 5 QTLs which were identified by either AUDPC or final % defoliation on chromosomes 2, 3, 5, 6 and 10. In F_8 the total phenotypic variation explained by the QTLs varied from 24% to 33% for the AUDPC and % defoliation data respectively. In F_9 the total phenotypic variation explained varied from 33% to 38% for 5 QTLs that were identified by both AUDPC and % defoliation data sets (Table 3-10). Epistatic interaction between QTLs was also observed in all three generations. The epistatic interactions were not consistent among different traits or years (Table 3-11).

Discussion

The disease evaluation scores show a continuous distribution in all three years of the experiment, indicating that EB resistance in the RIL population is quantitatively controlled. The quantitative inheritance of EB resistance was previously reported (Foolad et al. 2002a; Foolad et al. 2002b; Maiero et al. 1989, 1990a, b; Nash and Gardner 1988b; Thirthamallappa et al. 2000). Based on the results of the current study, there is little evidence supporting the existence of physiological races of *A. solani* characteristics of major gene inheritance of resistance reported by Bonde (1929).

The response of the cultivated breeding line parent (NCEBR-1) to EB was variable in different years of the study. The variable response of NCEBR-1 breeding line to EB can be attributed to environmental conditions, its inherent late maturity and/or experimental errors. Because in the growing season of the year 2005 (June 1st to Oct, 5th) the amount of rainfall was very limited (55.6 mm) (Pennsylvania State Climatologist 2006), EB incidence was much lower than in 2004 and 2006 with 445 mm and 114 mm rainfalls for the same period, respectively. This shows the clear effect of the environment on EB disease development in NCRBR-1. On the other hand, the difference in response to EB in wild type parent (PSLP125) and the F_1 was not statistically significant in different years. The results indicate the presence of genotype by environment interactions when it comes to EB disease infection in different genotypes. Furthermore, the PLSP125 was selected from among 270 wild type accessions of tomato (Foolad et al. 2000), therefore, the hypothesis that the resistance to EB in the wild type parent and F_1 is due to confounding effect of indeterminacy is not acceptable. The results of this experiment indicate that NCEBR-1 breeding line does not exhibit much resistance to EB, when environmental conditions are favorable for the disease. However, in the RIL population, some determinate (characteristic of NCEBR-1) to semi-determinate plants were observed with high level of resistance to EB. This suggests that contribution of the wild type parent (*L. pimpinellifolium*) has conferred enhanced resistance to EB and the linkage between indeterminacy and resistance to EB has broken in these specific lines. Whether this contribution is through the regulatory mechanisms or through introduction of new genes to determinate plants is not clear through this research. Another possibility is contribution of positive alleles for resistance from the susceptible

breeding line parent (NCEBR-1) that has caused transgressive segregation toward the resistance (see below).

The presence of transgressive segregants has been reported in a number of quantitative traits studies of tomato and other plant species (deVicente and Tanksley 1993; Monforte et al. 1997; Rieseberg et al. 1999). In the present study, transgressive segregations in both directions were observed in all three generations of the RIL population. The presence of extreme genotypes in the population made it possible to conduct a selective genotyping analysis. QTL analysis with 25% highest and lowest individuals in the population detected the same QTLs as to when all individuals were used in the analysis but LOD scores for the same QTLs were generally higher (Appendix B). Transgressive segregation for EB resistance was observed in both directions in an F_2 population of *L. esculentum* × *L. peruvianum* cross, whereas in F_3 families of the same cross, it was only toward resistance (Chaerani et al. 2007). Transgressive segregation was not observed in a BC_1 or BC_1S_1 populations of a cross between *L. esculentum* and *L. hirsutum* (Foolad and Lin 2001; Foolad et al. 2002b). This indicates that depending on the parents used in a cross to develop a population it is possible to observe transgressive segregation for EB resistance.

As it has been reviewed, EB resistance is considered as a low to moderate heritability trait (Chaerani and Voorrips 2006). Measuring heritability of the trait is important in predicting the degree to which progeny resemble their parents and to measure the extent to which the phenotypic value correspond to breeding value (Falconer and Mackay 1996). The family-based narrow-sense heritability (h^2) of EB resistance measured by Mixed model (h^2= 0.68) was very close to what previously reported (r = 0.69) by Foolad et al. (2002b) and ~2 times greater than the h^2 reported by Nash and Gardener (26% to 38%) (1988a). Both final % defoliation and AUDPC resulted in the same estimation for narrow-sense heritability. Estimation of narrow-sense heritability (h^2) of EB resistance based on GLM model, overestimated the heritability of EB by 10% (final % defoliation) and 50% (AUDPC) (data not shown). This indicates that the mixed model is a more accurate method to measure the heritability of EB than GLM model, in experimental designs similar to the one used in this study.

Because no selection against plant growth habit, low yield or late-in-maturity had been made during the development of the RIL population, variations in determinate/indeterminate,

early/late-in maturity, and high/low yield plants were observed. As a result, negative correlations between EB resistance and late-in-maturity, low-in-yield and indeterminacy were also observed. These observations were congruous with previous reports (Foolad and Lin 2001; Foolad et al. 2002a; Foolad et al. 2002b; Nash and Gardner 1988b). Furthermore, association of plant type (prostrate vs. upright) and plant size to the EB resistance was not previously investigated. Based on the current study, prostrate plants were more susceptible to EB than upright/upright-prostrate plants. With respect to the plant size, small plants were more susceptible to EB than large plants. Therefore, both plant type and plant size can be considered as the traits with negative correlation with EB resistance. One of the practical applications of these findings includes breeding and selection for upright plants to reduce the EB susceptibility in tomato. All of these observations clearly indicate that the resistance to EB in the RIL population was to some extent confounded by the effects plant growth habit and maturity related traits.

Correlation of fruit characteristics with EB resistance was not previously investigated. To the best of my knowledge, this is the first report to show the relationship between fruit weight, size, pH and SSC with EB resistance. Fruit characteristics seemed to have no or low correlation with EB resistance. In two out of the three years of evaluations of SSC, a low, negative but significant correlation with EB resistance was observed. However, the variability of pH and SSC of the fruits in different years hampered drawing any solid conclusion about the effects of these traits on EB resistance.

To date, characterization of resistance to *A. solani* at cellular level has been focused on studying a hand full of candidate genes (Fernandez et al. 1996; Lawrence et al. 1996; Lawrence et al. 2000; Schaefer et al. 2005). The use of resistance gene analogues (RGAs) as an alternative approach to better dissect the nature of EB resistance in tomato has been reported (Foolad et al. 2002b; Niño-Liu et al. 2003). However, in their study, chromosomal location of only seven RGAs coincided with the location of EB resistance QTLs and many of the RGAs were clustered together in chromosomal regions that were known to contain tomato R genes. Here, I have used QTL mapping approach, based on mapping candidate resistance/defense response genes, to identify co-localizations of ESTs for disease resistance/defense response with EB resistance in tomato. A similar approach was used in rice to identify genes for resistance to rice blast (Wang *et al.*, 2001). The induction of three candidate genes (linked EST markers to a QTL) after rice

plants were challenged with blast fungus (*Magnaporthe grisea*) was investigated. Northern blot analysis revealed that two of these were over-expressed in infected rice plants. These findings demonstrate that the power of QTL mapping - candidate gene approach to identify new resistance genes in a plant.

Co-localization of QTLs for EB resistance with well-characterized genes or previously identified QRLs was observed in this study. The QTLs on chromosome 1 were co-localized with *Cf* gene family (*Cf2.1*, *Cf2.2, Cf4* and *Cf9*) for resistance to leaf mold (*Cladosporium fulvum*) (Haanstra et al. 1999; Thomas et al. 1998) as well as *Cm.1* for resistance to bacterial canker (*Clavibacter michiganensis*) (Sandbrink et al. 1995). The identified QTL on chromosome 2 was co-localized with *Tm-1* gene for resistance to tomato mosaic virus (Ohmori et al. 1996). The QTL on chromosome 5 was co-localized with *rx* gene family for resistance to bacterial spot (Yu et al. 1995), *pto* and *prf* for resistance to bacterial speck (Martin et al. 1993b; Salmeron et al. 1996). The QTL for EB on chromosome 6 were co-localized with the *cf* gene family for resistance to leaf mould, QRLs for resistance to bacterial canker, *Ty-1* for resistance to yellow leaf curl virus (Zamir et al. 1994). The co-localization of the QTLs for EB resistance with these genes does not necessarily mean that the EB resistance in this population is the results of the action of these genes. However, the resistance genes products may play a role in EB resistance in this population.

More than 132 candidate resistance genes (ESTs) were included in the genetic map of the F_7-RIL population. The distribution and specification of the map were discussed in chapter 2. Co-localizations of the ESTs with identified QTLs were observed. Thus, it is likely that these ESTs are associated with or play a role in resistance to EB in this population. Clusters of ESTs on distal parts of chromosomes 1 and 2, indicated that these regions are rich in resistance/defense response genes. A cluster of resistant gene analogues (RGAs) was also observed in the distal part of the short arm of chromosome 2 in an F_2 map of the same cross (Foolad et al. unpublished data). Two strong and stable QTLs were observed on chromosome 5 and 6, respectively, which were co-localized with ESTs. Four other QTLs on chromosomes 3, 9, 11 and 12 were observed, but they were either not stable through the years or their LOD score was less than 2.4. The effect of all observed QTLs and their co-localization of these QTLs are discussed below.

The identified QTLs for EB on chromosomes 1 were not stable across the years / environments; the LOD scores were higher in 2004 and 2006 than in 2005 due to the rainfall and more disease incidence. The more severe the disease, the more significant difference between resistant or susceptible lines will be. Based on AUDPC data, on average the additive effect of this QTL was -157 *day-percent.* In another words, the AUDPC value of the RILs carrying this QTL was ~157 *day-percent* less than the RILs that did not have it. This QTL is tightly linked to EST markers cTOS16I16 (Acidic beta-1.3-glucanase), cLEC71F17 (Basic beta-1.3-glucanase), cLEC72D1 (Endo-1,4-beta-glucanase) and cLEI6L16 (Ribosomal protein S6 family protein - temporary annotated). Acidic and Basic-1.3-glucanases have previously shown to be over expressed in lines with EB resistance (Lawrence et al. 1996; Lawrence et al. 2000). The roles of the two other ESTs (cLEC72D1 and cLEI6L6) have not been investigated yet in tomato; therefore, they can be used as candidate genes in EB resistance. Another close EST marker to this QTL, cLEC34L10b, is a part of the *L. pimpinellifolium Cf-9* resistance gene cluster. *Cf-4* and *Cf-9* genes have been well characterized (Jones et al. 1994; Thomas et al. 1997) and most likely encode extracytoplasmic membrane-anchored glycoproteins comprised predominantly of LRRs (Dixon et al. 1996; Jones et al. 1994). The response of the lines carrying *Cf-4, Cf-9* or both to *A. solani* needs to be investigated to further confirm the reason(s) of the observed co-localization in the RIL population. Foolad et al. (2002b) identified a QTL on chromosome 1 based on a BC_1 and BCS_1 generations of a cross between *L. esculentum* and *L. hirsutum*. However, the position of identified QTL in their study was on the long arm of the chromosome whereas the identified QTL in the current study was on the short arm, exactly in the opposite side of chromosome. Because the effect of both QTLs was inherited from the wild type parents, it is evident that *L. pimpinellifolium* (PLSP125) carries a different QTL for EB than *L. hirsutum* (PI126445). Chaerani et al. (2007) identified a QTL on chromosome 1 in the same position as Foolad et al. (2002b) did, using *L. esculentum* cv. Sloentos and *L. peruvianum* (LA2157) in 157 $F_{2:3}$ families. Because this QTL was inherited from the wild type parent it is likely the *L. hirsutum* and *L. peruvianum* share similar QTL for EB resistance while they are both different from *L. pimpinellifolium*.

Of the two observed QTLs on chromosome 2, one was located on the short arm of the chromosome (*EB2.1*) in a more marker-saturated area and one was located in the long arm

(*EB2.2*) of the chromosome in an area with less markers. The QTL on the short arm of chromosome 2 has not been previously reported and it seems that it is specific to this population. The second QTL in the middle part of long arm was in the same position as reported by Chaerani et al. (2007). Foolad et al. (2002b) also observed a QTL with the same effect in a lower part of long arm of chromosome 2. It seems that their QTL is the same as the one observed here and the shifting toward the lower part of the chromosome is due to two large gaps (> 20 cM) in opposite sides of marker TG337 that might have caused a possible mapping error. The *EBR2.1* was identified by AUDPC data of F_8/2005 and the *EBR2.2* was detected by final % defoliation data of F_7/2004. At both ends of the *EBR2.2* interval (~10 cM), three ESTs were mapped, but the QTL peak was at markers TG463 and CG21. EST marker cLEY1K9 (pathogen-inducible alpha-dioxygenase) mapped to the right side of the QTL and EST marker cLEC72P14 (Acidic 27kDa endochitinase precursor) and cLET10E15 (Acidic 26kDa endochitinase precursor) were mapped to the left of the QTL. The role of Acidic and Basic chitinases were previously investigated in EB resistance (Lawrence et al. 1996). However, the role of pathogen-inducible alpha-dioxygenase needs yet to be determined. In the very distal part of short arm of chromosome 2 at least 7 ESTs were mapped in a 1.6 cM interval were *EB2.1* was identified. The *EBR2.1* was only significant (LOD>3.0) in F_9. In other years/generations the LOD score of this QTL varied from 0.5 to 2.0. Co-localization of the ESTs coded for Jasmonic acid, Xyloglucan-specific fungal endoglucanase inhibitor protein and several kinases with *EBR2.1* was observed. According to the results in this study, *EBR2.1* and *EBR2.2* are not stable QTLs through the years and their stability or their effects need to be verified in future by re-evaluation of the population.

A much stronger and important QTL (LOD = 4 to 8) was identified on chromosome 5 (*EBR5.1*). The effect of *EBR5.1* was consistent across the years and generations. In the same chromosomal position Foolad et al. (2002b) (LOD=3.7) and Chaerani el al. (2007) (LOD = 3.95 - 4.75) observed a QTL. However, in their studies the positive allele contributed from the disease-resistance parent while in the current study the positive allele was contributed from the susceptible parent (*L. esculentum* cv. NCEBR-1). Inheritance of resistance allele from the susceptible parent is not uncommon and has been reported in tomato and other plant species (Lefebvre and Palloix 1996; Pilet et al. 1998; Zhang et al. 2003). The presence of this QTL might

explain the transgressive segregation for EB in the RIL population as has been described before (deVicente and Tanksley 1993; Dirlewanger et al. 1994).

The QTL on chromosome 5 spans ~15 cM interval in which important ESTs such as disease resistance gene homolog *Mi*-copy1 (cTOC2J14), Ribosomal protein L6 (cTOA24J24), 60S ribosomal protein L6 (cTOC20J21), ethylene response factor-5 and metallothionein-like protein type-2 have been mapped. Co-localization of the peak of QTL with *Mi* gene further suggests that resistance to EB and root-knot nematodes (*Meloidogyne spp*.) may share a common pathway. Because the *Mi-1* gene encodes a protein sharing structural features with the nucleotide-binding site leucine-rich repeat containing (NBS-LRR) type of plant resistance genes (Vos et al. 1998) it is likely that it has dual functionality in the case of resistance to EB. The fact that *Mi-1* gene confers resistance to three unrelated plant pests, root-knot nematodes (Vos et al. 1998),potato aphid, *Macrosiphum euphorbiae* (Rossi et al. 1998), and whitefly, *Bemisia tabaci* (Nombela et al. 2000; Nombela et al.), raises the possibility that it may also confer resistance to EB. Challenging the lines carrying the *Mi-1* gene with *A. solani* can test this hypothesis.

The second most important QTL identified in this research was located on chromosome 6 (*EBR6.1*) spanning a ~20 cM region. A QTL in the same chromosomal position was identified by selective genotyping (Zhang et al. 2003) and in another study (Chaerani et al. 2007). Because the effect of this QTL was inherited from the resistance parents in all studies including this it is possible that *L. pimpinellifolium*, *L. hirsutum* and *L. peruvianum* share the same QTL for EB resistance.

For the QTL on chromosome 6, the Log Likelihood Ratio (LR) graph of QTL starts at marker cLEG32E10 for lipoxygenase B, and ends at marker (cLEN10H12) for alcohol dehydrogenase-2. In this chromosomal region two other ESTs were mapped for wound-induced protein-1 (cLEC76A13) and (cLEG49O24) for Phosphoenolpyruvate carboxylase kinase-2. Although these ESTs are not the only ESTs in this chromosomal region and it would a premature conclusion about their direct role in EB resistance, however, knowing their function is worthwhile. Lipoxygenase (LOX) activity has been identified during pathogen-induced defense responses (Kolomiets et al. 2000) and mechanical wounding and insect attack (Hildebrand et al. 1988). It has been suggested that LOX is involved in the development of an active resistance

mechanism known as the hypersensitive response (HR), a form of programmed cell death (Rusterucci et al. 1999). Mechanical wounding of potato leaves, stems, roots and tubers leads to a rapid increase of wound induced protein *wun1* mRNA (Logemann and Schell 1989). Because the peak of the QTL is at the EST marker for *wun1* it is likely that this gene is involved in EB resistance. Transforming susceptible tomato plant with this gene may confirm if this gene enhances the level of tomato resistance to *A. solani*. As an alternative, one can study the up-regulation of this gene in tomato plants challenged by *A. solani*. Alcohol dehydrogenase (ADH) is up-regulated in potato plants challenged with *Phytophthora infestans* and its role in fatty acid degrading in pathways of diseased potato is well characterized (Lyon 2002). Phosphoenolpyruvate carboxylase (PEPC), is a key enzyme of primary metabolism of higher plants and regulated by reversible phosphorylation, which is catalyzed by PEPC kinase (PPCK) (Fukayama et al. 2006). PEPC is up-regulating upon pathogen infection or wounding (Lyon 2002) but its role in metabolic pathway of disease resistance has not yet been characterized.

A number of ESTs were also co-localized with those QTLs that were not consistent in all three generations. For example, another copy of lipoxygenase (cLEC79A23) on chromosome 9 co-localized with a QTL in F_9 (*EB9.1*). In the same chromosomal location a stable QTLs was observed by Chaerani et al. (2007). However, Foolad et al. (2002b) found two QTLs on two distal parts of the chromosome whereas the QTL found in the current study is located in the middle of chromosome.

Diacylglycerol kinase (cLHT11J12), ferredoxin-I chloroplast precursor (cLER4F5a,b), NADH-ubiquinone oxidoreductase 23 kDa subunit (cTOF22M16), PR protein (cLEX10N16b), fructokinase (cLPP2M12), basic 30kDa endochitinase precursor (cLEC18O1), chloroplast ferredoxin I (cTOF30K21), all on chromosome 10 (*EB10.1*) identified only in F_7 with their effects inherited from NCEBR-1 breeding line. A QTL on chromosome 10 was observed on chromosome 10 by Foolad et al. (2002b) and Zhang et al. (2003) on the opposite side of the identified QTL in the current study. Therefore, by comparing these studies it is unlikely that *L. pimpinellifolium* and *L. hirsutum* share a QTL on chromosome 10.

A QTL (*EB11.1.*F_9) with a relatively significant effect (LOD~2.4) was only identified in F_9 generation that was co-localized with a COS (C2_Ag3g60830), SSR (SSR80) and a known

functional gene (KFG-1 for jointless trait) marker on chromosome 11. In the F_7-RIL map no EST was mapped in the QTL interval, however, in an F_2 map that current population was derived from (chapter 2) an EST (cTOF29F6, 10-hydroxygeraniol oxidoreductase) was mapped. The co-localized COS maker in the QTL region is not related to disease resistance or defense response but encodes an actin-related protein required for normal embryogenesis (*tair* 2007). Therefore, the role of actin-related protein can be investigated in EB resistance.

Co-localization of a QTL with WRKY transcription factor IId-2 (cTOS21D14), disease resistance protein RPP8 from *Arabidopsis thaliana* (cLEC80G6), and S-adenosyl-l-homocysteine hydrolysis (cLPT1G11) were observed on chromosome 12 (*EB12.1*) in F_9 and with a lower LOD score in F_8. The effect this QTL was inherited from the *L. pimpinellifolium* (PSLP125) parent and co-localized with a lycopene QTL (Chapter 4). The tight linkage of this QTL for enhanced EB resistance and high lycopene content would be promising for developing the tomato lines resistance to EB with high lycopene content.

Genetics and breeding of EB resistance in tomato, the number and effects of QTLs in different mapping populations have been reviewed by Foolad et al. (2005). BC_1- derived *L. hirsutum* population has been compared with an F_2 derived *L. pimpinellifolium* population. Comparative analysis between these two interspecific populations (*L. esculentum* × *L. hirsutum* or *L. esculentum* × *L. pimpinellifolium*) revealed 30% similarity between QTLs on chromosomes 3, 5, and 11 in the two populations. Presence of similar QTLs across species validates the authenticity of these QTLs for further exploitation in breeding for EB resistance. Other uncommon QTLs between the two populations were cross specific indicating that *L. hirsutum* and *L. pimpinellifolium* harbor different QTLs because both mapping populations have one *L. esculentum* parent in common (different accessions of the same species). The results of this study are complementary to our previous knowledge about EB resistance and are aimed to identify ESTs for disease resistance or defense response for EB resistance in a more classified manner.

The quantitative and complex nature of EB resistance has hindered our efforts to clone the QTLs for EB resistance. However, cloning of the fruit size QTL in tomato (Frary et al. 2000) opened a new window for cloning of other QTLs in tomato and other crop species. In this respect, mapping more than 130 ESTs to tomato genome for the first time and studying their co-

localizations with respect to EB resistance provides a unique opportunity to follow up the project to clone and transform susceptible lines to study the resistance mechanism at cellular level. The information provided here will help developing NILs and sub-NILs to map the genes for EB resistance by map based cloning.

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Tables

Table 3-1 Early Blight (EB) disease severity, based on final % defoliation and AUDPC, in three years for parental lines and F_1 progeny of the RIL population

Year	10 plants / Genotypes	Growth habit[a]	% defoliation		AUDPC	
			Mean±SE	Range	Mean±SE	Range
2004						
	P1 (NCEBR-1)	D	75±5.0	70-85	1570±100	1470-1670
	P2 (PSLP125)	I	32.5±2.5	30-35	514±16.0	498-530
	F_1	I	30±5.0	25-30	470±30.0	440-500
2005	P1 (NCEBR-1)	D	11±9.0	2-20	343±89.0	254-432
	P2 (PSLP125)	I	27.5±2.5	25-30	499±12.0	487-511
	F1	I	27.5±2.5	25-30	560±18.6	542.3-579.5
2006	P1 (NCEBR-1)	D	75±10	65-85	2045±460	1585-2505
	P2 (PSLP125)	I	25±5.0	20-30	509±141	368-650
	F_1	I	30±10.0	20-40	662±213	449-875
Grand total	P1 (NCEBR-1)	D	53.7±14.0	2.0-85.0	1349±334	254-2,505
of three	P2 (PSLP125)	I	28.33±2.11	20.0-35.0	507.3±36.9	367.5-650
years	F1	I	29.17±3.0	20.0-40.0	564.3±65.9	440-875

[a] D= determinate I= Indeterminate

Table 3-2 Early Blight (EB) disease severity (% defoliation) in three generations of the RIL population of *L. esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) cross

Generation / Year	% Defoliation†	Mean±SE	StDev	Variance	Min.	Max.	Range	Skewness
F_7 / 2004	Eva 1- Rep 1	28.67±1.73	21.84	477.15	2.00	95.00	93.00	1.24
	Eva 2- Rep 1	52.13±1.99	25.05	627.35	5.00	100.00	95.00	0.17
	Eva 3- Rep 1	73.63±1.85	23.28	541.77	13.00	100.00	87.00	-0.68
	Eva 1- Rep 2	29.45±1.66	20.82	433.40	3.00	90.00	87.00	1.05
	Eva 2- Rep 2	48.94±2.08	26.05	678.43	8.00	100.00	92.00	0.32
	Eva 3- Rep 2	65.06±2.12	26.59	707.21	15.00	100.00	85.00	-0.20
	Ave. FDP	69.32±1.83	22.92	525.52	15.00	100.00	85.00	-0.36
F_8 / 2005	Eva 1- Rep 1	10.67±0.74	9.691	93.906	0.0	50.000	50.000	1.76
	Eva 2- Rep 1	18.51±1.21	15.80	249.53	0.0	80.00	80.00	1.79
	Eva 3- Rep 1	28.86±1.63	21.26	451.89	0.0	95.00	95.00	1.24
	Eva 4- Rep 1	41.40±2.05	26.75	715.60	2.00	100.00	98.00	0.71
	Eva 1- Rep 2	11.36±0.80	10.469	109.597	0.0	50.000	50.000	1.83
	Eva 2- Rep 2	19.75±1.22	15.90	252.96	1.00	80.00	79.00	1.51
	Eva 3- Rep 2	31.33±1.74	22.73	516.74	2.00	95.00	93.00	0.89
	Eva 4- Rep 2	44.02±2.10	27.42	751.96	3.00	100.00	97.00	0.51
	Ave. FDP	42.71±1.90	24.86	618.00	6.00	100.00	94.00	0.59
F_9 / 2006	Eva 1- Rep 1	15.32±0.87	11.000	120.998	1.000	55.000	54.000	1.01
	Eva 2- Rep 1	36.84±1.64	20.72	429.40	3.00	95.00	92.00	0.85
	Eva 3- Rep 1	68.24±2.30	29.03	842.92	10.00	100.00	90.00	-0.43
	Eva 1- Rep 2	15.05±0.92	11.649	135.696	1.000	50.000	49.000	0.91
	Eva 2- Rep 2	38.51±1.86	23.59	556.40	5.00	95.00	90.00	0.73
	Eva 3- Rep 2	69.67±2.37	29.98	898.85	10.00	100.00	90.00	-0.56
	Ave. FDP	68.96±2.10	26.55	705.04	10.00	100.00	90.00	-0.47

Abbreviations: Eva = Evaluation, Ave = Average, Rep = Replication, Range = difference between minimum and maximum
† = Highlighted rows represent the % final defoliation

Table 3-3 Early Blight (EB) disease severity (AUDPC) in three generations of the RIL population of *L. esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) cross

Generation / Year	AUDPC	Mean±SE	StDev	Variance	Min.	Max.	Range	Skewness
F_7 /2004	Rep 1	1400.9±50.0	631.0	398207.8	176.0	2760.0	2584.0	0.23
	Rep 2	1311.2±53.7	672.5	452299.9	226.0	2692.0	2466.0	0.31
	Ave. R1 & R2	1356.0±48.8	611.6	374068.1	201.0	2720.0	2519.0	0.34
F_8 /2005	Rep 1	928.2±51.3	670.8	449950.0	12.0	3103.0	3091.0	1.30
	Rep 2	986.4±53.0	692.8	479949.8	74.5	3120.0	3045.5	0.99
	Ave. R1 & R2	957.3±49.1	641.5	411554.2	71.0	3085.0	3014.0	1.10
F_9 /2006	Rep 1	1283.7±47.1	595.6	354751.8	173.0	2802.5	2629.5	0.32
	Rep 2	1292.9±48.9	618.9	383008.1	175.0	2715.0	2540.0	0.26
	Ave. R1 & R2	1288.3±42.9	543.1	294906.8	208.3	2596.3	2388.0	0.32

