

Estimating genetic diversity among selected cotton genotypes and the identification of DNA markers associated with resistance to cotton leaf curl disease

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Abstract: To the extent of our knowledge, applications of DNA markers in marker-assisted breeding of cotton are handicapped due to low genetic diversity in cotton germplasm. Cotton leaf curl disease, a disease of viral origin, has substantially depressed cotton production in Pakistan, and this disease is also an emerging threat to the neighboring cotton-growing countries like China and India. The present study was designed to identify DNA markers, predominately simple sequence repeats (SSRs), associated with tolerance and/or resistance to the disease. Based upon 2 years of disease-screening field experiments, a total of 10 cotton genotypes (five highly tolerant, four highly susceptible, and one immune) of diverse origin were selected from the available cotton germplasm (~1200 accessions) of the National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan. In total, 322 SSRs derived from bacterial artificial chromosome end sequences of *Gossypium raimondii* (one of the progenitor species of cultivated tetraploid cotton) were screened. Out of these, 65 primer pairs were found polymorphic, and the extent of genetic similarity was in the range of 81.7% to 98.7%. A similarity matrix was used for studying their phylogenetic relationship using unweighted pair-group method with arithmetic means (UPGMA) analysis. The dendrogram showed the grouping of the genotypes into two distinct clusters comprising tolerant and susceptible genotypes, respectively. Out of the polymorphic markers, two SSR markers, PR-91 and CM-43, that were amplified only in tolerant genotypes showed significant association with resistance to the disease. These preliminary results set the stage for initiating in-depth marker-trait association studies, which will be instrumental for initiating marker-assisted breeding in cotton.

Key words: *Gossypium*, simple sequence repeats, tetraploid cotton, cotton leaf curl disease, genetic diversity, DNA marker, resistance

1. Introduction

Cotton is an important natural fiber crop grown in subtropical and temperate regions of about 80 different countries. Cotton fiber is used directly as a raw material in textile and cotton oil seed as a by-product. In Pakistan, cotton shares more than half of total foreign export earnings (Government of Pakistan, 2014), demonstrating high economic value for Pakistan (Rahman et al., 2012). Sustainability of cotton production is a major issue in many corners of the cotton-growing regions, which is largely threatened by the occurrence of biotic and abiotic stresses. Besides abiotic stresses, cotton leaf curl disease (CLCuD) alone limits cotton production by 20%–30% annually in Pakistan depending upon the severity of the disease (Briddon et al., 2000; Rahman et al., 2014). During the past few years, the disease has been constantly reported from a number of countries across Africa and South Asia, and more specifically in Pakistan and northwestern parts of India and recently in China (Cai et al., 2010).

Typical symptoms of the disease are greening of infected plants during the early stage of infection, leaf curling, vein darkening, vein swelling, and enation; in the case of severe infection, cup-shaped leaf-like structures appear on the undersides of the leaves (Figure 1a). In severely infected plants, internodal length shortens, resulting in stunted growth of the plant (Figure 1b). The disease is caused by a virus belonging to the genus *Begomovirus* of *Geminiviridae*, exclusively transmitted by a whitefly vector, *Bemisia tabaci* (Briddon, 2003).

Genomic tools have been found less applicable in cotton due to evolutionary bottlenecks during the process of domestication in cotton, which resulted in a narrow genetic base in the *Gossypium* lineage (Rahman et al., 2008). It has been comprehended very recently that the diverse genetic base of cultivars can play a central role to combat epidemics of diseases (Rahman et al., 2002). A broad genetic base of cultivars has been proven to be a key element in controlling the diseases in rice (Zhu et

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Figure 1. a. Leaf greening, vein thickening, and enations on the underside of leaf infected with CLCuD. **b.** Severe symptoms of disease showing stunting in growth of cotton plant.

al., 2000). Moreover, it has been widely acknowledged that forthcoming crop improvements would be based on the information about genetic diversity (Thormann et al., 1994). Genetic diversity for traits like resistance to various diseases or fiber quality is relatively limited in the elite cotton germplasm, a major compelling factor to look for novel alleles in other gene pools of the genus *Gossypium* (Becerra Lopez-Lavalle et al., 2011).

The rapid advances in molecular genetics have been promising for solving the shortcomings of traditional breeding approaches. In this regard, DNA marker technology offers a powerful tool to tag the genomic regions associated with important agronomic traits, which are difficult to analyze using traditional plant breeding methods (Prioul et al., 1997). Availability of tightly linked molecular markers for a trait could allow plant breeders to exercise marker-assisted selection to identify plants with desired traits in early generations, which will boost the efficiency of breeding programs. Molecular techniques not only make it possible to transfer desired genes between varieties but also allow introgression of novel genes from wild species into domesticated varieties (Tuberosa et al., 2002).

