MOLECULAR MAPPING OF QUANTITATIVE TRAIT LOCI CONFERRING RESISTANCE TO VERTICILLIUM WILT IN COTTON

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ABSTRACT

MOLECULAR MAPPING OF QUANTITATIVE TRAIT LOCI CONFERRING RESISTANCE TO VERTICILLIUM WILT IN COTTON

Cotton with its valuable natural fiber is an economically important field plant. Resistance against stress conditions and diseases are desired traits in cotton production. Verticillium wilt is one of the major factors resulting in huge cotton yield losses. Control of Verticillium disease has not been successful because of low genetic diversity and a complex disease mechanism. Moreover resistance against Verticillium wilt is a quantitative trait that is regulated by more than one gene and under environmental effects. Due to this complex inheritance, conventional breeding methods have not resulted in efficient resistance. Therefore, it is necessary to develop cotton lines resistant to Verticillium wilt using molecular breeding techniques. In this study we aimed to identify the quantitative trait loci which are responsible for Verticillium wilt resistance. For this purpose, we carried out a Verticillium wilt disease test in a natural population composed of 118 individuals. We inoculated the population with *Verticillium dahliae* in five replicates. After one month, the symptoms were examined and phenotypic variation was detected and scored. We then used 100 SSR markers for genotyping of the population. Population structure was determined with STRUCTURE 2.2.3. According to results, we determined two clusters. There were 44 and 34 individuals in the first and second clusters, respectively. A total of 40 individuals remained intermixed. Association analysis between phenotypic and allelic data was carried out with TASSEL 2.1. In this way we identified 30 SSR markers associated with Verticillium wilt resistance at a significance level of $p<0.05$. The most significant SSR locus was DPL080-238 ($p=0.0014$). Moreover, SSRs DPL188-130 and DPL223-251 showed the highest values for dominant and recessive allelic effects, respectively. We hope that our study will be helpful for the development of marker-assisted strategies for breeding of Verticillium wilt resistant cotton cultivars.

ÖZET

PAMUK'TA VERTİCİLLİUM SOLGUNLUĞUNA DAYANIKLILIK SAĞLAYAN KANTİTATİF KARAKTER LOKUSLARIN MOLEKÜLER HARİTALANMASI

Pamuk bitkisi birçok farklı sanayide ham madde olarak kullanılan doğal liflere sahip ekonomik değeri yüksek yenilebilir bir tarla bitkisidir. Pamuk üretiminde verimi etkileyen başlıca ana etmenlerden birisi Verticillium solgunluğu olarak tanımlanmaktadır. Verticillium hastalığını kontrol altına almak çeşitli sebeplerden dolayı henüz mümkün olmamıştır. Verticillium solgunluğuna karşı genetik çeşitliğinin yetersiz kalışı, hastalığa karşı direnç mekanizmaların yoksunluğu, dirençliliğin genetik altyapısının birden fazla gen bölgesi tarafından düzenlenmesi ve çevresel faktörlerden de önemli ölçüde etkilenmesinden dolayı ıslah çalışmaları ile başarılı bir sonuç alınamamıştır. Tüm bu durumlar göz önüne alındığında Verticillium hastalığına karşı dirençli yeni pamuk hatlarının geliştirilmesi kaçınılmaz bir noktaya gelmiştir. Bu çalışmamızın amacı moleküler markör teknolojileri kullanarak Verticillium solgunluğuna dayanıklı pamuk hatlarının geliştirilmesidir. Bu amaçla, birbirinden farklı 118 bireyden oluşan doğal pamuk popülasyonu Verticillium dahliae patojenine karşı 5 tekerrür halinde testlenerek hastalığa karşı fenotipik çeşitlilik gözlenmiştir. Popülasyonun polimorfizmin belirlenmesi amacıyla 100 SSR markörü bireylere uygulanmıştır. Popülasyonun yapısı STRUCTURE 2.2.3 ile belirlenmiştir. Analiz sonucunda popülasyon 2 gruba kümelenmiştir. Küme 1'de 44 birey varken küme 2'de 34 birey belirlenmiştir. 40 birey ise herhangi bir gruba atanamamıştır. Fenotipik çeşitlilik ile allelik veri arasındaki ilişki analizi TASSEL 2.1 ile gerçekleştirilmiştir. Çalışmamızın sonucunda, Verticillium solgunluğuna karşı dayanıklılık sağlayan gen bölgeleri ile ilişkili olan olası markörler belirlenmiştir. 30 SSR markörü Verticillium solgunluğu ile ilişkili bulunmuştur ($p<0.05$). En anlamlı p değerine sahip SSR lokusu $p=0.0014$ ile DPL080-238 olarak belirlenmiştir. En yüksek dominant allelik etki değerine sahip SSR lokusu DPL188-130 iken, en yüksek resesif allelik etkiye sahip DPL223-251 olarak bulunmuştur. Bu çalışmamız ile bulunan yüksek güvenirlilik değerine sahip SSR lokusları ileriki QTL çalışmalarında ve bu sayede Verticillium solgunluğuna dirençli hatların geliştirilmesi için kullanılacak moleküler ıslah yöntemleri çalışmalarına katkıda bulunacaktır.

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CHAPTER 1

INTRODUCTION

1.1. History of Cotton

Cotton (*Gossypium spp.*) belongs to the family *Malvaceae. Gossypium* is composed of 50 species including four domesticated species. Cotton generally refers to these four domesticated species: *G. arboreum L., G. herbaceum L., G.hirsutum* and *G. barbadense*. *G. arboreum* and *G. herbaceum* were domesticated in the Old World; and *G. hirsutum* and *G. barbadense* were domesticated in the New World. Archeological evidence shows that cotton was first cultivated 7,000 years ago. *G. arboretum* cultivation began in the Indus Valley of India and Pakistan and then spread to Africa and Asia. *G. herbaceum* was first cultivated in Arabia and Syria. According to archeological studies, *G. hirsutum,* upland cotton, was domesticated in the Tehuacan Valley in Mesoamerica whereas *G. barbadense* was cultivated in Ancon, a site on the Peruvian coast (Stephens et al., 1974; Brite et al., 2013).

Gossypium comprises 45 diploid and 5 allotetraploid species. Depending on meiotic pairing conditions, diploid species are classified into eight different genomic groups: A, B, C, D, E, F, G and K. Of the domesticated species, *G. hirsutum* and *G. barbadense* are tetraploids (AADD, 2n=4x=52) and *G. arboreum and* G. *herbaceum are* diploids (AD, 2n=2x=26) (Fryxell, 1992). Chromosome numbers 1 to 13 are assigned to the A subgenome (A1-A13); while chromosomes 14 to 26 belong to the D subgenome (D1-D13). These genome designations were suggested according to cytological features during meiotic pairing (Kohel, 1973). The allotetraploids, *G. hirsutum* and *G. barbadense,* were formed by interspecific hybridization between diploid species closely related to *G. herbaceum* and *G. raimondii* and/or *G. gossypioides* (Ulbrich) Standley (Kai et al., 2006). *G. herbaceum* and *G. raimondii* are generally thought to be the best ancestral candidates for the A and D-subgenomes (Endrizzi et al., 1985).

1.2. Properties of Cotton

Cotton is a very important plant that has had a crucial role in human life for thousands of years. It is used in many different areas. Each part of the cotton plant can be processed for different purposes. Its seed is used for oil, margarine, livestock feed, paper and other industries. Cotton fiber is used especially in the textile industry. Other parts of cotton are plowed into the soil to make it richer or they can be used for livestock feed. Cotton is very important because it is a source of valuable natural fibers. The use of cotton fibers is widespread. Cotton fiber is preferable to artificial (viscose rayon and acetates) and synthetic fibers (containing acrylics, polyesters and polyamides). Cotton fiber has features such as good absorbency, good strength, easy to handle and sew, color retention, air permeability, washability, and low transmission of static electricity. Fiber quality is an important trait for cotton as it directly affects the economic value of cotton production. Fiber quality depends on the physical features of cotton.

1.2.1. Fiber Quality Factors

Fiber quality is the main issue in the modern cotton industry. Fiber quality is composed of various parameters mainly: fiber length, short fiber index, uniformity index, fiber strength, elongation, color grade, reflectance degree, micronaire, trash, leaf grade, neps and spinning consistency (Peng et al., 2009; James et al., 2010; Johnson et al., 1996). Improvement of fiber quality is of great interest to industry. High quality fiber provides greater crop value and, thus, provides economic benefits to the different industries that make use of cotton. Fiber traits are under effects of environmental conditions and genetic background. It has been reported that most fiber traits show significantly genetic difference between cotton types suggesting that it is possible to manipulate these traits. For years, breeders have improved fiber quality by conventional methods. However, in recent years, modern genetic technology has become involved in breeding of cotton fiber quality (Ali et al., 2008).

1.2.1.1. Fiber Length

Fiber length is an economically important trait. It is defined as the average length of the longer half of the fiber. It is measured as mm or inch. Longer values are better for cotton fabric (Peng et al., 2009; James et al., 2010). For upland cotton species, fibers longer than 29 mm or 1 3/8 inch are acceptable. Longer fiber is important in the textile industry because it produces stronger yarn leading to higher-priced end products. Fiber length is controlled mostly by genetic variation. However, during fiber growth, different environmental conditions such as water stress and severe weather adversely affect fiber length in all cotton varieties. Heavy rainy periods affect boll opening and delay of harvesting for months negatively reduces fiber length. Moreover, it was reported that fiber length was longer in cotton exposed to far-red (FR) light during boll development. The cells that received FR were longer and thinner than those that received photosynthetic light (Kasperbauer, 1994).

1.2.1.2. Short Fiber Index

Short fiber index is defined as the percentage of fibers of length less than 12.7 mm. As the value decreases, the debris decreases and, thus, the cost of processing decreases. Therefore, a low value for short fiber index is desirable for high quality fiber (Peng et al., 2009; James et al., 2010). Because short fibers cannot be wrapped around each other as well as long fibers, short fibers increase the amount of trash and decrease the ability of yarn to be processed. Short fiber content is affected by the same factors as fiber length.

1.2.1.3. Uniformity Index

Length uniformity describes the ratio between the mean fiber length and the upper fiber length. It is indicated as a percentage. As this ratio decreases, it indicates that there is a high amount of short fibers causing low fiber quality for the textile industry (Peng et al., 2009; James et al., 2010). Since the uniformity index is about fiber length, the conditions that affect length also affect fiber uniformity.

1.2.1.4. Fiber Strength

Fiber strength is defined as the force necessary to break the fibers. This property is closely related to the diameter of the cotton fibers. It is measured in grams per denier (James et al., 2010; Johnson et al., 1996). The average acceptable value of cotton fiber strength is 3.0 to 4.9 gram per denier. It directly affects yarn strength. Strong yarn is processed efficiently and easily resulting in high yield. It is a very important trait for cotton production. Beside variety, fiber strength is affected negatively by any physical and external damage such as bad weather conditions, drying during the ginning process, over-ginning and microbial factors that decrease yield and quality.

1.2.1.5. Elongation

Elongation is defined as the percentage of difference between initial length and final length of fiber after fiber is elongated. For spinning ability a high elongation value is desired. As the value increases, the quality of cotton fiber increases (Peng et al., 2009; James et al., 2010).

1.2.1.6. Color Grade and Reflectance Degree

Color grade is determined by the degree of pigmentation. Color grade affects the suitability of processing for cotton fabric. According to the universal standard, there are five color groups which are white, light spotted, spotted, tinged and yellow stained. As the pigmentation value decreases, the fiber is better for textile processes (Peng et al., 2009; James et al., 2010). Reflectance is defined as the degree of light reflection. In other words, it indicates the brightness of cotton fibers. A higher value is better. Environmental factors have large effects (about 79%) on color grade and brightness in cotton. The amount of cellulose can result in decreased brightness; weathering can also affect brightness and color grade in the long term. Moreover, microorganisms or insects can cause discoloration due to their association with cotton boll rot.

1.2.1.7. Micronaire

Micronaire indicates both fiber fineness and maturity. It is a measure of air permeability of cotton fiber. Thinner fibers are more durable, brighter and softer. A low value of micronaire indicates finer fibers and is desired (Peng et al., 2009; James et al., 2010). Fine fibers form stronger yarn. Fineness directly affects processing stages. Maturity impacts the absorbency of dye in the processing stage. Increased maturity provides better dye absorbance. Fiber fineness is mainly controlled by variety whereas maturity is largely affected by environmental factors and management. Plant growth factors, fertility, date of planting, harvesting, moisture, temperature, plant nutrients, and insects play roles in micronaire.

1.2.1.8. Trash and Leaf Grade

Trash is considered as the amount of non-lint particles in a fiber sample. It is indicated as the percentage of surface area which is trash (Johnson et al., 1996). An acceptable value of trash should be in the range of 0 to 1.6 %. Leaf grade describes the amount of cotton plant particles within the cotton fiber samples. Lower values are better. One of the main reasons for trash is leaf particles. In the textile industry, leaf grade directly impacts quality, productivity and manufacturing. High leaf grade levels require extra processing to remove trash.

1.2.1.9. Neps

Neps are defined as small nodules on the fiber which are generated in the process of harvesting and ginning. A bigger nep is called a nap and a bigger nap is called a mote (Peng et al., 2009; James et al., 2010). Formation of neps is affected by adverse growth conditions, early harvesting, diseases and harmful factors. Neps are important factors because fibers carrying neps usually are broken during spinning. Also the thread of fiber with nep is not easy to dye (Pearson et al., 1944).

1.2.1.10. Spinning Consistency

Spinning consistency is described as the suitability of cotton fibers for yarn-spinning. A high value of spinning consistency is desired especially for the textile industry (Peng et al., 2009; James et al., 2010).

1.3. Production of Cotton

The demand for cotton is continually increasing due to increases in world consumption, the standard of living and industrial development. Although cotton has a very important economic position in world industry, cotton cannot be produced in all countries because of ecological limitations. Only eight countries provide up to 80% of worldwide cotton production. Cotton production of these countries is shown in Figure 1. In this list, China ranks first with 6.840.000.00 tonnes cotton production, while Turkey ranks eighth with 851.000.00 tonnes.

