DEVELOPMENT OF HIGH-YIELDING AND DISEASE RESISTANT PROCESSING TOMATO LINES USING MOLECULAR MARKER TECHNOLOGY

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ABSTRACT

DEVELOPMENT OF HIGH-YIELDING AND DISEASE RESISTANT PROCESSING TOMATO LINES USING MOLECULAR MARKER TECHNOLOGY

Fresh market and processing tomatoes are an important part of the daily diet. Processing tomatoes are used for tomato paste, ketchup, soup and drying. Processing tomatoes are grown under field and/or greenhouse conditions where abiotic (cold, drought, salt) and biotic (bacteria, fungi, viruses and nematodes) stress factors can affect yield and quality. Breeding programs aim to provide disease resistant lines with high quality fruits to farmers and the processing industry. Classical breeding and marker-assisted selection (MAS) are two important ways for development of novel inbred lines and tomato cultivars. Classical breeding is long term and based on phenotypic results while MAS provides genotypic information more easily and quickly. In this project, tomato lines were assessed for improved quality of various economic and agronomic features: fruit weight, external color, firmness, flowering, stem scar, fruit shape, wall thickness, yield, brix, internal color, number of flowers and number of fruits. F2 and F3 populations derived from high yielding tomato F1 hybrid MS1453 were scored for these phenotypic features. CAPs and SSR markers were used to genotype the plants and to determine QTL regions controlling the phenotypic features in the F2 population. In addition, disease resistance for Fusarium Crown and Root Rot, Tomato Mosaic Virus, Root Knot Nematode, Verticillium Wilt, and Tomato Yellow Leaf Curl Virus diseases were determined for 261 individuals to identify new sources of candidate disease resistance.

Keywords: Processing tomato, quantitative trait loci, marker-assisted selection, agronomic traits, disease resistance

ÖZET

MOLEKÜLER MARKÖR TEKNOLOJİLERİ UYGULAYARAK YÜKSEK VERİMLİ VE HASTALIKLARA DAYANIKLI VE TEKNOLOJİK ÖZELLİKLERİ İYİLEŞTİRİLMİŞ SANAYİLİK DOMATES ÇEŞİTLERİNİN GELİŞTİRİLMESİ

Sofralık ve sanayilik domatesler günlük diyetin önemli birer parçasıdır. Sanayilik domatesler salçalık, ketçaplık, çorbalık ve kurutmalık olarak kullanılmaktadır. Sanayilik domatesler tarla ve sera koşullarında yetiştirildikleri için abiyotik (soğuk, kuraklık, tuz) ve biyotik (bakteri, mantar, virüs ve nematod) stres faktörleri verim ve kaliteyi etkileyebilmektedir. Islah programları, çiftçiler ve sanayi kullanımı için hastalıklara dirençli yüksek kalitede meyveli hatlar geliştirmeyi amaçlamaktadır. Klasik ıslah ve markör destekli seleksiyon, yeni inbred hatların ve hibrit çeşitlerinin geliştirilmesinde iki önemli yoldur. Klasik ıslah uzun süreli ve fenotipik sonuçlara dayalı iken, moleküler destekli seleksiyon genotipik bilgiye dayalı olduğu için daha kolay ve hızlıdır. Bu çalışmada, çeşitli ekonomik ve agronomik (meyve ağırlığı, dış renk, sertlik, çiçeklenme, kabuk yarası, meyve şekli, duvar kalınlığı, verim, briks, iç renk, çiçek sayısı ve meyve sayısı) özellikleri arttırılmış domates hatları geliştirilmiştir. F2 ve F3 popülasyonları, yüksek verimli MS1453 F1 hibridinden geliştirilmiştir ve fenotipik özellikleri skorlanmıştır. CAPS ve SSR markörleri, bitkilerin genotiplerinin belirlenmesinde ve fenotipik özellikleri kontrol eden QTL bölgelerinin belirlenmesinde, F2 popülasyonunda kullanılmıştır. Ayrıca; Fusarium Kök ve Kök Boğazı Çürüklüğü, Domates Mozaik Virüsü, Kök-Ur Nematodları, Verticillium Solgunluğu ve Sarı Yaprak Kıvırcıklığı Virüsü hastalıkları, hastalığa dirençli yeni kaynakların belirlenmesi için 261 bireyde test edilmiştir.

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CHAPTER 1

INTRODUCTION

1.1. Tomato

1.1.1. Nutritional Importance And Consumption

The Solanaceae family is an economically important family that includes agricultural crops like cultivated tomato, *Solanum lycopersicum*. Tomato consumption can be grouped into two types: fresh fruits and various forms of processed products. Processed products of tomatoes are used as paste, diced tomatoes, whole peeled tomatoes, different forms of tomato juices or soups (Grandillo et al., 1999).Turkey has an important role in the total world production of tomato according to FAOSTAT 2012 statistics (Figure 1.1). Turkey is 4th for production of tomato as shown in Figure 1.

Tomato is grown in a large area of the world. Growing conditions of tomato can be adapted to the warmest and the coldest climates like the tropics and within a few degrees of the Arctic Circle.

The nutritional value of tomato is due to a wide variety of compounds including vitamin A, vitamin E and vitamin C, essential minerals,and antioxidants like lycopene which are important for protecting cells from oxidants (Foland et al., 2012; Sacco et al., 2013).

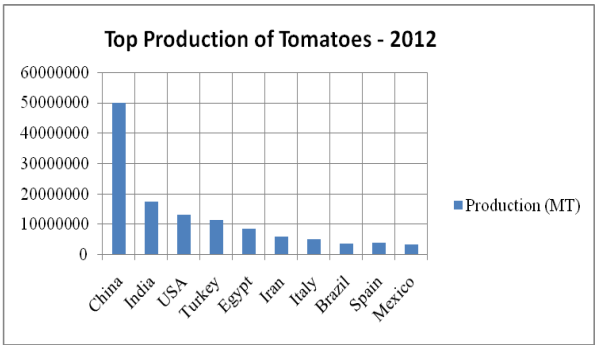

Figure 1.1. FAOSTAT values for total production of tomatoes in the world. (Source: FAOSTAT, 2012)

1.1.2. Origin

The origin of tomato is Western South America including Bolivia, Chile, Colombia, Ecuador and Peru. Domestication is thought to have occurred in Central America. The history of tomato domestication is not a clear process (Sims, 1979).

The advanced domestication stage of tomato occurred in Europe in the 15th century (Nowicki et al., 2013). The domestication process continued in the 18th and 19th centuries and *S. lycopersicum* has been produced since the 20th century. Since breeding of tomato started in the 20th century, different characteristics have been investigated with the aim to improve features (Bai and Lindhout, 2007) such as external and internal fruit color, firmness, fruit shape, fruit weight, stem scar, fruit size, locule number and fruit wall thickness. The closest wild ancestor of tomato, *S. pimpinellifolium,* has been used for improvement of modern tomato (*S. lycopersicum*). It has a mosaic genome from *S. lycopersicum* and *S. pimpinellifolium* (Pascual et al., 2013).

Growing in a wide area including different environmental conditions is an advantage for tomato (van der Knaap et al., 2014). However it has a lack of genetic variation in biodiversity because of the processes of domestication and breeding. In general, consumers prefer large fruits and mechanical harvesting requires proper shape and size; therefore, breeding has caused decreased biodiversity for domestication syndrome traits (Tanksley and McCouch, 1997).

1.1.3. Tomato As A Model Organism

The Solanaceae family contains more than 3000 species (Knapp, 2002) including tomato, potato, pepper, tobacco, and eggplant (Ranjan et al., 2012) and these species are found both in the Old World and the New World (Knapp, 2002). Information about the tomato genome can be used for the other species in the Solanaceae family.

Tomato is a diploid species and has a small genome (∼0.95 pg/1C, 950 Mbp) with 2n=24 chromosomes and this is an advantage for researchers (Fooland, 2007). Also *S. lycopersicum* members are easily transformed and tomato was the first genetically engineered and marketed variety in the US. The genomic sequence of

tomato adds a powerful resource for genetic studies. Thus, tomato has well-developed genetic and genomic tools (Sacco et al., 2013).

Tomato's short life-cycle and photoperiod insensitivity are also excellent traits for a model organism. The breeding potential of tomato is high and hybridization and pollination of tomato can be controlled (van der Knaap et al., 2014). Tomato has unique, agronomically important features such as climacteric fruit which is important for fruit development studies (Alexander and Grierson, 2002). Sympoidal shoot growth (sympodial shoot formation after 8-10 leaves causes a growth pattern with zigzag flower placement) (van der Knaap et al., 2014) and compound leaves are features that other model plant species do not have (Kimura and Sinha, 2008). All of these traits make tomato a model system for fleshy fruit development and composition (Giovannoni, 2004).

1.1.4 Tomato Species

Neutral forces which are based on genetic drift and gene flow may decrease cultivated plant diversity compared to wild relatives. Selection in nature often causes differential loss of genetic diversity in targeted genomic regions (Olsen and Wendel, 2013). When cultivated tomato lines are crossed with wild tomato lines, variation increases and the genetic pool is expanded (Tanksley and McCouch, 1997). Thus, interspecific introgression increases diversity (Olsen and Wendel, 2013). Wild species are important as reservoirs for disease resistance genes. Breeding programs for developing improved cultivars require genes from wild species for higher quality and by intercrossing, new cultivars can be developed (Kalia and Palanisamy, 2014; Bai and Lindhout, 2007).

S. lycopersicum has 12 close wild relatives (Sacco et al., 2013) but only *S. lycopersicum* is domesticated (Foolad and Panthee, 2012; Ranjan et al., 2012). These species can be both self-compatible and incompatible. Table 1.1 indicates self-compatible and incompatible species (Sacco et al., 2013).

S. pimpinellifolium is one of the small-fruited wild relatives of *S.lycopersicum* and mostly grows in Ecuador and Peru. It is a great resource for breeders since it is closely related with *S. lycopersicum*; therefore, it can be easily and successfully used in

hybridization. Diversification caused by environmental differences affect *S. pimpinellifolium* as smaller leaves, large leaf/mass area, and thicker leaves. These properties result in a higher transcription rate and are related with drought tolerance of this species (Nakazato et al., 2008; Zuriaga et al., 2009).

Table 1.1. Compatibility of wild tomato species.
(Source: Peralta and Spooner, 2000)

SELF-COMPATIBLE SPECIES	**SELF-INCOMPATIBLE SPECIES**	**SELF-COMPATIBLE AND SELF-INCOMPATIBLE SPECIES**
S. cheesmannii	*S. chilense*	*S. hirsutum*
S. chimielewskii	*S. peruvianum*	*S. pennellii*
S. lycopersicum		
S. parviflorum		
S. pimpinellifolium		

1.2. Marker-Assisted Selection

Classical breeding has been used for many years. However, it has a drawback in that the phenotype of some traits can be affected by environmental conditions. In such cases, classical breeding which is based on phenotypic selection can result in incorrect selection. Preventing this kind of mistake is possible with genetic information about individuals (genotype). Mapping with markers provides excellent information about the genetic background of a population (Gilmartin and Bowler, 2002). Selecting individuals with desired traits is easy and quick with marker-assisted selection (MAS). MAS uses molecular genetic markers which are linked to the trait of interest (Grandillo et al., 1999). Genetic variation of traits may be controlled by one gene (monogenic), few genes (oligenic) or multiple gene (polygenic) (Collard et al., 2005). It is easier to use monogenic traits for breeding because polygenic traits may not follow Mendelian segregation (Tanksley, 1993). Technologic and agronomic characters can be affected by alleles at multiple loci; therefore, identification and isolation of genes that plays role in domestication process is hard (Frary et al., 2000). By using molecular marker technology, genetic variations of individuals can be determined by linkage mapping and genome-wide association studies (Pascual et al., 2013).

Molecular markers include SSR, RFLP, RAPD, and AFLP markers, all of which are used in genome mapping. Molecular markers are mostly used for molecular linkage mapping (Gilmartin and Bowler, 2002). DNA markers can be used for determining polymorphism (Gilmartin and Bowler, 2002). Polymorphism between individuals is detected with co-dominant and dominant markers. Co-dominant markers have an advantage in detecting both heterozygote and homozygote individuals. But dominant markers can not distinguish heterozygote and dominant homozygote individuals (Collard et al., 2005). One of these co-dominant markers is cleaved amplified polymorphic sequences (CAPS). CAPS markers are based on single nucleotide polymorphisms. CAPS is a PCR based marker system and only a little amount of DNA is enough for analysis (Yeam et al., 2005). For tomato, two popular CAPS markers are COS (conserved ortholog set) and COSII (conserved ortholog set II) markers (Van Deynze et al., 2007).

Large numbers of Simple Sequence Repeats (SSR) are found in plant genomes. SSRs or microsatellites are tandem repeats with of 06 bp or less. SSR markers are co-

dominant, simple, PCR based and highly reproducible markers. However, they are expensive to establish, have a long development time and need specific primers (Jones et al., 2009)

InDels (insertion-deletion) or DIPs (deletion-insertion polymorphisms) are short length polymorphisms (Pepinski et al., 2013). Transposable elements, unequal crossover and slippage in simple sequence replication can result in the presence or absence of a short (typically 1-50 bp) sequence referred as InDels which are PCR-based, co-dominant markers (Britten et al., 2003).