DNA fingerprinting assays have been playing a key role in this regard by determining the structure of genetic diversity in a variety of crop species. Analysis of microsatellite or simple sequence repeat (SSR) loci is much more practical because of their reproducibility, transferability, codominant nature, etc. (Ghaffari and Hasnaoui, 2013). SSR markers have been extensively applied for the estimation of genetic diversity, monitoring of the introgression of novel alleles, QTL mapping, cultivar protection, and selective breeding (Blenda et al., 2006; Rahman et al., 2007; Saeed et al., 2014; Tyagi et al., 2014;

Zhao et al., 2014). Recent advances in plant genomics have made it possible to gain insight into population dynamics, targeted gene surveys, and precise selection of traits prior to domestication (Glaszmann et al., 2010).

The present study was conducted to estimate the extent of genetic divergence and preliminary marker-trait association in a set of ten diverse cotton genotypes selected out of ~1200 genotypes, followed by phylogenetic analysis. We have also identified potential DNA markers CM-43 and PR-91, which can differentiate between susceptible and resistant cotton genotypes. Thus, the present study would help in initiating marker-assisted selection in improving resistance to CLCuD, thus paving the way for more sustainable cotton production worldwide.

2. Materials and methods

2.1. Plant material

The plant material used in this study belonged to diverse genetic backgrounds consisting of a set of ten cotton genotypes/varieties/cultivars selected based upon 2 years of disease screening experiments from the National Institute for Biotechnology and Genetic Engineering (NIBGE, Faisalabad, Pakistan) cotton germplasm collection. For classification, we divided these genotypes into two groups (Table 1). Group one consisted of CLCuD-resistant/tolerant *Gossypium hirsutum* genotypes, including NIBGE-207, NIBGE-115, NN-3, VH-289, and MNH-886, whereas group two contained highly susceptible genotypes of *Gossypium hirsutum* including CAMD-E, Cascot-BR-1, Alcala-70-11, and FH-1000. One genotype of *Gossypium arboreum* 'Ravi', completely immune to disease, was kept as the control. The seeds of these genotypes were collected from the institutes of their origin.

Table 1. Parentage/origin of genotypes.

Sr. no.	Name	Genome	Parentage/origin
1	NIBGE-207	AD	CIM-448 × *Bollgard*; tolerant to the Burewala strain of CLCuD
2	NIBGE-115	AD	LRA-5166 × S-12; tolerant to the Burewala strain of CLCuD
3	NN-3	AD	LRA-5166 × S-12; tolerant to the Burewala strain of CLCuD
4	VH-289	AD	VH-144 × CP-15/2; tolerant to the Burewala strain of CLCuD
5	MNH-886	AD	FH-207 × MNH-770 × Bollguard-1; tolerant to the Burewala strain of CLCuD
6	Ravi	A2	465 D-selection; highly resistant/immune (Rahman et al., 2002)
7	CAMD-E	AD	Wild tetraploid genotype from CCRI Multan germplasm; susceptible to CLCuD
8	Albacala-70-11	AD	Wild tetraploid genotype from CCRI Multan germplasm; susceptible to CLCuD
9	CASCOT-BR-1	AD	Wild tetraploid genotype from CCRI Multan germplasm; susceptible to CLCuD
10	FH-1000	AD	CIM 448 × S 12; susceptible to CLCuD

*: Primary exotic transgenic source for the *CryIAC* insect resistance gene (MON531 event).

2.2. Field evaluation

Maximum CLCuD incidence has been reported in late-sown cotton from mid-June to early July (Rahman and Zafar, 2012). All selected genotypes were planted in the field during the last week of June 2010 and 2011 at the NIBGE, Faisalabad, Pakistan. A total of 150 plants of each genotype were planted in a randomized complete block design in three replications. A susceptible control cultivar, CIM-496 (Rahman and Zafar, 2012), was also planted. All control measures were taken, except that the population of whitefly was not controlled until 90 days after sowing. Fifty plants of each variety were tagged randomly and data on disease response were collected at 60, 90, and 120 days after sowing. The same experiment was repeated in the next planting season (2011), and the disease infection data were collected by following the standard protocol of the previous year's trials.

2.3. Disease scoring

The symptoms of disease were scored by combining the severity rating scale with the disease infection scale. The response of genotypes was monitored using a rating system (0–6) described in the review by Farooq et al. (2011). Percent disease index (PDI) and disease response of individual plant was calculated by using following formula.

$$PDI = \frac{\text{Sum of all disease ratings}}{\text{Total plants observed}} \times \frac{100}{6^*}$$

*: Maximum disease severity rating.

2.4. SSR analysis

2.4.1. DNA extraction

Leaf samples of each genotype were collected from 10 individual plants and bulked. These samples were taken to the laboratory in a container containing liquid nitrogen. Extraction of the total genomic DNA was carried out by following a modified CTAB protocol, described by Iqbal et al. (1997). After RNase treatment, the concentration of genomic DNA was measured by DyNA Quant (TM 200, Hoefer). DNA quality and quantity were further reconfirmed by running 30 ng of the genomic DNA of each genotype in 0.8% agarose gel. DNA samples with compact bands were selected for PCR while those giving smears were rejected. The purified total genomic DNA was later diluted in double-distilled water to make a working concentration of 15 ng/μL for PCR analysis.

