T.C.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
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MOLECULAR SCREENING OF VERTICILLIUM WILT RESISTANCE IN UPLAND COTTON USING SSR MARKERS

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Master Thesis

Supervisor
Assistant Professor Dr. EMİNUR ELÇİ

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Amna SAEED tarafından **Yrd. Doç. Dr. Eminur ELÇİ** danışmanlığında hazırlanan "**Molecular Screening of Verticillium Wilt Resistance in Upland Cotton using SSR Markers**" adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü **Bitkisel Üretim ve Teknolojileri** Ana Bilim Dalı'nda Yüksek Lisans tezi olarak kabul edilmiştir.

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THESIS CERTIFICATION

I certify that the thesis has been written by me and that, to the best of my knowledge and belief. All information presented as part of this thesis is scientific and in accordance with the academic rules. Any help I have received in preparing the thesis, and all sources used, have been acknowledged in the thesis.

Amna SAEED

ÖZET

VERTİCİLLİUM SOLGUNLUĞU HASTALIĞINA DAYANIKLI PAMUK GENOTİPLERİNİN SSR MARKÖRLERİ İLE MOLEKÜLER TESPİTİ

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Verticillium solgunluğu (VS) hastalığı, dünya çapında pamuk üretim alanlarında ciddi bir problem olarak kabul edilmekte olup önemli ürün kalite ve verim kayıplarına neden olmaktadır. Bu çalışmada, Türkiye'deki bazı Upland pamuk çeşitlerinin Basit dizi tekrarları (SSR) markörleri ile taranması amaçlanmıştır. Bu amaçla, farklı araştırma enstitülerinden 50 adet pamuk genotipi toplanmış ve markörler ile taranmıştır. Genomik DNA ekstrakte edilmiş ve SSR markörlerin çoğaltılması için polimeraz zincir reaksiyonu (PCR) analizleri yapılmıştır. Moleküler veri analizi için SSR markörlerin polimorfizm bilgi içeriği (PBİ) değerleri hesaplanmıştır. PBİ değerleri, DPL0022 ve DPL752 markörlerinin en iyi markörler olduğunu ortaya koymuştur. Testlenen markörler arasında VS'ya dayanıklılığı kontrol eden kantitatif karakter lokuslarına (QTL) bağlantılı olduğu tespit edilmiş JESPR65, GH215 ve DPL0022 markörlerinin PBİ yüksek olduğu ve marköre dayalı seleksiyon çalışmaları için umut verici oldukları belirlenmiştir. Ayrıca, analiz edilen elyaf kalite markörleri arasında CIR381, CIR246 ve DPL405 oldukça polimorfik bulunmuştur. Kümelenme analizleri sonuçlarına göre, dayanıklı çeşit olarak bilinen Julia ve N-87 çeşitleri, diğer tüm çeşitlerden belirgin olarak ayrılmış ve birbirleriyle yakından ilişkili olduğu bulunmuştur. Belirlenen bu çeşitlerin ileri ıslah çalışmalarında kullanılabileceği düşünülmektedir. Mevcut çalışmanın sonuçları, VS hastalığına dayanıklı pamuk çeşitlerinin ıslahında marköre dayalı seleksiyon stratejilerinin geliştirilmesine yardımcı olacaktır.

Anahtar Sözcükler: Gossypium hirsutum., Markör destekli seçim, Genetik çeşitliliği.

SUMMARY

MOLECULAR SCREENING OF VERTICILLIUM WILT RESISTANCE IN UPLAND COTTON USING SSR MARKERS

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Graduate School of Natural and Applied Sciences

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Verticillium wilt (VW) is one of the major factors resulting in huge cotton yield losses. The hiking problem of VW in cotton incurs heavy economic losses around the world as well as in Turkey. The objective of the current study was to screen upland cotton cultivars by simple sequence repeats (SSR) markers in Turkey. Fifty different cultivars collected from various research institutes were screened in the current study. Genomic DNA was extracted and polymerase chain reaction (PCR) was conducted to amplify the SSR markers. For the molecular data analysis, polymorphism information content (PIC) values of molecular markers were calculated. PIC values revealed that DPL0022, and DPL752 were the most informative markers. Among the tested markers that are linked to QTL for VW resistance, only the markers JESPR65, GH215 and DPL0022 were found to be very informative and promising for MAS studies. Moreover, among the analyzed fiber quality markers, CIR381, CIR246 and DPL405 were found to be very polymorphic. Based on the cluster analysis, cultivars Julia and N-87, which are known as resistance cultivars, were distinctly separated from all the cultivars and closely related with each other. The identified cultivars can be used in the further breeding programs. The current study will be helpful for the development of marker-assisted strategies for breeding of VW resistant cotton cultivars.

Keywords: *Gossypium hirsutum*, Quantitative trait, Marker-assisted-selection, Genetic diversity

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SYMBOLS AND ABBREVIATIONS

Symbols/Abbreviations	Descriptions
VW	Verticillium wilt
VD	*Verticillium dahliae*
MAS	Marker assisted selection
UC	Upland cotton
SSR	Simple sequence repeats
QTL	Quantitative trait loci
SIC	Sea island cotton
PCA	Principal component analysis
PCR	Polymerase chain reaction
Ml	Micro liter
°C	Degree celsius
PIC	Polymorphism information content

CHAPTER I
INTRODUCTION

Cotton (*Gossypium hirsutum* L.), the king of fibers, is most important natural textile fiber crop grown globally. Cotton belongs to the family Malvaceae. *Gossypium* genus contains 50 species out of which four species; *G. arboreum L., G. herbaceum L., G.hirsutum* and *G. barbadense* are cultivated. *G. arboreum* and *G. herbaceum* are called as Old World, whereas *G. hirsutum* and *G. barbadense* are cultivated in the New World. *Gossypium* encompasses 45 diploid and 5 allotetraploid species. Diploid species are categorized into eight different genomic groups; A, B, C, D, E, F, G and K on the basis of meiotic pairing. Cultivated cottons, *G. hirsutum* and *G. barbadense* are tetraploids (AADD, 2n=4x=52) and old world cotton are diploids (AD, 2n=2x=26) (Fryxell, 1992).

Recently cotton is cultivated on over 30 million hectares and almost 105 million bales are produced globally (USDA-FAS, 2016). India ranks first for cotton production followed by China and the United States. Turkey falls at 7th number in this ranking with 2.5% (2.6 million bales) of total global cotton production (USDA-FAS, 2016). Fiber yield and quality are highly affected by pathogens attacking the cotton crop, resulting in heavy economic losses.

VW incited by the soil inhabiting fungus *Verticillium dahliae* Kleb., having over 400 host plant species (Pegg and Brady, 2002) causes 1.5 million bales losses to the global cotton economy (Cai et al., 2009). The optimum temperature for *V. dahliae* as well as for cotton growth is 27°C. On the basis of pathogen virulence, VW pathogen is classified into defoliating (D) strains and non-defoliating (ND) strains. These pathotypes have been found in the cotton growing areas of the Mediterranean region of Turkey (Bicici and Kurt, 1998).

Several biotic and abiotic stress factors affect cotton yield due to lower genetic diversity for the traits of particular interest (i.e., disease resistance, high yield etc.). Repeated utilization of a few genetic backgrounds in the development of new cultivars has further reduced the genetic diversity of upland cotton (May et al., 1995; Rahman et al., 2002, 2005, 2008). Nonetheless, cultivation of the similar genotypes over large areas by the farmers has led to genetic homogeneity as well (Rahman et al., 2012). Various

strategies including crop rotation, chemical fumigation, use of resistant cultivars etc. are employed to manage the VW in cotton, however, these provide limited control (Wheeler and Woodward, 2016). Crop rotation with sorghum can delay the initial buildup of microsclerotia resulting in low wilt, higher yield and better economic returns (Wheeler et al., 2016).

The low efficiency of management options in controlling VW has urged the scientist to work on the genetic improvement programs. The cotton breeding programs are focused on synchronous improvements in fiber quality, yield and disease resistance, which are rather a challenging task (Zhao et al., 2014). Many cotton breeders are also frustrated due to the fact that a variety might be disease resistant, but is not high yielding. Therefore, synchronous improvements in these traits have also become inevitable. Recent developments in molecular quantitative genetics have made it possible to map the quantitative trait loci (QTL) for fiber, yield and disease resistance simultaneously and several QTLs have been identified to date (He et al., 2005; Zhao et al., 2014). The QTL mapping has facilitated the application of marker assisted selection (MAS) in genetic improvements of cotton (He et al., 2005).

Two different models (qualitative traits and quantitative traits) have been reported by the researchers for VW using different materials in traditional genetics (Cai et al., 2009). The development of molecular tools has enabled the breeders to map VW related QTLs in cotton, which provide more detailed and molecular level information regarding VW. More than 100 different QTLs conferring VW resistance in cotton have been reported on 22 different chromosomes during different growth stages with different *V. dahliae* isolates (Du et al., 2004; Bolek et al., 2005; Zhen et al., 2006). Considerable genetic diversity has been reported among the germplasm evaluated so far for VW resistance from different regions of the world, indicating enough scope of genetic improvements. Therefore, evaluation of existing germplasm may aid to local and regional breeding programs for development of VW resistant cotton cultivars.

Cotton is an important crop of Turkey and upland cotton is cultivated in three major regions, i.e., Aegean, Mediterranean and Southeastern Anatolian approximately 488.500 ha (Anonymous, 2012). VW is regarded as a notorious disease of cotton crop causing severe yield reduction and substantial economic losses in the country (Göre, 2007). The

first report of VW of cotton in Turkey dates back to 1941 (Iyriboz, 1941) however, unfortunately it was not considered as an important constraint ofcotton production up till 1971 (Karaca et al., 1971). Several factors such as climatic conditions, nature of cotton cultivars, growth stage and virulence of the strains are the major factors responsible for disease outbreaks in cotton in the country (Göre et al., 2009). The ever-changing climate, on the other hand worsens the situation with erratic distribution of precipitation.

In spite of the high virulence of VW in Turkey, VW resistant germplasm is very rare in the country. Moreover, the genetic improvement programs based on the induction of VW resistance in cotton are also limited. Cultural management options have long been applied to control WV in cotton, however, losses are consistent for past two decades. Modern upland cotton cultivars exhibit significant variation for important traits, including yield, fiber quality, pest resistance and tolerance to environmental adversities (Zhang et al., 2012; Zhou et al., 2014). A better understanding of genetic events of resistance against VW at the molecular level will increase our ability to utilize existing resistance in cotton germplasm to reduce these losses through conventional breeding.

Development of new cotton varieties has been proven the most effective and feasible way to control VW in cotton (Wang et al., 2008). Most of the upland cotton cultivars are either susceptible or have low resistance against VW; therefore, it is inevitable for breeders to improve disease resistance in upland cotton cultivars. The resistance can be induced by two ways; either by conducting introgression of resistance genes in sea island cotton, or gene pyramiding from different sources of resistance (Zhao et al., 2014). MAS have been effectively used for the introgression of resistance genes or gene pyramiding. Therefore, MAS can prove a valuable tool for genetic improvements in cotton.

Many VW-related QTLs have been tagged in two cotton cultivars differing in VW resistance (Bolek et al., 2005; Yang et al., 2008; Wang et al., 2008; Jiang et al., 2009; Ning et al., 2013; Fang et al., 2013; Li et al., 2014; Zhang et al., 2014a, b). Many *G. barbadense* genotypes are known to have high levels of resistance to VW (Wilhelm et al., 1974; Zhang et al., 2012; Zhou et al., 2014), but its resistance has not been successfully transferred into commercial upland cotton (Zhang et al., 2012, 2014a, b).

Simple sequence repeats (SSRs) are polymerase chain reaction (PCR) based markers which generally show high information content, simplicity, co-dominance, even distribution throughout the genome, reproducibility, and locus specificity, and have been widely applied in genetic diversity analysis, construction of linkage maps, QTL mapping and MAS.

Many of works have been accomplished to improve VW resistance in cotton using molecular tools; however, these tools have rarely been evaluated in Turkey for VW resistance improvement in upland cotton. Therefore, the current study was planned to screen the upland cotton cultivars with molecular markers linked to VW resistance QTLs. In addition to VW markers, fiber quality markers were also used. The studies will lay a strong foundation for breeding programs focused on improvements in VW resistance of upland cotton.

CHAPTER II
LITERATURE REVIEW

Verticillium wilt (abbreviated to "VW" throughout the chapter) is a destructive disease of cotton, which is caused by several isolates of *Verticillium dahliae* (abbreviated as "VD" hereafter). The disease has been causing disastrous effects on cotton in several regions of the world, and extensive studies have been conducted on the management and development of resistance in cotton germplasm against VW. These studies have extensively been reviewed in this chapter. As the objective of the present study is molecular screening of VW resistance in upland cotton (abbreviated as "UC" hereafter) cultivars in Turkey, the studies and literature are limited to UC to possible extent. Further, the emphasis is mostly kept on the molecular screening of VW using simple sequence repeats (abbreviated to "SSR" throughout the chapter) and marker assisted selection (abbreviated as "MAS" hereafter).

2.1 Global Distribution of Verticillium wi1t

The first report of the VW disease was from Virginia, United States on cotton crop (Carpenter, 1914) and later on okra was reported to be infested with the disease (Bell, 1992). The causal agent VD can be spotted in every cotton growing region of the world and disease was a major hurdle in cotton production till 1940. The fungus was reported to be spread all around the globe and was regarded as a serious restraint to cotton production.

The countries which suffered the most from disease epidemics are China, the United States and the former Soviet Union. El-Zik (1985) reported that Arkansas, Texas and the southwestern United States were infested with diseases and an estimated loss of 580,000 bales was reported in 1961 due to the disease (US Cotton Disease Council). Zhu (2007) reported that the cotton crop suffers from 40 types of different diseases from sowing to harvesting, which cause 15% production losses in China and VW and *Fusarium* wilt are the most important diseases in the country. The estimated losses due to VW only in the United States during 1990 to 2014 are estimated to approximately 480 million bales (Lawrence et al., 2016).

The global distribution of VW or VD is given in Figure 2.1. It is clear from the figure that the disease or its causal agent is spread all around the world except some countries.

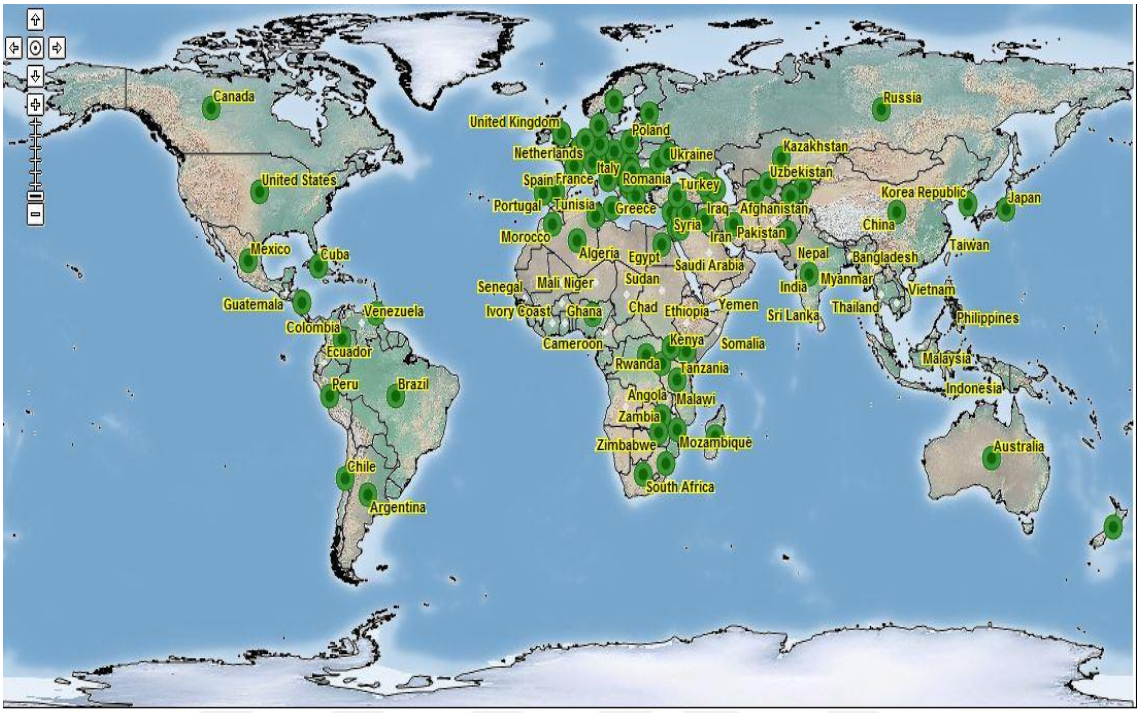

Figure 2.1. Global distribution of Verticillium wilt and *Verticillium dahlia* (Source: http://www.plantwise.org/KnowledgeBank/PWMap.aspx?speciesID=46312&dsID=56275&loc=global)

2.2 Verticillium wilt in Turkey and Associated Losses

The first report of the VW disease in Turkey dates back to 1941 (Iyriboz, 1941) however, it was ignored and not regarded as a significant threat to cotton production in the country until 1971 (Karaca et al., 1971). Cotton production in Turkey, like other cotton producing regions of the world, also suffers from VW infestation. The disease has been reported to become a serious restraint to the country's cotton industry (Dervis and Bicici, 2005a). The extensive distribution of VD is observed form Eastern Mediterranean region in the country which results in severe reduction of lint yield in the region (Dervis and Bicici, 2005b). Several factors such as climatic conditions, nature of cultivars, growth stage and virulence of the strains are the major factors responsible for disease outbreaks (Göre et al., 2009). Upland cotton is cultivated in Turkey on approximately 488,500 ha in three main regions (Aegean, Mediterranean and Southeastern Anatolia) with supplemental irrigation (Anonymous, 2012). The VW

incidence has progressively been increased in Turkey since the mid-1990s, which resulted in serious yield losses in cotton producing regions of the country (Göre et al., 2007). The VW epidemics in the Aegean region of the country were observed during 2004 (Göre et al., 2014).

In recent years, cotton production has been decreased in the country mainly due to the incidence of VW indifferent regions. Un-planned crop rotation, mono-culture and high nitrogen applications have been considered as the main contributing factors of disease development.

2.3 Current Status of Verticillium wilt Resistance in Cotton

Cai et al. (2009) wrote a comprehensive review on the molecular research and genetic engineering conducted to improve the VW resistance in cotton. The main conclusions of the review were; i) VW has become destructive for cotton, mainly for the UC, which occupies most of the cotton cultivated area globally. The main reason behind this disease infestation is the use of susceptible UC cultivars, ii) improving VW resistance through breeding has been proved the most effective option to cope with the disease, iii) exhaustive research has been conducted on the genetics, molecular mechanisms and biochemical mechanisms behind the VW resistance in cotton. With the technological advancements, identification of SSR markers linked to VW has become possible and several researchers have improved the VW resistance in UC through MAS and iv) identification and cloning of resistant genes could facilitate for rapid VW resistance improvement in upland cotton.

Zhang et al. (2014a) reviewed the current status of the VW resistance in cotton and identified several shortfalls. The main conclusions of their review were; i) there are many Acala and transgenic cotton cultivars developed in 1940s and 1990s which possess moderate to high VW resistance, however several difficulties are faced in VD inoculation which reported resistance in several sea island cotton (SIC) and UC cultivars, ii) the QTL mapping of several cultivars have reported the existence of VW resistance and all of the identified QTLs are located on chromosomes 5, 7, 8, 11, 16, 17, 19, 21, 23, 24 and 26, iv) an inefficiency has been reported in phenotypic selection of VW selection, whereas efficacy of MAS still needs to be explored and iv) better VD

inoculation and screening methods are needed on urgent grounds to fully uncover the genetic basis of VW resistance in cotton.

2.4 Field and Greenhouse Screening for Verticillium wilt Resistance in Cotton

The use of VW resistant cultivars is the best employment against VW; however data relating to VW resistance level in UC cultivars and transfer of VW resistance from SIC to UC is scarce. Therefore, Zhang et al. (2012) conducted greenhouse and field experiments in 2003, 2006 and 2007 to evaluate the VW resistance in commercial UC cultivars and germplasms. A total of 267 cultivars and germplasms and 357 genotypes were screened in greenhouse and field, respectively. The VW resulted in significant reduction of lint yield. Some of the commercial cotton cultivars had good level of VW resistance. The Acala cultivars also exhibited good resistance level, while all of the Acala cultivars were not resistant to VW. Pima cotton revealed higher level of VW resistance than UC. The cross between Pima and UC has a good level of VW resistance. It was concluded that VW resistance is controlled by few dominant genes.

Zhou et al. (2014) conducted an experiment in the United States to evaluate the level of VW resistance in all available germplasm of UC in the United States. They obtained 84 cultivars from seed companies, 52 breeding lines from the breeding program in the United States. Additionally, they also used 87 lines developed by New Mexico cotton breeding program from a cross between Acala 1517-99 9 and Pima PHY 76. All the cultivars and breeding lines were grown in grown house, had 10 replicates for each and were inoculated by defoliating VD isolate. The VW heritability varied from 0.58 to 0.80 having an average value of 0.67. The authors concluded that the genetic diversity among the tested germplasm is responsible for the variation of VW heritability. As a result of the greenhouse investigations, 6 UC cultivars (FM 9160B2F, FM 9170 B2F, NG 4010 B2RF, Nitro 44 B2RF, DP 1219 B2RF, and ST 4288 B2F), 5 breeding lines (Ark 0403-3, MD10-5, MD25, NC11AZ01, and PD 0504), 2 lines obtained through Mexico cotton development program (NM11Q1157 and 08N1618), and 4 Pima cultivars (COBALT, DP 357, PHY 800 and PHY 830) proved highly resistance to VW among 223 tested germplasm (i.e., cultivars, breeding lines and introgression lines). The highly resistant genotypes were re-evaluated for VW resistance and all of these genotypes proved resistance in repeated investigations. Therefore, authors concluded

that these highly resistance genotypes could contribute towards the development of improved VW resistance in upland cotton in the United States.