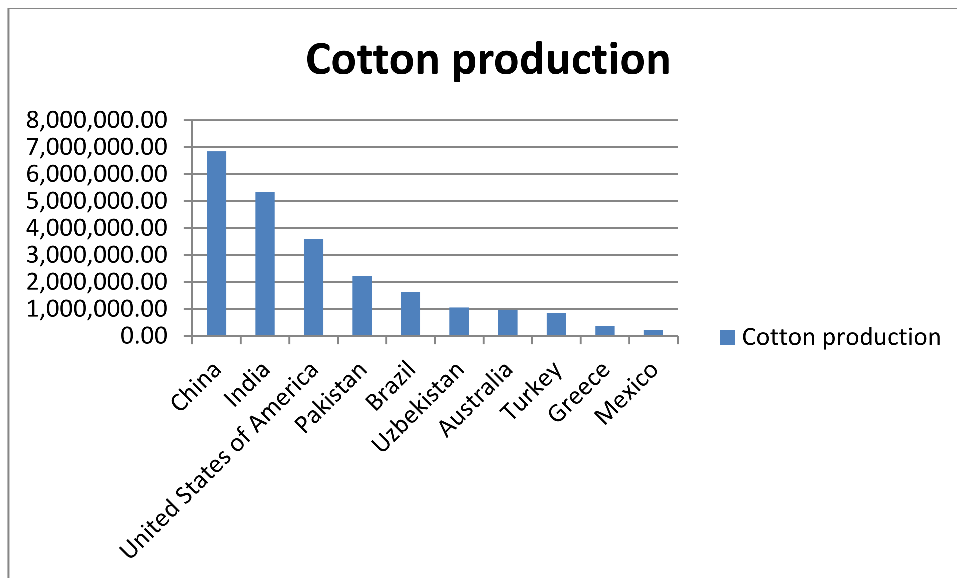

Figure 1.1. Cotton production around the world.
(Source: FAO 2012)

In Turkey, the history of cotton production dates back to 330 BC. The beginning of the main advances in cotton agriculture started during the Seljuk Empire (Gencer, 1999). In the 13th and 14th centuries, the Ottoman Empire expanded cotton agriculture in the Aegean and Çukurova regions by importing cotton seeds from Egypt. Major improvements in production started with the foundation of the Republic of Turkey. Cotton production is located in the Aegean, Antalya, Çukurova and Southeastern Anatolian regions in Turkey (Gencer, 1999). To date, the history of cotton production and yield in Turkey have actively developed. A timeline of cotton production in Turkey is shown in Figure 2. All cotton cultivars grown for agriculture in Turkey are *G. hirsutum L.* species.

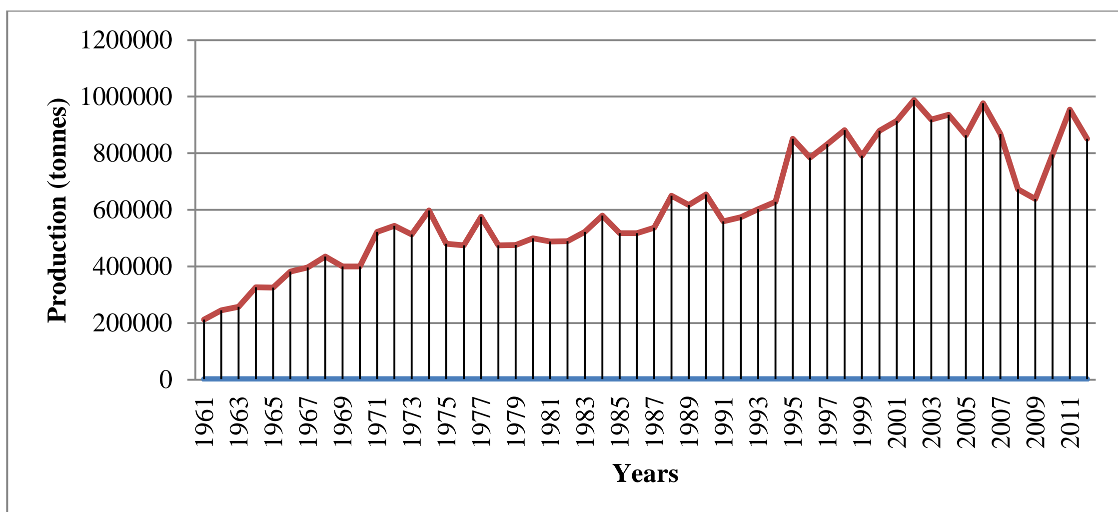

Figure 1.2. Cotton production of Turkey from 1961 to 2012
(Source: FAO)

Although Turkey has geographically and ecologically suitable conditions for cotton production, the desired quantity and quality of cotton yield and production have not yet been obtained. There are many reasons for this lack of optimum yield, quality and production. Some notable reasons are: the high cost of cotton production, insufficient education about cotton production and technical processing, insufficient experts for cotton standardization and insufficient improvement and development of cotton cultivars. In addition, cotton production in Turkey, like that in the rest of the world, suffers from many major ecological, biotic and abiotic stresses such as climate change and diseases. These stresses cause decreased cotton production and serious loss

of cotton yield (Saeed et al., 2011). Many abiotic stress factors arise from soil and weather conditions which give rise to significant damage (more than 50%). More specifically, high temperature, low light, salinity and drought are the main abiotic stresses on cotton production.

1.3.1. High Temperature

For best yield, cotton should be grown in hot climate regions; however, temperatures that are too high cause limitations in cotton growth resulting in yield loss. Cotton is sensitive to high temperatures for all growth stages. Generally the optimum temperature is reported to be 30 °C. High temperatures (above 35°C) negatively affect photosynthesis. Thus, production of carbohydrates is decreased resulting in reducing boll size and number. As a result, such conditions directly reduce cotton yield (Ossterhuis, 2011). When global warming is considered, this restrictive factor will possibly be an even more serious problem for cotton in the future (Ossterhuis, 2011). The degree of temperature sensitivity depends on cotton cultivar as a result of genetic differences. Several scientific studies have referred to this as genotypic thermotolerance (Cottee et al., 2007). Thus, breeders take advantage of genotypic thermotolerance to breed new cotton cultivars. However, according to trials for upland cotton, thermotolerance is not the best solution because of insufficient genetic diversity for this trait (Ossterhuis et al., 2009).

1.3.2. Low Light Stress

It is well-known that light is crucial for photosynthesis in plants. To receive sunlight perpendicularly to its leaves, *G. hirsutum* tracks the sun during the day. This case is explained by Lambert's Cosine Law. If the sunlight directly interacts with the leaf surface (meaning the angle of light is 0), the cosine is 1. This is shown in Figure 3. Although *G. hirsutum* tracks sunlight, *G. barbadense* does not. So while the cosine of incidence for *G. barbadense* changes, it remains around 1 for *G. hirsutum.* These movements increase the use of sunlight, effectively resulting in increased photosynthesis (Ossterhuis, 2011). The inability of such movements in certain cotton varieties affects the photosynthesis rate, especially on cloudy days and during cloudy

seasons. This may explain the fact that on cloudy days, a temporary carbohydrate shortage can cause leaf shedding in plants (Goodman, 1955). Previous studies showed that reduced sunlight significantly decreases photosynthesis rate, directly affecting cotton yield and quality (Zhao et al., 1998).

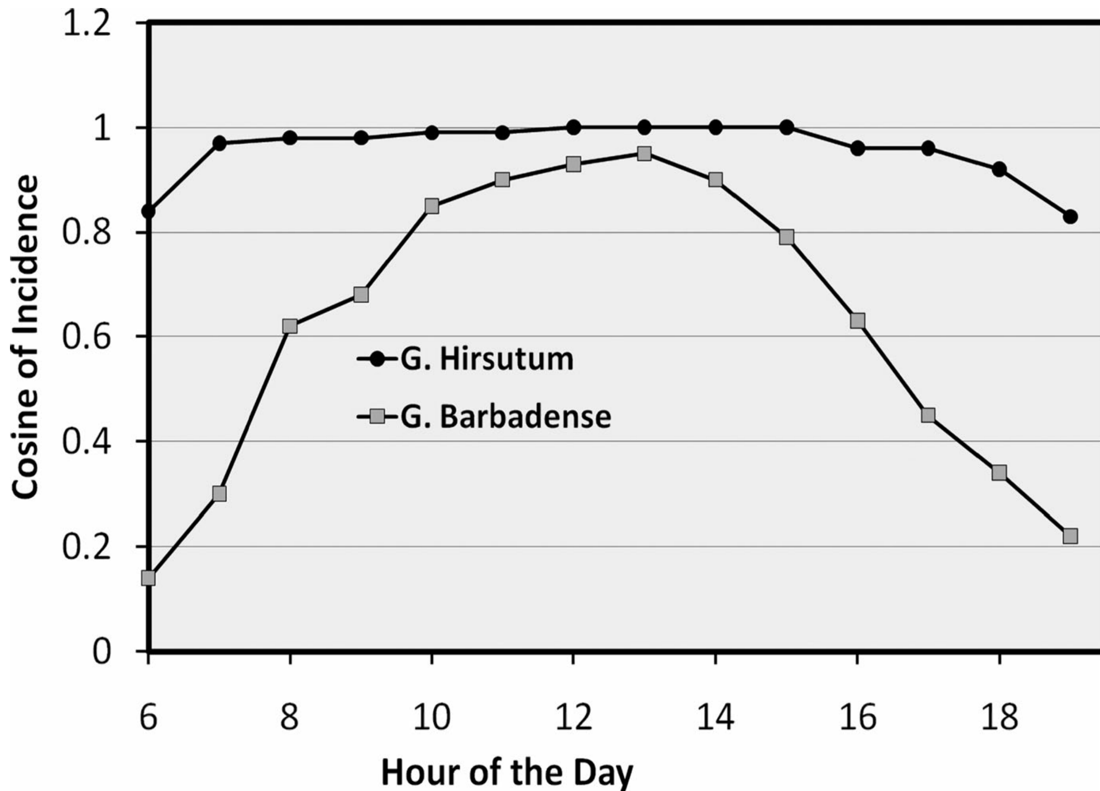

Figure 1.3. The angle of incidence for leaves of *G. hirsutum cv*. Stoneville 825, and *G. barbadense* cv. Pima S5. (Source : Ehleringer et al., 1987)

1.3.3. Salinity Stress

About 20% of cultivated land in the world suffers from salt stress (Desing et al., 2007). It is well-known that salinity leads to decreases in photosynthesis, respiration and normal metabolic activities. The ions that mainly cause salt stress are Ca, Mg, Na, C1, SO_4, HCO_3 and sometimes K and NO_3. Three major effects resulting from salt stress are: water stress, ion toxicity and imbalance of nutrients. Cotton can be considered as a relatively salt tolerant plant; however, under high salt concentrations, development and growth are restricted. (Zhong et al., 1993). Salinity shows its major effects especially at the seedling stage and directly on the plant’s roots. It adversely

affects the ability of root cells to adjust their water balance thereby altering root growth, elongation and morphology (Kurth et al., 1986).

1.3.4. Drought Stress

Drought stress is a very important problem worldwide since water is essential for plants. Around 33% of cultivated area is under the threat of high loss of crop productivity due to water deficiency. Because the roles of water in physiological and biochemical processes are crucial, water deficiency causes changes in anatomy and morphology (Ossterhuis, 2011; Massaci et al., 2008). According to studies, it is clear that water deficiency reduces photosynthesis. It has been debated that stomatal activities, decreased mesophyll conductance and metabolic causes lead to decreased photosynthesis in plants under drought stress. When stomata close, CO_2 diffusion decreases and this reduced CO_2 diffusion from outside to the carboxylation site is the critical reason for decreased photosynthesis (Flexas et al., 2007). There are several other factors that affect photosynthesis rate under drought stress. For example, feedback mechanisms for carbohydrate accumulation and leaf photochemistry can reduce photosynthesis.

Drought stress also induces oxidative stress because photosynthesis is prevented. This situation causes generation of toxic components such as reactive oxygen species. These components are dangerous for cellular structures containing lipids, proteins, carbohydrates and nucleic acids. To protect cells from this condition, antioxidants are produced (Monk et al., 1987). Because drought stress is an inductive chaos factor in plants, stress-induced proteins such as heat shock proteins are produced to survive (Ingram et al., 1996). Although plants have different ways to cope with the negative effects of drought stress, success is directly related with the extent and timing of the stress (Ossterhuis, 2011).

1.3.5. Diseases

Disease is a biotic stress that originates from bacteria, viruses, fungi and other microorganisms. Cotton diseases severely damage cotton production, yield and quality around the world. Sometimes the loss of yield is so high and large-scale that

productivity cannot be maintained. These cases result in massive costs in cotton agriculture (Smith et al., 1999).

1.3.5.1. Verticillium Wilt

Verticillium wilt is a severe cotton disease caused by *Verticillium dahliae*. *V. dahliae* is a fungal, soil-borne pathogen that affects economically important crops in warm temperature areas around the world. Verticillium wilt was firstly detected in Mentha, Michigan in 1924 (Sink et al., 1999). It was defined as a disease in 1927 by Herbert and Hubbard (Smith et al., 1999). Verticillium wilt spread to China from America in 1935 leading to huge losses of up to 30% in cotton production in the 1970s and 1980s (Bugbee, 1970). It is considered as the main threat to cotton production worldwide. The pathogen, *V. dahliae*, shows its effects by different mechanisms and its pathogenicity level is high. *V. dahliae* has a wide host range consisting of more than 300 woody and herbaceous plant species. The optimum temperature for *V. dahliae* is 27°C which is also the optimum temperature for cotton growth. The characteristic symptoms of Verticillium wilt are reduction in leaf surface, reduction in dry weight of root and stem leaf curl, vascular tissue wilt, and necrosis (Pegg, 2002)(Figure 4,5). Verticillium wilt is still a serious problem for cotton quality since there is no fungicide against it (Li et al., 2011). Because Verticillium wilt is a soil-borne disease when an affected plant dies, the parts of the plant that remain in the soil will cause its continued spread. There are two types of Verticillium wilt pathogen in terms of pathogen virulence effect: defoliating (D) strains and non-defoliating (ND) strains. Whereas D strains are highly virulent and can defoliate the whole plant, ND strains are mildly virulent types and lead to only wilt and local defoliation. D type strains cause earlier development, more severe effects on affected plants. These pathotypes have been found in the cotton-growing area of the Mediterranean region of Turkey (Bicici and Kurt 1998).

