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Association Mapping of Heat Tolerance SNPs in Upland Cotton During Vegetative Growth

Yusuf Güzel DEMİRAY [1]*, Remzi EKİNCİ [2], Adem BARDAK [3]

[1] *GAP International Agricultural Research and Training Center, Department of Field Crops, 21280, Diyarbakır, Türkiye*
[2] *Dicle University, Faculty of Agriculture, Department of Field Crops, 21280, Diyarbakır, Türkiye*
[3] *Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Agricultural Biotechnology, 46100, Kahramanmaraş, Türkiye*

✉ *Corresponding author: yusuf8255@hotmail.com*

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ABSTRACT

This study aimed to identify genomic regions associated with high-temperature stress tolerance in upland cotton, particularly during the vegetative period of this crop, which is increasingly affected by climate change. Understanding the genetic basis of thermotolerance is essential for improving resilience and sustaining yield under heat stress conditions. A total of 94 upland cotton genotypes were subjected to high-temperature stress in a low tunnel environment for four consecutive days during peak flowering. Physiological parameters, including relative cell injury (RCI), leaf temperature (LT), and SPAD chlorophyll values, were measured before and after the stress treatment to assess the genotypic response. Genotyping by sequencing (GBS) was used to detect single nucleotide polymorphisms (SNPs), and 6670 high-quality markers (MAF < 0.05) were retained for association mapping. Association analyses were conducted using general and mixed linear model (GLM-MLM) approaches. SNP markers associated with heat tolerance were identified using GLM and MLM models at significance levels of $p < 0.0001$–0.001 with a –Log10(P) threshold ≥ 2.5, and MLM results were validated using false discovery rate (FDR) correction. Four SNPs on chromosomes A07, A10, D03, and D09 (SNP1680, SNP2537, SNP4374, SNP6415) were linked to RCI; four SNPs on D05, D07, A10, and D08 (SNP5029, SNP5643, SNP2616, SNP5915) were associated with LT; and two SNPs on D12 and A08 (SNP7428, SNP1910) were related to SPAD chlorophyll content. These markers correspond to genomic regions encoding enzymes, proteins, and genes implicated in high-temperature stress responses in cotton (*Gossypium hirsutum* L.).

1. INTRODUCTION

Although cotton is a raw material for many industries, it is a strategic product worldwide, especially for fiber production. In 2022, a total of 24.9 million tons (MT) of cotton fiber was produced in the world. China (5.7 MT), India (5.2 MT), the USA (3.8 MT), Brazil (2.3 MT), Australia (1.2 MT), Pakistan (1.2 MT) and Turkey (0.8 MT) have the largest share in this production (ICAC, 2022). In addition to the fiber production that cannot meet the supply in the face of rapidly increasing population density, worse scenarios are expected with the climate crises experienced in the world. The IPCC (intergovernmental panel on climate change) reports indicate that global warming, an underlying cause of heat stress, is expected to raise air temperatures by about 0.2°C each decade, with a total projected increase of 0.5 to 5.4°C between 2020 and 2080 (IPCC, 2007; 2018). Research has demonstrated that rising temperatures expedite plant growth and developmental processes (Ziska and Bunce 1997). Nevertheless, these studies have also highlighted that when temperatures surpass favorable limits, they can exert adverse effects on overall crop performance (Ahmad et al., 2020). When plants exceed their ideal temperature range for their lifespan, researchers have stated that heat stress can cause some irreversible changes in plants. (Demiray et al., 2023). In the studies conducted, it was understood that the temperature requirements of different periods of cotton are various. Nevertheless, they emphasized that the optimal temperature values for the initial developmental stages of cotton are 30/22°C daytime/night (Demiray et al., 2023).

To meet the cotton fiber supply in today's technology, the importance of breeding varieties that are less affected by abiotic environmental conditions by using molecular techniques together with the classical breeding method is increasing. Although studies on the genetic basis of heat tolerance in cotton have increased in recent years, genome-wide association analyses specifically targeting the vegetative stage remain limited. Marker-based approaches such as QTL mapping and genome-wide association analysis have previously been shown to be effective tools for dissecting complex quantitative traits in cotton, enabling the linkage of phenotypic variation with underlying genetic regions (Semizer-Cuming et al., 2015). Rani et al. (2022) conducted QTL mapping in a *Gossypium hirsutum* F_2 population and identified 17 QTLs for 23 morpho-physiological traits related to heat stress, explaining 7.8–36.6% of the phenotypic variance. Salman et al. (2019) and Khan et al. (2017) highlighted the genetic effects contributing to high-temperature tolerance in critical physiological traits, such as relative cell injury (RCI), and thoroughly characterized their inheritance patterns. Bardak et al. (2021) identified 18 QTLs associated with leaf and stem disease indices in Verticillium wilt, demonstrating that these QTLs were linked to the small nucleolar RNA U3 gene, which plays a role in responses to both biotic and abiotic stresses. In a comprehensive GWAS and transcriptome study, Ma et al. (2021) analyzed 218 cotton accessions, identifying three heat tolerance loci and associated candidate genes (e.g., GhHRK1), with functional validation of these regions. In Turkey, Demiray et al. (2024) evaluated heat tolerance during the vegetative stage across different cotton genotypes, using indicators such as cell membrane thermostability and leaf temperature, and observed significant variation among genotypes. At the molecular level, previous studies have identified genes that contribute to heat tolerance in cotton and other plants. Yang et al. (2010) reported that the glycerol-3-phosphate 2-O-acyltransferase 6-like gene encodes an enzyme involved in forming a protective polyester layer on plant surfaces, enhancing resistance to environmental stress. Liao et al. (2017) demonstrated that the FERONIA (FER) gene functions in growth regulation, stress adaptation, and cell expansion. Chen et al. (2009) showed that callose synthase 5 contributes to mechanisms associated with both biotic and abiotic stress responses through callose production. Serine/threonine protein kinases, particularly the With-No-Lysine (WNK) kinase subfamily, are implicated in abiotic stress responses and circadian rhythms (Zhang et al., 2023). Wei et al. (1998) reported that the 26S proteasome interacts with the COP9 complex to regulate photomorphogenesis and various cellular signaling pathways. Luo et al. (2010) highlighted the role of GATA transcription factors in light-dependent processes, such as flowering, maturation, and petal development. Moreover, Gachomo et al. (2013) found that Angustifolia mutants with modified C-terminal binding proteins (CtBP) exhibit altered responses to both biotic and abiotic stresses. While these studies provide valuable insights into the genetic and molecular responses of cotton to high temperatures, high resolution SNP based genome wide analyses at the vegetative stage remain scarce. Therefore, the present GBS based GWAS on 94 cotton genotypes, evaluating key physiological traits such as RCI, leaf temperature, and SPAD, enables the identification of candidate gene regions and the discovery of SNP markers for marker assisted selection, representing a critical contribution to cotton breeding under heat stress conditions.

2. MATERIALS AND METHODS

The study included samples from 94 genotypes of cotton (*G. hirsutum* L.), including 88 domestic and foreign cultivars and 6 controls (C1: Tamcot Sphinx, C2: SJ-U86, C3: AGC 208, C4: ST 468, C5: ST 474, and C6: Carmen).

Table 1. Details on the genotypes of cotton used as plant materials.

