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Research Article
SSR-Based Genetic Diversity and Candidate Salinity-Response Associations in Uzbek Upland Cotton

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Abstract

Background and Objective: Soil salinity reduces cotton productivity and fiber quality in irrigated arid regions, while limited genetic diversity can restrict breeding progress. This study aimed to integrate field phenotyping, SSR-based genetic diversity and population structure analyses and candidate allele-trait screening to identify salinity-adapted upland cotton genotypes and potentially useful SSR loci. **Materials and Methods:** Seventeen Uzbek upland cotton (*Gossypium hirsutum* L.) genotypes were evaluated during 2025-2026 under non-saline, moderately saline (0.3-0.6% total soluble salts) and highly saline (0.6-0.9%) field conditions. Plant height, boll weight, seed cotton yield, upper half mean length (UHML), fiber strength and short fiber index (SFI) were measured. Twenty SSR loci were genotyped to assess genetic diversity, population differentiation and candidate allele-trait relationships. **Results:** Genotype, salinity and genotype × salinity effects were highly significant for all traits ($p<0.001$). From control to high salinity, mean plant height decreased from 77.26 to 49.04 cm, boll weight from 5.28 to 3.88 g and seed cotton yield from 26.74 to 8.72 g/plant. Orzu, Mehnat, T-1090 and T-2000 maintained comparatively higher productivity, whereas T-1001, L-217 and T-1024 showed greater stability for selected fiber properties. The SSR loci generated 53 alleles, averaging 2.65 alleles per locus; 19 of 20 loci were polymorphic, with mean He and PIC values of 0.488 and 0.436, respectively. The NAU2277 was the most informative locus (PIC = 0.761). Jaccard genetic distances ranged from 0.214 to 0.762. Three major genetic groups were identified, while AMOVA attributed 42% of molecular variation to differences among groups and 58% to differences within groups ($\Phi ST = 0.42$; $Nm = 0.35$). Seven nominal allele-trait relationships involving six loci were detected. Favorable allelic states were identified at NAU3588, BNL3545, BNL1421, NAU5434 and TMB1295, whereas the JESPR65 allele was associated with increased SFI. **Conclusion:** The integrated analysis identified genetically diverse and phenotypically complementary cotton genotypes suitable for breeding under saline conditions. The detected SSR allele-trait relationships represent promising candidate signals; however, they require false-discovery-rate correction and validation in larger populations and additional environments before routine use in marker-assisted selection.

Key words: Cotton germplasm, salinity tolerance, SSR markers, genetic diversity, population structure, marker-trait association, fiber quality, marker-assisted selection

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Upland cotton accounts for most global cotton production because of its productivity and broad adaptation. In Uzbekistan and other irrigated regions of Central Asia, however, salt accumulation reduces crop establishment, vegetative growth, boll development, yield and technological fiber properties. Salinity imposes osmotic limitation, ion toxicity, nutrient imbalance, oxidative stress and disturbed assimilate allocation. Although cotton is moderately salt tolerant as a species, the response varies substantially among cultivars and breeding lines, providing exploitable variation for genetic improvement[1,2].

Field phenotyping under naturally saline conditions is necessary for identifying locally adapted donors, but phenotypic selection alone is affected by environment and genotype×environment interaction. The SSR markers remain practical for germplasm characterization because they are reproducible, codominant, multiallelic and accessible to breeding laboratories. Their use can clarify genetic relationships, reduce parental redundancy and provide candidate alleles for subsequent marker-assisted selection[3,4].

Genetic diversity analysis and marker-trait association answer complementary questions. Diversity and population-structure analyses show how germplasm is related and which parents could broaden a breeding population; association screening links particular allelic states to useful or unfavorable phenotypes. Integrating both layers with multi-environment field responses is therefore more informative than treating them as separate studies. The objectives were to quantify agronomic and fiber responses to natural soil salinity, characterize allelic diversity and population differentiation using 20 SSR loci, identify candidate allele-trait relationships and nominate complementary parental materials for breeding cotton adapted to salt-affected environments.

MATERIALS AND METHODS

Plant material, site and experimental design: Seventeen upland cotton genotypes from Uzbek breeding programs were evaluated: Yulduz-2, T-1001, T-1002, T-1003, T-1004, T-1005, T-1024, T-1050, T-1080, T-1090, T-2000, Orzu, Ishonch, Mehnat, L-217, L-218 and L-421. Field trials were conducted in 2025 and 2026 at the experimental station of the Institute of Genetics and Plant Experimental Biology, Kibray District, Tashkent Region, Uzbekistan (41.389° N, 69.465° E), under a semiarid continental climate and irrigated sieroz soil. The study was conducted during 2025-2026.

A randomized complete block design[5] with two field replications was used within each of three naturally occurring salinity environments: Non-saline control, moderate salinity (0.3-0.6% total soluble salts) and high salinity (0.6-0.9%). Each plot contained four rows spaced 60 cm apart, with 10-15 cm between plants. Ten plants were sampled from the two central rows and plot means were treated as experimental units. Soil cores (0-30 cm) collected before planting were composited by plot for determination of total soluble salts. Crop management followed local recommendations.

Table 1: Primer sequences of the 20 SSR loci screened across 17 Uzbek upland cotton genotypes

Locus	Forward primer (5'-3')	Reverse primer (5'-3')
BNL1421	TGAAGATTTGGAGGCAATTG	GAAATCAAGCCTCAATTCGG
BNL1604	AGAGGGAGTAAAGATTTGGGG	TCCAGTTCTTTTTGCCTTGG
BNL1672	TGGATTTGTCCCTCTGTGTG	AACCAACTTTTCCAACACCG
BNL2634	AACAACATTGAAAGTCGGGG	CCCAGCTGCTTATTGGTTTC
BNL3280	GCAGAACTGCCACTTGTTTG	AGAAAATGGGTTGTGCTTGG
BNL3545	AGTCAGTTTTTTGTTAGCAATATGC	AACCATTAATTCCCTATTTAACCG
BNL4028	CTCGAATCACTGTTCCGACA	TTGTTGCGGATCATTGGATA
DPL0530	AGACTTACTTAAAGGCACCATTCG	GCAGACTCTTCTGGTGTAACAGTG
HAU0058	CCGAAGCATATTATGGAAGC	TGGCAGTAACACAACAGAGC
JESPR65	CCACCCAATTTAAGAAGAAATTG	GGTTAGTTGTATTAGGGTCGTTG
MUCS223	ACCTCCACTTTGTTTGTGGC	CACCTTGAATTTAGCACCCC
NAU2176	TGGCACTTGCTAAACCATAA	GGTTTGAGCAGAAGGGTATG
NAU2277	GAACTAGCCACATGATGCAC	TTGTTGAGGCATTAGTTTGC
NAU3011	CCTTCACCATTTGCTTACCT	CTCCGTTTCTATTCCCAAAA
NAU3377	TGCAAGGAATCAAGTTCACA	CTGATTGTACTTTGCGGGTA
NAU3588	CCCCATAGGGCATACTTCTA	GCCAACAAGAACAACAACAC
NAU5434	AAAAGAACTTACGGCACAGG	AAATCACTGGCACTGGAATC
NAU6315	CACTCACAAAATGTCCCTCA	CGGTTGATTCAAGCCTTATC
STV023	GACAAACTACAAAAGGATGAACATGG	AGGCTAGGAAGCTTCTTGTTTTCC
TMB1295	CTGATCCAAACACCCATGC	CGTGGAATTTGGTCATTTTG

