Research Article

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Microsatellite-based characterization of cotton genotypes for verticillium wilt and fiber quality traits

Pamuk Genotiplerinin Verticillium Solgunluğu ve Lif Kalitesi Özelliklerine Göre Mikrosatellit Temelli Karakterizasyonu

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Abstract: Cotton (Gossypium hirsutum L.) is the most important natural textile fiber crop grown worldwide. Several biotic and abiotic stress factors affect cotton yield due to lower genetic diversity for the traits of particular interest. Verticillium wilt (VW) is one of the major factors incurring huge cotton yield losses. The most effective management option against VW is the development of resistant cultivars. The resistant cultivars must also have superior fiber quality and yield traits. Therefore, the current study was aimed at screening some of the identified simple sequence repeats (SSR) markers for VW resistance and fiber quality traits of cotton genotypes in Turkey. Fifty different cultivars were screened with 30 SSR markers. Polymerase chain reaction (PCR) was conducted to amplify the SSR markers. The amplified bands were scored as 0 or 1 for absence and presence, respectively. For the molecular data analysis, polymorphism information content (PIC) values of molecular markers were calculated. Among tested SSR markers 13 were found polymorphic, which produced a total of 677 loci. The number of alleles per marker ranged from 1 to 4 and, overall average PIC values of markers ranged from 0.00 to 0.76, respectively. Principal component analysis executed on presence absence data yielded two distinct groups of cultivars screened. Hierarchical clustering revealed low genetic diversity among the tested cultivars. Based on the results TamcotCamdES, Gloria, Natalia, Lydia, Teks, Tamcot SP37H, N87 and BA525 are the promising cultivars for further breeding studies. The results of the current study also revealed that 4 SSR markers (DPL752 and DPL322 for fiber traits, DPL0022 and GH215 for VW resistance) out of 30 could be used for improving VW resistance and fiber quality in cotton through marker assisted selection.

Keywords: Gossypium hirsutum; Verticillium wilt; Fiber quality, SSR, Genetic diversity.

Özet: Pamuk (Gossypium hirsutum L.), dünya çapında yetişen en önemli doğal tekstil lif bitkilerinden birisidir. Çeşitli biyotik ve abiyotik stres faktörleri, genetik çeşitliliğin az olmasından dolayı, pamuk bitkisinin verimini azaltmaktadır. Verticillium solgunluğu (VS) hastalık, pamukta önemli verim kayıplarına neden olan en önemli hastalıklar arasında yer almaktadır. Hastalıkla mücadelede en önemli yöntem, VS’ na dayanıklı çeşitlerin geliştirilmesidir. Ayrıca geliştirilen dayanıklı çeşitlerin lif kalite ve verim özelliklerinin de iyi olması beklenmektedir. Bu nedenle çalışmada, pamuk genotiplerinin VS hastalığına dayanıklılığı ve lif kalitesi için tanımlanan “Basit dizi tekrarları (SSR)” markörleri ile tanınaması hedeflenmiştir. Çalışmada, eli farklı pamuk çeşitleri, PCR aracılığıyla 30 farklı SSR markörü ile tanımlanmıştır. PCR amplikonları var (1), yok (0) şeklinde skorlandıklar sonra moleküler markörlerin “Polimorfizm bilgi içerikleri (PBI)” hesaplanmıştır. Testlenen markörler içerisinde 13‘ü, toplamda 677 lokuslu bir polimorfik bulmuştu. PBI değerleri 0.00 ile 0.76 arasında değişkenlik gösterirken, markör başına düşen allele sayısını 1 ile 4 arasında değişikenlik göstermiştir. “Temel bileşen analizi”, testlenen örnekleri iki ana grup altında kümelemiştir. “Hiyerarşik küme analizi”, testlenen örnekler arasında genetik çeşitliliğin
Cotton (Gossypium hirsutum L.), the king of fibers, is most important natural textile fiber crop grown globally. Cotton is an important crop of Turkey, and upland cotton is cultivated in three major regions, i.e. Aegean, Mediterranean and Southeastern Anatolia on approximately 416.009 ha [1]. Fiber yield and quality are highly affected by pathogens attacking the cotton crop, resulting in heavy economic losses. Several biotic and abiotic stresses 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 [2–5].