Material Resource Institutions	Names of Cotton Varieties
USA	Tamcot Sphinx, SJU 86, AGC208
BASF Industry Trade L.C.	Fiona, Carla, ST 498, STV468, Carmen
Bayer Industry L.C.	Claudia, Gloria, Candia, Flora
Birlik Seed Industry Trade L.C.	Bir 781, Bir 949, Cosmos, Bir 138
Caso Seed Industry Trade L.C.	Caso 9048
DAGTAEM	Furkan
DATEM	TYA 193, Ceykot 340, TYA 366, ADN 701, MAY 355, MAY 455, MAY 505, TMK122, TMN18, MAY 344, Nihal, ADN 413, ADN 710, ADN 712, ADN 123, ADN 811, Gelincik, Sarıgelin, Çukurova 1518, Bossa 159, Teksa 415, Yıldırım 63, Ayzek 595, Gapkot 732, Ceykot 92
GAPTAEM	ZN 243
GAPUTAEM	Kartanesi
Golden West Seed Trade L.C.	Optasia, Esperia, Bomba, GW2345, Babylon, Famosa, Fantom, Penta (Golda), Primera
Livagro Agriculture Seed L.C.	Zara
May-Agro Industry Trade L.C.	Gaia, ST 474, MAY 404
Monsanto Industry Trade L.C.	DP 332, ST 478, DP 396, DP 499, SG 125
Özaltın Agri. Industry Trade L.C.	Lodos, Ozaltin 404, Ozaltin 112
Özbuğday Agri. Processing and Seed	Lider (Mig 119), Diva (Teks)
PAEM	SC 2009, SC 2079, Efe, Ergüven, Harem 1, Harem 2, ES 1, ES 2, Sezener 76, Özbek 105, İpek 607, Gürelbey, Aydın 110, Şahin 2000
Progen Seed Inc.	Kaira, Lima, Astoria, Edessa, BA 440, Carisma, PG 2018, BA 525, Flash
Tiriyo Seed L.C.	Zena 1010, Zena 1040, Zena 1018

DAGTAEM (East Mediterranean Transitional Zone Agricultural Research of Institute), DATEM (Eastern Mediterranean Agricultural Research Institute), GAPTAEM (GAP Agricultural Research Institute), GAPUTAEM (GAP International Agricultural Research and Training Center), PAEM (Cotton Research Institute)

The experiment was conducted in 2020 at the experimental area of the GAP International Agricultural Research and Training Center, using an augmented trial design with four blocks. The experiment was sown with plots of two rows each, 4 m long, 70 cm between rows, and 15-20 cm between plant rows. The experiment, which was established under field conditions, was taken into a low tunnel greenhouse for 4 days during the intense flowering period of cotton, and high-temperature shock treatment (HTST) was applied. A thermometer was placed inside the low tunnel. The maximum temperature inside the low tunnel was accepted as 50°C. When the temperature surpassed this threshold, the sides of the low tunnel were opened to help reduce the internal temperature. Phenotypic values for the traits examined in the study were taken as control before the application and stress after the application. The phenotypic data were analyzed using the augmented experimental design approach described. Genotyping by sequencing (GBS) analysis was conducted by Diversity Arrays Technology (Australia) following the protocols outlined by Elshire et al. (2011) and Poland et al. (2012). The conditional probabilities of population structure were calculated using the STRUCTURE software (version 2.3.4; Pritchard et al., 2000). To determine the appropriate number of clusters using the STRUCTURE 2.3.4 software, the K value was tested from one to ten. The permutation module was set between 50.000 and 100.000 iterations, and each K value was replicated three times to calculate the Delta K value. The ideal Delta K value was determined by loading Python-based software "Structure Harvester v.0.6.94" and Q-matrix data were obtained (Evanno et al. 2005). The association between SNP markers for the traits analyzed was determined using TASSEL 5.0 software (Bradbury et al., 2007). Association analyses were performed in GLM (Q matrix, phenotypic data and genotypic data) and MLM (Q matrix, phenotypic data, genotypic data and kinship data). Abdurakhmonov et al. (2009) stated that markers identified by both GLM and MLM approaches are suitable for use as QTLs. These highly significant and important SNP markers were determined using -log10(P-Value) ≥ 2.5 as defined by the researchers, based on previous association studies using comparable population sizes and marker densities in cotton (Shi et al., 2024; Meng-wei et al., 2023). Moreover, the threshold was supported by subsequent FDR correction (Benjamini and Hochberg, 1995) within the MLM model to minimize false positive associations. Therefore, this criterion was considered appropriate for the present dataset. The significance threshold for each identified marker was set as –log10(P-value) ≥2.5, based on the P-values derived from association analysis. The FDR (false discovery ratio) described by Benjamin and Hochberg (1995) was performed using the SAS Multisets procedure (SAS Institute Inc., 2020) to determine the statistical significance threshold (α=0.05). A blast search of the sequences of the associated

markers with major QTL at National Center for Biotechnology Information (NCBI) and their presence on protein coding genes in the cotton genome was detected (Anonymous, 2023).

3. RESULTS

In the study, those with a minor allele frequency value of less than 5% (MAF < 0.05) were extracted and high-quality robust SNPs were obtained using TASSEL 5.2.2, resulting in 6.670 reliable SNPs out of 8.708 SNPs. Cluster analysis was performed using the STRUCTURE 2. 3.4 program based on the Bayesian method (Pritchard et al., 2000). At the end of the analysis, the results obtained were uploaded to the "Structure Harvester" program and calculations were made, and it was seen that the most appropriate Delta K value was 5 (Figure 1).

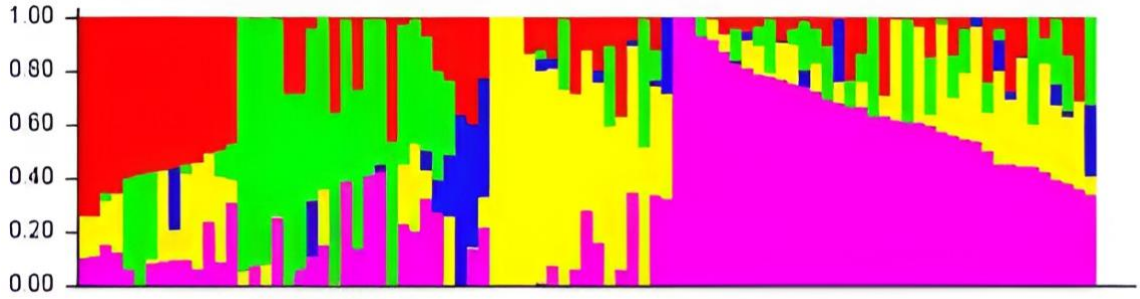

Figure 1. Population structure of 94 cotton genotypes based on 6670 strong SNPs (Delta K=5)

As a result of 22 million 247 thousand 785 comparisons, 0.70% at $P < 0.01$ and 0.59% at $P < 0.001$ were found to be highly significant. The number of R^2 0.1 in the total genome was 0.22% and $R^2 \geq 0.2$ was 0.05%. It can be said that 0.05% of the markers used were within 6-8 cM and 0.22% were within 10 cM of each other (Figure 2). Although Whitt and Buckler (2003) reported that LD decay decreases at a map distance of 25 cM at $R^2 \geq 0.1$, Abdurakhmonov et al. (2009) observed a 50 cM gap between microsatellites, which is in line with our results.