Wheeler and Woodward (2016) evaluated the relationship between VW incidence and leaf defoliation during boll filling stage. Trials were conducted in West Texas for 11 years. The cultivars were divided into 4 groups (A-D) for normalized wilt index and four classes on defoliation rate. A partially resistant (Fibermax 2484B2F) and a susceptible check (Deltapine 0912B2RF) were used to determine the classes using mix model analysis. The classes A and C were statistically similar to partially resistant and susceptible checks, respectively. A mixed model analysis using devised classes and lint yield was employed to infer the relationship. The lint yield was decreased as leaf defoliation was increased. When both VW infestation and defoliation classes were used in model with lint yield, Aa combination (lowest infestation and lowest defoliation) had the highest yield compared to rest of the classes' combinations. It was concluded that cultivars with low VW infestation and defoliation indices should produce higher yields when planted in VD infested field. It was recommended that these indices should be used and standardized to develop VW resistant cultivars.

The VW incidence is also reported to be linked with the irrigation frequency and soil type (Land et al., 2017). The authors evaluated several UC cultivars for VW incidence under six soil types and with irrigation or no-irrigation and found considerable variation among all tested cultivars. Among the tested cultivars, ST 4747 GLB2 proved to be the most tolerant with lowest disease incidence and highest yield. The disease incidence and severity was higher in the plants receiving irrigation compared with no-irrigated plants. Similarly, the highest disease incidence was noted on the plants grown on Decatur silt loam and Houston clay soils having the highest clay and silt content.

2.5 Field and Greenhouse Screening for Verticillium wilt Resistance in Cotton in Turkey

Göre et al. (2009) evaluated VW resistance of 28 most commonly grown UC cultivars in Turkey. The 6 week old plants were inoculated with defoliating or non-defoliating isolates of VD in greenhouse. The cultivars were visually assessed for their VW resistance level. Most of the tested cultivars were susceptible to VD isolates, while

susceptibility was more for defoliating isolates compared with non-defoliating isolates. Carmen and ST-373 proved to be the most promising cultivars during the study. A differential resistance was exhibited by the cultivar Carmen. It was found susceptible to defoliating pathotype, while proved resistant to non-defoliating pathotypes of VD. The cultivar ST-373 exhibited moderate susceptibility to both pathotypes. Six different parameters (plant height, number of nodes, leaf weight, stem weight, leaf-to-stem ratio, and total shoot weight) were used for phenotypic evaluation of resistance and data regarding these parameters were collected 13 days after inoculation. The percentage decrease in leaf–stem ratio and leaf weight were found to be the best indicators of resistance. The results provided valuable insights for evaluating phenotypic resistance and exploring the genetic mechanisms behind the resistance to both pathotypes.

Kheiri and Fatahi (2010) evaluated the yield and VW resistance response of 6 UC cultivars in Turkey during 2005 and 2006. On the basis of foliar symptoms, different indices such as disease percentage, disease index and disease severity were recorded. The lowest disease percent (20-29.38%), disease severity (1.42-1.62) and disease index (32.13-45.69) were noted for the cultivars Bakhtegan, 818 and B-557. The highest values of these indices were recorded for the cultivar Varamin (disease percentage 85.63%, disease severity 3.23 and disease index 278). Bakhtegan, 818 and B-557 produced significantly different yield from the rest of the cultivars.

Göre et al. (2011) grown 29 different cotton cultivars in VD infested plots in Turkey during 2008–2009. The cultivars ‘BA-151’, ‘Celia’, ‘Çukurova-1518’, ‘Flash’ and ‘Maraş 92’ proved more susceptible to VW with 85–95% disease index for all genotypes during each year of study. Different cultivars differed for the incidence of VD in seeds. For example VD incidence was 29.8% for Çukurova-1518, 27.6% for Flash, 24.6% for BA-151, 19.0% for Celia and 16.2% for Maraş 92. A total of 200 seeds of each cultivar (2 seeds in 1 pot) were grown in greenhouse to visually assess the disease symptoms. About 12–13 weeks after sowing, disease symptoms were noted. Maximum disease incidence values were; 3.3% for Celia, 4.5% for Maraş 92, 8% for BA-151, 9% for Flash and 9.5% for Çukurova-1518.

Dinler and Benlioğlu (2013) collected 47 different VD isolates from cotton growing regions of 12 towns in Aydin province of Turkey and assessed their diversity in

vegetative compatibility group. The authors assessed the pathogenicity of all collected pathotypes on cotton cultivar Acala SJ2 by growing in growth chamber. The pathogen virulence was observed in the range from 3.83 to 100%, whereas defoliation was observed between 0 to 100%. Nitrate non-utilizing (*nit*) mutants were used to assess the vegetative compatibility among 24 VD isolates. On the basis of *nit* mutants, 2 vegetative compatible groups were identified, one having 1 isolate, while the other group had 23 isolates. The authors concluded the presence of several VD isolates in the province capable of causing VW infestation in cotton.

Erdogan et al. (2013) conducted an experiment at Nazilli Cotton Research Institute, Turkey during 2006 and 2007 to correlate the VW severity and cotton earliness in maturity. A field trial under natural conditions with 10 cotton genotypes was conducted. They collected data on several parameters such as foliar disease index, vascular disease index, days to flowering, days to boll opening and seed cotton yield. Carmen, NGC, GSN 12 and M25-G, were proved the most tolerant genotypes on the basis of disease severity parameters, whereas NP Ozbek-100 proved to be the most sensitive genotype. Similarly, the other sensitive genotypes on the basis of these parameters were NMCH-11/4, NCCH-9/2 and NCCH-8/1. Two cultivars included in the study, i.e., Nazilli 84 S and Sayar 314 performed non-consistent for VW resistance. The disease severity parameters were negatively correlated with days to flowering and boll opening. However, days to flowering and boll opening were significantly positively correlated with seed cotton yield. The authors recommended that Carmen cultivar as most suitable for cultivation under the infestation of VW.

Göre et al. (2014) studied the prevalence of VD isolates in commercially available UC cultivars in Turkey. The study was conducted in two different steps. The first step comprised of collecting seed lots of cotton cultivars in Turkey and isolation of VD isolates from the collected seeds. In the second step, authors inoculated selected VD isolates in greenhouse and noted the incidence of VW. Total 104 seed samples were collected and VD was isolated from 67 samples (65%). The authors obtained 188 isolates from these samples and performed vegetative compatibility analysis using nitrate mutants. The vegetative compatibility analysis grouped the isolates into 4 groups named VCG1A, VCG2A, VCG2B and VCG4B which contained 105, 17, 64 and 2 VD isolates, respectively. The pathogenicity of selected 50 isolates was tested on 2 cotton

cultivars (DP 15–21 and Acala SJ-1) and all of the tested pathogens proved pathogenic on both tested cultivars. The authors made 3 distinct conclusions on the basis of their results; i) the commercially available seeds are infested with VD which serve as primary pathogen spread source, ii) The VD isolates distributed in Turkey belong to 4 groups and are widely distributed in seed lots and iii) the groups VCG1 and VCG2 are distinctive VD isolates and their virulence vary on cotton.

Erdoğan et al. (2015) performed an experiment to infer the yield, fiber quality traits and reactions of some cotton genotypes to VW disease. Firstly, 10 candidate varieties with susceptible Çukurova 1518, standard NP Özbek 100 cultivar and a tolerant Carmen cultivar were tested to determine reactions against VD in a pot experiment. Then, a field trial with four replications was conducted under natural infection of VW. Disease severity was determined in leaves, at 5-10% and 50-60% of boll opening periods, and in stem section after harvest. Some yield parameters and fiber quality properties data was also obtained. Although all candidate varieties were moderately resistant against VD11 (non-defoliating pathotype) isolate, they showed low level resistance against PYDV6 (defoliating pathotype) in the pot experiment. In the counting of the disease in three different periods, NMB 27/33 has come to the fore after Carmen variety.

Göre et al. (2017) evaluated 10 different upland cotton cultivars for VW resistance in Turkey. All the cultivars were inoculated with defoliating and non-defoliating pathotypes of VD with "pot immersion" method at 106 conidia/mL inoculum concentration. The external and internal disease symptoms were recorded for evaluating the resistance level using a scale of 0 to 4 (0 = healthy; 4 = dead plant). The symptoms were noted approximately 2 weeks after inoculation. The tested genotypes significantly differed for resistance levels. The lowest VW incidence level (1.0) was recorded of cultivar Maydos Yerlisi. Whereas, the highest incidence of VW (3.3) was recorded for the cultivar Nazilli NDT-15. It was concluded that 'Maydos Yerlisi' can be used in cotton breeding programs to develop VW resistant varieties.

2.6 Molecular Screening of Verticillium wilt Resistance in Cotton

Simple sequence repeats (SSRs) are polymerase chain reaction (PCR) based markers which generally show high information content, simplicity, co-dominance, even

distribution throughout the genome, reproducibility, and locus specificity, and have been widely applied in genetic diversity analysis, construction of linkage maps, QTL mapping, and marker-assisted breeding. The molecular works aimed at improving VW resistance in UC in several cotton growing regions of the world are briefly summarized as below;

Gao et al. (2003) believed that two major QTLs and one minor QTL control VW resistance in F_2 individuals of cross between UC and SIC.

Zhen et al. (2006) crossed susceptible UC cultivar CCRI-8 and resistant SIC cultivar Pima 90-53 and developed 182 F_2 individuals. Each F_2 individual descended its $F_{2:3}$ line through self-crossing. The F_2 individuals were evaluated in the disease nursery for VW resistance. Similarly, the $F_{2:3}$ lines were evaluated in growth chambers to validate the resistance of F_2 individuals. The results revealed 140 and 42 F_2 individuals as resistance and susceptible, respectively. Genomic DNA of 10 resistant and 10 susceptible individuals were extracted and screened against 782 SSR primer pairs. Among the tested SSR primer pairs, BNL2440 and BNL3255 were found to be polymorphic between resistant and susceptible DNA pools. Both these markers were also polymorphic in the parents. The marker BNL2440 was found to be not linked with VW resistant gene. A polymorphic fragment of 208 bp amplified by BNL3255 primer was labelled as BNL3255-208. The distance between VW-QTL and the marker BNL3255-208 marker was measured to be 13.7 cM and was located on chromosome 5. This marker could be used in MAS for VW resistance and gene cloning.

Wang et al. (2007a) derived F_2 population and $F_{2:3}$ lines through hybridization between VW resistant upland cotton and VW susceptible Luyan 343 cultivar. The VW resistance was evaluated by using SSR markers using phenotypic data collected at different developmental stages. A QTL *qVWR-16-1a* was detected from the vigorous developmental stage and is located between the markers, BNL2986 and NAU751 on chromosome with 5.73 cM distance between these markers. The QTL accounted for 16.53% of the variation and identified to be inherited from the resistant parent. Similarly, another QTL *qVWR-16-1b* related to VW was detected form the late developmental stage, located on the same interval of QTL *qVWR-16-1a* with 1.73 cM distance to the marker NAU751 and accounted 10.27% of the variation. Another QTL,

qVWR-16-2b was found to be located between the markers BNL1604 and BNL1395 on the same chromosome with 1.39 cM distance to BNL1395 and accounted for 10.8% of the variation. Similarly, QTL *qVWR-2-1b* was located between markers BNL3950 and BNL3971 with 0.01 cM to BNL3950 on chromosome 2, and accounted for 13.78% of the variation. Some offspring's of F_5 were evaluated for VW resistance and it was noted that pyramiding resistant genotypes of marker NAU751 and BNL1395 can significantly improve the VW resistance. It was concluded that these markers can successfully be used for improving VW resistance of UC.

Wang et al. (2007b) used AFLP and SSR markers to estimate the genetic diversity of 95 Chinese UC cultivars for *Fusarium* and/or Verticillium wilts. Fingerprinting was performed with 20 AFLP and 19 SSR primers with polymorphism. A total to 1480 bands were produced by AFLP markers and among these bands, 214 were found to be polymorphic. The number of bands for each primer pair ranged from 47 to 109, with an average of 74.0. Eighty nine bands were produced by 19 SSR primers and 61 of these bands were polymorphic. The total number of alleles per locus varied from 3 to 8, with an average of 4.7. The tested cultivars exhibited close relationship and narrow genetic diversity.

Molecular marker-assisted selection is effective for quickly breeding cultivars resistant to VW (Ge et al., 2008). The UC strain Chang 96, resistant to VW, and susceptible variety, Junmian 1 were used as experimental materials in the current study. A tagging population with 138 F_2 individual plants was developed. By artificially inoculating the strongly pathogenic fungi strain to the populations P1, P2, F1, and $F_{2:3}$, relative disease indices of each generation were estimated. A total of 1998 pairs of SSR primers, and 230 pairs of SRAP primers were screened and polymorphic loci were obtained from 148 SSR and 6 SRAP markers. As a result 1 resistant QTL was detected which localized on chromosome 9 between the interval of the NAU462 and JESPR114 markers and explained 13.8% phenotypic variations in $F_{2:3}$ individuals.

Yang et al. (2008) crossed tolerant cultivar of SIC with susceptible UC cultivar and developed two populations i.e., F_2 and BC_1. The leaf and vascular traits at seedling and maturity stages, respectively were used to quantify the disease reaction to the plants. Different types of molecular markers were used and two genetic linkage maps were

constructed. As a result of molecular analyses, 4 QTLs located on chromosomes 5, 7 and 8 of A sub-genome for leaf trait were identified, whereas 3 QTLs located on chromosomes 5, 7 and 9 of D sub-genome were identified for vascular trait in F_2 population which was inoculated with non-defoliating isolate of VD. The BC1S2 population was grown in three different disease nurseries inoculated with B_{P2}, V_{D8} and 592 VD isolates. In the plants inoculated with B_{P2}, 1 QTL located on chromosome 4 of D sub-genome was identified for leaf trait, while 2 QTLs located on chromosome 4 of D sub-genome and chromosome 8 of A sub-genome were identified for vascular tissue traits. Similarly, from the plants inoculated with V_{D8} isolate, 2 and 3 QTLs were detected for leaf and vascular tissue traits, respectively. The 592 inoculated plants yield 3 and 2 QTLs for leaf and vascular tissue trait, respectively. The QTLs were located on different chromosomes and at different growth stages of cotton. Therefore, these markers could significantly contribute towards the identification of resistance genes and improvement of VW resistance. Flanking markers with identified QTLS were qVL-A5-1BC1S2VD8 (NAU5273–NAU569b), qVL-A8-2BC1S2VD8 (JESPR232–NAU3201), qVV-D5-1BC1S2VD8 (NAU1042–NAU828b), qVV-D11-1BC1S2VD8 (NAU643–NAU3481), qVL-A5-1BC1S2592 (NAU3036–NAU2121), qVL-A5-2BC1S2592 (NAU3607–NAU1065a), qVL-A5-2BC1S2592 (NAU2513–BNL1878), qVV-D5-1BC1S2592 (BNL2656–BNL1671), qVV-D11-1BC1S2592 (NAU1640–BNL3279).

Wang et al. (2008) mapped the QTLs related to VW resistance genes in cotton and found 430 SSR mapped loci on 41 linkage groups. A total of 9 QTLs (q7.22-1, q7.22-2, q7.22-3, q7.22-4, q7.22-5, q8.24-1, q8.24-2, q8.24-3, and q8.24-4) were perceived grounded on the basis of disease severity index which explained 10.6-28.8% phenotypic variations in the tested lines. It was concluded that these flanking markers (BNL2441−BNL2766, BNL3867-3−BNL1605, BNL3368−BNL3537, BNL1706-2−BNL1706-1, BNL1673−BNL2894, BNL3017−JESPR305, BNL2441−BNL2766, BNL2766−BNL3065, and BNL1673−BNL2894) can be used for improving VW resistance in UC.

Jiang et al. (2009) crossed a resistant (60182) and susceptible cultivar (Junmian 1) for the identification of markers for VW resistant genes and validation of inheritance mode. Six different populations (P_1, P_2, F_1, B_1, B_2 and F_2) were developed from the cross between above mentioned cultivars and infested leaf percentage was used to perform

genetic segregation analysis of VW. Gene-polygene mixed inheritance model was used to evaluate the segregation. Two major genes were identified controlling the VW resistance (B_{P2}, V_{D8} and T_9 isolates) and inheritance was dominant. Additionally $F_{2:3}$ families were inoculated with different VD isolates and phenotypic data were collected at different growth stages to construct genetic linkage map. The constructed genetic linkage map had 1165 cM distance, covering 25.89% of cotton genome and comprised of 139 loci and 31 linkage groups. The average distance between two adjacent markers was 8.38 cM. From 60182, 4 QTLs were found on chromosome D7 and 4 on D9. Five QTLs on D7 and 9 on D9 were found for B_{P2}. Similarly, 4 QTL on D7 and 5 on D9 were found for V_{D8}. The results revealed that the QTLs linked to VW resistance are located on chromosomes D7 and D9. Hence these markers NAU3282, NAU3287, NAU2580-NAU5508, NAU2753-NAU2528, NAU2741, NAU1043, NAU808, NAU3200, and NAU1047 related to QTLs for VW resistance may facilitate the use of VW resistance genes in improving breeding programs for cotton.

Li et al. (2013) used two populations of UC and screened them against 39 SSR markers reported for VW resistance. The results revealed that polymorphism was observed in 12 SSR markers, no polymorphism was exhibited by 19 markers, whereas remaining 8 SSR markers failed to amplify. Among these polymorphic markers, co-dominant markers were; NAU5120, BNL3031, NAU1225, NAU1230, JESPR153, JESPR065, BNL2441, BNL1053 and BNL3255, whereas BNL3241, NAU4045 and NAU3201 were dominant markers. The markers which shown no polymorphism were; NAU3053, NAU5380, JESPR44, NAU2580, NAU2753, BNL3558, BNL1721, BNL3383, BNL3660, BNL3280, BNL1026, BNL2766, NAU3367, JESPR232, NAU2665, NAU5273, BNL3147, BNL1414 and DGAY1677. Similarly, the markers for which amplification was failed were; NAU5508, BNL3874, BNL2733, NAU2121, Y13, Y20, RGAY1032 and RGAY1833. The populations differed for disease grades from each other significantly for 4 SSR markers, i.e., BNL3241, NAU1225, NAU1230 and JESPR153, and highly significantly for the SSR marker BNL3031. Therefore the study concluded that these 5 SSR markers can be effectively used for target trait transfer in upland cotton.

Sun et al. (2013) used next generation sequencing to identify the genes linked to defense against VD in SIC and UC. Both cotton types were infested with several isolates of VD.

As a result, 77212 genes were observed which were subjected to BLAST searching and annotated using the GO and KO databases. A total of 6 sets of digital gene expression data were mapped to the reference transcriptome. The comparison between infested and non-infested cotton genotypes revealed 44 genes which were differentially expressed. The genes involved in the phenylalanine metabolism (PAL) pathway, hydroxycinnamoyl transferase gene was up regulated in upland cotton, whereas PAL, 4CL, CAD, CCoAOMT, and COMT were up regulated in SIC. The V991, D07038 isolates yielded no differentially expressed genes. The results provided novel data to explore the molecular basis of gene identification linked to VW resistance both in SIC and UC.

Fang et al. (2014) conducted 2 greenhouse experiments to analyze whether VW transmitted by a defoliating isolate of VD is heritable or not. For this purpose, the researchers performed 2 different greenhouse experiments and inoculated VD at two different times to ensure that all of the plants get infested by VW. Disease severity indices, and disease ratings were recorded at several times after the disease inoculation. The greenhouse experiments indicated that the disease is low to moderately heritable. Additionally a new linkage map of 882 SSRs, SNPs, RGA-AFLP markers (432 SSR, 414 SNP and 36 RGA-AFLP) was used to identify the QTLs linked to VW. As a result 21 QTLs, 11 chromosome and 2 linkage groups of VW were identified. The study concluded that the genetic basis of VW resistance in the population used are complex, however, markers SNP0315, SNP0159, DPL1022, SNP0405, TMB1637b, DC40113, HAU006, C2-052B, MUSB0979 and DPL0500b linked to the identified QTLs can help in VW resistance breeding in UC.

Kun et al. (2014) crossed Acala Prema and Chinese cultivar 86-1 to develop 161 recombinant inbred lines for QTL mapping of fiber strength and VW resistance. Markers were developed by the restriction-site associated DNA sequencing, using massively parallel and multiplexed sequencing of reduced-representation. The results identified 21.247 SNPs for the parents. Moreover, a genetic linkage map consisting of 3321 loci was constructed by using SSR and RAD markers. The fiber quality trials were conducted in 6 different environments, while VW resistance was evaluated in disease nursery using mixed isolates and greenhouse with individual isolates V991 and V_{D8}. A stable QTL for fiber strength *qFS-D3-1* was detected on chromosome 17 accounting for

2.56-18.35% of phenotypic variation. Similarly the major QTL linked to VW resistance *qVW-D9-1* was identified on chromosome 23 which accounted for 14.9-52.95% of phenotypic variation. The markers linked to QTLs identified in this study will facilitate the MAS for cotton breeding for improved VW resistance.