For linkage maps, normally all chromosomes are screened and this step is important for Quantitative Trait Loci (QTL) mapping (Gilmartin and Bowler, 2002). A comprehensive molecular linkage map of tomato enabled identification of QTLs to understand the genetic basis of a great number of quantitative traits (Ranjan et al., 2012). Different research groups from all over the world showed different QTLs for tomato fruit color (Bernacchi et al., 1998; Tanksley and Nelson, 1996; Paterson et al., 1990), fruit firmness (Bernacchi et al., 1998), fruit shape (Chen et al., 1999), fruit weight (Tanksley and Nelson, 1996; Goldman et al., 1995), soluble solid content (Bernacchi et al., 1998; Tanksley and Nelson, 1996; Goldman et al., 1995) and pH (Tanksley and Nelson, 1996).

As explained before there is a lack of polymorphism in cultivated tomato. In order to map a high number of traits in domesticated and wild type tomatoes, advanced backcross mapping populations are generated by breeders which also allowtransfer of favorable QTLs from wild type to cultivated tomato germplasm (Foolad and Panthee, 2012; Ranjan et al., 2012).

QTL identification is a powerful way to increase yield. The availability of increased genome sequence will allow easier QTL identification, mapping and cloning of genes (Ranjan et al., 2012). Molecular marker technology can be used for QTL identification and marker-assisted selection for breeding cultivars with economically important traits and disease resistant lines (Foolad and Panthee, 2012; Ranjan et al., 2012). In our project we used MAS features to determine the disease resistance of tomato lines with good technological features.

1. 3. Tomato Diseases

Several abiotic and biotic stresses affect productivity and yield of tomato plants (Kalia and Palanisamy et al., 2014). Pathogens that affect plants and cause biotic stress are viruses, bacteria, nematodes, fungi and oomycetes. Pathogens may attack the plant's fruits, leaves or roots and this attack may start below or above ground. Disease stages are affected by environmental conditions. Also pathogens may need certain stages of plant growth which show susceptibility. Generally older plants are more resistant to diseases. Physiologically, diseases cause symptoms by changing hormone regulation, photosynthesis level, water uptake and transport, reproduction and/or normal metabolism (Roberts, 2007).

Plant diseases cause economic losses, famine and environmental pollution all around the world. For a long time, diseases have been creating huge effects in different countries (Roberts, 2007). In Europe, two million people died and others migrated because of the biotic stress factor *Phytophthora infestans* which affects potato and 80% of potato fields were lost between 1845 and 1850 (Borém and Fritsche-Neto, 2012). *Helminthosporium maydis* caused important yield losses in corn fields in the 1970's by the disease corn leaf blight. Soybean Asian Rust is another example of the great effects that may cause reduction in crop production. In Brazil, 37-67% of soybean yield was lost because of this fungus and economically and environmentally negative effects were shown because of the disease (Borém and Fritsche-Neto, 2012).

These examples show that disease resistant lines need to be developed to prevent economic losses caused by decreased yield. It is also important for the environment to avoid negative effects of chemicals like pesticides (Borém and Fritsche-Neto, 2012). Moreover, chemical application provides limited protection because chemicals are expensive and wrong application techniques have negative effects on the environment. For economic and safety reasons, resistant crop breeding is a better alternative (Kaur et al., 2014).

Both classical breeding and molecular marker analysis can be used to increase disease resistance, improve yield traits and use cultivated tomato and wild relatives of tomato for new cultivars. Markers specific to a chromosome region can be used for developing varieties adapted to new agricultural and processing technologies (Grandillo et al., 1999).

1.3.1. Fusarium Crown And Root Rot

Fusarium Crown and Root Rot (FCRR) is caused by a fungus and is a serious soil borne disease. *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) causes FCRR. Resistance to FCRR is not a common feature for tomato lines. Only a few tomato lines or commercial tomato cultivars are resistant to FCRR (Foolad and Panthee, 2012). FORL causes major economic losses in tomato species in Turkey and worldwide. Chemicals againts Fusarium diseases are not practical (Arici et al., 2013).

The *Frl* gene was identified and mapped as a single resistance gene to provide protection to FCRR, on tomato chromosome 9, closely linked to the *Tm-2*^{2} gene (Foolad and Panthee, 2012). FORL is distinct from Fusarium Wilt agent *F.oxysporum* f. sp. *lycopersici*. Symptoms, disease development and physiology have different characteristics for these two agents. While FCRR is favoured below 20°C, Fusarium Wilt is favoured by temperatures ~27°C. FORL shows pathogenic effect on a wide spectrum of plant families: Chenopodiaceae, Cucurbitaceae, Leguminosae, and Solanaceae, but *F. oxysporum f. sp. lycopersici* is host-specific to members of the genus Solanum (Huang et. al, 2013).

1.3.2. Root-Knot Nematodes

Tomato production can be limited by Root-Knot Nematodes (RKN, *Meloidogyne* species) that prevent water and nutrient uptake and damage roots. Damaged roots are more susceptible to infection by other plant pathogens (Kaur et al., 2014).

Resistance to RKN can be detected by using the *Mi* gene specific marker *Mi23* (Danso et al., 2013). Resistance genes for RKN originate from wild tomato (*S. peruvianum* L.) and have a wild range for different RKN species like *M. incognita*, *M. javanica* and *M. arenaria*. These RKN species harm tomato cultivation worldwide (Kaur et al., 2014).

1.3.3. Tomato Mosaic Virus

Tomato Mosaic Virus (ToMV) is the most infectious virus disease in tomato. Three genes control ToMV: *Tm-1*, *Tm-2* and *Tm-2*^{2}. *Tm-1* has been mapped to chromosome 2 (Foolad and Panthee, 2012). *Tm-2* and *Tm-2*^{2} genes are found on chromosome 9 close to the centromere (Shi et al., 2011).

1.3.4. Tomato Yellow Leaf Curl Virus Disease

TYLCV disease is due to infection of a plant with different phylogenetically related Begomovirus species (Verlaan et al., 2013). These species produce similar symptoms but their host ranges are different. TYLCV can infect other crops like pepper and common bean (Luna et al., 2012).

One of the major resistance genes for Begomoviruses, *Ty-1*, was identified (Butterbach et al., 2014). *Ty-3* is an allele of *Ty-1*. *Ty-1* and *Ty-2* are dominant genes and provide high resistance to plants; however, *Ty-3*, *Ty-4* and *Ty-5* genes have partial effects on resistance to TYLCV (Hutton et al., 2013).

1.3.5. Verticillium Wilt

The Verticillium Wilt pathogens, *Verticillium dahliae* and *V. albo-atrum*, cause fungal vascular wilt disease and are soil borne pathogens. More than 200 dicotyledonous plant species suffer from Verticillium Wilt.

The *Ve* locus is responsible for resistance to Verticillium Wilt. The *Ve* locus includes the closely linked, inversely oriented genes *Ve1* and *Ve2* (Fradin et al., 2014). These genes were mapped in chromosome 9 in *S. lycopersicum* (Simko et al., 2003).

These genes are highly homologous and encode extracellular leucine-rich repeat containing cell surface receptors of receptor-like protein (eLRR-RLP) class (Fradin et al., 2014).

1.4. Technological And Agronomical Traits For Tomato

For consumers, the quality of tomato is defined by fruit flavor and attractiveness of fruit. Physical traits such as fruit color, shape, size and firmness provide attractiveness. Fruit flavor includes chemical traits like aroma and taste components which are based on the amount of sugars, acids and volatile compounds. For processing tomato, distributors and retailers care mostly about fruit soluble solids content and pH (Labate et al., 2007).

Chemical components of tomato include dry matter weight, sugar content, titratable acidity, pH, several aroma volatiles and pigments. More than 400 volatiles add taste as aroma to tomato fruit and aroma volatiles are one of the traits that change with environmental conditions (Causse et al., 2002).

Total soluble solids (TSS) is a quality trait of tomato and described with degrees Brix (Bx). Higher TSS amount is desired by processors and reduces the costs associated with processing tomatoes. Yield is inversely proportional with TSS. Breeding programs take into account other traits that negatively affect sugar content. Another negative effect arises from harvesting because fruits are collected before full-ripe stage and the sugar supply for the fruit is lost. Wild tomato species may have two or three times more sugar in their fruits (Sacco et al., 2013; Beckles et al., 2012). QTL regions for Brix are *Brix9-2-5*. *Brix9-2-5* genes increase sugar yield in tomatoes. Polymorphism of *Brix9-2-5* changes functionality by changing invertase enzyme kinetics (Fridman et al., 2004).

Technologically and agronomically important traits may be polygenic and, like fruit weight, the genes controlling such traits may have multiple effect on different aspects of fruit development (Alpert and Tanksley, 1996). Fruit weight is polygenic and 5-20 genes are thought to play role in this trait. One of the major genes for fruit weight is *fw2.2* (Alpert and Tanksley, 1996; Grandillo et al., 1999; Paran and van der Knaap, 2007). *fw2.2* is an important region and has different effects on physical traits. Fruit development, size, weight (Alpert and Tanksley, 1996) and shape (Nesbitt and Tanksley, 2001) can be affected by *fw2.2*. *fw2.2* is expressed early in floral development (Tanksley, 2004) encodes a member of the Cell Number Regulator gene family (Knapp et al., 2014). *fw2.2* is on tomato chromosome 2 (Alpert and Tanksley, 1996). It was identified and cloned as the first QTL in tomato by Frary et al. (Frary et al. 2000). Organ size and number are controlled by *fw.2.2* related genes in different plants. In

maize CRN1 is an ortholog to *fw2.2*, in avocado transcription of the *fw2.2*-like gene *Pafw2.2* controls organ cell number. Metal transportation is thought to be affected by *fw2.2*-like genes which also differentiate fruit shape and fruit mass (Zhang, 2012). Also fruit weight 11.3 (*fw11.3*) and fasciated (*fas*) were mapped to the same region of chromosome 11 and have major effects on fruit size of tomato (Huang et. al, 2011). Fruit size QTL have been identified using *S. lycopersicum* and *S. pimpinellifolium* and characterized (Ranjan et al., 2012). The other identified fruit weight QTL is *fw3.2* (Grandillo et al., 1999; Knaap et al., 2007). The allele of cultivated tomato has a dominant effect and gives rise to enlarged fruit. Studies showed that *fw3.2* primarily affects and controls fruit weight and also has minor effect on fruit shape. Effects of genes for fruit weight and shape are based on domestication and diversification process. Fruit mass is decreased by effect of the alleles from wild species (Alpert and Tanksley, 1996). Zhang et al. (2012) studied on identification of molecular mechanism of fruit weight by fine mapping of the *fw3.2* region which comprised seven candidate genes. *fw3.2* was identified by Chakrabarti et al. (2013). *fw3.2* encodes KLUH which is the ortholog of P450 enzyme (van der Knaap et al., 2014). *fw3.2* is on chromosome 3 (Zhang, 2012; Chakrabarti et al., 2013).

Another technological and agronomical trait is degree of fruit firmness. Marketable fruits have firmness value above 1.45 N mm^{-1}. Changes in firmness are related to fruit appearance in tomato and alter shelf life (Batu, 2004). Firmness is a polygenic trait that is due to cell wall structure, cuticle properties and turgor. Therefore, many genes are co-regulated to determine firmness. Firmness QTL were mapped to chromosome 2 by Chapman et al. (2012) in *Solanum pennellii*. Chapman et al. used *S. pennellii* introgression lines and tried to identify QTL regions for firmness and found several major regions. *Fir*$^{s.p.}$*QTL2.5* and *Fir*$^{s.p.}$ *QTL2.2* are some of these candidate genes (Chapman et al., 2012).

Yield improvement is achieved by a single over dominant gene (single flower truss) with heterosis in tomato (Ranjan et al., 2012). Yield is increased by heterosis from 30% to 400%. Heterosis also affects other quantitative and qualitative traits (Bhatt et al., 2001).

Heterosis (hybrid vigor) is the occurrence of hybrids with more vital, adaptive traits than their parent (Soliemana et al., 2013). Heterosis is based on the action or interaction of the genes or alleles (Georgiev, 1991). Tomato shows heterosis and

different investigators have studied it but the process is still unclear (Soliemana et al., 2013). Interaction and linkage between genes and the effects of the environment make it harder to understand the process (Georgiev, 1991). Heterosis breeding provides a great opportunity to get high yielding, high quality individuals with desired traits in one generation and may take less time than other breeding methods (Pemba Sherpa et al., 2014). In 2001, Bhatt et al. (2001) studied yield, ascorbic acid and total soluble solids and found positive highly significant heterosis for these traits. F1 hybrids are mostly preferred by breeders because of heterosis and also F1 hybrids also provide crop uniformity and protect against illegal reproduction (Bai and Lindhout, 2007).

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials

An F2 population including 167 individuals was developed by selfing the certified F1 hybrid MS1453. This population was used for QTL mapping of agronomic traits. The genotypes of the F2 lines screened and listed in Table 2.1 were grown in field conditions in Menemen (Izmir), in summer 2013 and tested for agronomic and technologic traits. F3 lines were also grown in the field in Manisa in summer 2014 and agronomic and technologic traits were measured.