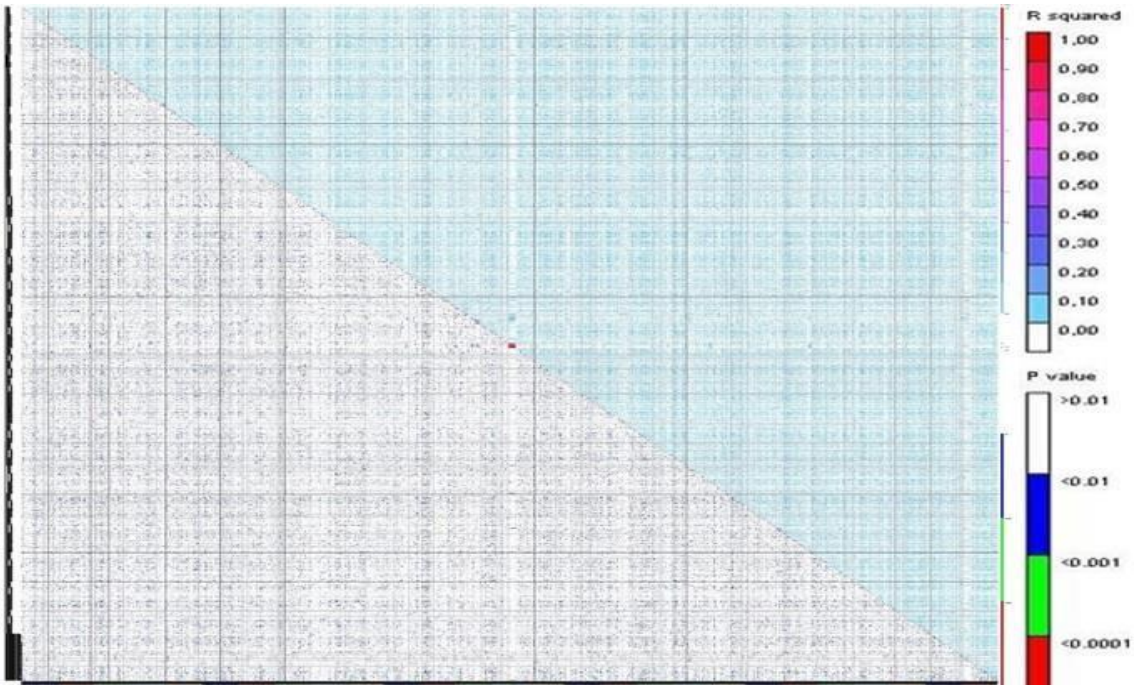

Figure 2. Linkage disequilibrium (LD) plot of SNPs Markers

Using the –log10(P-value) ≥2.5 criterion, SNP markers associated with high temperature tolerance across all traits were analyzed for significance within the range of P-values less than 0.001 and 0.0001. It was also analyzed by increasing the accuracy with false discovery ratio (FDR). According to GLM analysis results for the RCI trait, P values ranged between 0.0007 and 0.0070; R^2 values ranged between 0.0801 and 0.1391; -log10 (P-Value) ≥2.5 values ranged between 2.70 and 3.14. According to MLM results, P value varied between 0.0005 and 0.0067; R^2 values varied between 0.0105 and 0.1294; -log10 (P-Value) ≥2.5 values varied between 2.50 and 3.22. In terms of the trait analyzed, 4 markers were identified by GLM method and - log10 (P-Value) ≥2.5 condition, while 8 SNP markers were identified by the MLM method. In both methods, 2 strong SNPs (SNP1680, SNP2537 markers) that were common and met the –log10(P-Value) ≥2.5 condition were identified (Table 2). Chromosome, gene ID and nucleotide sequences of the detected markers are given in Table 3. In addition, Manhattan Plot and QQ Plot graphs related to high temperature tolerance determined by the GLM method according to the RCI trait are shown in Figure 3.

Table 2. Key attributes of SNP markers significantly associated with relative cell membrane injury (RCI, %) in cotton under high-temperature stress

Cell Membrane Injury (RCI) (%)									
Marker	Chr	Position (cM)	General Linear Model (GLM)			Mixed Linear Model (MLM)			
			P Value	R^2	-log10 (P-Value)	P Value	R^2	-log10 (P-Value)	FDR
SNP1680	A07	196	0.00161	0.10577	2.79	0.00317	0.010565	2.50	0.0031
SNP2537	A10	207	0.00105	0.11195	2.97	0.000598	0.08263	3.22	0.0031
SNP4374	D03	75	0.00374	0.12478	-	0.00284	0.1294	2.54	0.0031
SNP6415	D09	159	0.00701	0.09023	-	0.00267	0.12232	2.57	0.0031
SNP3092	A11	416	0.00605	0.08015	-	0.00248	0.06312	2.6	0.0031
SNP4311	D03	12	0.00332	0.09092	-	0.00218	0.015875	2.66	0.0031
SNP6534	D09	278	0.00198	0.13913	2.70	0.00583	0.12628	-	-
SNP5139	D05	426	0.000717	0.11985	3.14	0.00678	0.04578	-	-

R^2: Variation explained by the marker, P Value: Significant threshold of the markers, FDR: False discovery ratio, cM: Centimorgan, Chr: Chromosome

Table 3. SNP markers associated with relative cell membrane injury (RCI, %) in cotton leaves under high-temperature stress

Cell Membrane Injury (RCI) (%)				
Marker	Chr	Position (bp)	Gen ID	Series
SNP1680	A07	79351692	LOC107956390	TGCAGAAGACACTGGCATGTATCGAGCCTGGTCAGGGGACGACGACGATTACTTGACAGTTGCGGGACCTAG
SNP2537	A10	83870531	LOC107895542	TGCAGCAGATGATGATGGTATCTCTCTTACGCACTCCTCTCTCATTTTCAATGTGGTTCTTAGTTTTTTATCCTGGTT
SNP3092	A11	115618156	LOC107898822	TGCAGCCCGCTTCTTCTGGGCGAATTATTCATCATCGGCTCGCGCGAGGAGGTTCTTTGCAACCGATGGCTTACAGAT
SNP4374	D03	33711177	LOC107951183	TGCAGTGGACCACCGCGAACTTCCTTGGCGGTTTCAGCCAAAAGGAATGGATCTTTCAAGAGAGCTCCGCA
SNP4311	D03	577545	LOC107909460	TGCAGCCCTGTCAAACATGAGAAGACATTGGAGATGATTGGAAAGAAGTTGAAAAAGAACAGTGTA
SNP6415	D09	38872737	LOC107889491	TGCAGCGAAGAAGAATCCTCCCACACAACGTCGAACAAGTACACTACGGAATGGTCGAAGAGAGAGAGTCTGAC

Chr: Chromosome, bp: Base pair

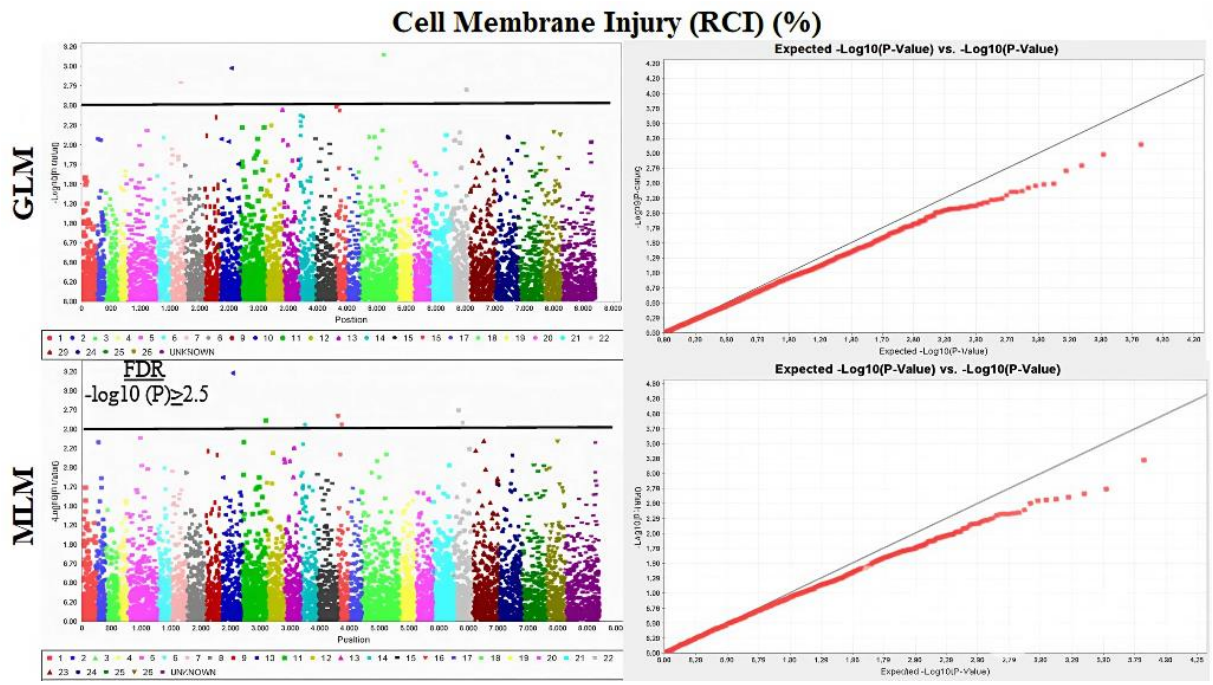

Figure 3. Manhattan and QQ Plots for the RCI trait related to high temperature tolerance determined by GLM- MLM method and satisfying the condition –log10(P-Value) ≥2.5