Table 3-4 Analysis of variance of final % defoliation for parental lines and F_1 generation of *L. esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) cross of the RIL population

S.O.V	DF	Seq SS	Adj SS	Adj MS	F	P
Year	2	2058.78	2058.78	1029.39	22.49	0.002
Rep(year)	3	474.83	474.83	158.28	3.46	0.092
Genotype	2	2485.44	2485.44	1242.72	27.15	0.001
Year*Genotype	4	3469.22	3469.22	867.31	18.95	0.001
Error	6	274.67	274.67	45.78		
Total	17	8762.94				

S = 6.77 RSq =96.87 R-Sq(adj) = 91.12 CV= 18.25%

Table 3-5 Analysis of variance for final % defoliation in the RIL population of *L. esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) cross for F_7, F_8 and F_9 generation grown from 2004 to 2006

S.O.V	DF	Seq SS	Adj SS	Adj MS	F	P
Year	2	119224.3	119224.3	59612.2	231.41	0.000
Rep. (Year)	3	4325.2	4325.2	1441.7	5.60	0.001
Accessions	133	347580.3	347580.3	2613.4	10.15	0.000
Acc. × Year	266	129016.7	129016.7	485.0	1.88	0.000
Error	399	102782.8	102782.8	257.6		
Total	803	702929.3				

S = 16.05 R-Sq = 85.35% R-Sq(adj) = 70.57% CV= 25.9%

Table 3-6 Simple linear correlations different generations of a RIL population of *L. esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) cross in three generations/years for early blight resistance

%defoliation	F_7 R1	F_7 R2	F_7 Ave. R1-R2	F_8R1	F_8R2	F_8 Ave. R1-R2	F_9R1	F_9R2
F_7 R2	0.68**							
F_7Ave R1-R2	0.91**	0.93**						
F_8R1	0.46**	0.36**	0.45**					
F_8R2	0.47**	0.46**	0.52**	0.69**				
F_8 Ave. R1-R2	0.51**	0.45**	0.53**	0.92**	0.92**			
F_9R1	0.50**	0.57**	0.59**	0.54**	0.60**	0.62**		
F_9R2	0.43**	0.38**	0.48**	0.44**	0.60**	0.56**	0.65**	
F_9 Ave. R1-R2	0.52**	0.53**	0.58**	0.54**	0.66**	0.65**	0.90**	0.91**

* $P < 0.05$; ** $P < 0.01$, ns= none significant correlation; R1= 1st replication, R2= 2nd replication Ave. R1-R2= the mean of R1 and R2

Table 3-7 Simple linear correlations among plant related traits and early blight resistance measure in a of *L. esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) RIL population in three generations/years

	Growth Habit	Plant Type	Plant Size	Maturity	Fruit Size	Yield	Fruit Weight	pH	Soluble Solids
% Defol.									
F_7 2004	-0.40**	-0.24**	-0.48**	-0.60**	0.16*	0.66**	-0.10^{ns}	0.01^{ns}	-0.08^{ns}
F_8 2005	-0.35**	-0.28**	-0.42**	-0.59**	-0.07^{ns}	0.52**	-0.13^{ns}	-0.21**	-0.16*
F_9 2006	-0.46**	-0.15*	-0.49**	-0.43**	-0.11^{ns}	0.33**	-0.01^{ns}	-0.01^{ns}	-0.32**
AUDPC									
F_7 2004	-0.40**	-0.28**	-0.55**	-0.66**	0.17*	0.67**	-0.07^{ns}	0.05^{ns}	-0.14^{ns}
F_8 2005	-0.32**	-0.31**	-0.44**	-0.61**	-0.07^{ns}	0.53**	-0.11^{ns}	-0.15*	-0.20**
F_9 2006	-0.33**	-0.19**	-0.37**	-0.56**	-0.20**	0.31**	-0.01^{ns}	-0.08^{ns}	-0.33**

* $P < 0.05$; ** $P < 0.01$, ns= none significant correlation

Table 3-8 Narrow-sense heritability estimates based on REML method for early blight resistance measured in a *L. esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) RIL population in three generations/years

	REML method /Mixed model	
	Plot basis	Family mean bases
Final % Defoliation	0.49 (0.043)*	0.68 (0.036)
AUPDC	0.49 (0.044)	0.69 (0.036)

*The numbers in parentheses are standard error of h^2 estimation

Table 3-9 QTLs detected for early blight based on composite interval mapping in a *L. esculentum*× *L. pimpinellifolium* F_7-RIL population. LOD, log-likelihood –; PVE, percent phenotypic variation explained; Additive effect; difference between E or PM alleles from the mean. Positive effect inherited from *L. esculentum* parent, Positive effect inherited from *L. pimpinellifolium* parent. For simplicity maximum LOD, Additive effect and PVE in the QTL interval has mentioned

	QTL Name	Marker Interval	Chr.	Position Interval (cM)	LOD	Additive	PVE [a]R^2
Final % defoliation F_7 - 2004	EB1.1	cTOS16I16 - cLEC34L10b	1	24.3-27.1	2.1	-5.3	0.04
	EB2.1	TG454- TG463	2	53.8-60.5	3.0	-6.00	0.06
	EB3.1	cLX12O16b	3	53.0	2.1	8.2	0.07
	EB5.1	cLEC76E11- cLEC76E11	5	65.0-78.9	4.8	9.1	0.12
	EB6.1	TG590	6	17.6	3.4	-6.4	0.07
	EB10.1	cLHT11J12- cLPP2M12	10	20.6-26.3	2.7	6.15	0.06
Final % defoliation F_8 - 2005	EB2.1	cLEC73K6b- cLC73I19a	2	0.1-3.6	2.2	-4.8	0.04
	EB5.1	cLEY18H8- cTOA24J24	5	70.1-80.1	5.1	8.0	0.11
	EB6.1	cLEG32E10- CT285	6	12.6-26.0	5.9	-8.0	
Final % defoliation F_9 - 2006	EB1.1	cLEI6D5- cLEC73K6b	2	0.5-0.13	2.5	-6.4-	0.04
	EB5.1	cTOA24J24	5	79.0	7.25	12.5	0.16
	EB6.1	TG590- CT285	6	17.6-26.0	2.7	-6.7-	0.05
	EB9.1	cLEC79A23	9	51.0	3.8	8.4	0.07
	EB11.1	C2_Atg60830	11	16.4	2.3	-6.5	0.04
	EB12.1	cLEC80G6 –TG473	12	73-77	2.3	-6.2-	0.04

[a] R^2 is the proportion of the variance explained by the QTL conditioned on the background markers and any explanatory variables.
*LOD thresholds were determined by 1000 permutation of markers for each trait. LOD of final % defoliation-F_7 = 3.78, final % defoliation-F_8 = 3.59, final % defoliation-F_9 = 3.28

Table 3-10 Total phenotypic, genetic variance and variation explained by QTLs detected for early blight resistance, revealed by multiple interval mapping (MIM) in a *L. esculentum*× *L. pimpinellifolium* RIL population.

Generation / Trait	Phenotypic variance	Genetic variance	Residual variance	R^2 Genetic	R^2 Residual
Final % defoliation					
F_7	522.2	201.2	320.9	0.39	0.61
F_8	513.7	120.8	393	0.24	0.76
F_9	830.1	272.5	557.7	0.33	0.67
AUDPC					
F_7	3.72E5	9.094e+004	2.815e+005	0.24	0.76
F_8	4.09E5	1.342e+005	2.75e+005	0.33	0.67
F_9	2.90E5	1.089e+005	1.809e+005	0.38	0.62

Table 3-11 QTLs detected and their interaction for early blight resistance based on multiple interval mapping (MIM) in a *L. esculentum* × *L. pimpinellifolium* RIL population.

Generation	QTL	Type	Chrom	Marker	Position	LOD	Effect	Effect (%)
F_7 % defoliation	1	A	1	cLEC34L10b	24.25	0.52	-4.33	2.5
	2	A	2	TG454	53.48	0.00	-0.60	0.5
	3	A	2	TG463	60.48	0.07	-3.43	2.9
	4	A	3	cLEX12O16b	53.03	2.43	11.82	9.4
	5	A	5	cLEC76E11	65.01	0.75	8.87	6.7
	6	A	5	cLEY18H8	72.92	0.00	-0.18	-0.1
	7	A	5	cTOA24J24	78.94	0.21	5.14	3.3
	8	A	6	TG590	17.58	1.93	-8.42	5.1
	9	A	10	cLHT11J12	20.61	0.31	6.53	5.2
	10	A	10	cLPP2M12	26.30	0.01	-1.03	-0.7
	3*6	AA	-	-	-	0.40	-4.40	3.9
F_7 AUDPC	1	A	1	cTOS16I16	14.08	1.17	-160.57	3.6
	2	A	2	cLPP13J1	0.19	0.03	-34.32	1
	3	A	5	TG351	75.81	1.94	268.48	9.9
	4	A	10	cLHT11J12	14.71	0.77	134.83	4.8
	2*3	AA	-	-	-	0.62	-149.30	5.2
F_8 % defoliation	1	A	2	cLEC73K6b	0.13	1.77	-7.45	7.5
	2	A	5	cLEY18H8	70.92	0.33	4.72	4.3
	3	A	5	cTOC20J21	80.94	0.24	4.45	3.4
	4	A	6	cLEG32E10	12.6	0.52	-4.69	4.6
	5	A	6	CT285	25.99	0.29	-3.86	3.8
	1*5	AA	-	-	-	0.18	2.35	-0.1
F_8 AUDPC	1	A	2	cLPP13J1	0.19	1.62	-184.06	6.2
	2	A	2	CG21	63.76	1.19	166.75	2.0
	3	A	5	cLEY18H8	70.92	0.34	132.56	4.3
	4	A	5	cTOC20J21	80.94	0.58	171.55	5.5
	5	A	6	TG590	17.58	1.26	-234.16	9.4
	6	A	6	CT285	25.99	0.18	-90.46	3.4
	2*5	AA	-	-	-	0.70	-129.19	2.1
F_9 % defoliation	1	A	2	cLEI6D5	0.05	1.29	-7.62	3.1
	2	A	5	cTOA24J24	78.9	2.96	13.34	10.9
	3	A	6	TG590	17.58	0.50	-6.68	3.3
	4	A	6	CT285	25.99	0.19	-4.62	2.7
	5	A	9	cLEC79A23	50.95	2.29	11.13	8.3
	6	A	11	C2_At60830	16.64	0.49	-4.88	1.5
	7	A	12	cLEC80G6	72.87	1.23	-7.74	1.5
	2*4	AA	-	-		0.73	6.73	1.5
F_9 AUDPC	1	A	1	cLEC34L10b	20.25	0.97	-116.09	1.9
	2	A	5	cLEY18H8	70.92	0.05	38.88	1.2
	3	A	5	cTOC2J14	78.58	0.88	182.61	5.6
	4	A	6	TG274	15.07	0.52	-117.97	6.1
	5	A	6	CT285	25.99	0.23	-102.35	5.1
	6	A	9	cLEC79A23	50.95	3.26	231.69	14.4
	4*5	AA	-	-		0.58	163.57	3.3

Figures

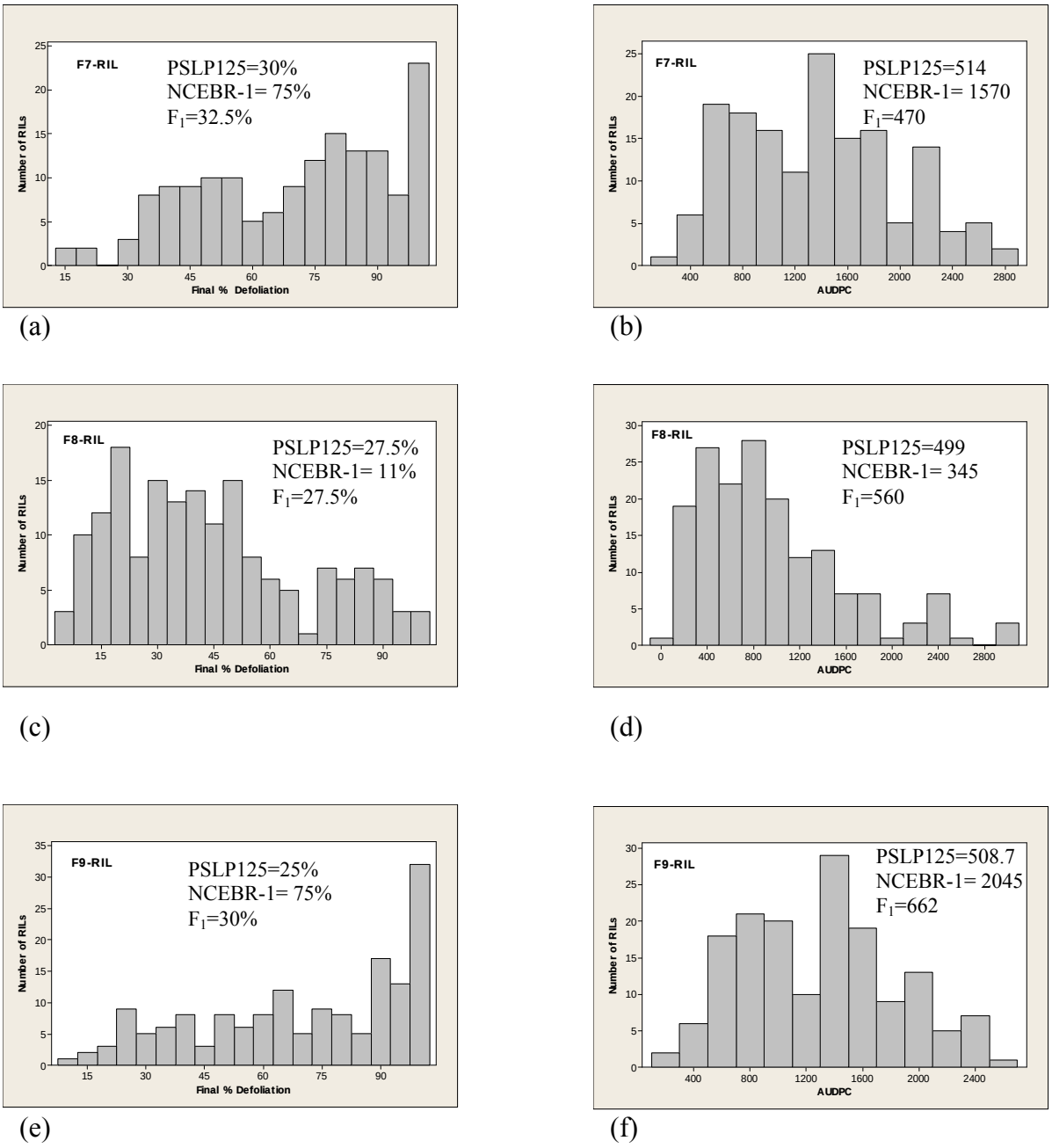

Figure 3-1 Frequency distribution of final % defoliation (left) and AUDPC (right) in three generations of a RIL population of a cross between *L. esculentum* (NCEBR-1)× *L. pimpinellifolium* (PSLP125) grown in Russell E. Larson Experimental Station, Horticultural Research Farm, Rock Spring, Pennsylvania.

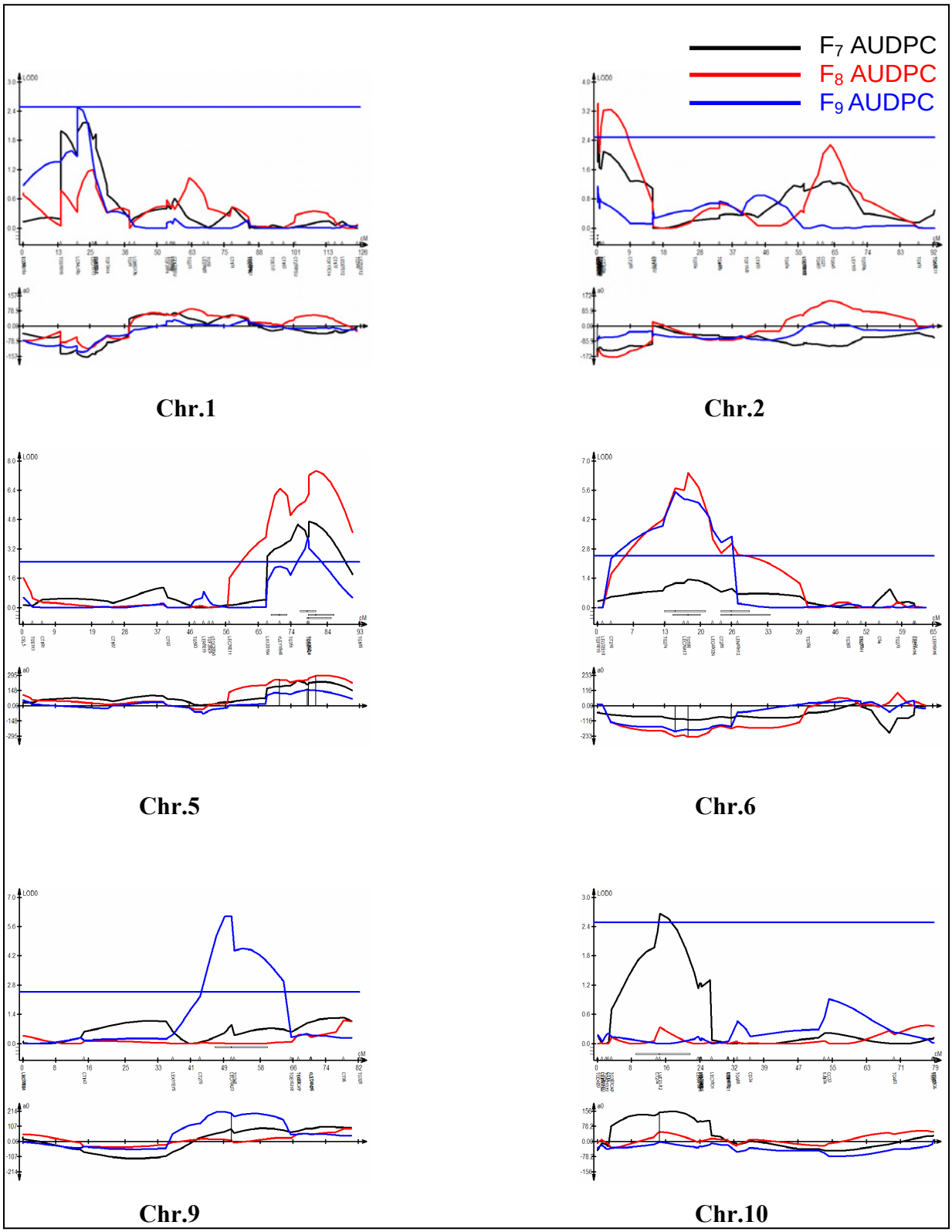

Figure 3-2 Identified QTLs for early blight resistance based on AUDPC data in three generations of a RIL population of a cross between *L. esculentum* (NCEBR-1)× *L. pimpinellifolium* (PSLP125).

Chapter 4 . Identification and characterization of QTLs for fruit quality characteristics in a RIL population of tomato

Abstract

In addition to yield and resistance to biotic and abiotic stresses, an important objective of most tomato breeding programs is to improve fruit quality of the crop. In the current competitive and demanding market, it is imperative to have high-quality fruit for both fresh market and processing tomatoes. However, the cultivated tomato has a narrow germplasm base and it is unlikely that hybridization of these cultivars generate much new variation. It is necessary to search for other sources of germplasm in order to introduce novel genes to the currently available elite lines. However, there are various problems associated with the use of exotic germplasm, including genetic drag, incompatibility, and pleiotrophic effects of exotic genes. These and other problems would prolong the breeding program by at least 3-4 years when using wild germplasm as gene resources. In addition, most of the desired traits in crop plants including tomato are inherited quantitatively, which are controlled by polygenes. An effective approach for studying complex traits is trough Quantitative Trait Locus (QTL) mapping based on the use of genetic markers (preferably DNA markers). Through QTL mapping the role of specific loci controlling a particular trait can be described, interaction between loci can be assessed and more ambitiously as the final goal, these loci can be cloned by fine mapping approaches.

In this respect, the potential of many wild relatives of tomato is still unexplored. In an attempt to find genetic sources of high fruit lycopene content in tomato, previously about 270 accessions of the wild tomato species *L. pimpinellifolium* were evaluated at Penn State tomato breeding program. One accession (PSLP125) had been identified to be a rich source of lycopene, as well as having several other desirable horticultural characteristics, including resistance to early blight. In 1998, PSLP125 was hybridized with an advanced tomato (*Lycopersicon esculentum*) breeding line (NCEBR-1) to develop a recombinant inbred line (RIL) population. As part of this thesis research, the process of developing the RIL population was completed in summer 2004, and a genetic linkage map was constructed based on a F_7-RIL population (n=172). The F_7-RIL population was selfed for two more generations to develop F_8 and F_9 generations in 2005 and

2006, respectively. The population was evaluated in the field and laboratory for different fruit quality characteristics, including fruit weight (FW), SSC, pH and colorimetric values of purée. In addition, in 2006 (F_9), HPLC and spectrophotometric assays were employed to measure the amount of lycopene in a sample (n=127) of RI lines. Based on the HPLC assay a simple regression model was developed to estimate the amount of lycopene in previous generations/years using colorimetric values as independent variables. The results of HPLC and spectrophotometric analyses clearly indicated that the lycopene content of the wild type parent was ~20 times of that of the cultivated parent. Correlation analysis revealed negative associations between fruit lycopene content and fruit FW and SSC. A positive and strong correlation was observed between lycopene content measured by HPLC and spectrophotometer. The wild type parent had higher SSC, and lower pH (although not significant difference) than the cultivated parent. A continuous variation was observed for all the traits in the RIL population in the three years of experiment, confirming the quantitative nature of the traits. Several QTLs for FW on chromosomes 1, 2, 3, 4, 7 and 11, pH on chromosomes 1, 2, 6, 8, 10 and 12, SSC on chromosomes 1, 3, 6, 8, 9 and 10, and lycopene on chromosomes 2, 4, 7, 11 and 12 were identified. The QTL analysis of fruit lycopene content as measured by different techniques, including HPLC, spectrophotometer, statistical model derived data and field visual evaluation, led to the identification of two new and consistent major QTLs on chromosomes 7 and 12, which seems to be promising for developing QTL-NILs and as well as for improving tomato lycopene content by marker-assisted breeding.

Introduction

The tomato, *L. esculentum* Mill., is the second most important fresh market vegetable crop in the U.S. and is one of the most important vegetable crops worldwide. Due to improved cultural practices and breeding efforts since 1970, the per unit area production of processing tomato has doubled and that of fresh market tomatoes has tripled (ERS-USDA 2006). In addition to increased yield, which is the main focus of most tomato breeding programs, improvement of quality of the tomato fruit has received much attention in the past and present. However, similar to most agronomically important traits such as yield, and tolerance to biotic and abiotic stresses, the inheritance of majority of fruit quality related characteristics is under the control of polygenes (Tanksley 1993) or quantitative trait loci, QTLs (Geldermann 1975). The distinct characteristic of polygenes is that they are highly influenced by environmental factors (Johanssen 1909; Nilsson-Ehle 1909). Therefore, studying these traits requires special methods and tools. Biometrical procedures have been proposed (Falconer and Mackay 1996; Mather and Jinks 1977) as a solution to study the complex traits. However, these methods have not completely addressed the questions regarding quantitative genetics (Grandillo and Tanksley 1996). The advent of molecular markers and developing genetic linkage maps has overcome many of the limitations of biometrical methods by dissecting the effect of quantitative traits into Mendelian factors (Paterson et al. 1988).

To date, molecular linkage maps have been developed for many crop species including tomato (Landry et al. 1987; Tanksley et al. 1992) and have been used to study the inheritance of many complex traits such as resistance to biotic (Kurti et al. 2006; van-Berloo et al. 2001) and abiotic stresses (Zhang et al. 2003), yield (Stuber 1994; Wanga et al. 2007), and fruit flavor (Jones and Scott 1983) and nutritional quality traits (Chen et al. 1999; Grandillo and Tanksley 1996; Paterson et al. 1991; Wanga et al. 2007). Molecular linkage maps have allowed us resolve the effects of minor and major QTLs and estimate the amount phenotypic variation explained at each locus. Molecular linkage maps have also helped a better understanding of genetic phenomena such as interloci (epistasis) and intralocus (dominant) interactions (Grandillo and Tanksley 1996), heterosis (Stuber et al. 1992) and identifying transgressive segregants (deVicente and Tanksley 1993; Paterson et al. 1988).

In tomato, most of the QTL mapping projects have been carried out using interspecific crosses between the cultivated and wild species, e.g. (Chen et al. 1999). This is due to the limited genetic variation in the cultivated tomato, which necessitates looking beyond the current commercial cultivars for novel sources of genes to increase the level of polymorphism and to generate more variation. By exploiting the wild species in tomato breeding, some of the biodiversity in tomato that was lost during its evolution and domestication can be restored (Gur and Zamir 2004 ; Tanksley and McCouch 1997). The most common approach to take advantage of wild relatives of any domesticated crop plant is through sexual hybridization of a cultivated genotype with a wild type accession. The ease of making interspecific crosses in tomato has pursued utilizing of wild type species, in nearly all QTL mapping studies of tomato (Chen et al. 1999; Tanksley and McCouch 1997).

The wild relatives of tomato are a rich source of genes for fruit quality such as lycopene, SSC and pH (Chen et al. 1999). In this respect and in practical tomato breeding, the utility of red-fruited wild tomato species such as *L. pimpinellifolium* or *L. cheesmanii* is more advantageous than using distantly related green-fruited wild species such as *L. hirsutum* or *L. pennellii* (Chen et al. 1999). Within the wild type *L. pimpinellifolium* species, accessions have been found with exceptional soluble solid content. The SSC of the tomato fruit is what makes tomato sauce thick and is largely composed of sugars and organic acids (Tanksley and Nelson 1996). Sugars, mainly glucose and fructose, account for about 65% of soluble solids of the tomato fruit. Citrate and malate acids are the two majors acids found in tomato fruit (Stevens and Rick 1986). The total soluble solids in the fruit range from 4% to 9% (Ho and J.D.Hewitt 1986) and can reach up to 15% of the fruit's fresh weight in some wild accessions of *Lycopersicon* (Garcia and Barrett 2006; Rick 1973), which is 2-3 times greater than cultivated tomatoes (Fridman et al. 2000). Higher SSC in tomato fruit is desirable not only to increase processed product yield but also to reduce the cost of dehydration of in processing tomato (Rick 1974). There is a high correlation between SSC and tomato flavor because flavor is determined to a large extent by the composition of sugar and acid contents of the fruit. It is believed that the amount of volatiles such as 2-isobutylthiazole, methyl salicylate and eugenol is also important in perception of good flavor (Stevens 1970).

