

GENETIC DIVERSITY AND ASSOCIATION ANALYSIS OF SALT TOLERANCE IN ASIATIC COTTON (*GOSSYPIUM ARBOREUM*) WITH MOLECULAR MARKERS

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ABSTRACT

Gossypium arboreum possesses favorable characteristics that are profitable for developing elite cotton cultivars. This study analyzed genetic diversity and identifies marker-trait association analysis related to salt tolerance using SSR markers for 215 accessions of *G. arboreum*. The salt tolerance-related traits like germination rate, fresh weight, stem length, water content, chlorophyll content, electric conductivity, and MDA of the accessions were identified using 150 mM NaCl for 7 days of seedling growth.

Two hundred fifteen accessions were categorized mainly into four groups based on the comprehensive index of salt tolerance. Twenty-four accessions were classified as highly tolerant to salt treatment (>2.5). The natural population was classified into 3 main groups by phylogenetic analysis. The classifications of phylogenetic analysis were largely congruent with the breeding history and ecological region.

Twenty-two strong marker-trait associations were obtained with strict significant P value i.e. $P < 0.01$ and four makers including NAU1023, NAU1099, JESPR222, and NAU2783 were significantly related to salt tolerance. The marker NAU2783 was highest associated ($P = 1.98E-12$) with relative electric conductivity, and with the highest phenotype variation of 20.87%. Some markers are significantly associated with more than two traits. MUSS020 was significantly ($P < 0.01$) related with relative fresh weight, and relative MDA, NAU1375 was associated with relative fresh weight and relative stem length, while NAU3468 was significantly associated with relative fresh weight, relative germination rate, and relative water content. The strong marker-trait association results might provide insights for marker-assisted selecting salt-tolerant varieties and will be useful for future cotton breeding programs.

Keywords *Gossypium arboreum*, salt tolerance, simple sequence repeats (SSR), phylogenetic analysis, association analysis

INTRODUCTION

High salinity is a significant environmental factor that not only hinders plant growth and productivity but has also emerged as a global concern [1]. Salinity stress impacts approximately one billion hectares of arid and semi-arid regions globally [2]. It has been estimated that soil salinity has had adverse effects on 80 million hectares of the world's cultivated land [3] and may encompass more than half of the world's arable land by 2050 [4, 5]. This issue is escalating due to climate changes, including variations in annual rainfall, evaporation, temperature, humidity, as well as unsustainable irrigation and excessive fertilization [2]. It is a widespread problem found in over 100 countries globally and is worsening in countries like the United States, China, Kuwait, the United Arab Emirates, Hungary, and Australia. Furthermore, it is expected to become more severe in regions such as North Africa, East Africa, the Middle East, East Asia, and South Asia [6, 7]. In China, salinity is a significant concern, with 6.6 million hectares of land reported as affected by various stages of salinization [8]. Additionally, around 17 provinces, including those in the northwest, northeast, north, and coastal areas, face annual salinity issues [9].

Scientists and agronomists have dedicated substantial efforts to enhance and maximize the utilization of saline soil through various approaches, including the use of chemicals, biological methods [10]), and the introduction of salt-tolerant plant [11]. The screening or breeding of high-salt-tolerant crops using modern molecular techniques presents an

economical and effective solution to the current situation [2]. Nevertheless, our understanding of the genetic basis of salt tolerance remains partial, owing to the diverse regulatory mechanisms and the intricate genetic architecture associated with salt tolerance [12]. Plants have developed a combination of biochemical and molecular mechanisms to adapt to salt stress, encompassing processes such as ion regulation and compartmentalization, the synthesis of compatible solutes, including proline, glycine betaine (GB), sugars, and polyols, as well as the induction of antioxidant enzymes like catalase, superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase [5, 13].

Conventional methods used for crop selection for salt tolerance are not consistent, because of the large effects of $G \times E$ interactions, low value of heritability [14], and difficulties in appropriate genotype selection, moreover these methods are time-consuming and labor-intensive with the low output [15, 16]. Genome-wide association studies (GWAS) are a potent method for dissecting complex traits by conducting genetic surveys across the entire genome to identify variants associated with a particular trait within natural populations. This approach has been highlighted for its effectiveness [17, 18]. Within the realm of GWAS, quantitative trait loci (QTL) and association mapping (AM) represent complementary strategies for pinpointing marker-trait associations. These investigations are carried out using diverse DNA-based molecular markers, including simple sequence repeats (SSR), single-nucleotide polymorphism (SNP) markers, and intron length polymorphisms (ILD) markers.

Simple sequence repeats (SSR), also known as microsatellites, are characterized by short tandem repeat nucleotides in DNA sequences, typically consisting of 2 to 6 base pairs (bp). These microsatellites are widespread in genomes, occurring across various organisms, both eukaryotes and prokaryotes, and can be found in different genomic regions, including protein-coding and non-coding regions. SSR markers are frequently used in studying genetic diversity, genome mapping, and pedigree analysis and marker-assisted selection due to their high reproducibility, multi-allelic nature, co-dominant mode of inheritance, abundance, and wide genome coverage (Pan et al., 2014). A population of 197 *G. arboreum* accessions was genotyped using 80 genome-wide SSR markers to establish patterns of the genetic diversity and population structure [19]. The A-genome of 189 F2 plants derived from the cross of two *G. arboreum* cultivars Jianglingzhongmian × Zhejiangxiaoshanlushu was analyzed, and generated a linkage map using 268 pairs of SSR primers with better polymorphisms [20].

