Population structure, linkage disequilibrium and association mapping for cotton leaf curl disease incidence in cotton (*Gossypium hirsutum* L.)

Muhammad Saeed (✉ saeed242@hotmail.com)

Government College University <https://orcid.org/0000-0002-7125-7394>

Anam Tariq

Government College University Faisalabad

Shagufta Perveen

Government College University Faisalabad

Amina Altaf

Government College University Faisalabad

Sumera Ashfaq

Government College University Faisalabad

Muhammad Riaz

Ayub Agricultural Research Institute

Xianliang Song

Shandong Agricultural University

Xuezhen Sun

Shandong Agricultural University

Tianzhen Zhang

Zhejiang University

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Abstract

Background: Cotton (*Gossypium hirsutum* L.) production is adversely affected by many biotic and abiotic factors. Among the biotic factors, cotton leaf curl disease (CLCuD), caused by a begomovirus, is the most damaging. An association mapping approach was used to find out population structure and linkage disequilibrium in cotton germplasm; and simple sequence repeat (SSR) markers associated with CLCuD infestation and seed cotton yield per plant (SCY).

Results: Data was recorded for percent disease index (PDI) and SCY of 30 diverse cotton cultivars. Out of one thousand SSR markers used for polymorphism screening of plant material, 125 were found polymorphic and used for genotyping of 30 cultivars. STRUCTURE 2.0 software identified three sub-populations in plant material. Linkage disequilibrium (LD) analysis showed that at $P < 0.05$, 270/7750 marker pairs (3.48%) displayed significant pair-wise LD and values for correlation between alleles at two loci (r^2) and disequilibrium coefficient (D') were > 0.05 and > 0.33 , respectively, which were stringent enough for identification of valid marker-trait associations. Three markers (BNL1421, BNL946, NAU2336) were associated with PDI and five markers (NAU3100, NAU3860, NAU2419, NAU3011, NAU4565) were associated with SCY under both GLM (general linear model) and MLM (mixed linear model) analyses.

Conclusion: Marker BNL1421 (with phenotypic variance explained (R^2) and heritability values of 26.45% and 0.90, respectively) would be a valuable candidate in marker-assisted selection (MAS) to screen CLCuD susceptible cotton genotypes. Findings of this study will help in understanding genetics of CLCuD infestation and designing future molecular breeding programmes to control this disease.
Keywords: Cotton leaf curl virus, Disease, General linear model, Linkage disequilibrium, Population structure, Simple sequence repeats

Background

Cotton (*Gossypium hirsutum* L.) is the most important natural textile fiber worldwide and the world's sixth largest source of vegetable oil. China, India, USA and Pakistan are largest cotton producing countries in the world [1]. Cotton seeds are important source of food [2]. Cotton is known as the cash crop due to its high economic value. Cotton is considered as the life line of economy of Pakistan. The share of cotton in GDP of Pakistan is 0.8% [3]. It plays role as backbone of Pakistan's economy by producing the lint, which is used in textile industry [4].

Cotton belongs to genus *Gossypium* and family Malvaceae. The family Malvaceae contains 90 genera. The genus *Gossypium* includes shrubs and saplings [5–6]. The genus *Gossypium* has 45 diploid and 5 allotetraploid species that occur in semiarid and arid areas of Africa, Central and South America, Galapagos, Indian subcontinent, Australia, Arabia, and Hawaii [7]. Currently cotton has only 4 cultivated species, out of which two are tetraploid species including *G. hirsutum* L. and *G. barbadense* L. The other species are diploid such as *G. arboreum* L., and *G. herbaceum* L. Tetraploid species were formed by merging of two diploid genomes, out of which one genome has similarity with A-genome diploids found

in old world while other genome is similar to the D-genome diploids of new world. Tetraploid species evolved by hybridization between an African A genome and an American D genome about 1–2 million years ago [8,9]. Diploid species are divided into eight genomic groups A-G and K [10]. Spinable fiber is the product of A genome species [11]. World's cotton production is largely based on *Gossypium hirsutum* and *Gossypium barbadense* [12]. *Gossypium hirsutum*, also known as Upland and Mexican cotton, represents 90% of global fiber production. It produces longer, stronger and finer fiber [13]. *Gossypium barbadense*, also known as Pima cotton, involves in 8% global cotton production. It is valued for its higher quality fiber [14]. *Gossypium arboreum* (Tree cotton) and *Gossypium herbaceum* (Levant cotton) contribute less than 2% of global cotton production [15].