In addition to SSC, flavor and other desirable characters, color is one the most important traits of tomato fruit. Tomato fruit can be yellow, orange, pink or red. However, there is a public interest in the tomatoes with red color. The red color of the tomato is primarily due to a member of carotenoids known as lycopene. It has been shown that lycopene, an important antioxidant, has anti-cancer properties by detoxifying the effect of free radicals in the blood (Andreassi et al. 2004; Riso et al. 2004). Its antioxidant activity is through inactivation of singlet oxygen molecules and to reduce carboxy radicals (Agarwal and Rao 2000; Britton 1995; Van den Berg H et al. 2000; Wang et al. 2007). Recently there has been an increasing interest in studying the effect of lycopene on cancer prevention in clinical studies. For example, the effects of lycopene on androgen-independent prostate cancer (Jatoi et al. 2007), breast cancer cell lines (Chalabia et al. 2007), insulin-like growth factor-1 (IGF-1) and binding protein-3 (IGFBP-3) (Graydon et al. 2007) have been studied. The biochemical pathway that leads to lycopene synthesis has been well studied (Hirschberg 2001), however, the mechanism by which lycopene production is regulated in tomato still is unknown. In fact, the inheritance of lycopene is similar to SSC and other fruit quality traits complex and quantitatively controlled. As lycopene receives more attention in the research, new and improved methodologies are needed to measure it accurately.

There are different methods and procedures to measure the carotenoids content of tomato. These procedures include colorimetric measurements, High Performance Liquid Chromatography (HPLC) (Arias et al. 2000; D'Souza et al. 1992; Hyman et al. 2004), spectrophotometric measurements (Davis et al. 2003), Attenuated Total Reflectance Infrared Spectroscopy (ATR-IS) (Halim et al. 2006) and Raman spectroscopy (Schulz et al. 2006). While colorimetric measurements are relatively easy to perform in many laboratories, the outputs which are color parameters such as a^*, b^*, L*, C* and h* so called Lab color parameters, (Commission International de'Eclairage) (Hunter and Harold 1987). These parameters need to be converted to lycopene content using a statistical model. In some cases the lab color parameters have been used directly and individually in QTL analysis (Liu et al. 2003), but it is not clear how reliable the results would be, if one use them a discrete entity. In order to convert these parameters to a more useful data, a model should be developed beforehand by more accurate methods such as HPLC. It should be noted that a model that is applicable in a particular study may not be appropriate for another. Therefore, finding a universal model that meets all expectations may be far from reality.

More accurate measurement methods such as HPLC have been used for lycopene content analysis. However, the HPLC technique has some inherent drawbacks. These include heavy use of toxic chemicals, expensive equipments/reagents, sensitivity to preparation of standards, and above all instability of carotenoids after being extracted from the fruit. The latter has a confounding effect on detecting the real variation among the samples. Recently, ATR-IS has been proposed as a simple method for lycopene measurement, but HPLC is still needed as a reference method to obtain a calibration model (Halim et al. 2006). Using spectrophotometer to measure the absorbance of carotenoids extracts is another simple method to measure the lycopene content of the tomato fruit, but it does not rule out the necessity of carotenoids extraction (an experiment in the current study). Apart from the techniques that are needed to measure the fruit lycopene, the trait itself is under the control of many regulatory mechanisms and is highly affected by environmental factors. Therefore, plant breeders need to compromise between the choice of technique and the level of precision needed, to evaluate their genetic materials. Precise measurements/evaluations techniques are needed to select superior lines, calculate response to selection in the breeding populations, identify reliable QTLs, parental selection for hybridizations and many other important decisions that need to be made in the course of breeding program.

In an attempt to screen a large collection of wild relatives of tomato at the Penn State tomato breeding program one *L. pimpinellifolium* accession (PSLP125) was selected from among 270 wild type accessions for its resistance a number of diseases including EB resistance (M.R. Foolad unpub.). Further screening determined that PSLP125 was also exceptional in having high fruit SSC and lycopene content (see results). The objectives of the current study were: (1) to map and characterize QTLs for a number of high fruit quality characteristic of PSLP125 using a Recombinant Inbred Line (RIL) population based on a cross between *L. esculentum* breeding line NCEBR-1 and *L. pimpinellifolium* accession PSLP125. (2) To determine the stability of QTLs across generations and years. (3) To apply different methods of lycopene measurement such as visual evaluation in the field, colorimetric, HPLC and spectrophotometric assays to: a) develop a statistical model to estimate the amount of lycopene in the population using colorimetric parameters and b) compare the HPLC and spectrophotometric assays.

Materials and methods

Plant Materials

A RIL population, composed of 172 lines at F_7, F_8 and F_9 generations from a *L. esculentum* (NCEBR1) × *L. pimpinellifolium* (PSLP125) cross, and the parents and F_1 progeny were field grown at the Horticulture Research Farm, Russell E. Larson Experiment Station, , Rock Springs, PA. Fruits of these lines were used for fruit quality analyses. In 2004 (F_7 generation) a single plant from each line was selected from the first replication of the experiment and all measurements were carried out on that single plant. The selected plants in the F_7 generation were the source of DNA for marker analysis and map construction as well as the source of seed for production of the next generation (F_8). In 2005 (F_8) and 2006 (F_9), fruits were collected from 10 plants of each RIL in bulk in two replications.

Fruit quality measurements

Fruit weight

The average fruit weight was determined by taking the mean weight of 10-50 randomly selected fruits of each RIL in each year.

Soluble solids content measurements

In summers of 2004, 2005 and 2006, the Brix indices of the F_7, F_8 and F_9 RILs, respectively, were measured using a Bausch & Lomb Abbé-3L refractometer (The Bausch and Lomb 1993) as follows. A total of 10-20 ripe fruits from each line were washed, weighed and thoroughly blended by a standard kitchen blender. Forty mL of the purée was transferred to a 50 mL Falcon tube and centrifuged at 3000 rpm for 10 minutes at room temperature. A 200-µL sample of the supernatant was applied on to the prism of the refractometer and the SSC was measured following manufacturer's instruction at the appropriate temperature.

pH measurement

The pH of the same fruit samples were measured using a standard lab pH meter. In most cases, the centrifuged purée consisted of three layers. To measure the pH, the pH meter probe was

inserted to the middle of liquid and left motionless for seconds to stabilize the reading and to measure the pH.

Colorimetric measurements

In all three years of the experiment (2004, 2005, and 2006), the following colorimetric procedure was employed to measure chromaticity values of the puree (see below). The Konica-Minolta CM-3500d reflectance based spectrophotometer was used to measure the *L**, *a**, *b**, *C** and *h* LAB color parameters (reflectance) for each accession at D65/10° (primary illuminant D65 and Observer at 10°). The machine was calibrating according to manufacturer's instruction prior to each run. To measure the chromaticity values, briefly, a portion of the purée (~3-4 mL) that was used for SSC and pH measurements was poured into a 5.0 cm wide Petri dish (BD Falcon Petri Dishes, 50x9mm, Tight-Fit Lid). The Petri dishes were placed on top of the unit (on a target mask) and SpectraMagic package was used to record the data. For each RIL, two samples were taken and the mean value of the chromaticity parameters was used for statistical analysis.

Carotenoid content measurements

Sampling

In September 2006 the fruits of 127 out of 172 F_9-RILs, including parents and F_1 were collected to measure the carotenoid content of the fruits by HPLC and absorbance based spectrophotometer (see below) to obtain a regression model for estimation of lycopene for the entire F_9-RIL population as well as for the previous generations (F_7 and F_8 RILs). The fruit quality characteristics such as FW, SSC and pH and colorimetric measurements were also measured for these 127 RILs, as described above.

Extraction of carotenoids

Non-polar carotenoids including lycopene and β-carotene were extracted using a methanol and tetrahydrofuran (THF) based solvent, as previously described elsewhere (Hyman 2001; Hyman et al. 2004) (see also Appendix C).

Chromatography (HPLC) conditions

A Waters 2695 Alliance separation module (Waters Corporation, Milford, Massachusetts, USA) was used for HPLC analysis. Carotenoids were separated on a SunFire reverse phase C_{18} analytical column (5.0 µm particle size, 4.6 x 250 mm column dimension, Part#: 186002560) coupled with a SunFire C_{18} guard column (5µm particle size, 4.6 x 20 mm column dimension, Part#: 186002684). Light absorption was measured with a Waters 2996 photodiode array detector (Waters Corporation, Milford, Massachusetts, USA) scanning from 300 nm to 600 nm. An isocratic mobile phase containing methanol (EMD Chemicals Inc, NJ, USA, CAT# UN1593) and stabilized THF with 250 ppm BHT (EMD Chemicals Inc, NJ, USA, CAT#UN2056) in a 9:1 ratio was chosen to maximize recovery of the carotenoids (Hyman 2001). A methanol-based mobile phase has shown to result in a higher recoveries than acentonitrile-based phase (Epler et al. 1992; Hyman 2001). The addition of 0.1% N,N-diisopropylethylamine (ICN Biomedical Inc, OH, USA Cat#150915) was recommended as a modifier to enhance recoveries and increase separation of isomers of carotenoids such as α and β-carotene (Hyman 2001; Kamber and Pfander 1984). Since the mobile phase reagents were HPLC grade, no additional purification was necessary, however, Hyman (2001) recommended filtering the mobile phase through 0.2 µm nylon filters and degassing with helium. To simplify the procedure, these steps were eliminated. Standard solutions were not purified due to their inherent purity, however, the RI lines extracts were passed through 4 mm single-use 0.2 µm PTFE membrane filters (Whatman International Ltd, Cat# 6783-402) using 3.0 mL disposable Luer-Lok tip syringes (BD Syringe, NJ, USA).

Carotenoids quantification via HPLC

Two separate stock solutions, each 100 µg/mL of pure lycopene (Sigma-Aldrich, MO USA Cat# L9878-1MG) and β-carotene (Sigma-Aldrich, MO USA Cat#C9750) were prepared by dissolving 1.00 mg of each component in 10.0 mL THF containing 0.1% BHT (Sigma-Aldrich, MO USA, Cat# B1378-100G). A series of five dilutions were made of the lycopene standard (100 µg/mL) using THF-0.1% BHT to obtain concentrations of 1, 5, 10, 20, and 30 µg/mL. With the same approach, a series of three concentrations of 1, 5, and 10 were made from stock solution of β-carotene (100 µg/mL). Preparation of the standards was carried out under dim light conditions and everything was kept in amber tubes and over dry ice to minimize degradation of carotenoids during the entire preparation process. Two standard curves were produced, for

lycopene and β-carotene, by regressing the concentration of standards (Y) on area or height of its corresponding peak (X). For both lycopene and β-carotene, area of the peak had a higher R^2 than the height of the peak. Therefore, the area of each peak was used to predict the amount of lycopene or β-carotene in 1.0 g of fresh purée from the RI lines.

Carotenoids quantification via spectrophotometer

To simplify the process of tomato fruit lycopene concentration analysis, a simple spectrophotometric measurement was conducted using a highly specialized 96-well quartz plate. Briefly, 200 µL of the standard solutions along with the stock solutions of the same carotenoid extracts that were used for HPLC analysis were loaded into each well of the quartz plate. SpectraMAX 190 plate reader (Molecular Devices Corp., Sunnyvale, CA) was used to measure the absorption of the extracts at 472 and 453.2 nm for lycopene and β-carotene respectively. The standard curves (were developed as described above) then were used to convert the absorption values to lycopene or β-carotene values.

Data Analysis

Means and Correlations

Means, standard errors, skewness and trait distributions were calculated for each trait in each year/generation using Minitab v14 software (MINITAB 2003). The Pearson correlation coefficients between measured lycopene concentration by HPLC and important fruit quality traits such as fruit weight, pH and SSC were calculated using SPSS v14 package (SPSS 2005). The mean of two replications in each year was used to correlation analysis. A stepwise regression model in Minitab was used to make a simple statistical model for the relationship between colorimetric data and actual lycopene concentration of the fruit. To determine the best model, several transformations of a^*, b^* and L^* values were examined.

QTL analysis

Different subroutines of Windows QTL Cartographer v2.5 software (Wang et al. 2006) were used to perform simple (SIM), composite (CIM) and multiple interval mapping (MIM) to find the position of QTLs. The mean of FW, pH and SSC for each RIL over the two replications of

the experiment within each year was used for QTL analysis. Lycopene concentration measured by HPLC and spectrophotometer only in F_9 in 2006 and the mean of two runs of HPLC or spectrophotometer for each sample was used for QTL analysis. In order to examine the usefulness of the estimated lycopene concentration by the statistical model, the lycopene concentrations of the RILs in previous generations, (F_7 and F_8, as well as F_9) were estimated by plugging in the L^*, $a^*$and b^* parameters (obtained by the colorimeter) into the developed statistical model. Therefore, in F_9 generation, two sets of data could be compared. The first set of data was the actual lycopene concentration of 127 RILs, measured by both HPLC and spectrophotometer, and the second set of data was the estimated concentrations based on the statistical model. To clarify this Chart 4.1 was developed to show the data types that were used for QTL analysis of lycopene concentration.

The amount of β-carotene was measured in the population; however, no QTL analysis was carried out for this trait. This was mainly because there was not a significant difference between the two parents for β-carotene concentration and the lack of variation in the population for this trait (Std. = 0.09).

Results

Trait Variation

Mean phenotypic values, standard errors, and ranges for traits measured in the parental lines and RIL population in three generations are displayed in Figure 4-1. The fruit quality related traits, FW, pH, SSC, and lycopene content, showed continues variation in the RIL population, characteristics of quantitative traits (Figure 4-1 *a-k*).

Fruit Weight (FW)

Over the three years of the experiment, the average fruit weight of *L. pimpinellifolium* (PSLP125), *L. esculentum* (NCEBR1) and F_1 were 2.26 g, 156.05 g, and 11.20 g, respectively. The FW of parents and F_1 generation were significantly different ($P < 0.001$). The average FW of the population varied from 12.97 g (F_7) to 14.19 g (F_9). However, there was a large variation within each generation for FW. For example, in F_9 the fruit weight ranged from 4.3 g to 42.6 g. the same trend also was observed in previous generations. ANOVA over three years of the experiment indicated that there was no significant differences among the three generations of the RIL population for tomato FW ($P > 0.05$) (Figure 4-1 *a-c*).

Fruit pH

Fruits of NCERB-1 had relatively higher pH in all three years of the experiment (4.22) and than PSLP125 (4.09). However, the differences between the two parents and F_1 progeny were not statistically significant ($P > 0.01$). *L. pimpinellifolium* (PSLP125) had the maximum variation in three years (SE mean = 0.11) and *L. esculentum* (NCEBR-1) had the lowest (SE mean = 0.05), while the F_1 was between the two parents (SE Mean = 0.03) (Figure 4-1 *d-f*).

Soluble Solid Content (SSC)

The mean SSC over three years of the experiment indicated that NCEBR-1 breeding line parent had lower SSC (4.65 °Brix) than PSLP125 parent (7.23 °Brix) while the F_1 progeny was intermediate (6.8 °Brix). The mean SSC of the population ranged from 4.96 °Brix in F_7 (2004) to 6.52 in F_9 (2006). This was further investigated by ANOVA (data not shown) and analysis of mean. Analysis of mean SSC of the RIL population showed that the F_7 was significantly

different ($P < 0.01$) from both F_8 and F_9 generations. Both negative and positive transgressive segregation for SSC were also observed in the RIL population in all three years of the experiment (Figure 4-1 *g-i*).

Lycopene Concentration

HPLC and spectrophotometric data: Fruit lycopene concentration of the parents, F_1, and F_9-RIL population was measured by HPLC in year 2006 only. As expected, fruits of PSLP125 were exceptionally rich in lycopene (LYC) content with an average of 208 ± 7.9 µg LYC per gram of fresh fruit, more than 10-fold higher than cultivated parent (18.3 ± 2.2 µg/g). In the F_9-RIL population, the LYC concentration was intermediate between the two parents ranging from 8.7 to 195.82 µg/g and an average of 80 ± 3.6 µg/g (based on HPLC data). The results of measuring lycopene content by absorbance-based spectrophotometer was very similar to HPLC analysis and there was a high correlation between the two measurement methods ($r = 0.92$, $P < 0.01$) (Figure 4-1 *j-k*).

Colorimetric data

A statistical model ($R^2 = 0.68$) was developed to convert the LAB color parameters to lycopene concentration as follows.

$$\text{Lycopene}_{(\mu g/g)} = 80.4 - 2.33\, L^* + 83.7\, a^*/b^*$$

Where:

L^*, a^* and b^* are lab colorimeter values obtained from reflectance spectrophotometer (colorimeter machine). The model was used to estimate the amount of lycopene in previous generations (F_7 and F_8) for which HPLC data were not available. The model was also used to estimate the lycopene content of fruits of two replications of the experiment in 2006 (F_9). The colorimetric measurement of these F_9-RILs was independent from the sample that was used for HPLC assay and model development. The estimated lycopene content in previous generations as well as F_9 were used for QTL analysis (see below).

Correlations among traits

In the F_9-RIL population, LYC concentration was negatively correlated with FW (r = -0.38, P < 0.01), consistent with the fact that larger fruit tend to have lower levels of lycopene. The LYC was positively correlated with pH (r = 0.22, P < 0.01) and there was no correlation between SSC and LYC (r = 0.08, P > 0.05). FW was strongly and positively correlated among the three generations of the RIL population (r = 0.88 – 0.93, P < 0.01). The same trend was also observed for SSC (0.48 – 0.70, P < 0.01) and pH (0.4 – 0.6, P < 0.01), which were moderately correlated in the three generations of the RIL population. A relatively weak, negative, but significant correlation was observed between SSC and FW in all three generations, for example in F8 generation the correlation between SSC and FW was -0.27 (P < 0.01). This was also consistent with the challenging problem to breed for larger fruits with higher SSC. SSC was also negatively correlated with pH, but it was not significant (Table 4-2).

Genetic Map

A genetic linkage map was constructed by employing 130 RFLP, 132 EST, and 13 PCR based markers, spanning 1066.1 cM of tomato genome with an average genetic distance between markers of 3.8 cM (as describe in Chapter 2). The order of the markers on this map were comparable with F_2 map of the same parents (Foolad et al. Unpublished data) as well as high-density RFLP map of tomato (Pillen et al. 1996).

QTL Analysis

The results of QTL analyses for different traits are summarized in Table 4-3 and Table 4-5. For all traits major QTLs (LOD > 2.4) were detected. Simple interval mapping (SIM), composite interval mapping (CIM) and Multiple Interval Mapping (MIM) were employed to detect QTLs. Due to a greater accuracy of CIM analysis compared to SIM the results of CIM is only presented. The MIM analysis combined the effects of all QTLs identified by CIM to calculate total phenotypic variation explained (PVE) by QTLs (Table 4-4).

Identified QTLs

Fruit Weight (FW)

Six QTLs on chromosomes 1, 2, 3, 4, 11 (*FW11.1* and *FW11.2*) were consistent across the years and generations (Figure 4-2). The effects of all QTLs were inherited from the cultivated parent (*L. esculentum* cv. NCEBR-1). The *FW2.1* reported here is most likely the QTL that was cloned by Frary et al. (2000). A strong QTL on chromosome 3 (LOD > 7.0) with a large phenotypic effect on fruit weight (~ 11% - 15%) identified by both SIM and CIM. This QTL could be a potential candidate for subsequent cloning. A few other generation-specific QTLs were identified which were rather not consistent across generations. A QTL on chromosome-1 at 77.8 cM position only identified in F_7 and another one on chromosome-12 at 3.9 cM position was specific to F_8.

The QTLs identified by CIM were used in the MIM analysis. According to MIM, the total phenotypic variation explained (PVE) by fruit weight QTL varied from 53% in F_8-RILs to 62% in F_9-RILs (Table 4-4). The final models for MIM analysis for FW trait in each year were refined several times to identify the most significant QTLs and the most significant interactive QTLs (Table 4-5). As it was shown in the CIM analysis, all of the QTL effects were inherited from *L. esculentum* parent. All interactions but one which is highlighted in Table 4-5 were not consistent through the years and generations.

Fruit pH

The mean pH of the cultivated parent over three years of the experiment was 0.1 units higher than that of the wild type parent. This difference was not statistically significant; therefore, the contribution of any QTL identified here was very low in increasing or decreasing the pH of the fruit. Because pH of the purée is a highly variable trait, it was not possible to identify consistent QTLs across all three generations. In spite of this inconsistency a few minor QTLs were identified on chromosomes 1, 2, 8, 10 and 12 (Figure 4-3; Table 4-3 and Table 4-5).

Soluble Solid Content (SSC)

Despite an inherent inconsistency of SSC in all three generation, a few minor QTLs on chromosomes 1, 3, 6 and 8 and one major QTLs on chromosome 10 were identified. The major QTL on chromosome 10 is promising for increasing the SSC of the fruit, because its additive effect ranged from 0.21 to 0.31 and inherited form the wild type parent and explained ~12% of total phenotypic variation.

The total PVE for all QTLs identified by CIM were calculated by MIM analysis. Total PVE varied from 17% in F_7, to 24% in F_8 and 31% in F_9 (Table 4-4). The MIM models were examined for possible epistasis interaction between the QTLs. In F_7-RIL no interactions between QTLs was found, however, in F_8 one interaction and in F_9 three interactions between paired QTLs were observed that none of them were repeated or consistent.

Lycopene Content

CIM analysis identified five major QTLs on chromosomes 2, 4, 7, 11 and 12 (Figure 4-5). As mentioned before, the HPLC carried out in two replications of the same sample extracts. Both HPLC replications, including spectrophotometer measurement data, identified the same QTLs on all chromosomes. However, the magnitudes of the identified QTLs were substantially different (Table 4-3). Four QTLs on chromosome 2, 7, 11 and 12 were consistent across the analyzing methods. Further, the QTL on chromosome 7 had the maximum LOD score in both HPLC and spectrophotometric data and could explain 15% of total phenotypic variation observed. The second most important and consistent QTL was located in distal part of the long arm of chromosome 12 explaining 8% of total phenotypic variation. These two QTLs were also identified; using the lycopene estimates by modeling data and visual evaluation of the fruits in the field (see below).

In order to verify the usefulness of the data obtained by the statistical model in QTL mapping, the fruit lycopene contents in previous RIL generations, F_7, F_8, as well as F_9, were estimated by using L^*, a^* and b^* parameters (measured by the colorimeter) in the model. In the case of F_9, two data sets were analyzed. One was the actual lycopene content based on either HPLC or spectrophotometer from an independent fruit sample of 127 individuals as described in the

previous section, and one was estimated lycopene content based on statistical model from the fruits collected in tow replication from the entire population . Using the statistical model, 2 out of 5 QTLs were consistent in all three generations. A QTL on chromosome 7 (9% -15% PVE) and one on chromosome 12 (14% - 27% PVE) (Table 4-3 to Table 4-5) were stable for lycopene content in the RIL population (Figure 4-6).

Analysis of visual fruit color measurements in the field resulted in identification of the same QTLs on chromosomes-7 and 12 in F_7 and F_8 generations (Figure 4-7). Possibly due to variation in scoring in the F_9 generation (2006), the QTL on chromosome 7 was not detectable. The effects of all identified QTLs were inherited from *L. pimpinellifolium* parent. In addition to these two major QTLs, a QTL on chromosome-1 was also identified in F_8 and F_9 generations that were not detected by any other data sets including HPLC and model derived data for lycopene.

MIM analysis measured the combined effects of all QTLs. The total phenotypic variation explained by the MIM analysis was ~11% to 34% for the field measurement data and F_7 model derived lycopene data, respectively. An interaction between two QTLs on chromosomes 11 and 12 was observed for HPLC derived data. The same interaction was also observed using spectrophotometer data; however, because it was not significant, it was removed from the final MIM model (Table 4-4 and Table 4-5).

Discussion

Although fruit quality characteristics of tomato were previously studied extensively (Chen et al. 1999; Eshed and Zamir 1995; Frary et al. 2004; Goldman et al. 1995; Paterson et al. 1991; Paterson et al. 1990; Paterson et al. 1988; Tanksley et al. 1982), none of those studies used a large RIL population or different methods of lycopene analyses. Here I am reporting the use of a RIL population and QTL mapping of fruit quality related traits in three generations and three successive years as follows.

Trait variation

Fruit weight: One the most contrasting traits between the two parents of the RIL population was fruit weight. The fruit of *L. pimpinellifolium* parent is naturally very small, averaging ~2.2 g, in contrast to that of the *L. esculentum* parent which is averaged ~165 g. When large- and small-fruited tomatoes are hybridized, the fruit size of F_1 progeny is more similar to the small-fruited parent (Grandillo and Tanksley 1996; MacArthur and Butler 1938). This was congruous with the fruit size of the F_1 progeny of NCEBR-1 breeding line and PLSP125, averaging ~14.0 g. Furthermore, no transgressive segregant in any direction was observed in the RIL population, similar to results of previous studies of this trait (Chen et al. 1999; MacArthur and Butler 1938; Paterson et al. 1988). With respect to the distribution of fruit size in the population, the RIL population was highly skewed toward the lower fruit size, suggesting the presence of many large fruit alleles in the cultivated parent. The observed skewed segregation toward the lower FW was also congruous with previous reports (Chen et al. 1999; Grandillo and Tanksley 1996; MacArthur and Butler 1938; Paterson et al. 1988). Skewed segregating toward the lower fruit weight was attributed to dominance effects in a BC_1 population of tomato (Chen et al. 1999), however, with respect to the RIL population all genetic effects are additive. Obviously, the utility of additive effects, characteristics of the RIL population, is much more in breeding than that of dominance effects to develop tomatoes with smaller size fruit. Therefore, the results presented here clearly support these hypotheses that the fruit weight in tomato is quantitatively controlled, transgressive segregation does not exist for fruit weight and *L. pimpinellifolium* alleles decrease the fruit size.

pH of purée: The striking difference that was observed between the two parents for FW did not exist for the fruit pH. The difference in pH of the two parents varied from 0.05 to 0.18 in different years. In fact, there was no significant difference between the two parents and any variation was likely due to non-genetic factors because of its inherent low heritability. This observation was in agreement with a previous report that the variation in tomato acidity was more likely related to maturity stage rather than the genetic differences (Stevens 1972)

Soluble Solid Concentration: Distribution of SSC was normal in the population and no skewed segregation was observed. Total soluble solid contents of the parents, F_1, as well as the entire population were variable in all three years of the experiment. However, the variability of SSC was less than pH, because of a moderate-to-high correlation for this trait across generations (r = 0.48-0.70, $P < 0.01$). Furthermore, the results and personal observations clearly indicated that SSC is highly affected by environmental factors such as excess of rain or drought, maturity stage of the fruit and harvesting time. However, one common feature of all experiments was that the wild type parent had a higher average SSC (7.23 °Brix) across all three years of the experiment than both the cultivated parent (4.65 °Brix) and the F_1 (6.80 °Brix). However, a higher variance was observed for the wild type parent than the cultivated parent. Whether this was due to smaller fruit size and pleiotropic effects of fruit size on SSC is unknown. A variable SSC for *L. pimpinellifolium* (PSLP125) in an experiment carried out during 1998 and 1999 was also reported by Hyman (2001). The analysis of SSC of the entire population showed that the maximum SSC could reach 9.4 °Brix for some RILs in F_9. This was higher than any of the parents and F_1 in that year, indicating a transgressive segregation for the trait. The same trend was observed in F_7 and F_8, however, the maximum SSC in those generations were 8.1 and 7.0 °Brix, respectively. The F_1 progeny had always lower SSC than the wild type parent. However, the SSC of the F_1 was in the same range as the wild type parent. This similarity can be attributed to the smaller fruit size of F_1 than *L. esculentum* parent (10 -15 times smaller).