In addition, using genome re-sequencing data, nine SNP rich regions revealed 143 polymorphisms that distributed 40 candidate genes associated with relative fresh weight, relative stem length, relative water content, and a comprehensive index of salt tolerance under salt conditions were reported across 215 *G. arboreum* accessions [1]

Using a dataset consisting of 57,413 high-quality single nucleotide polymorphisms (SNPs) and conducting a genome-wide association study (GWAS), researchers were able to pinpoint a total of 158 stable quantitative trait loci (QTLs) linked to three primary components of lint yield: single boll weight, lint percentage, and boll number per plant. This analysis encompassed 316 *Gossypium hirsutum* accessions studied under four different salt conditions over a span of two years. Additionally, by utilizing 17,264 single-nucleotide polymor-

phisms (SNPs), a set of twenty-three candidate SNPs associated with salinity stress-related traits was identified within a larger group of 419 *G. hirsutum* accessions [7]. To assess the genetic diversity in salt tolerance among various cotton species, researchers evaluated 17 diverse accessions of both allopolyploid (AD-genome) and diploid (A- and D-genome) *Gossypium* species. They assessed a total of 29 morphological and physiological traits linked to salt tolerance and ranked 17 *Gossypium* accessions based on their tolerance to moderate and high salt stresses using comprehensive salt tolerance index scores [5].

In a separate study, 215 accessions of *G. arboreum* underwent investigation for 11 seedling biomass-related traits. Genome-wide association studies (GWAS) were conducted using a dataset of 142, 5003 high-quality SNPs. This analysis resulted in the identification of 83 significant associations and the discovery of 69 potential candidate genes [21].

The genetic foundation of *G. arboreum* represents a crucial asset for genetics research aimed at improving key agronomic traits in cotton breeding. In light of this, the current study was conducted with the following objectives in mind: (i) to elucidate the genetic relationships among a range of geographically diverse accessions. (ii) to examine marker-trait associations employing SSR markers.

MATERIALS AND METHODS

Plant materials, Sample preparation, and Trait evaluation

The genetic materials included 215 accessions of *G. arboreum*, including 209 accessions belonging to China, 3 accessions from the United States, and 3 accessions from India, Pakistan, and Japan in the present study. The plant materials were assembled from the Germplasm Bank of the Institute of Cotton Research of the Chinese Academy of Agricultural