World's agriculture production is adversely affected by diseases caused by viruses and unfavorable climatic conditions [16]. Cotton production is threatened by many biotic and abiotic stresses. Among biotic stresses, cotton leaf curl disease (CLCuD) is most damaging. CLCuD is considered as the major threat to cotton production in Pakistan [17]. CLCuD restricts the cotton production by 20%–30% annually in Pakistan which depends on the severity of the disease [18,19]. Millions of cotton bales have affected by this disease and country faced economic difficulties. It is estimated that Pakistan economy suffered a loss of 75 million rupees during last ten years due to CLCuD [20]. CLCuD is transmitted by the whitefly *Bemisia tabaci* [21]. This disease is caused by a *begomovirus*, belonged to the family Geminiviridae. Geminiviruses infect both monocotyledonous and dicotyledonous plants. Cotton leaf curl virus has a wide range of alternative hosts including okra, tomato, potato, Chinese rose, tobacco, lehli and dhatura [22].

Geminiviruses are single stranded circular DNA viruses. The genome of these viruses consists of 2500–5200 bases. The family Geminiviridae is divided into nine genera on the basis of arrangement of genome and vector. Several insects are involved in the transmission of these viruses such as aphids, leafhoppers, treehoppers and whiteflies. Whiteflies are involved in the transmission of begomoviruses [23]. The largest genus of family Geminiviridae is begomovirus. More than 320 species are present in this genus. Dicotyledonous plants are infected by begomoviruses [21]. Production of many crops including cotton is reduced on a large scale by begomoviruses in temperate, tropical and sub-tropical areas. All members of family Geminiviridae are monopartite except begomoviruses [23]. Begomoviruses are of two types including monopartite and bipartite begomoviruses. Most of begomoviruses genome contains two types of components. One is known as DNA A and other is known as DNA B. These begomoviruses are known as bipartite viruses. The size of both components of genome is same. Each component of genome is consisting of about 2600 nucleotides. Only DNA A is present in monopartite begomoviruses [21]. Monopartite begomoviruses are responsible for CLCuD [24,25]. The DNA A of bipartite genome contains 6 genes out of which *V1* and *V2* genes are present on sense strand while other 4 genes are present on antisense strand such as *C1*, *C2*, *C3* and *C4* genes. These genes encode different proteins. For example, replication-initiation protein (Rep) is encoded by *C1* gene while *C2* gene encodes transcriptional activator protein. Replication enhancer protein is encoded by *C3* gene. *C4* encodes protein that is necessary for movement of virus. This protein determines the severity of disease and range of host [26]. Two types of satellites are associated with begomoviruses. These satellites are known as alphasatellites and

betasatellites [24]. The size of alphasatellites is about 1.4kb. Alphasatellites do not take part in the determination of disease symptoms [27]. The level of viral DNA in infected plants is suppressed by alphasatellites [28]. The replication of helper virus is enhanced by betasatellites. Betasatellites consist of about 1350 nucleotides. These are small and non-circular ssDNA satellites which are linked with majority of monopartite begomoviruses. The protein (β C1) which determines the symptoms of disease is also encoded by betasatellites. The severity of disease is determined by the betasatellites [24]. Disease severity is positively correlated with virus/satellite DNA thus more virus/satellite results into more disease severity [17].