Correlations between traits: The negative correlation observed between fruit weight and SSC in this research (r = -0.27, $P < 0.01$) was in good agreement with previous reports (Chen et al. 1999; Fulton et al. 1997; Goldenberg and von der Pahlen 1966; Hyman 2001; Paterson et al. 1991; Paterson et al. 1988). The weak correlation between fruit weight and SSC was attributed to the high variability of SSC relative to limited range in SSC (Hyman 2001). In contrast, a low but

positive association between FW and SSC was observed by Grandillo and Tanksley (1996) and because the positive relationship was observed in both indeterminate and indeterminate tomatoes they could not further explain the cause. In the current study, association of pH and SSC was also investigated, where a negative but non-significant correlation between pH and SSC was observed in all three years of the experiment. This included the F_9-RIL population from which the fruits of 127 individuals later in the season (Oct 2006) were collected for lycopene extraction analysis. In contrary to previous observations, in the sample of 127 individuals taken in F_9-RIL for lycopene analysis, a low, and positive but significant correlation between SSC and pH observed ($P < 0.05$). These results clearly indicate that SSC and pH are highly variable in a very limited range and that there are confounding effects of environmental factors and experimental errors possibly explaining such a mixed response. Fulton et al. (1997) reported both positive and negative correlations between SSC and pH, but did not discuss the reason. Chen et al. (Chen et al. 1999) and Hyman (Hyman 2001) reported a low, positive and significant ($P < 0.05$) correlation between SSC and pH. Thus, the observation of mixed responses of SSC and pH in previous studies makes drawing any conclusion about the real relationship between these traits in this population difficult. The positive association of FW and pH (in each year experiment) and negative correlation between FW and lycopene content resolved by HPLC, indicate that phenotypic selection for low pH or high lycopene concentration may result in smaller fruit size. This may not be desirable for fresh-market industry where larger fruits with higher lycopene are desirable. A negative correlation between lycopene and FW indicates that larger fruits tend to have less lycopene, however, this is not a cause and effect relationship, because there are small fruited tomatoes that have very little or no lycopene and vice versa.

No significant correlation between chromaticity parameters and fruit soluble solids or pH was found. However, FW was positively correlated with *L** and *h* and negatively correlated with *a**, *b** and *C**. Finding a positive correlation between FW and b* was in contrary to the report by Saliba-Colombani et al. (2001). A negative correlation between *L** and *a** observed that was in agreement with Liu et al. (2003). However, through this study no correlation between *L** and *b**was found. This was in opposite to Liu et al, (2003), who found a positive and relatively high correlation between *L** and *b** ($r = 0.77$). This might be because they did not use purée of the fruit to measure these parameters. The correlation analysis of lycopene content with *L*, b** and

*a** was highly congruous with the results of Liu et al. (2003). As *a** and *b** increase toward positive values, the color gets more intense toward red and orange, respectively. This explains the positive correlation between lycopene content and *a** and *b** values. The positive values of L* in all experiments, clearly indicates that tomato color is bright.

Heritability of the traits: The strong correlation between FW of different generations of the RIL population suggests that FW is a trait with high heritability. This was in good agreement with previous studies (Chen et al. 1999; Hyman 2001; Paterson et al. 1988) in which they reported a heritability of 0.75 to 0.79. The moderate correlation of SSC among the RIL populations in different years indicates that SSC is a trait with low to moderate heritability. A moderate heritability for SSC was reported also by Hyman (2001). Similar to SSC, pH seemed to be a highly variable trait, but interestingly moderate correlations were observed among all generations. The moderate heritability suggests that increasing or decreasing fruit pH in tomato is possible through phenotypic selection and QTL mapping and marker assisted selection.

QTL analysis

Fruit weight QTLs

The total PVE by QTLs for FW ranged from 60.1% to 88.00%, which is relatively a high R^2 for a quantitative trait. The results of SIM (data not shown) and CIM analyses were highly comparable across generations. Several stable and major QTLs for fruit weight on chromosomes 1, 2, 3, 4 and 11 were identified. Most likely these major QTLs are same as those that were previously reported for this trait (Chen et al. 1999; Eshed and Zamir 1995; Goldman et al. 1995; Grandillo and Tanksley 1996; Paterson et al. 1991; Tanksley et al. 1996). At the distal end of the short of chromosome 1, one interval was common in all generations and deemed to be stable. To the best of my knowledge, this QTL is novel and it has not been reported before. However, two minor QTLs were also detected on chromosome 1 in F_7 and F_9 generations, which most likely are those that were previously reported (Chen et al. 1999; Eshed and Zamir 1995; Paterson et al. 1991; Tanksley et al. 1996). Another major QTL detected on chromosome 2 that was previously cloned (Frary et al. 2000) and another one on distal part of the long arm of chromosome 11 is known to be responsible for carpel/locule number in tomato fruit. In addition to major QTLs, a few other

QTLs (less stable) were only identified in one generation as follows. Two minor QTLs on chromosome 1 as described above in the vicinity of chromosomal area that Grandillo and Tanksley (1996) and Eshed and Zamir (1995) and Goldman et al. (1995) reported a QTL. Whether or not the latter QTLs identified in the current study are stable needs to be investigated in future by re-evaluating the population in future. On chromosome 4, in addition to the stable QTL in the middle part of the chromosome which is a novel QTL, a QTL on distal part of the long arm of the chromosome was identified in F_7. The latter QTL is most likely the QTL that was reported before (Eshed and Zamir 1995; Goldman et al. 1995; Tanksley et al. 1996).

QTLs for fruit weight of tomato in crosses between *L. esculentum* and other wild type tomatoes, using different mapping populations have been identified in almost all tomato chromosomes. For instance, on chromosomes 2, 3, 4, 5, 7 and 9 (Tanksley et al. 1996), 1, 2, 8, 11(Grandillo and Tanksley 1996), 1, 2, 3, 4, 6, 7, 8, 9, 11 and 12 (Chen et al. 1999), 1, 2, 3 and 11(Lippman and Tanksley 2001), 2 and 3 (Doganlar et al. 2002) 1, 2, 3, 5, 6, 7, and 11 (Van-der-Knap and Tanksley 2003). The results of this study are very similar to Lippman and Tanksley (2001) in which they found four QTLs on chromosomes 1, 2, 3 and 11. The commonality of certain identified QTLs through this research with others indicates that these QTLs are not species specific, although their expression may affect by environmental conditions and genetic background.

QTLs for fruit pH

In total 8 QTLs for fruit pH were identified on chromosomes 1, 2, 6, 8, 10 and 12 (chromosome 1 and 2 had more than one QTL). However, not all identified QTLs were consistent in the three years. The frequency of the QTLs that were consistent in two years was much more than any QTL consistent in three years. QTLs for fruit pH have been reported in almost all chromosomes of tomato. On chromosomes 3, 6, 7, 8 and 10 (Paterson et al. 1988), 1, 3, 4, 6, 7, 8 and 10 (Paterson et al. 1991), 1, 3, 5, 7 and 12 (Tanksley et al. 1996), 2, 3, 5, 9, 10 and 12 (Fulton et al. 1997), 1, 2, 4, 5, 9 and 12 (Chen et al. 1999), 11 and 12 (Saliba-Colombani et al. 2001), and 2, 3, 4, 5, 7, 8, 9 ,10 ,11 and 12 (Fulton et al. 2000). However, in all of these studies QTL analysis was carried out in one year and one generation. Therefore, due to the lack of replication in previous studies it is not clear if these QTLs would be stable. Co-localization of a number of these QTLs

with either SSC or other horticulturally important traits has been reported. For example, Fulton, et al. (1997) observed two QTLs for pH on chromosome 2 and 9 that were co-localized with a SSC- yield derived trait (Brix*yield) and Brix respectively. The co-localization of the QTLs is important from a breeding point of view, since it facilitates improvement of fruits with desirable pH and SSC for better flavor.

QTLs for total SSC

Through this research, it was shown that SSC shows a continuous distribution and is quantitatively controlled. Similar to pH, the identified QTLs varied from generation to generation. In fact, the complex inheritance of SSC and the variation in a limited range explain the reason of instability of the QTLs.

Through the current study several QTLs for increasing SSC were identified, majority of them were inherited from the wild type parent, indicating the importance of this wild accession (PSLP125) to increase SSC of the tomato fruit. Through MIM analysis, the total phenotypic variation explained by all major and minor QTLs was 17% (F_7) to 31% (F_8) in the population. The low PVE by QTLs indicates the low heritability of the trait. The low heritability for SSC has also been reported (Chen et al. 1999; Hyman 2001). Co-localization of QTLs for SSC and FW was observed only in F_9 generation on chromosomes 3 (complete overlap) and 7 (partial overlap). This was in agreement with the previous reports (Chen et al. 1999; Grandillo and Tanksley 1996; Paterson et al. 1988) that QTLs for SSC unfavorably affected the QTLs that control fruit size.

SSC of tomato fruit has been extensively studied and many QTLs have been identified in interspecific crossed of tomato. For instance, using different types of populations derived from crosses between *L. esculentum* and *L. pimpinellifolium* accessions, QTLs for SSC were identified. Using BC_2 and BC_3 populations, 12 QTLs on chromosomes 2, 3, 4, 5, 6, 7, 8, 11 and 12 (Tanksley et al. 1996), in a BC_1 population, three QTLs on chromosomes 3, 6 and 9 (Grandillo and Tanksley 1996), by utilizing a BC_1S_1, 13 QTLs on chromosomes 1, 2, 3, 7, 10 and 12 (Chen et al. 1999), and by using a RIL population three QTLs on chromosomes 2 and 9

(Saliba-Colombani et al. 2001) were identified. An inspection of these reports shows that QTLs for SSC are identified on all chromosomes of tomato, indicating the complex nature of this trait.

QTLs for lycopene content

This is the first study in which lycopene content of the tomatoes has been measured and compared through utilization of colorimeter (reflective spectrophotometer), HPLC and absorbance based spectrophotometer. The measurement of lycopene content by all methods showed a continuous distribution indicating that the trait is quantitatively controlled. Since the two parents that were used to develop the population were contrasting for lycopene, it was possible to identify QTLs with minor and major effects. By regressing HPLC data on several chromaticity values (LAB color values) as independent factors, it was possible to develop a model to estimate the amount of lycopene in previous generations (F_7-RIL, F_8-RIL). In the proposed statistical model here, three important chromaticity values such as L^*, a^* and b^* are included. Previously developed models by D'Souza (1992) and Hyman (2004) for estimation of lycopene content were considering a^* and b^* or a^* alone. Application of these models to the data of the RIL population failed to detect any QTLs. This made it imperative to develop a new model for this RIL population. The LOD score values for the QTL analysis obtained from fitted data were higher than the actual measurements. This is due to not having a perfect model (R^2=68%). Therefore, some individuals have been over- or under-estimated for their lycopene content. However, this problem did not hinder the power to detect the two stable QTLs for lycopene on chromosomes 7 and 12. Another aspect of the current study was detections of the same QTLs on chromosome-7 (in two out of three years) and chromosome-12 (in all three years) through application of field evaluation data of fruit color. Therefore, the presence of these two QTLs was confirmed by HPLC, spectrophotometer, colorimeter and field assays.

Co-localization of lycopene QTLs with ESTs

The constructed genetic map that used in QTL mapping included 132 EST markers (Chapter 2) that were mainly selected from among 213,947 ESTs available for tomato to date (Computational Biology and Functional Genomics Laboratory 2007). These ESTs were primarily selected based on their function in disease resistance or defense response. However, a

handful of them are involved in carotenoid biosynthesis pathway in tomato. The two QTLs found on chromosome 7 and 12 were not co-localized with any of the mapped ESTs. However one of the ESTs (cTOF12F19 - lycopene ε cyclase) was mapped in another distal part of chromosome 12 just opposite to the interval that the major QTL for lycopene was identified and where the QTL for *Delta*, orange mature fruit color due to inhibition of lycopene and increase of δ-carotene (Ronen et al. 1999). Phytoene synthase catalyzes the formation of phytoene which is the precursor of ζ – carotene and lycopene. One out of three copies of the EST of phytoene synthase (cLEC73K6) was mapped on the distal part of short arm of chromosome-2. This copy of phytoene synthase was not co-localized with any of the identified QTLs, suggesting there are other genes or regulatory elements involved in tomato fruit color as suggested by (Liu et al. 2003).

Comparing methods of lycopene measurement

This is the first report on using four different methods of lycopene measurement in one study. While visual assessment of fruit color in the field is the simplest and least accurate method of fruit color evaluation, HPLC is the most laborious but precise method. Through this research it was shown that major QTLs for lycopene content will be identified even with less accurate measurement methods (field evaluation), however, identification of minor QTLs needs more accurate methods such as HPLC or spectrophotometric assays. However, easier, quicker and less expensive assays are always preferable while one is dealing with a large number of samples that need to be analyzed. Spectrophotometric (proposed here) and colorimetric methods have these characteristics. Because a large amount of samples can be analyzed in short time, they reduce the cost of assay as well as part of the variation between the samples, which is not due to genetics. In this respect, unlike the HPLC analyses where the variation between the first and the last analyzed samples in some circumstances (i.e., large amount of samples) may reach days or weeks, the spectrophotometric measurement proposed here is simple, nearly as accurate as HPLC and fast. Since all readings take place within a couple of minutes, the sample variation due to the analyzing time is virtually zero. In addition, during sample loading, the extracts could be kept cold on dry ice to minimize carotenoid degradation. The time for measuring the absorption of a plate containing 96 samples is less that one minute. Considering the time needed to load the wells, the whole procedure to analyze two plates does not take more than 1 or 2

hours. This analysis time is highly favorable as compared with the time needed for HPLC analysis, which, depending on the flow rate, takes approximately 20-60 minutes per sample. Furthermore, while colorimetric measurement needs a statistical model to convert LAB color parameters to lycopene concentration, the spectrophotometer assay is free from this constraint.

Implications for tomato breeding

The results of this study clearly showed that the wild type relative of tomato*, L. pimpinellifolium*, is a rich source of fruit quality traits such as lycopene and soluble solids. QTLs that are common in multiple studies or have shown stability in different generations/years are useful chromosomal regions for marker-assisted selection (MAS) (Edwards and Johnson 1994; Mohan et al. 1997; Robert et al. 2001; Stromberg et al. 1994). In the case of SSC, none of the identified QTLs were consistently co-localized with fruit weight. These QTLs may be useful in MAS for breeding large-fruited tomatoes with enhance SSC. Identification of two novel QTLs for lycopene, which were not co-localized with QTLs for FW in this population, is highly promising to improve the lycopene content of fresh market tomatoes, because they do not have any pleiotrophic effect on FW. Graphical genotyping (Young and Tanksley 1989) and the ability to track the desirable segments of the genome via MAS will help speed up the process of introgression of QTLs of interest to elite breeding lines that have low levels of lycopene. One practical aspect of QTL analysis of fruit quality traits in the RIL population was to find co-localization of QTLs of one trait with another or co-localization of the QTLs with candidate genes. Because the number of ESTs for lycopene synthesis was very limited in this study, adding more ESTs for fruit quality traits in the future to the map will be useful in basic research toward cloning the genes underlying lycopene or other fruit quality related traits. Another aspect of studying co-localization of QTL is to distinguish pleiotrophic effects of a single gene from effects of tightly linked loci as described above related to FW and SSC or lycopene content.

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Tables

Table 4-1 Mean phenotypic values, standard errors and ranges for traits measured for parents, F_1 and the RIL population in three years and generations of a *L. esculentum* (NCEBR-1)× *L. pimpinellifolium* (PSLP125) cross

Trait	NCEBR-1	PSLP125	F_1	F_7		F_8		F_9	
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Fruit Weight (g)	164.70±10.7	2.19±0.11	14.03±1.15	14.14±0.59	2.94 - 47.05	14.19±0.60	3.48 - 45.16	12.97±0.60	4.32 - 42.64
pH	4.22±0.05	4.09±0.11	4.10±0.03	4.06±0.01	3.70 - 4.49	4.09±0.01	3.63 - 4.73	4.20±0.01	3.89 - 4.65
Total Soluble Solids (°Brix)	4.65± 0.23	7.23±0.36	6.80±0.29	4.96±0.06	3.60 - 7.00	6.12±0.05	4.30 - 8.10	6.52±0.05	4.00 - 9.40
Lycopene Content (µg/g)									
HPLC	18.26 ±2.17	207.8±7.86	107.97±4.16	-	-	-	-	79.97±3.61	0.87-195.83
Spectrophotometer	24.07*	144.19*	72.10*	-	-	-	-	73.61±2.37	7.21-157.88

* Measurement was not replicated.

Table 4-2 Simple linear correlations among fruit quality related traits measure in a of *L. esculentum* (NCEBR-1) × *L. pimpinellifolium* (PSLP125) RIL population in three generations/years

Trait [a]	LYC (F_9)	FW(F_7)	pH(F_7)	SSC(F_7)	FW(F_8)	pH(F_8)	SSC(F_8)	FW(F_9)	pH(F_9)
pH(F_7)	-	0.11^{ns}							
SSC(F_7)	-	-0.20*	-0.17^{ns}						
FW(F_8)	-	0.92**	0.12^{ns}	-0.23*					
pH(F_8)	-	0.22*	0.40**	-0.12^{ns}	0.26**				
SSC(F_8)	-	-0.28**	-0.20*	0.59**	-0.27**	-0.07^{ns}			
FW(F_9)	-0.38**	0.88**	0.11^{ns}	-0.24**	0.93**	0.28**	-0.27**		
pH(F_9)	0.22**	0.09^{ns}	0.47**	-0.16^{ns}	0.09^{ns}	0.59**	-0.15^{ns}	0.15^{ns}	
SSC(F_9)	0.08^{ns}	-0.17^{ns}	-0.19*	0.48**	-0.14^{ns}	-0.13ns	0.70**	-0.20*	-0.08^{ns}

[a] LYC, lycopene concentration; FW, fruit weight; SSC, total soluble solid content; pH, fruit pH.
* $P < 0.05$; ** $P < 0.01$, ns= none significant correlation

Table 4-3 QTLs detected for fruit traits based on composite interval mapping in a *L. esculentum* × *L. pimpinellifolium* F_7-RIL population.

LOD, log-likelihood –; PVE, percent phenotypic variation explained; Additive effect; difference between E or PM alleles from the mean. Positive effect inherited from *L. esculentum* parent, Positive effect inherited from *L. pimpinellifolium* parent. For simplicity maximum LOD, Additive effect and PVE in the QTL interval has mentioned.

Trait	QTL Name	Interval	Chr.	Position (cM)	[t] LOD	Additive Effect	PVE [a] (R^2)
Fruit Weight (F_7)	*FW1.1*	TG301- cLEC34L10b	1	10.1 - 22.3	3.4	1.8	0.05
	FW1.2	CT191	1	77.9	2.8	1.5	0.04
	FW2.1	cLEY1K9- TG151	2	72.1-92.0	9.2	3.0	0.15
	FW3.1	cLEI4N5	3	75.3	7.2	2.6	0.12
	FW4.1	cTOS21D12	4	36.4	2.1	1.3	0.03
	FW4.2	CT50	4	84.0	4.4	2.0	0.07
	FW11.1	C2_Atg44790	11	34.6	2.5	1.5	0.04
	FW11.2	TG30	11	99.7	7.3	2.5	0.11
Fruit Weight (F_8)	*FW1.1*	TG301- cLEC34L10b	1	12.1-24.5	4.1	2.1	0.06
	FW2.1	CG21- cLEY1K9	2	63.8-72.1	9.5	3.2	0.14
	FW3.1	cLEI4N5	3	73.38	5.7	2.5	0.09
	FW4.1	cTOS21D12 - cTOF10N11	4	36.4-38.5	2.5	1.5	0.03
	FW11.1	KFG-J1	11	31.8	2.7	1.6	0.04
	FW11.2	TG400 - cTOF28I23	11	92.4-105.9	3.7	1.9	0.09
	FW12.1	TG68a - CT79	12	4.3-10.1	3.9	1.9	0.05
Fruit Weight (F_9)	*FW1.1*	TG301	1	2.1	3.6	1.6	0.06
	FW1.2	cTOF3A14- cLEC66G13b	1	39.4-41.5	4.7	2.0	0.08
	FW2.1	CT103 – TG599b	2	43.9-74.9	8.7	2.8	0.17
	FW3.1	cLEI4N5	3	71.4	7.2	2.7	0.15
	FW4.1	cTOS21D12- cLEF42D20	4	36.4-40.4	3.1	1.5	0.05
	FW7.1	cLEN14F9- cTOF21F12	7	17.9-31.8	2.7	1.6	0.05
	FW11.1	KFG-J1- C2_Atg44790	11	29.8-34.5	3.0	1.6	0.05
	FW11.2	TG400-COSOH57	11	86.4-96.5	4.0	1.8	0.07
	FW11.3	TG30-cTOF28I23	11	101.7-105.9	5.0	1.9	0.08
Fruit pH (F_7)	*pH1.1.*	cLES9N20-TG59	1	67.5-70.9	2.5	0.04	0.07
	pH1.2.	CT137	1	115.7	3.0	0.04	0.07
	pH2.1.	cLEI6D5	2	0.1	3.0	-0.04	0.07
	pH2.2.	cLEI6D5-TG645	2	56.5-68.5	3.2	0.05	0.08
	pH10.1	CT234	10	14.0	2.2	0.04	0.05
	pH10.2	TG403	10	77.5	2.1	-0.04	0.05
Fruit pH (F_8)	*pH1.1*	TG559a- TOE7J7	1	86.7-95.0	2.8	0.05	0.06
	pH2.1	TG559b	2	72.9	3.0	0.05	0.06
	pH6.1	TG274	6	13.1	2.4	0.04	0.05
	pH8.1	TG302CAPS- cLEN10H3	8	38.5-45.1	3.4	-0.05	0.07
Fruit pH (F_9)	*pH1.1*	cTOE7J7- CT163	1	93.0-99.2	3.2	0.04	0.08
	pH8.1	TG349	8	31.6	2.3	-0.03	0.06
	pH8.2	cLET16E21-cLEN10H3	8	44.8-49.1	2.8	-0.04	0.08
	pH10.1	cLHT11J12	10	16.6	2.6	0.04	0.07
	pH12.1	cTOF12F19-CT100	12	13.4-21.8	3.0	-0.04	0.08
Fruit SSC (F_7)	*SSC6.1*	cLEG32E10-TG590	6	10.6-17.6	2.6	-0.2	0.06
	SSC8.1	cLG61B21b-CT92	8	23.8-29.9	3.2	0.2	0.07
	SSC8.2	TG201- cTOE23J12	8	62.9-70.4	2.1	-0.2	0.05

Trait	QTL Name	Interval	Chr.	Position (cM)	[t] LOD	Additive Effect	PVE [a](R^2)
Fruit SSC (F_8)	*SSC3.1*	CT22	3	31.7	2.9	-0.2	0.06
	SSC9.1	CT143	9	19.0	2.2	-0.2	0.05
	SSC10.1	CT92	10	14.6	4.1	-0.2	0.08
Fruit SSC (F_9)	*SSC1.1*	cLEC34L10b-cTOF3A14	1	24.3-31.4	2.9	0.2	0.07
	SSC1.2	CT137	1	116.4	2.1	-0.2	0.05
	SSC3.1	cLEI4N5-CT85	3	67.4-79.3	3.1	-0.3	0.10
	SSC6.1	cLEG49O24	6	22.3	2.2	-0.2	0.06
	SSC10.1	CT234	10	14.0	4.8	-0.3	0.12
Lycopene (HPLC)	*Lyc2.1*	CT103-TG454	2	45.9-51.8	2.3	-12.3	0.06
	Lyc4.1	cLEN7N12a-CT50	4	75.19-78.0	2.2	-12.9	0.07
	Lyc7.1	cTOS19O5- cLEN14F9	7	8.01-17.9	5.0	-19.1	0.15
	Lyc11.1	C2_At60830	11	14.6	2.6	-13.0	0.07
	Lyc12.1	TG473	12	77.3	3.4	-14.7	0.08
Lycopene (Spectrophoto-meter)	*Lyc2.1*	CT103	2	43.9	2.5	-8.0	0.06
	Lyc7.1	cTOS19O5-cLEN14F9	7	6.01-19.9	3.8	-10.8	0.12
	Lyc11.1	C2_At60830	11	14.6	2.2	-7.5	0.06
	Lyc12.1	CT156-cLEC80G6	12	71.76-72.8	2.3	-7.7	0.06
F_7 Lycopene Statistical model	*Lyc7.1*	cTOS19O5	7	4.0	6.9	-8.3	0.15
	Lyc12.1	cLEZ15E8	12	77.0	13.3	-11.0	0.27
F_8 Lycopene Statistical model	*Lyc7.1*	cTOS19O5	7	0.01	6.4	-6.0	0.10
	Lyc12.1	cLEC80G6- TG473	12	76.8-77.3	12.7	-8.8	0.23
F_9 Lycopene Statistical model	*Lyc1.1*	cLEI6L6-cTOF3A14	1	31.2-39.4	4.5	-8.0	0.10
	Lyc7.1	cTOS19O5	7	2.0	3.7	-7.4	0.09
	Lyc12.1	CT156-TG473	12	67.8-77.3	7.3	--10.3	0.18
Fruit color visual evaluation F_7-RIL	*FC7.1*	cTOS19O5	7	2.0	5.1	-0.3	0.13
	FC7.2	cTOF18O1-CT226	7	56.0-63.7	2.4-2.3	-0.2	.07
	FC12.1	CT156-TG473	12	69.8-77.3	2.3-5.3	-0.	0.11
Fruit color visual evaluation F_8-RIL	*FC1.1*	cTOF20P4-TG273	1	53.7-61.9	3.2	-0.2	0.06
	FC7.1	TG113-cLEN14F9	7	10.3-15.8	3.5	-0.2	0.07
	FC11.1	TG30-cTOF28I23	11	101.7-105.9	4.7	0.22	0.10
	FC12.1	CT80-cLEZ15E8	12	76.9-77.0	4.7	-0.2	0.09
Fruit color visual evaluation F_9-RIL	*FC1.1*	cLEC66G13b - cLEC6O2	1	53.5-55.8	4.3	-0.2	0.12
	FC12.1	TG473	12	76.9	3.5	-0.2	0.07

[a] R^2 is the proportion of the variance explained by the QTL conditioned on the background markers and any explanatory variables, expressed as PVE.

[t] LOD thresholds were determined by 1000 permutation of markers for each trait. FW04 = 3.58, FW05RIRII = 3.5 and FW06RII = 3.58, pH04 = 3.97, pH05RIRII = 3.24 and pH06RIRII = 3.58, SSC04 = 3.39, SSC05RIRII = 4.0 and SSC06RIRII = 3.78; Lycopene (HPLC) Rep II = 3.8, Lycopene (Spectrophotometer) = 3.54; Lycopene F_7 (Modeling data) = 3.34, LOD F_8 (Modeling data) = 3.28, F_9 (Modeling data) = 3.42; In-field fruit color F_7 = 3.7, In-field fruit color F_8 = 3.6, In-field fruit color F_9 = 3.36

Table 4-4 Total phenotypic, genetic variance and variation explained by QTLs detected for fruit quality related traits, revealed by multiple interval mapping (MIM) in a *L. esculentum*× *L. pimpinellifolium* RIL population.