Wang et al. (2014) used interspecific chromosome segment introgression lines (CSILs) to identify QTLs associated with resistance to VW in cotton grown in greenhouse and inoculated with three defoliating VD isolates. A total of 42 QTL, including 23 with resistance-increasing and 19 with resistance decreasing, influenced host resistance against the three isolates. These QTL were identified and mapped on 18 chromosomes (chromosomes A1, A3, A4, A5, A7, A8, A9, A12, A13, D1, D2, D3, D4, D5, D7, D8, D11, and D12), with LOD values ranging from 3.00 to 9.29. Among the positive QTL with resistance-increasing effect, 21 conferred resistance to only one VD isolate, suggesting that resistance to VD conferred by most QTL is pathogen isolate-specific. The A sub-genome of cotton had greater effect on resistance VW than the D sub-genome. They conclude that pyramiding different resistant QTLs could be used to breed cotton cultivars with broad-spectrum resistance to VW.

Yinhua et al. (2014) evaluated 320 UC cultivars in disease nursery to identify the resistant genes. Association mapping was followed to detect the markers linked with VW resistance. Genetic diversity, population structure and linkage disequilibrium was evaluated with 106 different microsatellite markers. General linear models revealed significant association among polymorphic markers and VW resistance traits. Four and 13 loci exhibited positive and negative effect to VW, respectively indicating that 4 loci (NAU2265-382, NAU2277-60, BNL1695-415, NAU2741-284 and NAU5099-280) and association markers (NAU3419-252, NAU2265-382, NAU2277-260, BNL1694-235 and TMB1963-218) could promote VW resistance. The results displayed that association mapping could complement and enhance QTLs information for MAS in cotton breeding.

Zhang et al. (2014b) produced a $F_{2:3}$ generation of the population LHB226JM11. A greenhouse experiment was conducted and plants were inoculated with V991 a defoliating VD isolate to infer the QTLs conferring VW resistance. As a result of the greenhouse experiment, *qVW-c6-1* QTL, distributed on chromosome 6 was identified.

The authors further used the $F_{2:3}$ generation of population LHB226NNG to validate this QTL by growing the plants in a disease nursery for subsequent 2 years and inoculating with the defoliating isolate. Similarly, F_4 generation of the population grown in the field, severely infested with the isolate was also evaluated for the QTL. The alleles within the QTL were determined to be originated from the parent LHB22. The alleles from the resistant parent explained 23 to 27% phenotypic variation. The allele conferring resistance within the QTL *qVW-c6-1* with nearest marker MGHES18region originated from parent LHB22 and could explain 23.1–27.1% of phenotypic variation. Interestingly, another QTL (*qVW-c21-1*) with nearest marker DPL0050 linked to VW resistance, located on chromosome 21 was also mapped from the susceptible parent, JM11. The QTLs reported in the study can be used as viable tools for improving VW resistance in UC.

Zhao et al. (2014) conducted greenhouse and disease nursery experiments to identify the QTLs linked with VW in UC. A total of 158 UC germplasm was used in the experiments and 212 whole genome wide markers were used for genotyping. The genotyping revealed 42 marker loci distributed on 15 chromosomes. Ten of the identified markers were similar with the previously identified QTLs, whereas 32 QTLs were novel. Moreover, QTLs clusters were also identified on chromosome 16 in that study. The study reported candidate markers BNL2599, NAU5233, NAU3592, NAU3828, NAU3212, BNL3255, NAU3201, NAU3499, DPL0222, NAU3074, CIR196, NAU980, NAU3563, Gh454, NAU5463, BNL3649, BNL3646 and JESPR0001 for association mapping of VW resistance in UC.

Zhang et al. (2015a) conducted a 4 years trial on a backcross inbred line for identifying QTLs linked to VW resistance. A total 392 SSR markers covering a distance of 2895 cM were used in the study and 10 QTLs were identified. In the second step of the study authors used another already identified 182 QTLs linked to VW, 75 QTLS linked to red-knot nematodes, 27 to *Fussarium* wilt and 7 QTLs linked to reniform nematodes reported in different publications. The meta-analysis of all these QTLs yielded 28 QTL clusters. Among these clusters 13, 8 and 3 QTL hotspots belonged to VW, root-knot nematodes and *Fussarium* wilt, respectively.

Guo et al. (2016) used two resistant UC lines (5026 and 60182) to identify the elite QTLs linked to VW resistant in upland cotton. They used 13 SSR markers (NAU4045, NAU2741, NAU1225, NAU3569, NAU905, NAU2754, NAU1043, NAU3053, BNL3031, NAU5508, NAU2508, MUCS219 and NAU6598) linked to VW resistance and developed 155 cotton lines. They hybridized the above mentioned cotton lines by pyramiding different QTLs. As a result of the study 4 elite QTLs or alleles (*q-5/NAU905-2*, *q-6/NAU2754-2*, *q-8/NAU3053-1* and *q-13/NAU6598-1*) linked to VW resistance were identified. Pyramiding of these QTLs improved the VW resistance in inbred lines. Therefore it was concluded that these markers can be used as candidate markers for the development of VW resistance in UC.

Shi et al. (2016) developed 3 populations (BC_1F_1, BC_1S_1 and BC_2F_1) through an interspecific backcross between highly resistant line (Halil) of SIC and a susceptible UC variety (CCRI36). The populations BC_1S_1 and BC_2F_1 were evaluated in the field for scoring disease incidence, while the BC_2F_1 was inoculated with defoliating VD isolate in disease nursery to infer the incidence of VW. The authors used a high density SSR map from the population BC_1F_1 covering 2229 loci and 5115.16 cM distance of the AD cotton genome. Similarly, data related to QTLs related to VW resistance was obtained for one date only for the populations evaluated in the field and 4 dates for the population evaluated in nursery. The study identified 48 QTLs linked to VW resistance and among these QTLs, 37 were found to have a positive additive effect. The authors concluded that the alleles from SIC significantly improved the VW resistance in the tested populations. The identified QTLs were distributed on 19 chromosomes in AD cotton genome. Among 48 QTLs, 33 and 15 QTLs were located on A and D sub-genomes, respectively. Moreover, 6 of the identified QTLs were found to be stable. The stable QTLs were concluded to be consistent with the already identified, whereas 42 QTLs were novel. Besides, a meta-analysis identified 17 QTL hotspot regions and 10 were novel regions. The authors concluded that these regions need further investigations to better understand the molecular basis of VW resistance in upland cotton.

Yan et al. (2016) used back cross method to transfer VW resistance from SIC to UC using MAS approach. They used SSR marker BNL3255-208 for the targeted transfer of VW resistance. A total of 71 lines were developed and among these, 19 lines exhibited enhanced resistance to VW. Similarly, 11 and 4 lines were highly resistant and resistant,

respectively. The authors concluded that VW resistance can be transferred to new lines and SSR marker BNL3255-208 is most effective in this regard.

Palanga et al. (2017) identified the QTLs linked to VW resistance in UC by conducting experiments in greenhouse for 1 year (6 replications in total) and field experiments for consecutive 4 years (2 replications in each year). Two different disease parameters, i.e., disease incidence and disease index were considered in these evaluations. As a result of these experiments a total of 119 QTLs linked to both parameters considered on 25 different chromosomes (except chromosome 13) on the cotton genome. A total of 62 QTLs for disease index were mapped on the 24 chromosomes of the cotton genome (except 11 and 13) which explained 3.7 to 12.2% of the phenotypic variations noted during the experiment. Similarly, 59 QTLs were observed for disease incidence which explained 2.3 to 21.3% of the observed phenotypic variation and were distributed on 19 chromosomes (except 5, 8, 12, 13, 18, 19 and 26). A total of 7 QTLs for disease index proved to be stable and 6 of these had GK9708 alleles, whereas 28 stable QTLs of disease incidence was recorded during the study. A total 18 QTL clusters distributed on 13 chromosomes (1, 2, 3, 4, 6, 7, 10, 14, 17, 20, 21, 22, 24 and 25) having 40 QTLs were recorded. It was concluded that the results of the study can contribute towards gene cloning for improving VW resistance in UC and open a way towards understanding the complex molecular basis of VW resistance in upland cotton.

2.7 Molecular Screening of Verticillium wilt Resistance in Cotton in Turkey

Bölek et al. (2005) tested the polymorphism between susceptible and resistant cotton cultivars collected from Turkey using microsatellites. A total of 10 susceptible and 10 resistant progeny were screened against 225 SSR primers. Among these 225 pairs, 60 were used in mapping QTLs. The study resulted in a total of 11 linkage groups which consisted of 35 SSR markers. The QTL analysis revealed significant linkage of 15 SSR markers JESPR66, CIM162, CIM25, CIM76, JESPR291, CIM71-1, CIM71-2, CIM50, CIM209, CIM12, CIM23, CIM29, BNL3147-1, JESPR135-2, CM50-2 and JESPR270-1, while 9 SSR markers were distributed on chromosomes 10, 11, 12, and 25. It was concluded the VW resistance is controlled by 3 different large loci; CM12, STS1 and 3147-2.

Baytar et al. (2017) genotyped 108 elite UC cultivars for VW resistance. A total of 177 SSR markers were used in the study for identifying QTLs linked to VW resistance. The results revealed 967 loci divided into 4 groups having a mean genetic distance of 39%. General linear models and mixed linear models were used to perform association analysis for the identification of SSR marker loci linked to VW resistance. A total of 26 marker loci distributed on 14 chromosomes were associated with VW resistance. Eight of the 26 associated marker loci were highly significant. The phenotypic variation explained by individual markers ranged from 3.2% to 8.2%. Three (JESPR153, JESPR274 and CIR218) of the 26 marker loci were consistent with previous studies.

It is evident from the extensive review that VW is a global problem and most promising method to cope with the disease is the development of resistant cultivars. However, the available germplasm at regional scales have narrow genetic diversity and molecular data on the germplasm is often limited. The molecular screening of the available germplasm using SSR markers has become a promising technique to identify the QTLs linked to VW resistance in different parts of the world. Although some recent studies have assessed the molecular basis of VW resistance in the available cotton cultivars in Turkey, however, there are still plenty of cultivars which have never been molecularly screened for assessing the levels of VW resistance. This study was therefore designed to molecularly screen the available upland cotton cultivars for VW resistance using microsatellite markers.

CHAPTER III
MATERIALS AND METHODS

3.1 Experimental Site Description

The study regarding the molecular screening of Verticillium wilt resistance in commercial upland cotton cultivars available in Turkey was conducted at the Molecular Biology Laboratory of Department of Plant Production and Technologies, Faculty of Agricultural Science and Technology, Niğde Ömer Halisdemir University, Turkey.

3.2 Plant Material Collection

Forty nine (49) different upland cotton cultivars were genotypically tested along with Maydos Yerlisi, used as both resistant (Göre et al., 2017) and out-group control belonging to *G. herbaceum*, commercially marketed in Turkey. The seeds of the cultivars were collected from different research companies, private companies and research institutes in Turkey (Table 3.1). The plant material was leaves of these cultivars. Details regarding the names of the cultivars used in this study are summarized in Table 3.1. Among 50 tested cultivars, 4 are known to be tolerant, i.e., Carmen, N-m 503, N-87, Julia and 4 are sensitive i.e., Çukurova 1518, Şahin2000, Nata, Lacata (Baytar et al., 2017) to VW, while remaining 42 cultivars have unknown status of resistance against VW.

Table 3.1. The details of the commercial upland cotton cultivars used during the current study

Cultivar Code	Cultivar Name	Maintainer
T1	BA-151	Progen Tohum A.Ş.
T2	BA-525	Progen Tohum A.Ş.
T3	Carisma	Progen Tohum A.Ş.
T4	Çukurova 1518	Doğu Akdeniz Tarımsal Arş.Enst.Müd.
T5	Gloria	Bayer Türk Kimya San. Ltd. Şti.

Table 3.1. (Continue) The details of the commercial upland cotton cultivars used during the current study

Cultivar Code	Cultivar Name	Maintainer
T6	Lydia	ProGen Tohum A.Ş.
T7	Acala-1517V	New Mexico State Univ
T8	BA-308	Progen Tohum A.Ş.
T9	BA-320	Progen Tohum A.Ş.
T10	BA-811	Progen Tohum A.Ş.
T11	CIM-496	Central Cotton Research Institute
T12	DP-499	Monsanto Gıda ve Tarım Tic. Ltd. Sti.
T13	IH-4028	Progen Tohum A.Ş.
T14	IH-20	Progen Tohum A.Ş.
T15	IH-26-K-5	Progen Tohum A.Ş.
T16	IH-27-TYL	Progen Tohum A.Ş.
T17	IH-82-K-3	Progen Tohum A.Ş.
T18	IH-82-Y-1	Progen Tohum A.Ş.
T19	MCH-578	Progen Tohum A.Ş.
T20	Natalia	Bulgarian Agricultural Academy
T21	PG424-1	Progen Tohum A.Ş.
T22	PG426-4	Progen Tohum A.Ş.
T23	PG510-15	Progen Tohum A.Ş.
T24	PG510-7	Progen Tohum A.Ş.
T25	PG511-7	Progen Tohum A.Ş.
T26	PG518-11	Progen Tohum A.Ş.
T27	PG519-19	Progen Tohum A.Ş.
T28	PG520-7	Progen Tohum A.Ş.
T29	PG-2018	Progen Tohum A.Ş.
T30	PG-300	Progen Tohum A.Ş.
T31	PG-310	Progen Tohum A.Ş.
T32	PG-53-KT-2	Progen Tohum A.Ş.
T33	PG-53-YT-11	Progen Tohum A.Ş.
T34	Prema	New Mexico State Uni

Table 3.1. (Continue) The details of the commercial upland cotton cultivars used during the current study

Cultivar Code	Cultivar Name	Maintainer
T35	ST-468	May-Agro Tohum San.Tic. A.Ş.
T36	Tamcot CamdES	Texas A&M University
T37	Tamcot SP37H	Texas A&M University
T38	Tamcot Sphinx	Texas A&M University
T39	Taşkent-1	Uzbek Scientific Research Institute
T40	Taşkent-3	Uzbek Scientific Research Institute
T41	GW Teks	Golden West
T42	VD-4	Nazilli Pamuk Araştırma İstasyonu
T43	Şahin2000	Pamuk Araştırma İstasyonu Müdürlüğü
T44	Nata	May-Agro Tohum San.Tic. A.Ş.
T45	Lacata	May-Agro Tohum San.Tic. A.Ş.
T46	Carmen	Bayer Türk Kimya San. Ltd. Şti.
T47	N-m 503	Nazilli Pamuk Araştırma İstasyonu
T48	N-87	Nazilli Pamuk Araştırma İstasyonu
T49	Julia	Bayer Türk Kimya San. Ltd. Şti.
T50	Maydos Yerlisi	Nazilli Pamuk Araştırma İstasyonu

3.3 Molecular Analysis

3.3.1 Genomic DNA extraction

The DNA was extracted from the leaves by cetyl trimethylammonium bromide (CTAB) extraction method (Doyle and Doyle, 1987) with a few modifications. For each 100 mg of tissue, 300 µL of CTAB isolation buffer (2% hexadecyltrimethylammonium bromide, 1.4 M NaCl, 0.2% β-ME, 20 mM EDTA, 100 mM Tris-HCl, pH 8) was added to each tube, and homogenized by TissueLyser (Qiagen, Germany). More CTAB extraction buffer (450 µL) was added to each tube and the samples were incubated at 65 °C for 60 min with occasional mixing. Due to the high content of polyphenolic compounds in cotton tissues, 750 µL of phenol/chloroform/isoamyl alcohol (25:24:1 v/v) was added to

each sample, samples were vortexed and then centrifuged. The supernatants were transferred to a new tube and 500 µL of chloroform/isoamyl alcohol (24:1 v/v) solution was added. Next, 500 µL of ice-cold isopropanol was added to each tube and the tubes were incubated for 30 min at room temperature. The samples were centrifuged and the supernatants were discarded. The pellets were air-dried and then re-suspended in 100 µL of 10 mM Tris, pH 8.0, 1 mM EDTA buffer. Nucleic acids were measured quantitatively and qualitatively by spectrophotometer. The extracted DNA was stored at –20 °C.

3.3.2 PCR analysis

According to DNA quantity and quality results, all samples were diluted to a final concentration of 50ng/µl. Thirty (30) simple sequence repeats (SSR) (NAU, DPL, BNL, JESPR, CIR, CGR and GH) primer pairs linked to QTLs for VW resistance were used for polymerase chain reaction (PCR) analysis (Table 3.2). The details on the sequence of SSR markers are given in Table 3.2. The PCRs were carried out with 16.8 µL sterilized water, 0.5 µL of 10 µM dNTP mix, 2 µL of 25 mM $MgCl_2$, 2.5 µL of 10X Dream Taq buffer, and 0.5 µL of 10 µM of each primer with 0.20 µL of 5 Unit/µL Dream Taq DNA polymerase (Promega, Madison, WI, USA) and 2 µL of pure DNA. Total final reaction mixture of PCR was 25 µL. Reactions were incubated at 94 °C for 2 min and following 40 amplification cycles (30 s at 95 °C, 30 s at 50 °C, and 30 s at 72 °C) were performed. The final PCR products were visualized under UV light after electrophoresis on ethidium bromide-stained 3% agarose gels.

3.4 Data Analysis

For genetic analysis based on molecular data, each amplified band was scored based on the presence (1), absence (0) and (9) for missing of bands. The binary qualitative data matrix was used to construct similarity matrices based on Jaccard similarity coefficients (Jaccard, 1908) and to construct the dendrogram using JMP software (version 13.1; SAS Institute). For the molecular data analysis, polymorphism information content (PIC) values of molecular markers were calculated according to the following formula: $PIC = 1 - \Sigma P2i$, where P*i* is the frequency of the *i*th allele (Anderson et al., 1993). Principal component analysis was also performed using Microsoft®Excel/XLSTAT©-

Pro (Thierry Fahmy, 1993). To accomplish genetic diversity analysis of the cultivars we used DARwin6 (Dissimilarity Analysis and Representation for Windows) analysis program with presence/absence data and un-weighted Neighbor Joining (Perrier, 2006).

Table 3.2. Different SSR markers used to screen the Verticillium wilt resistance in upland cotton cultivars and their sequences

Marker Name	Type	Primer Sequence 5'*	Trait name	QTL	Chr. No.	References	PIC Value evaluated
DPL0022	Genomic	GGTGGGTTCTTCTGCAGGTATATT, CCCTTTCAATGCTAGAAAGAAGTTG	Verticillium wilt	*qVW-A5-1*	AD-05	Zhiyuan et al., 2013	0.76
BNL4108	Genomic	TCCACCATTCCCGTAAATGT, TGGCCAAGTCATTAGGCTTT	Fiber Uniformity	_	AD-06	_	0
GH215	Genomic	TCGGATACCACTTGTTGGAAGCA, GTGTTAGTTATAAAAAGAATAGCAG	Verticillium wilt	*qVW-A1-1*	AD-13	Zhiyuan et al., 2013	0.54
DPL0322	Genomic	AAACCTCGTAGTCATAGGCTCAAA, AACTATGCACACAGATTTGGTACG	Fiber elongation	*qFE-C15-1*	AD-15	Sun et al., 2011	0.63
CIR246	Genomic	TTAGGGTTTAGTTGAATGG, ATGAACACACGCACG	Fiber elongation	*qFL-D2-1*	AD-14	Wang et al., 2006	0.10
CIR381	Genomic	TTTCCATCCTTTTGTGA, AAGGAGAAGAACAAGCAA	Fiber elongation	-	AD-02, 14,4	-	0.63
DPL431	Genomic	CTATCACCCTTCTCTAGTTGCGTT, ATCGGGCTCACAAACATCA	DPL – SSR Markers	-	AD-10	-	0.57
JESPR-65	Genomic	CCACCCAATTTAAGAAGAAATTG, GGTTAGTTGTATTAGGGTCGTTG	Fiber elongation Fiber Fineness	*qVL-A5-1F2*	5(A5)	Yang et al., 2007	0.61
DPL0253	Genomic	TCACTATCTCAAGACCACCTTCAA, AGTTCAAAGGACTCACCTGATGAT	DPL-SSR-Markers	-	AD-11	-	0.0412
DPL0513	Genomic	AGACCCGGCTACTACATGTTATCTT, ACATACAGATGCTTCACACAAACA		-	AD-1	-	0
DPL0405	Genomic	GAGATCCATGCTAACGTCTTACAAA, ATGGGAGGAGGGAGTGGAA		-	AD-14	-	0.49

Table 3.2. (Continue) Different SSR markers used to screen the Verticillium wilt resistance in upland cotton cultivars and their sequences