Because most commercial cotton cultivars are sensitive to Verticillium wilt, it is very important to develop Verticillium wilt resistant upland cotton cultivars (Zhao et al., 2014). In cotton, breeding for resistance against Verticillium wilt is limited due to lack of immunity or a lack of high levels of genetic resistance. However, it is known that certain cotton lines show resistance against Verticillium wilt. Using these resistant lines, molecular or traditional breeding studies can be performed. Unfortunately, because of

the complexity of resistance mechanisms, conventional breeding methods are not very successful (Yang et al., 2008). Instead of conventional methods, molecular breeding methods are less labor intensive and are effective in improvement of cotton features and disease resistance. In recent years, molecular studies have provided quantitative trait loci (QTL) analysis for finding genes responsible for resistance to Verticillium wilt. At present there are several QTLs reported by different studies. (Bolek et al., 2005; Fang et al., 2013; Gao et al., 2003; Wang et al., 2008; Yang et al., 2008)

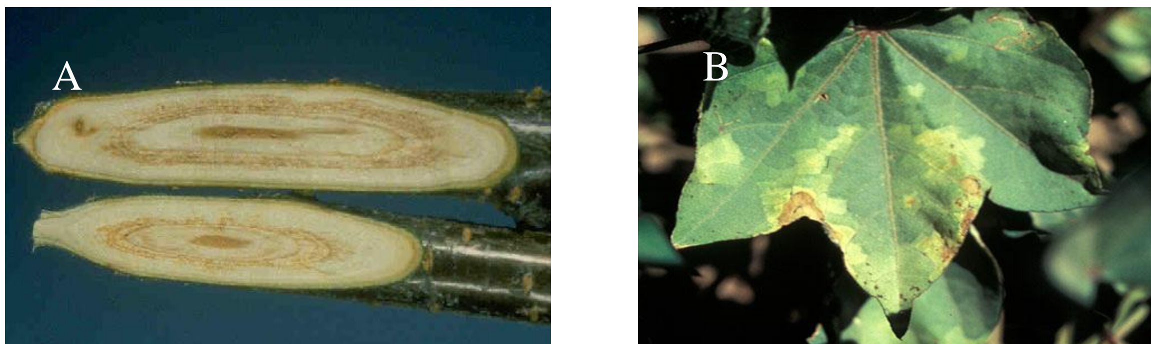

Figure 1.4. a) Vascular discoloration. b) Leaf necrosis due to Verticillium wilt.

1.4. Marker-Assisted Selection

For many years, breeders have applied conventional methods to develop or improve desired traits in agriculture. Although there have been very successful and abundant results using conventional techniques, these traditional methods have not completely fulfilled the requirements for improvement of complex traits. Molecular methods provide alternative, effective ways for successful breeding of complex traits. The use of DNA markers offers increased efficiency and speed in plant breeding. DNA markers which are useful for plant breeding are DNA regions showing variation between individuals that is associated with specific traits such as resistance to disease, salt, or drought. Markers associated with the desired trait can be used to select individuals at the molecular genetic level. This is called marker-assisted selection (MAS). MAS dispenses with the need for extensive growth of individuals, excessive effort for breeding and reduces time and cost (Collard et al., 2008).

There are many DNA markers that are used in molecular breeding such as sequence tagged site (STS), sequence characterized amplified region (SCAR), single

nucleotide polymorphism (SNP), simple sequence repeat (SSR), restriction fragment length polymorphism (RFLP), and amplified fragment length polymorphism (AFLP) markers. There are some concerns about the use of DNA markers in studies including reliability, DNA amount and quality, polymorphism, methodological procedure and cost. These considerations change according to DNA marker type. For high reliability, the marker must be as tightly linked to the trait as possible. Some DNA markers such as AFLP require high amounts of very pure DNA. To detect associations, the essential requirement is polymorphism between individuals. Therefore, the level of polymorphism for DNA markers is important. Another important consideration is technique. Methods which are simple and high-throughput are desired for analysis. However, the cost of the method is another important consideration which should be appropriate for the project resources. There are three main requirements for carrying out MAS: a segregating population, polymorphic markers and software analysis to process the data. MAS is summarized in Figure 5.

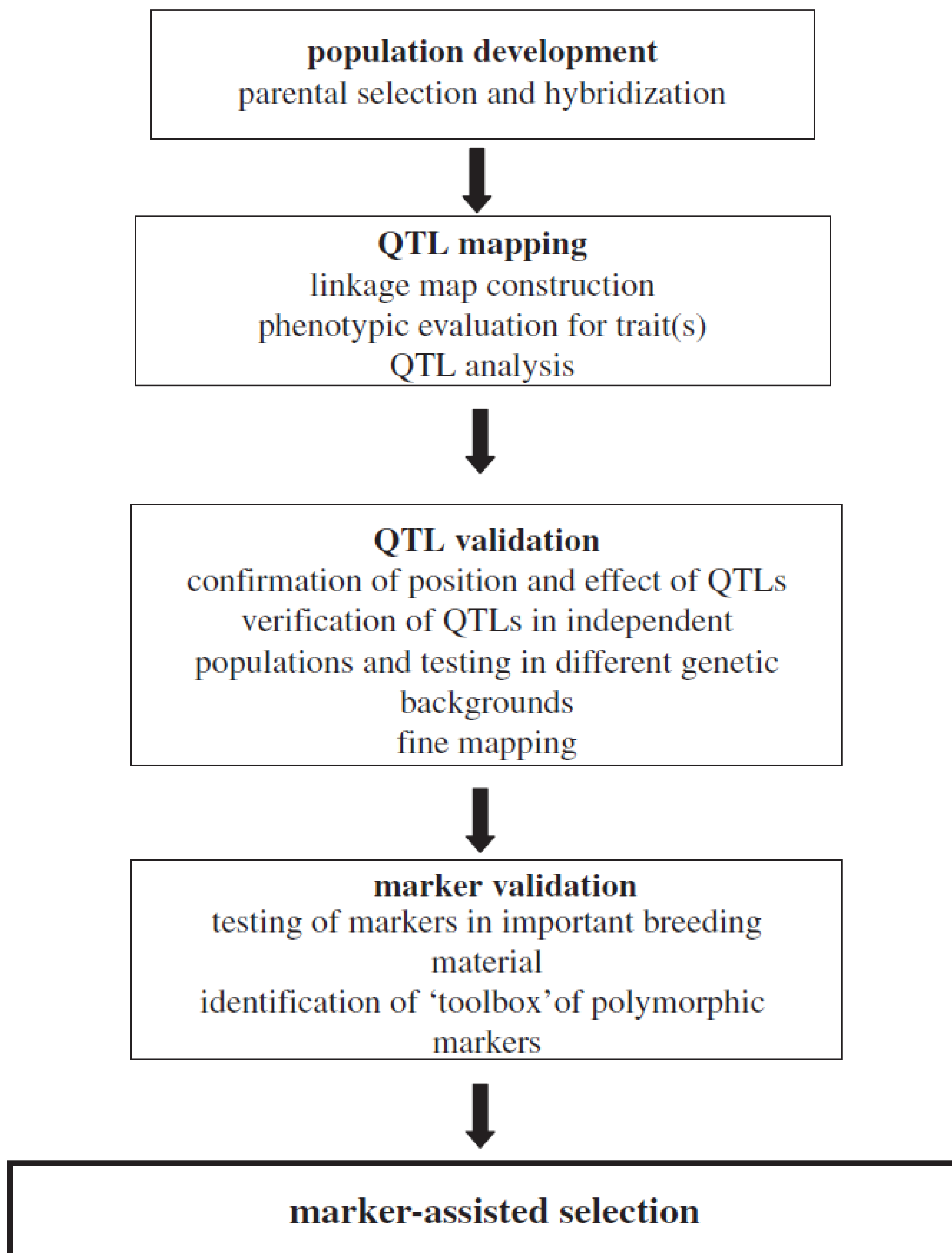

Figure 1.5. Flowchart of marker- assisted selection.
(Source : Collard et al., 2008)

1.5. Aim of the Study

Cotton is an essential, economical crop not only for the textile industry but also for other industries in the world. Almost every part of the cotton plant is exploited in different ways. Because of the importance of cotton, breeding to improve its yield, quality and resistance to diseases and stresses is essential. Verticillium wilt is a fungal disease and one of the major causes of huge losses in cotton production in the world. There are a limited number of studies on development of Verticillium resistance in cotton species. Although there have been several studies about QTL analysis of resistance to Verticillium wilt, there is no properly achieved result yet. In this study we aimed to find quantitative trait loci responsible for Verticillium wilt resistance using SSR markers in a natural population. To accomplish this we carried out phenotypic and genotypic analysis to find polymorphisms between cotton individuals for detection of QTLs.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials

In this study, a natural cotton population was used because in natural populations the possibility of sampling more different alleles is higher than in biparental populations. Plant materials (seeds) were provided by Nazilli Cotton Research Institute (NCRI) and The United States Department of Agriculture (USDA). A total of 118 different cultivars which are known to show variation in terms of resistance to Verticillium wilt were genotypically and phenotypically tested. The individuals used in this study are listed in Table 2.1. All materials used in this study were *G. hirsutum* cultivars.

Table 2.1. Plant individuals (*Gossypium hirsutum*) used in this study.

Sample No	Sample Name	Sample No	Sample Name	Sample No	Sample Name	Sample No	Sample Name
1	Sealand 542	31	Stoneville 453	62	Ngf-63	92	Elsa
2	Dpl 90	32	Stoneville 8751	63	Barut2005	93	Delta Diamond
3	Acola 1517	33	Tomcot Cabcs	64	Gsn12	94	Gloria
4	Acala 5	34	Tky 9309	65	Menderes 2005	95	Julia
5	Auburn M	35	Tky 9409	66	Napa122	96	Flora
6	Blightmaster	36	Tky3304 Gs316	67	Npege 2009	97	Pg2018
7	Cabu1cs2-1-83	37	Togo	68	Np Ozbek 100	98	Ba308
8	Caroline Queen	38	Somon	69	Şahin2000	99	Ba525
9	Coker 208	39	Carmen	70	Samarkant Uzbek	100	Ba119
10	Delta Opal	40	N727cc	71	Taskent-1		Carmen
11	Dp388	41	Nieves	72	Taskent-6		Sahin2000
12	Dpl6	42	Semu Ss/6	73	Taskent Uzbek		Ba119

(Cont. on the next page)

Table 2.1. (cont.)

13	Dpl5415	43	Siccla 312	74	152f		Gsn12
14	Dpl882	44	Sicola 33	75	Aleppo-1		Clandio
15	Dpl883	45	Sahel 1	76	S-9		St-373
16	Dpl20	47	Corona	77	Delcerro		St-488
17	Dpl886	48	Lachata	78	Delcerro Ms-30		Famosa
18	Dpl-C-37 Prima	49	Nata	79	Sindos 80		Prestige
19	Dpl Sr-383	50	Vulcano	80	Zeta 2		Teks
20	Gc-262	51	Eysan92	81	A2 31		Pg-Aksel
21	Gc 555	52	Sayar 314	82	Eva		Pg-Flash
22	Gsa 78	53	Ayhan 107	83	Gw Teks		Pg-2018
23	Lankort 57	54	Dak-6613	84	Niab 111		Ba-525
24	Mcnair 220	55	Ms-30/1	85	Niab 999		Ba119
25	Paymaster404	56	Nazilli 143	86	Tomcot 22		Ba308
26	Rex 1	57	Nazilli 84-S	87	Tomcot Sphinx		Ba151
27	Sj-V Visalia Elmer	58	Nazilli 87	88	Sj-U86		Ba468
28	Sg1001	59	Nazilli M39	89	Condia		Cukurova1 518
29	Sg125	60	Nazilli M 503 (97-3)	90	Celia		
30	Stoneville 213	61	Nazilli 503	91	Claudia		

2.2. Methods

2.2.1. Verticillium Dahliae Testing

Cotton lines were individually planted in pots with five replications of each line. They were grown in the growth chamber at 24 ±1 °C under 12 h daylight periods. When the plants reached the 4-5 leaf stage they were inoculated with *Verticillium dahliae* Kleb. fungal pathogen. For inoculation, a conidial suspension containing Tween-80 and both high virulent Vd11 (ND) and PYDV6 (D) isolates was used. These types are found in Mediterranean and Aegean regions in Turkey. One month later the inoculated plants were observed for their disease phenotypes. Leaves of each individual were examined and scored from 0 to 4 according to the degree of pathogen symptoms to determine resistant and sensitive phenotypes (Bejarano-Alcazar et al., 1996). While 0

represented resistant individuals with no visible symptoms, values up to 1 indicated tolerant individuals (1-33 % disease symptoms), 1-2 indicated moderate tolerance (34-66 % disease symptoms), 2-3 indicated sensitive individuals (67-97 % disease symptoms) and 3-4 highly sensitive individuals (dead individuals). Disease rate was calculated by averaging the disease scores across replicates. Inoculation and scoring process were carried out by Dr. Oktay Erdoğan, Nazilli Cotton Research Station.

2.2.2. DNA Extraction

Genomic DNA extraction of cotton plants was carried out from the youngest and the lightest green leaves by manual CTAB DNA isolation procedure (Doyle and Doyle, 1987). At the end of DNA extraction, all DNA samples were dissolved and homogenized in TE buffer. After that, the quantity and quality of DNA was measured using a Nanodrop ND-1000 spectrophotometer. All DNA samples were stored at -20°C.

2.2.3. Molecular Marker Analysis

2.2.3.1. SSR Analysis

According to DNA quantity and quality results, all samples were diluted to a final concentration of 25 ng/µl. For SSR analysis, 100 SSR primer pairs (DOW, DPL, BNL, and MUSS) were selected to apply to the cotton population. The primer information was obtained from www.cottonmarker.org. The cotton SSR markers are listed in Table 2.2.

Polymerase chain reactions (PCR) were carried out with following components in 25 µl volume:
2.5 µl 10X PCR buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl2, pH: 8.3), 1.5 µl MgCl2, 0.5 µl dNTP (0.2 mM), 0.5 µl forward and 0.5 µl reverse primers (10 pmol), 0.3 µl Taq polymerase (0.25 U), 17.2 µl sterile didistilled water, and 2 µl DNA (~50 ng/µl). To amplify DNA, PCR conditions were optimized as follows: one step of 3 min at 94°C for denaturation, 35 cycles with 1 sec at 94°C, 45 seconds at 55-60 °C annealing temperature (depending on primer pair), 1 min at 72°C for extension and a final

extension step of 10 min at 72°C in BIO-RAD Thermal Cycler™. After PCR reactions, a Fragment Analyzer™ Automated CE System was used to separate DNA fragments at high resolution and detect polymorphisms. This system has the capacity to separate amplified fragments with a minimum of 3 bp difference.

Table 2.2. Simple sequence repeat (SSR) markers which were used in this study.