2.4.2. Survey of SSR primer pairs

A total of 322 SSR primer pairs were used to identify polymorphisms among the 10 cotton genotypes. These primers predominantly (269 primer pairs) belonged to PR series, derived from bacterial artificial chromosome end genomic sequences of the *Gossypium raimondii* genome, developed at the PGMB (NIBGE, Faisalabad Pakistan) and PGML (UGA, USA) labs, and 50 primers of Monsanto series, two pairs of CM series, and one pair of JESPER series. Sequences of these primers were obtained from the Cotton Marker Database (<http://www.cottonmarker.org/>). PCR assay was performed in a 20-μL reaction volume, having 25 mM MgCl₂, 2.5 mM dNTPs, 30 ng/μL of each primer, 10X buffer [(750 mM Tris-HCl (pH 8.8), 200 Mm (NH₄)₂SO₄, and 0.1% Tween 20], 15 ng of template DNA, 5 U/μL Taq polymerase, and double-distilled water.

The amplification process was performed in a Mastercycler Gradient (Eppendorf, Germany) using the defined cycling program: one cycle of 94 °C for 5 min; 40 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min; and one cycle of 72 °C for 10 min, followed by holding at 4 °C until the tubes were shifted. Amplified products of PCR reactions were resolved on 2% agarose gel and detected by ethidium bromide staining. The products of polymorphic primers were also resolved on 2.5% Metaphor agarose gel.

2.4.3. Scoring of amplified products

All visible and unambiguously scorable (easily readable) fragments amplified by each primer were scored as 0 = absent or 1 = present. This scoring was used to assess the number of common amplicons (Nei and Li, 1979).

2.5. Data analysis

Principal component analysis (PCA) was performed to investigate the genotypic response toward CLCuD with the aid of STATISTICA software (StatSoft version 12, StatSoft, Inc., Tulsa, OK, USA). PCA converts multidimensional and complex correlated data into a relatively more simple, linearized axis while containing the original variation. The data were analyzed using PCA. The principal components were stated as eigenvalues and expressed in an eigenvector for the analyzed trait in each principal component axis. The first two principal components, showing maximum variations, were selected to construct the diagram.

Similarity coefficients were utilized to generate a dendrogram by unweighted pair-group method with arithmetic means (UPGMA) analysis (Sneath and Sokal, 1973) with the help of STATISTICA software. Allele frequency, minor alleles and major alleles, and similarity matrix were also calculated by Power Marker version 3.25.

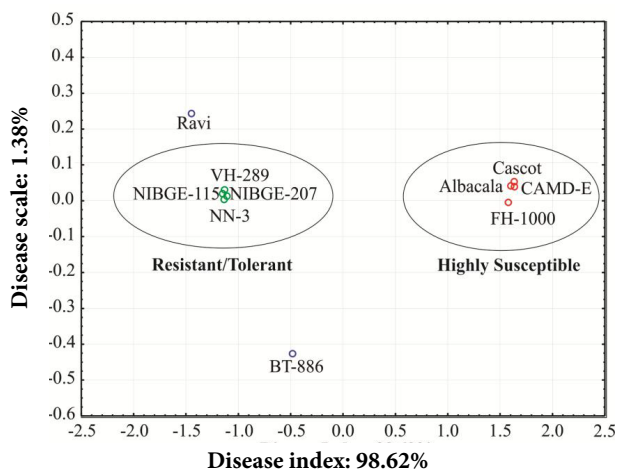


Figure 2a. Distribution of cotton genotypes based on two principal components in year 2010/11.

3. Results

3.1. PCA analysis

Data regarding the disease response of each genotype were analyzed using PCA. For the 2010 and 2011 field experiment, PC1 contributed 98.62% of the total variation while PC2 explained 1.37% of the total variation. The characteristics of both principal components were calculated on the basis of estimated factor loadings. The scatter diagram of the two principal components distributed the genotypes into two groups, ‘tolerant’ and ‘highly susceptible’ (Figure 2a). PCA was also used to analyze 2011 and 2012 disease data and resulted in scatter diagram similar to that for 2010 and 2011, demonstrating similar responses of genotypes against disease (Figure 2b).