Marker Name	Type	Primer Sequence 5'*	Trait name	QTL	Chr. No.	References	PIC Value evaluated
CGR5258		AACCAGTCTGGAAGATGGCA, CCTGAACGTCATCACCCTCT	Fiber elongation	*qFL03.2*	-	Wang et., 2016	0.00
GH527	Genomic	AGCTGGAGGATTTCAGCTTGATTC, ATGCCAGTTAACTTACCACGTTGG	Verticillium wilt	*qVW-A7-1*	7A7	Zhiyuan et al., 2013	0.00
CIR295	Genomic	ATCACGCCAAAGAAAC, TGTGGAGGCGTAAACT		*qVW-D2-1*	AD-14	Zhiyuan et al., 2013	0.00
NAU3700	EST	ATCACGGAAATCGGTAATGT, CTCTTCCTTCACCTCTTCCA		*qVW-D3-1*	AD-17	Zhiyuan et al., 2013	0.00
NAU5465	EST	TTTGGGGAAAAATCACATCT, ATGGTAGGTTGAGGGATGAA		*qVW-D2-1*	AD-14, 23	Zhiyuan et al., 2013	0.00
JESPR-12	Genomic	CCTAGACATCTGATTTAGCCAC, GAAGAAGAAGAATCCGACAG		*qVW-A7-1*	7A7	Zhiyuan et al., 2013	0.00
DC-20067	Genomic	ATGCAAACCATAAACATCT, TGGGTTTGTGTGCTATCT		*qVW-A5-1*	AD-19, 05,13	Zhiyuan et al., 2013	0.00
NAU3414	EST	CAACTTCCCAAGCTCGTATT, GTTCAACTTCTCTCTTCCCTCT		*qVW-D9-1*	AD-23, 09	Zhiyuan et al., 2013	0.00
NAU3669	EST	AAGGTAACCGGATTGTCAAA, TGTGGGAAGATTCAAAGTTCT		*qVW-A9-1*	-	Zhiyuan et al., 2013	0.00
HAU3303	EST	GCAGCCATCCGACAACTGGA, AAGTTCCTACGCGGGGGAGT		*qVW-D11-1*	AD_21	Zhiyuan et al., 2013	0.00
NAU2741	EST	AGTCCACGTCCACAGACTTT, GGCACCTTGGAATACATCTC		*qVW-A1-1*	AD-19, 01	Zhiyuan et al., 2013	0.00

Table 3.2. (Continue) Different SSR markers used to screen the Verticillium wilt resistance in upland cotton cultivars and their sequences

Marker Name	Type	Primer Sequence 5'*	Trait name	QTL	Chr. No.	References	PIC Value evaluated
NAU2354	EST	AATATCTCCGTCGCCAATTA, GAAACTTCCTCCTCCTTTCC	Verticillium wilt	*qRV07DF2-A9-2*	AD-9	Zhiyuan et al., 2013	0.00
NAU2954	EST	AAGGAAATGCTGCCAACTAC, AGACTTGCTCTGGTCTGCTT		*qVW-D9-1*	AD-23, 25	Zhao et al., 2014	0.00
DPL752	Genomic	CACATCACCTAATTACCATTGAAGC, TATCGTGAATATGTATGTGCGTGG		-	AD-01		0.76
DPL901	Genomic	GATGTGGTTAGGTGAGAAAGCA, CTTTCCAGCTGCAGGACT	DPL-SSR-Markers	-	AD-03, 14		0.00
DPL866	Genomic	AGAGTCAACTTCGACGCCAA, CTTGCTCACTTCGATATGCT		-	AD-26, 12		0.00
DPL890	Genomic	ACAGCATTAGCAGGCACCTT, TATGAACGATGTGCTAGCCG		-	AD-26		0.58
DPL 752	Genomic	CACATCACCTAATTACCATTGAAGC, TATCGTGAATATGTATGTGCGTGG		-	AD-24		0.00
DPL490	Genomic	AGTATCGTCACTTGTCAAAGTCCA, CTCATGCATGCTTATCACACATC		-	AD-01		0.58

*The first line in primer sequence is for forward primer, while the second line represents reverse primer sequence

CHAPTER IV
RESULTS

The results of the current study conducted for the genetic characterization of cotton genotypes through SSR markers are described in detail in this chapter. The results have been divided into 3 different sections, i) DNA extraction, ii) SSR markers polymorphism and iii) Classification of the cultivars through different statistical techniques.

4.1 DNA Extraction

DNA isolation was carried out with a manual CTAB extraction method. Genomic DNA was extracted from the leaves of 50 different cultivars, twice. The first extraction did not produce significant amount of DNA, therefore extraction was conducted twice to smoothly run the studies. The DNA concentration of the 2nd extraction run was considered for all the procedures selected in the study. The DNA was successfully extracted during the 2nd run from all of the cultivars, however the concentration and quality were varied. The DNA concentration varied from 350 ng/uL to 4287 ng/uL. The highest DNA concentration was extracted from the cultivars CIM496, whereas the lowest DNA concentration was obtained from the cultivar BA-308. The DNA concentration obtained from each of the cultivars is presented in Table 4.1.

Table 4.1. The concentration of extracted DNAs with 260(abs)/280(abs) ratio values from different cotton cultivars included in the study

Cultivar Code	Cultivar Name	DNA Concentration	
		ng/uL	260/280
T1	BA-151	3071.1	1.79
T2	BA-525	1077.8	1.71
T3	Carisma	1403.9	1.71
T4	Çukurova 1518	2143.1	1.72
T5	Gloria	1820.9	1.76
T6	Lydia	2354.8	1.74
T7	Acala-1517V	2611.2	1.73

Table 4.1. (Continue) The concentration of extracted DNAs with 260(abs)/280(abs) ratio values from different cotton cultivars included in the study

Cultivar Code	Cultivar Name	DNA Concentration	
		ng/uL	260/280
T8	BA-308	350	1.60
T9	BA-320	1279	1.75
T10	BA-811	1192	1.74
T11	CIM-496	4287	1.77
T12	DP-499	2163.4	1.70
T13	H-4028	397	1.55
T14	IH-20	1651.2	1.74
T15	IH-26-K-5	2443	1.73
T16	IH-27-TYL	2270	1.77
T17	IH-82-K-3	2204	1.74
T18	IH-82-Y-1	1371.6	1.74
T19	MCH-578	1353	1.76
T20	Natalia	2069	1.76
T21	PG424-1	742.6	1.60
T22	PG426-4	1950.8	1.68
T23	PG510-15	1423	1.66
T24	PG510-7	802	1.63
T25	PG511-7	887	1.65
T26	PG518-11	975.4	1.67
T27	PG519-19	1248.1	1.72
T28	PG520-7	947	1.66
T29	PG-2018	781.5	1.71
T30	PG-300	778.5	1.72
T31	PG-310	776.4	1.72
T32	PG-53-KT-2	994.5	1.76
T33	PG-53-YT-11	994.5	1.76
T34	Prema	2060.7	1.76
T35	ST-468	806.2	1.68
T36	Tamcot CamdES	964.8	1.75
T37	Tamcot SP37H	705.07	1.93
T38	Tamcot Sphinx	1059	1.69
T39	Taşkent-1	1354.9	1.71
T40	Taşkent-3	1151	1.68
T41	Teks	1215	1.60

Table 4.1. (Continue) The concentration of extracted DNAs with 260(abs)/280(abs) ratio values from different cotton cultivars included in the study

Cultivar Code	Cultivar Name	DNA Concentration	
		ng/uL	ng/uL
T42	VD-4	1498.2	1.62
T43	Şahin2000	480	1.53
T44	Nata	1086	1.55
T45	Lacata	834.7	1.76
T46	Carmen	564	1.61
T47	N-m 503	765.8	1.50
T48	N-87	453	1.70
T49	Julia	539.9	1.50
T50	Maydos Yerlisi	1098.7	1.50

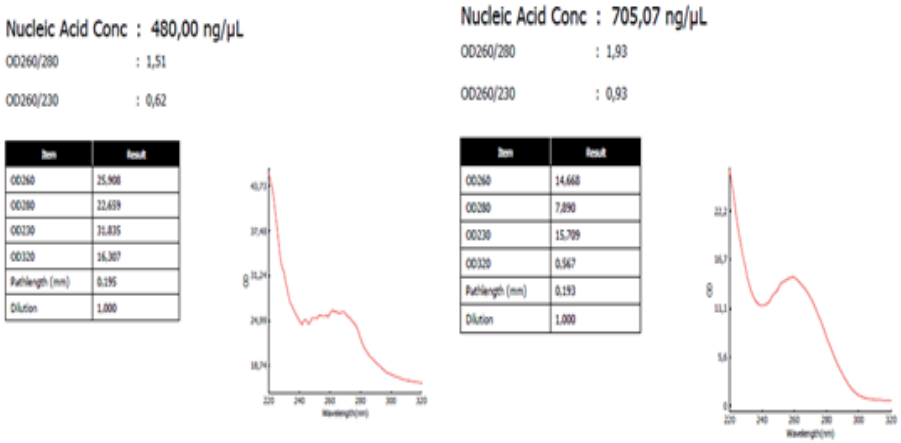

Figure 4.1. Spectrophotometer analysis results of extracted nucleic acids from cultivars Şahin2000 and Tamcot SP37H

4.2 Polymorphism of SSR Markers

The polymorphism of the SSR markers used during the current study is described separately for each of the markers in this section. The cultivar codes and their respective scores have been mentioned on the gel image of each of the SSR markers.

4.2.1 DPL0022 (SSR marker linked to QTL for VW resistance trait)

Different types of alleles were observed for the primer DPL0022. The primer is known to be linked to the QTL qVW-A5-1 located on chromosome AD-05 in cotton (Zhiyuan et al., 2013). The cultivars where polymorphism is observed are probably suitable for VW screening compared with the other cultivars of the study. It shows the highest PIC value 0.76, which shows that this marker is very informative and helpful for MAS (Figure 4.2).

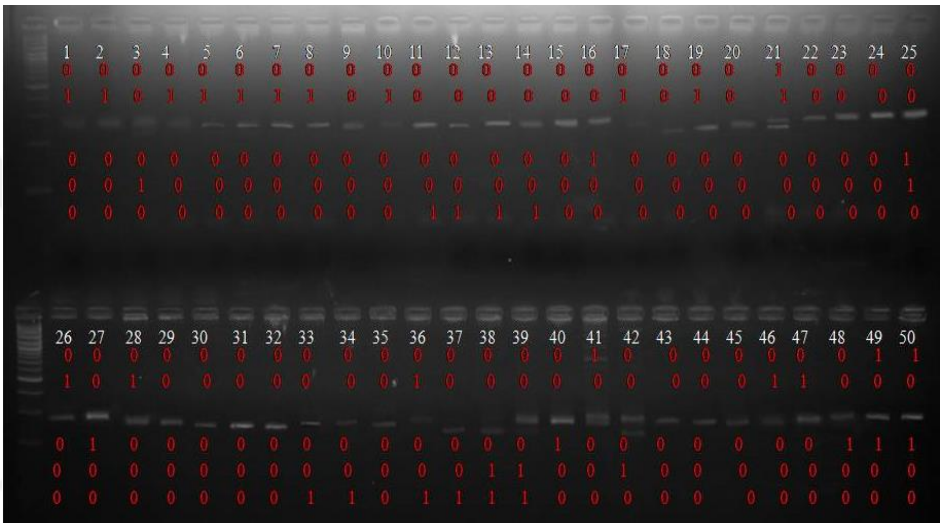

Figure 4.2. Agarose gel image of the SSR primer DPL0022. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar. (1 denotes presence, whereas 0 denotes absence of the respective allele)

4.2.2 BNL4108

This marker exhibited polymorphism and two types of different alleles were observed. This marker is located on AD-06 chromosome (Figure 4.3).

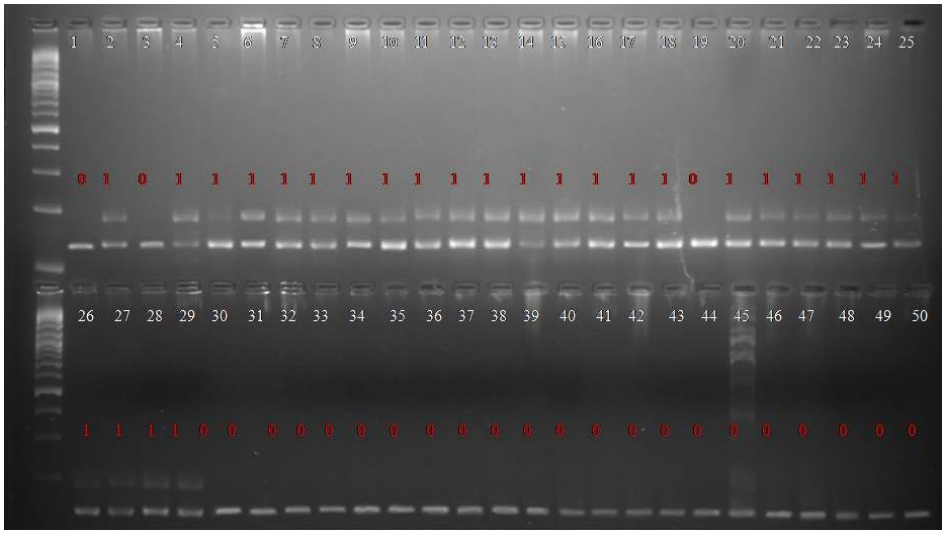

Figure 4.3. Agarose gel image of the SSR primer BNL4108. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar. (1 denotes presence, whereas 0 denotes absence of the respective allele)

4.2.3 GH215 (SSR markers linked to QTL for VW resistance trait)

Five different types of alleles were observed for the primer GH215. This SSR marker is located on chromosome AD-13 known to be linked on QTL *qVW-A1-1* (Zhiyuan et al., 2013). The calculated PIC value was 0.54 which seems to be good for MAS (Figure 4.4).

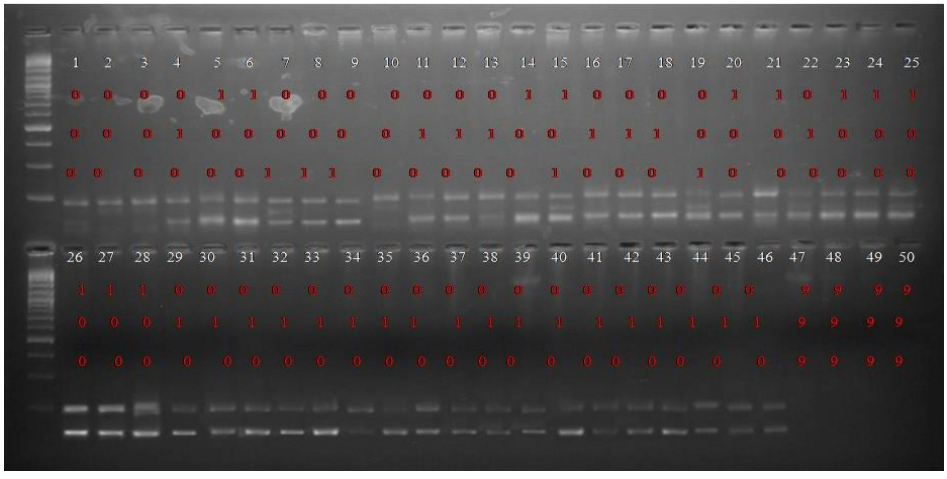

Figure 4.4. Agarose gel image of the SSR primer GH215. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar. (1 denotes presence, 0 denotes absence of the respective allele, while 9 represents missing data of the alleles)

4.2.4 DPL0322 (SSR marker linked to QTL for fiber quality traits)

Different types of alleles were observed for the primer DPL0322. This SSR marker is located on chromosome AD-15 known to be linked with QTL *qFE-C15-1* (Sun et al., 2011). This primer was informative with PIC value 0.63 related to VW resistance (Figure 4.5).

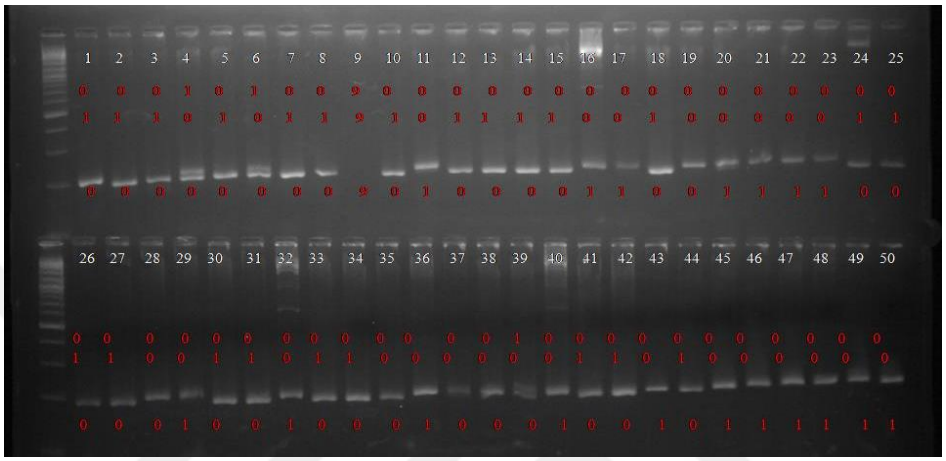

Figure 4.5. Agarose gel image of the SSR primer DPL0322. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar (1 denotes presence, whereas 0 denotes absence of the respective allele)

4.2.5 CIR246 (SSR marker linked to QTL for fiber quality traits)

Three Different types of alleles were observed for the primer CIR246. The primer is known to be linked to the QTL *qFL-D2-1* located on chromosome AD-14 (Wang et al., 2006). It exhibited lowest PIC value 0.10 which indicates that this marker is not informative for MAS. This might be due to poor gel electrophoresis, however, it can be improved by using capillary gel (Figure 4.6).

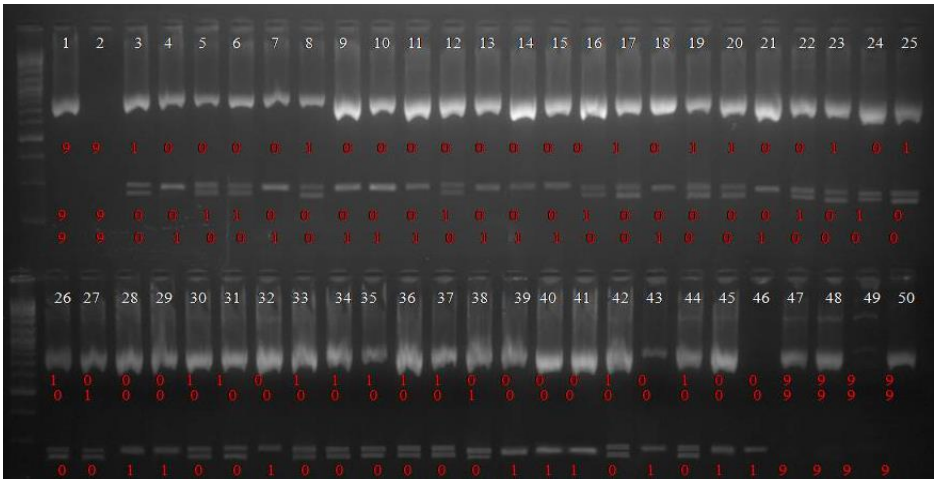

Figure 4.6. Agarose gel image of the SSR primer CIR246. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivars (1 denotes presence, 0 denotes absence of the respective allele, while 9 represents missing data of the alleles)

4.2.6 CIR381 (SSR marker linked to QTL for fiber length and fiber uniformity traits)

Four different types of alleles were observed for the primer CIR381. The primer is located on chromosome AD-02, 14, 4 on QTL *qMi-C11* (Wu et al., 2009). This marker produced 0.63 PIC value which indicate that it is an informative marker and can be used for MAS in future (Figure 4.7).

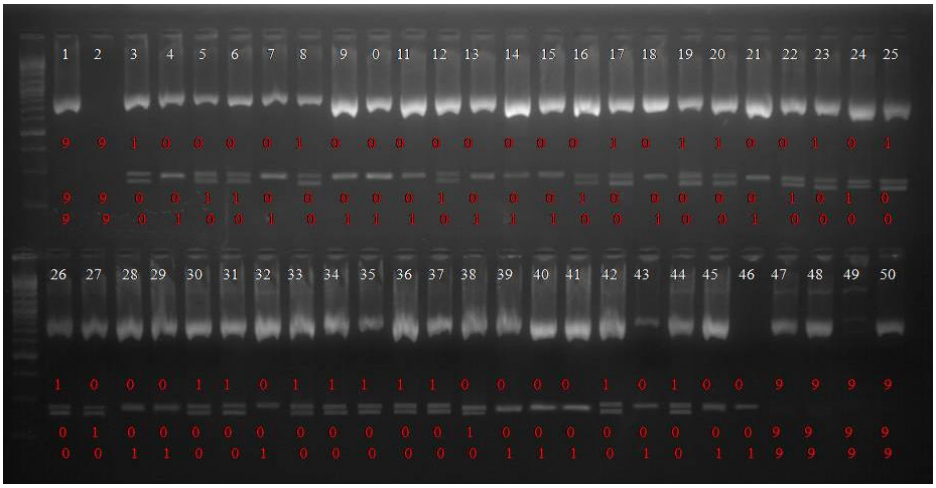

Figure 4.7. Agarose gel image of the SSR primer CIR381. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivars. (1 denotes presence, 0 denotes absence of the respective allele, while 9 represents missing data of the alleles)

4.2.7 DPL431

Different types of alleles were observed for the SSR marker DPL431. This SSR marker is located on chromosome number AD-10. Calculated PIC value was 0.57 which reveals that this marker can be further used for MAS (Figure 4.8).