In terms of the RCI trait, QTL were detected through 6 highly significant SNP markers by false discovery rate (FDR) analysis of P values obtained in the MLM method as a result of SNP marker matches in GLM and MLM models. Of these, marker SNP2537 is located on chromosome A10 of the cotton genome at position 83870531 bp and on the callose synthesis 5 (LOC107895542) gene, marker SNP1680 is located on chromosome A07 of the cotton genome at position 79351692 bp and on the receptor-like protein kinase FERONIA (LOC107956390) gene, SNP3092 marker on chromosome A11 of the cotton genome at position 115618156 bp and the 50S ribosomal protein L15, chloroplastic (LOC107898822) gene, SNP4374 marker on chromosome D03 of the cotton genome at position 33711177 bp and serine/threonine-protein kinase WNK8 (LOC107951183) gene, SNP4311 marker on chromosome D03 of the cotton genome at position 577545 bp and 26S proteasome non-ATPase regulatory subunit 4 homolog (LOC107909460) gene, The SNP6415 marker was found to be located on chromosome D09 of the cotton genome at position 38872737 bp and the kinesin-like protein KIN-7K, chloroplastic (LOC107889491) gene was found to match the cotton (*G. hirsutum* L.) genome (Table 3). According to the GLM analysis results for LT trait, P values varied between 0.0001 and 0.0012, R^2 values varied between 0.1130 and 0.1660, and –log10(P-Value) ≥2.5 varied between 2.91 and 3.87. According to MLM analysis results, P-value varied between 0.0006 and 0.0030, R^2 values varied between 0.1094 and 0.1693, and -log10(P-Value) ≥2.5 varied between 2.51 and 3.21. In terms of LT traits, 83 SNP markers were identified according to GLM analysis, 48 SNP markers were identified by the MLM method and 24 SNP markers were identified according to –log10(P-Value) ≥2.5 condition. But nine common markers were identified by both methods according to the condition of -log10(P-Value) ≥2.5 (Table 4). SNP6665, SNP5029, SNP7949, SNP5643, SNP642, SNP2616, SNP5915, SNP5037 and SNP3217 markers were identified by both methods. Some characteristics of these markers are given in Table 5. Manhattan and QQ Plot graphs related to high temperature tolerance determined by the GLM and MLM methods for the trait analyzed are shown in Figure 4.

Table 4. Key attributes of SNP markers associated with leaf temperature (LT, ℃) response in cotton under high-temperature stress

Leaf Temperature (LT) (℃)									
Marker	Chr	Position (cM)	General Linear Model (GLM)			Mixed Linear Model (MLM)			
			P Value	R^2	-log10 (P-Value)	P Value	R^2	-log10 (P-Value)	FDR
SNP6665	D10	100	0.0001333	0.1611	3.87	0.00064	0.15406	3.18	0.0021
SNP5029	D05	316	0.0001454	0.15225	3.83	0.00061	0.15012	3.21	0.0021
SNP7949	D13	149	0.0001836	0.14967	3.73	0.00069	0.14564	3.15	0.0021
SNP5643	D07	38	0.0002227	0.15225	3.65	0.00084	0.14833	3.07	0.0021
SNP642	A04	34	0.0004911	0.13135	3.3	0.00198	0.12064	2.7	0.0024
SNP2616	A10	286	0.0005550	0.16608	3.25	0.00155	0.16933	2.80	0.0024
SNP5915	D08	16	0.0005897	0.1268	3.22	0.00188	0.12188	2.72	0.0024
SNP5037	D05	324	0.0006441	0.12976	3.19	0.00152	0.1308	2.81	0.0033
SNP3217	A12	117	0.00121	0.11309	2.91	0.00302	0.10947	2.51	0.0033

R^2: Variation explained by the marker, P Value: Significant threshold of the markers, FDR: False discovery ratio, cM: Centimorgan, Chr: Chromosome

Table 5. SNP markers linked to leaf temperature (LT, ℃) variation in cotton under high-temperature conditions

Leaf Temperature (LT) (℃)				
Marker	Chr	Position (bp)	Gen ID	Series
SNP1680	A07	79351692	LOC107956390	TGCAGAAGACACTGGCATGTATCGAGCCTGGTCAGGGGACGACGACGATTACTTGACAGTTGCGGGACCTAG
SNP2537	A10	83870531	LOC107895542	TGCAGCAGATGATGATGGTATCTCTCTTACGCACTCCTCTCTCATTTTCAATGTGGTTCTTAGTTTTTTATCCTGGTT
SNP3092	A11	115618156	LOC107898822	TGCAGCCCGCTTCTTCTGGGCGAATTATTCATCATCGGCTCGCGCGAGGAGGTTCTTTGCAACCGATGGCTTACAGAT
SNP4374	D03	33711177	LOC107951183	TGCAGTGGACCACCGCGAACTTCCTTGGCGGTTTCAGCCAAAAGGAATGGATCTTTCAAGAGAGCTCCGCA
SNP4311	D03	577545	LOC107909460	TGCAGCCCTGTCAAACATGAGAAGACATTGGAGATGATTGGAAAGAAGTTGAAAAAGAACAGTGTA
SNP6415	D09	38872737	LOC107889491	TGCAGCGAAGAAGAATCCTCCCACACAACGTCGAACAAGTACACTACGGAATGGTCGAAGAGAGAGAGTCTGAC

Chr: Chromosome, bp: Base pair

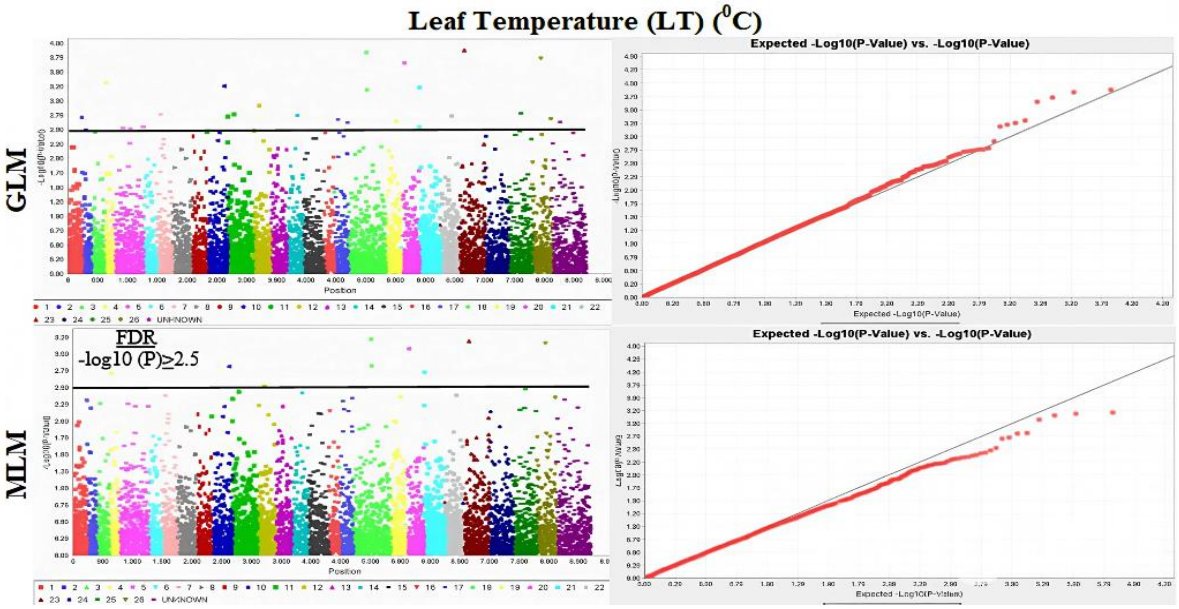

Figure 4. Manhattan and QQ Plots for LT trait related to high temperature tolerance determined by GLM-MLM method and satisfying the condition -log10 (P-Value) ≥2.5