Generation	Phenotypic variance	Genetic variance	Residual variance	R^2 Genetic	R^2 Residual
Fruit weight					
F_7	54.24	33.45	20.79	0.62	0.39
F_8	60.26	32.32	28.93	0.53	0.47
F_9	42.75	22.93	19.81	0.54	0.46
pH					
F_7	0.03	0.01	0.02	0.18	0.81
F_8	0.03	0.01	0.02	0.26	0.74
F_9	0.02	0.01	0.01	0.27	0.73
Total Soluble solids					
F_7	0.49	0.80	0.40	0.17	0.83
F_8	0.49	0.12	0.38	0.24	0.76
F_9	0.70	0.21	0.49	0.31	0.69
Lycopene F_9 (HPLC)	2332.00	593.40	1739.00	0.25	0.75
Lycopene F_9 (spectrophotometer)	919.70	217.60	702.10	0.24	0.76
Lycopene F_7 Modeling	437.80	148.70	289.10	0.34	0.66
Lycopene F_8 Modeling	331.70	107.80	223.9	0.33	0.67
Lycopene F_9 Modeling	566.30	154.40	411.90	0.27	0.73
Fruit color visual evaluation F_7	0.50	0.12	0.38	0.24	0.76
Fruit color visual evaluation F_8	0.47	0.05	0.41	0.11	0.88

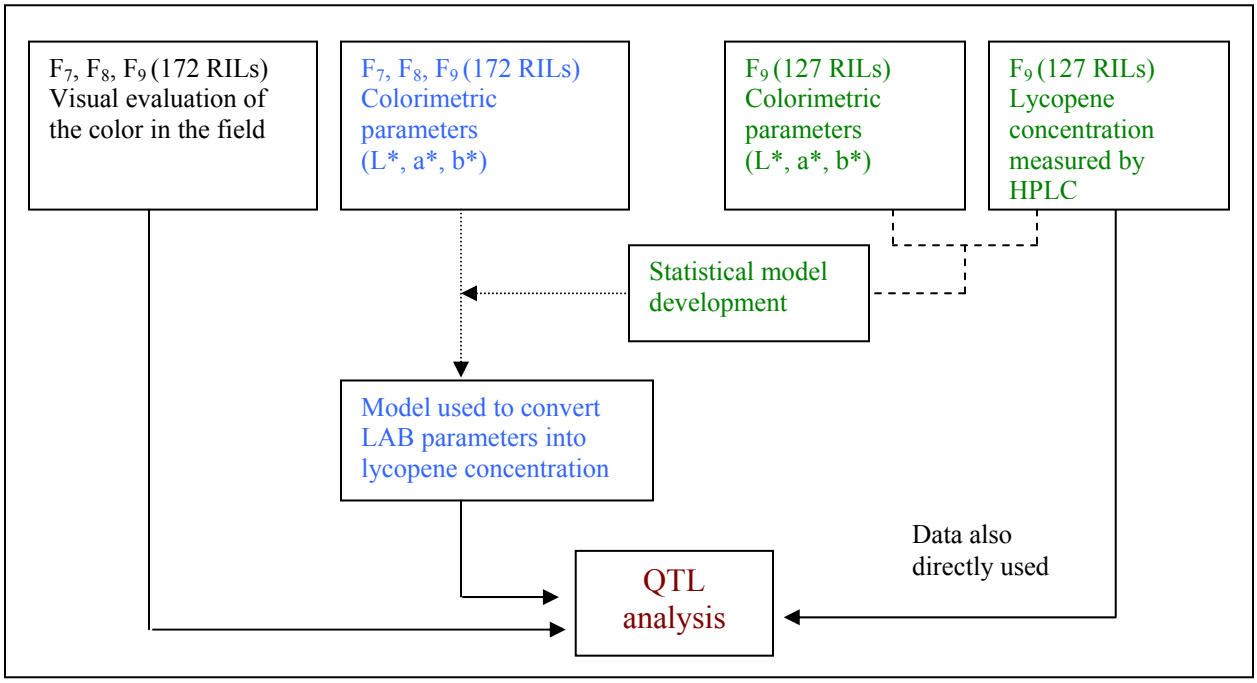
Table 4-5 QTLs detected and their interaction for fruit traits based on multiple interval mapping (MIM) in a *L. esculentum* × *L. pimpinellifolium* RIL population.

Trait/Generation	QTL(pair)	Type	Chr.	Marker	Position	LOD	Effect	Effect (%)
Fruit Weigh								
F_7	1	A	1	TG301	10.1	1.10	1.40	4.1
	2	A	1	CT191	78.0	1.60	1.70	4.5
	3	A	2	cLEC72P14	56.6	0.40	0.90	2.9
	4	A	2	cLEY1K9	72.1	0.00	0.50	1.9
	5	A	2	TG559b	78.9	0.30	1.60	6.2
	6	A	2	TG151	92.0	0.40	1.20	3.4
	7	A	3	CT85	75.3	4.00	2.80	11.9
	8	A	4	cTOS21D12	36.5	1.80	1.80	4.9
	9	A	11	C2_Atg44790	34.5	0.80	1.20	3.9
	10	A	11	TG30	99.8	1.70	1.80	7.3
	1×5	AA	1 , 2	-	-	0.70	1.30	1.9
	2×8	AA	1 , 8	-	-	0.90	1.30	4.0
	5×8	AA	2 , 4	-	-	1.00	1.40	1.9
	1×9	AA	1 , 11	-	-	0.60	1.20	2.8
F_8	1	A	1	TG301	12.1	0.30	1.10	3.8
	2	A	1	Clec34L10b	24.3	0.30	1.00	2.5
	3	A	2	CG21	63.8	0.10	0.70	1.9
	4	A	2	Cley1k9	72.1	2.10	3.00	10.5
	5	A	3	cLEI4N5	73.4	1.40	1.90	6.1
	6	A	4	cTOS21D12	36.5	0.90	1.50	4.1
	7	A	11	KFG-J1	31.8	0.20	0.80	2.2
	8	A	11	TG30	99.8	1.70	2.20	7.5
	9	A	12	TG68a	4.3	0.70	2.00	6.2
	10	A	12	CT79	10.2	0.10	0.90	2.4
	1×4	AA	1, 2	-	-	1.20	1.90	1.2
	4×8	AA	2, 11	-	-	0.80	1.40	1.9
	1×9	AA	1, 12	-	-	0.70	1.50	2.3
F_9	1	A	1	TG301	2.0	0.10	0.50	1.6
	2	A	1	cROF3A14	39.4	1.20	1.50	5.6
	3	A	2	CT103	44.0	0.50	1.00	4
	4	A	2	cLEY1K9	72.1	2.20	2.20	9.4
	5	A	3	cLEI4N5	71.4	1.70	1.80	7.8
	6	A	4	cTOS21D12	36.5	0.90	1.30	3.4
	7	A	7	cLEN14F9	17.9	0.80	1.30	4.7
	8	A	11	KFG-J1	29.8	0.20	0.80	3.4
	9	A	11	COSOH57	96.6	0.10	0.70	2.9
	10	A	11	TG30	101.7	0.30	1.60	7.5
	7×10	AA	7, 11	-	-	0.90	1.60	3.4
pH								
F_7	1	A	1	cLES9N20	67.5	0.90	0.04	2.9
	2	A	1	CT137	116.5	0.90	0.04	4.5
	3	A	2	cLEI6D5	0.1	0.70	-0.03	3.1
	4	A	2	cLET10E15	56.6	0.20	0.02	1.5
	5	A	2	TG645	68.5	0.20	0.02	1.4
	6	A	10	CT234	14.1	0.10	0.01	0.6
	7	A	10	TG403	77.5	0.70	-0.04	4.2
F_8	1	A	1	TG559a	86.7	1.60	0.06	7.3
	2	A	2	TG559b	72.9	1.10	0.04	3.6
	3	A	6	TG274	13.2	0.60	0.04	3.1
	4	A	8	TG302CAPS	38.1	0.05	-0.01	0.6
	5	A	8	cLEN10H3	45.1	1.00	-0.07	10.5
	1×2	AA		-	-	0.80	0.04	1.3

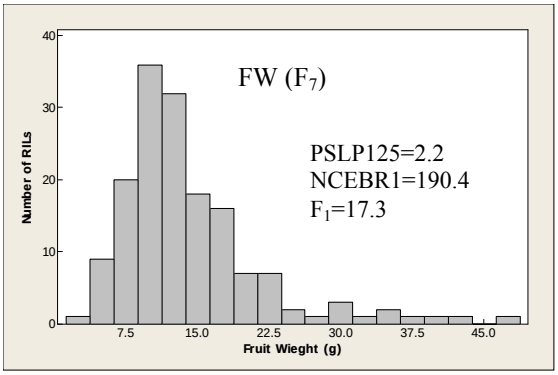
Trait/Generation	QTL(pair)	Type	Chr.	Marker	Position	LOD	Effect	Effect (%)
F_9	1	A	1	cTOE7J7	93.1	0.40	0.03	4.6
	2	A	1	CT163	99.2	0.00	0.00	-0.2
	3	A	2	TG559b	72.9	0.40	0.02	2.8
	4	A	4	cLEG32E10	24.6	0.30	0.02	1.8
	5	A	8	TG349	31.7	0.10	-0.01	2
	6	A	8	cLET16E21	44.8	0.50	-0.03	6.2
	7	A	10	cLHT11J12	16.6	0.30	0.03	2
	8	A	10	cLPP2M12	24.3	0.00	0.01	0.3
	9	A	12	cTOF12F19	13.4	0.10	-0.02	2.6
	10	A	12	CT100	21.8	0.40	-0.03	4.6
Total SSC								
F_7	1	A	6	cLEG32E10	10.6	0.00	-0.08	2
	2	A	6	TG590	17.6	0.00	-0.03	0.8
	3	A	6	cLEN10H12	31.2	0.10	-0.08	1.8
	4	A	8	CT92	29.9	1.20	0.21	5.6
	5	A	8	TG201	63.0	0.00	0.02	-0.4
	6	A	8	cTOE23J12	70.5	0.50	-0.23	6.7
F_8	1	A	3	CT22	31.7	0.80	-0.15	3.1
	2	A	6	TG477	61.7	0.20	-0.14	0.9
	3	A	8	cLET16E21	44.8	1.30	0.19	5.4
	4	A	9	CT143	19.0	0.80	-0.17	5.8
	5	A	10	cLHT11J12	14.7	1.20	-0.18	5.5
	4×5	AA	9 , 10	-	-	0.60	0.14	3
F_9	1	A	1	TG477	24.3	0.40	0.22	2.8
	2	A	1	cLEC34L10b	31.5	0.00	0.01	0.1
	3	A	1	cTOF3A14	116.5	0.70	-0.18	3.8
	4	A	3	cLEI4N5	67.5	0.90	-0.27	6.8
	5	A	3	CT85	79.3	0.00	0.01	-0.2
	6	A	6	cLEG32E10	12.6	0.60	-0.23	3.3
	7	A	6	cLEG49O24	22.4	0.00	0.01	-0.1
	8	A	10	CT234	14.1	2.50	-0.34	9.1
	1×3	AA	1 , 1	-	-	0.70	-0.19	1.3
	1×5	AA	1 , 3	-	-	0.50	0.17	2.4
	6×7	AA	6 , 6	-	-	0.70	0.25	1.4
F_9 Lycopene (HPLC)	1	A	1	cTOS16I16	20.2	0.00	-1.23	0.2
	2	A	2	CT103	45.9	0.10	-4.90	1.5
	3	A	4	cLEN7N12a	75.2	0.50	-9.55	3.1
	4	A	7	TG113	12.3	0.10	-9.50	4.0
	5	A	7	cLEN14F9	17.9	0.05	-3.77	1.2
	6	A	7	CT226	57.7	0.30	-7.59	1.9
	8	A	11	SSR80	14.6	0.50	-8.78	1.5
	9	A	12	CT79	77.4	1.20	-14.91	6.7
	10	8×9	AA	-	-	0.60	12.19	5.4
F_9 Lycopene (Spectro-photometer)	1	A	2	CT103	44.0	0.40	-5.50	3.4
	2	A	7	cTOS19O5	6.0	0.40	-7.95	6.2
	3	A	7	cLEN14F9	20.0	0.20	-5.51	3.3
	4	A	11	C2_At60830	14.6	1.00	-8.55	4.6
	5	A	12	CT156	71.8	1.10	-8.70	6.1
Lycopene/F_7 (Modeling data)	1	A	7	cTOS19O5	4.0	2.50	-7.94	12.3
	2	A	12	cLEZ15E8	77.1	4.70	-10.60	21.6

Trait/Generation	QTL(pair)	Type	Chr.	Marker	Position	LOD	Effect	Effect (%)
Lycopene/F_8 (Modeling data)	1	A	7	cTOS19O5	0.0	2.40	6.32	11.2
	2	A	12	cLEC80G6	76.8	4.50	-9.00	21.3
Lycopene/F_9 (Modeling data)	1	A	7	cTOS19O5	2.0	1.80	-8.76	11.2
	2	A	12	TG473	77.4	2.60	-10.38	16.0
Fruit color/F_7 (Field data)	1	A	7	cTOS19O5	2.0	3.20	-0.32	17.2
	2	A	12	TG473	77.4	1.50	-0.20	6.9
Fruit color/F_8 (Field data)	1	A	7	TG113	10.4	0.80	-0.16	4.5
	2	A	12	cLEZ15E8	77.0	1.30	-0.19	6.7

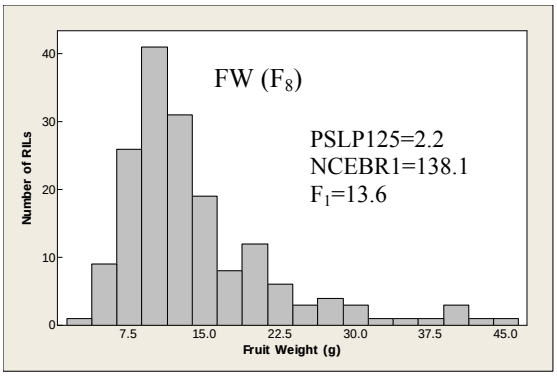
Chart 4.1 Diagram of data used in QTL mapping



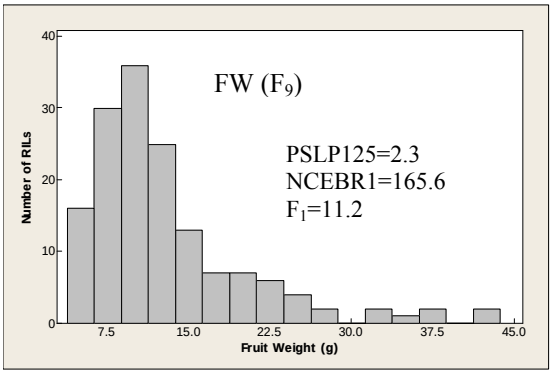
Figures



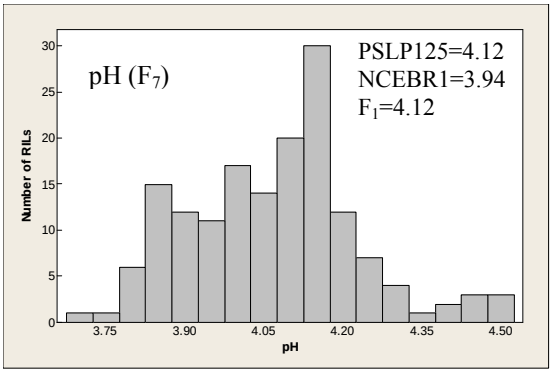
(a)



(b)



(c)



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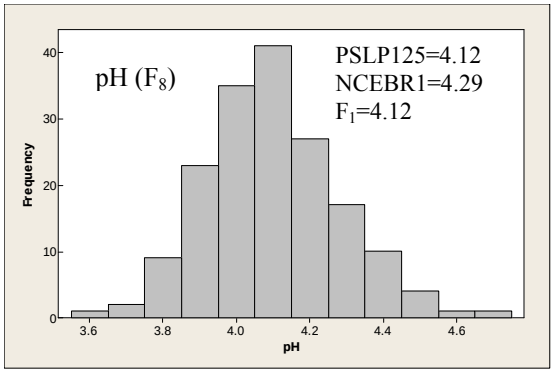

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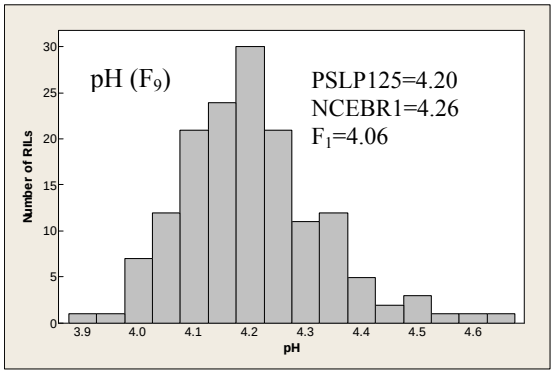
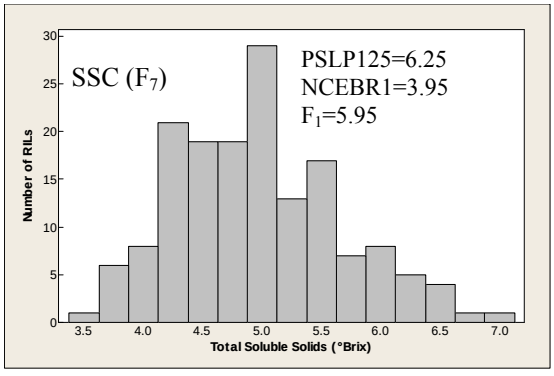


(f)



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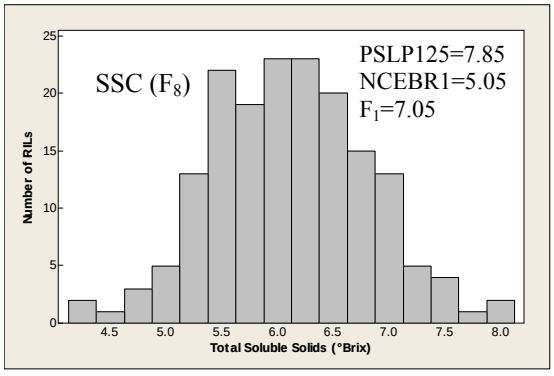

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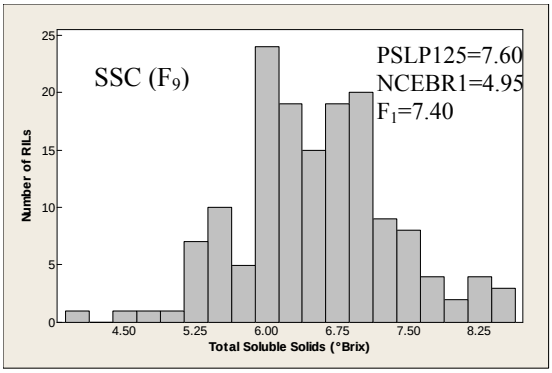

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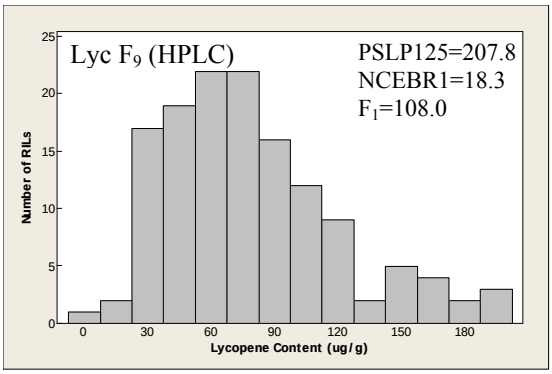

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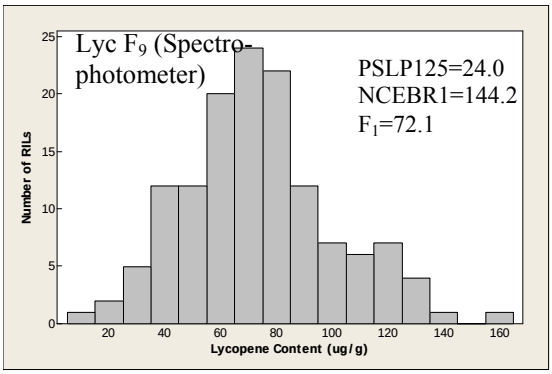


(k)

Figure 4-1 Frequency distributions for tomato fruit traits in a RIL population of *L. esculentum* (NCEBR-1, maternal parent) and *L. pimpinellifolium* (PSLP125, staminate parent) in three generations and years.

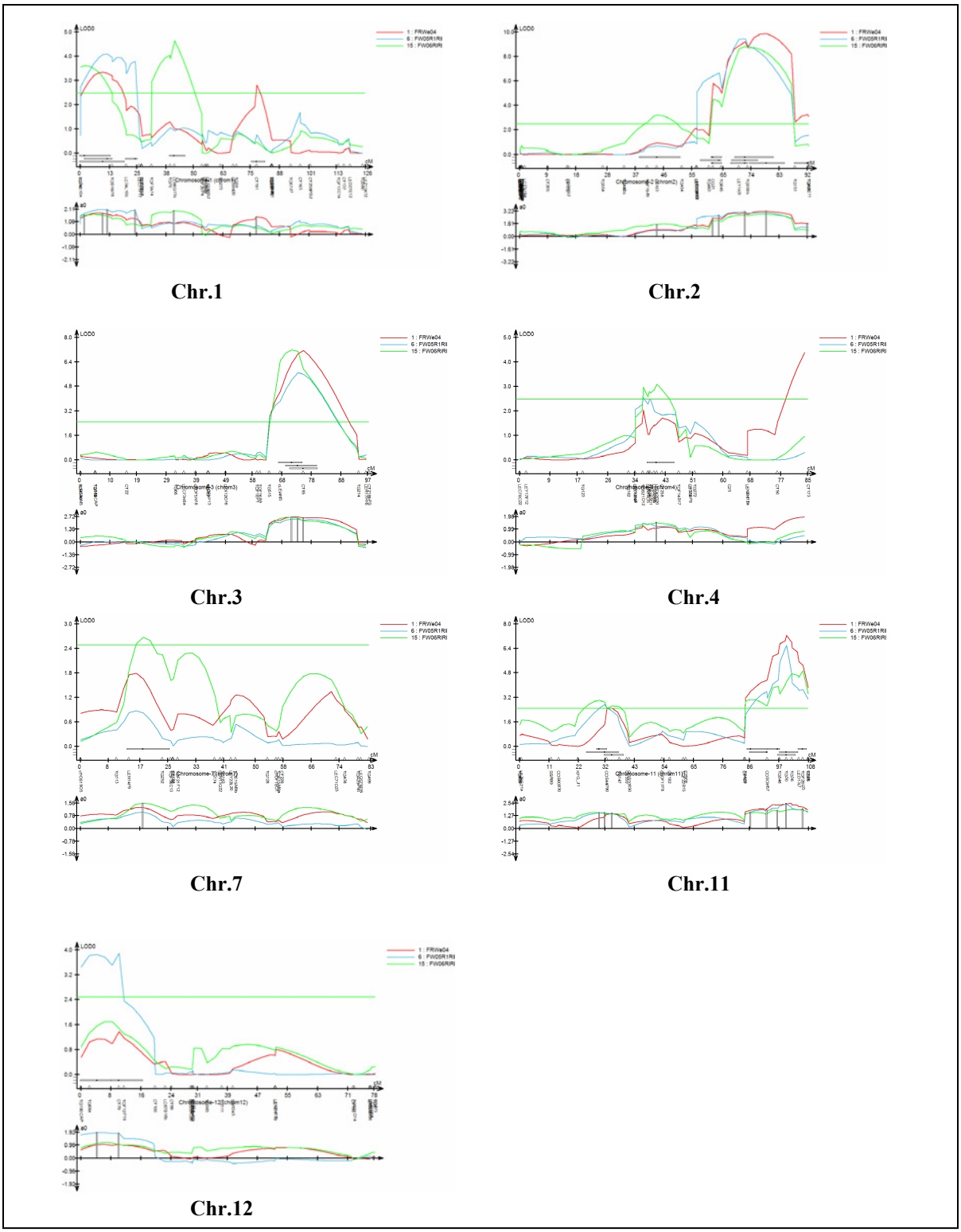
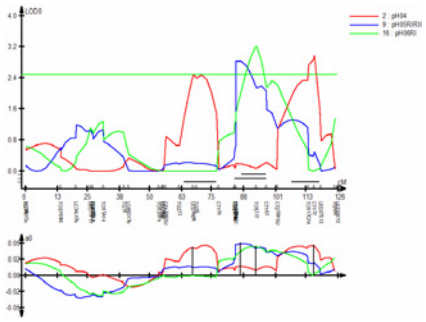
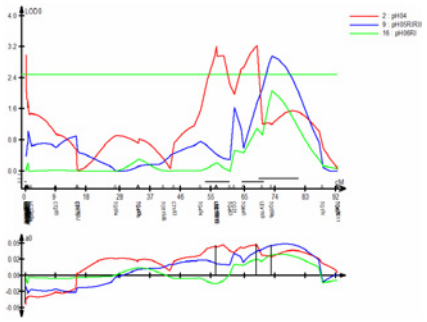


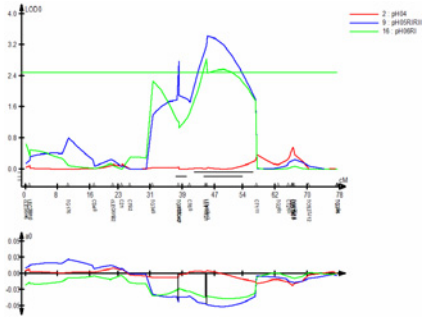
Figure 4-2 QTLs detected for fruit weight in three generations by CIM on tomato chromosomes.$F_{7,}$ F_8 and F_9 generations are depicted in red, blue, and green lines, respectively.