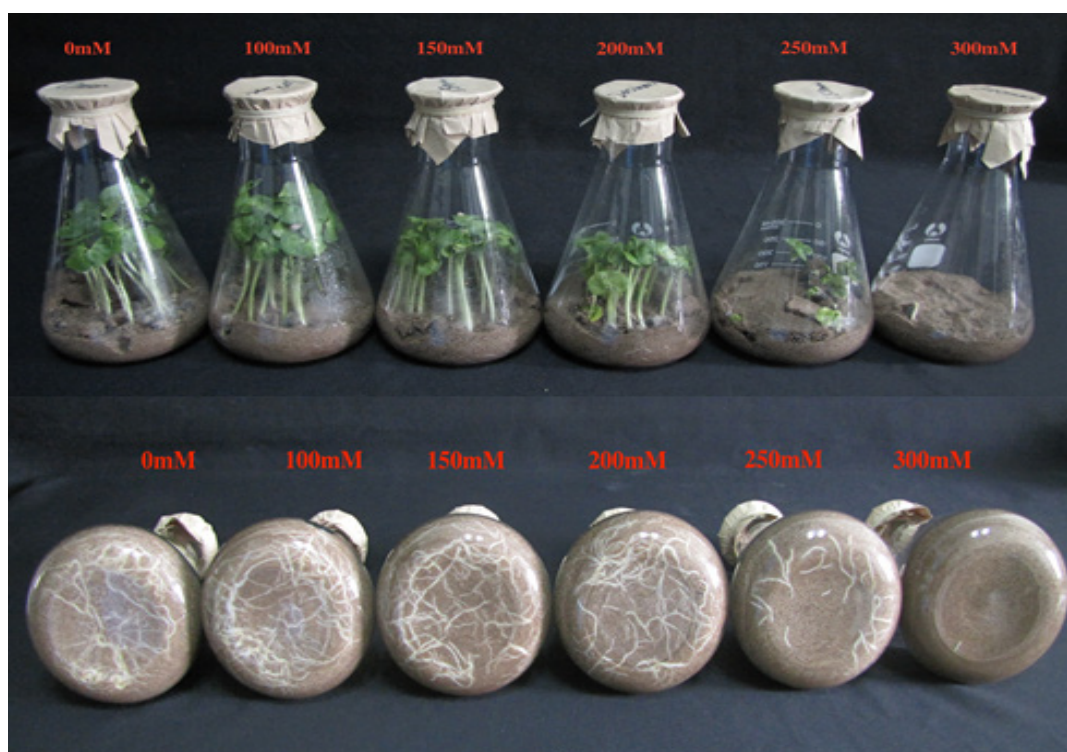


Figure -1 Germination of SiChuanSuiNing ZhongMianGuangZi under different NaCl

Sciences (CAAS), Anyang, China. The detailed list of accessions along with their origin was described in the S 1 Table. After seven days of the germination, the 215 *G. arboreum* accession seedlings grown in the soil were evaluated for seven physiological traits related to salt tolerance, such as germination rate (GR), fresh weight (FW), stem length (SL), water content (WC), chlorophyll content (ChlC), electric conductivity (EC), methylene dioxyamphetamine (MDA) under 0 mM (C) and 150 mM (S) NaCl treatment (Figure 1 and S1 Table). The phenotypic and genetic experiments were performed in the laboratory of the Cotton Research Institute of CAAS, Anyang, China. All Sample preparation and Trait evaluation methods were described in our previous study [1].

DNA extraction and marker amplification

Young leaves from five plants of each accession were collected and stored at -80°C . The DNA was isolated from frozen leaf using the CTAB method [22] with some modifications. DNA concentration was quantified using a NanoDrop2000 instrument (Thermo Scientific, USA), and normalized to 50ng/mL. Markers were analyzed by using PCR and 8% polyacrylamide gel electrophoresis (PAGE). PCR-amplification was performed in a total reaction mix of 10 μl containing 1 μl 10 \times PCR buffer, 0.2 μl dNTPs (10mM each), 0.35 μl labeled primers (Pfl0 μl), 0.2 μl DNA polymerase (5U/ μl), and 1.5-1.6 μl genomic DNA (50ng). Polymerase Chain Reaction (PCR) was conducted using Takara PCR thermo cycler with the first denaturation at 95°C for 3 min, followed by 30 cycles of 94°C for 45 sec, 57°C for 45sec (decreases of 0.5°C in each cycle), and 72°C for 1min and storage at 4°C . The chromosome-specific primer pairs were selected based on previously published papers and their sequence information was downloaded from the Cotton Marker Database (CMD; <http://www.cottonmarker.org>). SSR data were scored as a dominant marker type with “0” for absent alleles, “1”, for a present allele (?), and “-9” for the occasional non-amplification or missing data.

Data analysis

Phenotypic and Genetic diversity

Analysis of variance (ANOVA) and assessment of phenotypic correlations among various physiological traits related to salt stress were conducted using SAS 9.21. The relative value

for each trait and the comprehensive salt tolerance index were determined using the following formula: Relative value = (Value under stress treatment (S)) / (Value under control treatment (C)). Comprehensive index of salt tolerance = (Positive index (GR + SL + FW + ChlC + WC) / (Negative index (EC + MDA)). Based on the polymorphic bands of the SSR primers, the number of alleles, gene diversity, and the polymorphism information content (PIC) were estimated using Excel. P_i was used for first alleles frequency, $\sum P_i^2$ used for polymorphism information content value, also known as Simpson polymorphism index, $PIC=1-\sum P_i^2$; and the Shannon-Weaver diversity index was used, (H'), $H=1-\sum P_i \ln P_i$ [23].

Population structure analysis and association mapping

Allele frequencies were used to produce a bootstrap analysis of phylogeny where Nei's DA distance was generated through neighbor-joining (NJ) tree analysis with 100 bootstrap replications by POWER MARKER v3.25 software [24, 25]. The analyzed tree was exported to MEGA5 to generate a consensus tree [26]. A mixed linear model (MLM) was used to construct markers-trait association tests using the TASSEL 2.0.1 software package [27]. The MLM association test was performed by simultaneous accounting of multiple levels of population structure (Q-matrix) and relative kinship among the individuals (K-matrix) according to [28] method.

RESULTS

Phenotypic diversity

ANOVA analysis of seven salt tolerance traits as measured for genetic diversity shows significant difference among the accessions ($P<0.0001$). The positive correlation with significance ($P=0.01$), highly significance ($P = 0.0001$) and negative correlation ($P = 0.01$) were found between the traits. According to the comprehensive index (CIST) of salt tolerance, 215 accessions were categorized mainly into four groups. Group 1 contained 12 accessions that were sensitive to high salt treatment (<0.6), group 2 contained 26 accessions that were moderately tolerant to salt treatment ($0.6 \leq 1.5$), group 3 included 153 accessions that were tolerant ($1.5 \leq 2.5$), and group 4 had 24 accessions that were highly tolerant to salt treatment (>2.5). The result of ANOVA analysis, correlation analysis between the traits, and classification according to the