In the second part, 261 inbred lines collected from Prof. Dr. Sami Doğanlar (Table 2.2) were screened for disease resistance by CAPS markersThen 60 individuals were selected from the 261 individuals (Table 2.3) were measured for further disease resistance by High Resolution Melting (HRM) analysis.

Table 2.1. The F2 mapping population consisting of 167 individuals.

12T771-1	12T771-41	12T771-86	12T771-130	12T771-182
12T771-2	12T771-43	12T771-87	12T771-131	12T771-183
12T771-3	12T771-44	12T771-89	12T771-132	12T771-185
12T771-4	12T771-46	12T771-90	12T771-133	12T771-186
12T771-5	12T771-47	12T771-91	12T771-137	12T771-187
12T771-6	12T771-48	12T771-93	12T771-138	12T771-188
12T771-7	12T771-49	12T771-95	12T771-140	12T771-189
12T771-8	12T771-50	12T771-96	12T771-144	12T771-191
12T771-9	12T771-51	12T771-97	12T771-145	12T771-192
12T771-10	12T771-52	12T771-98	12T771-146	12T771-193
12T771-11	12T771-53	12T771-99	12T771-147	12T771-195
12T771-12	12T771-54	12T771-100	12T771-148	12T771-196
12T771-13	12T771-55	12T771-102	12T771-149	12T771-197
12T771-14	12T771-56	12T771-103	12T771-150	12T771-198
12T771-15	12T771-57	12T771-104	12T771-151	12T771-200
12T771-17	12T771-58	12T771-105	12T771-153	12T771-201
12T771-18	12T771-59	12T771-106	12T771-156	12T771-202
12T771-19	12T771-60	12T771-107	12T771-158	12T771-203
12T771-20	12T771-62	12T771-108	12T771-159	12T771-204
12T771-21	12T771-63	12T771-109	12T771-160	12T771-205
12T771-22	12T771-64	12T771-110	12T771-161	12T771-206
12T771-23	12T771-65	12T771-111	12T771-163	12T771-207
12T771-24	12T771-66	12T771-112	12T771-164	12T771-208
12T771-26	12T771-68	12T771-114	12T771-166	12T771-209
12T771-27	12T771-69	12T771-117	12T771-167	12T771-210
12T771-28	12T771-71	12T771-118	12T771-169	12T771-211
12T771-30	12T771-72	12T771-119	12T771-170	12T771-212
12T771-31	12T771-73	12T771-120	12T771-172	
12T771-32	12T771-74	12T771-122	12T771-173	
12T771-34	12T771-76	12T771-123	12T771-176	
12T771-35	12T771-79	12T771-124	12T771-177	
12T771-36	12T771-82	12T771-125	12T771-178	
12T771-37	12T771-83	12T771-126	12T771-179	
12T771-38	12T771-84	12T771-128	12T771-180	
12T771-40	12T771-85	12T771-129	12T771-181	

Table 2.2. List of 261 candidate donor materials to develop next generations.

12S337	12S426	12S497	12S575
12S338	12S427	12S498	12S576
12S339	12S428	12S500	12S577
12S340	12S429	12S502	12S578
12S341	12S430	12S503	12S579
12S342	12S431	12S505	12S580
12S343	12S432	12S506	12S581
12S344	12S433	12S507	12S582
12S345	12S434	12S508	12S583
12S346	12S435	12S509	12S584
12S347	12S436	12S510	12S585
12S348	12S437	12S511	12S587
12S349	12S438	12S514	12S588
12S351	12S439	12S520	12S589
12S352	12S440	12S521	12S590
12S353	12S441	12S522	12S591
12S354	12S442	12S523	12S592
12S355	12S443	12S524	12S593
12S356	12S444	12S525	12S594
12S357	12S445	12S526	12S595
12S358	12S446	12S527	12S596
12S359	12S447	12S529	12S597
12S360	12S448	12S530	12S598
12S361	12S449	12S531	12S599
12S362	12S450	12S532	12S600
12S363	12S451	12S533	12S601
12S364	12S452	12S534	12S602
12S365	12S453	12S535	12S603
12S366	12S454	12S536	12S604
12S367	12S455	12S537	12S606
12S368	12S456	12S538	12S607
12S388	12S457	12S539	12S609
12S389	12S458	12S540	12S610
12S390	12S459	12S541	12S612
12S391	12S460	12S542	12S613
12S392	12S461	12S543	12S614
12S393	12S465	12S544	12S615
12S394	12S466	12S545	12S616
12S395	12S467	12S546	12S617
12S396	12S468	12S547	12S618
12S397	12S469	12S548	12S619

(Cont. on the next page)

Table 2.2. (cont.)

12S398	12S470	12S549	12S620
12S399	12S471	12S550	12S621
12S400	12S472	12S551	12S623
12S401	12S473	12S552	12S624
12S402	12S474	12S553	12S625
12S403	12S475	12S554	12S626
12S404	12S476	12S556	12S627
12S405	12S477	12S557	12S628
12S406	12S478	12S558	12S629
12S407	12S479	12S559	12S630
12S410	12S480	12S560	12S631
12S411	12S481	12S561	12S632
12S412	12S482	12S562	12S633
12S413	12S483	12S563	12S635
12S414	12S484	12S564	12S636
12S415	12S485	12S565	12S638
12S416	12S486	12S566	12S639
12S417	12S487	12S567	12S640
12S418	12S490	12S568	12S641
12S420	12S491	12S569	12S642
12S421	12S492	12S570	12S644
12S422	12S493	12S571	12S645
12S423	12S494	12S572	
12S424	12S495	12S573	
12S425	12S496	12S574	

Table 2.3. List of 60 donor materials selected from 261 inbred lines to measure disease resistance with HRM analysis.

LINE	PEDIGREE NUMBER	DISEASE	LOCATION
12T065	SD1217	Tm2a, Fr1 NIL --> SD209	IYTE
12T066	SD1215	Ty-1 NIL --> SD209	IYTE
12T097	SD1170	I2, Pto, Tm2a NIL --> SD209	IYTE
12T099	SD1214	Ty-1 /Ty-3 NIL --> SD209	IYTE
12T112	SD2822	SD1138, j2, Py1, Tm2a, Pto, l2, og	IYTE
12T117	SD2993	SD523+SD11 60+j2+Tm2a+Pto	IYTE
12T118	SD3300	SD1138, Tm2a, l2, Pto, Sw5, J2	IYTE
12T119	SD3314	SD523, SD1138, l2, Tm2a, j2, Pto	IYTE
12T121	SD3299	RIN, SD1138, 12, Tm2a, Mi, J2	IYTE
12T124	SD3181	SD523, SD1138, Tm2a, J2, l2	IYTE
12T125	SD3000	SD523, SD1160, j2, Tm2a, Pto, l2	IYTE
12T129	SD2050	I2, Pto, Mi, j2, og NIL --> SD209	IYTE
12T130	SD1253	Tm2a, Pto, I2, Mi(long) --> SD209	IYTE
12T143	SD1168	I3 NIL --> SD209	IYTE
12T144	SD1216	Ty-1 --> NIL in SD209	IYTE
12T151	SD1154	Lv (long)--> SD209 NIL	IYTE
12T156	SD3007	SD523, SD1160, j2, Tm2a, Pto, Mi, l2	IYTE
12T158	SD2049	I2, Pto, Mi, j2, og NIL --> SD209	IYTE
12T165	SD1433	Tm2a, Pto, j2 NIL --> SD209	IYTE
12T166	SD1431	I2, Pto, j2 NIL --> SD209	IYTE
12T171	SD3315	Sw5, SD1138, Mi, Tm2a, Pto, J2, l2	IYTE
12T172	SD3188	Tm2a, SD1138, pto, J2, l2, Py1	IYTE
12T176	SD3191	SD1138, Mi, Tm2a, Sw5, Pto, J2	IYTE
12T196	SD3142	BW, Tm2a	IYTE
12T197	SD3188	Tm2a, SD1138, pto, J2, l2, Py1	IYTE
12T198	SD2828	SD1138 + j2, og, Py1, Tm2a, Mi	IYTE
12T199	SD1392	Tm2a/Tm2a; (j2/j2) NIL --> SD209	IYTE
12T201	SD1382	Mi (short) --> SD209	IYTE
12T204	SD1146	I2, Pto, Mi NIL --> SD209	IYTE
12T208	SD1386	Mi(s), I2, Pto, Tm2a --> SD209	IYTE
12T209	SD1147	Tm2a, Sw5 --> SD209	IYTE
12T213	SD1429	SD209 NIL: Tm2a, I2, Pto, j2	IYTE
12T214	SD1142	I2, Pto NIL --> SD209	IYTE
12T223	SD2827	SD1138 + j2, og, Py1, Tm2a, Mi	IYTE

(Cont. on the next page)

Table 2.3. (cont.)

12T224	SD1213	Ty-1 /Ty-3 --> NIL in SD209	IYTE
12T228	SD2814	SD1138, j2, Pto, l2, og, Py-1, Tm2a, Mi	IYTE
12T230	SD2831	SD1160 + j2, Sw5, Py1	IYTE
12T231	SD1430	SD209 NIL: I2, Pto, j2	IYTE
12T232	SD1153	Lv NIL --> SD209	IYTE
12T234	SD1596	SD1451+Pto, I2, j2 NIL	IYTE
12T246	SD2068	SD209 NIL + j2, I2, Pto, Mi, Sw5, og	IYTE
12T247	SD2115	SD1451 + I2, Pto, Mi, j2	IYTE
12T248	SD2083	SD209 NIL w/ og, j2, I2, Pto, Mi, Lv	IYTE
12T250	SD2048	I2, Pto, Mi, j2, og NIL --> SD209	IYTE
12T252	SD2086	SD209 NIL w/ og, j2, I2, Pto, Mi	IYTE
12T255	SD2093	SD1451 + Mi, j2, Pto, I2, og	IYTE
12T257	SD2518	SD1451 + j2, Pto, I2, Tm2a	IYTE
12T288	SD03-102-2	SD1138; Mi, Tm2a, Sw5, J2, Pto	IYTE
12T401	SD2856	SD1138; Tm2a, I2=3; Lv, Sw5	IYTE
12T034	MS1453 F1	Sanayilik Hibrit	POLEN
12T033	SD06-182	Sanayilik Safhat	IYTE
12T008	SD3047	Cf9	TGRC
12T011	SD4025	I, I2, I3	TGRC
12T019	SD3471	Frl, I, I2, Mi, pyI, Tm2a, Ve	TGRC
12T022	SD4026	I, I2, I3, j2	TGRC
12T029	SD4285	I, I2, Ph3, Sm, Tm2	TGRC
12T090	SD1168	I3 NIL --> SD209	IYTE
12T098	SD1169	(I2, Pto, Mi-short) NIL --> SD209	IYTE
12T203	SD1145	Pto-h NIL --> SD209	IYTE
12T211	SD1381	Mi (short) NIL --> SD209	IYTE

2.2. Methods

2.2.1. Phenotypic Characterization

F2 and F3 populations were scored for economically and agronomically important traits: number of fruits, total weight of fruits, fruit shape, external color, internal color, firmness, stem scar, wall thickness, brix, locule number and flower number. Fruit weight (FW) was measured as the average weight of each ripe fruit for each individual. Fruit shape was detected by the ratio of fruit length to diameter. External and internal color were determined as intensity of the color. Fruit firmness was determined by hand squeezing. Stem scar size, fruit wall thickness and locule number were detected by eye. Soluble solid content was analyzed from tomato juice with refractometer. Traits were scored as follows: fruit shape (1 = round, 5 = elongated), external color and internal color (1 = yellow or orange, 5 = most intense red), firmness (1 = soft, 5 = very firm), stem scar (1 = small, 5 = very large), wall thickness (1 = thin, 5 = very thick). These traits were then analyzed for QTL identification as described below.

2.2.2. DNA Extraction

Leaves of tomatoes were collected in Eppendorf tubes and DNA extraction was performed. The DNA isolation method of Bernatzky and Tanksley (1986) was used. DNA samples were diluted with distilled water to ~50 ng/µl and controlled with nanodrop spectrophotometer and agarose gel image.

2.2.3. Genomic Characterization

The parents and 167 individuals of the F2 population were screened using CAPS, SSR and InDel markers. PCR based CAPS markers were used for determination of genotypes and genetic mapping.

Disease resistance to Fusarium Wilt (*I2* and *I3* genes), Root-Knot Nematode (*Mi-1* gene), Bacterial Spot Disease (*Pto* gene) and VerticilliumWilt (*Ve5* gene) of the 60 individuals listed in Table 2.3 were measured by HRM analysis.

A total of 261 individuals Table 2.2 were screened for six different diseases with nine different markers (Table 2.4). SNP based CAPS assays for disease resistance genes were developed from SNP markers (Shi et al., 2011; Garcia et al., 2007). MAS assays were done for Fusarium Crown and Root Rot, Tomato Mosaic Virus (Shi et al., 2011), Root Knot Nematode (Garcia et al., 2007; Zengin S., Antalya Tarım, Inc.), Verticillium Wilt (Shi et al., 2011), Tomato Yellow Leaf Curl Virus diseases (Zengin S., , Antalya Tarım, Inc.). *Ve2* Snp1 and *Ve2* Snp3 markers give the same result for disease resistance; therefore, all individuals were not tested with both markers.