Verticillium wilt (VW) incited by the soil inhabiting fungus Verticillium dahliae Kleb., having over 400 host plant species [6], causes 1.5 million bales losses to the global cotton economy [7]. The low efficiency of management options in controlling VW has urged the scientists to work on the genetic improvement programs. The cotton breeding programs are focused on synchronous improvements in fiber quality, yield and disease resistance, which is rather a challenging task [8]. 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 [8–13]. The QTL mapping has facilitated the application of marker assisted selection (MAS) in genetic improvements of cotton [11].

VW is regarded as a notorious disease of cotton crop, causing severe yield reduction and substantial economic losses in Turkey [14]. The first report of VW in the country dates back to 1941 [15], however, it was not considered as an important constraint up till 1971 [16]. 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 the country [17]. In spite of the high virulence of VW, resistant germplasm is very rare in the country. Moreover, the genetic improvement programs based on the induction of VW resistance are also limited. Modern upland cotton cultivars exhibit significant variation for important traits, including yield, fiber quality, pest resistance and tolerance to environmental adversities [8, 18]. 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.

Different physical traits of cotton are associated with the proficient spinning and weaving processes, which change the fiber into fabrics. These physical traits like fiber length, length uniformity, strength, elongation, maturity, micronaire and fineness can be measured [19]. Consequently, it is very essential to make fiber quality better in locally dominating cotton cultivars to fulfill the necessities of the rising textile industry [20].

The QTLs, MAS, and simple sequence repeats (SSR) are valuable tools for improving VW resistance and fiber quality traits in cotton. Many of works have been accomplished to improve VW resistance and fiber quality in cotton using these tools; however, these have rarely been evaluated in Turkey. The aim of the present study was to determine the genetic diversity among cotton cultivars through molecular SSR markers. So far little information exists on the genetic diversity and composition of various resistant genotypes at DNA level. Therefore, the current study was planned to screen the cotton cultivars with molecular markers linked to VW resistance and fiber quality traits QTLs. The results of the study will lay a foundation for breeding programs focused on improvements in VW resistance of upland cotton.

Materials and methods

The current study was conducted at Molecular Biology Laboratory, Department of Plant Production and Technologies, Faculty of Agricultural Science and Technology, Niğde Ömer Halisdemir University, Niğde, Turkey.

Plant material collection

Forty nine (49) different upland cotton cultivars were genotypically tested along with MaydosYerlisi, used
as both resistant [21] and out-group control belonging to *G. herbaceum*, commercially marketed in Turkey. The seeds of the cultivars were collected from different research organizations, private companies and research institutes in Turkey. The plant material was leaves of these cultivars. Details regarding the names of the cultivars used are given in Table 1. Among 50 tested cultivars, 4 are known to be tolerant, i.e. Carmen, N-m 503, N-87, Julia,