Primer information was compiled from Lacape *et al.*[6] and Abdurakhmonov *et al.*[3]

Agronomic and fiber measurements: Plant height was measured at physiological maturity. Boll weight was calculated from 20 mature bolls per experimental unit and seed cotton yield was recorded from the sampled plants and expressed as g/plant. Lint samples were conditioned for 24 hrs at 21±1°C and 65±2% relative humidity. UHML (inches), fiber strength (g/tex) and SFI (%) were measured using an HVI 1000 system (Uster Technologies, Switzerland).

DNA extraction, SSR amplification and scoring: Genomic DNA was isolated from young leaves using a PureLink Genomic DNA Mini Kit. DNA concentration and purity were assessed with a NanoDrop 2000 and integrity was checked by agarose electrophoresis. Twenty SSR loci were selected from published cotton marker resources[3,6]. PCR reactions (20 µL) contained approximately 50 ng DNA, 10 µL 2×master mix, 0.5 µM of each primer and nuclease-free water. Cycling comprised 95°C for 5 min; 35 cycles of 94°C for 30 sec, 55-60°C for 30 sec and 72°C for 45 sec; and 72°C for 10 min. Products were separated on 3% agarose gels and clear reproducible alleles were scored as present (1) or absent (0).

Statistical and molecular analyses: Two-way ANOVA tested genotype (G), salinity (S) and G×S effects. Assumptions were assessed using Shapiro-Wilk and Levene tests and means were compared using Tukey HSD at $p<0.05$[5]. For each polymorphic allele, carriers and non-carriers were compared for phenotypic traits. Because multiple alleles were screened, Benjamini-Hochberg false-discovery-rate (FDR) adjustment is required before an association can be considered statistically validated; unadjusted $p<0.05$ signals are reported here as candidates[6].

Observed (Na) and effective (Ne) allele numbers, expected heterozygosity (He), PIC and percentage polymorphic loci were calculated from the SSR matrix[7]. Jaccard distances[8] supported UPGMA and Neighbor-Joining[9] analyses. Molecular principal component analysis summarized allelic differentiation[10]. AMOVA[11] used 999 permutations to partition variation among and within the three molecular groups; ΦST described differentiation and Nm approximated gene flow[12,13]. Analyses used R 4.3.0, PowerMarker 3.25[14], GenAlEx 6.5[15], DARwin 6.0, MEGA X and OriginPro 2024, as appropriate.

RESULTS

Agronomic response to salinity: Two-way ANOVA revealed highly significant effects of genotype (G), salinity (S) and their interaction (G×S) on plant height, boll weight and seed cotton yield ($p<0.001$; Table 2). For plant height, the F-values were 21.63 for genotype, 24.12 for salinity and 10.29 for the G×S interaction. Salinity exerted a particularly strong effect on boll weight ($F = 452.61$) and seed cotton yield ($F = 534.62$), compared with the corresponding genotype effects ($F = 24.83$ and 16.74, respectively). Significant G×S interactions were also observed for boll weight ($F = 4.32$) and seed cotton yield ($F = 4.39$), demonstrating that the magnitude of genotypic responses varied across salinity environments.

Consistent with these ANOVA results, mean plant height decreased from 77.26 cm under the non-saline control to 62.84 cm under moderate salinity and 49.04 cm under high salinity, representing reductions of 18.7 and 36.5%, respectively. The T-1050, Orzu, L-218 and T-1090 maintained comparatively greater plant height under salinity, whereas L-421 exhibited the strongest reduction. However, variation in plant height was not consistently associated with reproductive performance, indicating that maintenance of vegetative growth alone did not necessarily confer superior yield performance under salinity.

Mean boll weight declined progressively from 5.28 g under the control condition to 4.23 g under moderate salinity and 3.88 g under high salinity. Mehnat, Orzu and T-2000 maintained comparatively higher boll weights across saline environments. Seed cotton yield showed the strongest overall decline, decreasing from 26.74 g/plant in the control to 18.47 g/plant under moderate salinity and 8.72 g/plant under

Table 2: Two-way ANOVA for agronomic traits under natural soil salinity

Source	df	Plant height	Boll weight	Seed cotton yield
Genotype (G)	16	F = 21.63***	F = 24.83***	F = 16.74***
Salinity (S)	2	F = 24.12***	F = 452.61***	F = 534.62***
G×S	32	F = 10.29***	F = 4.32***	F = 4.39***
Error	102	-	-	-

***p<0.001

Table 3: Two-way ANOVA for fiber-quality traits under natural soil salinity

Source	df	UHML	SFI	Strength
Genotype (G)	16	F = 28.74***	F = 41.58***	F = 32.19***
Salinity (S)	2	F = 395.62***	F = 512.41***	F = 476.83***
G×S	32	F = 4.81***	F = 6.14***	F = 5.47***
Error	102	-	-	-