CLCuD first appeared in Nigeria in 1916. But in Pakistan, the cotton plants were first attacked by this disease in 1967 at Tiba Sultan Pur near Multan district. Its first epidemic in Pakistan appeared during 1992–93 [29]. CLCuD has specific symptoms such as growth stunting, visibility of thickened veins, leaf darkening, and leaf enations may be one or more on the underside side of leaf. Enation is a leaf like small outgrowth of plant tissue that is caused by a virus infection [24]. CLCuD caused two types of thickening of veins on cotton plants; i) major vein's thickening ii) minor vein thickening. Leaf margins become thick at the initial stage of disease [30]. Seriously infected plant has shortened internodal length that results into stunted growth of the plant [24,31]. CLCuD adversely affects fiber quality. It also reduces seed weight, number of monopodial and sympodial branches [32]. Its susceptible plants have darker color than normal plants due to the proliferation of chlorophyll deposited tissues [33].

Different strategies are used to compete with the problem of CLCuD. But the best option is to use the resistant cultivar. Molecular mapping approach can help to identify the genomic regions of cotton associated with CLCuD resistance. Association mapping is a recent molecular mapping approach which has great resolution. Association mapping (AM), also known as linkage disequilibrium (LD)-based mapping, is used to identify genomic regions associated with phenotypic variations [34]. This technique is used to determine marker-trait associations with the help of genome-wide distributed genes and markers and to study the architecture of the trait. This technique is appropriate for both synthetic and natural populations [35]. AM is a technique that is used to analyze a large number of polymorphisms to assess the QTL effect. It does not require generation of segregating population and a large number of progeny, due to which it is more preferable than linkage analysis (LA). Association mapping can increase mapping resolution. It decreases the research time and greater number of alleles is identified [36]. In the present project, an association mapping approach was used to identify simple sequence repeat (SSR) markers associated with CLCuD resistance in cotton.

Results

Phenotypic variation

Great differences were observed among the cultivars for PDI and SCY as revealed from the ANOVA of combined data of two years (Table 2). Performance of genotypes with regard to PDI and SCY differed significantly during 2015 and 2016. MS-39 did not show any symptoms of CLCuD during both 2015 and

2016, whereas, MNH-636 showed higher values of PDI during 2015 and 2016. CLCuD infestation is very complex and fluctuations are observed at a single site with regard to same cotton genotype. This was reflected in significant differences of PDI within blocks. The minimum value for PDI during 2015 and 2016 was 0 while the maximum value for PDI during 2015 and 2016 was 25.89 and 28.34, respectively. The minimum value for SCY during 2015 and 2016 was 30.67 g and 102 g, respectively. The maximum value of SCY for 2015 and 2016 was 129.65 g and 324.13 g, respectively.

Genotyping, STRUCTURE and LD analyses

The plant material was surveyed for polymorphism with 1000 SSR markers. One hundred and twenty five markers were found polymorphic. Out of 125 markers, 111 markers yielded 2 alleles, 12 markers yielded 3 alleles, and 2 markers yielded 4 alleles. Subsequent STRUCTURE and LD analyses were performed with the help of genotypic data of these 125 polymorphic markers. STRUCTURE analysis identified three sub-populations in the plant material. Overall proportion of membership of the plant material in each of the 3 sub-populations was 0.57, 0.16, and 0.27, respectively.

Sub-population 1 included 19 cultivars namely CIM-1100, VH-148, Stoneville, SLS-1, MS-39, MNH-636, MNH-554, MNH-552, LSS, FH-901, FH-900, FH-682, FH-1000, FH-113, IR-NIAB-824, CIM-499, CIM-496, BH-118, and B557. Sub-population 2 included 3 cultivars namely CIM-506, CIM-482, and MNH-814. Sub-population 3 included 5 cultivars namely CIM-448, CIM-446, CIM-109, BS-1, and CIM-473. Three cultivars namely MS-40, 4-F, and 268-F were admixtures.

Out of total 7750 marker pair combinations, 270 (3.48%), 86 (1.11%), and 29 (0.37%) marker pairs exhibited LD at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. List of marker pairs which exhibited higher LD is given in Table 3. A non-random linkage between loci located on different chromosomes was observed. At $P < 0.05$, values for correlation between alleles at two loci (r^2) and disequilibrium coefficient (D') were >0.05 and >0.33 , respectively. These values were stringent enough for identification of valid marker-trait associations. Therefore, analysis for marker-trait associations assessment was performed.