No	Marker Name	No	Marker Name	No	Marker Name
1	BNL1145	37	DPL049	73	DPL679
2	BNL1151	38	DPL068	74	DPL684
3	BNL2882	39	DPL071	75	DPL728
4	BNL3034	40	DPL075	76	DPL743
5	BNL3383	41	DPL080	77	DPL847
6	BNL3502	42	DPL100	78	DPL866
7	DOW003	43	DPL112	79	DPL885
8	DOW004	44	DPL119	80	DPL890
9	DOW006	45	DPL136	81	JESPR014
10	DOW036	46	DPL140	82	JESPR066
11	DOW038	47	DPL156	83	JESPR135
12	DOW044	48	DPL168	84	JESPR151
13	DOW051	49	DPL176	85	JESPR152
14	DOW053	50	DPL181	86	JESPR153
15	DOW054	51	DPL186	87	JESPR157
16	DOW055	52	DPL188	88	JESPR197
17	DOW056	53	DPL193	89	JESPR204
18	DOW057	54	DPL199	90	JESPR205
19	DOW058	55	DPL204	91	JESPR208
20	DOW059	56	DPL212	92	JESPR218
21	DOW062	57	DPL216	93	JESPR228
22	DOW069	58	DPL220	94	JESPR273
23	DOW070	59	DPL223	95	JESPR308
24	DOW073	60	DPL228	96	MUSS151
25	DOW074	61	DPL241	97	MUSS261
26	DOW075	62	DPL247	98	MUSS414
27	DOW077	63	DPL253	99	MUSS425
28	DOW082	64	DPL299	100	MUSS532
29	DOW083	65	DPL307		
30	DOW085	66	DPL322		
31	DOW093	67	DPL354		
32	DOW094	68	DPL490		
33	DOW100	69	DPL513		

(Cont. on the next page)

Table 2.2. (cont.)

34	DPL009	70	DPL541		
35	DPL019	71	DPL659		
36	DPL045	72	DPL674		

2.2.3.2. Association Analysis

Allelic data obtained from fragment analysis were scored according to band presence/absence. To detect the correct number of clusters reflecting the best population structure, STRUCTURE 2.2.3 was used (Pritchard et al., 2000). STRUCTURE is a clustering program. STRUCTURE gives a quantification of how likely it is that each individual belongs to each group. This information is used to detect the best cluster number of population showing ancestral background of the population. The analysis in STRUCTURE was run with parameters of burn-in period of 100,000 and 500,000 MCMC replications and a hoc statistic introduced by Evanno et al. was used to determine the correct estimated number of clusters (Evanno et al., 2005) with STRUCTURE HARVESTER online program. To obtain information about genetic diversity of the population, DARwin5 (Dissimilarity Analysis and Representation for Windows) was used. Association analysis was performed by TASSEL 2.1 (Trait Analysis by aSSociation, Evolution and Linkage) software program with MLM (mixed linear model) (Bradbury et al., 2007). For this aim, a Kinship matrix (K matrix) calculated by TASSEL program, Q matrix which showed the quantities of individuals for the best cluster number from STRUCTURE, allelic data and phenotypic data of the Verticillium wilt test were used in TASSEL. TASSEL associated the phenotypic and genotypic data to find significant links between the polymorphic markers used in this study and Verticillium wilt resistance.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1. Verticillium Wilt Test

Five plants of each genotype of 118 cotton individuals were inoculated with *V. dahliae*. After 1 month, the symptoms of the disease were examined and scored. The scoring results are shown in Table 3.1. According to intensity of pathogen symptoms, tolerant and susceptible individuals were observed. CUKUROVA1518 and Sealand-542 showed high sensitivity with scores of 3.24 and 3.1, respectively (Figure 3.1). However, PRESTIGE showed high tolerance with a score of 0.67 while CELIA showed moderate tolerance with a score of 1.28 (Figure 3.2). According to results, it was clear that there was variation in terms of response against *Verticillium dahliae* virulence. Obviously these differences resulted from differences in genetic background of cotton lines. Therefore this natural population was suitable for association analysis because it showed phenotypic segregation for the trait.

For Verticillium wilt resistance, population distribution is demonstrated in Figure 3.3. The graph showed expected distribution of phenotypic variation for Verticillium wilt resistance. It showed clearly that the population was suitable for further association analysis.

Figure 3.1. Sensitivity of Cukurova1518 and Sealand542. a) Cukurova1518 was very sensitive and almost completely defoliated. b) Sealand542 also showed high sensivity.

Figure 3.2. Resistance of Prestige and Celia. a) Prestige showed high tolerance. There was no defoliation and no disease spots on leaves. b) Celia also showed high tolerance.

Table 3.1. Scoring results of Verticillium wilt test with five plants of each genotype. 0 represents resistant individuals, values up to 1 indicate tolerant individuals, 1-2 indicates moderate tolerance, 2-3 indicates sensitive individuals and 3-4 highly sensitive individuals. Average values of five replication are given in last column with standard errors (SE).

Genotypes		**Repeat**					**Average ± SE**
Sample code	Sample name	**I**	**II**	**III**	**IV**	**V**	
18	DPL-C-37 PRIMA	3.5	3.4	1.8	1.2	1.6	2.30 ± 0.54
17	DPL886	2.0	2.0	2.8	2.2	1.6	2.12 ± 0.22
16	DPL20	3.2	2.6	2.1	2.6	3.0	2.70 ± 0.21

(Cont. on the next page)

Table 3.1. (cont.)

15	DPL883	1.6	2.5	1.8	2.6	3.2	2.34 ± 0.32
14	DPL882	3.4	1.5	1.8	2.7	-	2.35 ± 0.43
13	DPL5415	2.7	1.0	2.0	-	-	1.90 ± 0.43
12	DPL6	1.5	3.0	1.8	2.0	1.8	2.02 ± 0.29
11	DP388	3.0	3.2	3.0	-	-	3.07 ± 0.06
10	DELTA OPAL	1.0	3.0	2.0	-	-	2.00 ± 0.50
9	COKER 208	3.7	2.0	3.2	3.0	-	2.98 ± 0.36
8	CAROLİNE QUEEN	2.1	2.0	1.8	1.8	-	1.93 ± 0.08
7	CABU1CS2-1-83	3.4	2.8	2.1	1.2	1.3	2.16 ± 0.48
6	BLIGHTMASTER	2.0	2.5	3.0	2.4	1.4	2.26 ± 0.30
5	AUBURN M	1.5	1.6	1.8	1.8	-	1.67 ± 0.08
4	ACALA 5	1.0	1.2	2.3	2.0	1.6	1.62 ± 0.27
3	ACOLA 1517	2.1	2.6	3.5	1.1	2.1	2.28 ± 0.44
2	DPL90	1.6	1.6	1.6	-	-	1.60 ± 0.00
1	SEALAND 542	3.0	3.2	3.2	-	-	3.10 ± 0.06
36	TKY3304 GS316	4.0	4.0	1.0	4.0	3.0	3.20 ± 0.65
35	TKY 9409	3.2	4.0	4.0	3.0	3.0	3.44 ± 0.26
34	TKY 9309	1.4	3.0	3.0	3.2	3.2	2.76 ± 0.38
33	TOMCOT CABCS	2.1	3.0	3.0	3.0	1.6	2.54 ± 0.33
32	STONEVILLE 8751	1.2	1.5	1.5	1.8	1.6	1.52 ± 0.33
31	STONEVILLE 453	3.0	0.8	3.0	3.0	1.0	2.16 ± 0.58
30	STONEVILLE 213	2.0	1.0	1.0	1.0	0.5	1.10 ± 0.27
29	SG125	3.0	3.0	1.0	1.6	2.6	2.24 ± 0.45

(Cont. on the next page)

Table 3.1. (cont.)

28	SG1001	1.0	1.3	1.6	1.2	3.2	1.66 ± 0.44
27	SJ-V VISALIA ELMER	1.7	2	1.5	2.2	1.5	1.78 ± 0.16
26	REX 1	1.2	3.6	3.0	3.2	3.2	2.84 ± 0.47
25	PAYMASTER404	2.7	2.7	3.0	1.7	2.8	2.58 ± 0.25
24	MCNAIR 220	2.8	0.8	1.0	1.2	1.2	1.40 ± 0.40
23	LANKORT 57	1.0	1.0	1.2	1.0	-	1.05 ± 0.05
22	GSA 78	3.2	3.5	1.2	1.0	2.6	2.30 ± 0.57
21	GC 555	1.4	2.7	3.2	2.2	-	2.37 ± 0.38
20	GC-262	3.6	0.8	0.6	1.0	1.0	1.40 ± 0.62
19	DPL SR-383	0.8	1.0	1.0	1.2	0.7	0.94 ± 0.10
54	DAK-6613	1.1	3.0	3.2	3.2	0.7	2.24 ± 0.62
53	AYHAN 107	3.0	3.0	3.0	3.4	-	3.10 ± 0.10
52	SAYAR 314	2.8	3.0	3.2	1.2	1.2	2.28 ± 0.50
51	EYSAN92	3.5	1.1	1.0	3.0	1.2	1.96 ± 0.60
50	VULCANO	3.2	3.2	3.2	1.2	3.4	2.84 ± 0.46
49	NATA	3.2	1.6	3.4	3.0	0.8	2.40 ± 0.57
48	LACHATA	0.8	3.2	3.0	2.2	1.3	2.1 ± 0.52
47	CORONA	2.0	1.5	2.5	2.6	1.8	2.08 ± 0.23
45	SAHEL 1	1.2	1.4	2.5	2.7	1.6	1.88 ± 0.34
44	SICOLA 33	1.0	0.8	3.2	1.4	3.5	1.98 ± 0.64
43	SICCLA 312	1.1	0.7	1.3	2.0	3.0	1.62 ± 0.45
42	SEMU SS/6	1.1	1.8	0.8	2.2	3.6	1.90 ± 0.55
41	NIEVES	1.2	1.0	1.6	1.1	-	1.22 ± 0.13
40	N727CC	1.7	1.2	0.8	1.2	-	1.22 ± 0.18

(Cont. on the next page)

Table 3.1. (cont.)

39	CARMEN	1.2	1.0	1.0	1.0	1.3	1.10 ± 0.07
38	SOMON	3.2	0.4	0.5	1.8	3.2	1.82 ± 0.69
37	TOGO	3.4	3.2	3.0	3.2	3.2	3.20 ± 0.07
91	CLAUDİA	1.8	1.5	2.8	3.0	1.6	2.14 ± 0.35
90	CELIA	3.0	0.8	0.8	0.6	1.2	1.28 ± 0.49
89	CONDIA	2.8	2.7	1.2	0.8	0.8	1.66 ± 0.50
88	SJ-U 86	1.0	1.2	1.0	2.7	-	1.47 ± 0.41
87	TOMCOT SPHINX	1.0	0.8	1.0	3.0	-	1.45 ± 0.52
86	TOMCOT 22	3.0	0.8	2.8	0.8	0.8	1.64 ± 0.58
85	NIAB 999	1.2	1.2	1.2	1.2	1.8	1.32 ± 0.13
84	NIAB 111	1.3	1.3	1.3	1.1	1.0	1.20 ± 0.07
83	GW TEKS	0.8	0.8	2.0	3.0	1.8	1.68 ± 0.46
82	EVA	1.6	1.2	1.0	2.1	-	1.47 ± 0.24
81	A2 31	3.2	2.8	2.7	1.0	1.8	2.30 ± 0.44
80	ZETA 2	1.7	1.5	1.0	1.2	1.0	1.28 ± 0.16
79	SINDOS 80	3.0	0.8	3.1	3.1	1.0	2.20 ± 0.59
78	DELCERRO MS-30	3.0	1.1	3.1	1.4	3.2	2.36 ± 0.51
77	DELCERRO	2.0	3.2	0.6	1.1	3.2	2.02 ± 0.59
76	S-9	1.0	3,3	3.3	3.0	3.0	2.57 ± 0.53
75	ALEPPO-1	3.6	3.4	3.2	1.0	1.7	2.58 ± 0.58
74	152F	1.2	0.6	1.6	3.0	3.0	1.88 ± 0.54
ST-373	ST-373	1.1	1.0	1.5	1.2	1.1	1.18 ± 0.10
ST-488	ST-488	1.5	1.3	1.1	1.1	1.1	1.22 ± 0.09

(Cont. on the next page)

Table 3.1. (cont.)

FAMOSA	FAMOSA	1.5	1.5	1.5	2.0	1.0	1.50 ± 0.18
BA-119	BA-119	1.2	1.2	2.6	0.8	1.5	1.46 ± 0.34
GSN-12	GSN-12	1.1	1.2	1.4	3.0	1.4	1.62 ± 0.39
S.2000	S.2000	1.0	1.1	0.7	1.0	1.0	0.96 ± 0.08
CARMEN	CARMEN	0.8	0.6	0.6	0.6	0.8	0.68 ± 0.05
CLANDIO	CLANDIO	1.8	1.0	1.0	3.0	0.8	1.52 ± 0.46
100	BA119	1.4	1.8	2.0	1.3	2.8	1.86 ± 0.30
99	BA525	2.1	1.8	1.1	1.0	0.8	1.36 ± 0.28
98	BA308	1.2	1.5	0.8	0.7	0.6	0.96 ± 0.19
97	PG2018	1.4	2.4	1.3	1.6	3.0	1.94 ± 0.37
96	FLORA	1.0	1.0	1.2	1.4	1.0	1.12 ± 0.09
95	JULIA	3.5	3.5	2.0	2.2	-	2.80 ± 0.41
94	GLORIA	1.4	1.2	1.6	2.4	2.0	1.72 ± 0.24
93	DELTA DIAMOND	1.1	1.2	1.2	2.7	1.0	1.44 ± 0.35
92	ELSA	1.4	2.2	1.1	3.0	1.5	1.84 ± 0.38
62	NGF-63	1.3	1.8	2.1	2.6	1.4	1.84 ± 0.38
PRESTIGE	PRESTIGE	0.6	0.6	0.8	0.7	-	0.68 ± 0.05
TEKS	TEKS	1.0	1.0	1.1	1.4	1.1	1.12 ± 0.08
PG-Aksel	PG-Aksel	1.6	3.0	2.0	2.0	-	2.15 ± 0.30
PG-Flash	PG-Flash	1.7	1.0	0.8	1.6	2.5	1.52 ± 0.33
PG-2018	PG-2018	0.8	1.1	1.1	1.1	1.1	1.04 ± 0.07
73	TASKENT UZBEK	2.0	1.5	1.6	3.0	2.5	2.12 ± 0.32
72	TASKENT-6	2.0	2.3	1.4	1.6	1.0	1.66 ± 0.25

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Table 3.1. (cont.)