3.2. SSR markers data

Of the 322 surveyed SSR primer pairs, 279 produced reproducible fragments, and 65 of these primers were used to draw information regarding their association and genetic diversity assessment among ten genotypes of cotton. In total, 325 alleles/fragments with an average of 1.16 bands per primer pair were amplified. Out of these, 137 amplified alleles were unique. The average number of fragments amplified by each polymorphic primer was 2.1 (Figure 3). The average gene diversity was 0.0522, varying from 0.1800 (PR-70) to 0.6400 (PR-519) (Figure 4). Maximum DNA fragments (5) were amplified by the SSR primer pair PR-505. The average molecular weight ranged from 70 to 1100 bp. The majority of the primers amplified polymorphic DNA fragments among a few genotypes, but none of the primers differentiated all the genotypes. The maximum number of DNA fragments (328) were amplified from the genomic DNA of NIBGE-115, whereas the minimum number of DNA fragments (294) were amplified

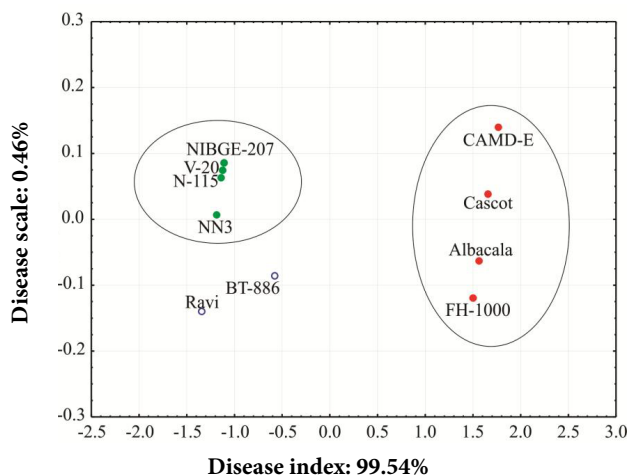


Figure 2b. Distribution of cotton genotypes based on two principal components in year 2011/12.

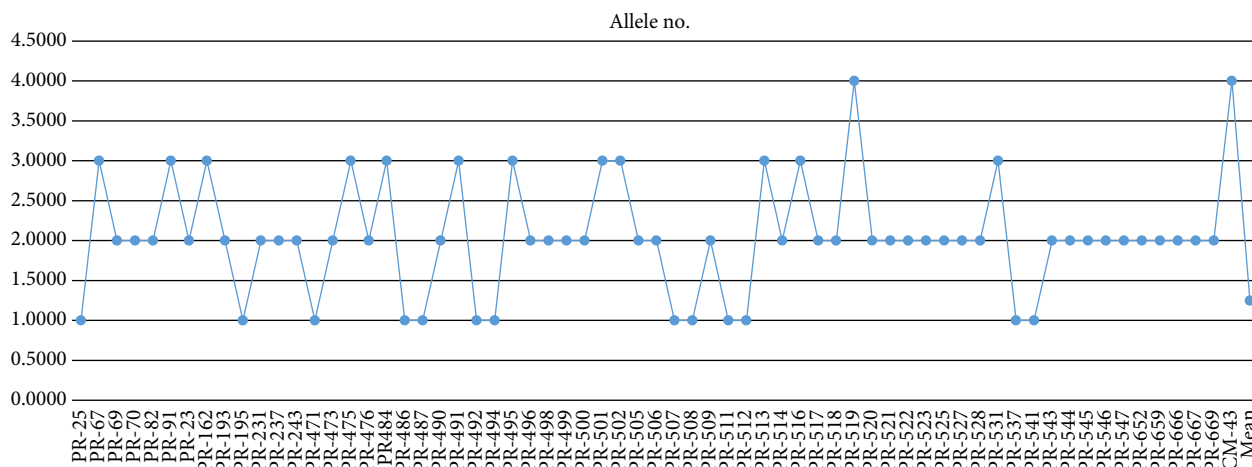


Figure 3. Allelic variations by individual polymorphic primers.

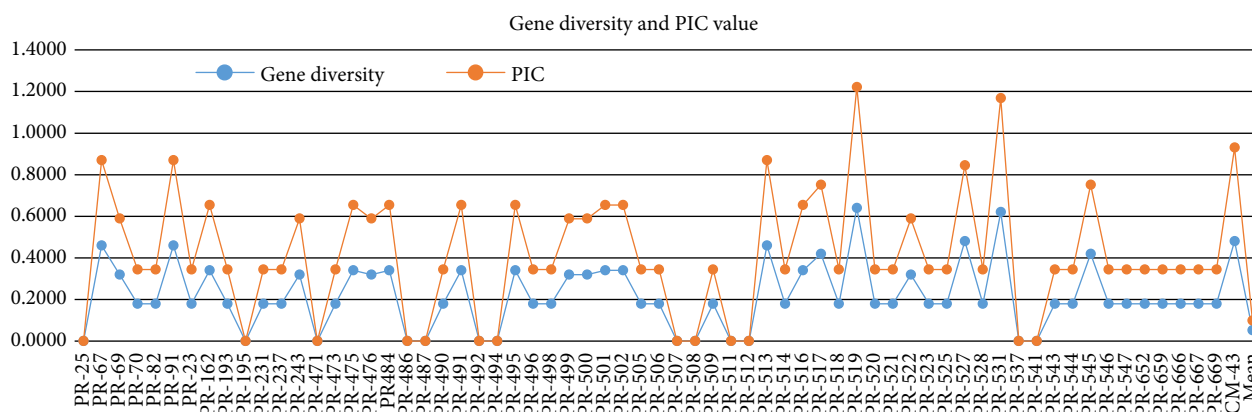


Figure 4. Estimated PIC values and gene diversity.

