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QTL MAPPING AND CANDIDATE GENE ANALYSIS FOR DROUGHT TOLERANCE AT THE SEEDLING STAGE IN SORGHUM USING BSA-SEQ

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Abstract

To elucidate the genetic basis of drought tolerance at the seedling stage in sorghum, we used the drought-tolerant restorer line LNR-6 and the drought-sensitive restorer line LR-2381 as parents to construct an F_2 segregating population. Drought stress was carefully applied at the six-leaf seedling stage, and two extreme phenotype bulks (highly tolerant and highly sensitive) were established based on phenotypic evaluation. Bulked segregant analysis (BSA) combined with high-throughput sequencing was then employed to map quantitative trait loci (QTL) associated with seedling drought tolerance. The results showed that two significantly associated genomic regions were detected on chromosomes 9 and 10, designated *qKH9* (29.677 kb) and *qKH10* (400.118 kb), respectively. The *qKH9* region contained 231 variant sites involving 17 transcripts, whereas *qKH10* harbored 4,039 variant sites involving 144 transcripts. Functional annotation indicated that *qKH9* includes candidate genes related to zinc finger proteins and protease-mediated protein degradation, while *qKH10* contains putative drought-responsive genes involved in peroxidase activity and kinase-mediated signal transduction. These QTL provide key genomic intervals for dissecting the molecular mechanisms underlying seedling drought tolerance in sorghum and lay an important foundation for fine mapping, marker development and molecular breeding for drought tolerance.

Key words: Sorghum; Seedling drought tolerance; BSA-seq; SNP; QTL mapping

Introduction

Sorghum (*Sorghum bicolor*) is an important dual-purpose crop used for both grain and forage, as well as a key raw material for brewing. It is widely cultivated in northern China and other semi-arid and dryland agricultural regions (Zheng *et al*., 2011). Compared with other cereal crops such as maize, sorghum possesses stronger drought tolerance potential and exhibits clear production advantages in water-limited environments (Borrell *et al*., 2014; Wang *et al*., 2025a). However, during the seedling stage, the root system of sorghum is not yet fully developed, making plants highly sensitive to fluctuations in soil moisture. Drought stress at this stage can lead to seedling wilting and growth inhibition, which may severely affect population establishment and subsequent yield formation (Pinto *et al*., 2023; Wang *et al*., 2025b). Therefore, drought tolerance at the seedling stage is a critical trait in sorghum stress-resistance breeding and an essential factor for ensuring stable production in dryland agriculture (Belete, 2018; Min *et al*., 2025).

Drought tolerance in crops is a typical quantitative trait that is usually controlled by multiple genes acting in coordination. Its genetic mechanism is complex and strongly influenced by environmental conditions (Kiriga *et al*., 2016; Liu *et al*., 2023). Traditional drought-resistance breeding mainly relies on field phenotypic selection; however, this approach is greatly affected by annual climatic fluctuations and environmental variability, resulting in relatively low selection efficiency (Cattivelli *et al*., 2008; Kiriga *et al*., 2016). With the development of molecular breeding technologies, identifying drought-related quantitative trait loci (QTLs) and developing reliable molecular markers for rapid screening at the seedling stage have become important strategies to improve breeding efficiency (Tuberosa *et al*., 2007; Khan *et al*., 2013; Hassan *et al*., 2023).

In recent years, bulked segregant analysis combined with high-throughput sequencing (BSA-seq) has been widely applied in the mapping of quantitative traits (Habila *et al*., 2025; Hu *et al*., 2025). This method constructs DNA pools from individuals with extreme phenotypes and detects allele frequency differences across the genome to rapidly identify genomic regions significantly associated with target traits. Compared with traditional linkage mapping, BSA-seq requires smaller population sizes and offers faster mapping speed and higher resolution, making it particularly suitable for the preliminary identification of complex traits such as drought tolerance (Klein *et al*., 2018; Majeed *et al*., 2022; Esposito *et al*., 2025).

Currently, most studies on sorghum drought tolerance have focused on physiological response mechanisms, agronomic trait evaluation, and cultivation management strategies. In contrast, genome-level mapping studies targeting drought tolerance at the seedling stage remain relatively limited. In particular, further research is needed to precisely define candidate genomic regions, evaluate the resolution of mapped intervals, and explore their potential for molecular marker development. Therefore, taking advantage of the available sorghum genomic resources, the application of BSA-seq technology to map loci associated with seedling-stage drought tolerance is of great

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significance for narrowing target intervals and improving the efficiency of marker development.