Genes mapped to SNP markers were found to match, respectively, SNP5029 marker kinesin-like protein KIN-14R (LOC107905143), SNP7949 marker glycerol- 3- phosphate 2- O- acyltransferase 6- like (LOC107961543), SNP5643 marker protein EMBRYONIC FLOWER 1-like (LOC107900517), SNP642 marker receptor-like protein EIX2 (LOC121217273), SNP2616 marker with caffeoyl shikimate esterase (LOC107897770), SNP5915 marker with small nucleolar RNA U3 (LOC121220675) genes. The leaf chlorophyll density (SPAD) trait, P values varied between 0.0003 and 0.0047 by the GLM method; R^2 values varied between 0.0903 and 0.1426 and -log10(P-Value) ≥2.5 condition varied between 2.62 and 3.49. According to the MLM method, P values varied between 0.0009 and 0.0032; R^2 values varied between 0.1323 and 0.1687 and –log10(P-Value) ≥2.5 condition varied between 2.50 and 3.03. The SPAD trait, 60 SNP markers, was identified by GLM analysis and 43 SNP markers were identified by the MLM method. However, 8 SNP markers meeting the condition of –log10(P-Value) ≥2.5 were defined as highly significant according to the MLM method. According to both methods and conditions, 5 markers (SNP7623, SNP7492, SNP4621, SNP4403 and SNP3385) were significant (Table 6). Abdurakhmonov et al. (2009) indicated that markers detected by both GLM and MLM approaches are suitable for use as QTLs. These highly significant and important SNP markers (Shi et al., 2024; Meng-wei et al., 2023) were identified using -log10(P-Value) as described by the researchers. To avoid false genetic variants associated with the SPAD trait, 4 highly significant and important markers (SNP1910, SNP4621, SNP7263, SNP7428) were identified by performing the –log10(P-Value) ≥2.5 condition in the MLM method (Table 6). However, it was determined that only SNP1910 and SNP7428 markers were characterized in the cotton genome. Some characteristics of the co-determined markers are given in Table 7. Manhattan and QQ Plot graphs related to high temperature tolerance determined by the GLM and MLM methods for the SPAD trait are shown in Figure 5.

Table 6. Key attributes of SNP markers influencing SPAD-measured leaf chlorophyll content in cotton under high-temperature stress

Leaf Chlorophyll Density (SPAD)									
Marker	Chr	Position (cM)	General Linear Model (GLM)			Mixed Linear Model (MLM)			
			P Value	R^2	-log10 (P-Value)	P Value	R^2	-log10 (P-Value)	FDR
SNP7263	D11	255	0.00237	0.10664	2.62	0.00183	0.15011	2.73	0.0028
SNP7492	D12	89	0.00199	0.10952	2.7	0.000931	0.16036	3.03	0.0028
SNP4621	D04	138	0.00166	0.11093	2.77	0.00127	0.1687	2.89	0.0028
SNP4403	D03	104	0.00079873	0.12547	3.09	0.00245	0.13233	2.61	0.0028
SNP3385	A12	285	0.00052452	0.13589	3.28	0.0022	0.14304	2.65	0.0028
SNP3386	A12	286	0.00032231	0.14262	3.49	0.00177	0.14557	2.75	0.0028
SNP7428	D12	25	0.00479	0.09032	-	0.00323	0.13822	2.50	0.0032
SNP1910	A08	159	0.00335	0.0973	-	0.00174	0.1439	2.75	0.0028

R^2: Variation explained by the marker, P Value: Significant threshold of the markers, FDR: False discovery ratio, Chr: Chromosome

Table 7. SNP markers influencing SPAD-measured leaf chlorophyll content in cotton under high-temperature stress

Leaf Chlorophyll Density (SPAD)				
Marker	Chr	Position (bp)	Gen ID	Series
SNP1910	A08	98957833	LOC107900870	TGCAGCAATTTCCCACCAGCAGCACAAGATCGTAGACCAATTGTCTCCTGAAAAAAATATGCAAAAAAAAAAT
SNP4621	D04	33431400	LOC121209746	TGCAGTAGTTTGACTTTGAAAATTCACCAAAAATTGTAGAAATTGAATTAGAGGTTACAGATCGGAAGA
SNP7263	D11	50962474	LOC107961858	TGCAGCTACTCGAAACCTTCTTTGCAGATCAAGCCGTATCGCATGGGTTACGGGACTCTGCTCTACTTTCT
SNP7428	D12	2080513	LOC107947383	TGCAGTTACATAATGATCGGCAATGAACCATATATATACAGTTGTCAAACGAACTCAATTATTACAACAAA

Chr: Chromosome, bp: Base pair

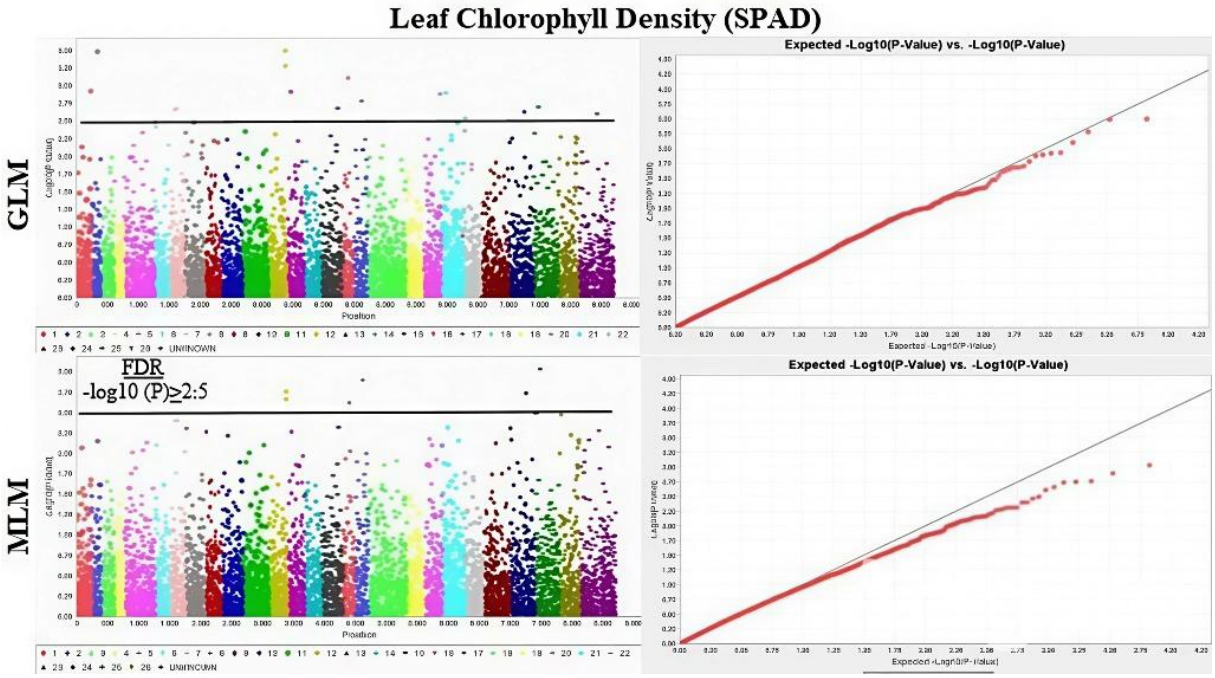

Figure 5. Manhattan and QQ Plots of the SPAD trait related to high temperature tolerance determined by GLM- MLM method and satisfying the condition -log10(P-Value) ≥2.5

In this context, 2 QTL gene matches, such as the SNP1910 marker CtBP (LOC107900870) and the SNP7428 marker GATA transcription factor 24 (LOC107947383), were identified. The SNP1910 marker was located on chromosome A08 of the cotton (*G. hirsutum* L.) genome at position 98957833 bp and mapped to the CtBP gene (LOC107900870). The CtBP gene, identified from the genomic sequence NC_053444.1 through Gnomon and confirmed by expressed sequence tag (Gnomon EST) data (Anonymous, 2023), matched the SNP1910 marker and was positioned within the coding region of the genome.