from Ravi (the genotype representing *G. arboreum*). The PIC values per locus ranged from 0.1638 for the PR-70 locus to 0.5812 for PR-519, with an average of 0.0464 for all loci (Figure 4). Polymorphic primers CM-43 and PR-91 amplified polymorphic fragments in resistant genotypes. PR-91 amplified two loci (a1 and a2) in NIBGE-207 and NIBGE-115 with fragments sizes of 140 bp and 170 bp, respectively (Figure 5). The second polymorphic primer, CM-43, amplified two loci in all resistant genotypes. The estimated size of the amplified loci was 125 bp (b_1) and 150 bp (b_2).

3.3. Phylogenetic analysis

The genotyping data of each primer were subjected to UPGMA analysis, which grouped all the genotypes into two major clusters, A and B (Figure 6). Genotypes NIBGE-207, NIBGE-115, NN-3, and VH-289 grouped in cluster A and were highly tolerant to CLCuD, while the genotypes included in cluster B showed high susceptibility to the disease under natural conditions. The analysis of

data also revealed a great level of similarity between two genotypes of cluster B, Albacala-70-11 and Cascot-BR-1 (98.70), and minimum similarity between NIBGE-115 and Ravi (81.70%). Genotype Ravi, representing the A genome (diploid species), did not group with either of the clusters representing *G. hirsutum* species. Two genotypes, MNH-886 (tolerant) and FH-1000 (susceptible), were also placed in distinct positions. In the present study, we report 81.7%–98.7% genetic similarity among 10 genotypes with an average of 88.5% genetic similarity by employing 322 SSRs (Table 2).

4. Discussion

Breeding for broad-spectrum and durable resistance against diseases is the principal goal of many crop improvement programs (Michelmore et al., 1991). The increasing deployment of genomic tools has significantly contributed to mitigate plant diseases by augmenting definition of and access to germplasm resources available

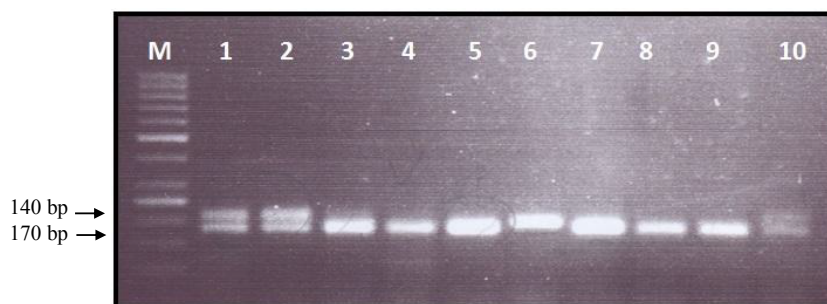


Figure 5. Amplification profile of 10 cotton genotypes with SSR primer PR-91.

M = Marker, 1 = NIBGE-207, 2 = NIBGE-115, 3 = NN-3, 4 = VH-289, 5 = MNH-886, 6 = *Gossypium arboreum* ‘Ravi’, 7 = CAMD-E, 8 = Cascot-BR-1, 9 = Albacala-70-11, 10 = FH-1000.

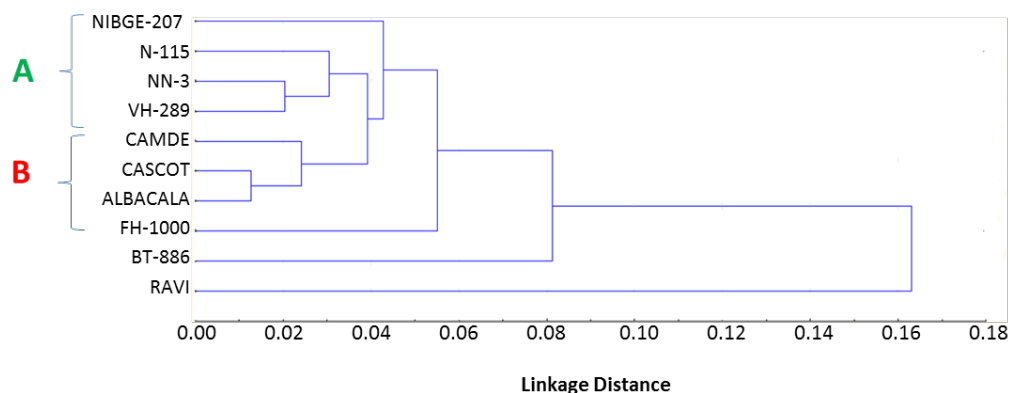


Figure 6. Dendrogram of 10 cotton cultivars/genotypes constructed from SSR data using UPGMA. The scale is based on Nei and Li's coefficients of similarity. Cluster A contains resistant/highly tolerant genotypes while cluster B represents the group of highly susceptible genotypes.