| Cultivar code | Cultivar name     | Maintainer                                      |
|---------------|-------------------|------------------------------------------------|
| 1             | BA151             | Progen Seed Company                             |
| 2             | BA525             | Progen Seed Company                             |
| 3             | Carisma           | Progen Seed Company                             |
| 4             | Cukurova1518      | East Mediterranean Agriculture Research Station |
| 5             | Gloria            | Bayer Company                                   |
| 6             | Lydia             | Progen Seed Company                             |
| 7             | Acala-1517V       | New Mexico State University                     |
| 8             | BA308             | Progen Seed Company                             |
| 9             | BA320             | Progen Seed Company                             |
| 10            | BA811             | Progen Seed Company                             |
| 11            | CIM496            | Central Cotton Research Institute, Pakistan     |
| 12            | DP499             | Monsanto Company                                |
| 13            | IH-4028           | Progen Seed Company                             |
| 14            | IH-20             | Progen Seed Company                             |
| 15            | IH-26-K-5         | Progen Seed Company                             |
| 16            | IH-27-TYL         | Progen Seed Company                             |
| 17            | IH-82-K-3         | Progen Seed Company                             |
| 18            | IH-82-Y-1         | Progen Seed Company                             |
| 19            | MCH-578           | Progen Seed Company                             |
| 20            | Natalia           | Bulgarian Agricultural Academy                  |
| 21            | PG424-1           | Progen Seed Company                             |
| 22            | PG426-4           | Progen Seed Company                             |
| 23            | PG510-15          | Progen Seed Company                             |
| 24            | PG510-7           | Progen Seed Company                             |
| 25            | PG511-7           | Progen Seed Company                             |
| 26            | PG518-11          | Progen Seed Company                             |
| 27            | PG519-19          | Progen Seed Company                             |
| 28            | PG520-7           | Progen Seed Company                             |
| 29            | PG-2018           | Progen Seed Company                             |
| 30            | PG-300            | Progen Seed Company                             |
| 31            | PG-310            | Progen Seed Company                             |
| 32            | PG-53-KT-2        | Progen Seed Company                             |
| 33            | PG-53-YT-11       | Progen Seed Company                             |
| 34            | Prema             | New Mexico State University                     |
| 35            | ST468             | May Seed Company                                |
| 36            | TamcotCamdES      | Texas A&M University                            |
| 37            | Tamcot SP37H      | Texas A&M University                            |
| 38            | Tamcot Sphinx     | Texas A&M University                            |
| 39            | Tasjkent-1        | Uzbek Scientific Research Institute             |
| 40            | Tasjkent-3        | Uzbek Scientific Research Institute             |
| 41            | GW Teks           | Golden West Company                             |
| 42            | VD-4              | Nazilli Cotton Research Station                 |
| 43            | Şahin2000         | Cotton Research Station, Turkey                 |
| 44            | Nata              | May Seed Company                                |
| 45            | Lacata            | May Seed Company                                |
| 46            | Carmen            | Bayer Company                                   |
| 47            | N-m 503           | Nazilli Cotton Research Station                 |
| 48            | N-87              | Nazilli Cotton Research Station                 |
| 49            | Julia             | Bayer Company                                   |
| 50            | Maydos Yerlisi    | Nazilli Cotton Research Station                 |
Genomic DNA extraction

The DNA was extracted from the leaves by cetyl-trimethyl ammonium bromide (CTAB) extraction method [23] with few modifications. For each 100 mg of tissue, 300 μL of CTAB isolation buffer (2% hexadecyl-trimethyl ammonium 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, and samples were then vortexed followed by centrifugation. 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.

PCR analysis

According to DNA quantity and quality results, all samples were diluted to a final concentration of 50 ng/μL. Thirty SSR primer pairs (NAU, DPL, BNL, JESP, CIR, CGR and GH) linked to QTLs for VW resistance and fiber quality traits were used for Polymerase chain reaction (PCR) analyses. The details on the sequence of SSR markers are given in Table 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 MgCl₂, 2.5 μL of 10X Dream Taq buffer, and 0.5 μL of 10 μM of each primer with 0.20 μL of 5 U/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 50–65°C, and 30 s at 72°C). The final PCR products were visualized under UV light after electrophoresis on ethidium bromide-stained 3% agarose gels.

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 bands. The binary qualitative data matrix was used to construct similarity matrices using WARD’s method, which involves an agglomerative clustering algorithm based on Jaccard similarity coefficients [32], 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: \[ \text{PIC} = 1 - \sum P_i^2 \], where \( P_i \) is the frequency of the \( i \)th allele [33]. Principal component analysis was also performed using XLSTAT statistical package. To accomplish genetic diversity analysis of the cultivars DARwin6 (Dissimilarity Analysis and Representation for Windows) analysis program was used with presence/absence data and un-weighted Neighbor Joining (NJ) [34].