The PCR protocol for CAPS markers was performed in a 25 µl reaction mix that included: 2 µl DNA (~50 ng/µl), 2.5 µl 10X PCR buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, pH: 8.3), 0.5 µl dNTP (0.2 mM), 0.5µl for each forward and reverse primers (10 pmol), 0.25 µl *Taq* polymerase enzyme (0.25U) and 18.75 µl distilled H_2O. Figure 2.1 shows the PCR profile for CAPS.

The PCR protocol (Figure 2.2) for SSR and InDel markers was performed in 20 µl reaction mix that included: 1 µl DNA (~50 ng/µl), 2.0 µl 10X PCR buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, pH: 8.3), 0.6 µl dNTP (0.2 mM), 0.45µl for each forward and reverse primers (10 pmol), 0.25 µl *Taq* polymerase enzyme (0.25U) and 15.25µl distilled H_2O. Figure 2.2 shows the PCR profile for SSR (Long et al., 2013) and InDel markers.

After PCR, the appropriate restriction enzyme was used for restriction digestion and to reveal polymorphic results for the CAPS markers. For this purpose 15 µl PCR product, 1.5 µl 10X restriction buffer (1X), 0.5 µl restriction enzyme and 3.0 µl distilled water were added to each amplified product. Incubation time was 4 hours and proper temperatures for each enzyme were applied. Table 2.5, Table 2.6 and Table 2.7 show the markers and restriction enzymes which were used for genetic mapping assays.

Restriction digestion products were separated on 3% agarose gels prepared with 1X TBE buffer and ethidium bromide and visualized under UV light.

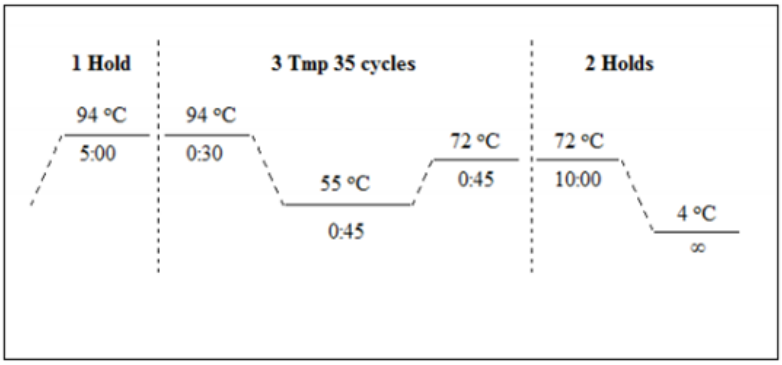

Figure 2.1. PCR profile for CAPS

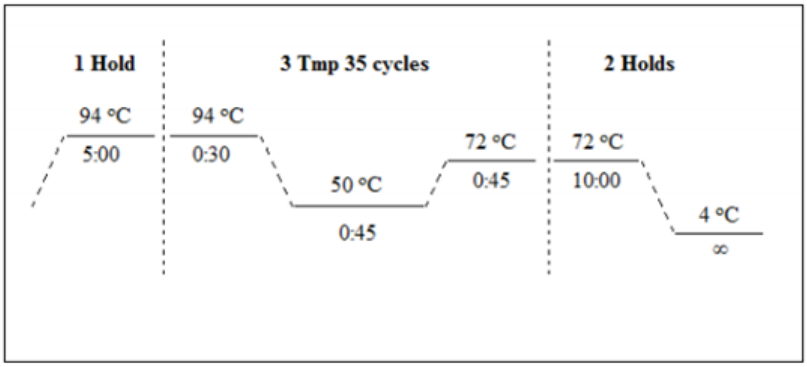

Figure 2.2. PCR profile for SSR and InDel

Table 2.4. CAPS markers and restiriction enzymes for screening disease resistance.

MARKER	ENZYME
Forl	PCR
Mi23	HpyCH4IV
TM2 Snp1	AluI
TM2 Snp2	BslI
Ty1	PCR
Ty3	PCR
Ve1 Snp3	Hpy166II
Ve2 Snp1	Hpy188III
Ve2 Snp3	Bsp1286I

Table 2.5. CAPS markers, primer sequences and restriction enzymes for genetic mapping.

MARKER	ENZYME	FORWARD SEQUENCE	REVERSE SEQUENCE
TG302	AluI	ATGGTTGCATTACTCAGTCAT TGCACTCTCCGGGTGGCTATT ACATTTTTGAAAGGAGTAATT ATTTCCCGCAAGAGAAGCACT TCCCTTATGTATAGAAATCAG TGACGTAAAATTTATAATCAA CATAAAACTCTAAAGTTTGGT TCTGGTGAAGTAACTGGAGTT AAATAATCCTACTAACACTCA TATCAGAGGGTTGGCTCCAAA CAAATTTCTCGGTCATGAAAA ACACCCATATAAGAGAAAGA ATAGACATCCTTATTTTTGGG GAAGGATTTTGGTTCCTATCA GTGAAATTTGGAATCATAGTT ATAAGATGAAAAAAATGAGT GGAAAATTACTTATTAAAAAA GAAAACAGGGGTGATACCAG CTTAGATGGATCGATTTAAGT GCTATAAGATGTAGAAGTATT TGCAAGGTGAAAGGACTCTCC CGCGAATTTATTAAAATAAAT TTACATTTATTGGTCACAA	AAAGAAGCAAGAACCATTTTAA TTAGGAAAGCATCTAGCTGAAA TTAGCGGCCAGAGGCACCACCT CTTGCAAGAAAAGATACTATAA CCCTAGCTAGAAAAAGTGCAAA TTTTTATGTAAACAGCATTGTCC AACAAAAATAAAGGAAGACTAA AAACAACATAAATCTAACTTGT TTGTCAGAACTAGATATCTCAA ATAAATGAAAGTTTGTCTTATTT CTACTAATTATCATCTGGCAACT GGCATTTCTATCCTAGTTGAATA ACCTGCAAGTTTATGAGCAAAT CTGATTGTTATCTTTTTATTTCTT TTTGGTGTAAATTGAATGAGTA AATGATTAGGAACTCCAATTAC AGTTCTTAGAAGCTTTTGCATAT GCTAGTCTTGGGACTCCTCCTTT TCTTTCCTTTTTTCTGTTCTCTTT TGGATGTAATGACAGCACTTTCT TAGTGCTAATTTTGACATCAATA ATATTAACCCTTCATAAA
At1g05350	ApoI	TGAACGAACCCTAAAGCGTGA AGG	TCCGAACTTCAACAAGTACTTCA ATGTG
At1g48300	ApoI	AAGAAGATGAAATTACTTAAG GGTTTG	TTTAGTGTTGCATTCTCAAGTGC TCG
At3g47990	PCR	AGAGAAGCAGTGGAGGCACT CATTC	AGAAAACCTTGCAACCTCAGCA G
At2g42750	BstuI	TCCAGTGCAAAGGAGAGTTTA TGATG	ACTCTAGCTCTTCCAAAGTCTTC CTC
At1g19530	DraI	AAACAGGCGAATATTGTATTT GAGG	AGCCATGTTGGCTACGTGAAAT TGTG
At1g19140	PCR	AGGCCCTTGTACTCAGTGCCT CTC	TCATGGCGGTTTCAGTCCATCC
At2g01720	DraI	ACAAATTGGTACATGCTGGTG CTC	TGGCCTGTTAGACTGATATTCAA C
At2g20860	DraI	ATTGAAGCCACATATACTCAT AGAAGC	TCCAGATTTTGCAACTTTCTCTA CAC
At1g55870	PCR	AAGTTCGCCGTCGTTCAGTTC G	ATTGGTTGCTACCAGGAATTTCT TG

Table 2.6. SSR markers used for genetic mapping.

MARKER	FORWARD SEQUENCE	REVERSE SEQUENCE
SSR14	TCTGCATCTGGTGAAGCAAG	CTGGATTGCCTGGTTGATTT
SSR276	CTCCGGCAAGAGTGAACATT	CGACGGAGTACTTCGCATTT
SSR3.171.1	CTAATATAGTAGAGTAGGAG TAAG	GCTCTAATGATAAGGAGAGA GTCTG
SSR3.7.1	GCTTTATTTG TGTTTCCTG	CAACTATCAC ATGATAATAA TTC
SSR320	ATGAGGCAATCTTCACCTGG	TTCAGCTGATAGTTCCTGCG
SSR7.54.1	CCATTTAGTA GAGATTTCAA TC	GCTCAAGTGT ATTTGTGAGT TTC
SSR8.87.5	GTGAAGTGGTAGCTCTTCAA TG	CTTACACATG CATTAGCATT CC
SSR9.94.1	CGGTGGAAACCTAGTATGTA TG	GTTAATTATC ATATTATTAT TCG

Table 2.7. InDel markers used for genetic mapping.

MARKER
Ychr11F2C
Ychr34
Ychr36
Ychr82
Ch1232
Ch715

2.2.4. Statistical Analysis

For genetic mapping to detect QTL regions, phenotypic (average fruit weight, external color, firmness, flowering, stem scar, fruit shape, wall thickness, yield, brix, internal color, number of flowers and number of fruits) and genotypic data for the F2 population were analyzed by QGENE (Nelson, 1997) program using single marker regression algorithm.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. QTL Identification Of Agro-Morphological Characteristics

F2 and F3 populations were scored for economically and agronomically important traits: number of fruits, total weight of fruits, fruit shape, external color, internal color, firmness, stem scar, wall thickness, brix, locule number and flower number. The mean for MS1453 F1 is shown with a star in histograms of F2 population. Phenotypic characterization of agronomically and economically important traits showed continuous distribution in the F2 and F3 populations.

Figure 3.1 shows fruit weight which varied from 18.7 g to 136.0 g in F2 lines. Average FW was 46.7 g. The trait had a normal distribution as expected for a quantitative trait. In the F3 lines, average fruit weight varied from 24 g to 155 g. Average FW was 58.1 g. F3 lines also had a normal distribution with a skew toward higher FW than observed in the F2. Fruit weight of MS1453 was measured as 67.8 g. Therefore, the F2 and F3 lines had transgressive segregation indicating that fruit weight can be increased in future candidate hybrids using these populations. FW of typical processing tomato lines sold in Turkey vary from 50 to 120 g with most weighing 80-90 g. Thus, our candidate lines show distribution among desired values.

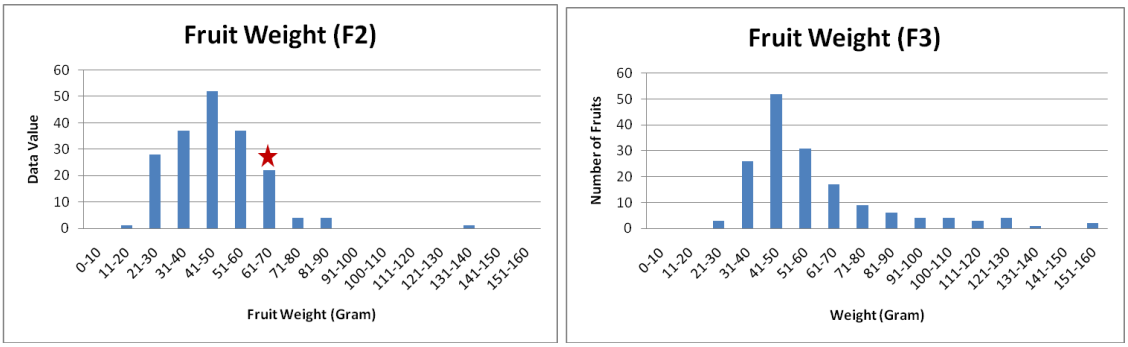

Figure 3.1. Segregation of fruit weight of F2 and F3 populations

Fruit shape varied from 1 to 4 in F2 lines (Figure 3.2). Mean value of individuals was 2.1. Therefore, most of the fruits were round. MS1453 had an average value of 2 meaning that fruit were slightly elongated. Shape of F3 fruits ranged between 1 and 5 with a mean value of 3.0. Compared to the F2, fewer fruits in the F3 population were round. Processing tomatoes can be round, smooth plum- or pear-shaped (Díez and Nuez, 2008).