UHML: Upper half mean length, SFI: Short fiber index and ***p<0.001

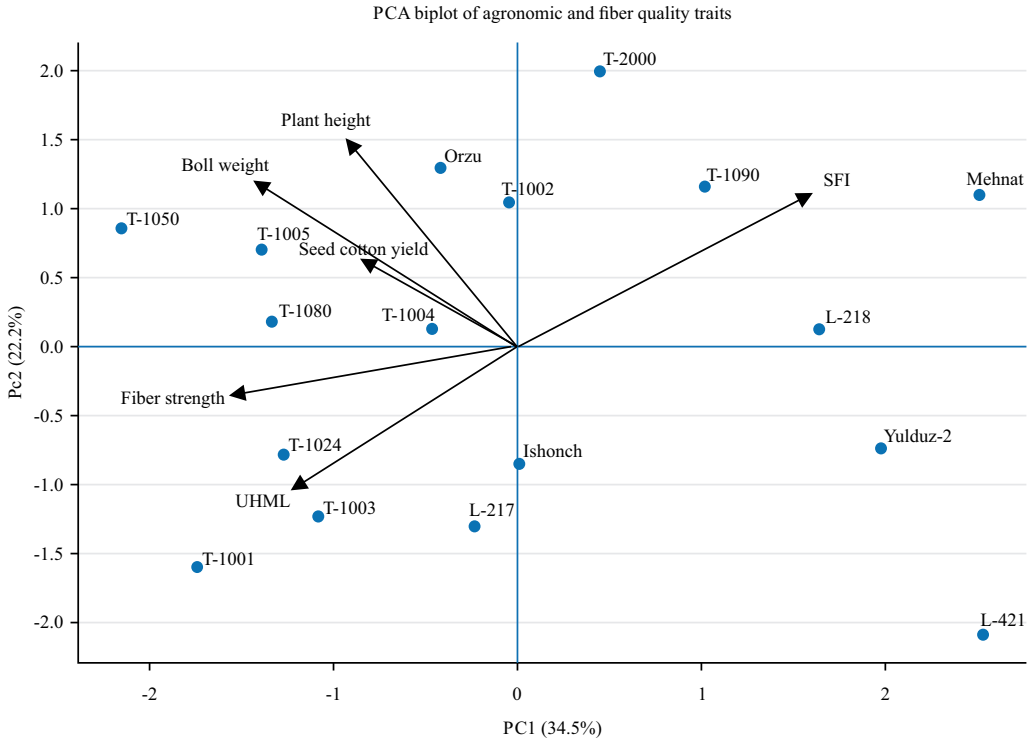


Fig. 1: PCA biplot integrating agronomic and fiber-quality traits across the 17 genotypes. PC1 and PC2 explain 34.5 and 22.2% of phenotypic variation, respectively

high salinity. The latter represented a 67.4% reduction relative to the control. Despite the substantial yield penalty imposed by salinity, Orzu, Mehnat, T-1090 and T-2000 exhibited comparatively better yield maintenance, indicating their potential value as parental materials for improving productivity under salt-affected conditions.

Fiber-quality response: Two-way ANOVA revealed highly significant effects of genotype (G), salinity (S) and their interaction (G×S) on all three fiber-quality traits, including Upper Half Mean Length (UHML), Short Fiber Index (SFI) and fiber strength ($p<0.001$; Table 3). Salinity exerted the strongest effect on these traits, with F-values of 395.62 for UHML, 512.41 for SFI and 476.83 for fiber strength. Significant genotypic effects were also detected for UHML ($F = 28.74$), SFI ($F = 41.58$) and fiber strength ($F = 32.19$). Moreover, the significant G×S interactions for UHML ($F = 4.81$), SFI ($F = 6.14$) and fiber strength ($F = 5.47$) indicated that the magnitude of fiber-quality responses to salinity differed among genotypes.

Consistent with these ANOVA results, SFI generally increased with increasing salinity, indicating a deterioration in fiber uniformity and quality. T-1003, T-1001 and T-1024 maintained comparatively stable SFI values, whereas Mehnat, L-218, T-1002 and T-1005 exhibited larger increases. The UHML generally decreased under salinity; however, T-1024, T-1050, T-1001 and L-217 maintained fiber length more effectively than the other genotypes. Fiber strength also declined with increasing salinity, although T-2000, T-1002, L-217 and T-1003 showed comparatively greater stability. These genotype-specific responses, together with the significant G×S interactions, demonstrate that tolerance to salinity cannot be evaluated on the basis of a single fiber trait and that simultaneous selection for productivity, fiber length, strength and short-fiber content is necessary when developing cotton cultivars for salt-affected environments.

Integrated phenotypic variation: Principal component analysis of the combined agronomic and fiber dataset separated productivity-related and fiber-related response patterns (Fig. 1). PC1 explained 34.5% and PC2 explained 22.2% of the phenotypic variation (56.7% jointly). Plant height, boll weight and seed cotton yield loaded in broadly similar directions, whereas UHML and fiber strength were opposed to SFI. T-2000 was positioned toward greater plant height, while T-1001 and T-1003 were aligned more closely with UHML and fiber strength. Mehnat and L-421 were displaced toward higher SFI, illustrating that productive or vigorous genotypes do not necessarily retain favorable fiber profiles under salinity.

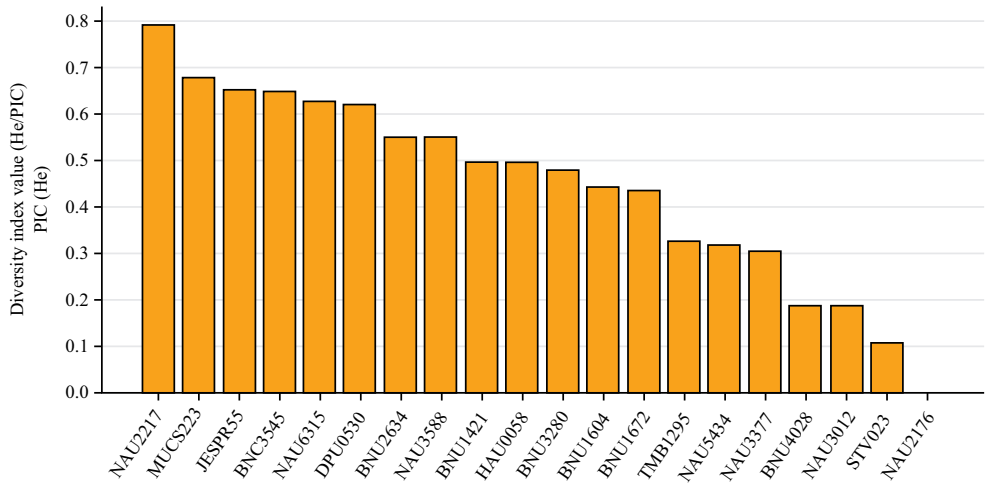


Fig. 2: Distribution of expected heterozygosity (He) and polymorphism information content (PIC) across SSR loci

Table 4: Genetic diversity parameters for the SSR loci

Locus	Na	Ne	He	PIC	PPL (%)
BNL1421	2	1.68	0.4	0.35	100
BNL1604	3	2.15	0.5	0.45	100
BNL1672	2	1.82	0.45	0.39	100
BNL2634	2	1.74	0.42	0.37	100
BNL3280	3	2.2	0.52	0.47	100
BNL3545	4	2.98	0.66	0.58	100
BNL4028	2	1.55	0.36	0.32	100
DPL0530	3	2.58	0.6	0.55	100
HAU0058	2	1.95	0.47	0.42	100
JESPR65	3	2.9	0.65	0.58	100
MUCS223	4	3.12	0.68	0.61	100
NAU2176	1	1.0	0.0	0.0	0
NAU2277	5	4.85	0.79	0.76	100
NAU3011	2	1.72	0.41	0.36	100
NAU3377	2	1.85	0.44	0.38	100
NAU3588	2	1.67	0.39	0.34	100
NAU5434	2	1.88	0.45	0.4	100
NAU6315	4	2.65	0.61	0.55	100
STV023	2	1.9	0.46	0.41	100
TMB1295	3	2.05	0.49	0.43	100

Na: Observed alleles, Ne: Effective alleles, He: Expected heterozygosity, PIC: Polymorphism information content and PPL: Locus polymorphism status