4. DISCUSSION

In this study, a series of genes and markers were identified in cotton (*G. hirsutum* L.) exposed to high-temperature stress through SNP based association analyses. In particular, genes associated with growth, development, and stress tolerance, such as Callose synthase 5 (LOC107895542), the FERONIA receptor-like kinase (LOC107956390), WNK8 kinase (LOC107951183), several kinesin family proteins, the GATA24 transcription factor, and an Embryonic Flower 1-like protein stood out. The identification of these genes contributes to a deeper understanding of the mechanisms underlying high-temperature stress responses in cotton.

Callose synthase 5, a key enzyme regulating callose deposition in the cell wall during stress responses, showed a strong association with relative cell injury (RCI). Callose is known to limit membrane damage by forming a barrier in the cell wall under stress conditions (Akman et al., 2012). Members of the CalS (Callose synthase) family in cotton have been reported to undergo expression changes under high-temperature and other abiotic stress conditions (Feng et al., 2018). The association of SNP2537 with Callose synthase 5 suggests that this gene may contribute to stress tolerance by preserving plasma membrane integrity. Considering that high RCI values indicate disruption of membrane stability through increased electrolyte leakage, callose synthesis may act as a repair or protective mechanism. This finding aligns with the well-established role of callose in stress responses. Studies in model plants have shown that CalS genes increase callose deposition at plasmodesmata under high temperature, thereby regulating intercellular communication and developmental processes. These observations support the hypothesis that Callose synthase 5 may function similarly in cotton. Likewise, the FERONIA receptor-like kinase, which senses cell wall integrity and regulates signal transduction, also emerges as a key candidate.

Markers such as SNP1680, associated with the FERONIA receptor-like kinase and Serine/Threonine-protein kinase WNK8, were linked to membrane damage and RCI. Abiotic stresses have been shown to induce substantial physiological and biochemical disruptions in a range of field crops, emphasizing the importance of stress-related traits such as membrane stability and physiological homeostasis (Karim et al., 2022; Zirak-Qoturbulagh et al., 2025). FERONIA is a central protein that functions as a sensor of cell wall integrity and interacts with the RALF1

peptide to regulate cell expansion and stress signaling (Haruta et al., 2014). Genetic studies in cotton have shown that certain gene combinations enhance membrane stability, likely involving members of the RLK family. In this study, the FERONIA signaling pathway may have contributed to stabilizing the cell wall plasma membrane interface under high-temperature stress, thereby improving RCI values. However, it should be noted that FERONIA plays context dependent roles and may also trigger cell death in certain cases (Chen et al., 2016). This dual functionality underscores the importance of elucidating FERONIA's precise role in cotton in future research.

Phenotypes observed under high-temperature stress such as leaf tissue degradation, boll desiccation, and impaired boll opening indicate disruptions in cell wall integrity and intracellular trafficking. In tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*), the xylanase (EIX) genes and their receptors, which play critical roles in plant immune responses and are internalized via endocytosis, have been well characterized (Ron and Avni, 2004). This system is closely linked to the perception of cell wall integrity and signal transduction. Although an EIX-like pathway has not yet been reported in cotton, similar receptor kinases and endocytic mechanisms for monitoring cell wall homeostasis are likely conserved. Our findings suggest that the physical and chemical deterioration of cell wall structure caused by high-temperature stress such as loosening of the pectin and hemicellulose matrix may adversely affect these conserved signaling networks.

The SNP5643 marker corresponds to an EMBRYONIC FLOWER 1-like gene, which encodes a VEFS domain protein involved in regulating vegetative growth and reproductive development. The gene's role in developmental transitions provides a plausible explanation for the phenotypic abnormalities observed under heat stress, including leaf deformation and floral defects.

Kinesin-like proteins (KIN-7K and KIN-14R), which encode microtubule associated motor proteins, were also associated with high-temperature stress. Temperature stress is known to affect microtubule dynamics, directly influencing cell division and intracellular transport. Proteomic studies in cotton have reported changes in the abundance of cytoskeletal proteins under high temperatures, suggesting that kinesin genes may play important roles in the stress response. Microtubules are highly sensitive to temperature and can depolymerize under heat stress, disrupting cell division, cytoskeletal organization, and intracellular transport. In cotton, Rikin et al. (1975) demonstrated a link between microtubule stabilization and cold tolerance. Our findings suggest that a similar mechanism may apply to heat stress. We hypothesize that kinesins may play a vital role during heat stress by transporting essential components (e.g., heat shock proteins or repair factors) or removing damaged structures. However, the specific functions of these kinesin genes in cotton and the cargo proteins they transport under high temperature remain unclear and require functional studies. Additionally, transcription factors such as GATA24 and Embryonic Flower 1-like proteins act at the intersection of development and stress responses. GATA transcription factors are known to be associated with light signaling, growth, and stress adaptation. In cotton, high-temperature induced changes in fiber development and sucrose metabolism may reflect the regulatory potential of these genes. Functional validation studies including expression analyses and promoter fusion experiments could further support these associations; phenotypic screenings may also evaluate impacts on flowering, fiber development, and membrane stability.

Caffeoyl-shikimate esterase (CSE), identified as a gene in the lignin biosynthesis pathway, emerges as another candidate. Lignin accumulation (lignification) is thought to be a response to abiotic stresses, potentially strengthening the cell wall (Moura et al., 2010). Under heat stress, enhanced lignification could contribute to the leaf desiccation symptoms observed, suggesting a link between cell wall reinforcement and thermal damage responses. Markers corresponding to GPAT family genes, including GPAT6, highlight pathways related to lipid metabolism and membrane structure. GPAT6 is associated with endoplasmic reticulum function and glycerolipid synthesis, both essential for maintaining membrane stability under abiotic stress. The identification of GPAT associated loci in cotton offers new insights into species specific mechanisms regulating heat tolerance. Vanholme et al. (2013) demonstrated that CSE is a key enzyme in lignin biosynthesis in *Arabidopsis*. The heat induced leaf desiccation and cell collapse observed in our study may be consistent with increased lignification. However, excessive lignification may also reduce cell flexibility and restrict growth, making it necessary to determine whether CSE's overall effect on heat stress responses in cotton is beneficial or detrimental.