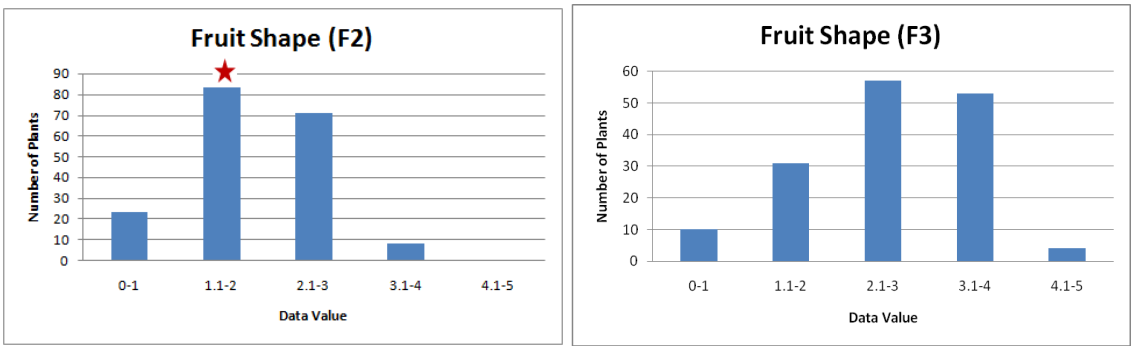

Figure 3.2. Segregation of fruit shape of F2 and F3 population

F2 lines had fruit firmness between 1 and 4 (Figure 3.3). Mean value of F2 lines was 2.4. MS1453 had firmer fruit with a mean value of 3.5. Fruit firmness of the F2 population showed continuous distribution. Firmness for the F3 lines segregated between 2 and 5. Mean value was 3.5, identical to the parental hybrid. Thus, firmness increased in the F3 population, a trait which is desired in processing tomatoes.

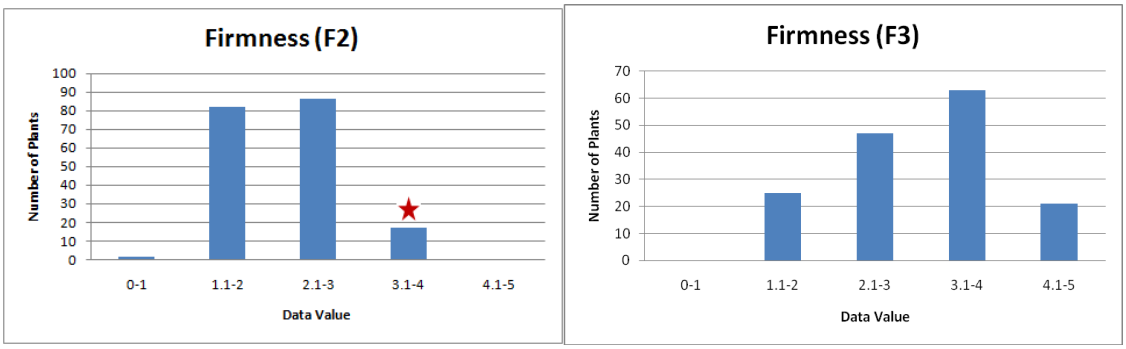

Figure 3.3. Segregation of firmness of F2 and F3 population

Stem scar value ranged from 1 to 5 with a mean value 1.7 in the F2 lines compared to 2.5 for MS1453 (Figure 3.4). For the F3 lines, mean value was 2.7 and individuals segregated between 1 to 5. Although stem scar was small in both F2 and F3

lines as expected for processing tomatoes, the increase in average scar size in the F3 was probably due to the overall increase in fruit weight in that population.

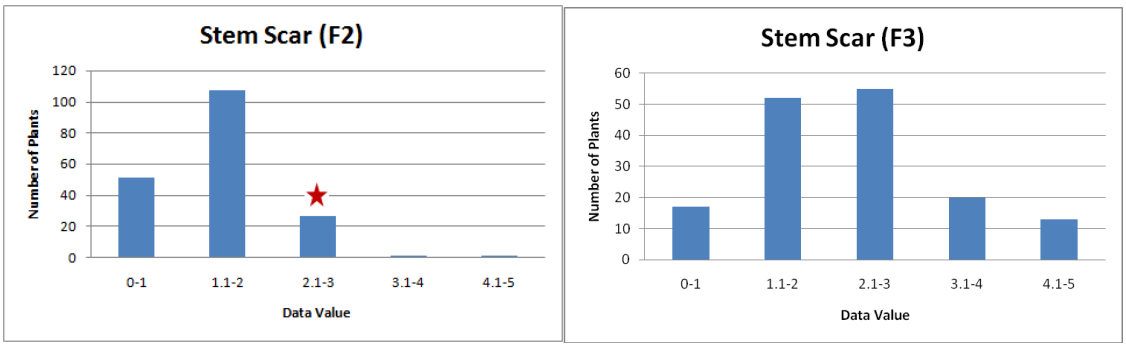

Figure 3.4. Segregation of stem scar of F2 and F3 population

MS1453 had scores of 4.5 for external (Figure 3.5) color and 4 for internal (Figure 3.6) color. In the F2 population, external color varied from 3 to 5 with a mean value of 4.6 while internal color ranged from 2.5 to 4.5 (mean 4.3). For the F3 lines, external color values ranged from 2 to 5 with a mean of 4.4 and internal color varied from 2 to 5 with a mean value of 3.7 in F3 lines. Thus, most F2 and F3 lines had red and dark-red color fruits. We expected this because the parents of MS1453 and MS1453 itself have good red color. External and internal color must be red for processing tomatoes because even a small amount of green tomatoes is enough to disturb aroma of tomato paste. Therefore, the tomato paste industry needs fully-grown, fresh, healthy, dark red tomatoes.

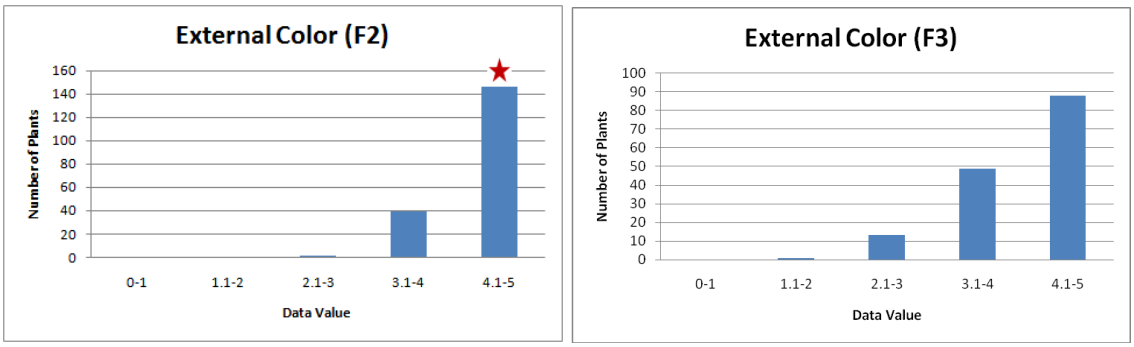

Figure 3.5. Segregation of external color of F2 and F3 population

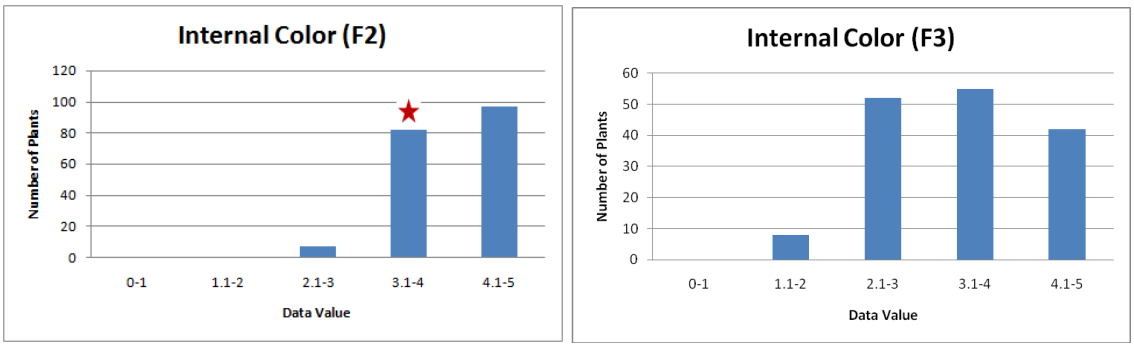

Figure 3.6. Segregation of internal color of F2 and F3 population

Mean value of locule number was 3.1. MS1453 has 3 locules (Figure 3.7). Locule number shows normal distribution and was low as expected for processing tomatoes.

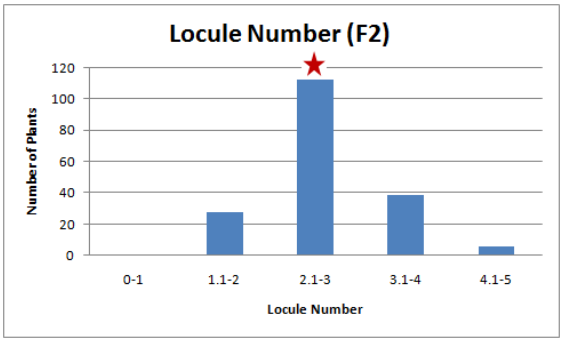

Figure 3.7. Segregation of locule number of F2 population

Wall thickness, also known as pericarp thickness, of the F2 lines varied from 1.5 to 4.5 (Figure 3.8). Mean value of the F2 was 2.8. F3 lines varied from 1 to 5 with a mean value of 2.9. Wall thickness showed continuous variation for both F2 and F3 populations. MS1453 had a moderately thick wall (average 3.5) and the results indicated that wall thickness can be increased using these populations. Thick pericarp provides a 'meatier' tomato and also protects fruit from damage during mechanical harvesting (Garcia and Barrett, 2006).

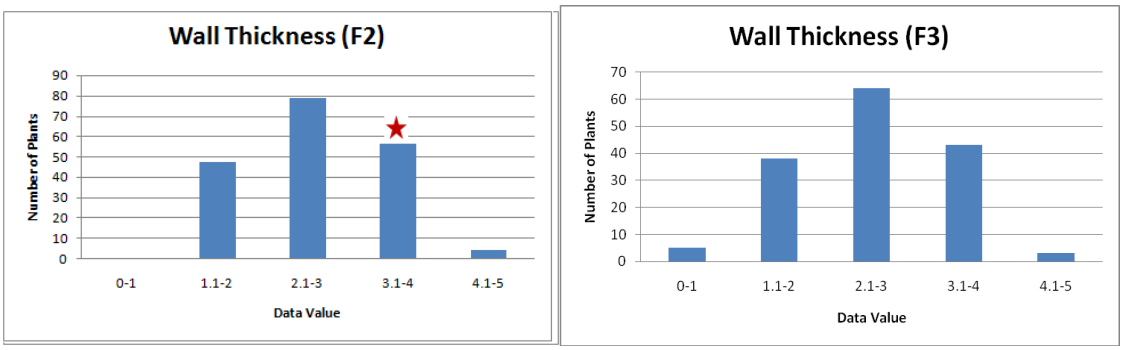

Figure 3.8. Segregation of wall thickness of F2 and F3 population

Brix varied from 3.8 to 6.2 in the F2 population and 4.0 to 6.4 in the F3 population (Figure 3.9). Mean values of F2 and F3 populations were 4.6 and 5.1. Both F2 and F3 populations showed normal distribution; however, there were more individuals with higher soluble solids in the F3 lines. A high brix value is necessary for decreasing evaporation time during tomato processing and for increasing product aroma. Average brix value for processing tomatoes in Turkey is about 5 and maximum is about 6.5. Most of the sugars that cause increased brix value are reducing sugars which play roles in the Maillard reaction which gives aroma to tomato paste (Porretta and Sandei, 1991). It is known that the brix value of MS1453 is 6.0 and in the next generations this value can be increased because we have lines with higher soluble solids.

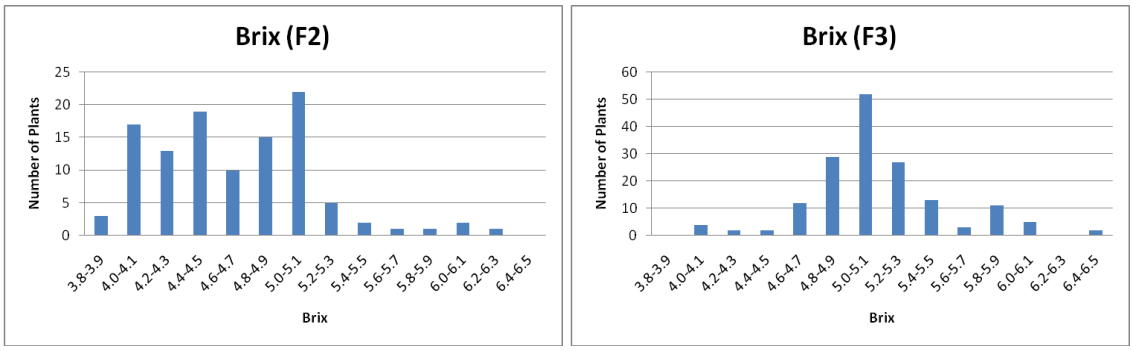

Figure 3.9. Segregation of brix of F2 and F3 population

Flower numbers per truss was counted in the F3 lines for determining flowering capacity which is related to yield (Figure 3.10). Flower number of the F3 population showed a normal distribution with a minimum of 3.5, maximum of 7.1 and a mean of 5.2 flowers per truss.

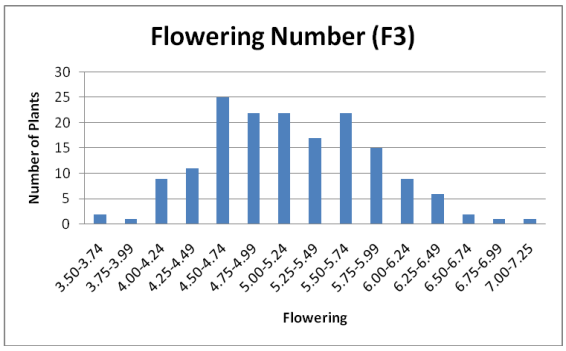

Figure 3.10. Segregation of flowering number of F3 population

Yield was measured for the F3 lines and had a normal distribution (Figure 3.11). Minimum yield was 3.4 tons per decare and maximum yield was 12.9. Mean value for yield was 7.7. Yield of MS1453 was 15-17 tons per decare which was higher than seen in the F3 lines.