In this study, the drought-tolerant restorer line LNR-6 and the drought-sensitive restorer line LR-2381 were used to construct an F_2 segregating population. Extreme drought-tolerant and drought-sensitive individuals were selected under controlled drought stress at the seedling stage to perform BSA analysis and identify QTL regions associated with drought tolerance. The objective of this study was to determine the physical intervals of drought-related loci and characterize the distribution of genetic variants within these regions, thereby providing a theoretical basis for subsequent fine mapping and molecular marker development, as well as technical support for molecular breeding of drought-tolerant sorghum varieties.

Material and Methods

Experimental design: The experiment was conducted on May 15, 2023, at the experimental field of the Liaoning Academy of Agricultural Sciences. The drought-tolerant sorghum restorer line LNR-6, the drought-sensitive restorer line LR-2381, and 419 F_2 individuals derived from their cross were used as experimental materials. The experiment was carried out using a pot-culture system. Thirty seeds were evenly sown in each pot. At the three-leaf stage, seedlings were thinned to maintain 20 healthy and uniformly growing plants per pot. Normal irrigation was maintained before the seedlings reached the six-leaf stage, with soil moisture controlled at approximately 70% of field capacity. When the plants reached the six-leaf stage, drought stress treatment was initiated by withholding water, and soil moisture was reduced and maintained at approximately 40% of field capacity for a duration of seven days. After that, leaf samples were collected for DNA extraction, and BSA was performed for preliminary gene mapping.

DNA extraction from leaf samples: In this study, 50 extremely drought-tolerant and 50 extremely drought-sensitive individuals were selected from the F_2 segregating population, together with the two parental lines. The selection was based on their post-stress survival rate, leaf wilting severity, and overall growth performance after drought treatment, with individuals representing the two extreme phenotypic groups. Young leaf tissues were collected and immediately frozen in liquid nitrogen for preservation. Genomic DNA was extracted using the DNAsecure Plant Genomic DNA Extraction Kit (Tiangen, DP320). The integrity and quality of the extracted DNA were first assessed by 1.0% agarose gel electrophoresis to ensure the absence of degradation. DNA concentration and purity were then preliminarily evaluated using a NanoDrop ND-2000 UV-Vis spectrophotometer. Samples with OD_{260}/OD_{280} ratios between 1.8 and 2.0 and OD_{260}/OD_{230} ratios greater than 2.0 were considered to have acceptable purity. After confirming DNA quality, equal amounts of DNA from selected individuals were pooled according to their drought-tolerance phenotype to construct the extreme phenotype pools. The total DNA quantity in each pool was adjusted to meet the requirements for subsequent library construction.

Library construction and high-throughput sequencing: Qualified DNA samples were randomly fragmented into approximately 350 bp fragments using a Covaris ultrasonic sonicator. Sequencing libraries were constructed using the Illumina TruSeq DNA Nano Library Preparation Kit. The library preparation procedure included end repair of DNA fragments, addition of a 3′ A-tail, ligation of Illumina-specific sequencing adapters, purification of ligation products using AMPure XP magnetic beads, and PCR amplification to enrich adapter-ligated DNA fragments. The constructed libraries were first quantified using a Qubit 4.0 Fluorometer. The size distribution of library insert fragments was then evaluated using an Agilent 2100 Bioanalyzer to ensure that the library quality met the expected standards. Libraries that passed quality control were mixed at equimolar concentrations based on their effective concentrations and the required sequencing depth. The pooled libraries were sequenced on the Illumina NovaSeq 6000 platform using the paired-end 150 bp (PE150) sequencing strategy.

Sequencing data processing and variant detection: Raw sequencing data generated from the platform were first subjected to quality control using the Fastp software. The filtering steps included removing low-quality reads, eliminating reads containing adapter sequences, and discarding reads with more than 5% ambiguous bases (N). After quality filtering, high-quality clean reads were obtained. These clean reads were aligned to the sorghum reference genome (BTx623T2T-AGI) using the BWA software with default parameters. The resulting alignment files were processed using Samtools for sorting, format conversion, and removal of PCR duplicates to ensure data accuracy. Variant detection for single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) was performed using the UnifiedGenotyper module in the GATK software package. The Variant Filtration module was applied to filter the raw variant dataset. The filtering criteria for SNPs were:--cluster Window Size 4, --filter Expression "QD < 4.0 || FS > 60.0 || MQ < 40.0", and -G_filter "GQ < 20".For InDels, the filtering criterion was:--filter Expression "QD < 4.0 || FS > 200.0" (Hu *et al*., 2024).