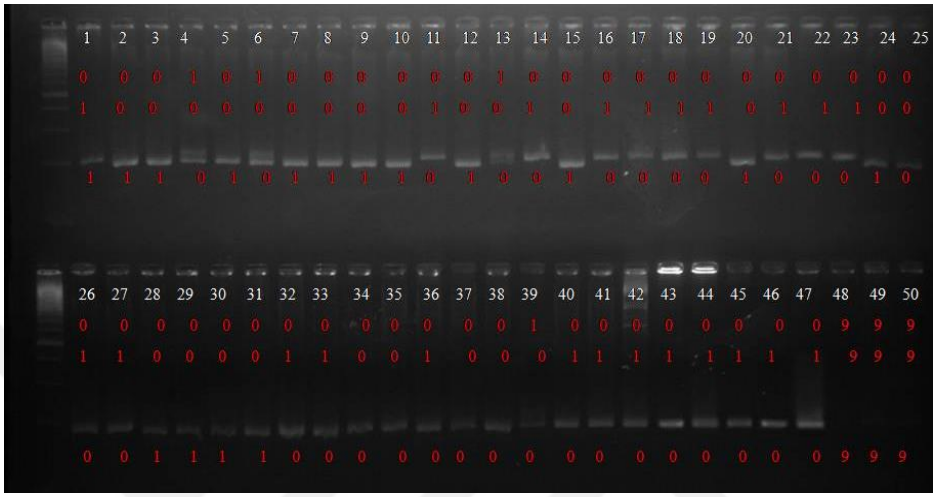

Figure 4.8. Agarose gel image of the SSR primer DPL431. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivars. (1 denotes presence, 0 denotes absence of the respective allele, while 9 represents missing data of the alleles)

4.2.8 JESPR-65 (SSR marker linked to QTL for VW resistance trait)

Many different types of alleles were observed for the primer JESPR-65. The primer is linked to the QTL *qVV-A5-1F2* located on chromosome AD-05 (Yang et al., 2007). The marker found to be polymorphic with PIC value 0.61, which shows the importance of this marker in MAS (Figure 4.9).

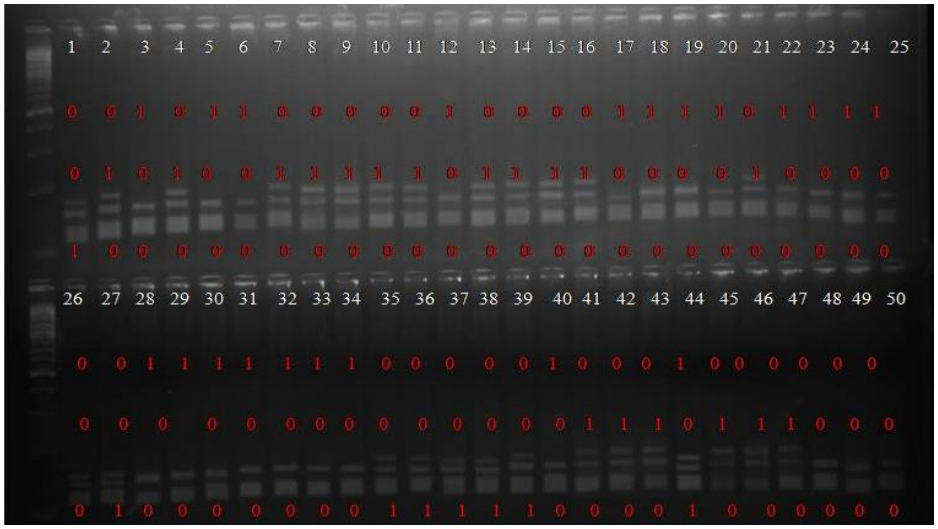

Figure 4.9. Agarose gel image of the SSR primer JESPR-65. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar (1 denotes presence, 0 denotes absence and 9 represent missing data of the respective allele)

4.2.9 DPL0253

Different types of alleles were observed for the primer DPL0253. The primer is located on chromosome AD-11. This marker displayed PIC value of 0.0412 (Figure 4.10).

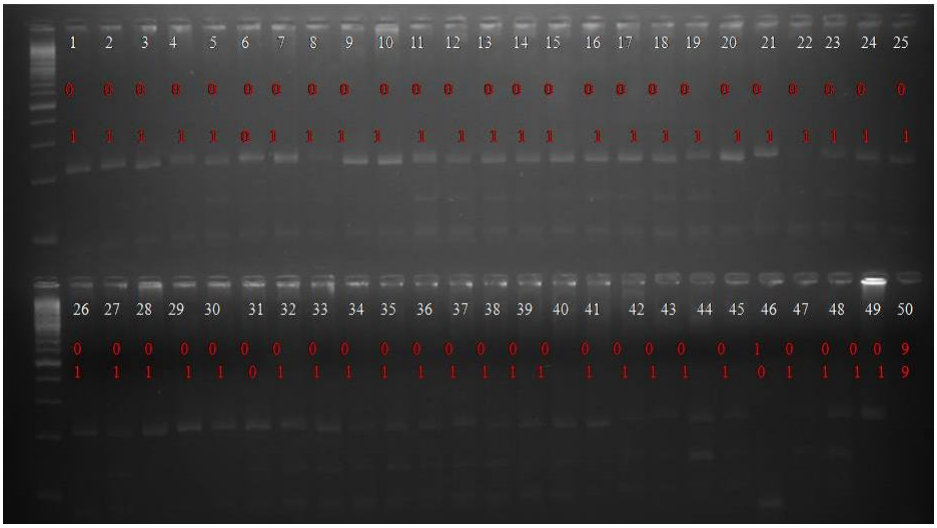

Figure 4.10. Agarose gel image of the SSR primer DPL0253.The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar. (1 denotes presence, 0 denotes absence and 9 represent missing data of the respective allele)

4.2.10 DPL0513

The primer DPL0513 resulted no polymorphism for all the tested cultivars. No different allele types were observed as a result of the PCR product of this primer. This might be due to agarose gel electrophoresis, however, use of Capillary electrophoresis can improve these results (Figure 4.11).

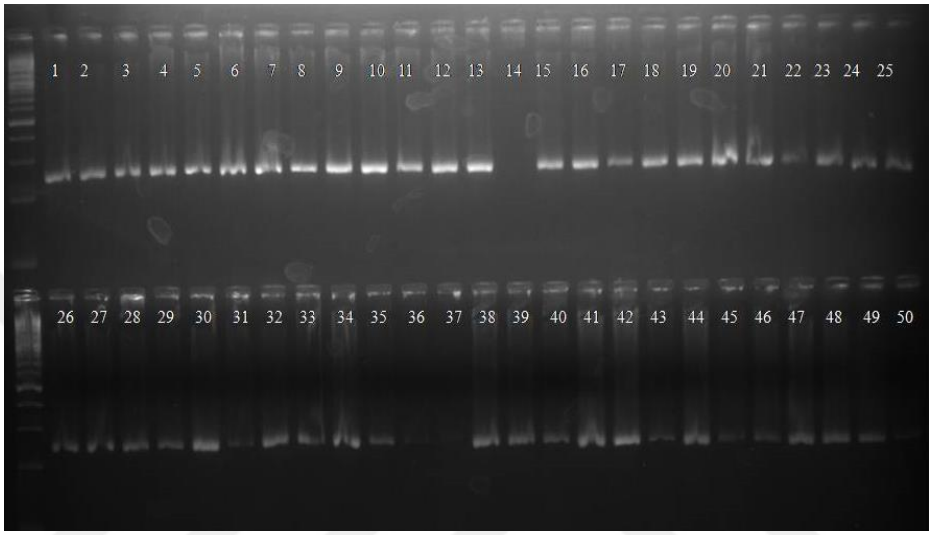

Figure 4.11. Agarose gel image of the SSR primer DPL0513.The digits in white represent the cultivar codes

4.2.11 DPL0405

Eight different types of alleles were observed for the SSR marker DPL0405. The primer is located on chromosome AD-14. The calculated PIC value for this cultivar was 0.49 (Figure 4.12).

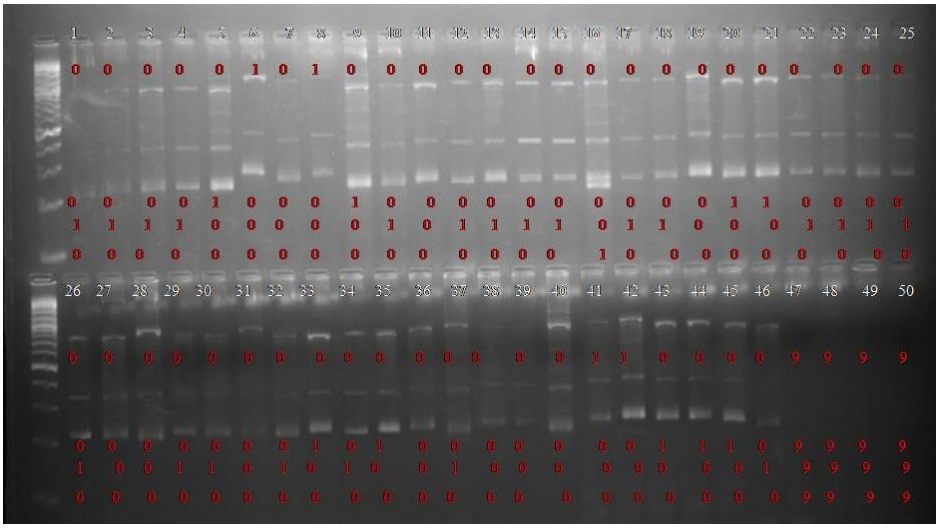

Figure 4.12. Agarose gel image of the SSR primer DPL0405. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar. (1 denotes presence, 0 denotes absence and 9 represents missing (data of the respective allele)

4.2.12 CGR5258 (SSR marker linked to QTL for VW resistance and fiber quality traits)

The primer CGR5258 resulted no polymorphism at all for all the tested cultivars. No allele types were observed as a result of the PCR product of this primer (Figure 4.13).

Figure 4.13. Agarose gel image of the SSR primer CGR5258. The digits in white represent the cultivar codes

4.2.13 GH527 (SSR Marker linked to QTL for VW resistance trait)

The primer GH527 displayed no polymorphism at all for all the tested cultivars. It seems that this primer has no correlation with VW resistance (Figure 4.14).

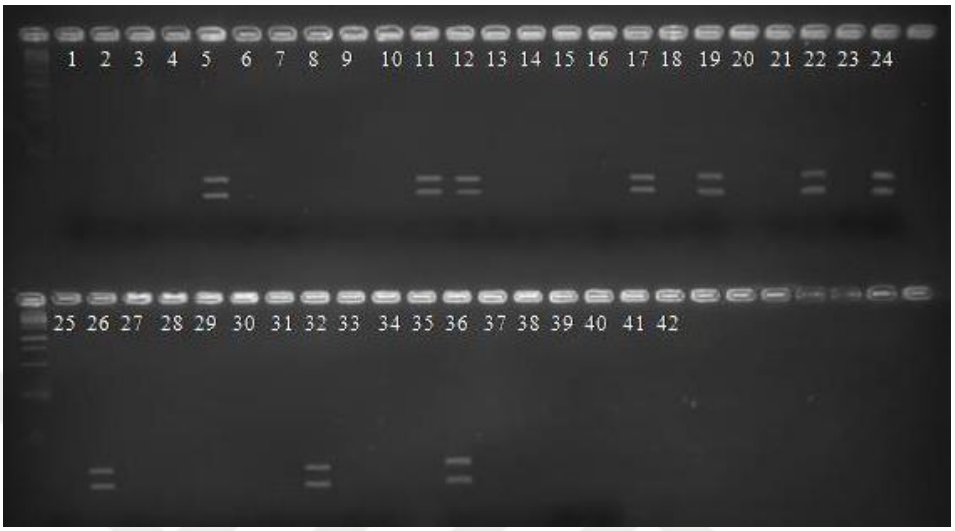

Figure 4.14. Agarose gel image of the SSR primer GH527. The digits in white represent the cultivar codes

4.2.14 CIR295 (SSR marker linked to QTL for VW resistance trait)

The primer CIR295 resulted no polymorphism for all the tested cultivars. No allele differences were observed as a result of the PCR product of this primer (Figure 4.15).

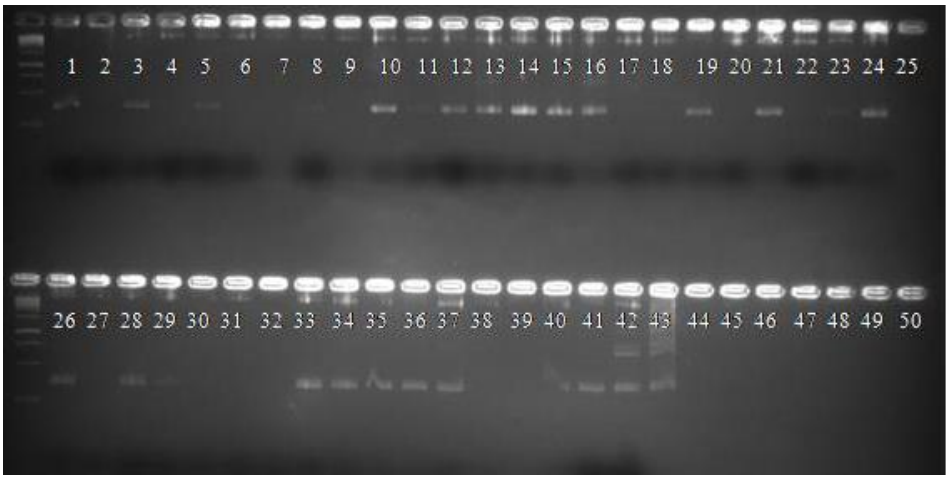

Figure 4.15. Agarose gel image of the SSR primer CIR295. The digits in white represent the cultivar codes

4.2.15 NAU3700 (SSR marker linked to QTL for VW resistance trait)

The primer NAU3700 resulted no polymorphism for all the tested cultivars. No different allele types were observed as a result of the PCR using with the primer NAU3700 (Figure 4.16).

Figure 4.16. Agarose gel image of the EST primer NAU3700. The digits in white represent the cultivar codes

4.2.16 NAU5465 (SSR marker linked to QTL for VW resistance trait)

The primer NAU5465 displayed no polymorphism for all the tested cultivars. No different allele variety was observed as a result of the PCR product of this primer (Figure 4.17). Capillary electrophoresis could be better for this analysis.

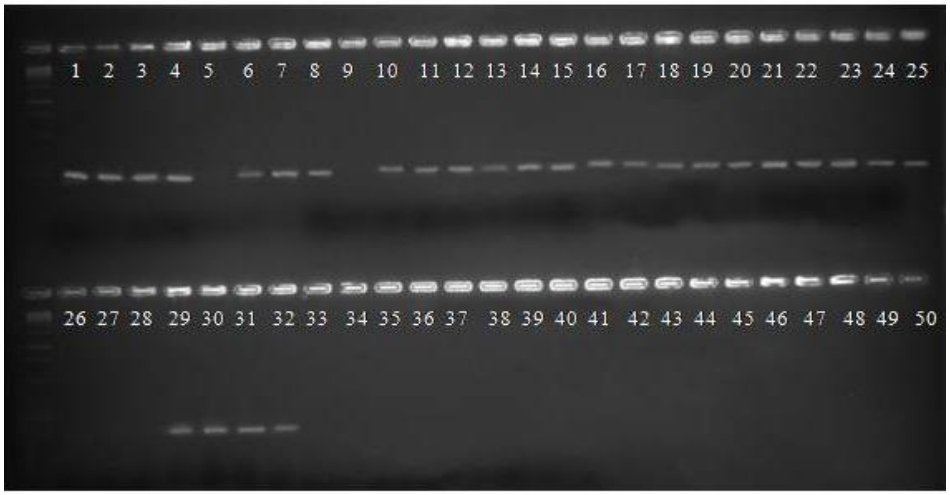

Figure 4.17. Agarose gel image of the EST primer NAU5465. The digits in white represent the cultivar codes

4.2.17 JESPR-12 (SSR marker linked to QTL for VW resistance trait)

The SSR marker JESPR-12 failed to amplify for all the tested cultivars. No different allele types were observed as a result of the PCR product of this primer (Figure 4.18).

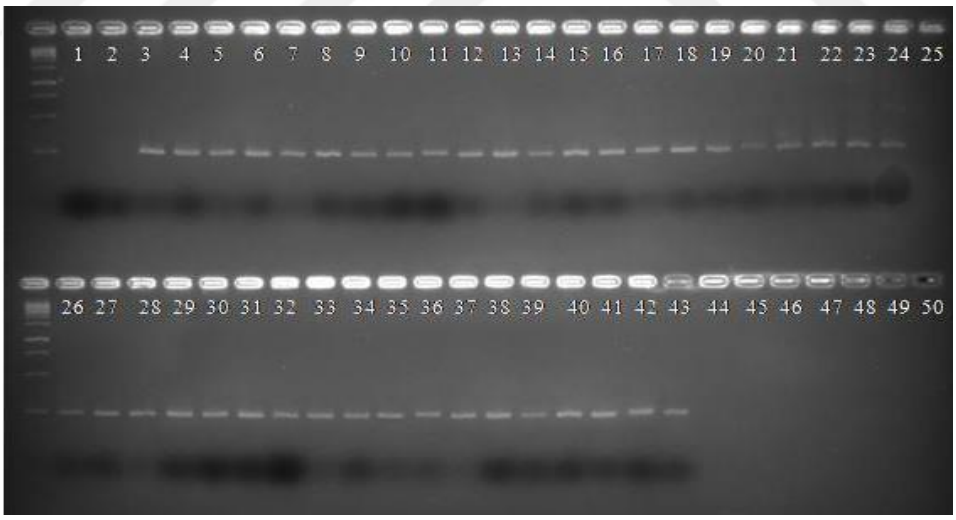

Figure 4.18. Agarose gel image of the primer JESPR-12. The digits in white represent the cultivar codes

4.2.18 DC20067 (SSR Marker linked to QTL for VW resistance trait)

The primer DC20067 exhibited no polymorphism for all the tested cultivars. No dissimilar allele variety was observed as a result of the PCR product of this primer. This

primer is known to be linked QTL *qVW-A5-1* (Zhiyuan et al., 2013) (Figure 4.19). It is not suggested for MAS studies.

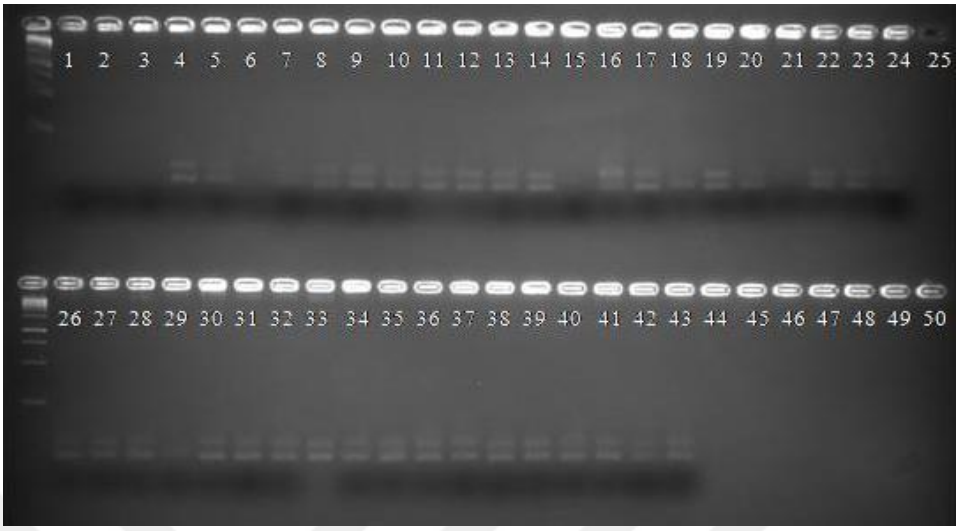

Figure 4.19. Agarose gel image of the primer DC20067. The digits in white represent the cultivar codes

4.2.19 NAU3414 (SSR marker linked to QTL for VW resistance trait)

The primer NAU3414 demonstrated polymorphism for all the cotton cultivars used in this study. This primer is linked with QTL *qVW-D9-1* located on chromosome AD-9 (Zhiyuan et al., 2013; Figure 4.20).

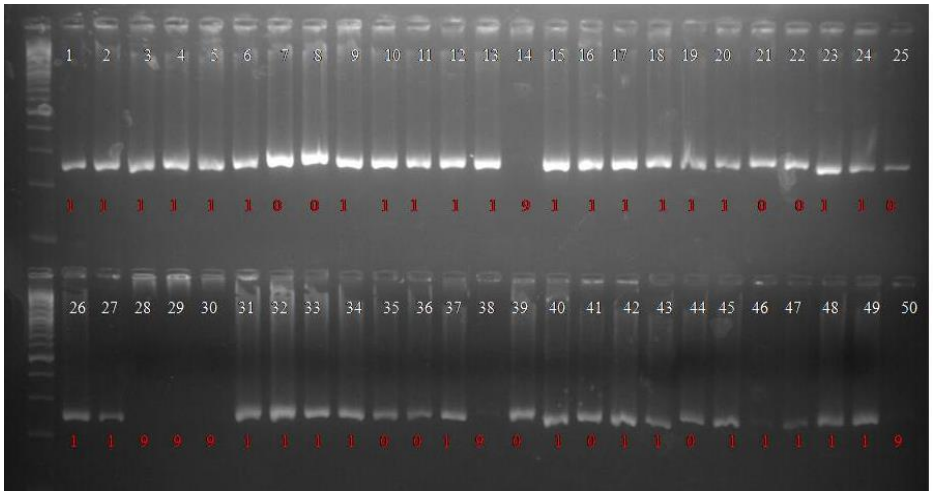

Figure 4.20. Agarose gel image of the primer NAU3414. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar. (1 denotes presence, 0 denotes absence and 9 represents missing data of the respective allele)

4.2.20 NAU3669 (SSR marker linked to QTL for VW resistance trait)

The primer NAU3669 resulted in no polymorphism at all for the tested cultivars. No allele types were observed as a result of the PCR product of this primer (Figure 4.21).

Figure 4.21. Agarose gel image of the primer NAU3669. The digits in white represent the cultivar codes

4.2.21 HAU3303 (SSR markers linked to QTLs for VW resistance trait)

The primer HAU3303 showed no polymorphism for all the cotton cultivars used in this study. No unlike allele variety was observed as a result of the PCR product of this primer (Figure 4.22).