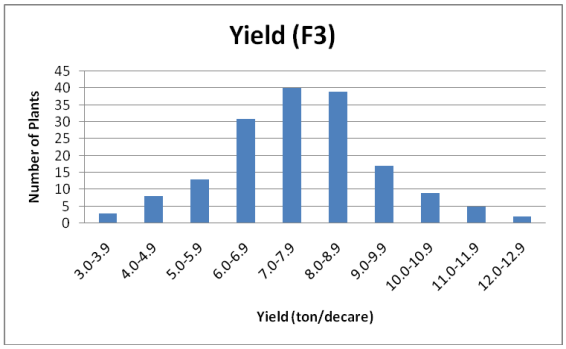

Figure 3.11. Segregation of yield of F3 population

Number of flowers and number of fruits were measured for each F3 plant in June 2014 (Figure 3.12). F3 plants had a minimum of 35 and maximum of 72 flowers. Mean value was 52 for this trait. Fruit number varied from 32 to 255 for F3 individuals. Mean value for number of fruits was 108. This mean exceeded what is normally expected for processing tomato lines which usually have 50-100 fruits. Flower number in June was less than the final number of fruits indicating that flowering was not uniform and that more flowers were produced after flower number was determined. Non uniform flowering can be a problem for harvesting if fruit ripening is not uniform. Both F2 and F3 lines showed continuous variation and transgressive segregation indicating that the population has individuals which can be used to improve upon the performance of MS1453.

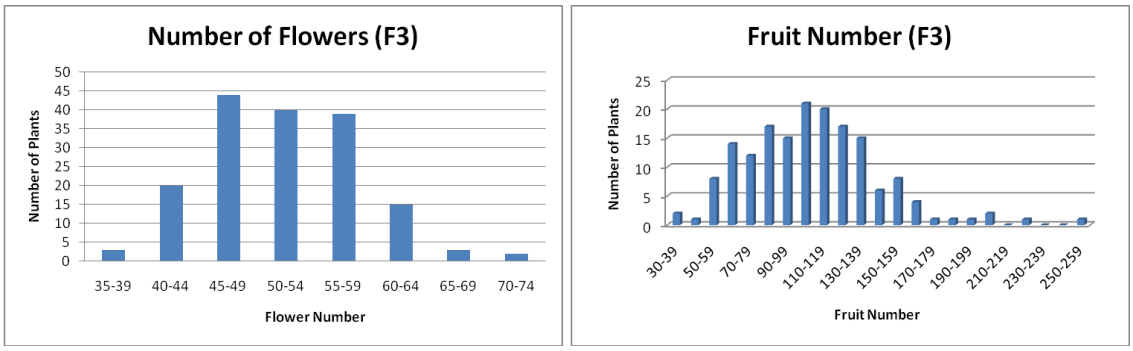

Figure 3.12. Segregation of number of flowers and number of fruits for F3 population

Correlations between argo-morphological characteristics were determined by SPSS program. Positive correlations between fruit weight and fruit number, external color and internal color were observed. Table 3.1 shows correlations between traits. Red colored values indicate statistically significant relations between traits ($P<0.05$). Internal color is correlated with external color as expected while higher lycopene levels provide intense red color in external and internal parts of tomato fruits. Correlations are low for most traits. Fruit number and fruit shape had negative correlations with stem scar. Thus, plants which produced more fruit and elongated fruit tended to have smaller scars. Fruit number and external color, fruit number and internal color, fruit weight and stem scar, fruit weight and wall thickness, firmness and fruit shape, firmnes and stem scar, stem scar and wall thickness, wall thickness and external color, brix and internal color had low positive correlations.

For QTL regions for technologically important traits, a total of 27 markers including SSR, InDel and COS II markers were used for scanning. Table 3.2 shows our results for LOD (logarithm of odds) score analysis for markers. LOD values > 3 are indicating possible QTL regions for related trait.

Selected traits which were used for our phenotypic characterization are polygenic and environmental conditions also affect the phenotype of plants. Unfortunately, our results did not allow detection of many QTL. SSR63 (in chromosome 8) had a LOD score of 2.25 for stem scar and marker Ychr34 had a LOD of 4.38 for internal color.

Table 3.1. Correlation values of agronomically important traits.

	FRUIT NUMBER	FRUIT WEIGHT	FIRMNESS	FRUIT SHAPE	STEM SCAR	EXTERNAL COLOR	INTERNAL COLOR	WALL THICKNESS	BRIX
FRUIT NUMBER	1								
FRUIT WEIGHT	.780	1							
FIRMNESS	-.046	.031	1						
FRUIT SHAPE	.130	-.034	.158	1					
STEM SCAR	-.210	.205	.144	-.415	1				
EXTERNAL COLOR	.158	.057	-.094	-.085	-.020	1			
INTERNAL COLOR	.186	.032	-.069	-.113	-.072	.735	1		
WALL THICKNESS	-.034	.209	-.093	.009	.275	.143	.034	1	
BRIX	.104	.078	.050	-.212	-.002	.108	.189	-.154	1

Table 3.2. QTL identified for agronomic traits, their location in the tomato genome and LOD scores for these QTL regions.

Trait	QTL Symbol	Chromosome	Marker	LOD Score	R^2
Fruit Weight	*Fwx*	?	Ychr34	1.004	0.049
External Color	*ECol7*	7	At1g19140	1.577	0.082
	ECol3	3	SSR320	1.813	0.094
	EColx	?	Ychr34	1.46	0.076
Internal Color	*ICol3*	3	SSR320	1.27	0.066
	IColx	?	Ychr34	4.381	0.211
Firmness	*Firm12*	12	At1g48300	1.212	0.059
Stem Scar	*Scar12*	12	At1g48300	1.13	0.055
	Scar8	8	SSR63	2.252	0.107
	Scarx	?	Ychr11F2C	1.146	0.056
	Scary	?	Ychr82	1.236	0.06
Wall Thickness	*Wall7*	7	At2g42750	1.892	0.09
	Wall8.1	8	SSR63	1.529	0.075
Brix	*Wall8.2*	8	TG302	1.368	0.066
	Brix12	12	At1g48300	1.43	0.071
Flowering	*Flw3*	3	At3g47990	1.473	0.072
	Flwx	?	Ch715	1.165	0.057

(Cont. on the next page)

Table 3.2. (cont.)

Yield	*Flw8*	8	SSR8.87.5	1.505	0.073
	Flwy	?	Ychr34	1.049	0.052
	Yield3	3	At3g47990	1.239	0.06
	Yield8	8	SSR63	1.301	0.062
Number of Flowers	*Yieldx*	?	Ychr11F2C	1.68	0.08
	NFlo3	3	At3g47990	1.046	0.051
	NFlox	?	Ch715	1.376	0.067
Number of Fruits	*NFlo8*	8	SSR8.87.5	1.817	0.087
	NFloy	?	Ychr34	1.183	0.057
	NFrux	?	Ychr34	1.161	0.056

Although the populations that were used in this study were suitable for QTL analysis in terms of showing suitable segregation for agro-morphological traits, the populations were deficient in DNA polymorphism. More than 300 COS II marker-enzyme combination were tested in parents and were not polymorphic. Therefore, only a limited number of markers could be used for mapping. Indeed, lack of sufficient DNA polymorphism is a major limitation of intraspecific tomato populations. Use of a different marker systems may solve this problem. Thus, markers with a higher efficiency for detecting smaller changes such as SNPs should be applied to our population.

3.2. Genomic Characterization Of Disease Resistance

A total of 261 individuals (Table 3.3) were screened for five different diseases with nine different markers. SNP based CAPS assays for disease resistance genes were developed from SNP markers (Shi et al., 2011; Garcia et al., 2007). MAS assays were done for Fusarium Crown and Root Rot, Tomato Mosaic Virus (Shi et al., 2011), Root Knot Nematode (Garcia et al., 2007; Zengin S., Antalya Tarım, Inc.), Verticillium Wilt (Shi et al., 2011), and Tomato Yellow Leaf Curl Virus diseases (Zengin S. , Antalya Tarım, Inc.). *Ve2* SNP1 and *Ve2* SNP3 markers gave the same result for disease resistance, therefore, all individuals were not tested with both markers.

Table 3.3. Disease resistance of donor materials. (Individuals homozygous for disease resistance alleles are highlighted with green and heterozygous individuals are highlighted with yellow. "na" is used for non-amplified scores and "nd" is used for non-determined scores.)

Pedigree Number	***TM2* SNP1**	***TM2* SNP2**	***Ve1* SNP3**	***Ve2* SNP123**	***Ty3***	***Ty1***	***Mi***	***Frl***	***Mi23***
12S337	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	na
12S338	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2 /mi-1.2*
12S339	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*na*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S340	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S341	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S342	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S343	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S344	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S345	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S346	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S347	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S348	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2 /mi-1.2*
12S349	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2 /mi-1.2*
12S351	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2 /mi-1.2*
12S352	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S353	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2 /mi-1.2*
12S354	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S355	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*na*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S356	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S357	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S358	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S359	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S360	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*

(Cont. on the next page)

Table 3.3. (cont.)

12S361	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S362	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S363	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S364	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2 /mi-1.2*
12S365	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S366	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S367	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S368	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S388	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S389	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S390	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S391	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S392	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S393	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*na*	*na*	*na*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S394	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S395	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S396	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*Ty3/Ty3*	*Ty1/ty1*	nd	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S397	na	na	na	na	*ty3/ty3*	nd	*Mi/Mi*	na	na
12S398	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	na
12S399	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S400	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S401	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S402	*tm-2/tm-2*	na	na	na	*ty3/ty3*	nd	*Mi/mi*	na	*mi-1.2/ mi-1.2*
12S403	na	*tm-2/tm-2*	na	*Ve2/Ve2*	na	*ty1/ty1*	*mi/mi*	na	na
12S404	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*na*	*ty1/ty1*	*mi/mi*	*na*	*mi-1.2/ mi-1.2*
12S405	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*na*
12S406	na	na	na	*Ve2/Ve2*	na	na	na	na	*mi-1.2/ mi-1.2*
12S407	*Tm-2/tm-2*	*Tm-2/tm-2*	*Ve1/ve1*	*Ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/frl*	na
12S410	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	na	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S411	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*

(Cont. on the next page)

Table 3.3. (cont.)

12S412	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*na*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S413	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	nd	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S414	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	nd	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S415	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	na	na	na	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S416	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	nd	*Mi/Mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S417	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	na	*ty1/ty1*	*mi/mi*	*frl/frl*	na
12S418	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	na	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S420	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	na	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S421	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	na	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S422	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S423	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	nd	*Mi/Mi*	na	na
12S424	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	na	na	na	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S425	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	na	*Ty1/ty1*	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S426	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	nd	*Mi/Mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S427	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*Ty1/ty1*	nd	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S428	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	na	na	na	*Frl/Frl*	na
12S429	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	na	nd	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S430	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	na	*ty1/ty1*	*mi/mi*	*Frl/Frl*	na
12S431	*tm-2/tm-2*	*tm-2/tm-2*	na	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S432	*tm-2/tm-2*	*tm-2/tm-2*	na	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S433	*tm-2/tm-2*	*tm-2/tm-2*	na	*Ve2/Ve2*	na	na	na	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S434	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	nd	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S435	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*na*	*ty1/ty1*	*mi/mi*	*na*	*mi-1.2/ mi-1.2*
12S436	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S437	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S438	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S439	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*na*	*ty1/ty1*	*mi/mi*	*na*	*mi-1.2/ mi-1.2*
12S440	*na*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S441	*tm-2/tm-2*	*tm-2/tm-2*	*na*	*na*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*

(Cont. on the next page)

Table 3.3. (cont.)

12S442	*tm-2/tm-2*	*tm-2/tm-2*	*na*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S443	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S444	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S445	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S446	*Tm-2/tm-2*	*Tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/frl*	*mi-1.2/ mi-1.2*
12S447	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S448	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S449	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S450	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S451	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S452	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S453	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S454	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S455	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S456	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S457	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S458	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*na*
12S459	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S460	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S461	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*na*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S465	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S466	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*na*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S467	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*na*	*mi-1.2/ mi-1.2*
12S468	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S469	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S470	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*na*
12S471	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S472	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*Ty3/Ty3*	*Ty1/ty1*	*nd*	*na*	*mi-1.2/ mi-1.2*
12S473	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S474	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*

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Table 3.3. (cont.)

12S475	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S476	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*Mi/mi*	*na*	*na*
12S477	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S478	*tm-2/tm-2*	*tm-2/tm-2*	*na*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S479	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S480	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S481	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S482	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S483	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S484	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S485	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*na*
12S486	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*na*
12S487	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*na*
12S490	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S491	*Tm-2/tm-2*	*Tm-2/tm-2*	*na*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/frl*	*mi-1.2/ mi-1.2*
12S492	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S493	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S494	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S495	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S496	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S497	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S498	*tm-2/tm-2*	*na*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S500	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S502	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S503	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S505	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S506	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S507	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S508	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*

(Cont. on the next page)

Table 3.3. (cont.)