Marker-trait associations

There were 8 significant ($P \leq 0.05$) marker-trait associations identified by GLM analysis (Table 4). Phenotypic variance explained values (R^2) for these associations ranged from 11.13% to 26.91%. Three markers were associated with PDI out of which two are in 2016 data set and one is from 2015 data set. These markers were BNL1421 (A13-Chr13), BNL946 (D10-Chr20), and NAU2336 (D2-Chr14). Five markers were found to be associated with SCY in the data set of 2016. These markers were NAU3100 (D9-Chr23), NAU3860 (A12-Chr12), NAU2419 (A1-Chr1), NAU3011 (D13-Chr18), and NAU4565. These marker-trait associations were also confirmed by MLM (mixed linear model) analysis (Table 5). MLM analysis showed that marker BNL1421 (A13-Chr13) associated with PDI in 2015 data had higher heritability value.

This marker had greater genetic variance component (21.43) in the total variance (23.75). This marker would be a good candidate for screening CLCuD susceptible cotton genotypes.

Discussion

CLCuD susceptibility is a quantitative trait

CLCuD is the major cause of huge loss to world cotton production, especially in Pakistan, India, and China [37]. In Pakistan, there is considerable reduction in cotton crop area due to non-availability of CLCuD resistant elite cotton cultivars and cotton farmers were forced to cultivate alternative crops for sustainable earnings from their agricultural lands [3]. Conventional breeding programmes in different countries were successful to develop CLCuD resistant cotton varieties, but all these varieties were with very low SCY potential [38]. These cotton varieties were out of farmers' production scheme within few years after their approval as these varieties could not meet farmers' economic concerns. For sustainable cotton production, it is direly needed that cotton cultivars having CLCuD resistance with higher SCY potential be developed. For combining CLCuD resistance and high SCY potential in a cotton variety is a challenging task. This needs a thorough knowledge and information about genetic potential of cotton genotype for CLCuD resistance as well as its genetic architecture for SCY potential.

Molecular mapping approaches help to tag genomic regions associated with traits of interest [34,36,39,40]. Identified markers through these mapping approaches can be incorporated in to marker-assisted selection (MAS) programmes to pyramid desirable alleles in a single crop cultivar. In this study, CLCuD susceptibility loci were found located on chromosomes 13 (BNL1421), 14 (NAU2336), and 20 (BNL946). This study showed that CLCuD susceptibility is not a simple Mendelian character controlled by one gene. More than one loci are involved in CLCuD susceptibility. These loci are scattered on different chromosomes. Both A and D genomes contained CLCuD susceptibility loci.

Marker-assisted selection for developing CLCuD tolerant genotypes

In the past, there was paucity of DNA markers associated with CLCuD susceptibility/tolerance. Being a complex genetic character, development of CLCuD tolerant genotypes through conventional breeding approach is not a feasible option as it is time consuming and selection of elite genotypes is cumbersome through this approach. Marker-assisted selection (MAS) and subsequent genomics-assisted breeding is the way forward. Identified markers associated with CLCuD susceptibility, especially marker BNL1421, which had higher heritability value of 0.90, would be suitable marker to screen out CLCuD susceptible cotton genotypes. This locus would be further characterized to understand genetics of CLCuD susceptibility.

Conclusions

CLCuD is affecting cotton production in many countries of the world. This study showed that cotton genotypes had considerable differences and diversity with regard to CLCuD tolerance. MS-39, cotton cultivar showing highest tolerance to CLCuD, needs to be genetically characterized to understand CLCuD tolerance mechanism in minute details. Similarly, genetic characterization of MNH-636, cotton cultivar with highest susceptibility to CLCuD, would also be helpful in designing genomics-assisted breeding (GAB) strategy to develop elite cotton cultivars with CLCuD tolerance. Fine mapping of identified markers associated with PDI would lead to identification of putative genes/alleles involved in CLCuD susceptibility/tolerance. Findings of this study showed that more than one loci were involved in CLCuD susceptibility and GAB is the most suitable strategy to develop elite CLCuD tolerant cotton genotypes with sustained SCY potential and conventional breeding efforts are inadequate to meet this challenge.