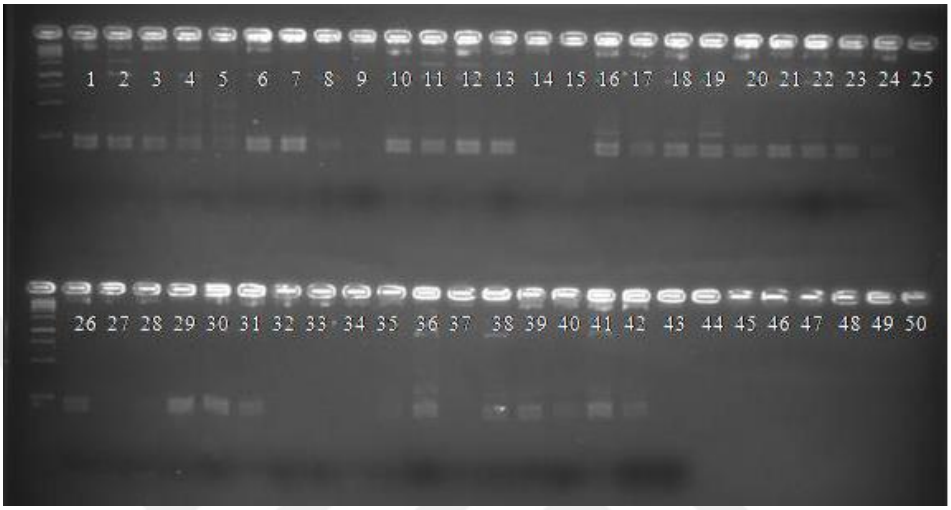

Figure 4.22. Agarose gel image of the primer HAU3303. The digits in white represent the cultivar codes

4.2.22 NAU2471 (SSR marker linked to QTL for VW resistance trait)

The primer NAU2471 exhibited no polymorphism for all the tested cultivars. No dissimilar allele variety was observed as a result of the PCR product of this primer (Figure 4.23).

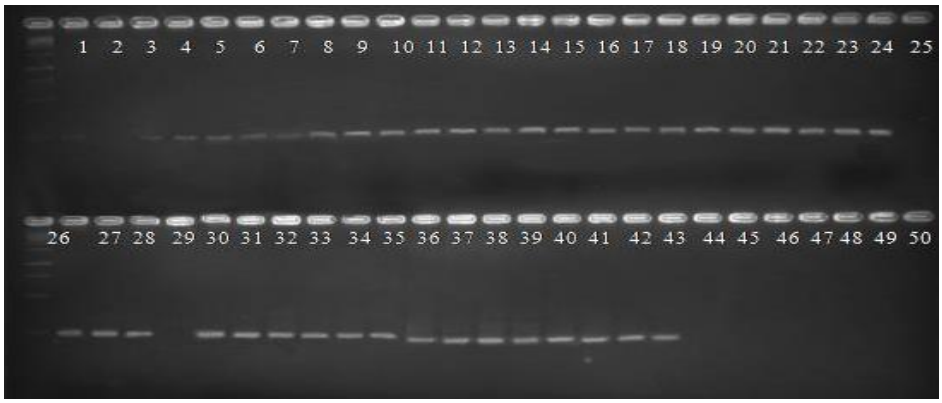

Figure 4.23. Agarose gel image of the primer NAU2471. The digits in white represent the cultivar codes

4.2.23 NAU2354 (SSR marker linked to QTLs for VW resistance trait)

The SSR marker NAU2354 displayed no polymorphism for all the tested cultivars. No different allele types were observed as a result of the PCR product of this primer (Figure 4.24).

Figure 4.24. Agarose gel image of the primer NAU2354. The digits in white represent the cultivar codes

4.2.24 NAU2954 (SSR marker linked to QTL for VW resistance trait)

The primer NAU2954 exhibited no polymorphism for all the cotton cultivars which used in this study. No unlike allele variety was observed as a result of the PCR product of this primer (Figure 4.25).

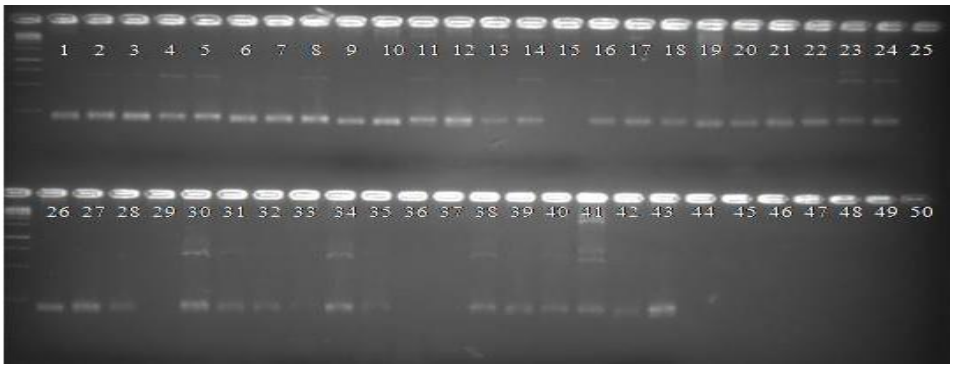

Figure 4.25. Agarose gel image of the primer NAU2954. The digits in white represent the cultivar codes

4.2.25 DPL901

The primer DL901 demonstrated that there were no polymorphism for all the cotton cultivars used in this study. This primer is located on chromosome AD-03, 14. This can be improved by using capillary electrophorese rather than Agarose gel (Figure 4.26).

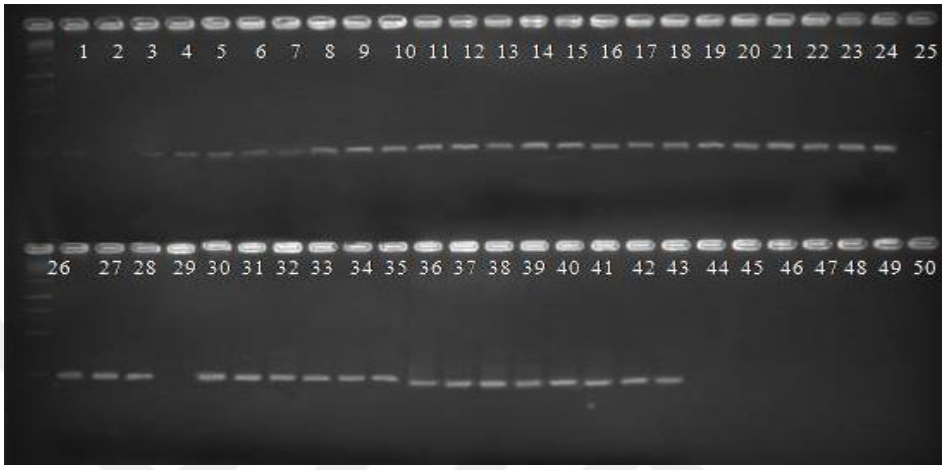

Figure 4.26. Agarose gel image of the primer DPL901. The digits in white represent the cultivar codes

4.2.26 DPL0866

Two different types of alleles were observed for the SSR marker DPL0866. The primer is located on chromosome AD-26, 12 (Figure 4.27).

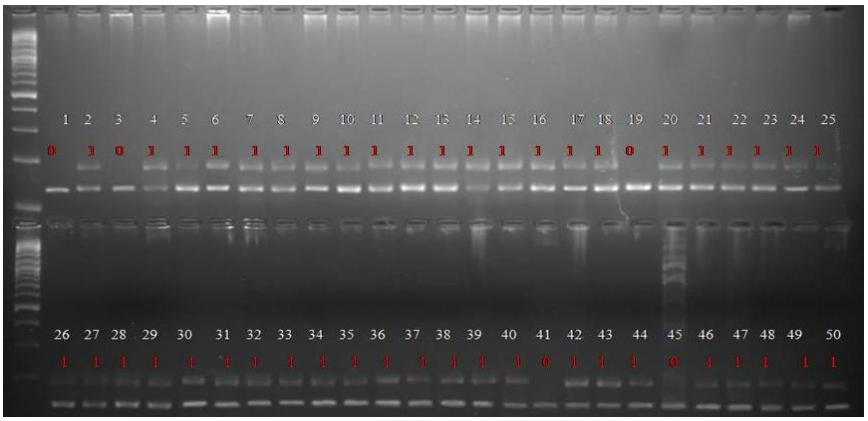

Figure 4.27. Agarose gel image of the SSR primer DPL0866. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar. (1 denotes presence, 0 denotes absence and 9 represents missing data of the respective allele)

4.2.27 DPL890

Two dissimilar types of alleles were observed. The cultivars where polymorphism is monitored probably are useful for VW resistance. The marker is located on chromosome AD-26. Its PIC value was 0.58 (Figure 4.28).

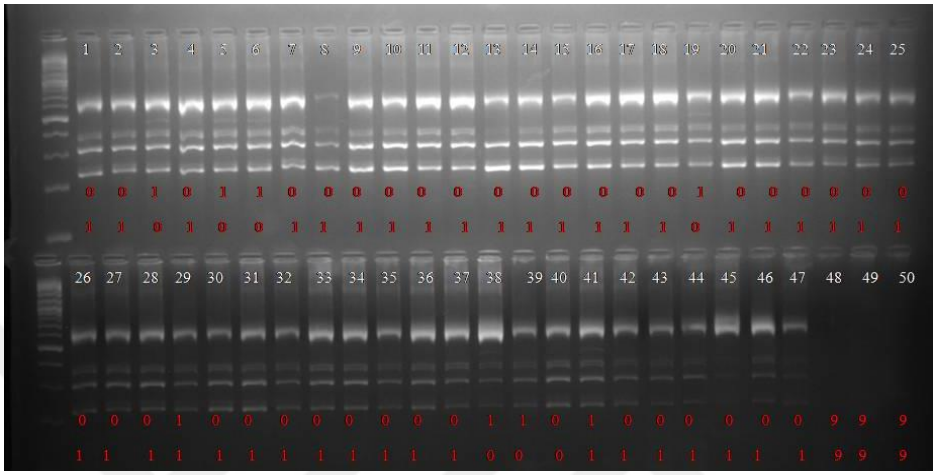

Figure 4.28. Agarose gel image of the SSR primer DPL890. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar. (1 denotes presence, whereas 0 denotes absence and 9 represents missing data of the respective allele)

4.2.28. DPL752

Different types of alleles were observed, but this marker is not informative with 0 PIC value (Figure 4.29).

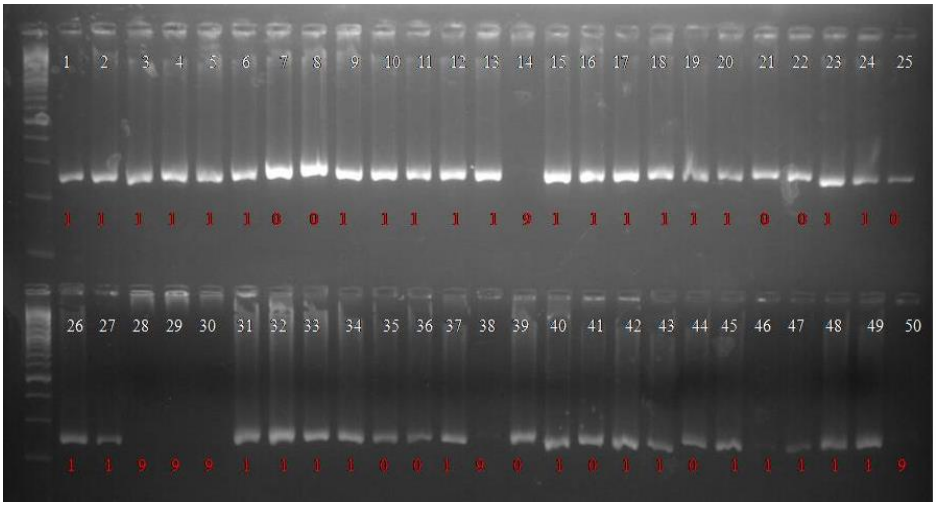

Figure 4.29. Agarose gel image of the SSR primer DPL752. The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar. (1 denotes presence, whereas 0 denotes absence and 9 represents missing data of the respective allele)

4.2.29 DPL490

Different types of alleles were observed for the SSR marker DPL0490. The primer is located on chromosome AD-01. Its calculated PIC value was 0.58 (Figure 4.30).

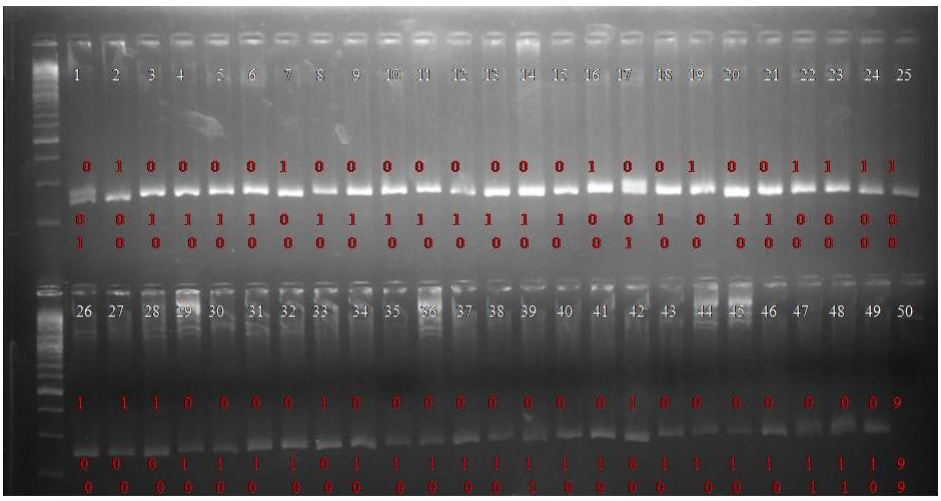

Figure 4.30. Agarose gel image of the SSR primer DPL490.The digits in white represent the cultivar codes, whereas colored digits correspond to the allele scores of the respective cultivar. (1 denotes presence, whereas 0 denotes absence and 9 represents missing data of the respective allele)

4.2.30 DPL307

The primer DPL307 resulted in no polymorphism at all for all the tested cultivars. No different allele types were observed as a result of the PCR product of this primer. The results of the PCR product can be improved by using Capillary electrophoresis (Figure 4.31).

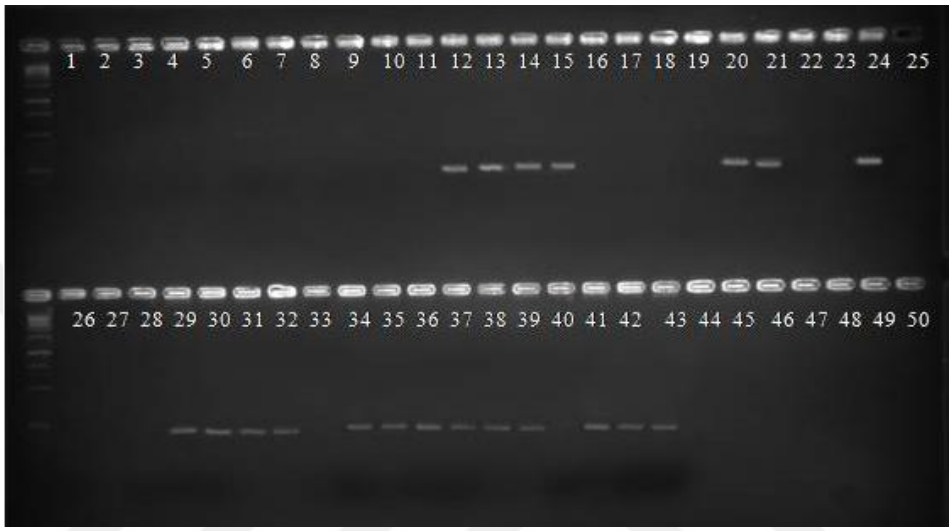

Figure 4.31. Agarose gel image of the SSR primer DPL307. The digits in white represent the cultivar codes

4.3 Classification of the Cultivars

The cultivars tested during the current study were classified on the basis of their dissimilarity to each other with several classification techniques. The results obtained through each of the classification technique are described in the following sections.

4.3.1 Principal component analysis of cotton cultivars

Principal Component Analysis (PCA) signifies the associations of genotypes in a more consequential form showing that PCA should be used along with the dendrogram to increase a better understanding of relationships among genotypes.

Table 4.2. The factor loading of the first 9 Principal Components obtained through Principal Component Analysis executed on the scoring data of 30 SSR primers and 50 cultivars included in the study

	F1	F2	F3	F4	F5	F6	F7	F8	F9
DPL0022a	-0.552	-0.056	-0.193	-0.144	-0.268	0.141	-0.125	-0.244	0.224
DPL0022b	0.072	-0.212	0.545	-0.080	-0.345	0.105	0.063	0.377	0.043
DPL0022c	0.128	0.242	-0.150	0.012	0.224	-0.088	0.480	0.093	0.621
DPL0022d	0.165	0.526	-0.336	0.567	0.030	0.333	0.169	0.013	-0.282
DPL0022e	0.165	0.526	-0.336	0.567	0.030	0.333	0.169	0.013	-0.282
GH215a	**0.942**	0.019	-0.090	-0.138	-0.108	-0.051	0.063	-0.048	-0.004
GH215b	**0.933**	0.140	-0.117	0.011	-0.119	-0.053	-0.053	-0.113	0.023
GH215c	**0.949**	-0.031	-0.085	-0.029	-0.154	-0.086	0.000	-0.085	-0.002
DPL431a	**0.940**	-0.009	0.134	0.055	-0.103	0.043	0.040	0.094	0.014
DPL431b	**0.896**	-0.015	0.190	0.023	-0.113	0.022	-0.005	0.013	0.029
DPL431c	**0.918**	-0.096	0.157	0.014	-0.137	-0.054	0.032	0.158	-0.056
CIR 246a	0.081	-0.037	-0.096	-0.363	0.352	-0.091	0.598	-0.202	0.083
CIR 246b	-0.239	0.416	0.250	0.221	-0.169	-0.350	-0.328	0.338	0.224
DPL890a	**0.930**	0.021	0.130	0.041	-0.112	-0.043	-0.001	0.103	0.031
DPL890b	**0.925**	-0.066	0.141	0.030	-0.111	0.060	0.045	0.082	0.002
DPL890c	**0.934**	-0.062	0.135	0.017	-0.132	0.001	0.061	0.111	0.018
DPL68	-0.047	-0.010	-0.357	-0.056	0.230	0.474	0.035	0.480	0.222
DPL490a	**0.949**	0.035	-0.069	-0.059	-0.156	-0.062	-0.008	-0.088	0.046
DPL490b	**0.846**	0.062	-0.157	0.024	-0.100	-0.126	0.058	-0.125	0.001
DPL490c	**0.931**	0.048	-0.058	-0.094	-0.122	-0.058	0.097	-0.090	-0.082
DPL490d	**0.955**	0.035	-0.080	-0.053	-0.141	-0.063	0.015	-0.100	-0.003
NAU3414	0.164	-0.203	0.402	0.090	0.347	0.029	-0.045	-0.444	0.037
DPL405a	**0.614**	-0.084	0.428	0.073	0.500	0.056	0.026	0.039	0.040
DPL405b	0.526	-0.034	0.334	0.341	0.494	0.169	-0.277	-0.023	-0.031
DPL405c	0.496	-0.040	0.535	0.300	0.543	0.071	-0.017	0.040	0.033
BNL4108	0.336	-0.437	-0.125	-0.386	0.050	0.386	0.321	0.247	-0.211
DPL0322a	-0.038	**0.736**	0.380	-0.409	-0.077	0.292	-0.007	-0.042	0.048
DPL322b	0.056	**0.660**	0.379	-0.423	-0.109	0.251	0.033	-0.072	-0.047
DPL322c	-0.213	**0.619**	0.349	-0.448	-0.014	0.310	-0.070	-0.117	0.015
CIR381a	**0.806**	0.128	-0.372	-0.155	0.147	0.009	-0.242	-0.066	0.119
CIR381b	0.405	0.112	-0.536	-0.209	0.335	0.183	-0.416	0.074	0.190
CIR381c	**0.805**	0.022	-0.390	-0.124	0.077	0.142	-0.297	-0.062	0.122
JESPR-65a	0.268	0.213	-0.116	-0.498	0.358	-0.470	-0.067	0.203	-0.330
JESPR-65b	0.118	-0.511	0.145	0.172	-0.308	0.618	-0.068	-0.186	0.179
JESPR-65c	0.086	0.454	0.035	0.568	-0.136	-0.269	0.204	-0.040	0.229
Eigenvalue	14.27	3.03	2.77	2.49	1.96	1.83	1.36	1.10	1.03
Variability (%)	40.78	8.65	7.90	7.12	5.60	5.22	3.89	3.13	2.94
Cumulative %	40.78	49.43	57.33	64.45	70.06	75.27	79.17	82.30	85.24

The bold digits represent the SSR markers possibly influenced the respective axis of the PCA

Principal component analysis executed on the scoring data of SSR primers yielded 9 principal components (PCs) with eigen values ≥1 (Table 4.2). The first two PCs accounted for 49.43% of variation in the data. Similarly, the cumulative variability explained by first three and four axis was 57.33% and 64.45%, respectively. The axis having eigen values more than 1 collectively explained 85.24% of variation in the data. The 1st axis was possibly affected by 16 different SSR markers (with correlation

coefficients ≥0.6), whereas the 2nd was influenced by 3 SSR markers. However, 3rd and fourth axis did not contain any SSR primers having correlation coefficients ≥0.6 (Table 4.2). These explained variation by the first two axis and correlation coefficients of the primers indicate that SSR primers, GH215, DPL431, DPL890, DPL490, DPL405, DPL322 and CIR381 are the probable drivers of the variation in the scoring data of the tested cultivars.