Table 2. Similarity matrix for Nei and Li's coefficient for 10 cotton genotypes.

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
V1	1									
V2	0.964	1								
V3	0.959	0.975	1							
V4	0.954	0.964	0.98	1						
V5	0.909	0.904	0.914	0.924	1					
V6	0.827	0.817	0.832	0.843	0.8223	1				
V7	0.949	0.944	0.959	0.964	0.9188	0.843	1			
V8	0.957	0.952	0.967	0.967	0.9315	0.85	0.977	1		
V9	0.959	0.954	0.97	0.97	0.9289	0.848	0.975	0.987	1	
V10	0.931	0.926	0.942	0.942	0.9213	0.85	0.947	0.959	0.967	1

V1 = NIBGE-207, V2 = NIBGE-115, V3 = NN-3, V4 = VH-289, V5 = MNH-886, V6 = *Gossypium arboreum* ‘Ravi’, V7 = CAMD-E, V8 = Cascot-BR-1, V9 = Albacala-70-11, V10 = FH-1000.

for genetic enrichment of crops (Paran and Michelmore, 1993; Rahman et al., 2012). However, these genomic tools are handicapped because limited genetic diversity is available in the cotton gene pools of cultivated types (Bertini et al., 2006; Guang et al., 2006; Khan et al., 2009; Tyagi et al., 2014). Molecular markers, predominantly SSR markers, have been extensively utilized in the genetic studies of cotton, such as molecular tagging of important traits, diversity analysis, population structure studies, and construction of molecular maps (Han et al., 2006; He et al., 2007; Shen et al., 2007; Wu et al., 2009; Doğan et al., 2010).

In the present study, we initially screened the cotton genotypes for the disease for two consecutive years; we demonstrated marked differences among their responses to CLCuD. The data grouped the genotypes into two different clusters (tolerant vs. susceptible) with the help of PCA.

The repeated utilization of the same genetic material has considerably reduced the natural genetic diversity reported in cotton (Rahman et al., 2002; Hinze et al., 2012). Our results are also in accordance with previous studies, showing high levels of genetic similarity among cotton genotypes ranging from 81.1% to 98.7%. In a study of elite cotton cultivars of Pakistan that showed significant resistance to CLCuD, genetic resemblance of 81.41%–94.90% was observed (Rahman et al., 2002). RFLP assay of *Gossypium hirsutum* cultivars also provided confirmatory evidence for the conservation of genetic similarity in cotton as compared to other plant taxa (Brubaker and Wendel, 1994; Chen et al., 2006; Bardak and Bolek, 2012). Similar findings were reported by other cotton researchers (Iqbal et al., 1997; Surgun et al., 2012) using RAPD markers. Among the cotton genotypes used in this study, few are elite cultivars/lines (NIBGE-115, NN-3, VH-289, MNH-886). Hence, these results on their genetic relationship is in accordance with previous reports.

Deployment of linkage disequilibrium-based association mapping studies in cotton expedites efficient utilization of prevailing natural genetic diversity in *Gossypium* germplasm (Abdurakhmonov et al., 2007; Hall et al., 2010). In the present study, marker-trait association was sought among the cotton genotypes using the molecular data of 65 polymorphic primer pairs. UPGMA analysis resulted in the formation of two distinct clusters (A and B) containing resistant and susceptible genotypes respectively, demonstrating contrasting genetic

differences between genotypes of both clusters. Among the examined genotypes of cluster A, NIBGE-115 and NN-3 are 97% genetically related. These two genotypes are potential sources of resistance against the disease, developed at NIBGE (Rahman and Zafar, 2007, 2012). It was reported that the genetics of resistance to the Multan strain of CLCuD was controlled by two resistant genes and one suppressor gene (Rahman et al., 2005). We also found that despite Ravi ($2n = 2x = 26$, A2 genome species) forming an outgroup due to its phylogenetic divergence, two other genotypes, MNH-886 and FH-1000, also formed an outgroup, reflecting significant genetic differences. We identified two new polymorphic markers, PR-91 and CM-43, which were amplified only in resistant genotypes. These markers showed significant association with resistance and their further validation in large-scale germplasm screening can provide valuable information to establish conclusive evidence of marker-trait association, thus providing a way forward to get insight into chromosomal regions linked with resistance.

SSR analysis revealed a limited level of genetic diversity among most of the genotypes. Breeding for CLCuD resistance demands the introduction of highly diverse genotypes into existing cultivars. Moreover, our studies suggest that detection of marker-trait associations have potential benefits for identifying novel alleles for crop improvement. The identified SSR markers have shown significant association with CLCuD resistance in this limited set of cotton germplasm. However, these markers need validation by surveying them on large sets of cotton germplasm. These markers will then be useful in future endeavors by fueling marker-assisted breeding for disease resistance and screening of cotton germplasm for discovering novel alleles.