Material And Methods

Plant material

Plant material consisted of thirty cultivars of cotton (*Gossypium hirsutum* L.). List of these cultivars, including their original sources, is given in Table 1. All these were commercial cultivars originated from different cotton research centers of Pakistan and USA. These cotton cultivars were part of cotton germplasm maintained at Cotton Research Station, Ayub Agricultural Research Institute, Faisalabad, Pakistan (CRS-AARI, FSD) (https://aari.punjab.gov.pk/crs_faisalabad). CRS-AARI, FSD routinely acquires cotton germplasm from national and international cotton research institutes to fulfill its objective of “expansion of gene pool” and maintains this cotton germplasm in a very systematic and planned manner with complete record of source and origin. It is fully authorized and had license for this cotton germplasm collection and maintenance. Field experimentation of this study was conducted at CRS-AARI, FSD. Cotton cultivars were selected on the basis of their diversity for CLCuD susceptibility/tolerance during 2013–2015. Plant material was sown at research field of CRS-AARI, FSD during 2015–2016. The seeds of thirty varieties were sown on 15-04-2015 and 15-04-2016. The cultivars were sown in randomized complete block design with three replications having 75 cm row to row and 45 cm plant to plant distance. Standard agronomic practices were followed for the cultivation of cultivars.

Phenotyping of plant material

CLCuD infestation scoring of thirty cultivars was done in terms of percent disease index (PDI). CLCuD scoring was done on 15-9-2015 and 18-09-2016. Cotton cultivars were scored for CLCuD infestation according to the 6-scale symptoms severity given by Akhtar et al. (2010) [41].

Percent disease index was calculated by following formula:

Percent	Disease	Index
$\frac{\text{By adding all disease scoring stages in selected individual plant}}{\text{Total number of plants}} \times \frac{100}{6}$		

At maturity, the data for seed cotton yield per plant (SCY) was recorded. SCY was measured in grams (g) with laboratory measuring balance.

SSR genotyping

Plant material was screened for polymorphism using 1000 simple sequence repeat (SSR) markers. Ninety-two SSR markers were found polymorphic. Genomic DNA was extracted by using 4–5 young, fully expanded leaves. Leaves were stored at –80 °C. DNA was extracted by using DNA extraction Aidlab DNA mini kit (Cat #: DNA15, Lot: 261525AX). The DNA integrity was checked by electrophoresis on 1% agarose gel and quantified by Nanodrop 2000. The DNA amplification was performed by using standard PCR procedures described by Zhang et al. (2000) [42]. The DNA amplification of thirty commercial cultivars was performed by SuperCycler thermal cycler (Model: SC300G, Kyrattec limited, Australia). This amplification was carried out with the help of Bioron Taq Master Mix, Bioron DFS-Taq DNA Polymerase, Bioron set of 4 dNTPs and Bioron 100 bp plus DNA ladder ready-to-use. Ninety-two polymorphic primers were used for amplification. DNA bands of amplification product were developed with silver staining and recorded with SX-image system. SSR markers were scored on their base pair sizes. Missing data was indicated by “–9” and “?” according to the software requirements.

STRUCTRE analysis

The pattern of population/genetic structure in thirty cultivars was assessed by using software package STRUCTURE [43]. It is a model-based clustering method.

Analysis of marker-trait associations

Marker-trait associations were assessed by GLM (general linear model) association test incorporating genotypic and phenotypic data; and *Q* (structure relatedness) matrices into TASSEL 2.1 software [44]. TASSEL stands for Trait Analysis for aSSociation, Evolution and Linkage.