The scatter diagram of the first two axis yielded 2 distinct group of the tested cultivars. The group 1 was affected by 17 SSR markers, while the 2nd group was affected by 2 SSR markers only (Figure 4.32). The group 1 (on right side) of the scatter plot contained 4 cultivars, while the group 2 (on left side) was represented by 46 cultivars included in the study. The PCA scatter diagram, thus identifies that 4 cultivars of the group 1 have mutual similarity, while the remaining 46 cultivars of the 2nd group are similar to each other.

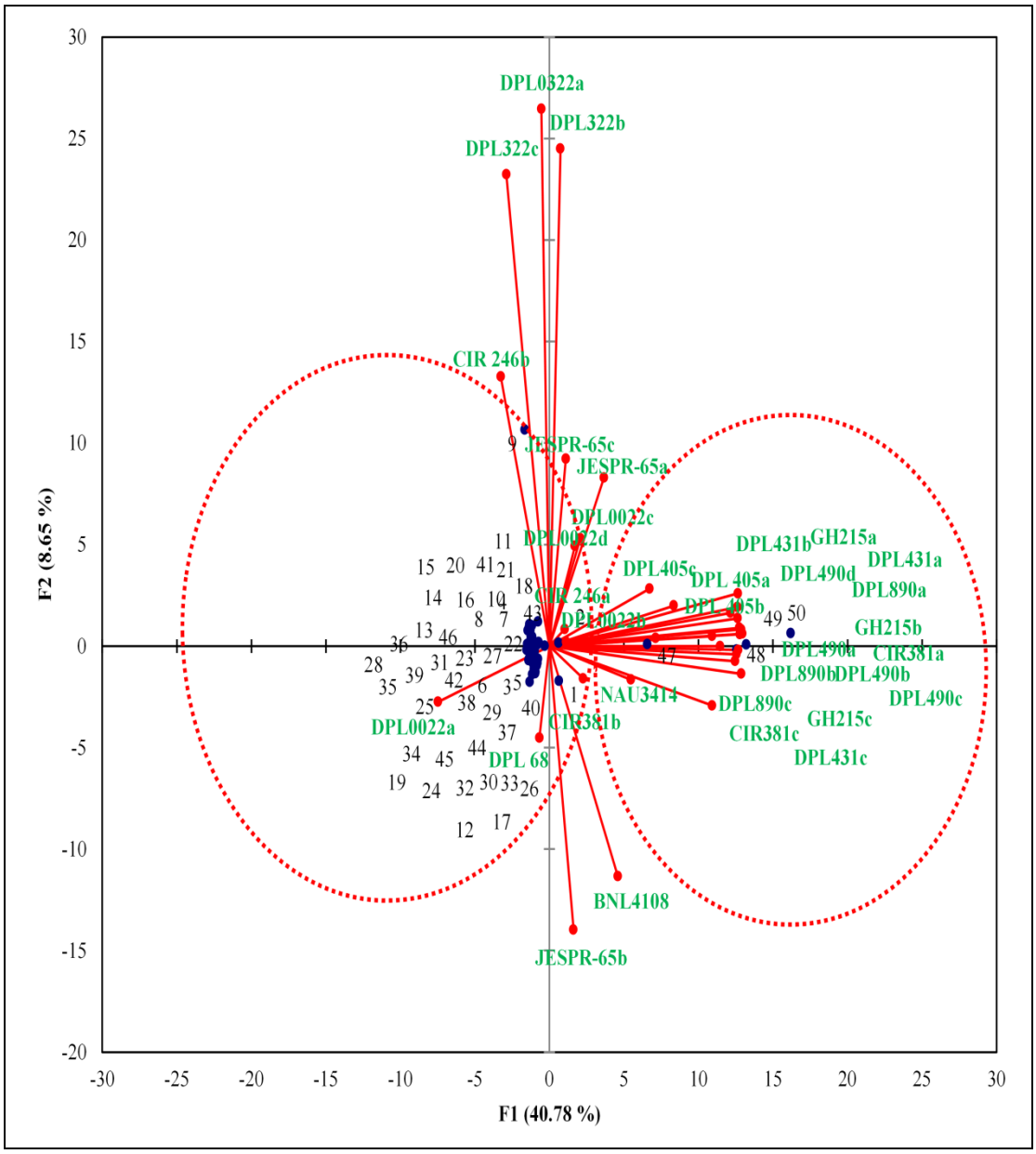

Figure 4.32. The biplot of first two axis obtained through principal component analysis executed on the scoring data of 30 SSR primers and 50 cotton cultivars included in the study. Red points represent SSR markers while blue points show cultivars. The effect size of the SSR marker is explained by the length of red lines

4.3.2 Hierarchical clustering of cotton cultivars

The hierarchical clustering divided the cultivars into two major groups. The first group contained 3 cultivars, while the second group was composed of 47 cultivars. The first group was sub-divided into many subsets (Figure 4.33). The sub-group 2 was initially sub-divided into two main groups, one containing 6 cotton cultivars, while the other group was composed of 41 different cotton cultivars included in the study. This sub-

division indicated that the cultivars of first group (IH-20, Tamcot Sphinx, PG520-7, PG-2018, PG-300 and Carmen are different from the cultivars of the other group. Maydos Yerlisi, Julia and N-7 cultivars were distinctly separated from all the remaining cultivars. It is expected that the cultivar Maydos Yerlisi was separated due to the different genus (*G. herbaceum*). It can be concluded that the cultivars Julia and N-7 have different genetic background and would be useful for breeding of VW resistant genotypes. Overall results also demonstrate that most individuals were genetically close to each other, such as IH-26-K-5/IH-27 TYL, PG2018/PG300/PG310/PG520-7 and BA151/BA525 due to their similar breeding programs. Therefore bringing in greater genetic diversity into breeding programs might be helpful.

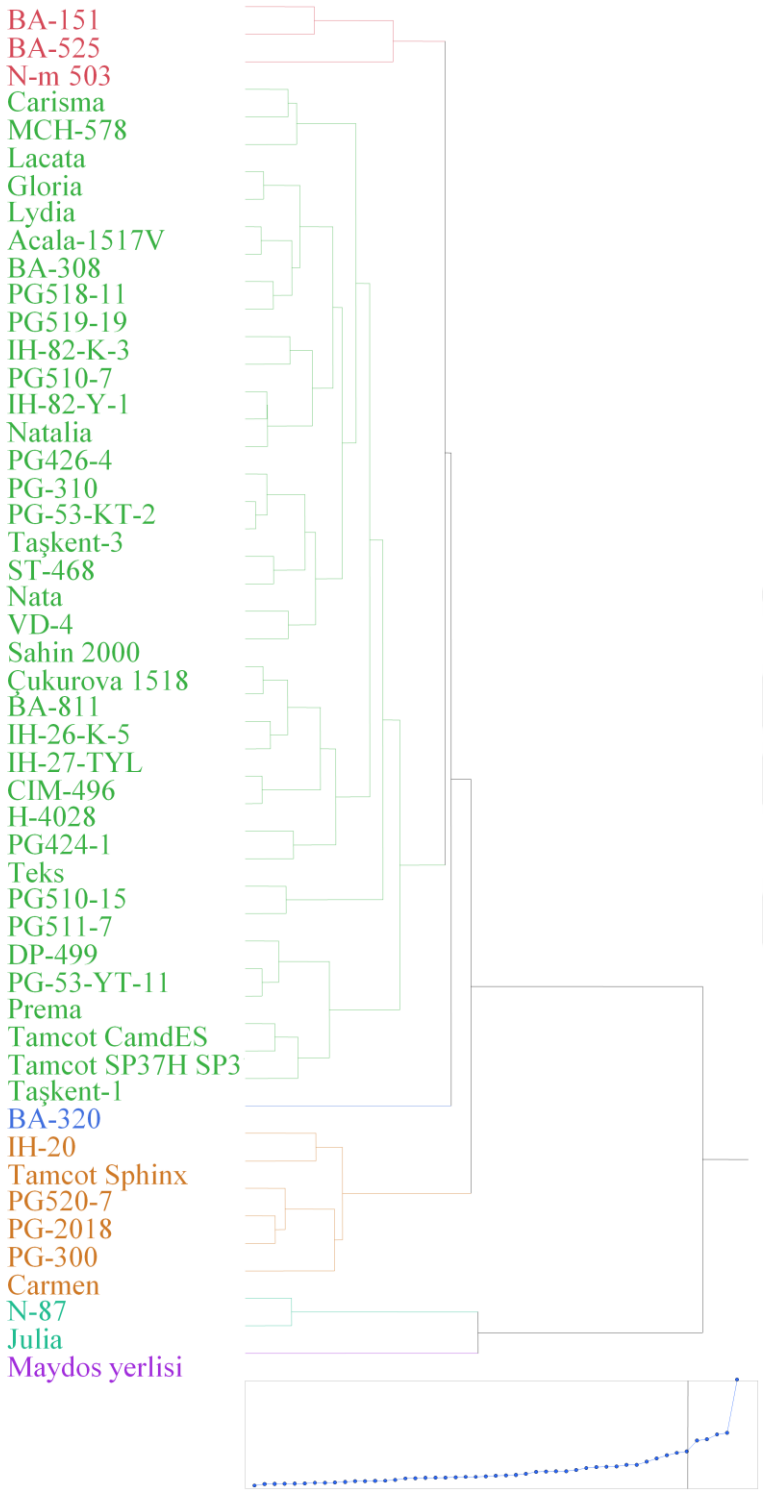

Figure 4.33. The dendrogram obtained through hierarchical clustering analysis executed on the scoring data of 30 SSR primers and 50 cotton cultivars included in the study

From the Figure 4.33 it is clear that Maydos yerlisi, and also Julia and N-87 were closely related with each other. All of them were resistance cultivars. Moreover,

Carmen and N-m-503 which were also our positive controls were related to BA525 and Şahin2000, respectively. Çukurova 1518 and Nata both were sensitive genotypes related with each other, but Şahin2000 that is also sensitive cultivar, was closely related to our resistance control i.e., N-m-503 which might be due to similar genetic background. Red color cultivars were similar to each other while green color genotypes were closely related to each other, in a same way cultivars with brown color were belongs to similar genetic background. Maydos Yerlisi was represented by different color due to dissimilarity with other cultivars. BA320 also exhibited dissimilarity with other cultivars.

4.3.3 Neighbor joining analysis of cotton cultivars

To accomplish genetic diversity analysis of the cultivars we used DARwin6 analysis program with presence/absence data and UnWeighted Neighbor Joining algorithm.

The Neighbor Joining method is a way for reconstructing phylogenetic trees with different branch lengths. The two nearest nodes of the tree are chosen and defined as neighbors in our tree. Neighbor joining is better option than distance tree. It is considered more reliable. In total, 50 genotypes were analyzed with this program. According to the results of the neighbor joining analysis, two clusters were obtained (Figure 4.34). In cluster A there were 16 individuals, whereas cluster B was composed of 34 individuals. Maydos Yerlisi, the out-group genotype showed dissimilarity with other cultivars and similarity to Julia and N-87 which were both resistance and positive controls. It indicates that they are genetically similar to each other and different from rest of the cultivars.

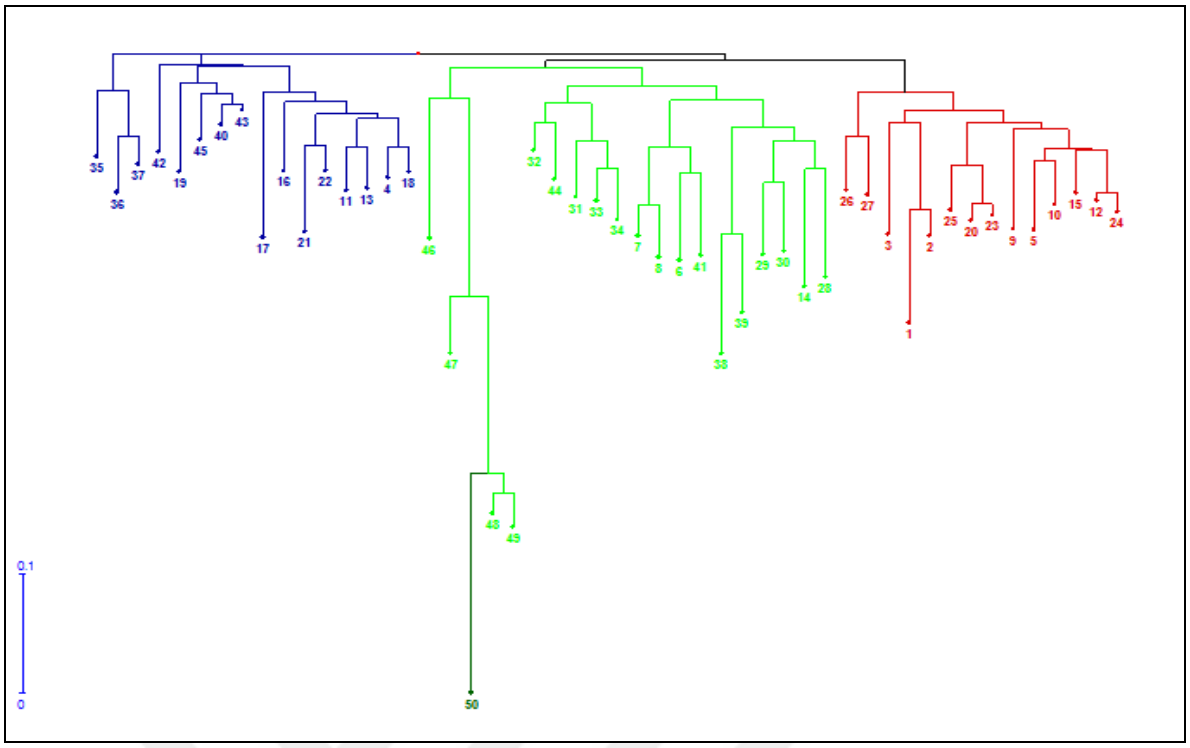

Figure 4.34. The dendrogram obtained through Neighbor-joining analysis executed on the scoring data of 30 SSR primers and 50 cotton cultivars included in the study

Maydos Yerlisi was different from remaining cultivars. Prema, the resistance cultivars showed dissimilarity with other resistance genotype. All sensitive cultivars were dissimilar from each other. Julia, Carmen, N-87, N-m-503 all were resistant cultivars and showed similarity with each other. All these cultivars were joined to a same node but the branch connecting one population to the node were different from the others which represented genetic distance of these cultivars. Şahin2000, Çukurova 1518, Nata, and Lacata all were susceptible cultivars hence showed dissimilarity with each other.

Both WARD tree and NJ tree revealed that Maydos Yerlisi belong to different group. Most of results obtained from these two clustering techniques were similar. The NJ algorithm allowed branch lengths to vary, because it represented more accurate genetic distance matrix. However UPGMA tree didn't show different branch lengths hence all cultivars belonging to same node were assigned same branch length.

CHAPTER V
DISCUSSION

Verticillium wilt (VW) is the most destructive disease of cotton all around the world. Due to the low efficiency of management options in controlling VW, it has urged the scientist to focus on the genetic improvement programs. VW is a global problem and most promising method to cope with the disease is the development of resistant cultivars. However, the available germplasm at regional scales has narrow genetic diversity and molecular data on the germplasm is often limited. The molecular screening of the available germplasm using SSR markers has become a promising technique to identify the QTLs linked to VW resistance. Evaluations of phenotypic and genotypic variability among plant varieties are very important for plant breeding and genetic diversity studies. Even though phenotypic diversity has been exemplified for many years before, but genotypic characterization studies depend on the recent studies through molecular marker technology. Therefore the current study has been accomplished for the genetic characterization of cotton genotypes through SSR markers in Turkey. Although some recent studies have assessed the molecular basis of VW resistance in the available cotton cultivars in Turkey, however there are still plenty of cultivars which have never been molecularly screened for assessing the levels of VW resistance.

A total of 49 upland cottons along with 1 from *Gossypium herbaceum* were selected on the basis of agronomic characteristics to identify molecular markers for VW resistance. A total of 30 SSR markers were assayed on the cotton materials which produced 677 loci that were used for genetic diversity analysis. As a result of the molecular analyses, 13 SSR markers proved to be polymorphic, and few of them are persistent with previous studies. The number of alleles per marker ranged from 2 to 48. The observed number of alleles per marker is important for germplasm diversity as well as for marker type and the method used for fragment separation and resolution (Lacape et al., 2007). This study will be helpful in MAS for improving VW resistance in upland cotton. It is complicated to produce reliable and reproducible data from QTL analyses of VW resistance because of highly complex inheritance of the trait, inoculation scheme and disease scoring as well as the risk of environmental impact on disease symptoms (Zhang et al., 2014). To summarize, we used an assorted collection of elite upland cotton genotypes to identify

30 SSR marker loci linked to VW resistance with positive and negative effects. Of the identified loci, some were consistent with the previous works. The Maydos Yerlisi was distinctly separated from all the genotypes as this was expected because of different genus and it is evidently dissimilar from other upland genotypes. Julia and N-87 were closely related to Maydos Yerlisi as these were resistance and positive controls. Prema, N-m-503 were also resistant cultivars and exhibited dissimilarity with each other. This may be attributed to their different genetic backgrounds. Çukurova 1518 and Nata both were used as sensitive resistance and were closed to each other. Lacata and Şahin2000 were also sensitive genotypes but both were very distant from each other. Although upland cotton has a very contracted genetic structure resulting from its evolutionary history, and breeding (Paterson et al., 2004), however remarkable genetic diversity was observed among the analyzed commercial Turkish cotton varieties exposed by PCA, WARD and Neighbor Joining analysis. According to diversity analysis, certain varieties from the same source had low diversity. The result obtained through hierarchical clustering analysis demonstrated that most individuals from the similar breeding program were genetically close to each other such as IH-26-K-5/IH-27 TYL, PG2018/PG300/PG310/PG520-7 and BA151/BA525. According to Neighbor Joining analysis Maydos Yerlisi was different from remaining cultivars and same result was obtained through WARD tree. All sensitive cultivars were dissimilar from each other unlike WARD clustering result. Julia, Carmen, N-87 and N-m-503 all were resistant cultivars and showed similarity with each other. Similar results were obtained through WARD tree. All these cultivars were joined to a same node but the branch connecting one population to the node were different from the others, which represented genetic distance of these cultivars. Şahin2000, Çukurova 1518, Nata and Lacata all were susceptible cultivars however, showed dissimilarity with each other. According to phenotypic characterization analysis that carried out by Agricultural Faculty of Mustafa Kemal University (TÜBİTAK Project No: 214O086) , the cultivars Tamcot Camd-ES, Gloria, Natalia, Lydia, Teks, Tamcot SP37H, ST468 and BA525 are the resistance cultivars, while CIM-496, H-4028 and DP499 are susceptible ones to VW.

Introducing greater genetic diversity into breeding programs might be advantageous. PCA signifies the associations of genotypes in a more consequential way therefore PCA should be used along with dendrogram better visualize and understand the relationship among diverse genotypes. PCA indicated that Maydos Yerlisi, Julia, N-87 and N-m-503

showed different analysis compared to other cultivars and these all were resistance cultivars. Polymorphism information content (PIC) values are used to get information of a genetic marker either these markers are useful for linkage analysis or not. For this purpose PIC value of the molecular markers were calculated. Average PIC value was 0.56, whereas the normal PIC value of a core set of SSR markers for *Gossypium* species varied from 0.0 to 0.82 (Yu et al., 2012). In the current study, markers DPL0022, DPL0253 and DPL0752 proved to be the most informative marker with highest PIC value 0.76, whereas CIR246 proved as the least significant marker with 0.10 PIC value.

Some of the SSR markers were consistent with previous studies which verified the effectiveness of these SSR markers for MAS for VW resistance. One of these markers DPL0022 was significantly linked to VW resistance with 0.76 PIC value in the current study. DPL0022 has also been reported to be associated with VW on QTL *qVW-A5-1* by Zhiyuan et al. (2013). GH215 was also found to be linked with VW on QTL *qVW-A1-1* by (Zhiyuan et al., 2013). Likewise, in the current study a connection was found between GH215 and VW resistance. Yang et al. (2007) performed association mapping analysis and found JESPR-65 on QTL *qVL-A5-1F2* and was linked to VW resistance. In the current study, similar marker was found to be associated with VW. One SSR marker pair, NAU3414 was also found to be linked to VW as observed in the previous study (Zhiyuan et al., 2013). Some of the markers related to fiber elongation and fiber quality traits are linked to QTL s*qMi-C11, qFE-C15-1, qFL-D2-1, qFL03.2, qRV07DF2-A9-2* (Wang et al., 2006, Wu et al., 2009, Sun et al., 2011). Among the tested marker for these traits, CIR381, CIR246, DPL322 demonstrated polymorphism in the current study. It was also observed that some markers (GH527, CIR295, NAU3700, NAU5465, DC20067, NAU3669, HAU3303, and NAU2741) related to VW show polymorphism, however contrasting results were obtained in the current study. In conclusion, most of the identified markers linked to QTL for VW resistance investigated under this study found to be ineffective. The markers NAU2954, NAU2354, NAU2471, HAU3303, NAU3669, NAU3414, DC20067, NAU5465, NAU3700, GH527, CGR5258 and CIR295 were non-informative for all the tested cultivars with agarose gel electrophoresis system. Among them, only the markers JESPR65, GH215 and DPL0022 were found to be very informative and promising for MAS studies. These results could be improved by using Capillary electrophoresis. Moreover, among the analyzed fiber quality markers, CIR381, CIR246 and DPL 405 were found to be very polymorphic.