Table 5: Summary of genetic distance and molecular differentiation

Parameter	Value
Minimum genetic distance	0.214
Maximum genetic distance	0.762
Mean genetic distance	0.487
Broad molecular groups	3
Most similar pair	T-2000×T-1005
Most divergent pair	T-1004×Mehnat

SSR polymorphism and marker informativeness: The 20 SSR loci produced 53 alleles, averaging 2.65 alleles per locus. Nineteen loci were polymorphic (95%), whereas NAU2176 was monomorphic (Table 4). Across all loci, the mean expected heterozygosity (He) was 0.488 and the mean polymorphism information content (PIC) was 0.436. NAU2277 showed the greatest allelic richness (Na = 5) and the highest informativeness (PIC = 0.761), followed by MUCS223, BNL3545, JESPR65, NAU6315 and DPL0530. The distribution of He and PIC across the SSR loci further illustrates the differences in marker informativeness (Fig. 2). These results demonstrate moderate SSR diversity within the evaluated upland cotton germplasm, although marker informativeness itself does not establish a functional association with salinity tolerance.

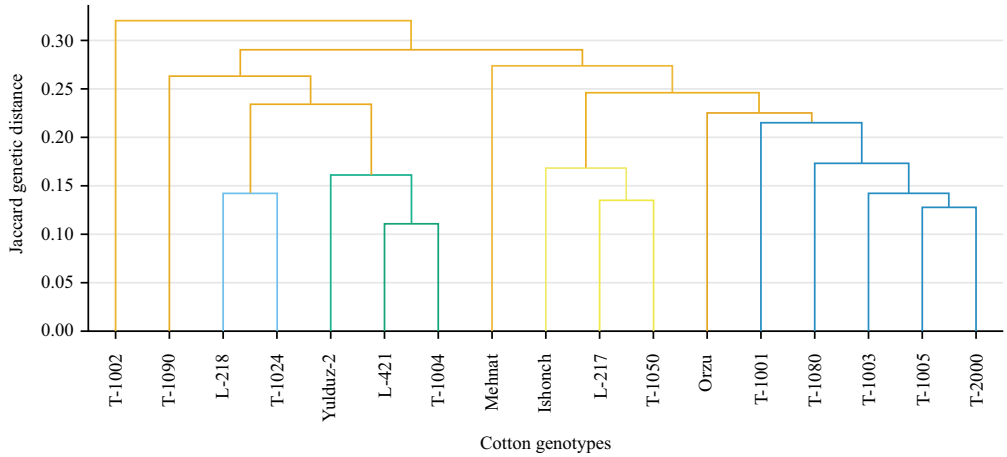


Fig. 3: UPGMA dendrogram of 17 upland cotton genotypes based on Jaccard distances from binary SSR profiles

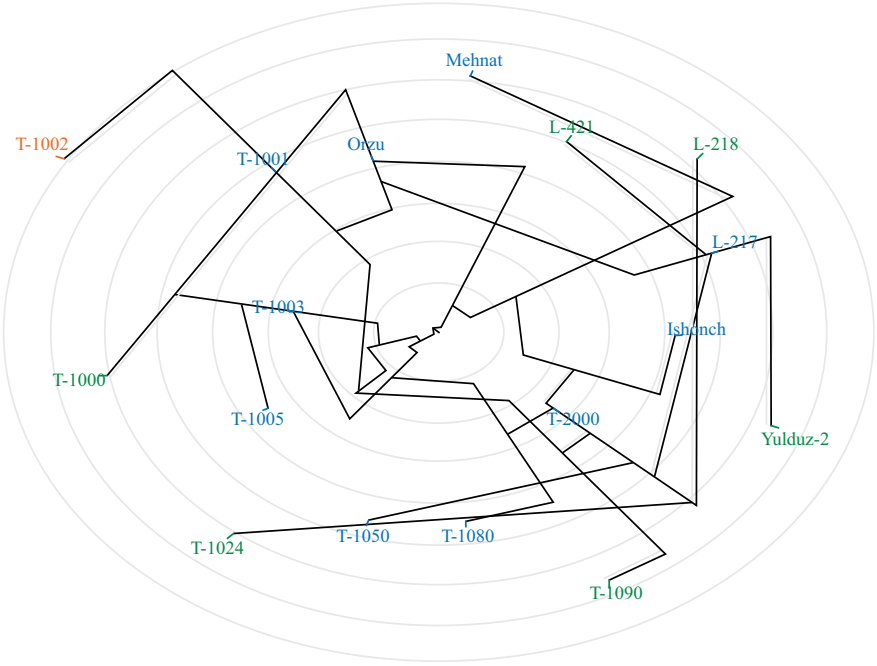


Fig. 4: Radial Neighbor-Joining representation of genetic relationships among the 17 genotypes

Genetic relationships and population structure: Pairwise Jaccard genetic distance averaged 0.487 and ranged from 0.214 to 0.762 (Table 5). T-2000×T-1005 was the most similar pair, followed by T-1080×T-2000 and T-2000×T-1003. T-1004×Mehnat was the most divergent pair, followed by T-1001×Orzu. UPGMA separated the panel into three broad groups. T-2000, T-1003, T-1005 and T-1080 occupied the core molecular group, while T-1002, Mehnat, Yulduz-2 and T-1004 were among the more peripheral materials. The UPGMA dendrogram (Fig. 3) and radial Neighbor-Joining representation (Fig. 4) supported broad differentiation, although the exact membership of branches varied with the ordination or clustering method.

The molecular PCA (Fig. 5) showed that the first two principal components explained 34.7% of allelic variation (PC1 = 20.4%; PC2 = 14.3%; Table 6). T-1002 occupied an isolated position, while several core genotypes clustered more closely. AMOVA assigned 42% of the molecular variation to differences among the three groups and 58% within groups (Table 7). The fixation index was high (ΦST = 0.42, $p<0.001$) and estimated gene flow was low (Nm = 0.35), consistent with differentiated breeding backgrounds.