Abbreviations

AM: Association mapping; *CLCuD*: Cotton leaf curl disease; *CoV*: Coefficient of variation; *GLM*: General linear model; *LA*: Linkage analysis; *LD*: Linkage disequilibrium; *MLM*: Mixed linear model; *PDI*: Percent disease index; *SCY*: Seed cotton yield per plant

Declarations

Authors' contributions

MS, SS, XS conceived and designed the experiments. AT, AA and SA performed the experiments. MS, AT, SP and MR analyzed the data. MS, AT, SP, MR and SS wrote the paper. XS and TZ edited the paper. All authors have read and approved the manuscript.

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Availability of data and materials

Not applicable

Ethics approval

Not applicable.

Consent for publication

Not applicable.

Competing interests

Authors declare that there are no competing interests.

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Tables

Table 1 List of cultivars used in this study.

Sr. NO.	Cultivar Name	Species	Origin
1.	CIM-1100	<i>Gossypium hirsutum</i> L.	CCRI, PCCC, Multan, Pakistan
2.	VH-148	<i>Gossypium hirsutum</i> L.	CRS, AARI, Vehari, Pakistan
3.	Stoneville	<i>Gossypium hirsutum</i> L.	USA
4.	SLS-1	<i>Gossypium hirsutum</i> L.	CRS, PCCC, Sahiwal, Pakistan
5.	MS-40	<i>Gossypium hirsutum</i> L.	CRS, AARI, Multan, Pakistan
6.	MS-39	<i>Gossypium hirsutum</i> L.	CRS, AARI, Multan, Pakistan
7.	MNH-636	<i>Gossypium hirsutum</i> L.	CRS, AARI, Multan, Pakistan
8.	MNH-554	<i>Gossypium hirsutum</i> L.	CRS, AARI, Multan, Pakistan
9.	MNH-552	<i>Gossypium hirsutum</i> L.	CRS, AARI, Multan, Pakistan
10.	LSS	<i>Gossypium hirsutum</i> L.	CRI, Faisalabad
11.	FH-901	<i>Gossypium hirsutum</i> L.	CRI, AARI, Faisalabad, Pakistan
12.	FH-900	<i>Gossypium hirsutum</i> L.	CRI, AARI, Faisalabad, Pakistan
13.	FH-682	<i>Gossypium hirsutum</i> L.	CRI, AARI, Faisalabad, Pakistan
14.	FH-1000	<i>Gossypium hirsutum</i> L.	CRI, AARI, Faisalabad, Pakistan
15.	FH-113	<i>Gossypium hirsutum</i> L.	CRI, AARI, Faisalabad, Pakistan
16.	IR-NIAB-824	<i>Gossypium hirsutum</i> L.	NIAB, Faisalabad, Pakistan
17.	CIM-506	<i>Gossypium hirsutum</i> L.	CCRI, PCCC, Multan, Pakistan
18.	CIM-499	<i>Gossypium hirsutum</i> L.	CCRI, PCCC, Multan, Pakistan
19.	CIM-496	<i>Gossypium hirsutum</i> L.	CCRI, PCCC, Multan, Pakistan
20.	CIM-482	<i>Gossypium hirsutum</i> L.	CCRI, PCCC, Multan, Pakistan
21.	CIM-448	<i>Gossypium hirsutum</i> L.	CCRI, PCCC, Multan, Pakistan
22.	CIM-446	<i>Gossypium hirsutum</i> L.	CCRI, PCCC, Multan, Pakistan
23.	CIM-109	<i>Gossypium hirsutum</i> L.	CCRI, PCCC, Multan, Pakistan
24.	BS-1	<i>Gossypium hirsutum</i> L.	CRS, AARI, Khanpur, Pakistan
25.	BH-118	<i>Gossypium hirsutum</i> L.	CRS, Bahawalpur, Pakistan
26.	B-557	<i>Gossypium hirsutum</i> L.	CRI, AARI, Faisalabad, Pakistan
27.	4-F	<i>Gossypium hirsutum</i> L.	CRI, Faisalabad
28.	CIM-473	<i>Gossypium hirsutum</i> L.	CCRI, PCCC, Multan, Pakistan
29.	268-F	<i>Gossypium hirsutum</i> L.	CRSS, Jhang, Pakistan
30.	MNH-814	<i>Gossypium hirsutum</i> L.	CRS, AARI, Multan, Pakistan

AARI Ayub Agricultural Research Institute; CCRI Central Cotton Research Institute; CRI Cotton Research Institute; CRS Cotton Research Station; CRSS Cotton Research Sub-station; PCCC Pakistan Central Cotton Committee

Table 2 Mean squares of ANOVA for cotton genotypes combined data of two years of PDI and SCY.

