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ANALYSIS OF CANDIDATE GENES AND THEIR FUNCTIONS BASED ON QTLS ASSOCIATED WITH ABIOTIC STRESS TOLERANCE IN *GOSSYPIUM HIRSUTUM* L. VARIETIES

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Abstract. In this study, we identified quantitative trait loci (QTL) associated with drought-tolerance traits in *Gossypium hirsutum* L. and performed functional evaluation of candidate genes within these QTL regions. Specifically, genes for transcription factors **(**GhWRKY27**,** GhWRKY70**,** GhDREB2**)** and other stress-responsive genes **(**GhCAT1**,** GhCIPK6**,** SnRK2.6**)** were analyzed in QTL located on chromosomes D01, A05, A11, and D08. The presence of ABRE elements in promoters, consistency with previous transcriptomic data, and detected epistatic interactions support the involvement of these genes in drought stress tolerance. Additionally, novel QTL regions on chromosomes A12 and D05 were identified for the first time and confirmed to be associated with tolerance traits. Analysis revealed that most drought-enhancing alleles originated from the wild *G. tomentosum* genotype. These candidate genes are promising targets for marker-assisted selection, genome editing (CRISPR/Cas9), and transgenic studies. The findings contribute to the genetic understanding necessary for developing drought-tolerant cotton cultivars.

Keywords: *Gossypium hirsutum*, Drought stress, QTL mapping, Candidate genes, Transcription factors, GhWRKY70, GhDREB2, CRISPR/Cas9, Genetic selection, Wild cotton germplasm (*G. tomentosum*)

Introduction: Under the accelerating impact of climate change, enhancing plant tolerance to abiotic stresses—particularly drought—has become a critical

research priority. Cotton (*Gossypium hirsutum* L.), a globally important fiber crop, is highly vulnerable to water deficiency, which significantly impairs its growth, fiber quality, and yield. Drought tolerance is a complex quantitative trait controlled by various genetic components including transcription factors and signaling pathways. In recent years, Quantitative Trait Loci (QTL) mapping has emerged as a robust approach for detecting genomic regions linked with stress-related traits. Within these regions, candidate genes can be scrutinized through molecular biology and bioinformatics techniques to elucidate their functional roles in stress responses. Transcription factor families such as WRKY**,** DREB**,** SnRKCIPK, and CAT are key regulators of physiological adaptations to drought stress. Moreover, the presence of cis-regulatory motifs like ABRE in promoter regions suggests a gene's involvement in abiotic stress responses. In this study, we analyzed genes located in QTL segments identified from a *G. hirsutum* × *G. tomentosum* F_2 and F_3 hybrid population. Our goal was to pinpoint promising candidate genes—in particular, transcriptional regulators and stress signal mediators—and to uncover the genetic mechanisms underlying drought tolerance to enhance marker-based selection.

Study Object. The research object comprises F_2 and F_3 progenies derived from hybrids between cultivated *G. hirsutum* and its wild relative *G. tomentosum*. This interspecific population was used to study QTLs and candidate genes governing drought tolerance traits.

Candidate Genes and Their Functions: Analysis of Genes Within QTL Segments. An analysis of the genes located within the QTL segments revealed which of them could be recommended as candidate genes. As an example, within the ~0.75 Mb genomic interval of the D01 chromosome cluster, a total of 15 genes were identified, of which 5 are of unknown function, while the remaining 10 encode known proteins. Interestingly, this region harbors the transcription factor gene GhWRKY27, whose homolog in *Arabidopsis* has been reported to regulate reactive oxygen species (ROS) production in response to drought stress (Hayano-Kanashiro et al., 2009). Additionally, among the genes in this cluster, GH_D01G0182, GH_D01G0205, and GH_D01G0218 are of unclear function; however, our promoter analysis results revealed that these genes possess a relatively high number of ABRE (ABA-responsive element) motifs, which are associated with drought response. Therefore, we have conditionally included these genes in the list of candidate genes potentially involved in drought tolerance. Another important QTL region is located on chromosome A05, within the 89.0–90.6 Mb interval. In a study by Li et al. (2019), a significant association was

identified in this region with leaf wilting scores. They identified the WRKY70 gene in this interval and reported that its expression significantly increases under drought conditions. WRKY70 is known to participate in ABA hormone signaling and regulate stress response pathways (T. Mahmood et al., 2020). In our own population, a QTL was also detected in this same A05 segment, affecting the EWS trait (early wilting score). Therefore, GhWRKY70 is considered a strong candidate gene potentially playing an important role in drought tolerance.