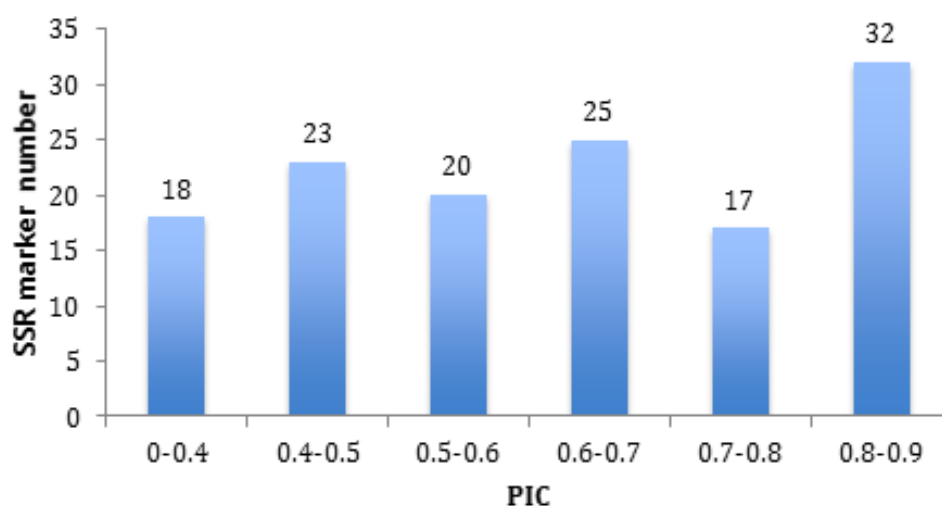


Figure - 2 PIC frequency of SSR markers used in the study

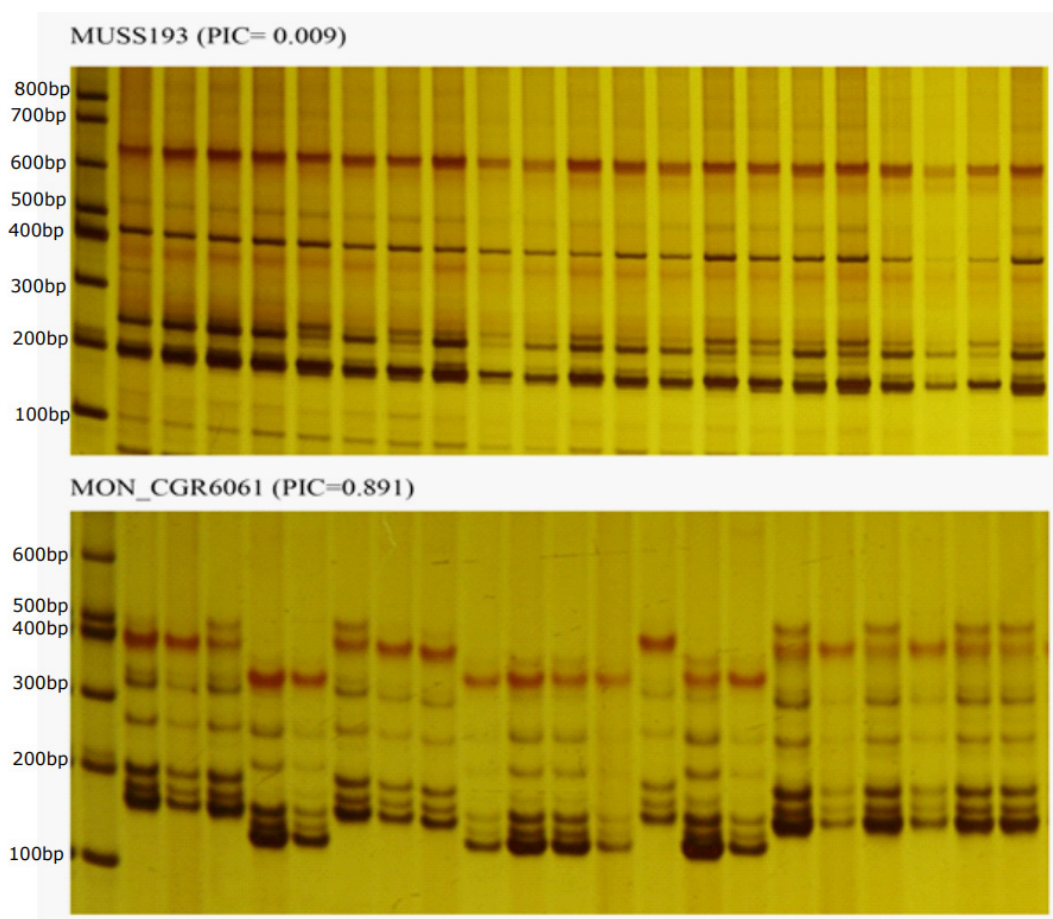


Figure - 3 SSR primers have the lowest (MUSS193) and the highest PIC (MON_CGR6061) values

comprehensive index (CIST) of salt tolerance of accessions were interpreted in our previous study [1].