71	TASKENT-1	1.1	1.2	1.4	0.8	1.3	1.16 ± 0.12
70	SAMARKANT UZBEK	1.6	3.2	1.8	1.5	-	2.03 ± 0.40
69	SAHIN2000	1.5	2.0	1.8	3.0	2.6	2.18 ± 0.30
68	NP OZBEK 100	1.0	1.2	1.2	1.0	1.2	1.12 ± 0.05
67	NPEGE 2009	1.5	2.2	1.4	2.7	1.3	1.82 ± 0.30
66	NAPA122	1.6	1.6	0.8	1.2	-	1.30 ± 0.19
65	MENDERES 2005	1.3	1.5	1.2	1.6	3.5	1.82 ± 0.48
64	GSN12	3.2	0.8	1.0	1.4	3.2	1.92 ± 0.59
63	BARUT2005	3.0	1.2	1.0	1.0	1.2	1.48 ± 0.43
61	NAZILLI M 503	0.8	1.0	1.4	0.8	1.4	1.08 ± 0.15
60	NAZILLI M 503-(93-7)	1.0	0.6	0.6	1.1	1.0	0.86 ± 0.12
59	NAZILLI M39	3.2	1.6	1.1	1.6	1.1	1.72 ± 0.43
58	NAZILLI 87	1.0	0.8	0.6	0.6	1.0	0.80 ± 0.10
57	NAZILLI 84-S	3.4	1.0	3.4	3.4	0.8	2.40 ± 0.69
56	NAZILLI 143	1.0	1.4	2.6	0.6	1.0	1.32 ± 0.38
55	MS-30/1	2.1	1.1	1.3	1.2	1.0	1.34 ± 0.22
BA-525	BA-525	1.1	1.7	1.8	2.0	3.7	2.06 ± 0.49
BA-119	BA-119	1.4	2.8	3.0	1.8	1.8	2.16 ± 0.35
BA-308	BA-308	3.0	2.8	1.2	1.0	1.5	1.90 ± 0.47
BA-151	BA-151	1.6	2.2	3.0	2.0	-	2.20 ± 0.29
BA-468	BA-468	2.5	1.8	1.8	1.0	1.2	1.66 ± 0.29
CUKUROVA1518	CUKUROVA1518	3.8	3.0	3.2	3.0	3.2	3.24 ± 0.16

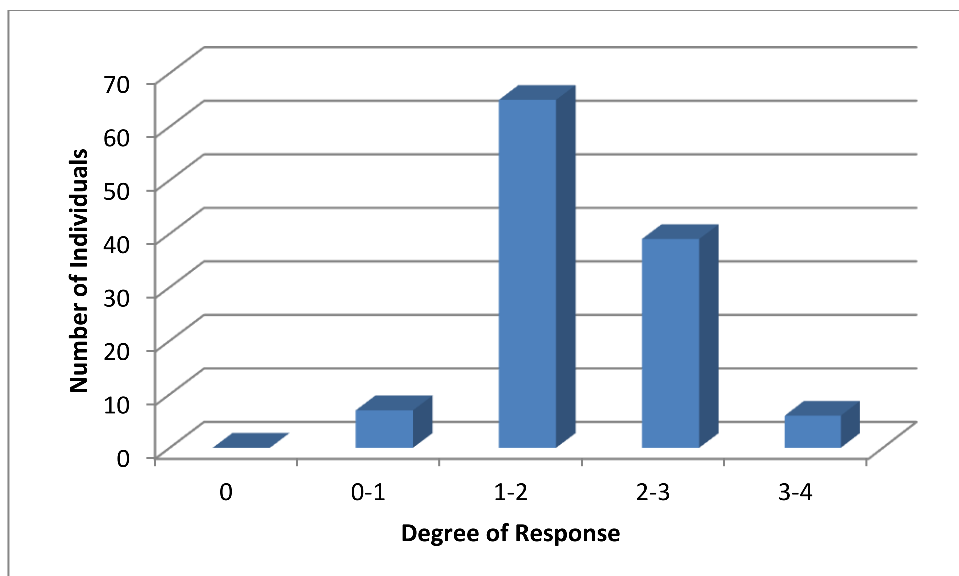

Figure 3.3. Distribution of the population representing phenotypic variation for Verticillium Wilt resistance. 0 represents completely resistant individuals (none), 0-1 represents high tolerant individuals (7 cultivars) and 1-2 represents moderately tolerant individuals (65 cultivars), 2-3 represents susceptible individuals (39 cultivars) and 3-4 represents high susceptible individuals (7 cultivars).

3.2. DNA Extraction

DNA isolation was carried out with a manual CTAB extraction method. Quality and quantity values of stock DNA samples were measured with Nanodrop ND-1000 spectrophotometer. Nanodrop results are shown in Table 3.2. DNA quantities and qualities were suitable for further experiments.

Table 3.2. Stock DNA quantity results with 260 (abs)/280(abs) ratio values. 260/280 values show quality of DNA. Good quality values are between 1.8-2.0.

Genotype	ng/uL	260/280
Sealand-542	4938,8	1.18
Dpl-90	4662,0	1.65
Acola1517	4699,9	1.39
Acala-5	4345,6	1.70
Auburn M	4972,8	1.19
Blightmaster	3744,7	1.90
Cabu1cs2-1-83	4610,1	1.62
Carolina Queen	3440,6	1.76
Coker-208	4875,1	1.44
Delta opal	4997,1	1.11
DP-388	4854,1	1.33
DPL-66	4395,6	1.82

Genotype	ng/uL	260/280
Nazilli M503	2230,0	1.95
Ngf-63	4800,0	1.17
Barut-2005	1787,0	1.99
Gsn-12	1447,0	1.94
Menderes-2005	1860,0	1.97
Napa-122	3681,0	1.86
Npege-2009	3864,0	1.80
Np ozbek-100	4691,0	1.36
Sahin-2000	2021,0	1.86
Samarkant Uzbek	4278,0	1.72

(Cont. on the next page)

Table 3.2. (con.)

DPL-5415	4565,9	1.56
DPL-882	2814,8	1.95
DPL-883	2913,1	2.07
DPL-20	4957,4	1.12
DPL-886	4901,7	1.36
DPL-c-37prima	3752.1	1.64
DPL SR-383	1926,5	2.04
GC-262	2299,7	1.94
GC-555	3822,2	1.93
Gsa-78	3463,5	1.93
Lankort	4916,5	1.16
Mcnaire-220	1974,1	2.02
Paymaster-404	2064,1	1.57
Rex-1	4275,4	1.86
Sj-V visalia elmer	4995,7	1.18
Sg-1001	4999,5	1.10
Sg-125	5066,2	1.12
Stoneville-213	3307,0	1.90
Stoneville-453	5008,2	1.21
Stoneville-8751	4895,8	1.46
Tomcot cabcs	4909,8	1.40
Tky-9309	5037,9	1.17
Tky-9409	2468,9	1.73
Tky-3304 Gs316	4998,5	1.10
Togo	2474,2	2.02
Somon	4021,0	1.84
Carmen	4963,1	1.14
N-727-cc	1710,0	1.1
Niaves	4795,0	1.32
Semu SS/6	4899,0	1.13
Sıccla-312	1841,0	2.00
Sıcola-33	3623,1	1.97
Sahel-1	4286,2	1.82
Corona	4618,1	1.60
Lachata	4790,0	1.37
Nata	4052,0	1.87
Vulcano	3081,4	2.09
Eysan-92	4553,0	1.13
Sayar-314	4940,0	1.24
Ayhan-107	4728,4	1.36
Dak-6613	4860,0	1.30
Ms-30/1	2545,0	1.95
Nazilli-143	2906,0	1.91
Nazilli-84-S	1425,0	1.95
Nazilli-87	1949,0	1.80
Nazilli M39	2753,0	1.88
Nazilli M503-(93-7)	2062,0	1.90

Taskent-1	1883,0	1.89
Taskent-6	2942,0	1.86
Taskent Uzbek	3158,0	1.76
152-F	4502,0	1.63
Alleppo-1	3688,0	1.85
S-9	2385,0	1.92
Delcerro	4178,0	1.70
Delcerro MS-30	4600,0	1.70
Sindos-80	2420,0	1.95
Zeta-2	2825,0	2.00
A2 31	1830,0	1.01
Eva	3260,0	1.91
Gw-teks	3800,0	1.74
Niab-111	4780,0	1.35
Niab-999	2017,0	2.04
Tomcot-22	2356,0	1.97
Tomcot Sphinx	1355,0	1.86
Sj-U-86	4074,0	1.48
Condia	2700,0	1.82
Celia	1304,0	1.99
Claudia	4431,0	1.62
Elsa	4900,0	1.23
Delta Diamond	4837,0	1.07
Gloria	3075,0	1.88
Julia	4600,0	1.62
Flora	2361,0	2.03
Pg-2018	4645,0	1.29
Ba-308	3044,0	1.96
Ba-525	4900,0	1.77
Ba-119	2260,0	2.04
BA-119	4658,0	1.61
BA-119	3593,0	1.91
BA-151	3794,0	1.71
BA-308	4789,0	1.58
BA-468	5018,0	1.10
BA-525	4169,0	1.82
Carmen	4900,0	1.21
Clandio	4700,0	1.49
Cukurova1518	2138,0	1,82
Famosa	4294,0	1.68
GSN-12	4717,0	1.70
PG-2018	4899,0	1.06
PG-Aksel	4950,0	1.08
PG-Flash	4841,0	1.55
PRESTIGE	4618,0	1.64
ST-373	3398,0	2.01
ST-488	2980,0	2.01
S.2000	3235,0	1.92
TEKS	3291,0	1.98

3.3. Molecular Marker Analysis

To determine allelic polymorphisms between the 118 individuals we applied 100 SSR markers to the population. We used Fragment Analyzer™ Automated CE system to separate the fragments. One of the polymorphic markers was DPL080. The fragment lengths for DPL080 ranged from 224 to 241 bp. The capillary gel result is shown in Figure 3.4 as an example.

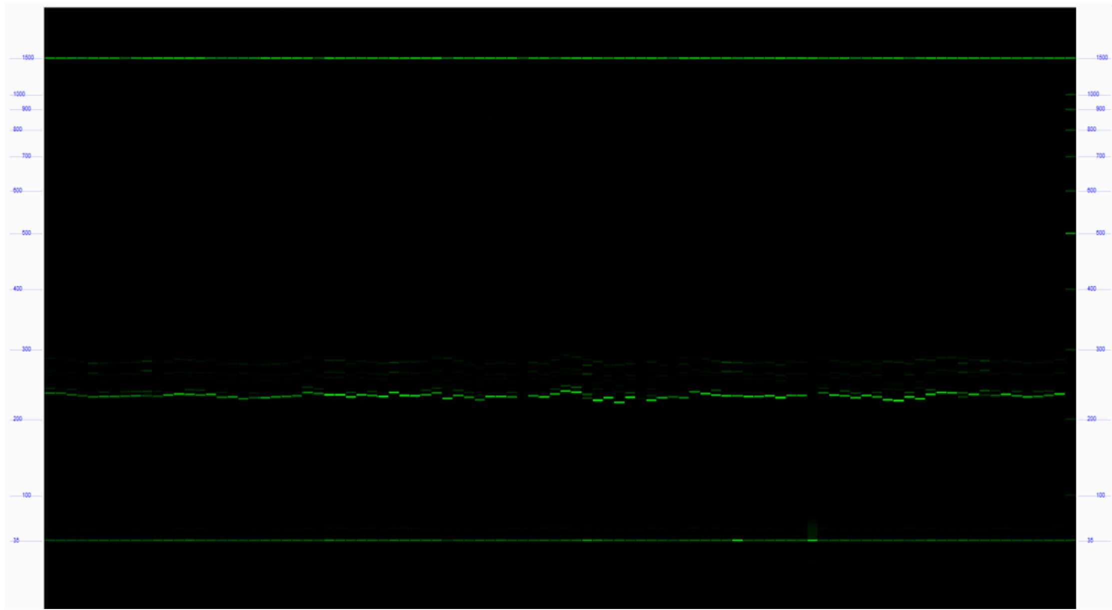

Figure 3.4. DPL080 capillary electrophoresis gel result for 96 individuals.At the end of the PCR analysis, 100 SSR markers resulted in 421 polymorphic loci for the 118 individuals. The fragments were scored in presence/absence (1/0) format. This allelic data was used in further software analysis for diversity and association.

3.3.1. Genetic Diversity and Association Analysis

3.3.1.1. Diversity Analysis

To carry out genetic diversity analysis of the population we used DARwin5 analysis program with Presence/Absence data, Dice coefficient and UnWeighted Neighbor Joining algorithm. A total of 111 individuals were analyzed with this program. Seven individuals were excluded from the analysis because of too much

missing data. According to the results of neighbor joining analysis, three clusters were obtained (Figure 3.7). In cluster A there were 53 individuals, in cluster B there were 38 individuals and in cluster C there were 20 individuals. The minimum genetic dissimilarity was determined as 0.134 (13%) and maximum genetic dissimilarity was determined as 0.582 (58%). Minimum genetic diversity was between Sample 12 (DPL6) and Sample 36 (TKY3304GS316) in cluster A. The maximum genetic diversity was between Sample 1 (SEALAND 542) in cluster A and Sample 81 (A231) in cluster B. The 111 units gave 6105 distance values and mean value was determined as 0.351 (Figure 3.5). According to MANTEL test the cophenetic r was 0.9396 which means that there was high correlation between the dissimilarity matrix and dendrogram.

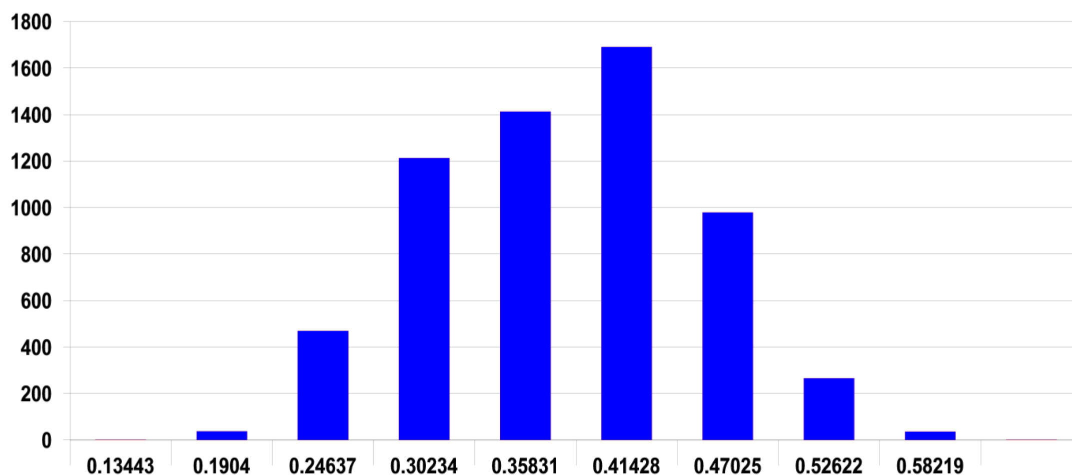

Figure 3.5. Genetic dissimilarity between individuals of the cotton population. Y axis represents numbers of values and x axis represents dissimilarity values of individuals.