In the same study by Li and colleagues (2019), several other important candidate genes were identified, including GhCIPK6 (a calcium-signaling kinase), SnRK2.6 (an ABA-dependent kinase), and NET1A (a cytoplasmic protein), all of which were shown to activate various defense mechanisms in plants under drought stress. These genes were found to be upregulated under drought in tolerant genotypes and systems, while downregulated in sensitive ones. In other QTL segments identified in our study, we also found numerous genes involved in cellular responses to drought-induced stress, including those encoding antioxidant enzymes and proteins involved in the synthesis of osmoprotectants. For instance, the GhDREB2 transcription factor is located within a QTL region on chromosome A11. Its homologs in *Arabidopsis* and cotton are considered “master regulators” that activate protective genes in response to drought stress. Another example is a QTL identified near the GhCAT1 gene on chromosome D08. This gene encodes a catalase enzyme that becomes active under drought and ABA treatment, helping to maintain ROS homeostasis (T. Mahmood, 2020). These examples demonstrate that the results obtained through QTL mapping are supported at the molecular level and suggest the presence of genes contributing to drought tolerance within the analyzed genomic regions. In the future, the precise functions of these genes can be validated through CRISPR/Cas9-based genome editing and tested in transgenic lines. Moreover, integrating full genome sequencing data with large-scale datasets such as transcriptomics, proteomics, and epigenomics will provide deeper insights into the mechanisms of drought tolerance in cotton (T. Mahmood, 2020).

Discussion of Results. A comparative analysis was conducted between the QTLs identified in this study and findings from previous research. As noted earlier, some of the QTLs confirmed earlier results in terms of their genomic location and effect — for example, those found on chromosomes A05 and D01**.** At the same time, several novel QTLs were identified for the first time. Notably, a QTL influencing leaf wilting resistance on chromosome A12 has not been previously reported in the literature and is considered a new finding. Similarly, the QTL we detected on D05 (chromosome 19) associated with dry stem biomass also

represents new information. These results suggest that there are alleles contributing to drought tolerance located in previously unexplored regions of the cotton genome. We believe that these alleles originated specifically from the wild *G. tomentosum* gene pool, as they were absent in the cultivated parent lines (i.e., the sensitive genotypes in our population lacked these alleles). In fact, genetic analysis revealed that out of the 30 stable QTLs, 18 carried positive (tolerance-enhancing) alleles from the wild parent (tomentosum), 7 from the cultivated parent, and in the remaining cases, the heterozygous genotype conferred the highest phenotypic performance. This demonstrates the rich genetic potential of G. tomentosum for drought adaptation and supports strategies to introgress its alleles into cultivated varieties (Magwanga R.O. et al., 2020). Furthermore, heterosis (hybrid vigor) is often observed in wild × cultivated hybrid populations — and in our $F_{2:3}$ generation, several heterozygous lines outperformed both parents under stress conditions in terms of growth and yield, indicating the presence of epistatic interactions.

Indeed, epistatic relationships between certain QTLs were detected. For example, the combination of loci on A05 and D08 resulted in a higher-than-expected tolerance index (positive epistasis), whereas A07 and D13 together led to a reduced phenotype (negative epistasis). These findings emphasize that drought tolerance is a complex trait that cannot be fully explained by the effects of individual QTLs alone. Instead, it is essential to consider their interactions within genetic networks (Lopes et al., 2014).

Conclusion. Using QTL mapping, we identified a number of genetic determinants that contribute to drought stress tolerance in cotton. While many of these aligned with previously known loci, several represent novel discoveries. Further investigation into the functional significance of the identified QTLs will be crucial, particularly for their application in marker-assisted selection (MAS). These future studies will enhance the potential for breeding more drought-resilient cotton varieties.

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