Genetic diversity

The genetic relationship and genetic diversity of 215 *G. arboreum* were analyzed by SSR primer pairs. In total 135 primer pairs produced 552 alleles with an average of 4.09 alleles per marker. The effective number of alleles (N_e) varied from 1.009 to 9.163; The Shannon-Weaver diversity index (H') was 0.266 (ranging from 0.0433 to 0.367); the PIC value was 0.591 (ranging from 0.00925 to 0.891). Forty-eight markers (36% of the 135 SSRs) were identified as possessing a high PIC value (i.e. > 0.7). The PIC value of thirteen primer pairs was more than 0.8, which markers were MON_CGR6061, NAU945, BNL2634, MON_CGR5876, JESPR0157, BNL2921, NAU761, BNL1673, BNL3031, BNL1034, NAU1241, GH58, and BNL4053. Zhou et al. (2013b) also reported that BNL1673 and BNL1034 revealed higher PIC of more than 0.8 in the genetic diversity study of *G. arboreum*. Seventy-five primer pairs had PIC value of more than 0.6 accounting for 55.55% of the total SSR primer. The primer pair MUSS193 has the lowest PIC value (i.e. 0.009) with two polymorphic bands at about 250 and 260bp, while primer pair MON_CGR6061 has the highest PIC value (i.e. 0.891) with 10 bands distributed between 100 and 500bp (Figure 2, Figure 3, and S2 Table).

Phylogenetic relationship

Phylogenetic relation analysis allowed us to divide the accessions into three groups with 85, 34, and 96 accessions respectively (Figure 4 and S1 Table). Group 1 (G1) contained

85 accessions, a mixed group including 22 accessions from the Yangtze River Region, 15 accessions from the Yellow River Region, 38 accessions from South China, and 4 accessions from North China. Group 2 (G2) had 34 accessions and represents the Yellow River accessions, 73.5% (25 accessions of 34) of this group was from the Yellow River Region, four accessions from North China, and four accessions from South China, and only one accession from the Yangtze River Region. Group 3 (G3) included 96 accessions, representing Yangtze River accessions, 87.5% (84 accessions of 96) of this group was from the Yangtze River Region, and six accessions from the south China area that is close to the Yangtze River Region (Figure 4 and S1 Table). These results further indicated that Asiatic cotton accessions possessed extensive genetic diversity in different growing areas.

Association mapping

Association between SSR loci with seven salt tolerance traits (RGR, RFW, RSL, RWC, RChC, REC, and RMDA) of 215 accessions was performed using the MLM analyses in TASSEL 2.0.1 software package (Bradbury et al., 2007). This analysis showed 90 marker-trait associations with significant level $P < 0.05$ (S3 Table). Twenty-two marker-trait associations with strict significant P-value i.e. $P < 0.01$ were focused on future analysis. Among these associations, 5 markers were associated with relative germination rate (RGR), 3 markers were associated with relative fresh weight (RFW), 4 markers were associated with relative stem length (RSL), 4 markers were associated with relative chlorophyll content (RChC), 3 markers were associated with relative methylene dioxyamphetamine (RMDA), 2 markers were associated with rela-