According to diversity analysis, certain varieties from the same source had low diversity (Figure 3.7). For example, most of the varieties registered by Nazilli Cotton Research Station (NCRS) clustered together. These included Nazilli-143, Nazilli-84S, Nazilli-87, Nazilli-M39, Barut-2005 and Menderes-2005 (coded 56, 57, 58, 59, 63 and 65, respectively, in blue). Two other varieties registered by NCRS, Sealand-542 and Delcerro (1 and 77, respectively, in blue), were genetically distinct from each other and from the other NCRS cultivars. Two varieties registered by Bayer Turk Kimya San. Ltd. Şti. were Julia and Flora (95 and 96, respectively, in red) which clustered together. Similarly, Niab-111 and Niab-999 (84 and 85, respectively, in pink) were clustered

together. DPL-6, DPL5415 and DPL882 (12, 13 and 14, respectively, in green) clustered together indicating limited genetic diversity. Thus, these results suggest that cotton cultivars from the same breeding program are often highly similar to each other. This highlights the importance of introduction of genetic diversity into such programs. Moreover, the dendrogram provides an indication of which varieties can be selected to increase genetic diversity in a given breeding program.

When we combined DARwin results and Verticillium wilt test results (Figure 3.8) we see that 41.5% of Group A were moderately tolerant individuals. In contrast, for Group B, 68.4% of the individuals were moderately tolerant individuals while 65 % of Group C were moderately tolerant (Table 3.3). These results indicated that while susceptible individuals tended to cluster in group A, tolerant individuals tended to cluster in groups B and C (Figure 3.6).

Table 3.3. Percentage values of presence in DARwin groups for tested individuals.

	Tolerant Individuals	Moderately Tolerant Individuals	Sensitive Individuals	Highly Sensitive Individuals
Group A	5.7%	41.5%	43.4%	9.4%
Group B	5.3%	68.4%	23.7%	2.6%
Group C	10%	65%	25%	0%

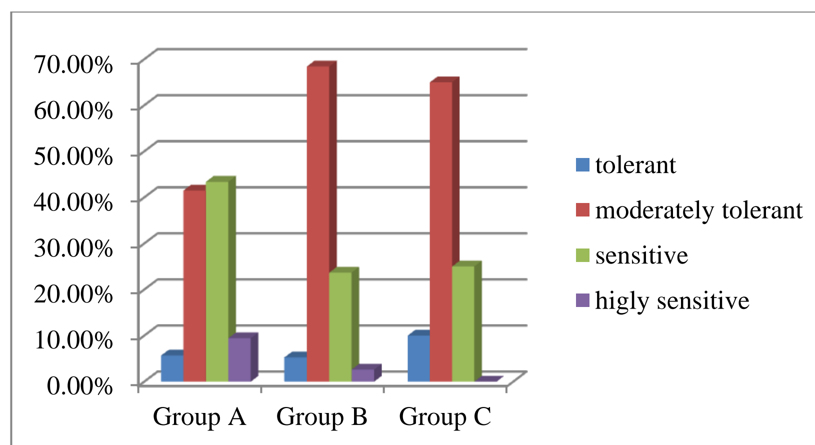

Figure 3.6. Showing presence of tested individuals with responses in their assigned groups by DARwin 5 program.

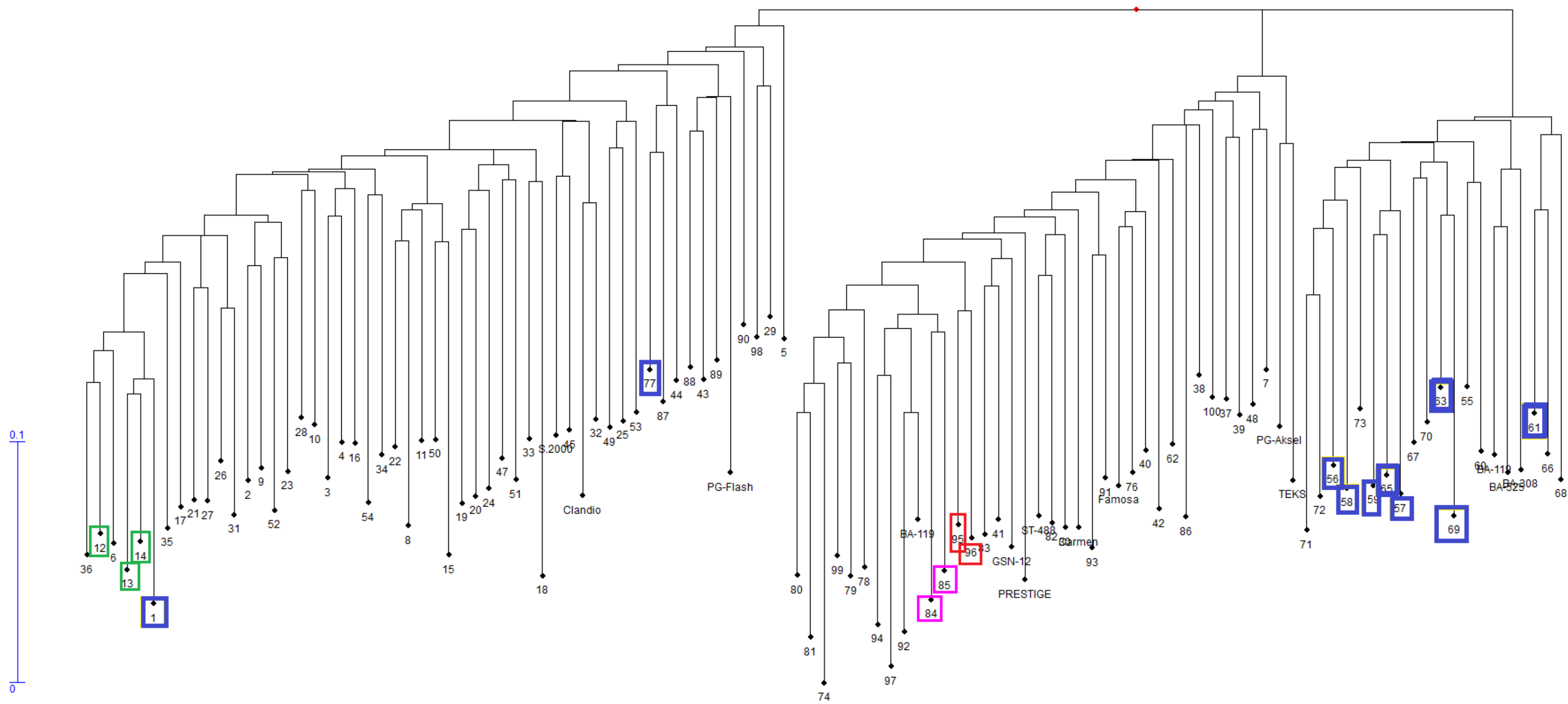

Figure 3.7. Dendrogram showing genetic diversity of the population resulting in 3 clusters. 53 cultivars were clustered in Group A, 38 cultivars in Group B and 20 cultivars in Group C.

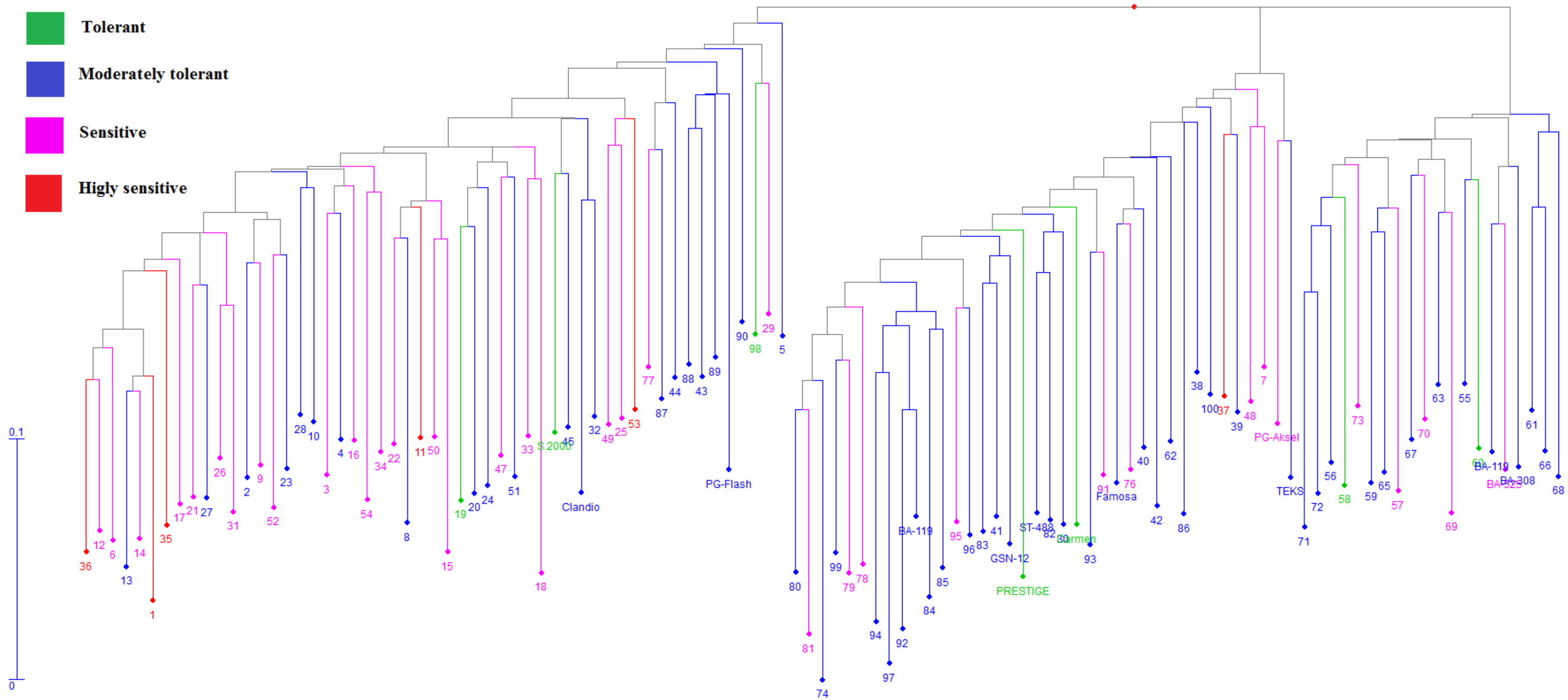

Figure 3.8. Verticillium wilt responses of the cultivars as clustered by neighbor-joining analysis. Each color represents each disease response in three clusters from DARwin.

3.3.1.2. Structure Analysis

Population structure was determined with STRUCTURE program. It analyzed the population from group (K) 2 to 10 with 20 iterations for each group to find the most correct population cluster number. The STRUCTURE results were processed with STRUCTURE HARVESTER online program. To determine the best cluster number for the population, delta K (ΔK) was used (Evanno et al. 2005). High value of ΔK shows the most correct number of clusters in the population. Delta K is calculated based on the second order rate of change of the likelihood:

L(K)= The log likelihood for each K

L'(K) = L(K)n – L(K)n-1

L''(K) = L'(K)n – L'(K)n-1

Delta K = [L''(K)]/Stdev

According to the results, the maximum ΔK value was determined for 2 groups (K=2) (Figure 3.9). However, we can also consider K= 3 as significant cluster number because of its high relative ΔK value.

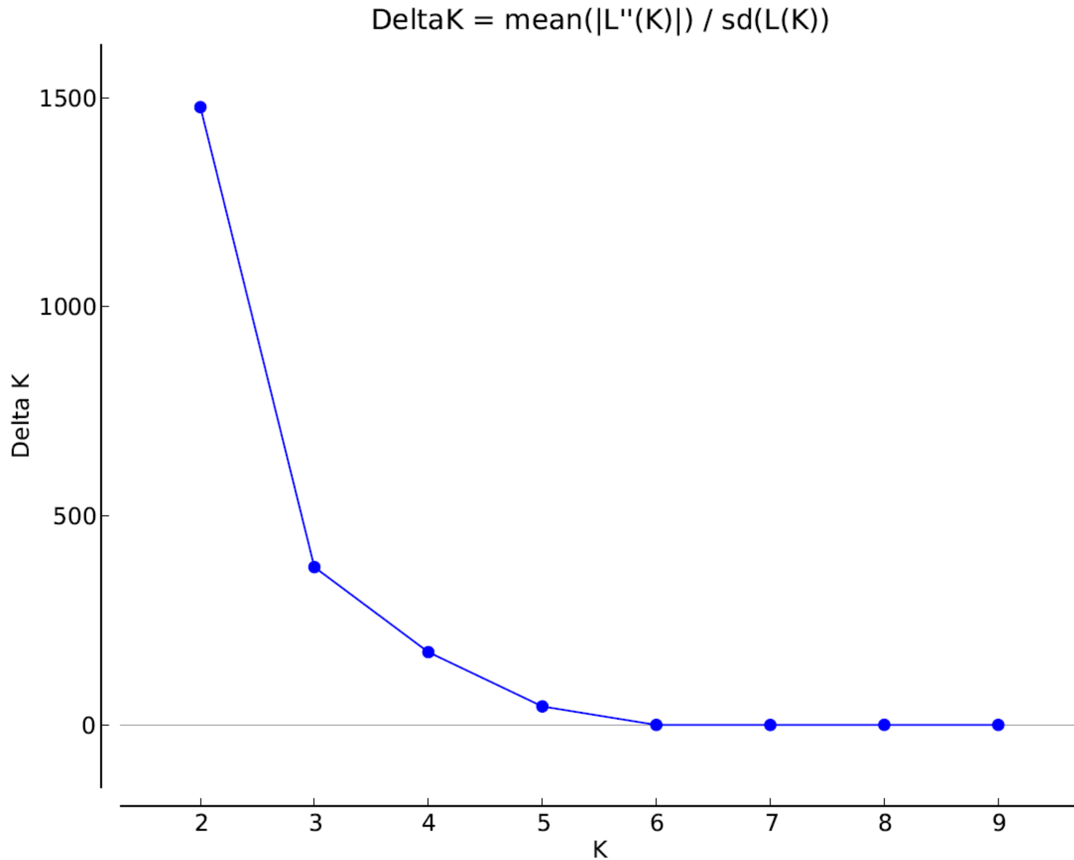

Figure 3.9. Delta K values for each value of K for the cotton population. High value of ΔK shows the most correct number of clusters in the population. In this graph we can see the highest value belongs to 2 groups but for 3 it is still significantly high value.