A complementary principal coordinate analysis based on the same Jaccard matrix (Fig. 6) showed Mehnat, T-1002 and several L-series materials at relatively isolated positions, while T-2000, T-1003, T-1005 and Orzu occupied a more

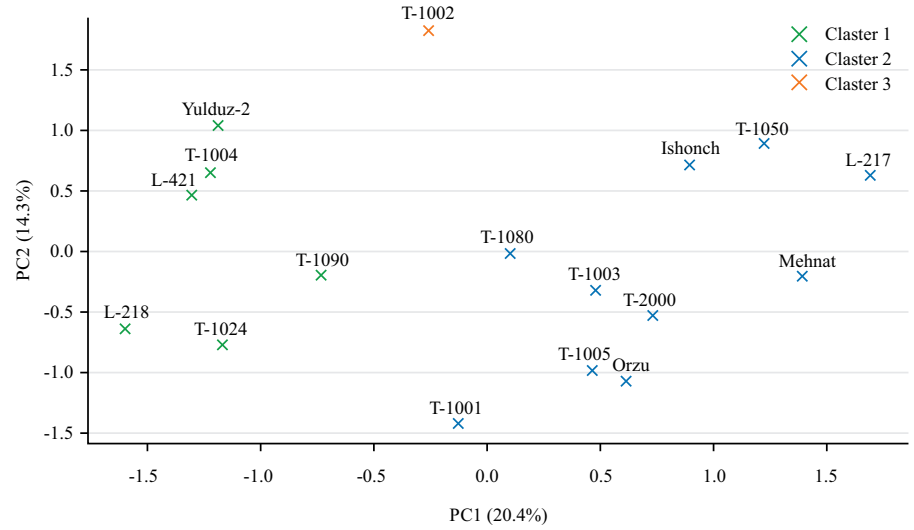


Fig. 5: Molecular PCA of 17 upland cotton genotypes; PC1 and PC2 explain 20.4 and 14.3% of variation, respectively

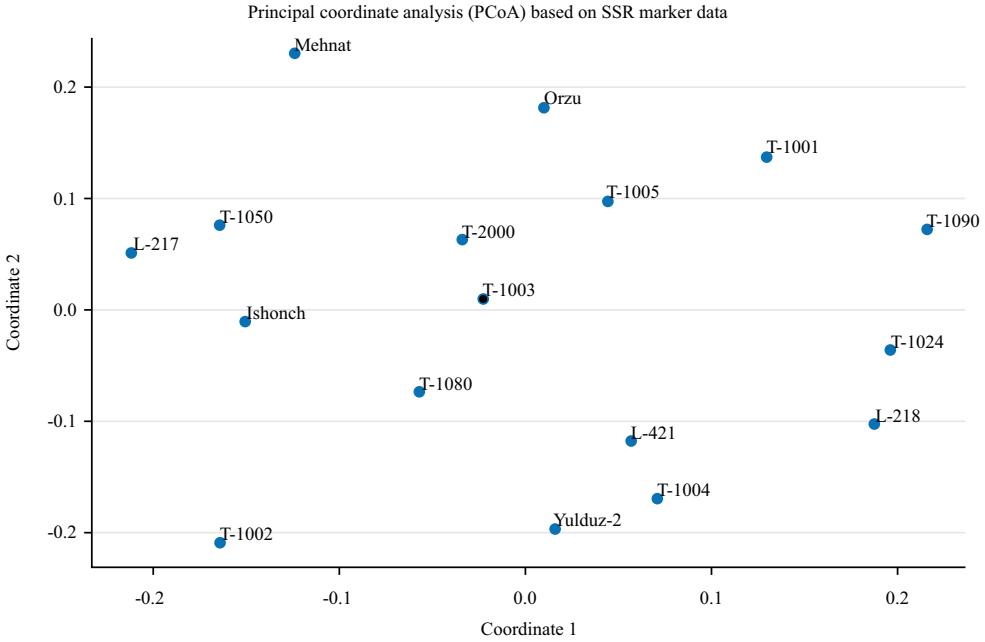


Fig. 6: Principal coordinate analysis of the 17 genotypes based on Jaccard distances calculated from binary SSR allele profiles

Table 6: Molecular principal components

Component	Eigenvalue	Variance (%)	Cumulative (%)
PC1	4.08	20.4	20.4
PC2	2.86	14.3	34.7
PC3	2.14	10.7	45.4
PC4	1.68	8.4	53.8

Table 7: Analysis of molecular variance among SSR-defined groups

Source	df	Sum of squares	Variance component	Variation (%)	Φ-statistic
Among groups	2	86.74	4.21	42.0	ΦST = 0.42***
Within groups	14	81.57	5.81	58.0	-
Total	16	168.31	10.02	100	-

***p<0.001 based on permutation testing

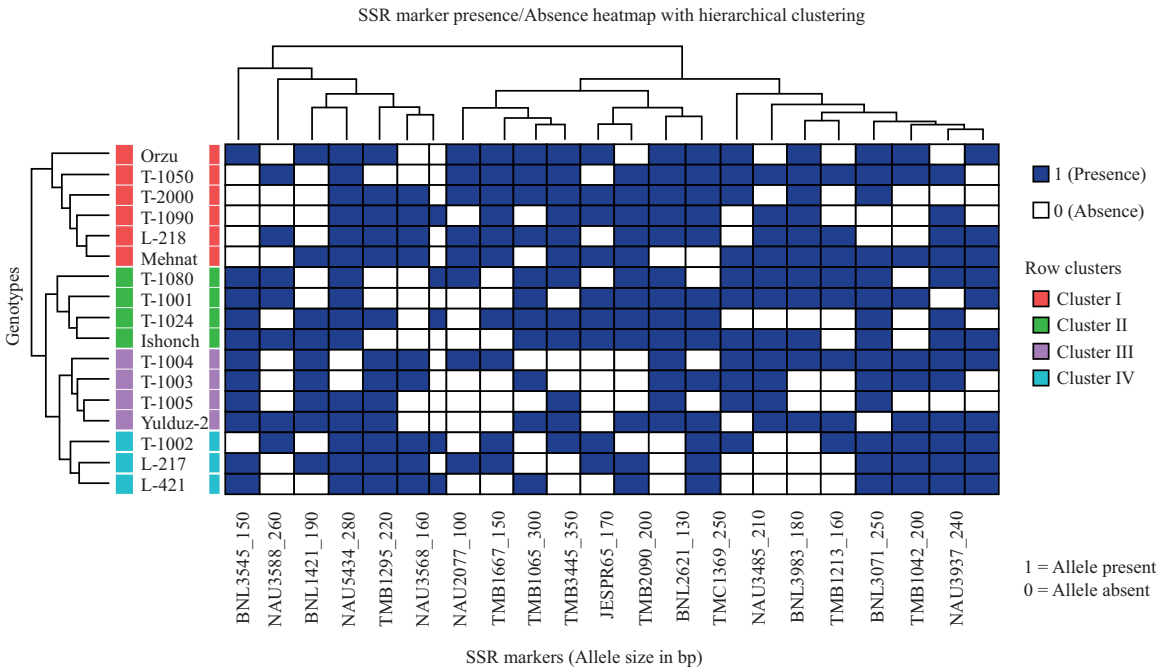


Fig. 7: Hierarchical heatmap of SSR allele presence (blue) and absence (white), showing four fine-scale genotype subclusters