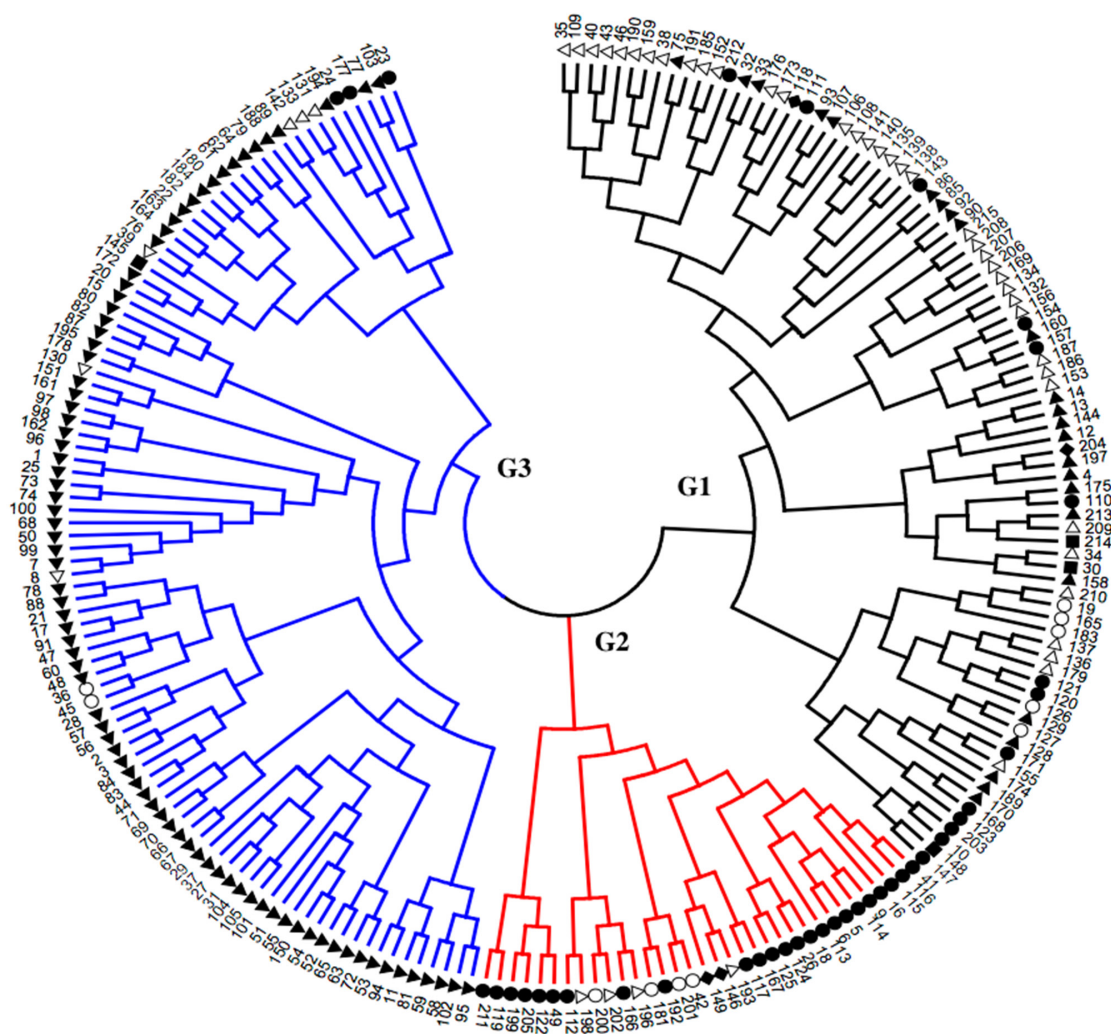


Figure 4 - Phylogenetic analysis of 215 *G. arboreum* accessions.

▲ Yangtze River Region; △ South China; ● Yellow River Region; ◻ Nouth China; ◆ Abroad

tive electric conductivity (REC), and 1 marker was associated with relative water content (RWC) (Table 1). In this study, highly significant marker-trait associations were found which includes, NAU1023, NAU1099 (both of these were associated RGR), and JESPR222 (associated RChIc) with higher P-value ($P > 3$), especially, the marker NAU2783 was highest associated to relative electric conduct (REC) ($-\log P = 11.7$), and also with the highest phenotype variation of 20.87% (Table 1). Some SSR markers were simultaneously associated with two or three traits of interest. Among the 18 significant asso-

ciated markers, 15 markers (BNL1034, BNL1122, BNL2440, BNL2921, BNL4108, DPL417, JESPR222, NAU1007, NAU1023, NAU1099, NAU1215, NAU2156, NAU2783, NAU2934, and NAU4036) were significantly ($P < 0.01$) associated with only one trait. Two markers were significantly ($P < 0.01$) associated with two traits, MUSS020 was significantly ($P < 0.01$) associated RFW and RMDA, NAU1375 was RFW, and RSL. Moreover, the marker NAU3468 was significantly ($P < 0.01$) associated with RFW, RGR, and RWC (Table 1).

Table 1 Association of SSR markers with phenotypic traits and candidate genes in *G. arboreum* accessions ($P < 0.01$)

Trait*	Locus	Polymorphic band	F value	P-value	$-\log P$	R ²
		Number Size (bps)				
RGR	NAU1023	2 a150, b200	17.52	4.17E-05	4.379	0.0763
	NAU1099	2 a175, b500	12.93	4.17E-04	3.379	0.067
	NAU3468	2	8.1	0.0031	2.508	0.0463
	BNL2921	6 a170, b180, c200 d210, e230, f250	8.7495	0.0035	2.455	0.0411

	NAU2156	5	a125, b140, c160, d175, e190	7.8429	0.0056	2.251	0.0367
RFW	NAU1375	3	a200, b210, c240	9.78	0.0021	2.677	0.0518
	NAU3468	2		8.081	0.005	2.301	0.0419
	MUSS020	5	a250, b253, c225, d270, d300	7.3329	0.0074	2.131	0.0394
RSL	NAU1375	3	a200, b210, c240	9.73	0.0021	2.677	0.0515
	BNL1034	8	a175, b180, c190, d200, e210, f250, g300, h400	8.7549	0.0035	2.455	0.0452
	BNL4108	5	a100, b110, c120, d130, e150	8.1287	0.0049	2.309	0.0441
	DPL417	5	a150, b190, c235, d245, e300	7.5271	0.0067	2.174	0.0381
RWC	NAU3468	2		7.4051	0.0071	2.148	0.0385
RChIC	JESPR222	7	a75, b80, c90, d95, e100, f110, g210	11.39	9.15E-04	3.038	0.0632
	BNL1122	5	a160, b175, c180, d190, e210	10.99	0.0011	2.958	0.0531
	BNL2440	4	a190, b225, c275, d300	10.61	0.0013	2.8868	0.0481
	NAU4036	4	a70, b80, c165, d170	7.1987	0.008	2.097	0.0385
REC	NAU2783	8	a180, b200, c210, d215, e225,	55.91	1.98E-12	11.703	0.2087
	NAU1007	4	a150, b160, c175, d185	9.15	0.0028	2.552	0.0412
RMDA	NAU1215	2	a175, b185	7.3955	0.0071	2.148	0.0336
	NAU2934	2	a300, b350	6.846	0.0096	2.018	0.0364
	MUSS020	5	a225, b250, c253, d270, e300	6.7045	0.0104	1.983	0.0361