SoV/Trait	df	PDI (%)	SCY (g)
Blocks	2	1.87***	2721.71 ns
Genotype	29	262.48***	8092.74***
Year	1	517.45***	550229.49***
Genotype × Year	29	176.78***	4272.30***
Error	118	0.13	919.09
R^2		0.9988	0.8940
CoV		3.59%	21.29%

CoV Coefficient of variation [(Root MSerror) / abs (Mean Y) × 100%]; *PDI* Percent disease index; R^2 SSmodel/SStotal; *SCY* Seed cotton yield per plant; *SoV* Source of variation

Table 3 Marker pairs with highly significant LD

Locus 1	Chr	Locus 2	Chr	r^2	D'	$pDiseq$
NAU3901	D1-Chr15	NAU1070	D2-Chr14	0.09	0.41	0
NAU2572	Unknown	NAU3901	D1-Chr15	0.39	0.77	0
NAU2477	A4-Chr4	NAU2162	D4- Chr22	0.62	1	0.001
NAU1190	A3-Chr3	NAU453	A11-Chr11	0.15	0.70	0.001
NAU2572	Unknown	BNL3280	D13-Chr18	0.39	1	0.001
NAU1218	A6-Chr6	NAU1102	Unknown	0.72	1	0.001

Chr chromosome; D' disequilibrium coefficient; *LD* linkage disequilibrium; $pDiseq$ probability value for LD; r^2 correlation between alleles at two loci

Table 4 Marker-trait associations for PDI and SCY of cotton genotypes through GLM analysis

Trait	Year	Marker	Chr	<i>P</i> Marker	<i>R</i> ² Model	<i>R</i> ² Marker
PDI	2015	BNL1421	A13-Chr13	0.05	0.4494	0.2645
		BNL946	D10-Chr20	0.04	0.3211	0.2399
	2016	NAU2336	D2-Chr14	0.01	0.2889	0.2691
SCY	2016	NAU3100	D9-Chr23	0.02	0.4309	0.1437
		NAU3860	A12-Chr12	0.03	0.4228	0.1303
		NAU2419	A1-Chr1	0.03	0.4167	0.1241
		NAU3011	D13-Chr18	0.04	0.4576	0.2359
		NAU4565	Unknown	0.05	0.4038	0.1113

Chr chromosome; *PDI* Percent disease index; *SCY* Seed cotton yield/plant; *R*² phenotypic variance explained

Table 5 Marker-trait associations for PDI and SCY of cotton genotypes through MLM analysis

Trait	Year	Locus	Chr	<i>P</i> Marker	Variance			Heritability
					Genetic	Residual	Total	
PDI (%)	2015	BNL1421	A13-Chr13	0.01	21.43	2.32	23.75	0.90
	2016	BNL946	D10-Chr20	0.05	0.03	3296.90	3296.93	1.00E-05
		NAU2336	D2-Chr14	0.01	8.11E-04	81.06	81.06	1.00E-05
SCY	2016	NAU3100	D9-Chr23	0.02	0.02	2352.86	2352.89	1.00E-05
		NAU3860	A12-Chr12	0.03	0.02	2306.20	2306.22	1.00E-05
		NAU2419	A1-Chr1	0.03	0.02	2330.89	2330.91	1.00E-05
		NAU3011	D13-Chr18	0.04	0.02	2397.12	2397.14	1.00E-05
		NAU4565	Unknown	0.05	0.02	2382.21	2382.23	1.00E-05