Results

Level of SSR polymorphism

Within the group of 50 cotton cultivars, total 30 SSR markers were assayed which produced 677 loci, which were used for genetic diversity analysis. As a result of the molecular analyses, 13 SSR markers proved to be polymorphic. Four DPL SSR analyzed markers, picked from microsatellite-enriched genomic libraries of \( G. \) *hirsutum* (data provided by Dr. David Fang, Delta and Pine Land Company, Winterville, MS, USA), DPL513, DPL307, DPL901, DPL866 were found to be the least informative marker (PIC value = 0.0). Markers DPL752 and DPL0022 proved to be the most informative markers, with 0.76 PIC value. The number of alleles per marker ranged from 1 to 4. Overall average PIC values of markers ranged from 0.0 to 0.76 (Table 2).

Classification of the cultivars

Several classification techniques were used to test the similarity between the tested cultivars. The results obtained
Table 2: Different SSR markers information used to screen the Verticillium wilt resistance and fiber quality traits in upland cotton genotypes.

| Marker name | Type     | Primer sequence 5' | Number of alleles | PIC value evaluated | Melting temp (°C) | References |
|-------------|----------|--------------------|-------------------|---------------------|-------------------|-------------|
| DPL431      | Genomic  | CTATCACCCTTCTCTAGTTGCGTT | 3                  | 0.57                | 58                | [24]        |
| DPL253      | Genomic  | CTATCACCCTTCTCTAGTTGCGTT | 2                  | 0.04                | 60                | [24]        |
| DPL1531     | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL513      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
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| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
| DPL752      | Genomic  | AGACCCGCTACCTGATTT   | 4                  | 0.76                | 60                | [24]        |
| DPL901      | Genomic  | AGACCCGCTACCTGATTT   | 1                  | 0.00                | 59                | [24]        |
| DPL405      | Genomic  | AGACCCGCTACCTGATTT   | 3                  | 0.49                | 60                | [24]        |
Table 2 (continued)

| Marker name   | Type    | Primer sequence 5’* | Trait name          | QTL     | Chr. no. | References | PIC value evaluated | Melting temp (°C) | Number of alleles |
|---------------|---------|---------------------|---------------------|---------|----------|------------|---------------------|--------------------|------------------|
| NAU3700       | EST     | ATACGGAATACCCGTAATGA TCTCTCCCTACACCTCTGCA | Verticillium wilt | qVW-D3-1 | AD-17    | [30]       | 0.00                | 56                 | 1                |
| NAU5465       | EST     | TTTGGGAATAATCATACATCT ATGGTAGGTTGAGGATGAA |                     | qVW-D2-1 | AD-14, 23| [30]       | 0.00                | 56                 | 1                |
| JESPR12       | Genomic | CCACTACATCTGATTTAGCCAC GAGAAGAAGAATCCGACAG |                     | qVW-A7-1 | 7A7      | [30]       | 0.00                | 54                 | 1                |
| DC20067       | Genomic | ATGCCAAACAAACATCT TGGTTGTTGTGCTATCT |                     | qVW-A5-1 | AD-19, 05, 13 | [30]       | 0.00                | 50                 | 1                |
| NAU3414       | EST     | CAACCTCCACGCTCTGATTTGTC TGGTTGTTGTGCTATCT |                     | qVW-D9-1 | AD-23, 09| [30]       | 0.00                | 55                 | 1                |
| NAU3669       | EST     | AAGGTACCCGAGTTGCAA TGTGGGAAGATTCAGAAGTCT |                     | qVW-A9-1 | –        | [30]       | 0.00                | 64                 | 1                |
| HAU3303       | EST     | GCACCGATCCGACACGTGGA AAGTTGATATCAGGAGGT |                     | qVW-D11-1 | AD-21    | [30]       | 0.00                | 64                 | 1                |
| NAU2741       | EST     | AGTCAAGCATCTCAGACATTT GCCACCTGAGAATACATCT |                     | qVW-A1-1 | AD-19, 01| [29]       | 0.00                | 57                 | 1                |
| NAU2954       | EST     | AAGGAAATGTCGCCAATCACA GACTTGCTGTCCTGCTGC |                     | qVW-D9-1 | AD-23, 25| [31]       | 0.00                | 65                 | 1                |
| DPL0022       | Genomic | GTTGGGTCCTCTCAGTGCTATTT CCCCCTCAGTCTAGAAGAGT |                     | qVW-A5-1 | AD-05    | [30]       | 0.76                | 61                 | 4                |
| GH215         | Genomic | TCGTACCTGTTGGAGCA GTTATGATATAAAGAAGACAG |                     | qVW-A1-1 | AD-13    | [29]       | 0.54                | 51                 | 3                |