Determination of the correct number of groups for use in TASSEL analysis program is important. If there is more than one significant cluster number, the lowest cluster number is selected for natural populations. Here, we considered K=2 and K=3 as significant cluster numbers. The standard deviation results showed that standard deviations (SD) were high with more than 5 clusters. However, the SDs for K=2 and K=3 were at an acceptable level (Figure 3.10). SD values are given in Table 3.4. These results were consistent with each other and indicated that K=2 was suitable to represent population cluster number. We used cluster number 2 in further analysis in TASSEL.

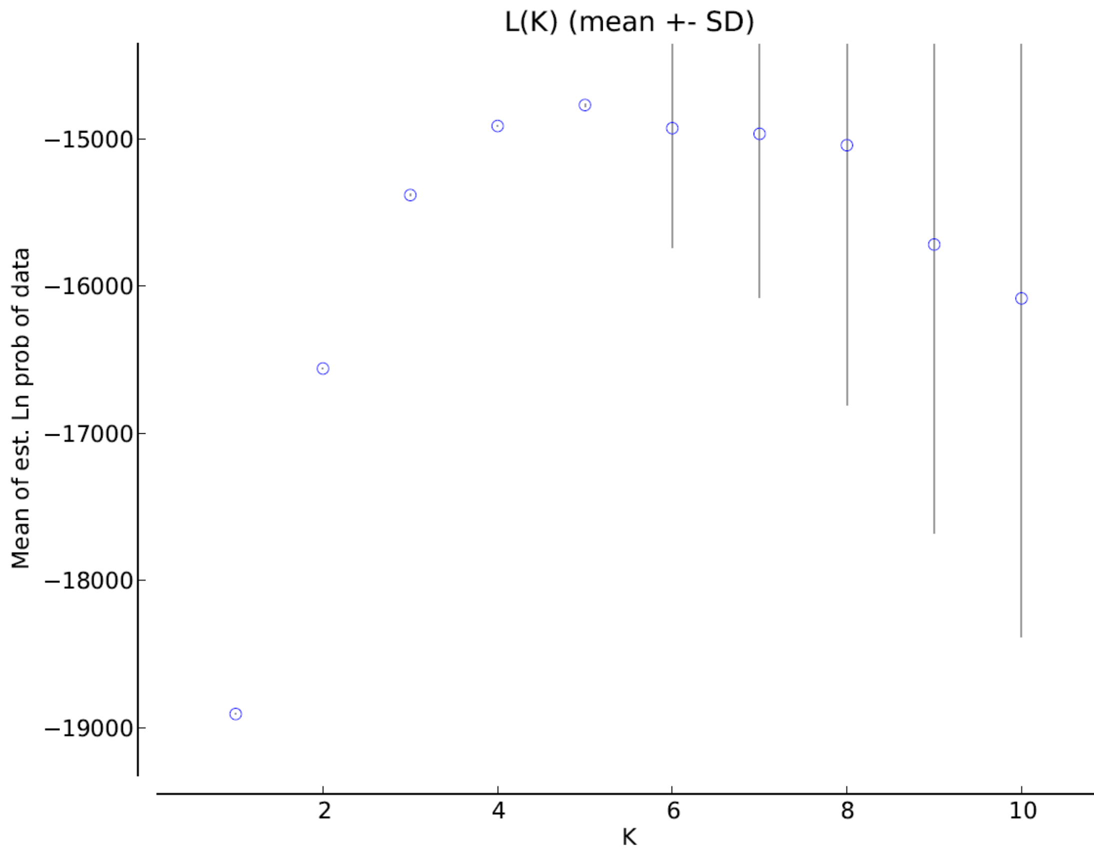

Figure 3.10. Standard deviation levels for each K. For cluster number 2 and 3 the values are acceptable.

Table 3.4. Estimation of Ln probability, standard deviations, Ln values and ΔK values.

K	**Mean LnP(K)**	**Stdev LnP(K)**	**Ln'(K)**	**|Ln''(K)|**	**Delta K**
1	-18.906.945.000	0.173129	—	—	—
2	-16.559.800.000	0.790736	2.347.145.000	1.168.495.000	1.477.731.185
3		1.879.670	1.178.650.000	709.665.000	377.547.730
4	-14.912.165.000	1.873.436	468.985.000	326.480.000	174.268.012
5	-14.769.660.000	6.758.418	142.505.000	299.790.000	44.358.016
6	-14.926.945.000	806.886.954	-157.285.000	118.875.000	0.147325
7	-14.965.355.000	1.109.556.384	-38.410.000	38.830.000	0.034996

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Table 3.4. (cont.)

8	-15.042.595.000	1.765.357.685	-77.240.000	597.520.000	0.33847
9	-15.717.355.000	1.960.985.830	-674.760.000	308.790.000	0.157467
10	-16.083.325.000	2.299.396.633	-365.970.000	—	—

The bar plots of the results show the structure of the population (Figure 3.11 and Figure 3.12). Each bar along the x-axis shows an individual in the population. Different groups are color-coded. The length of each colored bar shows the percentage of probability for assignment of each individual to a group. For cluster number 2 (K=2), the percentage probability of each individuals are shown in Figure 3.13.

Figure 3.11. The bar plot image for K=2 of cotton population. Red color represent cluster 1 and green color represents cluster 2.

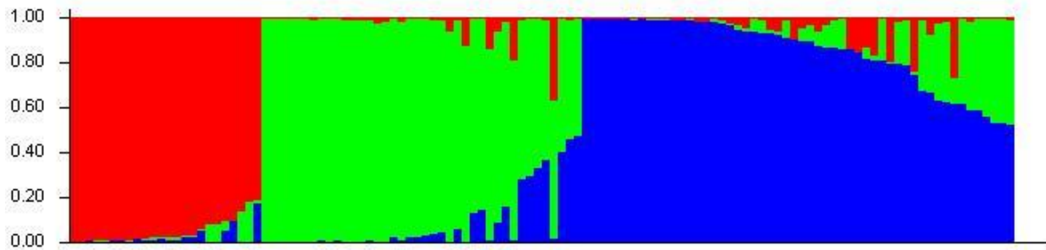

Figure 3.12. The bar plot image for K=3 of cotton population. Red color represent cluster 1, green color represents cluster 2 and blue color represents cluster 3.

Individuals were assigned to related clusters with cut-off 70% probability value. The individuals under this threshold were not assigned to any cluster and remained as intermixed individuals. The cluster assignment values of each individual are shown in Table 3.5. There were 44 individuals in cluster 1 and 34 individuals in cluster 2. However, 40 individuals remained as intermixed because they were not assigned to any cluster.

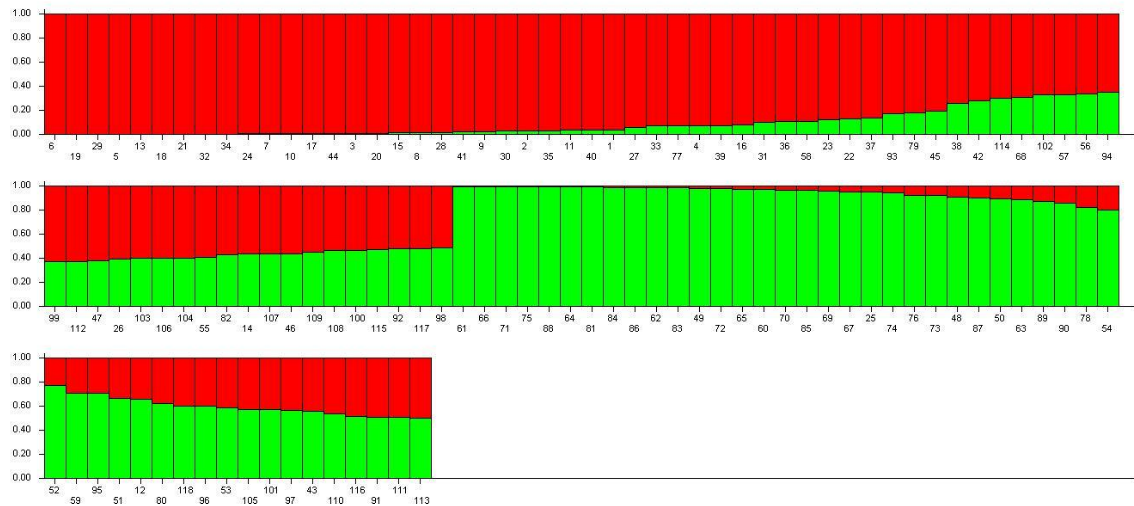

Figure 3.13. The cluster assignment values of each individual. For K=2 the bars show the percentage of probability of assignment to related 2 groups. Red color represent cluster 1 and green color represents cluster 2.

Table 3.5. Assignment values (%) of inferred ancestry of individuals are listed.

No	**Genotype**	**Label**	**Inferred Clusters**		**Assignment cluster**
1	Dpl-C-37 Prima	18	0.961	0.039	1
2	Dpl886	17	0.968	0.032	1
3	Dpl20	16	0.988	0.012	1
4	Dpl883	15	0.924	0.076	1
5		14	0.994	0.006	1
6	Dpl 5415	13	0.996	0.004	1
7	Dpl 6	12	0.991	0.009	1
8	Dp388	11	0.981	0.019	1
9	Delta Opal	10	0.975	0.025	1
10	Coker 208	9	0.991	0.009	1

(Cont. on the next page)

Table 3.5. (con.)

11	Caroline Queen	8	0.962	0.038	1
12	Cabu1cs2-1-83	7	0.34	0.66	intermixed
13	Blightmaster	6	0.994	0.006	1
14	Auburn M	5	0.564	0.436	intermixed
15	Acala 5	4	0.985	0.015	1
16	Acola 1517	3	0.916	0.084	1
17	Dpl 90	2	0.99	0.01	1
18	Sealand 542	1	0.994	0.006	1
19	Tky3304 Gs316	36	0.996	0.004	1
20	Tky 9409	35	0.987	0.013	1
21	Tky 9309	34	0.994	0.006	1
22	Tomcot Cabcs	33	0.867	0.133	1
23	Stoneville 8751	32	0.876	0.124	1
24	Stoneville 453	31	0.992	0.008	1
25	Stoneville 213	30	0.049	0.951	2
26	Sg125	29	0.602	0.398	intermixed
27	Sg1001	28	0.94	0.06	1
28	Sj-V Visalia Elmer	27	0.98	0.02	1
29	Rex 1	26	0.996	0.004	1
30	Paymaster404	25	0.969	0.031	1
31	Mcnair 220	24	0.899	0.101	1
32	Lankort 57	23	0.994	0.006	1
33	Gsa 78	22	0.927	0.073	1
34	Gc 555	21	0.994	0.006	1
35	Gc-262	20	0.965	0.035	1
36	Dpl Sr-383	19	0.887	0.113	1
37	Dak-6613	54	0.859	0.141	1
38	Ayhan 107	53	0.736	0.264	1
39	Sayar 314	52	0.922	0.078	1

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Table 3.5. (con.)

40	Eyşan92	51	0.962	0.038	1
41	Vulcano	50	0.976	0.024	1
42	Nata	49	0.715	0.285	1
43	Lachata	48	0.436	0.564	intermixed
44	Corona	47	0.99	0.01	1
45	Sahel 1	45	0.803	0.197	1
46	Sicola 33	44	0.56	0.44	intermixed
47	Siccla 312	43	0.621	0.379	intermixed
48	Semu Ss/6	42	0.088	0.912	2
49	Nieves	41	0.016	0.984	2
50	N727cc	40	0.101	0.899	2
51	Carmen	39	0.332	0.668	intermixed
52	Somon	38	0.227	0.773	2
53	Togo	37	0.408	0.592	intermixed
54	Claudia	91	0.197	0.803	2
55	Celia	90	0.589	0.411	intermixed
56	Candia	89	0.659	0.341	intermixed
57	Sj-U 86	88	0.669	0.331	intermixed
58	Tomcot Sphinx	87	0.887	0.113	1
59	Tomcot 22	86	0.286	0.714	2
60	Niab 999	85	0.024	0.976	2
61	Niab 111	84	0.005	0.995	2
62	Gw Teks	83	0.013	0.987	2
63	Eva	82	0.111	0.889	2
64	A2 31	81	0.007	0.993	2
65	Zeta 2	80	0.023	0.977	2
66	Sindos 80	79	0.005	0.995	2
67	Delcerro Ms-30	78	0.048	0.952	2
68	Delcerro	77	0.692	0.308	intermixed
69	S-9	76	0.041	0.959	2

(Cont. on the next page)

Table 3.5. (con.)

70	Aleppo-1	75	0.033	0.967	2
71	152F	74	0.006	0.994	2
72	St-373	ST-373	0.017	0.983	2
73	St-488	ST-488	0.078	0.922	2
74	Famosa	Famosa	0.054	0.946	2
75	Ba-119	BA-119	0.006	0.994	2
76	Gsn-12	GSN-12	0.074	0.926	2
77	S.2000	S.2000	0.926	0.074	1
78	Carmen	Carmen	0.178	0.822	2
79	Clandio	Clandio	0.818	0.182	1
80	Ba 119	100	0.374	0.626	intermixed
81	Ba525	99	0.007	0.993	2
82	Ba308	98	0.568	0.432	intermixed
83	Pg2018	97	0.014	0.986	2
84	Flora	96	0.012	0.988	2
85	Julia	95	0.033	0.967	2
86	Gloria	94	0.012	0.988	2
87	Delta Diamond	93	0.094	0.906	2
88	Elsa	92	0.006	0.994	2
89	Ngf-63	62	0.123	0.877	2
90	Prestige	PRESTIGE	0.14	0.86	2
91	Teks	TEKS	0.487	0.513	intermixed
92	Pg-Aksel	PG-Aksel	0.515	0.485	intermixed
93	Pg-Flash	PG-Flash	0.827	0.173	1
94	Pg-2018	PG-2018	0.65	0.35	intermixed
95	Taşkent Uzbek	73	0.29	0.71	2
96	Taşkent-6	72	0.4	0.6	intermixed
97	Taşkent-1	71	0.433	0.567	intermixed
98	Samarkant Uzbek	70	0.508	0.492	intermixed
99	Şahin2000	69	0.624	0.376	intermixed

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Table 3.5. (con.)