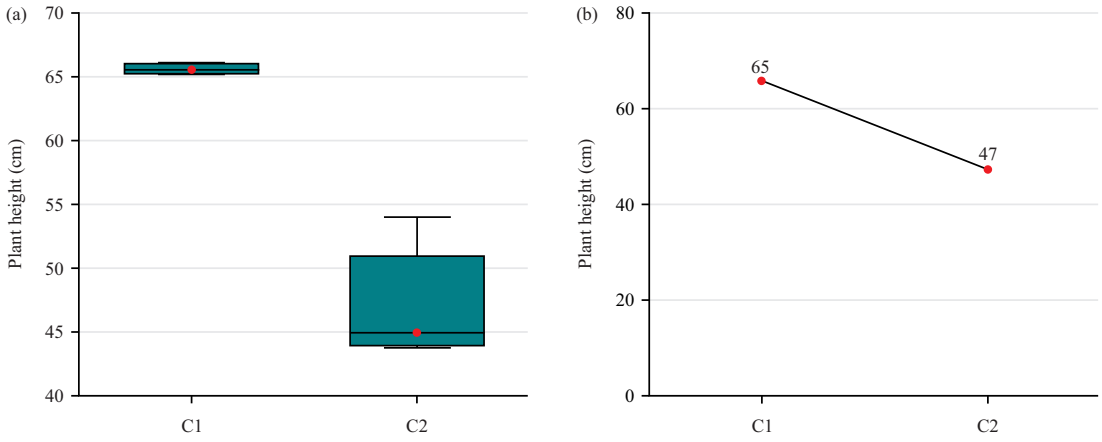


Fig: 8(a-b): (a) Distribution of plant height for genotypes carrying (C1) or lacking (C2) the NAU3588 260-bp allele and (b) Mean comparison of plant height between genotypes carrying (C1) and lacking (C2) the NAU3588 260-bp allele

central region. Hierarchical analysis of the allele-by-genotype matrix resolved four finer subclusters within the three broad groups (Fig. 7). This distinction reflects analytical resolution: The broad UPGMA/NJ structure comprised three major groups, whereas the heatmap subdivided the collection at a finer cut level. Allele-presence patterns at BNL3545, NAU3588, BNL1421, NAU5434 and TMB1295 contributed visibly to genotype separation.

Candidate SSR allele-trait relationships: Seven nominal relationships involving six loci connected SSR allelic states with agronomic or fiber measurements under saline conditions (Table 8). The NAU3588 260-bp allele was associated with both greater plant height (65.0 vs 47.0 cm) and fiber strength (31.0 vs 28.0 g/tex). BNL3545 150 bp was associated with greater boll weight, while BNL1421 190 bp and NAU5434 280 bp were associated with greater seed cotton yield. The TMB1295 180/220-bp profile was associated with a small increase in UHML. Conversely, JESPR65 170 bp was associated with a higher SFI and was unfavorable in this panel.

NAU3588 associations with plant height and fiber strength: Carriers of the NAU3588 260-bp allele averaged 65.0 cm in plant height under salinity, compared with 47.0 cm in

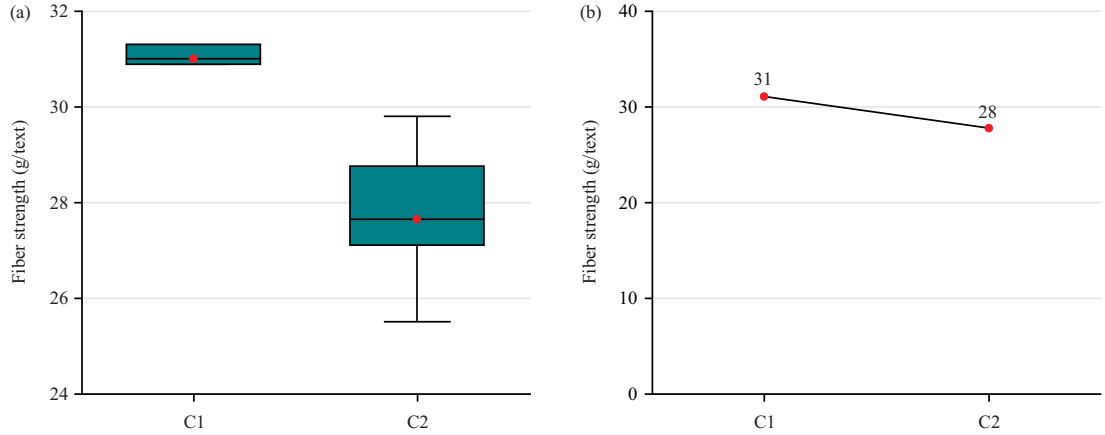


Fig. 9(a-b): (a) Distribution of fiber strength for genotypes carrying (C1) or lacking (C2) the NAU3588 260-bp allele and (b) Mean comparison of fiber strength between genotypes carrying (C1) and lacking (C2) the NAU3588 260-bp allele

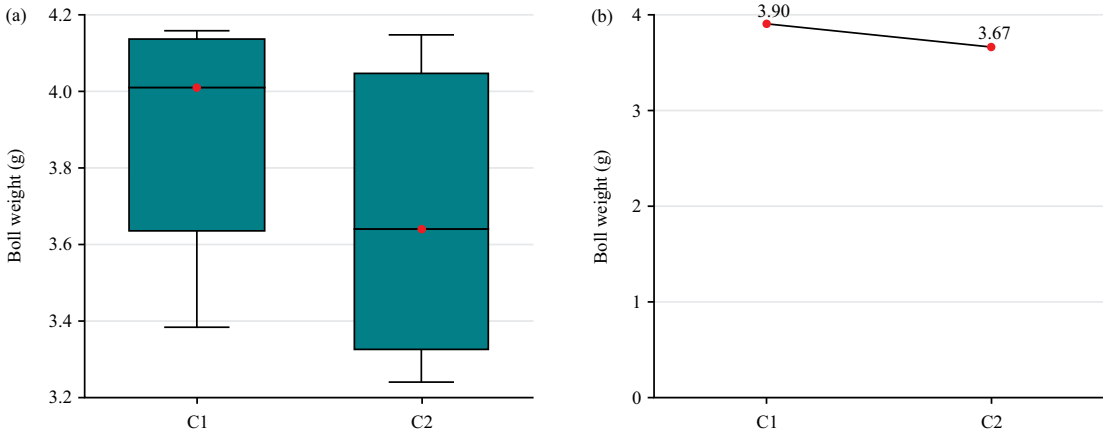


Fig. 10(a-b): (a) Distribution of boll weight for carriers (C1) and non-carriers (C2) of the BNL3545 150-bp allele and (b) Mean comparison of boll weight between carriers (C1) and non-carriers (C2) of the BNL3545 150-bp allele

Table 8: Candidate allele-trait relationships detected in the 17-genotype panel

Locus	Allelic state	Trait	Carrier vs non-carrier mean	Direction
NAU3588	260 bp	Plant height	65.0 vs 47.0 cm	Favorable
BNL3545	150 bp	Boll weight	3.90 vs 3.67 g	Favorable
BNL1421	190 bp	Seed cotton yield	9.3 vs 6.9 g/plant	Favorable
NAU5434	280 bp	Seed cotton yield	10.5 vs 8.3 g/plant	Favorable
JESPR65	170 bp	Short fiber index	9.4 vs 8.0%	Unfavorable
NAU3588	260 bp	Fiber strength	31.0 vs 28.0 g/tex	Favorable
TMB1295	180/220 bp	UHML	1.07 vs 1.03 inches	Favorable

Relationships were detected at the nominal threshold (unadjusted $p<0.05$) and FDR-adjusted P-values were not available in the source analyses; therefore, these signals are candidates, not validated diagnostic markers

non-carriers, an absolute difference of 18.0 cm (38.3%; Fig. 8a-b). The same allelic state was associated with mean fiber strength of 31.0 g/tex versus 28.0 g/tex (Fig. 9a-b). The parallel favorable pattern makes NAU3588 a priority candidate, but the two responses must be validated independently because the signal may reflect linkage or genetic background rather than pleiotropy.