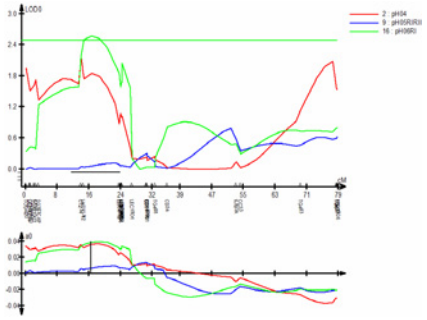
Chr.1



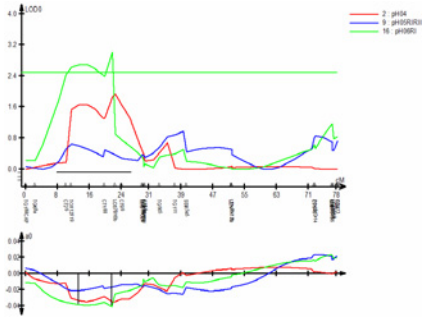
Chr.2



Chr.8

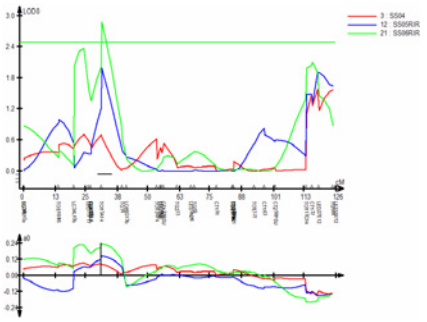


Chr.10

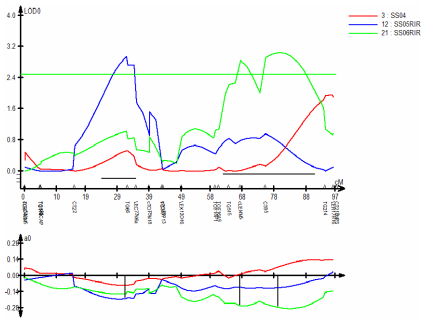


Chr.12

Figure 4-3 QTLs detected for pH in three generations by CIM on tomato chromosomes. F_7, F_8 and F_9 are depicted with red, blue and green colors respectively.



Chr.1



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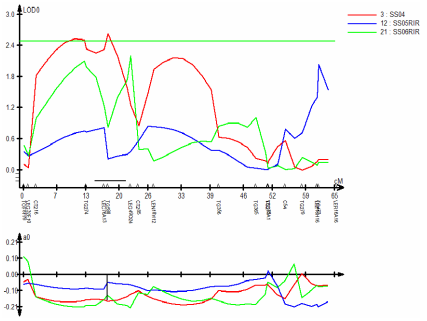
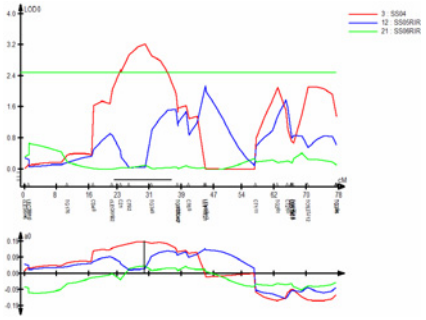
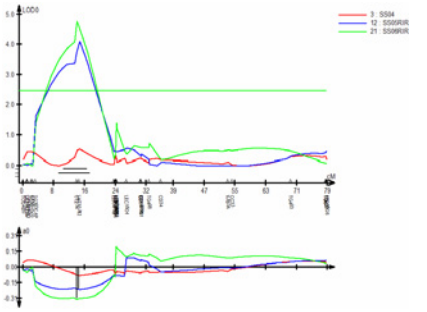


Chr.6

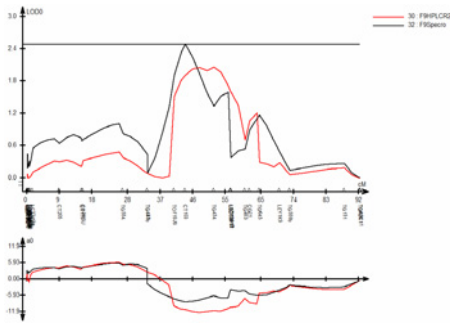


Chr.8

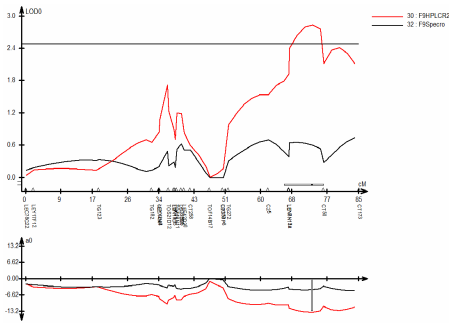


Chr.10

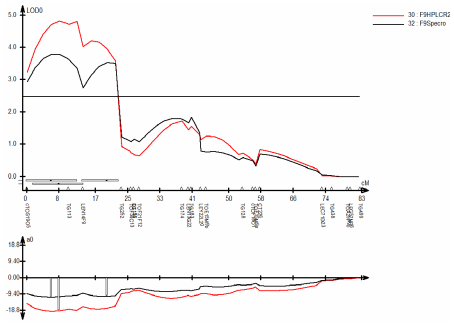
Figure 4-4 QTLs detected for SSC in three generations by CIM on tomato chromosomes. F_7, F_8 and F_9 are depicted with red, blue and green colors respectively.



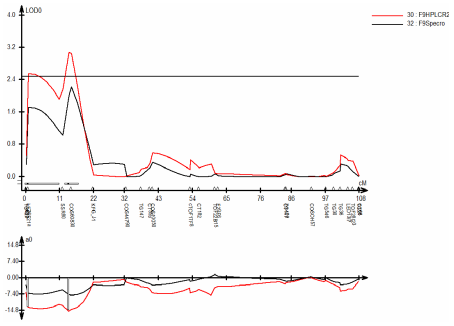
Chr.2



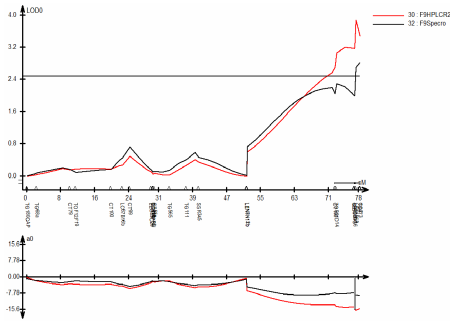
Chr.4



Chr.7



Chr.11



Chr.12

Figure 4-5 QTLs detected for Lycopene in F_9 generation by CIM analysis using HPC and Spectrophotometer data. HPLC and spectrophotometer data are depicted in red and black colors, respectively.

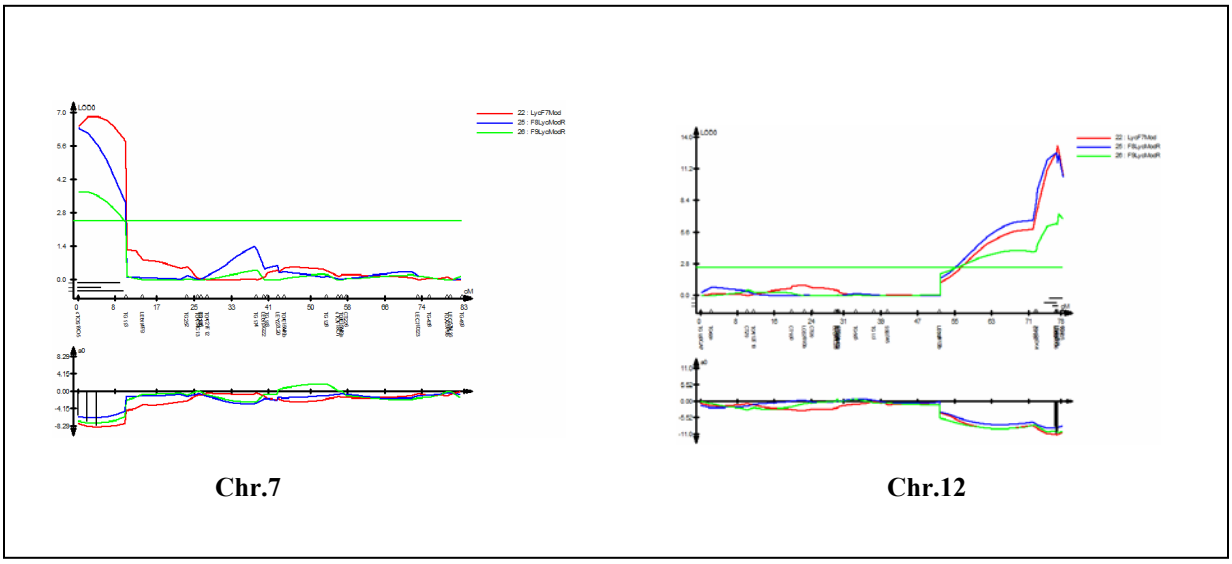


Figure 4-6 QTLs detected for Lycopene content in $F_{7,}$ F_8 and F_9 generations by applying the statistical model to LAB color parameters. QTLs depicted for F_7 as red, F_8 as blue and F_9 as green

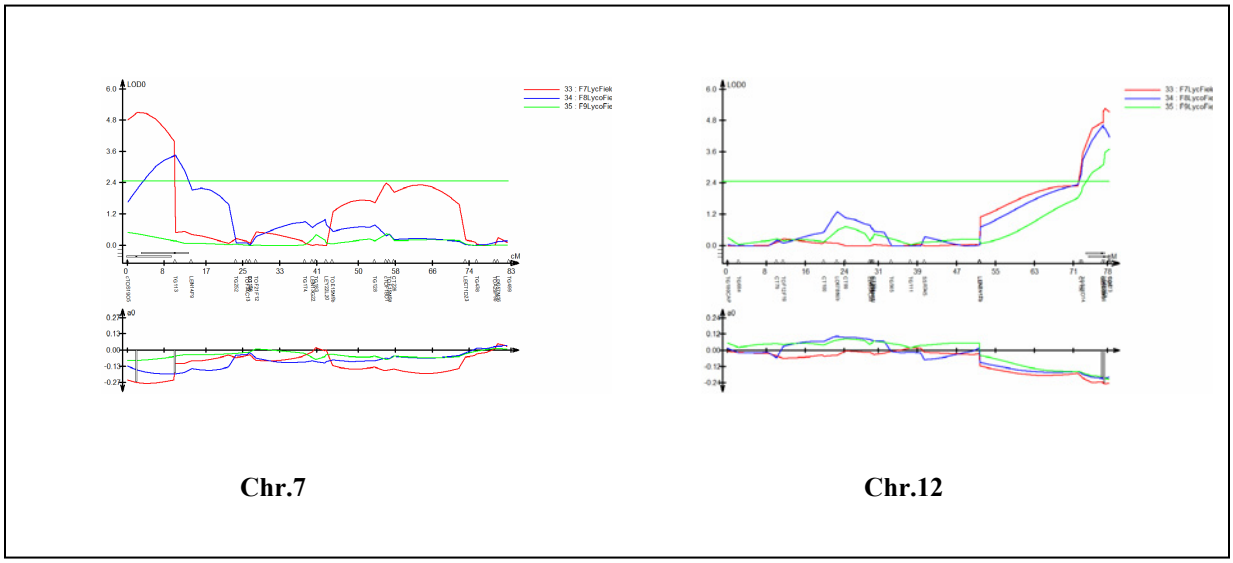


Figure 4-7 QTLs detected for Lycopene content in $F_{7,}$ F_8 and F_9 generations by visual evaluations of fruit color in the field. QTLs depicted for F_7 as red, F_8 as blue and F_9 as green

CHAPTER 5. Conclusions and future prospects

Genetic Mapping

Despite a relatively low level of DNA polymorphism between the two closely related species of tomato, a medium density molecular linkage map was constructed based on a cross between NCEBR-1 breeding line (*L. esculentum*) and PSLP125 (*L. pimpinellifolium*). This map will be useful in studies for QTLs identification for different traits as well as for MAS and breeding. Because the RIL population is a permanent population, the constructed map can be shared with other laboratories and even more markers can be added to the map in order to make it more specific for candidate genes that were not the focus of this research. The current genetic map is also useful for chromosome landing and ultimately cloning major genes or QTLs controlling important traits in tomato.

One of the distinct features of the constructed map is that more than 130 EST markers, which were not previously mapped onto tomato genetics maps and which putatively involve in disease resistance, defense response or lycopene synthesis, have been located on tomato chromosomes. Co-localization of these ESTs or candidate genes with QTLs will be useful in marker assisted selection programs and for introgression of stable QTLs to elite tomato lines. Mapping of more than 35% of ESTs to chromosomes 1, 2, 4 and 10 suggested that these four chromosomes are rich in disease resistance/defense response genes. On the other hand, clustering of these ESTs on the short arms of chromosome 1, 2 and the long arm of chromosome 10, further suggests the importance of these regions. Despite the low level of polymorphism between the two parents, a relatively high rate of polymorphism for ESTs was observed. This provides a unique opportunity to add more EST markers to the map in future. Additional ESTs can be added to the map based on their function or involvement in different traits such as high lycopene and resistance to extreme salinity or drought stress for which it is speculated this population is appropriate for. Similarities and dissimilarities observed between this map (E×PM-1) and F_2 (E×PM-2) map and other published maps was useful to find the hot spots of the chromosomes which are more prone to recombination.

Early blight resistance

Statistical analysis of collected data from three years of the experiment indicated that resistance to early blight (EB) resistance in the RIL population was a polygenic trait. Two QTLs emerged as stable, evidenced by their detection in three generations of the RIL populations and by using different QTL mapping approaches. The identification of QTLs for EB resistance in the closely-related, red-fruited wild species *L. pimpinellifolium* is highly valuable because it reduces the pre-breeding efforts to transfer both traits to elite lines at the same time. Furthermore, the inheritance of resistance QTLs from both parents may help identification of transgressive segregants and augmentation of resistance by MAS. Co-localization of QTL intervals with ESTs suggested the potential involvement of these candidates with EB resistance. Furthermore, many ESTs mapped to the locations of known resistance or defense-response genes in the tomato genome, suggesting that they may be involved in disease resistance. These associations may provide scope for further investigations on molecular mechanisms of resistance to EB. Fine mapping of the stable QTLs through production of NILs and sub-NILs may facilitate clean transfer of QTLs via marker assisted selection and map-based cloning of underlying resistance genes. Transformation of tomato plants with the candidate genes in future can confirm the effectiveness of candidate gene strategy combined with QTL analysis to breeding for EB resistance. The genetic map and the identified QTLs are also useful for marker-assisted exploitation of genetic variability within and between *L. pimpinellifolium* and *L. esculentum.* Through this research it was speculated that the candidate-gene approach could be an effective approach to identifying and mapping new *R* as well as other genes in wild type tomato accession PSLP125. For that reason, the identified QTLs on chromosomes 5 and 6 need to be fine mapped in future and eventually cloned by taking a map-based cloning approach. Adding more candidate resistance genes in the regions that other minor QTLs were located can help to identify potential QTLs for EB that were not identified through this research.

Fruit quality assays and QTL mapping

This is the first study in which fruit quality traits such as FW, pH and SSC has been studied in a RIL population of a cross between *L. esculentum* and *L. pimpinellifolium* in three years. By utilizing CIM analysis stable QTLs were identified at least in two generations in the case of fruit pH and SSC and in all generations in the case of FW and lycopene content. Because, the QTLs

for pH and SSC were not all consistent across the years or generations, optimization experiments are needed to carry out in future to find the most influential variables in the measurements. Reducing the number of variables will help to design the experiments and improve the analyses methods for future experiments. For example, collection of tomato fruits in a time course manner and measuring pH , SSC and lycopene from the same line in a replicated experiment in the same season may partially reveal the effects of maturity progression on the these traits.

The QTLs identified for lycopene in this study did not co-localize with any other measured trait and this is the first report on finding a major QTL for lycopene in distal parts of chromosomes 7 and 12. The two QTLs found on chromosome 7 and 12 were not co-localized with any of ESTs involved in carotenoids pathway too. This suggested that "There is more to tomato fruit color than candidate carotenoid genes" (Liu et al. 2003) and more candidate genes are needed to map in future, especially in the regions that QTLs on chromosome 7 and 12 were mapping to. By utilizing the graphical genotyping method it became possible to identify the individuals that carry the QTLs, have high lycopene content based on both visual evaluations in the field in three years and HPLC assay with maximum genome proportion of *L. esculentum* parent. These individuals will be used in a MAS breeding program to transfer the QTLs to the cultivated tomato.

Through this research three methods of lycopene content measurements were also compared. HPLC and spectrophotometer assays appeared to be more accurate than colorimetric measurement. A simple and as accurate as HPLC assay to measure lycopene and β-carotene from purée extract in a short time was suggested here. However, application of this method does not eliminate the need to extract the lycopene via one of the conventional methods of carotenoid extraction. This experiment needs to be repeated in future at least one more time to verify the applicability of this method.

Based on an HPLC assay a statistical model was also developed to estimate the amount of lycopene in previous generations for which only colorimetric measurements were available. This model could explain 68% of the variation in population for lycopene content. This R^2 is very close to the R^2 of the model (R^2 = 74%) proposed by (D'Souza et al. 1992) but less than the R^2 of Hyman's model (2004). The proposed model is advantageous over (D'Souza et al. 1992) or

(Hyman et al. 2004) models in a sense that all *L**, *a** and *b** parameters are involved in the model. Through repetition of the experiment using the fruits of the entire individuals (n=172) instead of a sub sample of the population and collection of the fruits on the same day it may be possible to improve the statistical model and it is likely that the model can be useful for other populations or laboratories.

References

D'Souza MC, Singha S, Ingle M (1992) Lycopene concentration of tomato fruit can be estimated from chromaticity values. HortScience 27:465-466

Hyman JR, Gaus J, Foolad M (2004) A rapid and accurate method for estimating tomato lycopene content by measuring chromaticity values of fruit puree. Journal of American Society for Horticutural Science 129:717-723

Liu Y-S, Gur A, Ronen G, et al. (2003) There is more to tomato fruit colour than candidate carotenoid genes. Plant Biotechnology Journal 1:195-207

Appendix A. Supplemental materials of chapter 2

Table A-1 The EST markers mapped only in the F_2 population, their putative function and chromosomal location (from A. Sharma, MR Foolad et al., unpublished data.)

EST Clone Name	Putative function	Chromosome No.
cTOA9E13	Squalene synthase, *C. annuum*	1
cLEC14N19	Similar to Pathogenesis-related genes transcript activator PTI5. *L. esculentum*	2
cLEW11E20	Resistance complex protein I2C-3, *L. Esculentum*	2
cTOE2F15	Catalase isozyme 1, *L. Esculentum*	3
cTOF29J22	4-coumarate--coA ligase 1, *S. tuberosum*	3
cLEX10F20	Ethylene response factor 1, *L. Esculentum*	3
cLEW24M21	TMV disease resistance protein-like protein, *Cicer arietinum*	4
cLEW22D11b	4-coumarate:coenzyme A ligase, *N. tabacum*	4
cTOE7J7b	Endo-1,4-beta-glucanase, *L. Esculentum*	5
cTOF29B13	Metallothionein-like protein type 2 a, *L. esculentum*	5
cTOF33C3	Serine/threonine protein kinase Pto, *L. esculentum*	5
cTOF23J19	Heat shock protein 90, *L. esculentum*	5
cLED11A2	Mitogen-activated protein (MAP) kinase 3, *C. annuum*	6
cLEW22D11a	4-coumarate:coenzyme A ligase, *N. Tabacum*	6
cLEY21L21	Disease resistance gene homolog Mi-copy1, *L. esculentum*	6
cLEW22N22	Ethylene-responsive element binding factor 6- N. sylvestris	6
cLEG34O20	UDP-glucose:salicylic acid glucosyltransferase, *N. tabacum*	7
cLED27C20	DNADPH oxidase; gp91- phox homolog, *L. esculentum*	8
cLER14J12	WRKY transcription factor IId-1 splice variant 2, *L. esculentum*	8
cTOF2L16	Phenylalanine ammonia-lyase (PAL), *L. esculentum*	8
cTOD3N7	Endo-1,4-beta-glucanase, *L. Esculentum*	8
cLEC6M14	PR-protein sth-2, *S. Tuberosum*	9
cLER14J6	Hexose transporter, *L. esculentum*	9
cTOF19O3	Hydroxyproline-rich glycoprotein homolog, *A. thaliana*	9
cTOF28P11	Phenylalanine ammonia-lyase (PAL)	9
cTOF31H10	Catechol O-methyltransferase, *N. tabacum*	10
cLEN9P2	Multi resistance protein homolog, *A. thaliana*	10
cLEZ6E21a	Ubiquitin, *L. esculentum*	11
cLEC14I18a	Resistance complex protein I2C-2, *L. Esculentum*	11
cLEC14I18b	Resistance complex protein I2C-2, *L. Esculentum*	11
cLEM22K17	9-cis-epoxycarotenoid dioxygenase, *L. Esculentum*	11
cLED23K21	Resistance complex protein I2C-5, *L. Esculentum*	11
cLES18N16	Phosphatidylinositol 4-kinase, *S. tuberosum*	11
cLEW25D9	Glutamine synthetase, *L. Esculentum*	12

Table A-2 Restriction enzymes used to identify CAPS polymorphisms

No.	Enzyme	Recognition Site (5′→3′)
1	AluI	AG▾CT
2	BstNI	CC▾W[a]GG
3	BstUI	CG▾CG
4	CfoI	GCG▾C
5	DpnII	▾GATC
6	DraI	TTT▾AAA
7	EcoRI	G▾AATTC
8	HaeIII	GG▾CC
9	HinfI	G▾AN[b]TC
10	Hspn92II	CATG▾
11	MspI	C▾CGG
12	RsaI	GT▾AC
13	ScrfI	CC▾NGG
14	ScaI	AGT▾ACT
15	TaqI	T▾CGA

[a]W = A or T [b] N= A or C or G or T

Table A-3 Restriction enzymes used to identify COS or KFG polymorphisms

No.	Enzyme	Supplier	Recognition Site (5′→3′)	No.	Enzyme	Supplier	Recognition Site (5′→3′)
1	AluI	Promega	AG▾CT	14	HindIII	Promega	A▾AGCTT
2	BfaI	NEB	C▾TAG	15	HinfI	Promega	G▾AN[b]TC
3	BsaBI	NEB	GATNN▾NNATC	16	Hspn92II	Promega	CATG▾
4	BsaJI	NEB	C▾NNGG	17	MspI	Promega	C▾CGG
5	BstNI	NEB	CC▾W[a]GG	18	RsaI	Promega	GT▾AC
6	BstUI	NEB	CG▾CG	19	SacI	Promega	GAGCT▾C
7	BsaWI	NEB	W▾CCGGW	20	ScrfI	NEB	CC▾NGG
8	CfoI	Promega	GCG▾C	21	ScaI	Promega	AGT▾ACT
9	DpnII	NEB	▾GATC	22	TaqI	Promega	T▾CGA
10	DraI	Promega	TTT▾AAA	23	Tth111I	Promega	GACN▾NNGTC
11	EcoRI	Promega	G▾AATTC	24	Tsp45I	NEB	▾GTS[c]AC
12	EcoRV	Promega	GAT▾ATC	25	Tsp509I	NEB	▾AATT
13	Hae III	Promega	GG▾CC	26	XbaI	Promega	T▾CTAGA

[a]W = A or T
[b] N= A or C or G or T
[c] S = C or G

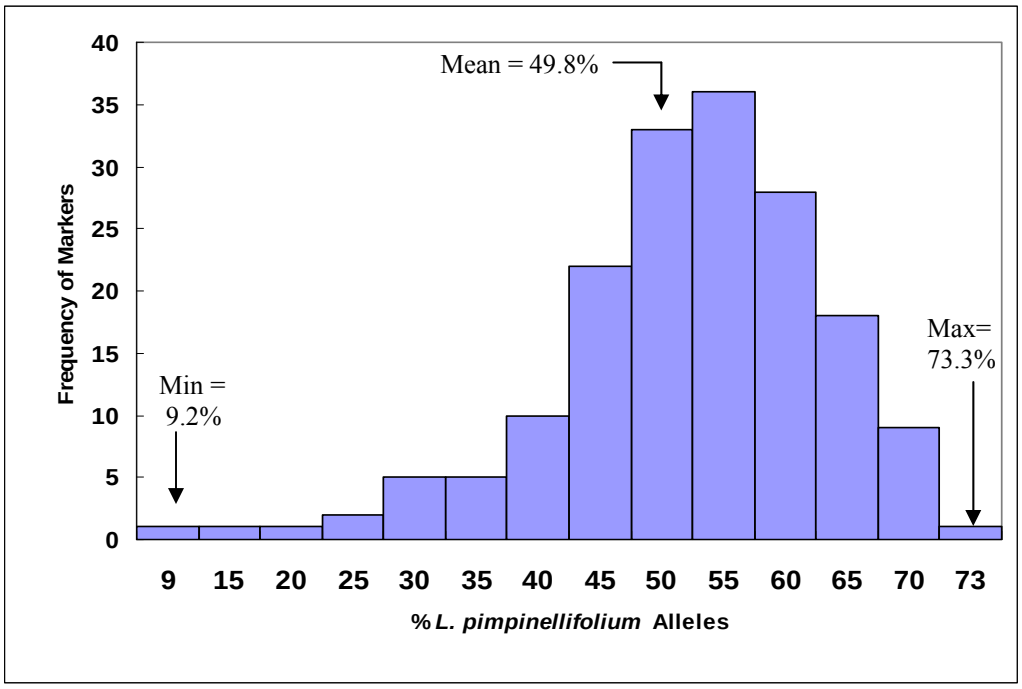


Figure A-1 Distribution of *L. Pimpinellifolium* (PSLP125) alleles based on 275 DNA markers

Graphical Genotyping

The graphical genotypes for all other 170 RIL were obtained using the GG32 program (data not shown). However, two screen shots of the graphical genotypes of RILs 131 and 21, which had the maximum number of E and P alleles, respectively, are depicted in Figure A-2 and Figure A-3. Graphical genotyping is useful for fine mapping of QTLs and speeding up the process of recovering the genome of the recurrent parent via back crossing procedure.

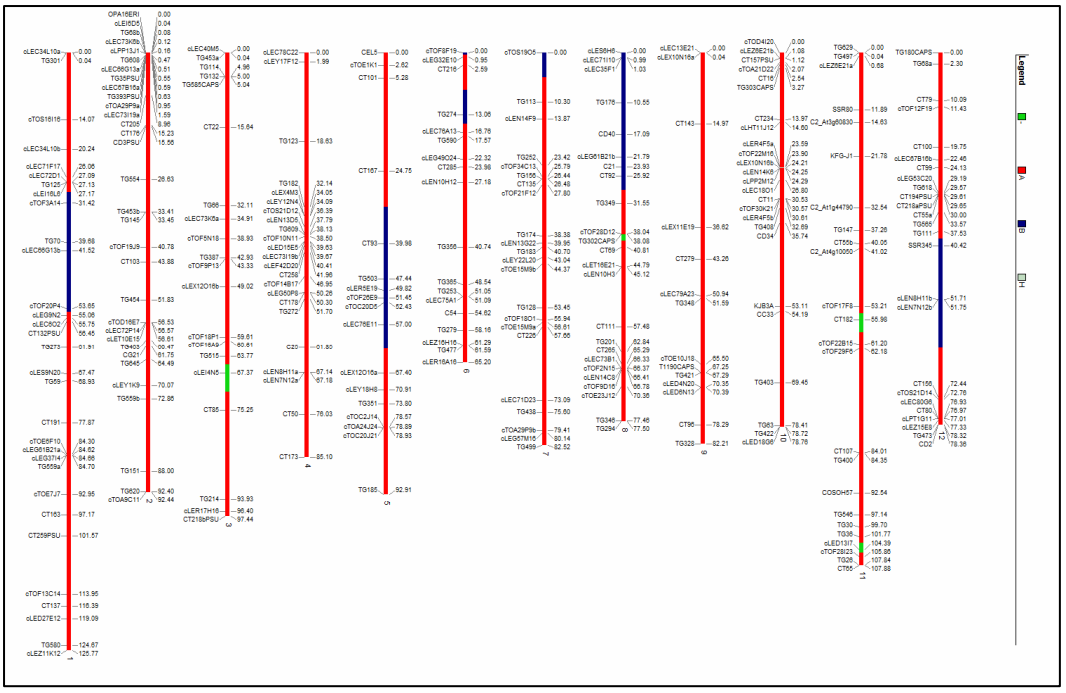


Figure A-2 A screen shot of GG32 Software for RIL #131 with the maximum number of *L. esculentum* parent alleles (*E* alleles). E allele are depicted in Red and P alleles are depicted in Blue, Heterozygote regions are in light blue and missing data are in dark green.