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References

- Abdurakhmonov IY, Buriev ZT, Saha S, Pepper AE, Musaev JA, Almatov A, Shermatov SE, Kushanov FN, Mavlonov GT, Reddy UK et al. (2007). Microsatellite markers associated with lint percentage trait in cotton, *Gossypium hirsutum*. *Euphytica* 156: 141–156.
- Bardak A, Bolek Y (2012). Genetic diversity of diploid and tetraploid cottons determined by SSR and ISSR markers. *Turk J Field Crops* 17: 139–144.

- Becerra Lopez-Lavalle L, Matheson B, Brubaker CL (2011). A genetic map of an Australian wild *Gossypium* C genome and assignment of homoeologies with tetraploid cultivated cotton. *Genome* 54: 779–794.
- Bertini CH, Schuster I, Sedyama T, Barros EGD, Moreira MA (2006). Characterization and genetic diversity analysis of cotton cultivars using microsatellites. *Genet Mol Biol* 29: 321–329.
- Blenda A, Scheffler J, Scheffler B, Palmer M, Lacape JM, Yu JZ, Jesudurai C, Jung S, Muthukumar S, Yellambalase P et al. (2006). CMD: A cotton microsatellite database resource for *Gossypium* genomics. *BMC Genomics* 7: 132.
- Briddon RW (2003). Cotton leaf curl disease, a multicomponent begomovirus complex. *Mol Plant Pathol* 4: 427–434.
- Briddon RW, Markham PG (2000). Cotton leaf curl virus disease. *Virus Res* 71: 151–159.
- Brubaker CL, Wendel JF (1994). Reevaluating the origin of domesticated cotton (*Gossypium hirsutum*; Malvaceae) using nuclear restriction fragment length polymorphisms (RFLPs). *Am J Bot* 81: 1309–1326.
- Cai C, Ye W, Zhang T, Guo W (2014). Association analysis of fiber quality traits and exploration of elite alleles in Upland cotton cultivars/accessions (*Gossypium hirsutum* L.). *J Integr Plant Biol* 56: 51–62.
- Cai JH, Xie K, Lin L, Qin BX, Chen BS, Meng JR, Liu YL (2010). Cotton leaf curl Multan virus newly reported to be associated with cotton leaf curl disease in China. *Plant Pathol* 59: 794–795.
- Doğan B, Ünal M, Özgökçe F, Martin E, Kaya A (2011). Phylogenetic relationships between *Malcolmia*, *Strigosella*, *Zuvanda*, and some closely related genera (Brassicaceae) from Turkey revealed by inter-simple sequence repeat amplification. *Turk J Bot* 35: 17–23.
- Farooq A, Farooq J, Mahmood A, Shakeel A, Rehman KA, Batool A, Riaz M, Shahid MTH, Mehboob S (2011). An overview of cotton leaf curl virus disease (CLCuD) a serious threat to cotton productivity. *Aust J Crop Sci* 5: 1823–1831.
- Ghaffari S, Hasnaoui N (2013). Microsatellite amplification in plants: optimization procedure of major PCR components. In: Kantartzi SK, editor. *Microsatellites: Methods and Protocols*. New York, NY, USA: Humana Press, pp. 139–146.
- Glaszmann JC, Kilian B, Upadhyaya HD, Varshney RK (2010). Accessing genetic diversity for crop improvement. *Curr Opin Plant Biol* 13: 167–173.
- Government of Pakistan (2014). *Economic Survey of Pakistan: Finance Division*. Islamabad, Pakistan: Government of Pakistan.
- Guang C, Xiong-Ming DU (2006). Genetic diversity of source germplasm of Upland cotton in China as determined by SSR marker analysis. *Acta Genetica Sinica* 33: 733–745.
- Hall D, Tegstrom C, Ingvarsson PK (2010). Using association mapping to dissect the genetic basis of complex traits in plants. *Brief Funct Genomics* 9: 157–165.
- Han Z, Wang C, Song X, Guo W, Gou J, Li C, Chen X, Zhang T (2006). Characteristics, development and mapping of *Gossypium hirsutum* derived EST-SSRs in allotetraploid cotton. *Theor Appl Genet* 112: 430–439.
- He DH, Lin ZX, Zhang XL, Nie YC, Guo XP, Zhang YX, Li W (2007). QTL mapping for economic traits based on a dense genetic map of cotton with PCR-based markers using the interspecific cross of *Gossypium hirsutum* × *Gossypium barbadense*. *Euphytica* 153: 181–197.
- Hinze LL, Dever JK, Percy RG (2012). Molecular variation among and within improved cultivars in the US cotton germplasm collection. *Crop Sci* 52: 222–230.
- Iqbal MJ, Aziz N, Saeed NA, Zafar Y, Malik KA (1997). Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. *Theor Appl Genet* 94: 139–144.
- Khan AI, Fu YB, Khan IA (2009). Genetic diversity of Pakistani cotton cultivars as revealed by simple sequence repeat markers. *Communications in Biometry and Crop Science* 4: 21–30.
- Michelmore RW, Paran I, Kesseli RV (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *P Natl Acad Sci USA* 88: 9828–9832.
- Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *P Natl Acad Sci USA* 76: 5269–5273.
- Paran I, Michelmore RW (1993). Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theor Appl Genet* 85: 985–993.
- Paterson AH, Wendel JF, Gundlach H, Guo H, Jenkins J, Jin D, Llewellyn D, Showmaker KC, Shu S, Udall J et al. (2012). Repeated polyploidization of *Gossypium* genomes and the evolution of spinnable cotton fibres. *Nature* 492: 423–427.
- Prioul JL, Quarrie S, Causse M, de Vienne D (1997). Dissecting complex physiological functions through the use of molecular quantitative genetics. *J Exp Bot* 48: 1151–1163.
- Rahman M, Hussain D, Malik TA, Zafar Y (2005). Genetics of resistance to cotton leaf curl disease in *Gossypium hirsutum*. *Plant Pathol* 54: 764–772.
- Rahman M, Hussain D, Zafar Y (2002). Estimation of genetic divergence among elite cotton cultivars-genotypes by DNA fingerprinting technology. *Crop Sci* 42: 2137–2144.
- Rahman M, Rahmat Z, Mahmood A, Abdullah K, Zafar Y (2014). Cotton germplasm of Pakistan. In: Abdurakhmonov IY, editor. *World Cotton Germplasm Resources*. Rijeka, Croatia: InTech. DOI: 10.5772/58620.
- Rahman M, Shaheen T, Tabbasam N, Iqbal MA, Ashraf M, Zafar Y, Paterson AH (2012). Cotton genetic resources. A review. *Agron Sustain Dev* 32: 419–432.
- Rahman M, Ullah I, Ahsraf M, Stewart JM, Zafar Y (2008). Genotypic variation for drought tolerance in cotton. *Agron Sustain Dev* 28: 439–447.