The SNP7428 marker, aligned with GATA Transcription Factor 24 (LOC107947383), underscores the potential contribution of transcriptional regulation to heat induced phenotypic variation. GATA transcription factors in cotton have not yet been fully characterized, but Reyes et al. (2004) reported that GATA factors regulate various light related processes in other plant species. This information suggests that GATA factors may influence SPAD related traits and leaf physiological responses following heat shock. Collectively, SNPs mapping to genes involved in cell wall organization, membrane integrity, cytoskeletal structure and motility, hormone related signal transduction, and gene expression regulation highlight the multifaceted nature of heat tolerance in cotton. Similar phenotypic variation in response to temperature-related stress has also been reported in other field crops, where physiological and developmental traits were shown to be strongly influenced by thermal conditions (Sümer, 2023). The genes identified within six QTLs associated with leaf temperature are located in protein coding regions; some

are consistent with known stress related pathways in other plant species, yet newly reported in cotton. These findings underscore the complexity of heat tolerance in *G. hirsutum* L. and identify genomic regions requiring further functional characterization.

Genes such as C-terminal Binding Protein (CtBP) and GATA Transcription Factor 24, which are linked to energy status and light signaling, also appear noteworthy. CtBP is sensitive to NAD(H) levels and can regulate transcription according to the cellular redox state (Zheng et al., 2007). High temperature disrupts cellular redox balance by increasing energy consumption and reactive oxygen species (ROS) production. CtBP may modulate the expression of stress response genes in accordance with this altered metabolic state. The leaf chlorosis and elevated SPAD values observed after heat stress in our study may be related to disruptions in the photosynthetic apparatus and chlorophyll metabolism. GATA factors are likely involved in regulating photosynthesis and carbon metabolism related genes under heat stress. Notably, the roles of CtBP-like NAD(P) binding proteins, GATA transcription factor 24, and kinesin-like proteins in cotton remain largely unexplored, representing promising targets for advancing our understanding of thermotolerance in this economically important crop.

This study identifies novel candidate genetic regions associated with heat tolerance in cotton, yet functional analyses are necessary for validation. Gene editing approaches such as CRISPR/Cas9, through gene silencing or overexpression, provide powerful means to confirm the roles of these genes in heat tolerance. Targeting genes associated with cell wall architecture, membrane stability, and signal transduction may help clarify the effects of heat stress on key physiological processes. Because cotton is an allotetraploid species, insights gained from model plants may not always translate directly. Thus, functional characterization of candidate genes in cotton will contribute significantly to both basic research and breeding efforts.

In conclusion, this study provides valuable insights into the genetic basis of heat tolerance in cotton. Experimental validation of the candidate genes will establish a strong scientific foundation for developing heat tolerant cotton cultivars.

5. CONCLUSION

This study provides insights into the potential genetic components of high-temperature stress tolerance in cotton, identifying a set of candidate genes and markers through association mapping. The distribution of these QTLs across specific chromosomes in the A and D subgenomes suggests a polygenic basis for thermotolerance, consistent with the observed physiological complexity of the trait. Findings from this work point to the possible involvement of various biological processes such as cell wall fortification mediated by callose, cell wall integrity sensing via pathways including FERONIA, intracellular transport involving kinesins, and transcriptional regulation in the cotton heat stress response. The phenotypic alterations observed following high-temperature treatment may be linked to disruptions in these systems. The QTLs and candidate genes identified here, while requiring further functional validation, present a valuable resource for future investigation. They offer a preliminary framework for subsequent research aimed at functional characterization, for instance through gene editing, and could inform longer term strategies for developing climate tolerant cotton varieties.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

REFERENCES

Abdurakhmonov, I. Y., Saha, S., Jenkins, J. N., Buriev, Z. T., Shermatov, S. E., Scheffler, B. E., Pepper, A. E., Yu, J. Z., Kohel, R. J., & Abdukarimov, A. (2009). Linkage disequilibrium-based association mapping of fiber quality traits in *G. hirsutum* L. variety germplasm. *Genetica*, 136(3), 401-417. https://doi.org/10.1007/s10709-008-9337-8.

Ahmad A, Ilyas MZ, Aslam Z, Roman M, Ali A, Naeem S, Nazar M, & Rehman, S.U. (2020). Physiological screening of cotton (*Gossypium hirsutum* L.) genotypes against drought tolerance. *Pure ApplBiol* 9:140-147. http://dx.doi.org/10.19045/bspab.2020.90017.

Akman, Y., Ketenoglu, O., Kurt, L., & Yigit, N. (2012). Plant Ecology-Ecological Synthesis. Palme Publishing, Ankara p 322.

Anonymous, (2023). National Center for Biotechnology Information. https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome, (Accessed July 10, 2023)

Bardak, A., Celik, S., Erdogan, O., Ekinci, R., & Dumlupinar, Z. (2021). Association Mapping of Verticillium Wilt Disease in a Worldwide Collection of Cotton (*Gossypium hirsutum* L.). *Plants* (Basel, Switzerland), 10(2), 306. https://doi.org/10.3390/plants10020306.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B, *Methodological* 57(1): 289-300, https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics* 23:2633-2635, https://doi.org/10.1093/bioinformatics/btm308.

Chen, X. Y., & Kim, J. Y. (2009). Callose synthesis in higher plants. *Plant signaling & behavior*, 4(6), 489-492, https://doi.org/10.4161/psb.4.6.8359.

Chen, J., Yu, F., Liu, Y., Du, C., Li, X., Zhu, S., ... & Luan, S. (2016). FERONIA interacts with ABI2-type phosphatases to facilitate signaling cross-talk between abscisic acid and RALF peptide in Arabidopsis. Proceedings of the National Academy of Sciences, 113(37), E5519-E5527.

Demiray, Y. G. (2024). Determination of DNA markers associated with tolerance/resistance to high temperature stress in cotton (*Gossypium hirsutum* L.) (Unpublished doctoral dissertation, 237 pages). Dicle University, Diyarbakır, Turkey.

Demiray, Y. G., Ekinci, R., & Bardak, A. (2023). Investigation of Vegetative High Temperature Tolerance of Some Cotton (*Gossypium hirsutum* L.) Varieties. *Turkish Journal of Nature and Science*, 12(2), 111-118, https://doi.org/10.46810/tdfd.1287892.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLOS One*, 6(5), e19379. https://doi.org/10.1371/journal.pone.0019379

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology*, 14(8), 2611- 2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.

Feng, W., Kita, D., Peaucelle, A., Cartwright, H. N., Doan, V., Duan, Q., ... & Dinneny, J. R. (2018). The FERONIA receptor kinase maintains cell-wall integrity during salt stress through Ca2+ signaling. Current Biology, 28(5), 666-675.

Gachomo, E. W., Jimenez-Lopez, J. C., Smith, S. R., Cooksey, A. B., Oghoghomeh, O. M., Johnson, N., ... & Kotchoni, S. O. (2013). The cell morphogenesis ANGUSTIFOLIA (AN) gene, a plant homolog of CtBP/BARS, is involved in abiotic and biotic stress response in higher plants. BMC Plant Biology, 13(1), 79. https://doi.org/10.1186/1471-2229-13-79.

Haruta, M., Sabat, G., Stecker, K., Minkoff, B. B., & Sussman, M. R. (2014). A peptide hormone and its receptor protein kinase regulate plant cell expansion. Science, 343(6169), 408-411.

ICAC, (2022). International Cotton Advisory Committee, Washington DC, USA, Cotton data book. DATABOOK-2022-ss.pdf.pdf.pdf (icac.org), (Accessed August 8, 2024)

IPCC, (2018). Intergovernmental Panel on Climate Change 2018. Synthesis report. Version ingles. Page Climate Change 2018: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. (Accessed June 26, 2023)

IPPC, (2007). Intergovernmental Panel on Climate Change (Climate Change 2007: Working Group I: The Physical science basis (online), http://www.ipcc.ch/publications_and_data/publications_ipcc_fourth_assessment_report_wg1_report_the_phyyssıcal_science_basis.htm, (Accessed June 26, 2023).

Karim, A. M. A., Sarker, U. K., Hasan, A. K., Islam, N., & Uddin, M. R. (2022). Physiological and biochemical responses of chickpea genotypes to different moisture stress in Bangladesh. Turkish Journal of Field Crops, 27(1), 1-9.