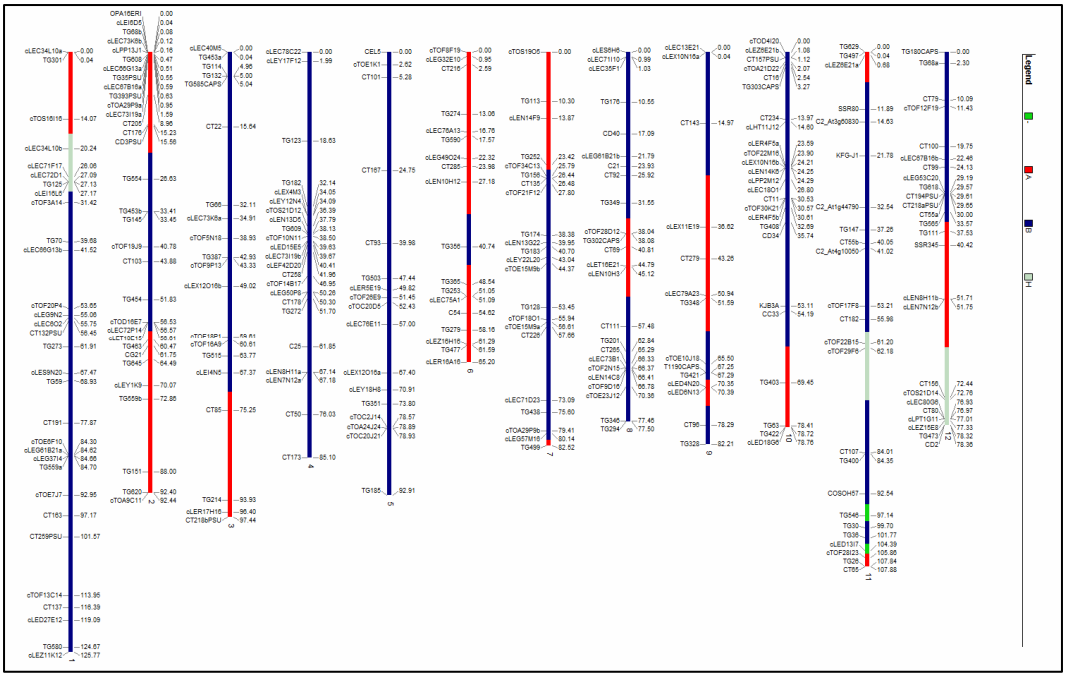


Figure A-3 A screen shot of GG32 Software for RIL #21 with the maximum percentage (67.9%) of *L. pimpinellifolium parent* (*PM* alleles). E allele are depicted in Red and P alleles are depicted in Blue, Heterozygote regions are in light blue and missing data are in dark green.

Marker Interval Comparison of the F_7-RIL map with the F_2 map

Because F_2 and F_7-RIL populations are derived from the same parents, the paired comparisons are more informative than comparisons with other populations whose parents are different. Therefore, marker interval comparisons are presented separately in Table A-4. A short comparison of RFLP anchor markers was made between E × PM1 and E × PM2 map in chapter 2. However, that comparison did not compare all common markers between the two maps as in that case comparisons were made for marker intervals common to all four maps. In this section, a more comprehensive comparison between all common marker intervals of the two maps is described. In total 162 markers (100 RFLPs and 62 ESTs) were common between the E × PM1 and E × PM2 maps. A good agreement in the order of the markers between the two maps was observed. As a result, a set of 138 separate marker interval comparisons were made between the F_7-RIL and F_2 maps. A side-by-side comparison of E × PM1 and E × PM2 maps was made by aligning the two maps by their common markers (Figure 4). The only inconsistency in marker order was on chromosome 11 for marker cTOF29F6, the position which on E× PM1 map was ~25 cM below maker TG145 while in E × PM2 map it was 12 cM above.

A significant correlation ($r = 0.93$, N = 139, $P < 0.000$) was observed between marker intervals in the E × PM1 and E × PM2 maps. In 18 marker intervals the distances between the adjacent markers were expanded by at least two-fold in E × PM1 map compared to the E × PM2 map. In 13 marker intervals the ratio of marker intervals was larger by two-fold in the E × PM2 map compared to E × PM1 map. In 6 marker intervals the distances were less than 1.0 cM and in 7 marker intervals the distances were between 3 and 12 cM. Overall the RIL population genetic map (E × PM1) was ~100 cM longer than F_2 (E × PM2) map.

Table A-4 Comparison of map distances based on common RFLP or EST marker intervals between F_7-RIL and F_2 Map

Interval	Chr.	Marker interval map distance (cM)		
		(E× PM-1) F_7-RIL	(E × PM-2) F_2	(E× PM-1) RIL-F_7 / (E × PM-2) F_2
TG125-cTOF3A14	1	4.3	2.0	2.2*
cTOF3A14-TG70	1	8.3	6.1	1.4
TG70-cTOF20P4*	1	13.9	16.4	0.8
cTOF20P4-cLEG9N2	1	1.4	0.4	3.5*
cLEG9N2-cLEC6O2	1	0.7	1.4	0.5
cLEC6O2-TG273	1	6.2	6.0	1.0
TG273-cLES9N20	1	5.6	7.1	0.8
cLES9N20-TG59	1	1.5	1.6	0.9
TG59-CT191	1	8.9	10.0	0.9
CT191-cTOE6F10	1	6.4	6.7	1.0
cTOE6F10-TG559(a)	1	0.3	0.7	0.4
TG559(a)- cTOE7J7	1	8.3	8.9	0.9
cTOE7J7-CT163	1	4.2	1.6	2.6*
CT163-CT137	1	19.2	17.7	1.1
CT137-cLED27E12	1	2.7	1.1	2.5*
cLED27E12-TG580	1	5.6	5.3	1.1
TG580-cLEZ11K12	1	1.1	4.2	0.3
TG608-CT176	2	17.6	11.1	1.6
CT176-TG554	2	11.4	13.4	0.9
TG554-TG453b	2	6.8	6.6	1.0
TG453b-TG145	2	0.0	0.4	0.4
TG145-cTOF19J19	2	7.3	7.3	1.0
cTOF19J19-CT103	2	3.1	3.0	1.0
CT103-TG454	2	7.9	12.8	0.6
TG454-cLET10E15	2	4.7	3.4	1.4
cLET10E15-TG463	2	3.9	2.4	1.6
TG463-cLEY1K9	2	9.6	9.1	1.1
cLEY1K9-TG620*	2	22.4	20.1	1.1
TG620-cTOA9C11	2	0.0	0.0	0.0
TG114-TG132	3	0.0	0.0	0.0
TG132-TG66	3	27.1	22.4	1.2
TG66-cLEX12O16b	3	16.9	15.0	1.1
cLEX12O16b-cTOF18P1	3	10.6	11.3	0.9
cTOF18P1-cTOF16A9	3	1.0	0.0	1.0
cTOF16A9-TG515	3	3.2	3.0	1.1
TG515-CT85	3	11.5	12.1	1.0
CT85-TG214	3	18.7	20.7	0.9
TG214-cLER17H16	3	2.5	1.0	2.5*
TG123-TG182	4	13.5	12.5	1.1
TG182-cTOS21D12	4	4.2	4.9	0.9
cTOS21D12-TG609	4	1.7	0.6	2.8*
TG609-cTOF10N11	4	0.4	0.3	1.3
cTOF10N11-cLED15E5	4	1.1	0.4	2.8*
cLED15E5-cTOF14B17	4	7.2	6.2	1.2
cTOF14B17-CT178	4	3.3	3.0	1.1
CT178-C25	4	11.5	13.3	0.9
C25-CT50	4	14.1	18.6	0.8
CT50-CT173	4	9.1	9.2	1.0

Table A-4 (Contd.)

Interval	Chr.	Marker interval map distance (cM)		
		(E× PM-1) F_7-RIL	(E × PM-2) $^{-}$ F_2	(E× PM-1) RIL-F_7 / (E × PM-2) $^{-}$ F_2
CEL5-cTOE1K1*	5	2.6	1.3	2.0*
cTOE1K1-CT101*	5	2.7	2.7	1.0
CT101-CT167	5	19.5	15.9	1.2
CT167-CT93	5	15.2	19.3	0.8
CT93-TG503	5	7.5	4.6	1.6
TG503-cTOF26E9	5	4.0	2.5	1.6
cTOF26E9-TG351	5	22.4	10.6	2.1*
TG351-cTOC2J14(b) †	5	4.8	1.2	4.0*
cTOC2J14(b)-TG185	5	14.3	9.5	1.5
cTOF8F19- cLEG32E10	6	0.9	0.8	1.1
cLEG32E10- CT216	6	1.6	0.6	2.7*
CT216-TG274	6	10.5	10.9	1.0
TG274-TG590	6	4.5	3.4	1.3
TG590-CT285	6	6.5	6.9	0.9
CT285-TG356	6	16.8	14.1	1.2
TG356-TG365	6	7.8	11.0	0.7
TG365-TG253	6	2.5	4.1	0.6
TG253-C54	6	3.5	7.4	0.5
C54-TG279	6	3.5	4.0	0.9
TG279-cLEZ16H16	6	3.1	6.0	0.5
cLEZ16H16-TG477	6	0.3	0.3	1.0
TG113-cLEN14F9*	7	3.6	1.3	2.8*
cLEN14F9-cTOF34C13	7	11.9	17.5	0.7
cTOF34C13-TG156	7	0.6	0.6	1.0
TG156-cTOF21F12	7	1.3	1.5	0.9
cTOF21F12-TG174	7	10.6	9.3	1.1
TG174-cLEN13G22	7	1.6	2.3	0.7
cLEN13G22-TG183	7	0.8	1.3	0.6
TG183-cLEY22L20	7	2.3	3.2	0.7
cLEY22L20-TG128	7	10.4	12.5	0.8
TG128-cTOE15M9(a)	7	3.2	2.4	1.3
cTOE15M9(a) – CT226	7	1.0	0.7	1.4
CT226-TG438	7	17.9	16.7	1.1
TG438-TG499	7	6.9	0.0	6.9*
TG176-CD40	8	6.5	8.5	0.8
CD40-CT92	8	8.8	6.2	1.4
CT92-TG349	8	5.6	6.7	0.8
TG349-TG302	8	6.5	7.3	0.9
TG302-CT69	8	2.7	2.4	1.1
CT69-cLET16E21	8	4.0	2.6	1.5
cLET16E21-cLEN10H3	8	0.3	0.7	0.4
cLEN10H3-TG201	8	17.8	19.0	0.9
TG201-CT265	8	2.4	4.1	0.6
CT265-cTOF-2N15	8	1.0	1.3	0.8
cTOF-2N15-cLEN14C8	8	0.0	0.0	0.0
cLEN14C8-cTOF9D16	8	0.4	0.6	0.7
cTOF9D16-cTOE23J12	8	3.6	1.5	2.4*
cTOE23J12-TG346	8	7.1	9.4	0.8
TG346-TG294	8	0.0	0.0	0.0

† cTOC2J14 in the F_7- RIL map is the same locus as cTOC2J14b in F_2 map

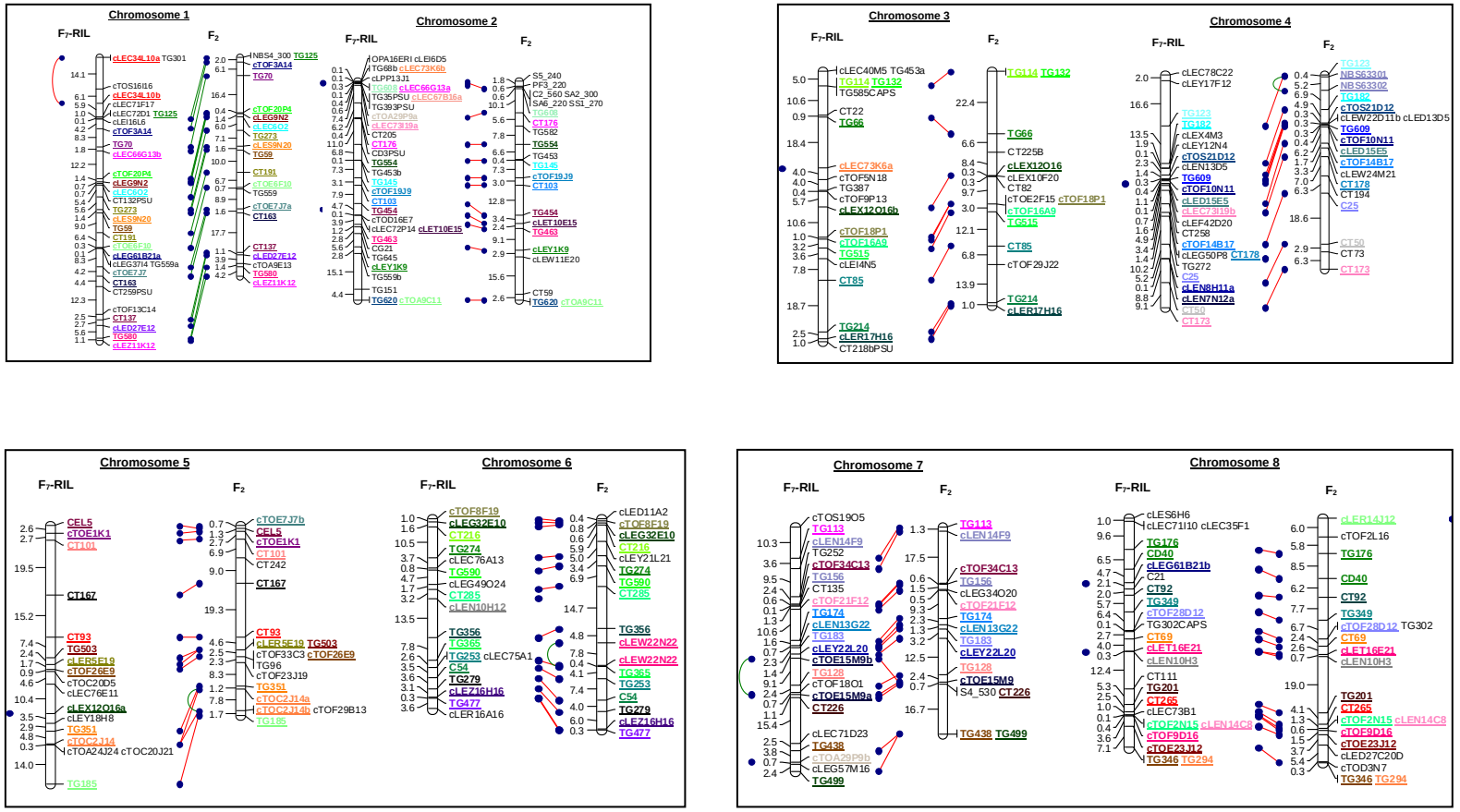
Table A-4 (Contd.)

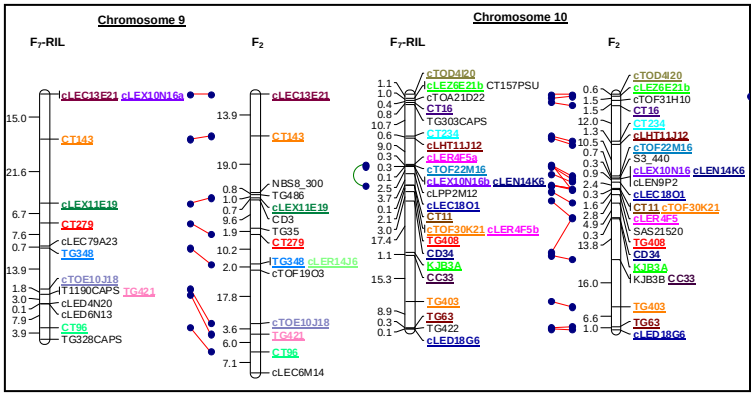
Interval	Chr.	Marker interval map distance (cM)		
		(E× PM-1) F_7-RIL	(E × PM-2) $^{-}F_2$	(E× PM-1) RIL-F_7 / (E × PM-2) $^{-}F_2$
cLEC13E21-CT143	9	14.9	13.9	1.1
CT143- cLEX11E19	9	21.7	19.8	1.1
cLEX11E19-CT279	9	6.6	12.2	0.5
CT279-TG348	9	8.3	10.2	0.8
TG348- TG421	9	15.6	14.9	1.0
TG421-CT96	9	10.9	6.3	1.7
cTOD4I20-cLEZ6E21b*	10	1.1	0.6	1.8
cLEZ6E21b-CT16*	10	1.4	3.0	0.5
CT16-CT234	10	11.4	12.0	1.0
CT234-cTOF22M16	10	9.9	11.8	0.8
cTOF22M16-cLEX10N16(b)	10	0.3	1.0	0.3
cLEX10N16(b)-cLEN14K6	10	0.0	0.0	0.0
cLEN14K6-CT11	10	6.2	3.3	1.9*
CT11-cTOF30K21	10	0.0	0.0	0.0
cTOF30K21-cLER4F5(b)	10	0.0	0.3	0.0
cLER4F5(b)-TG408	10	2.1	4.4	0.5
TG408-CD34	10	3.1	4.9	0.6
CD34- CC33	10	18.5	14.1	1.3
CC33-TG403	10	15.3	15.7	1.0
TG403-TG63	10	9.0	8.0	1.1
TG63-cLED18G6	10	0.3	1.0	0.3
TG629-TG497	11	0.0	0.3	0.0
TG497-TG147	11	35.1	40.3	0.9
TG147-CT55(b)	11	2.8	2.8	1.0
TG400-TG546	11	12.8	16.7	0.8
TG546-TG36	11	2.6	3.6	0.7
TG36-TG30	11	2.1	2.6	0.8
TG30-cLED13I7	11	2.6	9.0	0.3
cLED13I7-CT65*	11	3.5	4.7	0.7
CT65-TG26*	11	0.0	0.4	0.0
TG68-CT79	12	7.8	3.3	2.4*
CT79-CT100	12	9.6	14.1	0.7
CT100-CT99	12	4.4	2.7	1.6
CT99-TG618	12	5.5	5.0	1.1
TG618-TG565*	12	4.0	2.1	1.9*
TG565-TG111*	12	4.0	3.2	1.3
TG111-CT156	12	34.9	39.3	0.9
CT156-cTOS21D14	12	0.3	0.7	0.4
cTOS21D14- cLEZ15E8*	12	4.5	3.1	1.5
cLEZ15E8-TG473	12	1.0	1.7	0.6
TG473-CD2	12	0.0	0.0	0.0

*Different in interval length by at least two-fold or close to two-fold

Where the marker intervals are two-fold in PM2 relative to PM1 it has marked with red font

Figure A-4 Side-by-side-comparison of the order of the markers in F_7-RIL and F2 genetic map of a cross *between L. esculentum* (NCEBR-1) and *L. pimpinellifolium* (PSLP125)

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Chromosome 10
F7-RIL
F2
F7-RIL
F2
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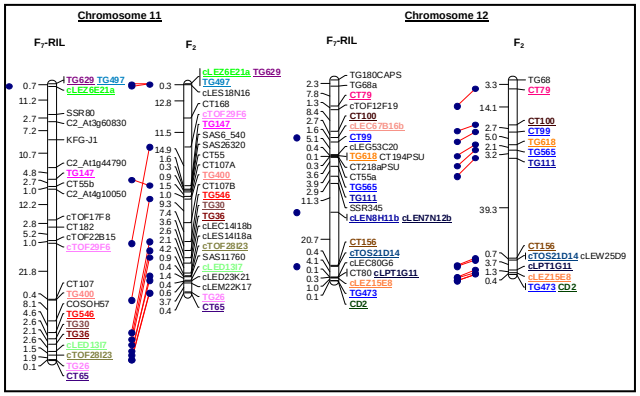
CT143
cLEX11E19
CT279
cLEC79A23
TG348
cTOE10J18
T1190CAPS TG421
cLED4N20
cLED6N13
CT96
TG328CAPS
cLEC13E21
CT143
NBS8_300
TG486
cLEX11E19
CD3
TG35
CT279
TG348 cLER14J6
cTOF19O3
cTOE10J18
TG421
CT96
cLEC6M14
cTOD4I20
cLEZ6E21b CT157PSU
cTOA21D22
CT16
TG303CAPS
CT234
cLHT11J12
cLER4F5a
cTOF22M16
cLEX10N16b cLEN14K6
cLPP2M12
cLEC18O1
CT11
cTOF30K21 cLER4F5b
TG408
CD34
KJB3A
CC33
TG403
TG63
TG422
cLED18G6
cTOD4I20
cLEZ6E21b
cTOF31H10
CT16
CT234
cLHT11J12
cTOF22M16
S3_440
cLEX10N16 cLEN14K6
cLEN9P2
cLEC18O1
CT11 cTOF30K21
cLER4F5
SAS21520
TG408
CD34
KJB2A
KJB3B CC33
TG403
TG63
cLED18G6


Chromosome 11
Chromosome 12
F7-RIL
F2
F7-RIL
F2
TG629 TG497
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SSR80
C2_At3g60830
KFG-J1
C2_At1g44790
TG147
CT55b
C2_At4g10050
cTOF17F8
CT182
cTOF22B15
cTOF28F8
CT107
TG408
COSOH57
TG546
TG30
TG36
cLED13I7
cTOF28I23
TG29
CT65
cLEZ6E21a TG629
TG497
cLES18N16
CT168
cTOF29F6
TG147
SAS6_540
SAS26320
CT55
CT107A
TG400
CT107B
TG546
TG30
TG36
cLEC14I18b
cLES14I18a
cTOF28I23
SAS11760
cLED13I7
cLED23K21
cLEM22K17
TG29
CT65
TG180CAPS
TG68a
CT79
CTOF12F19
CT100
cLES57B16b
CT99
cLEG53C20
TG618 CT194PSU
CT218aPSU
CT55a
TG565
TG111
SSR345
cLEN8H11b cLEN7N12b
CT156
cTOS21D14
cLEC80G6
CT80 cLPT1G11
cLEZ15E8
TG473
CD2
TG68
CT79
CT100
CT99
TG618
TG565
TG111
CT156
cTOS21D14 cLEW25D9
cLPT1G11
cLEZ19E9
TG473 CD2

Appendix B. Supplemental materials of chapter 3

Table B-1 Analysis of variance for final % defoliation – F_7 (2004)

S.O.V	DF	Seq SS	Adj SS	Adj MS	F	P
Accessions	158	164208.4	164105.9	1038.6	5.09	0.000
Replication	1	5692.9	5692.9	5692.9	27.91	0.000
Error	156	31818.6	31818.6	204.0		
Total	315	201719.9				

S = 14.28 R-Sq = 84.23% R-Sq(adj) = 68.15% CV= 20.6%

Table B-2 Analysis of variance for final % defoliation – F_8 (2005)

S.O.V	DF	Seq SS	Adj SS	Adj MS	F	P
Accessions	170	210119.8	210119.8	1236.0	5.34	0.000
Replication	1	584.2	584.2	584.2	2.52	0.114
Error	170	39364.3	39364.3	231.6		
Total	341	250068.3				

S = 15.21 R-Sq = 84.26% R-Sq(adj) = 68.42% CV= 35.63

Table B-3 Analysis of Variance for FPD – F_9 (2006)

S.O.V	DF	Seq SS	Adj SS	Adj MS	F	P
Accessions	159	224202.4	224202.4	1410.1	4.25	0.000
Replication	1	162.5	162.5	162.5	0.49	0.485
Error	159	52738.6	52738.6	331.7		
Total	319	277103.4				

S = 18.21 R-Sq = 80.97% R-Sq(adj) = 61.82% CV= 26.4%

Table B-4 Tukey's test of mean comparison of final % defoliation and AUDPC of the parents in 2004 to 2006

	Genotypes		
	PSLP125	F_1	NCEBR-1
Mean (Final % defoliation)	28.75	30.0	75.0
Mean (AUDPC)	511.4	566	1808
Group	(a)	(a)	(b)

Table B-5 Tukey's test of mean comparison of final % defoliations of the RIL population parents in three years

	Year		
	2004	2006	2005
Mean (final % defoliation)	45.8	43.3	22.0
Mean (AUDPC)	1072	851	467.6
Group	(a)	(a)	(b)

Table B-6 Correlation of the AUDPC over the years for different experiments

AUDPC	**F_7 R1**	**F_7 R2**	**F_7 Ave. R1-R2**	**F_8R1**	**F_8R2**	**F_8 Ave. R1-R2**	**F_9R1**	**F_9R2**
F_7 R2	0.748**							
F_7Ave R1-R2	0.931**	0.939**						
F_8R1	0.547**	0.445**	0.529**					
F_8R2	0.523**	0.509**	0.529**	0.736**				
F_8 Ave. R1-R2	0.574**	0.512**	0.580**	0.932**	0.932**			
F_9R1	0.489**	0.582**	0.574**	0.514**	0.544**	0.568**		
F_9R2	0.451**	0.415**	0.462**	0.360**	0.535**	0.480**	0.596**	
F_9 Ave. R1-R2	0.526**	0.557**	0.579**	0.488**	0.604**	0.586**	0.891**	0.896**

** Correlation is significant at the 0.01 level

R1= 1st replication, R2= 2nd replication Ave. R1-R2= the mean of R1 and R2

Table B-7 Correlation among of the final % defoliation and AUDPC over the years for different experiments

% Defol. / AUDPC	F_7 R1	F_7 R2	F_7 Ave. R1-R2	F_8R1	F_8R2	F_8 Ave. R1-R2	F_9R1	F_9R2	F_9 Ave. R1-R2
F_7 R1	0.92**	0.71**	0.88**						
F_7 R2	0.67**	0.96**	0.89**						
F_7Ave R1-R2	0.83**	0.90**	0.95**						
F_8R1	0.42**	0.35**	0.43**	0.92**	0.71**	0.89**			
F_8R2	0.41**	0.40**	0.46**	0.70**	0.93**	0.89**			
F_8 Ave. R1-R2	0.45**	0.40**	0.48**	0.86**	0.87**	0.95**			
F_9R1	0.42**	0.53**	0.52**	0.53**	0.58**	0.61**	0.86**	0.57**	0.79**
F_9R2	0.34**	0.36**	0.39**	0.31**	0.54**	0.46**	0.53**	0.81**	0.74**
F_9 Ave. R1-R2	0.42**	0.50**	0.51**	0.47**	0.60**	0.59**	0.77**	0.77**	0.85**

** Correlation is significant at the 0.01 level
R1= 1st replication, R2= 2nd replication Ave. R1-R2= the mean of R1 and R2

Table B-8 The SAS System output for components of variance based on mixed procedure for final % defoliation data set