- Rahman M, Yasmin T, Tabbasam N, Ullah I, Asif M, Zafar Y (2007). Studying the extent of genetic diversity among *Gossypium arboreum* L. genotypes/cultivars using DNA fingerprinting. *Genet Resour Crop Ev* 55: 331–339.
- Rahman M, Zafar Y (2007). Registration of NIBGE-115 cotton. *Journal of Plant Registrations* 1: 51–52.
- Rahman M, Zafar Y (2012). Registration of NN-3 cotton. *Journal of Plant Registrations* 6: 342–347.
- Saeed M, Wangzhen G, Tianzhen Z (2014). Association mapping for salinity tolerance in cotton (*Gossypium hirsutum* L.) germplasm from US and diverse regions of China. *Aust J Crop Sci* 8: 338–346.
- Shen X, Guo W, Lu Q, Zhu X, Yuan Y, Zhang T (2007). Genetic mapping of quantitative trait loci for fiber quality and yield trait by RIL approach in Upland cotton. *Euphytica* 155: 371–380.
- Sneath PH, Sokal RR (1973). *Numerical Taxonomy. The Principles and Practice of Numerical Classification*. 1st ed. London, UK: W.H. Freeman & Co.
- Surgun Y, Çöl B, Bürün B (2012). Genetic diversity and identification of some Turkish cotton genotypes (*Gossypium hirsutum* L.) by RAPD-PCR analysis. *Turk J Biol* 36: 143–150.
- Thormann CE, Ferreira ME, Camargo LEA, Tivang JG, Osborn TC (1994). Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. *Theor Appl Genet* 88: 973–980.
- Tuberosa R, Salvi S, Sanguinetti MC, Landi P, Maccaferri M, Conti S (2002). Mapping QTLs regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives in drought-stressed maize. *Ann Bot* 89: 941–963.
- Tyagi P, Gore MA, Bowman DT, Campbell BT, Udall JA, Kuraparthy V (2014). Genetic diversity and population structure in the US Upland cotton (*Gossypium hirsutum* L.). *Theor Appl Genet* 127: 283–295.
- Wu J, Gutierrez OA, Jenkins JN, McCarty JC, Zhu J (2009). Quantitative analysis and QTL mapping for agronomic and fiber traits in an RI population of upland cotton. *Euphytica* 165: 231–245.
- Zhao Y, Wang H, Chen W, Li Y (2014). Genetic structure, linkage disequilibrium and association mapping of Verticillium wilt resistance in elite cotton (*Gossypium hirsutum* L.) germplasm population. *PLoS One* 9: e86308.
- Zhu Y, Chen H, Fan J, Wang Y, Li Y, Chen J, Fan JX, Yang S, Hu L, Leung H (2000). Genetic diversity and disease control in rice. *Nature* 406: 718–722.