12S509	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S510	*Tm-2/tm-2*	*na*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S511	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S514	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S520	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S521	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S522	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*na*
12S523	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S524	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*Mi-1.2/ mi-1.2*
12S525	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*Mi-1.2/ mi-1.2*
12S526	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*na*	*mi-1.2/ mi-1.2*
12S527	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*na*
12S529	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*Frl/frl*	*mi-1.2/ mi-1.2*
12S530	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S531	*tm-2/tm-2*	*na*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S532	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*Frl/Frl*	*na*
12S533	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S534	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S535	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S536	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*Frl/Frl*	*na*
12S537	*Tm-2/tm-2*	*Tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*na*
12S538	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/frl*	*mi-1.2/ mi-1.2*
12S539	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*na*	*mi-1.2/ mi-1.2*
12S540	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S541	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2 /mi-1.2*
12S542	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S543	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*na*	*mi-1.2/ mi-1.2*
12S544	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S545	*tm-2/tm-2*	*tm-2/tm-2*	*na*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S546	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S547	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*

(Cont. on the next page)

Table 3.3. (cont.)

12S548	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S549	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S550	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S551	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S552	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*Ty3/Ty3*	*Ty1/ty1*	*nd*	*frl/frl*	*na*
12S553	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S554	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S556	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*na*
12S557	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*na*	*na*	*frl/frl*	*na*
12S558	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*na*
12S559	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S560	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*na*	*na*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S561	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*na*	*na*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S562	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*na*	*na*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S563	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*na*	*na*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S564	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*na*	*na*	*frl/frl*	*mi-1.2/ mi-1.2*
12S565	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S566	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*na*
12S567	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S568	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S569	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S570	*tm-2/tm-2*	*tm-2/tm-2*	*na*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S571	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S572	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S573	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S574	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*na*	*ty3/ty3*	*nd*	*Mi/Mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S575	*tm-2/tm-2*	*tm-2/tm-2*	*na*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*Frl/Frl*	*na*
12S576	*tm-2/tm-2*	*tm-2/tm-2*	*na*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*na*	*na*
12S577	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*na*
12S578	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S579	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*na*
12S580	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*

(Cont. on the next page)

Table 3.3. (cont.)

12S581	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S582	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*na*
12S583	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S584	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S585	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S587	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*na*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S588	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S589	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*Ty3/Ty3*	*nd*	*Mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S590	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*Ty3/Ty3*	*nd*	*Mi/mi*	*frl/frl*	*Mi-1.2/ Mi-1.2*
12S591	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*Mi-1.2/ Mi-1.2*
12S592	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*Ty3/Ty3*	*nd*	*Mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S593	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*Ty3/Ty3*	*nd*	*Mi/mi*	*frl/frl*	*Mi-1.2/ Mi-1.2*
12S594	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*Ty3/Ty3*	*ty1/ty1*	*mi/mi*	*na*	*Mi-1.2/ Mi-1.2*
12S595	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*Ty3/Ty3*	*ty1/ty1*	*mi/mi*	*na*	*mi-1.2/ mi-1.2*
12S596	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S597	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S598	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S599	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S600	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S601	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S602	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S603	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S604	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S606	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S607	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S609	*Tm-2/tm-2*	*Tm-2/tm-2*	*Ve1/Ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*na*	*mi-1.2/ mi-1.2*
12S610	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S612	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*

(Cont. on the next page)

Table 3.3. (cont.)

12S613	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S614	*Tm-2/tm-2*	*Tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/frl*	*mi-1.2/ mi-1.2*
12S615	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S616	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S617	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S618	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S619	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S620	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*nd*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S621	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S623	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S624	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S625	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*na*	*na*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S626	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*na*	*na*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S627	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S628	*Tm-2/Tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S629	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S630	*tm-2/tm-2*	*na*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S631	*tm-2/tm-2*	*tm-2/tm-2*	*ve1/ve1*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S632	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*na*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S633	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*-*	*Mi/Mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S635	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S636	*tm-2/tm-2*	*tm-2/tm-2*	*na*	*na*	*ty3/ty3*	*na*	*na*	*na*	*mi-1.2/ mi-1.2*
12S638	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*ve2/ve2*	*ty3/ty3*	*na*	*na*	*frl/frl*	*mi-1.2/ mi-1.2*
12S639	*tm-2/tm-2*	*tm-2/tm-2*	*Ve1/Ve1*	*na*	*na*	*ty1/ty1*	*mi/mi*	*na*	*mi-1.2/ mi-1.2*
12S640	*tm-2/tm-2*	*tm-2/tm-2*	*na*	*na*	*ty3/ty3*	*na*	*na*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S641	*Tm-2/Tm-2*	*Tm-2/Tm-2*	*Ve1/Ve1*	*na*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*frl/frl*	*mi-1.2/ mi-1.2*
12S642	*tm-2/tm-2*	*tm-2/tm-2*	*na*	*Ve2/Ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*Frl/Frl*	*mi-1.2/ mi-1.2*
12S644	*Tm-2/tm-2*	*Tm-2/tm-2*	*na*	*ve2/ve2*	*ty3/ty3*	*ty1/ty1*	*mi/mi*	*na*	*mi-1.2/ mi-1.2*
12S645	*tm-2/tm-2*	*tm-2/tm-2*	*na*	*na*	*ty3/ty3*	*na*	*na*	*Frl/Frl*	*na*

Three individuals: 12S536, 12S551, 12S554 were homozygous for six different markers related with four different disease resistances: Fusarium Crown and Root Rot, Tomato Mosaic Virus, Root-Knot Nematodes, and Verticillium Wilt. A total of 18 individuals: 12S355, 12S423, 12S449, 12S457, 12S458, 12S475, 12S484, 12S485, 12S486, 12S487, 12S492, 12S514, 12S526, 12S556, 12S565, 12S578, 12S620, 12S633 were homozygous for five different markers related with three different diseases (Tomato Mosaic Virus, Root-Knot Nematodes and Verticillium Wilt) and a total of six individuals: 12S425, 12S541, 12S548, 12S577, 12S582, 12S585 were also homozygous for five different markers related with three different diseases (Fusarium Crown and Root Rot, Tomato Mosaic Virus and Verticillium Wilt). These disease resistant lines are useful stocks for the transfer of disease resistance into other tomato lines.

A total of 251 individuals were tested with both *Tm*2 SNP1 and *Tm*2 SNP2. Results for these two markers were exactly the same except in one individual. *Tm*2 SNP1 and *Tm*2 SNP2 are closely linked markers and a low recombination frequency is expected. Based on our population of 251 individuals, recombination frequency between these two markers was 3.98×10^{-3}. Only homozygous individuals were considered for calculation.

A total of 240 individuals were considered with both *Ve*1 SNP3 and *Ve*2 SNP123. *Ve*1 SNP3 and *Ve*2 SNP123 were also closely linked and a low recombination frequency was expected. A total of 16 recombinant individuals were identified between *Ve*1 SNP3 and *Ve*2 SNP123. Thus, recombination frequency was 6.67×10^{-2}.

A total of 169 individuals were tested with both *Ty 1* and *Ty 3*. Only four individuals showed discrepancy in the results for *Ty 1* and *Ty 3*. Recombination frequency was 2.37×10^{-2}. Reduced susceptibility for different type of tomatoes are available for TYLCV but no cultivar with complete resistance to TYLCV is available. Therefore asmall numberof resistant individual was expected and these individuals were precious with their resistance genes.

A total of 211 individuals were considered with both *Mi* and *Mi23.* Recombination was detected in 31 individuals with results different for *Mi* and *Mi23.* Low recombination frequency was expected because *Mi* and *Mi23* are closely linked markers. Recombination frequency was 1.47×10^{-1}.

Marker PMI is supposed to test for both *Ty 1* and *Mi* gene (Zengin S., Antalya Tarım, Inc.); however, in our study the marker could not detect homozygous resistance

for both genes. This indicates preferential amplification of one gene template over another. Moreover when *Mi* was heterozygous, *Ty 1* could not be determined and vice versa. Thus, our results show that PMI marker can not be used efficiently for determining *Ty 1* and *Mi* genes together.

A total of 60 inbred lines were selected from 261 inbred lines by using MAS and CAPS results for disease resistance. DNA quantities for 60 inbred lines were measured with nanodrop spectrometer and results are listed in Table 3.4. DNA quality and quantities were suitable for HRM analysis for determining Fusarium Wilt (*I2* and *I3* genes), Root-Knot Nematode (*Mi-1* gene), Bacterial Spot Disease (*Pto* gene) and Verticillium Wilt (*Ve5* gene) disease resistance genes in the lines. Table 3.5 and Figures 3.13-3.17 show HRM results for different disease resistance genes. Disease resistance results are shown with "R" for homozygous resistant individuals, "S" for homozygous susceptible individuals, "H" for heterozygous resistant individuals and "nd" for not determined results.

Table 3.4. DNA and concentration quality for 60 inbred lines.

DNA	ng/µl	DNA	ng/µl	DNA	ng/µl	DNA	ng/µl	DNA	ng/µl
12T008	359.8039	12T112	468.4314	12T151	502.8431	12T203	246.2745	12T231	521.2745
12T011	506.9608	12T117	256.2745	12T156	286.7647	12T204	395.7843	12T232	399.8039
12T019	421.6667	12T118	286.4706	12T158	200.2941	12T208	341.7647	12T234	549.7059
12T022	334.7059	12T119	246.2745	12T165	440.4902	12T209	343.2353	12T246	457.9412
12T029	457.6471	12T121	321.8627	12T166	667.9412	12T211	388.3333	12T247	345.1961
12T065	457.8431	12T124	579.8039	12T172	509.8039	12T213	294.4118	12T248	408.9216
12T066	495.2941	12T125	606.3725	12T196	451.1765	12T214	118.6275	12T250	383.3333
12T090	580.0000	12T129	222.1569	12T197	257.6471	12T223	316.6667	12T252	239.7059
12T097	532.5490	12T130	333.0392	12T198	258.4314	12T224	429.1176	12T255	366.0784
12T098	505.9804	12T143	239.5098	12T199	334.0196	12T228	551.9608	12T257	294.3137
12T099	526.8627	12T144	287.5490	12T201	367.1569	12T230	434.5098	12T401	423.4314

Table 3.5. Disease resistance results of HRM analysis for 60 inbred lines.

	***I2* gene**	***I3* gene**	***Mi1* gene**	***Pto* gene**	***Ve5* gene**
12T008	S	S	S	S	S
12T011	R	R	S	S	R
12T019	R	S	R	S	R
12T022	R	S	S	S	R
12T029	R	S	S	S	R
12T065	S	S	S	S	R
12T066	S	S	R	S	R
12T090	S	R	S	S	R
12T097	R	S	S	R	R
12T098	R	S	R	R	R
12T099	S	S	R	R	R
12T112	R	S	S	H	R
12T117	S	S	S	R	R
12T118	R	S	H	H	R
12T119	R	S	S	S	R
12T121	R	S	R	S	R
12T124	R	S	S	S	R
12T125	R	S	S	R	R
12T129	R	S	R	R	R
12T130	R	S	R	R	R
12T143	S	S	S	S	R
12T144	S	S	R	H	R
12T151	S	S	S	nd	R
12T156	R	S	R	nd	R
12T158	R	S	R	nd	R
12T165	R	S	S	nd	R
12T166	R	S	S	nd	R
12T172	R	S	S	nd	R
12T196	S	S	S	nd	R
12T197	R	S	S	nd	R
12T198	R	S	R	nd	R
12T199	nd	S	S	nd	R
12T201	nd	S	R	nd	R
12T203	nd	S	S	nd	S
12T204	nd	S	R	nd	R
12T208	nd	S	R	nd	R
12T209	nd	S	S	nd	nd
12T211	nd	S	R	nd	nd