The Mixed Procedure

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	7725.55431899	
1	1	7235.82501979	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
year	216.21
rep(year)	8.8369
entry	354.73
year*entry	113.71
Residual	257.60

Fit Statistics

-2 Res Log Likelihood	7235.8
AIC (smaller is better)	7245.8
AICC (smaller is better)	7245.9
BIC (smaller is better)	7241.3

Table B-9 The SAS System out put for components of variance based on mixed procedure for AUDPC data set

The Mixed Procedure

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	12719.31824062	
1	1	12276.95523968	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
year	42719
rep(year)	471.06
entry	202458
year*entry	85389
Residual	124177

Fit Statistics

-2 Res Log Likelihood	12277.0
AIC (smaller is better)	12287.0
AICC (smaller is better)	12287.0
BIC (smaller is better)	12282.4

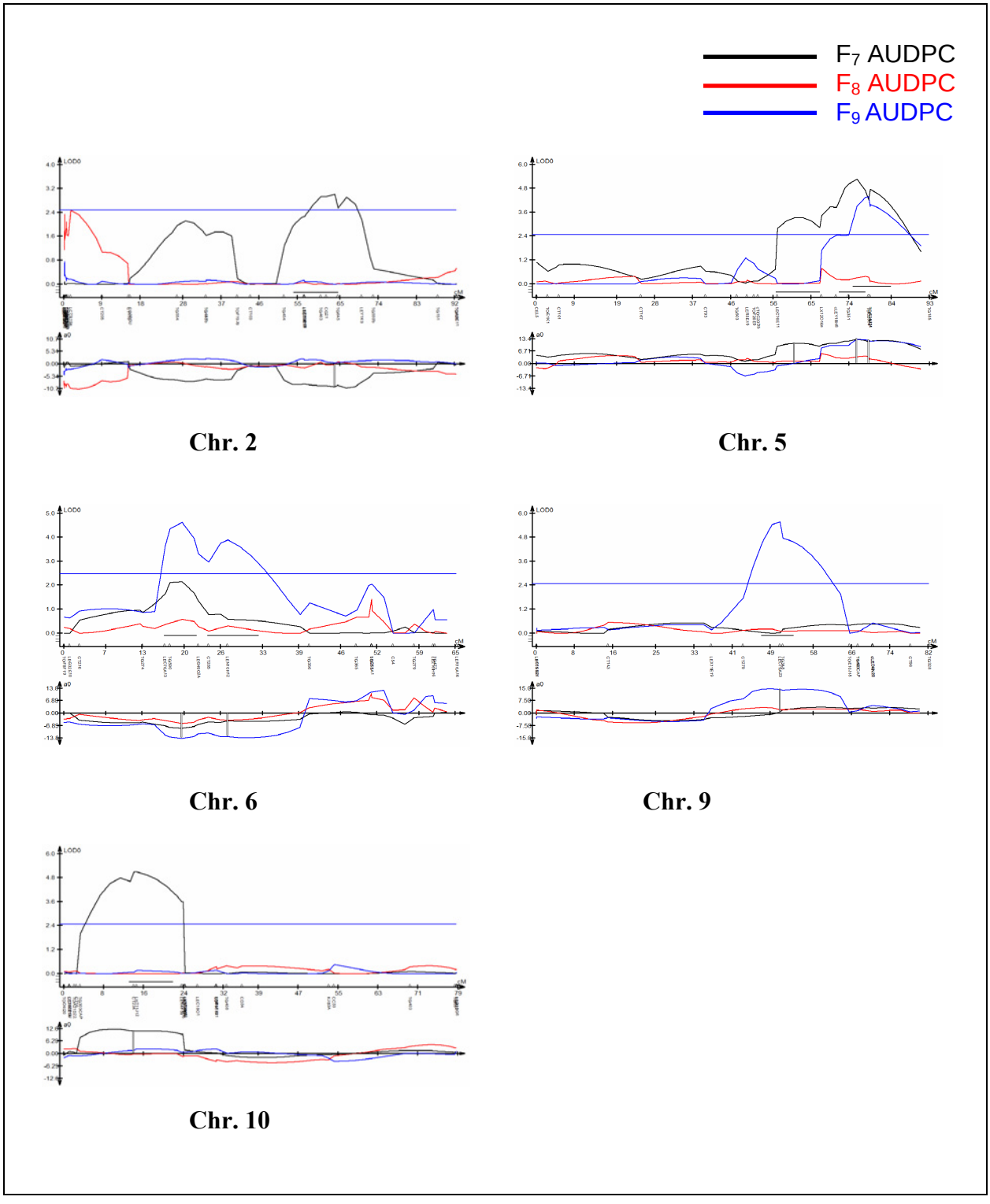

Figure B-1 Identified QTLs for early blight resistance based on AUDPC data and using selective genotyping in three generations of a RIL population of a cross between *L. esculentum* (NCEBR-1)× *L. pimpinellifolium* (PSLP125).

Appendix C Supplemental materials of chapter 4

Table C-1 Analysis of Variance for the fruit weight trait in 2005 (F_8)

Source	*DF*	*SS*	*MS*	*F*	*p*
RILs	170	20946.59	123.22	23.88	0.000
Replication	1	34.47	34.47	6.68	0.011
Error	170	877.33	5.16		
Total	341	21858.40			

S = 2.27173 R-Sq = 95.99% R-Sq(adj) = 91.95% CV= 16.0%

Table C-2 Analysis of Variance for the fruit weight trait in 2006 (F_9)

Source	*DF*	*SS*	*MS*	*F*	*p*
RIL line	152	16696.7	109.847	10.19	0.000
Replication	1	52.5	52.460	4.87	0.029
Error	152	1638.7	10.781		
Total	305	18387.8			

S = 3.28340 R-Sq = 91.09% R-Sq(adj) = 82.12% CV= 25%

Table C-3 Analysis of Variance for Fruit Weight for the RILs in 2004, 2005, 2006

Source	*DF*	*Seq SS*	*AdJ. SS*	*Adj. MS*	*F*	*p*
Year	2	278.43	278.43	139.21	2.29	0.102
Rep(Year)	2	86.93	86.93	43.47	0.71	0.490
Error	802	48783.86	48783.86	60.83		
Total	806	49149.22				

S = 7.79922 R-Sq = 0.74% R-Sq(adj) = 0.25%

Table C-4 ANOVA table of FW of the parents and F_1 in three years

S.O.V	*DF*	*Seq SS*	*AdJ. SS*	*Adj. MS*	*F*	*p*
Year	2	524	524	262	1.22	0.387
Genotype*	2	49250	49250	24625	114.49	0.000
Error	4	860	860	215		
Total	8	50634				

S = 14.6660 R-Sq = 98.30% R-Sq(adj) = 96.60%

*Genotype = Parents and F_1

Table C-5 Summary Statistics for the mean fruit weight of population in each generation and parents in 2004, 2005 and 2006

Fruit Weight	Genotype	Mean	SE Mean	St. Dev.	Variance	Coef. Var.	Minimum	Median	Maximum	Range	Skewness
2004	Population (F7)	14.14	0.586	7.39	54.590	52.25	2.94	12.360	47.05	44.11	1.87
Single	NCEBR	190.35	-	-	-	-	-	-	-	-	-
plant	PSLP125	2.17	-	-	-	-	-	-	-	-	-
evaluation	F1	17.30	-	-	-	-	-	-	-	-	-
2005	Population (F8)	14.19	0.60	7.85	61.600	55.31	3.48	11.86	45.16	42.13	1.78
Mean of two replications	NCEBR*	138.10	16.10	22.80	518.100	16.48	122.00	138.10	154.20	32.20	-
	PSLP125*	2.16	0.42	0.60	0.361	27.89	2.16	2.16	2.58	0.85	-
	F1*	13.59	0.82	1.15	1.328	8.48	12.77	13.59	14.40	1.63	-
2006	Population (F9)	12.97	0.60	7.41	54.920	57.16	4.32	11.05	42.64	38.32	1.86
Mean of two replications	NCEBR*	165.63	9.57	13.54	183.400	8.18	156.05	165.63	175.20	19.15	-
	PSLP125*	2.26	0.07	0.01	0.008	4.08	2.19	2.26	2.32	o.13	-
	F1*	11.20	0.40	0.57	0.330	5.11	11.20	11.20	11.61	0.80	-

* = The values are calculated based on the mean of two replications, however the original value of the each replication mean itself obtained by averaging fruit weight of 10-20 fruits.

Table C-6 Analysis of variance for the pH of purée in 2005 (F_8)

Source	*DF*	*SS*	*MS*	*F*	*p*
RILs	170	11.3073	0.06651	1.38	0.018
Replication	1	1.7082	1.70815	35.45	0.000
Error	170	8.1921	0.04819		
Total	341	21.2076			

S = 0.2195 R-Sq = 61.37% R-Sq(adj) = 22.52% CV=5.0%

Table C-7 Descriptive statistics for pH in Rep 1, Rep II and the mean of two replications for RIL-F_8 (2005)

Variable	Mean	St. Dev.	Variance	Coef.Var.	Min.	Median	Max.	Range	Skewness
pH Rep I	4.023±0.0179	0.2337	0.055	5.81	3.53	4.00	5.17	1.64	1.15
pH Rep II	4.16±0.0182	0.2452	0.0601	5.89	3.61	4.17	4.80	1.19	0.16
Mean of Two Reps	4.09±0.0139	0.1824	0.033	4.45	3.63	4.08	4.73	1.10	0.45

Table C-8 Analysis of variance for the pH of purée in 2006 (F_9)

Source	*DF*	*SS*	*MS*	*F*	*p*
RILs	152	4.94765	0.032550	4.93	0.000
Replication	1	0.23890	0.23890	36.17	0.000
Error	152	1.00395	0.006605		
Total	305	6.19050			

S = 0.08127 R-Sq = 83.78% R-Sq(adj) = 67.46% CV=1.9%

Table C-9 Descriptive statistics for pH in Rep 1, Rep II and the mean of two replications for RIL-F_9 (2006)

Variable	Mean	St.Dev	Variance	Coef.Var	Min.	Median	Max.	Range	Skewness
pH Rep I	4.17±0.0115	0.1421	0.0202	3.41	3.80	4.16	4.60	0.80	0.53
pH Rep II	4.23±0.0111	0.1377	0.0189	3.26	3.93	4.22	4.69	0.76	0.68
Mean of Two Reps	4.20±0.0103	0.1276	0.0163	3.04	3.89	4.19	4.65	0.75	0.71

Table C-10 Analysis of Variance for pH in 2004, 2005 and 2006 using Adjusted SS for Tests

S.O.V	*DF*	*Seq SS*	*AdJ. SS*	*Adj. MS*	*F*	*p*
Year	2	2.5665	2.5665	1.2833	34.83	0.000
Rep(Year)	2	1.9471	1.9471	0.9735	26.42	0.000
Error	802	29.5484	29.5484	0.0368		
Total	806	34.0620				

S = 0.191946 R-Sq = 13.25% R-Sq(adj) = 12.82% CV=4.64%

Table C-11 ANOVA table of pH of the parents and F_1 in three years

S.O.V	*DF*	*Seq SS*	*AdJ. SS*	*Adj. MS*	*F*	*p*
Year	2	0.025689	0.025689	0.012844	4.85	0.085
Genotype	2	0.050239	0.050239	0.025119	9.48	0.030
Error	4	0.010594	0.010594	0.002649		
Total	8	0.086522				

S = 0.0514647 R-Sq = 87.76% R-Sq(adj) = 75.51%

*Genotype = Parents and F1

Table C-12 Summary Statistics for the mean pH of population in each generation and parents in 2004, 2005 and 2006

pH of Puree	Genotype	Mean	SE. Mean	St. Dev.	Variance	Coef. Var	Min.	Median	Max.	Range	Skewness
2004	Population (F7)	4.06	0.013	0.16	0.026	3.93	3.70	4.09	4.49	0.79	0.33
Single	NCEBR	3.94	-	-	-	-	-	-	-	-	-
plant	PSLP125	4.12	-	-	-	-	-	-	-	-	-
evaluation	F1	4.12	-	-	-	-	-	-	-	-	-
2005	Population (F8)	4.09	0.014	0.182	0.033	4.45	3.63	4.08	4.73	1.10	0.45
Mean of	NCEBR*	4.29	0.125	0.177	0.031	4.13	4.16	4.28	4.41	0.25	-
two	PSLP125*	4.12	0.370	0.523	0.274	12.70	3.75	4.12	4.49	0.74	-
replications	F1*	4.12	0.095	0.134	0.018	3.26	4.02	4.12	4.21	0.19	-
2006	Population (F9)	4.20	0.010	0.128	0.016	3.04	3.89	4.19	4.65	0.75	0.71
Mean of	NCEBR*	4.26	0.075	0.106	0.011	2.49	4.18	4.33	4.33	0.15	-
two	PSLP125*	4.20	0.105	0.148	0.022	3.53	4.10	4.31	4.31	0.21	-
replications	F1*	4.06	0.050	0.070	0.005	1.74	4.01	4.11	4.11	0.10	-

* = The values are calculated based on the mean of two replications, however the original value of the each replication mean itself obtained by averaging fruit weight of 10-20 fruits.

Table C-13 Analysis of variance for the SSC of purée in 2005 (F_8)

Source	*DF*	*SS*	*MS*	*F*	*p*
RILs	170	169.341	0.99612	4.76	0.000
Replication	1	1.540	1.57380	7.52	0.007
Error	170	35.566	0.20921		
Total	341	206.481			

S = 0.4574 R-Sq = 82.78% R-Sq(adj) = 65.45% CV=6.33%

Table C-14 Descriptive statistics for SSC in Rep 1, Rep II and the mean of two replications for RIL-F_8 (2005)

Variable	**Mean**	**St. Dev.**	**Variance**	**Coef.Var.**	**Min.**	**Median**	**Max.**	**Range**	**Skewness**
SSC Rep I	6.047±0.0601	0.7857	0.6173	12.99	4.1	6.0	8.7	4.6	0.15
SSC Rep II	6.183±0.0586	0.7668	0.5880	12.40	4.5	6.1	8.1	3.6	0.11
Mean of Two Reps	7.215± 0.0540	0.7057	0.4981	9.78	5.4	7.2	9.2	3.8	0.09

Table C-15 Analysis of variance for the SSC of purée in 2006 (F_9)

Source	*DF*	*SS*	*MS*	*F*	*p*
RILs	152	200.601	1.31974	4.34	0.000
Replication	1	0.252	0.25250	0.83	0.364
Error	152	46.221	0.30409		
Total	305	247.074			

S = 0.5514 R-Sq = 81.29% R-Sq(adj) = 62.46% CV=8.46%

Table C-16 Descriptive statistics for SSC in Rep 1, Rep II and the mean of two replications for RIL-F_9 (2006)

Variable	**Mean**	**St.Dev**	**Variance**	**Coef.Var**	**Min.**	**Median**	**Max.**	**Range**	**Skewness**
SSC Rep I	6.5454± 0.0738	0.9123	0.8323	13.94	4.00	6.60	9.40	5.40	0.20
SSC Rep II	6.4879± 0.0719	0.8897	0.7915	13.71	4.00	6.50	9.20	5.20	0.02
Mean of Two Reps	6.5166±0.0657	0.8123	0.6599	12.47	4.00	6.55	8.60	4.60	0.05

Table C-17 ANOVA table of SSC of the parents and F_1 in three years (separated analysis over the years)

S.O.V	*DF*	*Seq SS*	*AdJ. SS*	*Adj. MS*	*F*	*p*
Year/Generation	2	3.2089	3.2089	1.6044	40.68	0.002
Genotype*	2	11.4839	11.4839	5.7419	145.57	0.000
Error	4	0.1578	0.1578	0.0394		
Total	8	14.8506				

S = 0.198606 R-Sq = 98.94% R-Sq(adj) = 97.88%

* Genotype = parents and F_1

Table C-18 Analysis of Variance for SSC in 2004, 2005 and 2006 using Adjusted SS for Tests

Source	*DF*	*Seq SS*	*AdJ. SS*	*Adj. MS*	*F*	*p*
Year	2	257.211	257.211	128.606	194.98	0.0000
Rep(Year)	2	1.826	1.826	0.913	1.38	0.251
Error	802	528.975	528.975	0.66		
Total	806	788.013				

S = 0.812139 R-Sq = 32.87% R-Sq(adj) = 32.54% CV=13.28%

Table C-19 Summary Statistics for the mean SSC of population in each generation and parents in 2004, 2005 and 2006

SSC of Purée	**Genotype**	**Mean**	**SE. Mean**	**St. Dev.**	**Variance**	**Coef. Var.**	**Min.**	**Median**	**Max.**	**Range**	**Skewness**
2004	Population (F7)	4.96	0.055	0.69	0.4889	14.10	3.60	5.00	7.00	3.40	0.47
Single	NCEBR	3.95	-	-	-	-	-	-	-	-	-
plant	PSLP125	6.25	-	-	-	-	-	-	-	-	-
evaluation	F1	5.95	-	-	-	-	-	-	-	-	-
2005	Population (F8)	6.12	0.054	0.71	0.498	11.54	4.30	6.10	8.10	3.80	0.09
Mean of	NCEBR*	5.05	0.150	0.21	0.045	4.20	4.90	5.05	5.20	0.30	-
two	PSLP125*	7.85	0.250	0.35	0.125	4.50	7.60	7.85	8.10	0.50	-
replications	F1*	7.05	0.250	0.35	0.125	5.01	6.80	7.05	7.30	0.50	-
2006	Population (F9)	6.52	0.052	0.90	0.810	13.81	4.00	6.50	9.40	5.40	0.11
Mean of	NCEBR*	4.95	0.050	0.07	0.005	1.43	4.90	4.95	5.00	0.10	-
two	PSLP125*	7.60	0.600	0.85	0.720	11.16	7.00	7.60	8.20	1.20	-
replications	F1*	7.40	0.100	0.14	0.020	1.91	7.30	7.40	7.50	0.20	-

Extraction of carotenoids

Nonpolar Carotenoids including lycopene and β-carotene were extracted based on the following protocol (Hyman 2001). In this procedure, 10-20 newly collected fruits from the field were washed, surface-dried, weighted and thoroughly blended with a standard kitchen blender, until the skin parts become less than 2 mm^2 in size. Samples were processed no longer than 24 hours after collection. The smaller the skin fragments in the purée the more efficient the extraction of carotenoids will be, because it is almost impractical to extract all carotenoids from the skin in a short time while processing a large number of samples. It is also advisable to use a Polytron brand homogenizer or an equivalent instrument. Under dim light conditions, about 1.0±0.05g of purée was weighed in a 30mL clean glass test tube. It is also advisable to avoid seeds (which confound the lycopene measurement) and large skin fragments, since it is difficult to extract all the lycopene from the skin. Ten mL of extraction solvent consisting of methanol and tetrahydrofuran (THF) in a 1:1 ratio containing 0.1% w/v BHT (butylated hydroxytoluene; a preservative agent) was added to the purée, thoroughly mixed and kept in a -80°C freezer for no more than 24-36 hours until further carotenoid extraction. Where possible, it is better to complete the extraction right away. The purée and the extraction solvent were then poured into a Buchner funnel and filtered through a 1.6µ GF/A filter (Whatman Inc., Clifton, NJ, USA) into a glass Erlenmeyer flask. The pulp on the filter paper was washed 2-3 times with 5-10 mL extraction solvent and scraped from the filter, using the same glass test tube, back into the test tube. More extraction solvent then was added (~10 mL) to the pulp, shook for approximately one minute and poured back into the funnel, never allowing either the paper filter or the pulp to dry out. This was repeated 3-4 times until no more color was detectable in the solvent.

Lycopene and β-carotene are non-polar carotenoids; therefore, the undesirable polar carotenoids need to be removed from the extract. They have much lower solubility in the organic phase than they do in the aqueous phase. Thus, they will segregate into the aqueous phase when saltwater is added. Since lycopene and β-carotene are nonpolar carotenoids they will do the opposite. In order to remove any polar carotenoids, the extract was washed with 10% NaCl water. About 50mL salt water was thoroughly mixed with the extract, 10mL dichloromethane (DCM) was added and everything in the flask was vigorously agitated again. The mixture was then poured

into a separating funnel (Nalgene). The cap was tightening up, the funnel was shook and inverted, and the stopcock was opened to allow gases to escape. The cocktail was allowed to separate for 10-15 minutes into an aqueous and an organic phase. DCM, the major component of the organic phase, is heavier than 10% saltwater. However, methanol is somewhat soluble in DCM although it is even more soluble in water. It will tend to be drawn into the aqueous phase until there is a balance in concentration/solubility between the two phases. As it is less dense than saltwater, a high enough concentration of methanol in the DCM may make DCM plus methanol less dense than 10% saltwater. This will cause DCM to rise above the saltwater. By adding more DCM or saltwater as needed, this will correct the density issue and the organic phase will then fall to the bottom. In general, two to three rounds of extraction-separation were performed for each accession.

Round 1: "The denser organic phase containing the nonpolar carotenoids was removed leaving the polar carotenoids and chlorophyll in the aqueous phase." The DCM (the lower phase) with mainly nonpolar carotenoids (lycopene plus β-carotene) and only trace amounts of polar carotenoids is drained into a flask and saved. The remaining saltwater in the separating funnel has mainly polar carotenoids and only trace amounts of nonpolar carotenoids (lycopene + β-carotene).

Round 2: "An additional 10 ml of DCM was added to the aqueous phase and a second round of agitation and separation was performed to remove any residual nonpolar carotenoids from the aqueous phase. The organic phase was removed again and the aqueous phase (containing polar carotenoids) was discarded." Once again, drain the DCM into the same flask from round 1 and save it. All of the nonpolar carotenoids should now be in the DCM which was saved but the DCM still contains trace amounts of polar carotenoids, methanol, and water which need to be removed. Remember cleaning out the separating funnel with a small amount of methanol and letting it dry out briefly before the next step.

*Round 3**:*** "The organic phase was transferred back into the separating funnel and washed with 2X volume of fresh saltwater solution to remove any remaining polar carotenoids. This also reduced the amount of methanol and residual water in the organic phase." Essentially, pour the saved DCM from the flask back into the clean separating funnel and wash it again with saltwater

to remove the trace amounts of polar carotenoids left in the DCM during the first two rounds of washing. The concentration of polar carotenoids, methanol, and water are now much lower in the saltwater so they migrate to the saltwater. This leaves the DCM and nonpolar carotenoids in the lower phase and the remaining junk in the upper phase. Once again, drain the lower phase into a flask and save it for analysis. Dispose of the upper phase.

It is difficult to drain all of the lower phase into the flask without getting any of the upper phase. Watch the liquid carefully as it drains through the stopcock. The boundary between the phases should be easily visible. The final washed samples were then dried down under a stream of pressurized nitrogen to almost complete dryness using an N-EVAP instrument (Organomation Associate, Inc., MA, USA). Presence of saltwater in the samples significantly prolongs the evaporation process. Therefore it is advisable to avoid saltwater passing through the stopcock during drainage of the lower phase. The dried extracts were re-suspended in 4mL of THF containing 0.1% w/v BHT, poured in amber glass tubes (Processed VWR TraceClean 40ml vials with fluoropolymer resin-lined solid-top cap) and stored at -80°C. HPLC assay of each sample was taken as soon as possible within three days.

Protocol for extraction of lycopene from tomato Based on (Hyman 2001)

Adapted by Matthew Kinkade

IMPORTANT: All steps of this protocol should be executed in a fume hood and in dim lighting. Wear proper protection when working with the chemicals in this procedure.

1. Have the following solutions made prior to extraction:

    Extraction solvent: 1:1 methanol:tetrahydrofuran (THF) containing 0.1% butylated hydroxytoluene (BHT)

    Washing solution: 10% w/v NaCl (in H_2O)

    Dicholoromethane (DCM; for separation)

    Resuspension solution: THF containing 0.1% BHT

    Mobile phase: 9:1 methanol:THF containing 0.1% N,N-diisopropylethylamine (DIE)

2. Grind fruit using a Polytron (or equivalent) until skin fragments are 1 mm or less. Weigh out 1.0 g of puree and place into a large glass test tube. Add 10 mL extraction solvent to the puree and mix thoroughly. Cover tube tightly with parafilm and place puree mixture in -80°C overnight.

3. Next day, filter mixture through a Buchner funnel containing a 1.6 um GF/A filter (Whatman cat# 1820 055) into a 250 mL Erlenmeyer flask. Scrape pulp back into test tube, resuspend in 4-5 mL extraction solvent for ~1 min and pour back into funnel. Repeat as needed until pulp/solution is colorless. (Note: it is impossible to extract everything from the skin.)

4. Pour 50 mL 10% w/v NaCl into test tube, then pour into funnel to wash remaining carotenoids into the flask. Remove funnel and add 10 mL DCM. Mix thoroughly.

5. Pour mixture into a separating funnel suspended on a ring clamp. Mix, and invert funnel and open stopcock to release gasses. Agitate solution for ~30 s and allow to separate. If cloudy phase (NaCl) is orange or if orange solution remains on top, add more DCM and mix again. If orange color is at the bottom but solution isn't separating, add more NaCl.

6. Place clear phase (should be at bottom of funnel) into a clean test tube by opening stopcock. Discard the NaCl phase. (NOTE: <u>avoid</u> NaCl from getting into the tube. This greatly increases the drying time.

7. Dry samples under a stream of nitrogen w/ N-Evap. Resuspend dried extracts in 4 mL THF w/ 0.1% BHT. Store at -80°C in dark. According to literature, these extracts are stable @ -80 for ~30 days, but it is recommended that HPLC analysis be conducted asap.

8. When ready to conduct HPLC, filter the final extract with a syringe (through a 0.2 um PTFE syringe filter) into an amber HPLC vial.

9. Chromatographic conditions:

Use a 4.6 x 250 mm reverse-phase C_{18} (for total lycopene; for separation of *cis* and *trans* isomers, use C_{30}) Sunfire HPLC column (Waters; or equivalent) with a SunFire guard column (or equivalent) w/ guard column holder.

Injection volume: 10 uL.
Flow rate: 0.4 mL/min.
Column temp: 30°C.
Sample run time: 10 min (retention for lycopene & b-carotene is 7-8 min for C_{18})
Spectrum: 300 nm – 550 nm (lycopene should max absorb at ~480 nm.)
Mobile phase: 9:1 MeOH:THF (with 0.1% v/v N,N-diisopropylethylamine as additive)

Reference

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Curriculum Vitae
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Education

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University of Karaj- Iran, **M.Sc**, Plant breeding, 1993-1996
University of Azad, Esfahan – Iran, **B.Sc.**, in Plant breeding and agronomy 1990-1992
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Research Experience

Jan,2002 – Jul, 2002 Concordia University, Montreal - Canada
Molecular Biology Laboratory – Research associate

June,1999- Dec, 2001 National Research Center for Genetic Engineering and Biotechnology of Iran (NRCGEB), Tehran-Iran
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May,1993- Sep,1996 Seed and Plant Improvement Institute of Iran (SPII) Karaj-Iran
Research staff in wheat breeding program, breeding for wheat rust resistance.

Teaching Experience

- Plant Breeding and Genetics (Hort-407) Teaching Assistant, Spring Semesters 2003, 2004, 2005 and 2006 The Pennsylvania State University
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