100	Np Ozbek 100	68	0.529	0.471	intermixed
101	Npege 2009	67	0.426	0.574	intermixed
102	Napa122	66	0.671	0.329	intermixed
103	Menderes 2005	65	0.595	0.405	intermixed
104	Gsn12	64	0.594	0.406	intermixed
105	Barut2005	63	0.424	0.576	intermixed
106	Nazilli M 503	61	0.595	0.405	intermixed
107	Nazilli M 503- (93-7)	60	0.561	0.439	intermixed
108	Nazilli M39	59	0.531	0.469	intermixed
109	Nazilli 87	58	0.547	0.453	intermixed
110	Nazilli 84-S	57	0.461	0.539	intermixed
111	NAZILLI 143	56	0.492	0.508	intermixed
112	MS-30/1	55	0.624	0.376	intermixed
113	BA-525	BA-525	0.493	0.507	intermixed
114	BA-119	BA-119	0.693	0.307	intermixed
115	BA-308	BA-308	0.523	0.477	intermixed
116	BA-151	BA-151	0.481	0.519	intermixed
117	BA-468	BA-468	0.515	0.485	intermixed
118	Cukurova1518	Cukurova1518	0.393	0.607	intermixed

Moreover the results of DARwin showed there were 3 clusters in the population. To see how STRUCTURE results correspond to the phylogenetic analysis we colored the neighbor-joining dendrogram. The third cluster in DARwin corresponded to the intermixed group identified by STRUCTURE (Figure 3.14). Group A of the dendrogram and Cluster 1 of STRUCTURE had 83% correspondence; Group B and Cluster 2 of STRUCTURE had 81% correspondence and there was 95% correspondence of Group C to the intermixed cluster of STRUCTURE. A total of 9 individuals for Group A, 7 individuals for Group B and only one individual for Group C did not show correspondence with DARwin dendrogram. Overall, these results indicated that both distance-based (neighbor-joining) and model-based (STRUCTURE) methods gave similar assessment of genetic relationships in the cotton population.

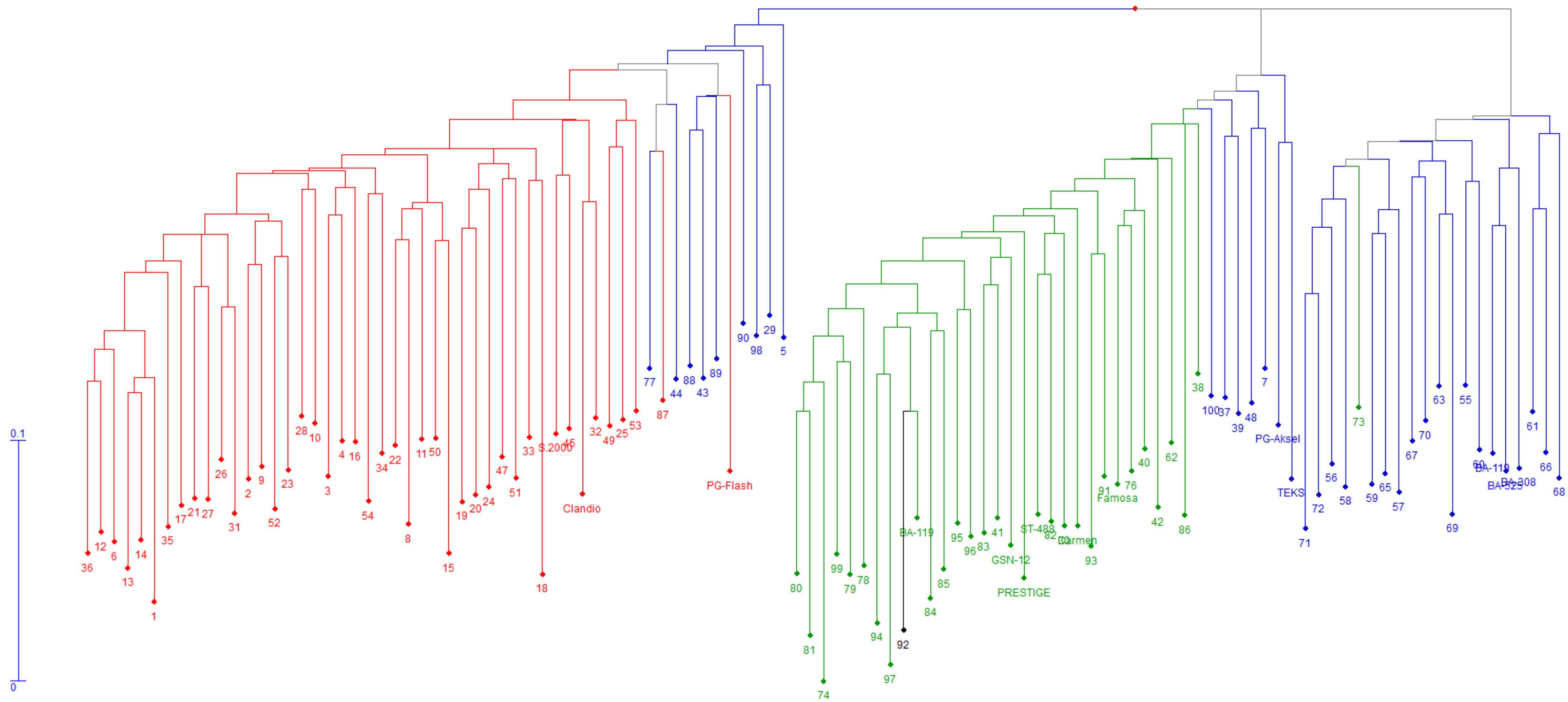

Figure 3.14. Correspondence between two estimates from DARwin and STRUCTURE analysis. This dendrogram was result of DARwin. Red color represents the individuals of Cluster1 from STRUCTURE, green color represents the individuals of Cluster2 from STRUCTURE and blue color represents the intermixed individuals from STRUCTURE.

3.3.1.3. Association Analysis

Association analysis between Verticillium wilt resistance and marker genotypes was carried out with TASSEL 2.1. The results were assessed according to significance levels of $p<0.05$ and $p<0.01$. We found that 30 of the SSR markers were significantly associated to Verticillium wilt resistance (R) (Table 3.6). At $p< 0.01$ significance level, 14 of SSR loci (shown in bold in the table) were considered as significantly associated with resistance. The chromosomal positions of the SSR markers were determined according to the high resolution consensus map of cotton (Blenda et al., 2012) and are shown with the allelic effects of the loci in Table 3.6.

Table 3.6. SSR loci significantly associated with Verticillium wilt resistance (R). Associated SSR loci are listed. However, for significant level $p<0.01$ allelic effects were considered as high significantly associated markers (shown in bold).

Locus	**P values p<0.05**	**Dominant allelic effect**	**Recessive allelic effect**	**Chr**
BNL2882-223	0,0489	0,1800	-0,1800	D2 (C14)
BNL3034-162	0,0252	0,3787	-0,3787	A3 and D2 (C14)
BNL3502-150	0,0081	-2,564	0,2564	D2 (C14)
DOW006-240	0,0064	0,2103	-2,1026	D13 (18)
DOW006-264	0,0447	0,3585	-0,3585	D13 (18)
DOW51-302	0,0035	-5,953	0,5953	D11 (21)
DOW53-391	0,0021	0,2405	-0,2405	D5 (19)
DOW59-337	0,0472	-0,1598	0,1598	D10 (20)
DOW59-338	0,0312	0,1921	-0,1921	D10 (20)
DOW83-227	0,0042	-0,9604	0,9604	A12
DOW83-230	0,0021	0,3153	-0,3153	A12
DPL009-206	0,0225	0,1521	-0,1521	A7
DPL080-238	0,0014	0,9051	-0,9051	A6
DPL100-163	0,0055	4,5131	-4,5131	UNKNOWN
DPL100-199	0,0196	-1,0729	1,0729	UNKNOWN
DPL140-251	0,0334	-0,5473	0,5473	D5 (19)
DPL156-252	0,0036	-0,4203	0,4203	A5
DPL156-284	0,0292	0,2887	-0,2887	A5
DPL156-291	0,0117	0,1599	-0,1599	A5
DPL168-213	0,0035	-0,3595	0,3595	D7 (16)
DPL176-214	0,0063	-0,4322	0,4322	A8
DPL186-170	0,0140	-0,865	0,865	A7
DPL188-130	0,0074	5,0686	-5,0686	UNKNOWN

(Cont. on the next page)

Table 3.6. (con.)

DPL204-177	0,0493	0,1999	-0,1999	A12
DPL223-251	0,0022	-1,809	1,809	UNKNOWN
DPL228-175	0,0346	0,363	-0,363	D11 (21)
DPL253-266	0,0078	2,768	-2,768	A11
DPL307-213	0,0484	-0,2060	0,2060	D9 (23)
DPL885-223	0,0271	-0,1735	0,1735	A9
DPL885-248	0,0466	-0,1881	0,1881	A9

According to the results, the most significant association was for DPL080 with p=0.0014. This marker is located on A6 chromosome. In terms of dominant allelic effect, DPL188-130 showed the highest effect while DPL223-251 showed the highest recessive allelic effect but the chromosome information of DPL188 and DPL223 is not known. The second highest mean value for dominant allelic effect belonged to DPL100-163 which also has an unknown chromosome position.

3.4. Correspondence of QTL with Previously Identified Loci

In recent years there have been many efforts to determine QTLs/genes responsible for VW resistance using molecular markers in cross populations derived from *G. barbadense* (resistant against VW) and *G. hirsutum* lines (susceptible against VW).

In one study, Bolek, et al. (2005) generated F2 population derived from *G. barbadense* cultivar Pima S-7 and *G. hirsutum* cultivar Acala 44 and found three QTLs regions associated with VW resistance on A11 with large effects. In this study we found that SSR locus DPL253-266 (p=0.0078) on A11 was associated with resistance.

In another study, Yang et al. (2008) generated F2 and BC1 populations from crossing *G. hirsutum* and *G. barbadense* for QTL studies. They determined QTL regions on D5 and D11. In this study, we found DOW053-391 (p=0.0021), DPL140-251 (p=0.0334) on D5 and DOW051-302 (p=0.0035), DPL228-175 (p= 0,0346) on D11.

In 2009, Jiang et al. used $F_{2:3}$ population derived from upland cotton Jumnian1 and *G. barbadense* line 60182, they found 41 QTLs which were associated with VW.

Of 41; 16 QTLs were found on D7, 25 were found on D9. In this study we found, DPL168-213 ($p=0.0035$) on D7 and DPL307-213 ($p=0,0484$) on D9.

In 2008, Wang et al. generated an intercross population from *G. barbadense* and *G. hirsutum.* They detected 9 QTL regions and 6 of them were determined on D-subgenome including D2 (chr14) and D7 (chr16). Similarly, in our study we found BNL3502-150 ($p=0.0081$) on D2 and DPL168-213 ($p=0.0035$) on D7.

In another study Fang et al., in a RIL population using of sensitive upland TM-1 and resistant upland NM 24016 with G. barbadense introgression, detected 21 QTLs for VW resistance on chromosomes A8, A11, A13, D1, D3, D5, D6, D8, D10, D11 , and D12. Here, we detected associated loci on A8, A11, D5, D10 and D11.

In 2013, Ning et al., carried out both greenhouse and field experiments. They found 7 QTLs on A1, A3, A5, A7, D2, and D9 in the field and 5 QTLs on A9, D3, D9, and D11 in the greenhouse. They also detected a major broad spectrum resistant-QTL on D9. Similarly, we found SSR loci on A3, A5 A7, A9, D2, D9 and D11.

Most studies reveal that D-subgenome has many QTLs responsible for resistance for VW. Mostly resistance QTLs were reported on A5, A7, A8, A11, D3, D5, D7, D8, D9, D11 and D12 (Zang et al., 2014). Because of small contributions of QTLs to the phenotype, to detect highly reliable QTLs is difficult. Moreover, Verticillium wilt resistance is highly affected by environmental conditions and differences. As a result, QTL loci/linked markers found in previous studies show differences depending on different populations.

Overall, we determined that 30 SSR loci on 15 chromosomes were linked to Verticillium wilt resistance at a significance level of $p<0.05$. The identified loci should be further analyzed with more DNA markers which are linked to related regions. As the resolution of candidate regions increases, more correct results can be obtained and used to identify appropriate markers for selection.

CHAPTER 4

CONCLUSION

Cotton has high economic value in the world thanks to its usefulness in different industries. High cotton yield and low yield losses are desired. Verticillium wilt is one of the major threats to cotton production. Most commercial cotton lines are known to be susceptible to Verticillium wilt. To cope with Verticillium wilt, classic, conventional breeding methods have not yet been successful because of the low genetic variability in cotton germplasm. Molecular marker technologies provide an alternative way to fight with this disease. Identification of responsible QTLs can help to manage the disease in cotton lines by the development or improvement of resistant cotton lines.

In this study, a natural population consisting of 118 cotton individuals was tested against *Verticillium dahliae* and used for genotypic analysis in order to identify QTL regions that have roles in resistance. A total of 100 SSR markers were used to identify polymorphisms within the population. For phylogenetic analysis, DARwin5 was performed to show diversity within the population. According to DARwin results, the population was divided into 3 clusters: Group A with 53 cultivars, Group B with 38 cultivars and Group C with 20 cultivars. We detected that there was low genetic distance between the individuals which were resulted from same breeding program. STRUCTURE analysis was used and the best cluster number of the population was found as 2 (cut-off 70) (Cluster1 with 44 cultivars, Cluster2 with 34 cultivars and 40 cultivars were intermixed). Group C of Darwin showed very high correspondence with admixture cluster of STRUCTURE. It was detected that there was very good agreement between two estimates. The allelic data were then analyzed to find associations between Verticillium resistance (R) and SSR loci. We found 14 associated SSR loci at the $p<0.01$ significance level while 30 SSR loci were determined to be associated with Rat significance level $p<0.05$ which were widely distributed on 15 chromosomes. The SSR marker which had most significant value was determined as DPL080 on A6. Resistance QTL which showed most significance were found on chromosomes A5, A6, A8, A11, A12, D2, D5, D7, D11 and D13. A5 and D5; and A11 and D11 are known to be homologous to each other. Further analysis and marker saturation of these regions will

help to further locate these R QTL. In this way, our results can contribute to future studies for development of Verticillium wilt resistant cotton lines.

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