Yield-component associations: The BNL3545 150-bp allele was associated with a modest increase in boll weight: Carriers averaged 3.90 g and non-carriers 3.67 g (6.3% difference; Fig. 10a-b). For seed cotton yield, BNL1421 190-bp carriers averaged 9.3 g/plant compared with 6.9 g/plant in non-carriers (Fig. 11a-b). NAU5434 280-bp carriers averaged 10.5 g/plant compared with 8.3 g/plant (Fig. 12a-b). These effects identify loci for targeted validation in segregating populations rather than markers ready for direct deployment.

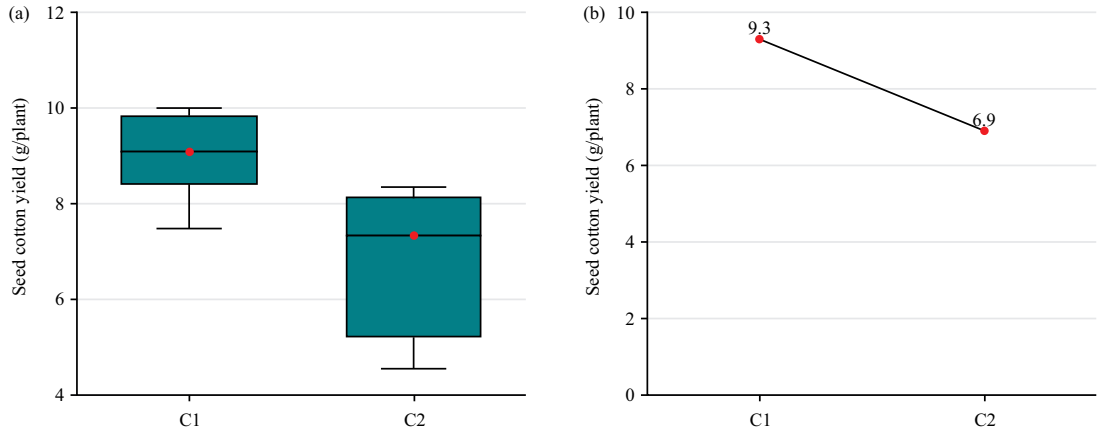


Fig. 11(a-b): (a) Distribution of seed cotton yield for carriers (C1) and non-carriers (C2) of the BNL1421 190-bp allele and (b) Mean comparison of seed cotton yield between carriers (C1) and non-carriers (C2) of the BNL1421 190-bp allele

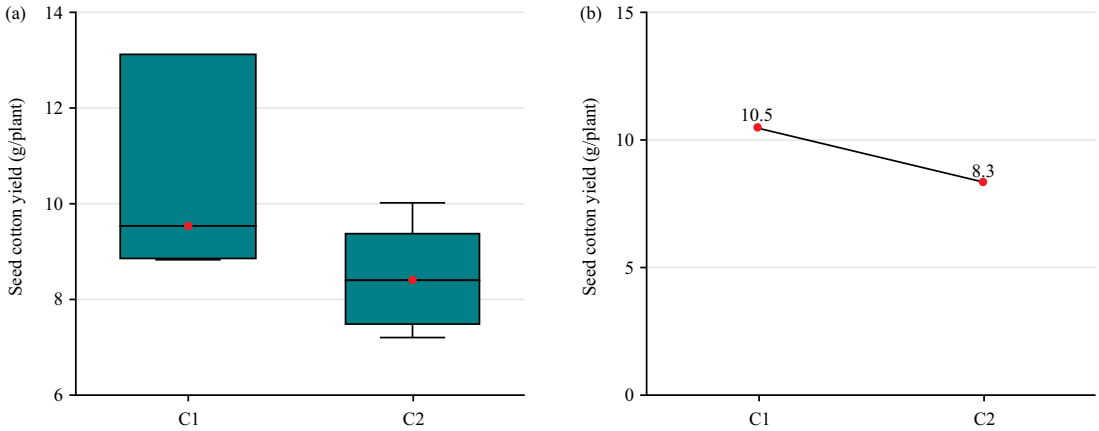


Fig. 12(a-b): (a) Distribution of seed cotton yield for carriers (C1) and non-carriers (C2) of the NAU5434 280-bp allele and (b) Mean comparison of seed cotton yield between carriers (C1) and non-carriers (C2) of the NAU5434 280-bp allele

Fiber-length and short-fiber associations: The TMB1295 180/220-bp profile was associated with UHML of 1.07 inches in carriers and 1.03 inches in non-carriers (approximately 3.9% difference; Fig. 13a-b). Although small, this difference may be technologically relevant if stable across environments. In contrast, carriers of JESPR65 170 bp had an SFI of 9.4%, compared with 8.0% among non-carriers, an unfavorable increase of 17.5% (Fig. 14a-b). Absence of this JESPR65 allele was therefore the favorable state in the current panel.

DISCUSSION

The sharp reduction in yield relative to plant height shows that reproductive processes were more sensitive to salinity than vegetative stature. Osmotic restriction and ion imbalance can limit assimilate supply to developing bolls and reduce both boll filling and final yield[1,2,16]. Significant G×S effects confirm that genotype rankings depend on salinity intensity. Orzu, Mehnat, T-1090 and T-2000 merit further evaluation as productivity donors, whereas T-1001, L-217 and T-1024 contribute fiber stability. Crosses between these groups may combine yield retention with acceptable technological quality.

The detection of 53 alleles and mean PIC of 0.436 indicates moderate SSR diversity comparable to cultivated upland cotton panels reported elsewhere[3,4,17-19]. NAU2277 and MUCS223 were especially informative for genotype discrimination. However, informativeness should not be confused with trait linkage: NAU2277 was the best diversity marker but was not among the candidate trait-associated loci.