*aThe first line in primer sequence is for forward primer, while the second line represents reverse primer sequence.
Principal component analysis

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 3: The factor loading of the first nine principal components obtained through principal component analysis executed on the scoring data of 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   | 0.892 | 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  | 0.890 | -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 |
| DPL307   | -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 |
| NAU3414a | 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 |
| JESP-R65a | 0.268 | 0.213 | -0.116 | -0.498 | 0.358 | -0.470 | -0.067 | 0.203 | -0.330 |
| JESP-R65b | 0.118 | -0.511 | 0.145 | 0.172 | -0.308 | 0.618 | -0.068 | -0.186 | 0.179 |
| JESP-R65c | 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.
correlation coefficients ≥0.6 (Table 3). 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 axes yielded two distinct groups 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 1). 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. The PCA scatter diagram thus identifies that 4 genotypes of the group 1 have mutual similarity, while the remaining 46 cultivars of the 2nd group are similar to each other.

Hierarchical clustering

The hierarchical clustering divided the cultivars into two major groups. The first group contained three cultivars, while the second group was composed of 47 cultivars. The first group was sub-divided into many subsets (Figure 2). The sub-group 2 was initially sub-divided into two main groups, one containing six cultivars, while the other group was composed of 41 different cultivars included in the study. This sub-division indicated that the cultivars of first group (IH-20, Tamcot Sphinx, PG520-7, PG2018, PG300 and Carmen are different from the cultivars of the other group. Maydos Yerlisi, Julia and N-7 are distinctly separated from rest of the cultivars. 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 demonstrated that most individuals were genetically close to each other, such as IH-26-K-5, IH-27, TYL, PG2018, PG300, PG310, PG520-7, BA151 and BA525 due to their similar breeding programs. Therefore bringing in greater genetic diversity into breeding programs might be helpful.

It is evident that Maydos Yerlisi, Julia and N-87 are closely related with each other (Figure 2), which are resistant cultivars. Moreover, the positive controls; Carmen and N-m-503 were related to BA525 and Şahin2000, respectively. Çukurova1518 and Nata both were sensitive genotypes.
related with each other, but Şahin2000, which is also sensitive cultivar was closely related to resistant control i.e. N-m-503. This might be due to similar genetic background. The cultivars in red, green and brown fonts are closely related to each other within each font (Figure 2). Maydos Yerlisi is represented by different color due to dissimilarity with other cultivars. BA320 was also dissimilar to the other cultivars.

Neighbor joining analysis

The NJ 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 the tree. NJ is better than distance tree and considered more reliable. According to the results of the NJ analysis; two clusters were obtained (Figure 3). The clusters A and B were represented by 16 and 34 cultivars, respectively. Maydos Yerlisi, the out group genotype, exhibited dissimilarity with other cultivars and similarity to Julia and N-87, which were both resistance and positive controls, respectively. It indicates that they are genetically similar to each other and different from rest of the cultivars.

Maydos Yerlisi was different from remaining cultivars. Prema, the resistance cultivar, 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 exhibited similarity with each other. All these cultivars were joined to a same node but the branch connecting one population to the node was different from the others, which represented genetic distance of these cultivars. Şahin2000, Çukurova1518, Nata and Lacata all were susceptible cultivars, and displayed 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 are similar. The NJ algorithm allowed branch lengths to vary and represented more accurate genetic distance matrix. In contrast, WARD tree did not show different branch lengths and all cultivars belonged to same node.