Khan, N., Ahmad, I., & Azhar, M. T. (2017). Genetic variation in relative cell injury for breeding upland cotton under high temperature stress. Turkish Journal of Field Crops, 22(1), 152-159.

Liao, H., Tang, R., Zhang, X., Luan, S., & Yu, F. (2017). FERONIA Receptor Kinase at the Crossroads of Hormone Signaling and Stress Responses. *Plant & cell physiology*, 58(7), 1143-1150. https://doi.org/10.1093/pcp/pcx048.

Luo, X. M., Lin, W. H., Zhu, S., Zhu, J. Y., Sun, Y., Fan, X. Y., Cheng, M., Hao, Y., Oh, E.,Tian, M., Liu, L., Zhang, M., Xie, Q., Chong, K., & Wang, Z. Y. (2010). Integration of light- and brassinosteroid-signaling pathways by a GATA transcription factor in *Arabidopsis*. *Developmental cell*, 19(6), n872-883. https://doi.org/10.1016/j.devcel.2010.10.023.

Ma, Y., Min, L., Wang, J., Li, Y., Wu, Y., Hu, Q., ... & Zhang, X. (2021). A combination of genome-wide and transcriptome-wide association studies reveals genetic elements leading to male sterility during high temperature stress in cotton. New Phytologist, 231(1), 165-181. https://doi.org/10.1111/pbi.13597

Meng-wei, L., Yu-hua, H., Rong, L., Guan, L., Dong, W., Yi-shan, J., Xin, Y., Shu-xian, H., Chen-yu, W., Yu, M., Bei, L., Tao, Y., & Xu-xiao, Z., (2023). Construction of SNP genetic map based on targeted next-generation sequencing and QTL mapping of vital agronomic traits in faba bean (*Vicia faba* L.), *Journal of Integrative Agriculture*, volume 22, issue 9, pages 2648-2659, September 2023, https://doi.org/10.1016/j.jia.2023.01.003.

Moura, J. C. M. S., Bonine, C. A. V., de Oliveira Fernandes Viana, J., Dornelas, M. C., & Mazzafera, P. (2010). Abiotic and biotic stresses and changes in the lignin content and composition in plants. Journal of integrative plant biology, 52(4), 360-376.

Poland, J. A., & Rife, T. W. (2012). Genotyping-by-sequencing for plant breeding and genetics. *Plant Genome* 5: 92-102. https://doi.org/10.3835/plantgenome2012.05.0005.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945-959. https://doi.org/10.1093/genetics/155.2.945.

Rani, S., Baber, M., & Naqqash, T. (2022). Identification and genetic mapping of potential QTLs conferring heat tolerance in cotton (*Gossypium hirsutum* L.) by using micro satellite marker's approach. Agron. 2022; 12 (6): 1381.

Reyes, J. C., Muro-Pastor, M. I., & Florencio, F. J. (2004). The GATA family of transcription factors in Arabidopsis and rice. Plant physiology, 134(4), 1718-1732. https://doi.org/10.1104/pp.103.037788.

Rikin, A., Waldman, M., Richmond, A. E. & Dovrat, A. (1975). Hormonal regulation of morphogenesis and cold resistance. I. Modifications by abscisic acid and gibberellic acid in alfalfa (*Medicago sativa* L.) seedlings. *Journal of Experimental Botany*, 26, 175- 183. https://doi.org/10.1093/jxb/26.2.175.

Ron, M., & Avni, A. (2004). The receptor for the fungal elicitor ethylene-inducing xylanase is a member of a resistance-like gene family in tomato. *The Plant cell*, 16(6), 1604-1615. https://doi.org/10.1105/tpc.022475.

Salman, M., Zia, Z. U., Rana, I. A., Maqsood, R. H., Ahmad, S., Bakhsh, A., & Azhar, M. T. (2019). Genetic effects conferring heat tolerance in upland cotton (*Gossypium hirsutum* L.). *Journal of Cotton Research*, 2, 1-8. https://doi.org/10.1186/s42397-019-0025-2.

SAS. (2020). Version 9.3. SAS Institute Inc., Cary, NC, 1989-2020

Semizer-cumıng, D., Altan, F., Akdemır, H., Tosun, M., Gurel, A., & Tanyolac, B. (2015). QTL analysis of fiber color and fiber quality in naturally green colored cotton (*Gossypium hirsutum* L.). Turkish Journal of Field Crops, 20(1), 49-58.

Shi, Y., Huang, K., Chen, J., Huda, M. N., Niu, J., Sun, Z., ... & Zhu, A. (2024). Genomic variation and candidate genes dissect quality and yield traits in *Boehmeria nivea* (L.) Gaudich. *Cellulose*, 31(3), 1449-1465. https://doi.org/10.21203/rs.3.rs-3012123/v1.

Sümer, F. Ö. (2023). The effects of different sowing times on the phenological characteristics and seed yield of the pea. Turkish Journal of Field Crops, 28(2), 301-312.

Vanholme, R., Cesarino, I., Rataj, K., Xiao, Y., Sundin, L., Goeminne, G., Kim, H., Cross, J., Morreel, K., Araujo, P., Welsh, L., Haustraete, J., McClellan, C., Vanholme, B., Ralph, J., Simpson, G. G., Halpin, C., & Boerjan, W. (2013). Caffeoyl shikimate esterase (CSE) is an enzyme in the lignin biosynthetic pathway in *Arabidopsis*. *Science* (New York, N.Y.), 341(6150), 1103-1106. https://doi.org/10.1126/science.1241602.

Wei, N., Tsuge, T., Serino, G., Dohmae, N., Takio, K., Matsui, M., & Deng, X. W. (1998). The COP9 complex is conserved between plants and mammals and is related to the 26S proteasome regulatory complex. *Current biology*, 8(16), 919-922. https://doi.org/10.1016/s0960-9822(07)00372-7.

Whitt, S. R., & Buckler, E. S. (2003). Using natural allelic diversity to evaluate gene function. *Methods in molecular biology* (Clifton, N.J.), 236, 123–140. https://doi.org/10.1385/1-59259-413-1:123.

Yang, W., Pollard, M., Li-Beisson, Y., Beisson, F., Feig, M., & Ohlrogge, J. (2010). A distinct type of glycerol-3-phosphate acyltransferase with sn-2 preference and phosphatase activity producing 2-monoacylglycerol. *Proceedings of the National Academy of Sciences*, 107(26), 12040-12045. https://doi.org/10.1073/pnas.0914149107.

Zhang, Q., Zhang, C., Pan, Z., Lin, H., Li, Z., Hou, X., Liu, J., Nie, X., & Wu, Y. (2023). "Genome- Wide Identification and Analysis of the WNK Kinase Gene Family in Upland Cotton." *Plants* 12, no. 23: 4036. https://doi.org/10.3390/plants12234036.

Zheng, X., Dai, X., Zhao, Y., Chen, Q., Lu, F., Yao, D., Yu, Q., Liu, X., Zhang, C., Gu, X., & Luo, M. (2007). Restructuring of the dinucleotide-binding fold in an NADP(H) sensor protein. *Proceedings of the National Academy of Sciences*, 104(21), 8809-8814. https://doi.org/10.1073/pnas.0700480104.

Zirak-Qoturbulagh, M. A., Mehri, S., Soleimanzadeh, H., & Ansari, M. H. (2025). Co-Inoculation of Bradyrhizobium and Arbuscular Mycorrhizal Fungus Alleviates the Effects of Drought Stress in Soybean (*Glycine max* L.). Turkish Journal of Field Crops, 30(1), 235-248.

Ziska, L. H. & Bunce, J. A. (1997). Influence of increasing carbon dioxide concentration on the photosynthetic and growth stimulation of selected C4 crops and weeds. Photosynthesis Research, 54.3: 199-208. https://doi.org/10.1023/A:1005947802161.