Concordance among genetic distances, UPGMA, Neighbor-Joining, PCA and AMOVA supports real differentiation within the collection. The relatively high among-group component and low Nm suggest distinct breeding backgrounds, while the larger within-group component preserves opportunities for selection. Crosses should balance divergence and phenotype. For example, a

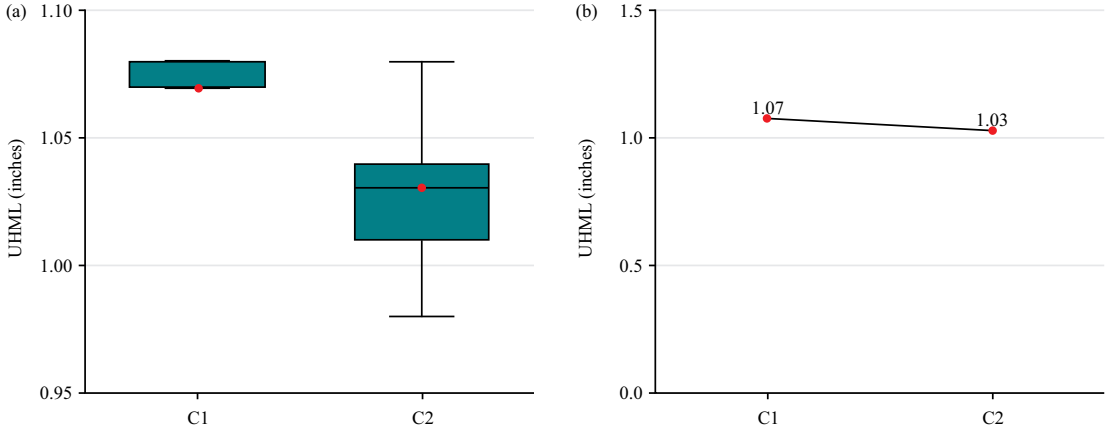


Fig. 13(a-b): (a) Distribution of UHML for genotypes with (C1) or without (C2) the TMB1295 180/220-bp profile and (b) Mean comparison of UHML between genotypes with (C1) or without (C2) the TMB1295 180/220-bp profile

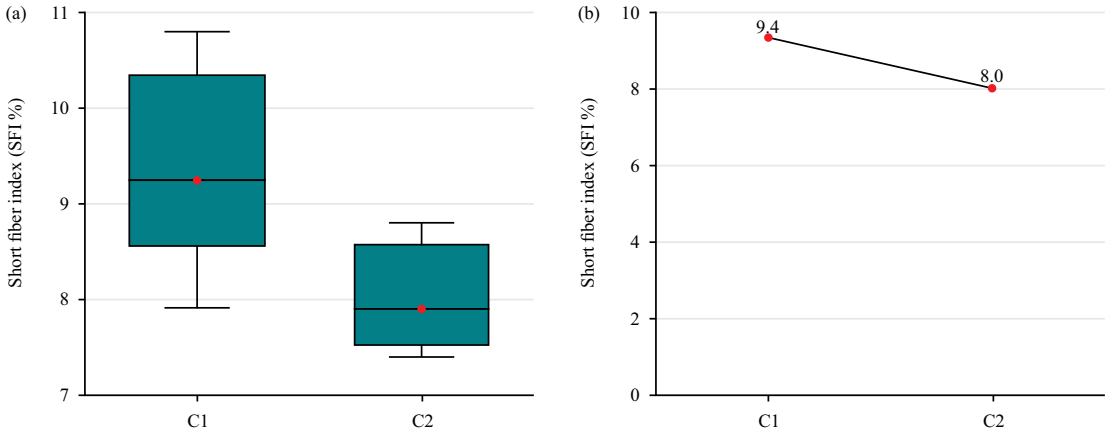


Fig. 14(a-b): (a) Distribution of SFI for genotypes carrying (C1) or lacking (C2) the JESPR65 170-bp allele and (b) Mean comparison of SFI between genotypes carrying (C1) and lacking (C2) the JESPR65 170-bp allele

high-yield-maintaining parent such as T-2000 or Mehnat could be combined with a fiber-stable parent such as T-1001, L-217, or T-1024. T-1002, T-1004, Mehnat and Yulduz-2 may broaden the genetic base, but divergence alone does not guarantee favorable salinity response.

The candidate alleles provide hypotheses for validation rather than ready-to-use selection assays[20-24]. NAU3588 is notable because the 260-bp allele was related to two distinct traits, yet pleiotropy cannot be inferred from this small panel. The pattern could reflect linkage disequilibrium, population structure, or shared ancestry. Similarly, the yield effects at BNL1421 and NAU5434 and the fiber effects at TMB1295 and JESPR65 require confirmation in segregating populations and independent environments. Multiple-testing correction is particularly important: Nominal $p<0.05$ associations can include false positives when many alleles and traits are examined.

A practical validation pipeline would genotype the candidate alleles in larger biparental or association populations, fit mixed models accounting for kinship and structure, report both raw and FDR-adjusted probabilities and test allele effects across locations and years. Markers that retain direction and effect size should then be converted to robust PCR or KASP assays and evaluated for selection efficiency in early generations.

The study used only 17 genotypes and two field replications, limiting statistical power and control of genetic background. Natural field salinity improves agronomic realism but may include spatial heterogeneity and correlated soil factors. The 20 candidate SSR loci do not constitute genome-wide coverage. Group definitions used in AMOVA were inferred from the same marker data and estimated Nm is therefore descriptive rather than a direct measure of contemporary migration. Finally, FDR-adjusted association statistics were not available. These limitations do not negate the observed diversity and field contrasts, but they constrain causal and predictive interpretation.

CONCLUSION

Integrated phenotypic and SSR analyses revealed substantial variation in the response of Uzbek upland cotton germplasm to natural soil salinity. Orzu, Mehnat, T-1090 and T-2000 retained comparatively higher productivity, while T-1001, L-217 and T-1024 contributed stability for selected fiber characteristics. The marker panel detected 53 alleles, with NAU2277, MUCS223, BNL3545, JESPR65, NAU6315 and DPL0530 providing high discrimination. Three broad genetic groups, ΦST = 0.42 and Nm = 0.35 demonstrated differentiated breeding backgrounds.

Candidate favorable signals were detected at NAU3588, BNL3545, BNL1421, NAU5434 and TMB1295, whereas JESPR65 170 bp was associated with an unfavorable increase in SFI. These alleles should not yet be used as diagnostic markers; larger structured populations, multiple environments and FDR-controlled analyses are required. The strongest immediate breeding strategy is to cross phenotypically complementary and genetically divergent parents, followed by validation of the candidate loci in their segregating progeny.

SIGNIFICANCE STATEMENT

This study provides integrated phenotypic and molecular evidence for salinity adaptation in Uzbek upland cotton genotypes. The identified diverse and comparatively stable genotypes, together with candidate SSR allele-trait relationships, provide useful preliminary resources for cotton breeding under saline conditions. Further validation across larger populations and environments is needed to confirm their breeding value.

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