Discussion

Evaluation of phenotypic and genotypic variability among plant genotypes is essential for plant breeding and genetic diversity analysis [24]. Examining the diversity of genetic resources for disease resistant cotton cultivars provides indication not only for parental selection of breeding program, but also for prophecy of off-spring distinction and exploitation of heterosis [35]. Diversity in plant genetic resources gives prospects to plant breeders for developing new and improved cultivars with desirable traits [36]. VW is the most destructive cotton disease all around the world [7, 37]. The available germplasm at regional scales has narrow genetic diversity and molecular data on the germplasm is often scarce. SSRs are co-dominant molecular markers that differentiate homozygotic and heterozygotic characters and also have many numbers of alleles. The molecular screening of the available germplasm using SSR markers has become a promising tool to identify the QTLs linked to the traits of particular interest [38].
In the current study 13 SSR markers proved to be polymorphic, and few of them are persistent with the earlier studies. The number of alleles per marker ranged from 1 to 4. The observed number of alleles per marker is important for germplasm diversity, marker type and the method used for fragment separation and resolution [19]. PIC values are used to get information are used to assess the usefulness and linkage analyses of genetic markers. The normal PIC value of a core set of SSR markers for *Gossypium* species varied from 0.0 to 0.82 [39]. PCA indicated that the resistant cultivars i.e. Maydos Yerlisi, Julia, N-87, N-m-503 were dissimilar to the rest of the cultivars. According to clustered base classification, Maydos Yerlisi was distinctly separated from all other genotypes. This distinction was expected because of different genus of the cultivar than upland cultivars. Julia and N-87 were closely related to Maydos Yerlisi as these were resistance and positive controls. Prema and N-m-503 were also resistant cultivars, however exhibited dissimilarity with each other. This may be attributed to their different genetic backgrounds. Çukurola1518 and Nata were used as sensitive resistant and were close to each other. Lacata and Şahin2000 were also sensitive genotypes, but they were very distant from each other. According to diversity analysis, certain cultivars from the same source exhibited too low diversity. The low genetic diversity in region cotton cultivars has also been reported in previous studies [40, 41].

Introducing higher genetic diversity in breeding programs might be advantageous. In the current study, markers DPL0022, DPL752 proved to be the most informative marker with highest PIC value of 0.76, whereas some markers proved as the least informative markers with 0.00 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 associated with VW on QTL *qVW-A5-1* [30]. GH215 was also found to be linked with VW on QTL *qVW-A1-I* [30]. Likewise, in the current study a connection was found between GH215 and VW resistance. Yang et al. [42] performed association mapping analysis and found JESPR65 on QTL *qVL-A5-1F2* linked to VW resistance. In the current study, similar marker was found associated with VW. One SSR marker, NAU3414, was also linked to VW as observed in the previous study [30]. Some of the markers related to fiber elongation and fiber quality traits are linked to QTL.
sqMi-C11, qFE-C15-1, qFL-D2-1, qFL032 and qRV07DF2-A9-2 [25, 42]. Among the tested marker for these traits, CIR381, CIR246 and DPL322 demonstrated polymorphism in the current study. It was also observed that some of the markers related to VW (such as GH527, CIR295, NAU3700, NAU5465, DC20067, NAU3669, NAU3303 and NAU2741) display polymorphisms, however contrasting results were obtained in the current study. The contrasting results are attributed to the nature and genetic background of the cultivars used in the current study.

In conclusion, most of the identified markers linked to QTL for VW resistance investigated in this study were proved ineffective. The markers NAU2954, NAU2354, NAU2471, HAU3303, NAU3669, NAU3414, DC20067, NAU5465, NAU3700, GH527, CGRS258, and CIR295 were not proved informative for all the tested cultivars with agarose gel electrophoresis system. Among tested markers, only GH215 and DPL0022 were found informative and promising for MAS studies. Moreover, among the analyzed fiber quality markers, CIR381, CIR246, DPL405, JESP65, and DPL752 were found polymorphic. The consequences of PIC value of these markers exhibit their relationship with fiber quality and yield components in cotton [43]. All of the indicated markers should be considered as a good candidate for MAS studies.

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