The contrasting results are attributed to the nature of genotypes used in the current study. All of the indicated markers should be considered as a good candidate for marker assisted selection in the future.

CHAPTER VI
CONCLUSION

Cotton is the most desired crop all around the world due to its worth in different industries. More cotton production and low yield losses are needed. Verticillium wilt is a global problem and most promising method to cope with the disease is the development of resistant cultivars. Most of commercial cotton genotypes are susceptible to VW. Classic, conventional breeding methods have not yet been successful to cope with VW, because of the low genetic diversity in cotton germplasm. Due to the problems in VW evaluation, selection for VW resistance has been actually ineffective and unproductive. MAS may aid the selection process; however, validation of markers to be used in MAS remains a big problem. In this study, a natural population consisting of 50 cotton individuals was tested against *Verticillium dahliae* through genotypic analysis in order to identify SSR markers responsible for resistance. A set of 30 SSR markers (DPL, NAU, CIR and JESPR) were used to recognize polymorphisms within the genotype out of which 13 markers displayed polymorphism. For the molecular data analysis, polymorphism information content (PIC) values of molecular markers were calculated, which revealed that DPL0022 and DPL752 were the most informative markers. Principal component analysis was carried out on the scoring data of SSR primers. The first two PCs accounted for 49.43% of variation in the data. Dendrograms were constructed based on phenotypic and genotypic data using WARD on JMP which categorized the genotypes into two sets, which demonstrated that there was low genetic distance between the individuals which were resulted from same breeding program. The current study will be supportive for the development of marker-assisted strategies for breeding of VW resistant in cotton cultivars. The main conclusions of the current study are;

i) The available commercial cotton cultivars in Turkey have low genetic diversity as most of these cultivars have been produced from same breeding program
ii) Some of the SSR markers linked to VW in other studies in the world were failed to amplify against Turkish cotton cultivars
iii) The genetic diversity, although low in the Turkish cotton cultivars, can be further used to improve VW resistance.

LITERATURE CITED

Akkose, A., Molecular Mapping of Quantitative Trait Loci Conferring Resistance to Verticillium wilt in Cotton, M.Sc. (Hons.) Thesis, ***Izmir Institute of Technology***, 2014.

Anderson J.A., Churchill, G.A., and Autrique., J.E., "Optimizing parental selection for genetic linkage maps", **Genome** 36, 181–186, 1993.

Annonymous Agricultural structure, State Institute of Statistics, Prime Ministry of Republic of Turkey, ***Data Dissemination Div.*** Publ. no. 3942, Ankara, 2012.

Baytar, A.A., Erdogan, O., Frary, A., Frary, A. and Doganlar, S., "Molecular diversity and identification of alleles for Verticillium wiltresistance in elite cotton (*Gossypium hirsutum* L.) germplasm", ***Euphytica*** 213, 31, 2017.

Bell, A.A., Verticillium wilt. In: Hillocks, R.J. (Ed.), Cotton Diseases. ***CAB International, Wallingford***, UK, pp. 87-126, 1992.

Bicici, M. and Kurt, S., Etiology, incidence and prevalence of cotton wilt disease and strains of the wilt pathogen in Cukurova. In, Gillham F. M. (editor) ***Proc. World Cotton Res. Conf. – 2, New Frontiers in Cotton Research***, Athens, Greece, p. 914-918, 16-18 May, 1998.

Bolek, Y., El-Ziki, K.M., Pepper, A.E., Bell, A.A., Magill, C.W., Thaxton, P.M. and Reddy, O.U.K., "Mapping of Verticillium wilt resistance genes in cotton", ***Plant Sci*** 168, 1581–1590, 2005.

Cai, Y., Xiaohong, H., Mo, J., Sun, Q., Yang, J. and Liu, J., "Molecular research and genetic engineering of resistance to Verticillium wilt in cotton: a review", ***Afr J Biotechnol*** 8(25), 7363-7373, 2009.

Carpenter, C.W., "The Verticillium wilt problem", ***Phytopathology*** 4, 393, 1914.

Dervis, S. and Bicici, M., "Distribution of Verticillium wilt in cotton areas of southern Turkey", ***Plant Pathol J*** 4(2), 126-129, 2005a.

Dervis, S. and Bicici, M., "Vegetative compatibility groups in *Verticillim dahlie* isolates from cotton in Turkey", ***Phytoparasitica*** 33, 157-168, 2005b.

Dinler, H. and Benlioğlu, S., "Aydın İli'nde pamuk alanlarından elde edilen *Verticillium dahliae* Kleb.izolatlarının vejetatif uyum grupları", ***Bitki Koruma Buletini*** 53, 85-99, 2013.

Doyle, J.J. and Doyle, J.L., "A rapid DNA isolation procedure for small quantities of fresh leaf tissue", ***Phytochem Bull*** 19, 11–15, 1987

Du, W.S., Du, X.M. and Ma, Z.Y., "Studies on SSR markers of resistance gene of Verticillium wilt in cotton", ***J. Northwest Sci- Tech Univ Agric For.*** 32, 20–24, 2004

Elçi, E., Akışcan, Y. and Akgöl, B., "Genetic diversity of Turkish commercial cotton varieties revealed by molecular markers and fiber quality traits", ***Turk J Bot*** 38(6), 1274-1286, 2014.

El-Zik, K.M., "Integrated control of Verticillium wilt of cotton", ***Phytopathology*** 6, 1025-1032, 1985.

Erdoğan, O., Bölek, Y., Dündar, H. and Bardak, A., "Screening of cotton genotypes for resistance to *Verticillium dahlia* Kleb. under greenhouse and field conditions", ***Rom Agric Res*** 32, 53-61, 2015.

Erdogan, O., Ozbek, N., Aydın, U. and Gore, M.E., "The determination of relationship between Verticillium wilt (*Verticillium dahliae* Kleb.) and early maturity in cotton (*Gossypium hirsutum* L.)", ***Turk J Field Crops*** 18, 8-12, 2013.

Fang, H., Zhou, H., Sanogo, S., Flynn, R., Percy, R.G., Hughs, S.E., Ulloa, M., Jones, D.C. and Zhang, J., "Quantitative trait locus mapping for Verticillium wilt resistance in

a backcross inbred line population of cotton (*Gossypium hirsutum* × *Gossypium barbadense*)", ***Euphytica*** 194, 79-91, 2013.

Fang, H., Zhou, H., Sanogo, S., Lipka, A.E., Fang, D.D., Percy, R.G., Hughs, S.E., Jones, D.C., Gore, M.A. and Zhang, J., "Quantitative trait locus analysis of Verticillium wilt resistance in an introgressed recombinant inbred population of Upland cotton", ***Mol Breed*** 33, 709-720, 2014.

Fryxell, P.A., "A revised taxonomic interpretation of *Gossypium* L. (Malvaceae)", ***Rheedea*** 2, 108-165, 1992.

Gao, Y.Q., Nie, Y.C. and Zhang, X.L., "QTL mapping of genes resistant to Verticillium wilt in Cotton", ***Cotton Sci*** 15(2), 73-78, 2003.

Ge, H., Wang, Y., Guo, W. and Zhang, T., "Inheritance and molecular tagging of resistance against Verticillium wiltin Upland cotton", ***Cotton Sci*** 20, 19-22, 2008.

Göre, M. E., Erdoğan, O. and Altın, N., "Searching for resistance sources to Verticillium wilt of cotton in seedlings from *Gossypium* spp.", ***Trop Plant Pathol*** 2, 1-4, 2017.

Göre, M., Erdoğan, O., Altin, N., Aydın, M., Caner,Ö., Filizer, F. and Büyükdöğerlioğlu, A., "Seed transmission of Verticillium wilt of cotton", ***Phytoparasitica*** 39, 285-292, 2011.

Göre, M.E., "Vegetative compatibility and pathogenicity of *Verticillium dahliae* isolates from the Aegean Region of Turkey", ***Phytoparasitica*** 35, 222-231, 2007.

Göre, M.E., Caner, Ö.K., Altın, N., Aydın, M.H., Erdoğan, O., Filizer, F. and Büyükdöğerlioğlu, A., "Evaluation of cotton cultivars for resistance to pathotypes of *Verticillium dahliae*", ***Crop Prot*** 28, 215–219, 2009.

Göre, M.E., Erdoğan, O., Caner, Ö.K., Aydın, M.H. and Berk, S., "VCG diversity and virulence of *Verticillium dahliae* from commercially available cotton seed lots in Turkey", ***Eur J Plant Pathol*** 140(4), 689-699, 2014.

Guo, X.H., Cai, C.P., Yuan, D.D., Zhang, R.S., Xi, J.L. and Guo, W.Z., "Development and identification of Verticillium wilt-resistant upland cotton accessions by pyramiding QTL related to resistance", ***J Integr Agr*** 15, 512-520, 2016.

He, D.H., Lin, Z.X., Zhang, X.L., Nie, Y.C., Guo, X.P., Feng, C.D. and Stewart, J.M., "Mapping QTLs of traits contributing to yield and analysis of genetic effects in tetraploid cotton", ***Euphytica*** 144(1), 141-149, 2005.

Iyriboz, N., Cotton diseases, Turkish ministry of Agriculture and Rural Affairs (Vol. 237). ***Marifet Press***. Izmir, Turkey, 1941.

Jaccard P., "Nouvelles recherches sur la distribution lorale", ***Bulletin de La Societe Vaudoise des Sciences Naturelles*** 44, 223-270, 1908.

Jiang, F., Zhao, J., Zhou, L., Guo, W. and Zhang, T., "Molecular mapping of Verticillium wilt resistance QTL clustered on chromosomes D7 and D9 in Upland cotton", ***Sci China C Life Sci*** 52, 872–84, 2009.

Karaca, İ., Karcilioğlu, A. and Ceylan, S., "Wilt disease of cotton in the Ege region of Turkey", ***J Turk Phytopathol*** 1, 4–11, 1971.

Kheiri, A. and Fatahi, M., "Evaluation of Verticillium wilt tolerance in different cotton cultivars*",* ***J Res Agric Sci*** 6, 57-61, 2010.

Kun, Y., QTL mapping for fiber strength and Verticillium wilt resistance with RAD-seq in upland cotton, ***Washington State University,*** Washington, United States, 2014.

Lacape, J.M., Dessauw, D., Rajab, M., Noyer, J.L. and Hau, B., "Microsatellite diversity in tetraploid *Gossypium* germplasm: assembling a highly informative genotyping set of cotton SSRs", ***Mol Breed*** 19(1), 45-58, 2007.

Land, C., Lawrence, K., Burmester, C. and Meyer, B., "Cultivar, irrigation, and soil contribution to the enhancement of Verticillium wilt disease in cotton", ***Crop Prot*** 96, 1-6, 2017.

Lawrence, K., Hagan, A., Olsen, M., Faske, T., Hutmacher, R., Mueller, J., Wright, D., Kemerait, R., Overstreet, C., Price, P., Lawrence, G., Allen, T., Atwell, S., Thomas, S., Goldberg, N., Edmisten, K., Bowman, R., Young, H., Woodward, J. and Mehl, H., "Cotton disease loss estimate committee report, 2015", ***Proceedings of the 2016 Beltwide Cotton Conference, vol. 1.*** Memphis, United States, p. 113-115, 20-23 June, 2016.

Li, C.Q., Liu, G.S., Zhao, H.H., Wang, L.J., Zhang, X.F., Liu, Y., Zhou, W.Y., Yang, L. L., Li, P.B. and Wang, Q.L., "Marker-assisted selection of Verticillium wilt resistance in progeny populations of upland cotton derived from mass selection-mass crossing", ***Euphytica*** 191, 469-480, 2013.

Li, F., Fan, G., Wang, K., Sun, F., Yuan, Y., Song, G., Qin, Li., Ma., Z., Lu, C., Zou, C., Chen, W., Liang, X., Shang, H., Liu, W., Shi, C., Xiao, G., Gou, C., Ye, W., Xu, X., Zhang, X., Wei, H., Li, Z., Zhang, G., Wang, J., Liu, K., Kohel, R.J., Percy, R.G., Yu, J.Z., Zhu, Y., Wang, J. and Yu, S., "Genome sequence of the cultivated cotton *Gossypium arboretum*" ***Nat Genet*** 46, 567-72, 2014.

May, O.L., Bowman, D.T. and Calhoun, D.S., "Genetic diversity of U.S. upland cotton cultivars released between 1980 and 1990", ***Crop Sci*** 35(6), 1570-1574, 1995.

Ning, Z.Y., Zhao, R., Chen, H., Ai, N.J., Zhang, X., Zhao, J., Mei, H., Wang, P., Guo, W. and Zhang T., "Molecular tagging of a major quantitative trait locus for broad-spectrum resistance to Verticillium wilt in Upland cotton cultivar Prema", ***Crop Sci.*** 53, 2304-2312, 2013.

Palanga, K.K., Jamshed, M., Gong, J., Li, J., Iqbal, M.S., Liu, A., Shang, H., Shi, Y., Chen, T. and Ge, Q., "Quantitative trait locus mapping for Verticillium wilt resistance in an upland cotton Recombinant Inbred Line using SNP-Based high density genetic map", ***Front Plant Sci*** 8, 382, 2017.

Paterson, A.H., Saranga, Y., Menz, M., Jiang, C.X., and Wright, R., "QTL analysis of genotype× environment interactions affecting cotton fiber quality." ***Theor Applied Genets*** 106, 384-396, 2003.

Pegg G.F. and Brady B.L., Verticillium Wilts. ***CABI Publishing***, Wallingford, United Kingdom, 2002.

Perrier, X., and Jacquemoud-Collet, J.P., "**DARwin software**." 2006.

Rahman, M., Hussain, D. and Zafar, Y., "Estimation of genetic divergence among elite cotton (*Gossypium hirsutum* L.)cultivars/genotypes by DNA fingerprinting technology", ***Crop Sci*** 42, 2137-2144, 2002.

Rahman, M., Hussain, D., Malik, T,A. and Zafar, Y., "Genetics of resistance to cotton leaf curl disease in *Gossypium hirsutum*", ***Plant Pathol*** 54, 764-772, 2005.

Rahman, M., Yasmin, T. and Tabassum, N., "Studying the extent of genetic diversity among *Gossypium arboreum* L. genotypes/cultivars using DNA fingerprinting", ***Genet Resour Crop Evol*** 55, 331-339, 2008.

Shi, Y., Zhang, B., Liu, A., Li, W., Li, J., Lu, Q., Zhang, Z., Li, S., Gong, W. and Shang, H., "Quantitative trait loci analysis of Verticillium wilt resistance in interspecific backcross populations of *Gossypium hirsutum*×*Gossypium barbadense*", ***BMC Genomics*** 17, 877, 2016.

Sun, Q., Jiang, H., Zhu, X., Wang, W., He, X., Shi, Y., Yuan, Y., Du, X. and Cai, Y., "Analysis of sea-island cotton and upland cotton in response to *Verticillium dahliae* infection by RNA sequencing", ***BMC Genomics*** 14, 852, 2013.

Thierry, F., "Addinsoft", 40 rue Damremant, 75018, Paris, France@XLSTAT.com, 1993.

USDA-FAS, "United States Department of Agriculture", Foreign Agricultural Service, US,http://apps.fas.usda.gov/psdonline/psdReport.aspx?hidReportRetrievalName=Table?

04?Cotton?Area%2c?Yield%2c?and?Production&hidReportRetrievalID=851&hidReportRetrievalTemplateID=1. Accessed 01 June 2016.

Wang, F., Liu, R., Wang, L., Zhang, C., Liu, G., Liu, Q., Ma, X. and Zhang, J., "Molecular markers of Verticillium wilt resistance in upland cotton (*Gossypium hirsutum* L.) cultivar and their effects on assisted phenotypic selection", ***Cotton Sci*** 19, 424-430, 2007a.

Wang, X., Ma, J., Yang, S., Zhang, G. and Ma, Z., "Assessment of genetic diversity among Chinese upland cottons with Fusarium and/or Verticillium wilts resistance by AFLP and SSR markers", ***Frontiers of Agriculture in China*** 1, 129-135, 2007b.

Wang, H.M., Lin, Z.X., Zhang, X.L., Chen, W., Guo, X.P., Nie, Y.C. and Li, Y.H., "Mapping and quantitative trait loci analysis of Verticillium wilt resistance genes in cotton", ***J Integr Plant Biol*** 50, 174–182, 2008

Wang, P., Ning, Z., Lin, L., Chen, H., Mei, H., Zhao, J., Liu, B., Zhang, X., Guo, W. and Zhang, T., "Genetic dissection of tetraploid cotton resistant to Verticillium wilt using interspecific chromosome segment introgression lines", ***The Crop J*** 2, 278-288, 2014.

Wheeler, T.A. and Woodward, J.E., "Field assessment of commercial cotton cultivars for Verticillium wilt resistance and yield", ***Crop Prot*** 88, 1-6, 2016.

Wilhelm, S., Sagen, J.E. and Tietz, H., "Resistance to Verticillium wilt in cotton: sources, techniques of identification, inheritance trends, and the resistance potential of multiline cultivars", ***Phytopathology*** 64, 924–931, 1974.

Wu, J., Gutierrez, O.A., Jenkins, J.N., McCarty, J.C. and Zhu, J., "Quantitative analysis and QTL mapping for agronomic and fiber traits in an RI population of upland cotton". ***Euphytica***, 165(2), 231-245, 2009.

Yan, Z., Liqiang, W., Zhikun, L., Guiyin, Z., Xingfen, W. and Zhiying, M., "Targeted transfer of trait for Verticillium wiltresistance from *Gossypium barbadense* into *G. hirsutum* using SSR markers", ***Plant Breed*** 135, 476-482, 2016.

Yang, C., Guo, W., Li, G., Gao, F., Lin, S. and Zhang, T., "QTLs mapping for Verticillium wiltresistance at seedling and maturity stages in *Gossypium barbadense* L", ***Plant Sci*** 174, 290–298, 2008.

Yinhua, J., Xiwen, W., Junling, S., Zhongli, Z., Zaoe, P., Shoupu, H., Baoyin, P., Liru, W. and Xiongming, D., "Association mapping of resistance to Verticillium wilt in *Gossypium hirsutum* L. germplasm", ***Afr J Biotechnol*** 13(31), 3165-3172, 2014.

Yu, J., Yu, S., Fan, S., Song, M., Zhai, H., Li, X. and Zhang, J., "Mapping quantitative trait loci for cottonseed oil, protein and gossypol content in a *Gossypium hirsutum*× *Gossypium barbadense* backcross inbred line population". ***Euphytica***, 187(2), 191-201, 2012.

Zhang, B., Li, J., Liu, A., Shi, Y., Shao, Y., Zhang, J., Jia, L. and Yuan, Y., "QTL Analysis of resistance to Verticillium wilt using *Gossypium hirsutum*× *G. barbadense* backcross populations" ***The World Cotton Research Conference-4***, 23-26 October, 2007.

Zhang, J., Yu, J., Pei, W., Li, X., Said, J., Song, M. and Sanogo, S., "Genetic analysis of Verticillium wilt resistance in a backcross inbred line population and a meta-analysis of quantitative trait loci for disease resistance in cotton", ***BMC Genomics*** 16, 577, 2015.

Zhang, J.F., Fang, H., Zhou, H.P., Sanogo, S. and Ma, Z.Y., "Genetics, breeding, and marker-assisted selection for Verticillium wilt resistance in cotton", ***Crop Sci*** 54, 1289–303, 2014a.

Zhang, J.F., Percy, R.G. and McCarty, Jr. J.C., "Introgression genetics and breeding between Upland and Pima cotton- a review", ***Euphytica*** 198, 1–12, 2014b.

Zhang, J.F., Sanogo, S., Flynn, R., Baral, J.B., Bajaj, S., Hughs, S.E. and Percy, R.J., "Germplasm evaluation and transfer of Verticillium wilt resistance from Pima (*Gossypium barbadense*) to Upland cotton (*G. hirsutum*)", ***Euphytica***. 187, 147–160, 2012.

Zhao, Y., Wang, H., Chen, W. and Li, Y., "Genetic structure, linkage disequilibrium and association mapping of Verticillium wilt resistance in elite cotton (*Gossypium hirsutum* L.) germplasm population", ***PloS One*** 9(1), e86308, 2014.

Zhen, R., Wang, X.F., Ma, Z.Y., Zhang, G.Y. and Wang, X., "An SSR marker linked with the gene of Verticillium wilt resistance in *Gossypium barbadense*", ***Cotton Sci*** 18(5), 269-272, 2006.

Zhiyuan, N., Zhao, R., Chen, H., Ai, N., Zhang, X., Zhao, J., Mei, H., Wang, P., Guo, W. and Zhang, T., "Molecular tagging of a major quantitative trait locus for broad-spectrum resistance to Verticillium wilt in upland cotton cultivar Prema", ***Crop Sci*** 53, 2304-2312, 2013.

Zhou, H.P., Fang, H., Sanogo, S., Hughs, S.E., Jones, D.C. and Zhang, J.F., "Evaluation of Verticillium wilt resistance in commercial cultivars and advanced breeding lines of cotton", ***Euphytica*** 196, 437–448, 2014.

Zhu, H. Q., Feng, Z. L., Song, X. X. and Liu, X. Y., "Inhibition on *V. dahliae* Kleb. of 22 kinds extracts of Chinese traditional medicine" ***Cotton Sci***19(6), 489-492, 2007.

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