*RGR, Relative germination rate; RFW, Relative fresh weight; RSL, Relative stem length; RWC, Relative water content; RChIC, Relative chlorophyll content; REC, Relative electric conductivity; RMDA, Relative methylene dioxymphetamine

DISCUSSION

Genetic variation in salt tolerance traits of *G. arboreum* accessions

Global scarcity of the water resources, ecological pollution and enlarged salinization of soil and water became noticeable problem in the beginning of the 21st century. But numerous environmental stresses, like high temperatures, excessive winds, flood, drought and soil salinity have predominately disturbed the yield and cultivation of the important agricultural crops [29]. Indeed, salinity is a major abiotic stress limiting the growth and development of cotton plants at the germination and seedling stages [30]. *G. arboreum* has valuable favorable characteristics for the development of premium cotton varieties [31]. ANOVA analysis, the result of Correlation analysis between the traits, and classification based on the comprehensive index (CIST) of salt tolerance of accessions were discussed deeply in our previous study [1]. Our current study mainly focused on genetic relationships and marker-trait associations using SSR markers. Based on phylogenetic tree analysis, all 215 accessions were assembled into 3 main groups and were largely congruent with the breeding history and ecological region, which includes three main cotton-producing areas (Yangtze River, Yellow River, and South China) respectively. Our report also shows the presence of high genetic diversity among the accessions that belong to the different groups, with varied geographical origins. This result was in agreement with the previous studies that found that accessions of *G. arboreum* from different geographical regions commonly clustered together elsewhere [31-33]. *G. arboreum* has 5,000 years of cultural history, has been domesticated and

cultivated for almost 2000 years in China since it was first introduced from India. So, it is acceptable that the widely different environments of China contributed significant difference to the diversity of *G. arboreum* [34].

Marker-trait association analysis of salt tolerance traits using SSR markers

In conventional breeding, it is difficult to breed varieties with elite salt tolerance by hybridization of different salt-tolerance lines. By screening molecular markers that are related to salt tolerance traits, and a combination of molecular and traditional breeding, the efficiency of salt-tolerant breeding in cotton can be greatly improved [35]. QTL mapping and association mapping for salt tolerance related traits have been developed for understanding and using the molecular basis of salt tolerance in *G. hirsutum* [36]. The SSR genotype analysis illustrated that 215 accessions possessed extensive genetic diversity. Therefore, *G. arboreum* accessions might be an important source of new genes that are advantageous for the molecular and genetic breeding of cotton. Perhaps for confirmation of these results, more polymorphic markers and germplasms were screened to identify the potential alleles. A larger size of accessions helps to increase the detection power and allows the quantification of more alleles that would have enough counts for association analysis [36].

In this study, significant marker-trait associations were found which including NAU1023, NAU1099 (both of these were associated with RGR), JESPR222 (associated with RChIC), and NAU27 (associated with REC) had high P-value ($P > 3$). We identified that the NAU3468 was associated with RGR, RFW, and RWC with a highly significant correlation

value ($P = 0.0001$) between the three traits, while this marker was reported to be associated with the germination rate of *G. hirsutum* under 0.3% salt concentration by [37]. In our study, BNL3255 ($P < 0.5$) was associated with RMDA among the accessions, while BNL3255 was found to be associated with the germination rate of *G. hirsutum*, under 0.3% salt concentration [38]. Remarkably, these markers, which are significantly correlated with salt tolerance, can provide fundamental information for future molecular breeding of novel salt-tolerant cultivars in cotton.

CONCLUSION

Acknowledge on the genetic diversity of *G. arboreum* can facilitate the efficient use of these resources in the development of premium cotton varieties with favorable agronomic traits. From our knowledge, these SSR markers in this study may be used as references for studies of salt tolerance in cotton.

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Authors' contributions XD, GW, and DT conceived and designed the experiment. DT performed the experiment and drafted the manuscript. PZ and DP helped to collect and analyze the data. XD and SM edited the final draft of the manuscript.

Competing interest The authors declare that they have no competing interests.

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МОЛЕКУЛЯРЛЫҚ МАРКЕРЛЕРДІҢ КӨМЕГІМЕН АЗИЯЛЫҚ МАҚТАНЫҢ (*GOSSYPIUM ARBOREUM*) ГЕНЕТИКАЛЫҚ ӘРТҮРЛІЛІГІН ЖӘНЕ ТҰЗҒА ТӨЗІМДІЛІК БЕЛГІЛЕРІМЕН АССОЦИАЦИЯЛЫҚ БАЙЛАНЫСЫН ЗЕРТТЕУ

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ТҮЙІН

Gossypium arboreum мақтаның жоғары сорттарын дамыту үшін құнды болып табылатын қолайлы сипаттамаларға ие. Бұл зерттеу жұмысы *Gossypium arboreum*-ның 215 түрі үшін SSR маркерлері мен тұзға төзімділікке қатысты 7 белгісін және бір тұзға төзімділік индексі арасындағы маркер-белгі ассоциациялық қатынасты анықтау мақсатында жүргізілді. Мақта 215 түрінің тұқы 150 mM тұз концентрациясында 7 күн өскеннен кейін тұқымның өну жылдамдығы, тұтас өсімдіктің салмағы, сабақ ұзындығы, жапырақтың су мөлшері, хлорофилл мөлшері, жапырақтың электр өткізгіштігі және метилendioксиамфетамин (MDA) сынды тұзға төзімділікке байланысты белгілерді зерттей отырып скринингтен өтті.