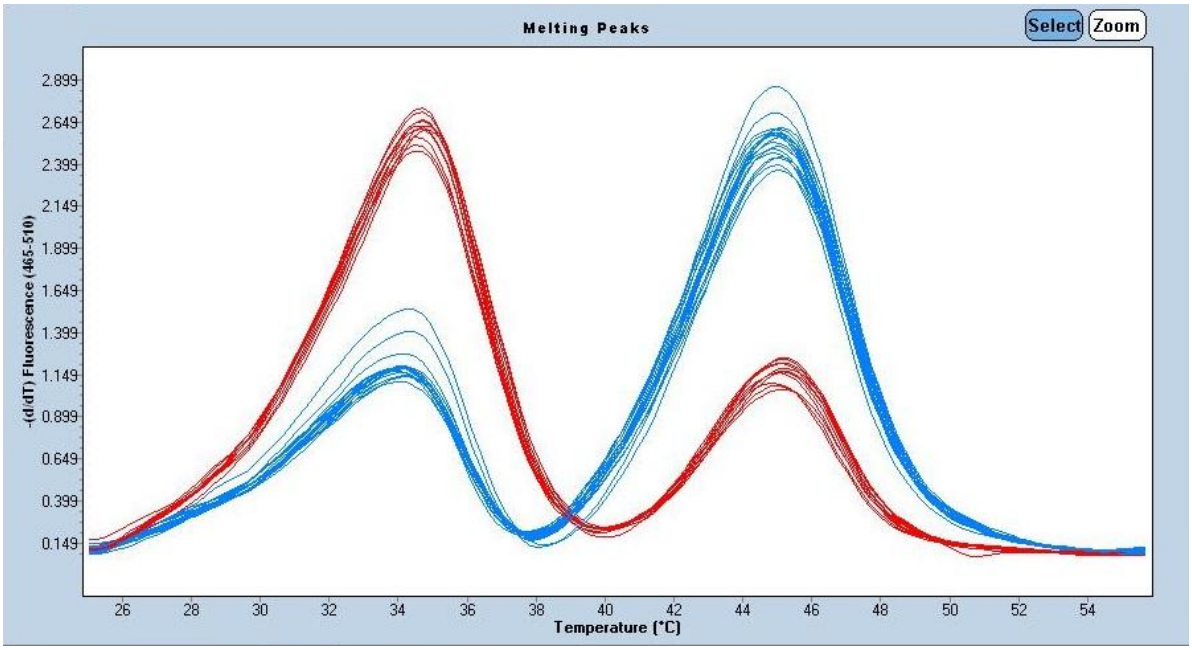

Figure 3.13. High Resolution Melting Curve for Fusarium Wilt (*I2* gene)

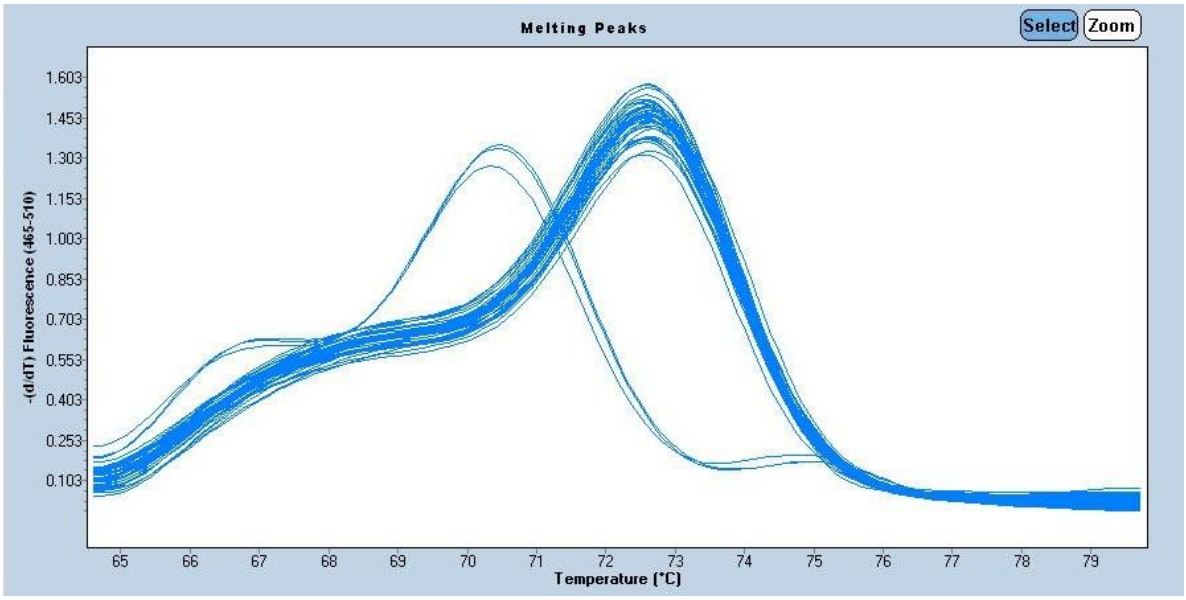

Figure 3.14. High Resolution Melting Curve for Fusarium Wilt (*I3* gene)

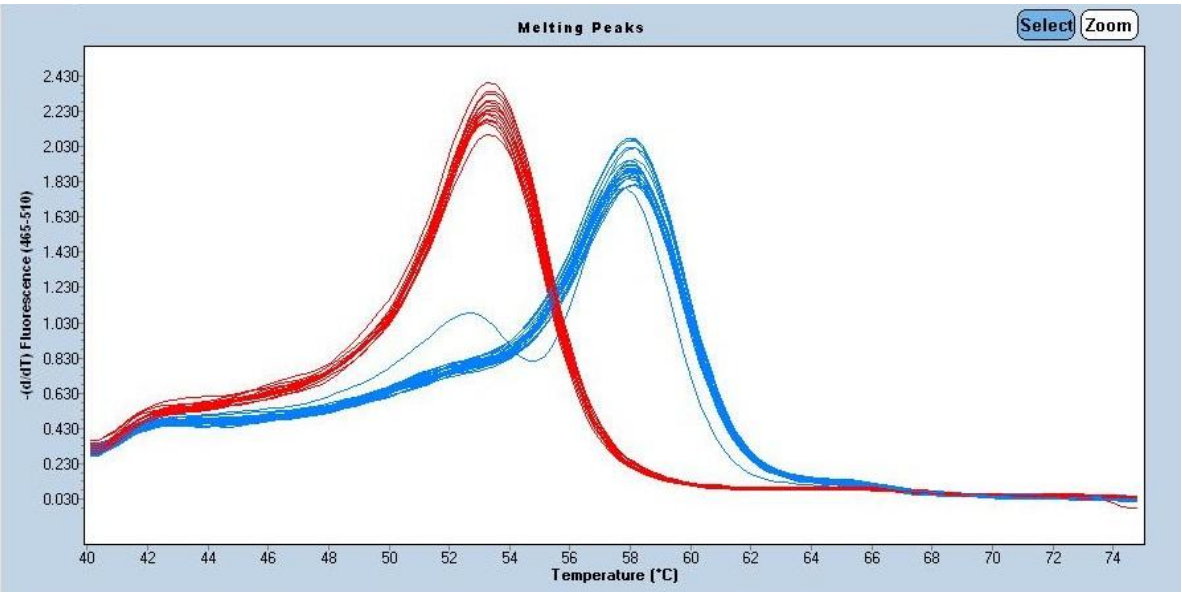

Figure 3.15. High Resolution Melting Curve for Root-Knot Nematode (*Mi1* gene)

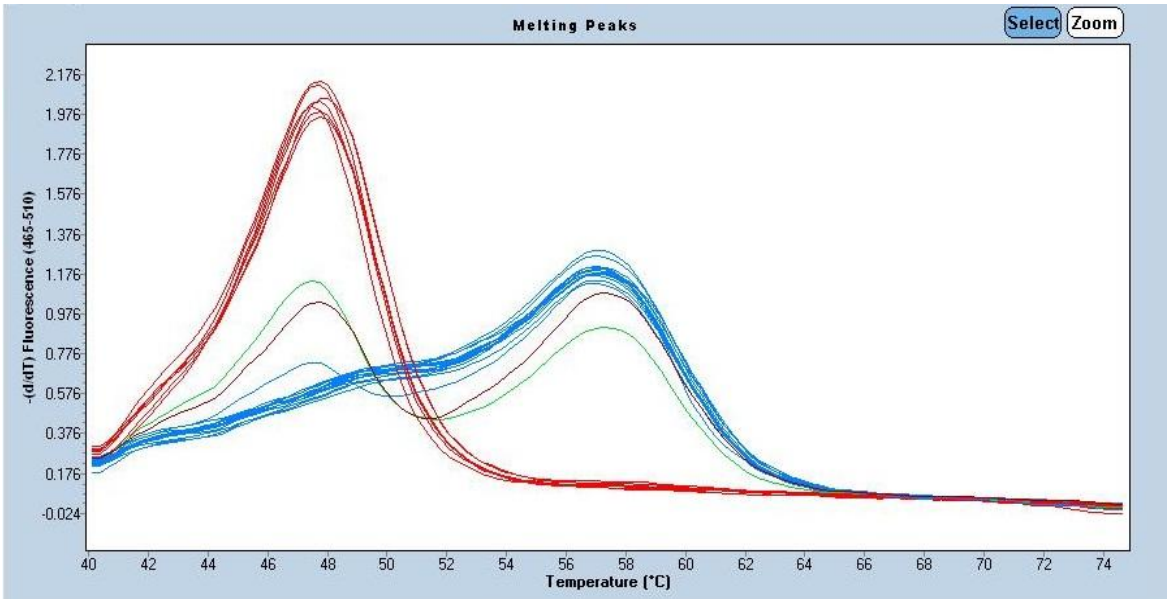

Figure 3.16. High Resolution Melting Curve for Bacterial Spot Disease (*Pto* gene)

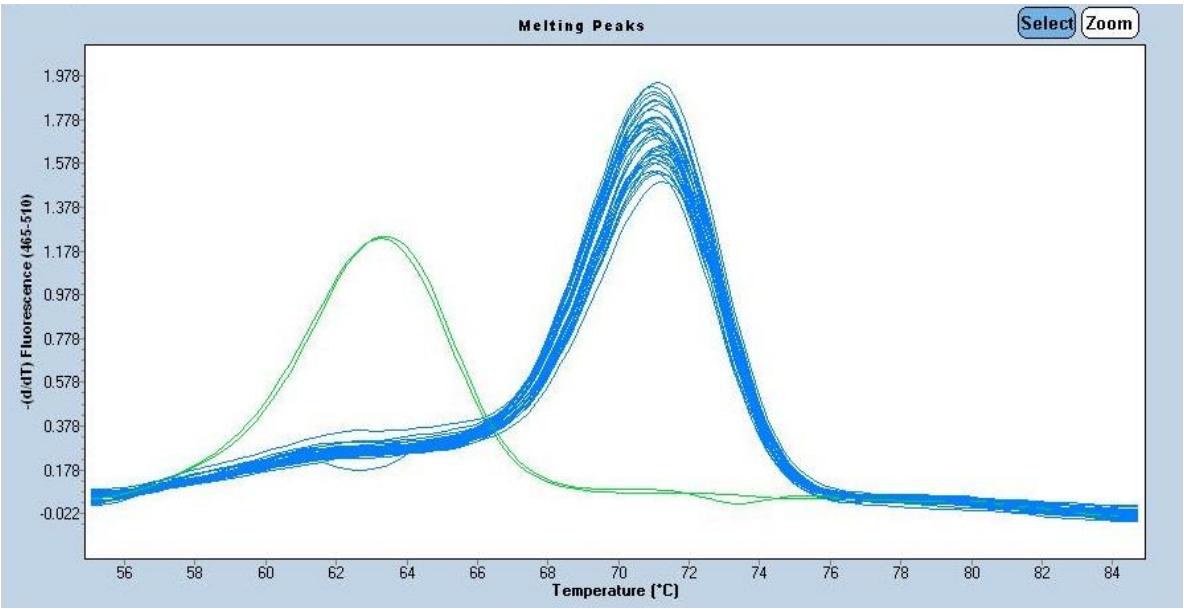

Figure 3.17. High Resolution Melting Curve for Verticillium Wilt (*Ve5* gene)

Overall, 60 candidate hybrids that were highly resistant to diseases were developed for processing tomato industry. In future, phenotypic characterization of these 60 candidate hybrids can be measured.

CHAPTER 4

CONCLUSION

Tomatoes are important dietary sources of nutrients and various tomato products like ketchup, tomato paste, and tomato juice are used worldwide. The main goal of this study was to explore the use of the wild tomato species, *Solanum pimpinellifolium* as a source for tomato improvement and to map genes controlling agronomically important traits (number of fruits, total weight of fruits, fruit shape, external color, internal color, firmness, stem scar, wall thickness, brix, locule number and flower number) in a population derived from this wild species. The F1 hybrid of *S. lycopersicum* X *S. pimpinellifollium*, MS1453, has high brix and favorouble processing features. These traits are polygenic and environmental conditions also affect their expression in plant phenotype. The agronomic performance of F2 and F3 populations developed from MS1453 was tested in field conditions in İzmir (Summer, 2013) and Manisa (Summer, 2014) and environmental differences were avoided by two different field experiment. Correlations between agro-morphological characteristics were determined by SPSS program. Positive correlation between fruit weight and fruit number, external color and internal color were observed. F2 and F3 populations showed continuous distribution and indicated that the populations might be useful to identify the genes that control these traits. Unfortunately, the population had low DNA polymorphism and only 27 markers including SSR, InDel and COS II markers were used for scanning the genome for QTLs using the QGENE program. Because of limited polymorphism, very few QTL were detected. A different marker system such as SNPs which has higher efficiency for detecting smaller changes should be used to identify more polymorphisms and to increase the power of QTL mapping.

In the second part of the thesis, 261 inbred lines were screened for five different diseases with nine different markers. Three individuals were homozygous for six different markers related with four different disease resistances: Fusarium Crown and Root Rot, Tomato Mosaic Virus, Root-Knot Nematodes, and Verticillium Wilt. Then 60 inbred lines were selected from by using MAS and CAPS results for disease resistance. Further disease resistance analysis were done by HRM analysis. Thus, 60 candidate hybrids with high resistance to diseases were developed for processing tomato industry.

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