Тұзға төзімділіктің кешенді индексі бойынша 215 мақта түрі негізінен төрт топқа жіктелді. Тұзға жоғары төзімді (>2, 5) 24 түр анықталды. *G. arboreum* мақтасының 215 түрлерінің табиғи популяциясы филогендік талдау бойынша 3 негізгі топқа жіктелді. Филогендік талдау нәтижелері негізінен түрлердің топтастырылуы өсіру тарихымен және экологиялық аймақпен сәйкес келді және *G. arboreum* түрлердің фенотипі мен генотипі бойынша әртүрлілігін көрсетті.

Біз күшті ассоциацияны табу үшін қатаң P ($P < 0,01$) мәнін қолдандық, және 22 маркер-белгілік ассоциациялық байланысты алдық. Осының ішінде төрт маркер NAU1023, NAU1099, JESPR222 және NAU2783 тұзға төзімділік белгілермен жоғары байланыста болды. NAU2783 маркері салыстырмалы электр өткізгіштікпен жоғары ассоциациялық байланысты ($p=1.98E-12$), сондай-ақ фенотиптің ең жоғары вариациясымен (20,87%) байланысты көрсетті. Кейбір маркерлер екіден көп белгілермен жоғары ассоциациялық байланыста екені анықталды. MUSS020 салыстырмалы жаңа салмақпен жоғары ассоциациялық байланысты ($P<0,01$) көрсетті. NAU1375 салыстырмалы салмақпен салыстырмалы сабақ ұзындығымен және салыстырмалы MDA мөлшерімен байланысты болды, ал NAU3468 салыстырмалы салмақпен, салыстырмалы өну жылдамдығымен және салыстырмалы су мөлшерімен айтарлықтай байланысты. Күшті маркер-белгілік ассоциациялық нәтижелері тұзға төзімді сорттарды таңдауда жаңа түсініктер бере алады және мақта өсірудің болашақ бағдарламалары үшін пайдалы ақпарат береді.

Негізгі сөздер: *Gossypium arboreum*, тұзға төзімділік, қарапайым реттілік қайталануы (SSR), филогенетикалық талдау, ассоциацияны талдау

АНАЛИЗ ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ И АССОЦИАЦИИ СОЛЕУСТОЙЧИВОСТИ АЗИАТСКОГО ХЛОПЧАТНИКА (*GOSSYPIUM ARBOREUM*) С ПОМОЩЬЮ МОЛЕКУЛЯРНЫХ МАРКЕРОВ

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АННОТАЦИЯ

Gossypium arboreum обладает благоприятными свойствами, которые ценны для выведения превосходных сортов хлопчатника. Это исследование было проведено с целью анализа генетического разнообразия и определения ассоциаций маркеров и признаков, связанного с солеустойчивостью, с использованием SSR-маркеров для 215 образцов *G. arboreum*. Признаки, связанные с солеустойчивостью, такие как скорость прорастания, масса в свежем виде, длина стебля, содержание воды, хлорофилла, электропроводность и MDA данных образцов хлопчатника, были определены с использованием 150 мм соли в течение 7 дней роста проростков.

Согласно комплексному показателю солеустойчивости 215 образцов были разделены в основном на четыре группы. Двадцать четыре образца были классифицированы как высокоустойчивые к солевой обработке (>2,5). Естественная популяция была разделена на 3 основные группы с помощью филогенетического анализа. Классификации филогенетического анализа в значительной степени соответствовали истории размножения и экологическому региону, что указывает на обширное генетическое разнообразие образцов *G. arboreum* как по фенотипу, так и по генотипу.

Двадцать две сильные ассоциации между маркерами и признаками были получены со строго значимым значением P

($P < 0,01$). Включая NAU1023, NAU1099, JESPR222 и NAU2783, были в значительной степени связаны с устойчивостью к соли в этом исследовании. В частности, маркер NAU2783 был наиболее тесно связан ($P = 1,98E-12$) с относительной электропроводностью, а также с самой высокой изменчивостью фенотипа - 20,87%. Было обнаружено, что некоторые маркеры достоверно связаны более чем с двумя признаками, например, MUSS020 был достоверно ($P < 0,01$) связан с относительным свежим весом и относительным MDA, NAU1375 был связан с относительным свежим весом и относительной длиной стебля, в то время как NAU3468 был достоверно связан с относительным свежим весом, относительной скоростью прорастания, и относительное содержание воды. Результаты сильной ассоциации маркеров с признаками могут дать новое представление о маркерах-помощниках при отборе солеустойчивых сортов и будут полезны для будущих программ селекции хлопка.

Ключевые слова *Gossypium arboreum*, солеустойчивость, простые повторы последовательности (SSR), ассоциативный анализ