BSA analysis and QTL mapping: BSA was conducted based on the filtered high-quality SNP dataset. Using the two parental lines as reference genotypes, allele frequencies at each SNP locus were calculated separately for the drought-tolerant bulk and the drought-sensitive bulk. The SNP-index was defined as the proportion of reads harboring the target allele at a given SNP locus within a bulked sample. The ΔSNP-index was calculated as the difference between the SNP-index of the drought-tolerant bulk and that of the drought-sensitive bulk. To minimize the influence of sequencing errors and alignment biases, additional filtering was applied. SNP loci were removed if the sequencing depth in both bulks was less than 7×, if the SNP-index in both bulks was less than 0.3, or if the SNP-index value was missing in either bulk. Significant QTL regions were identified based on the smoothed ΔSNP-index values and the 95% confidence interval generated through 1,000 permutation tests. Continuous chromosomal regions where the absolute value of ΔSNP-index exceeded the 95%

confidence threshold were defined as significant QTL intervals. Genes located within these candidate regions were subsequently subjected to functional annotation.

Results and Analysis

Quality assessment of sequencing data: High-throughput sequencing was performed on four samples: the parental lines LNR-6 (MP) and LR-2381 (FP), the drought-tolerant bulk (F1-D), and the drought-sensitive bulk (F1-ND). High-quality raw sequencing data were obtained (Table 1). After stringent quality control using Fastp, the proportion of valid data for each sample exceeded 98%, indicating reliable sequencing quality. The sequencing error rates of the clean data were all below 0.03%. Regarding base quality, the proportion of bases with a quality score of Q20 (base-calling accuracy ≥99%) ranged from 95.69% to 96.13%, while Q30 values (base-calling accuracy ≥99.9%) ranged from 90.25% to 90.87%, meeting the requirements for subsequent analyses. The GC content ranged from 43.76% to 44.61%, which is consistent with the average GC content of the sorghum genome and showed no abnormal bias. Overall, the sequencing data from all samples exhibited sufficient sequencing depth, high quality, and a normal GC distribution, indicating that library construction and sequencing were successful and providing a reliable data foundation for subsequent variant detection and QTL mapping.

Read mapping to the reference genome: Clean sequencing data were aligned to the sorghum reference genome, and PCR duplicates were subsequently removed. The results are presented in Table 2. The number of reads aligned to the reference genome for the four samples was 78,035,504, 76,143,715, 62,700,468, and 55,134,520, respectively. The mapping rate exceeded 98% for all samples, indicating good suitability of the reference genome and reliable data quality. The average sequencing depth ranged from 10.74× to 14.89×. Specifically, the average depths for the two bulks were 14.89× and 14.22×, while the parental lines had depths of 11.81× and 10.74×. Regarding coverage, the 1× coverage (proportion of the genome covered by at least one base) exceeded 90% for all samples. The 5× coverage (depth ≥5×) was between 79.86% and 86.84%, and the 10× coverage (depth ≥10×) ranged from 44.54% to 68.48%. Overall, the sequencing depth and coverage for all samples met the requirements for BSA analysis, and the alignment results were suitable for subsequent variant detection and association analysis (Table 3).

SNP and InDel detection and annotation: A substantial number of genetic variants were detected across the four samples (Fig. 1). The majority of SNP variants in all four samples were located in intergenic regions, accounting for over 74% of the total. SNPs located in exons ranged from 3.65% to 3.94%, while those in introns ranged from 7.53% to 7.78%. SNPs located upstream or downstream of genes constituted between 4.14% and 5.46%. Among the SNP variants located in exons, non-synonymous mutations were predominant. Specifically, sample F1-D contained 52,367 non-synonymous mutations, 745 variants causing premature termination, and 170 variants resulting in stop codon loss. Sample F1-ND had 51,135 non-synonymous mutations, 721 premature termination variants, and 163 stop codon loss variants. Sample FP showed 44,519 non-synonymous mutations, 640 premature termination variants, and 140 stop codon loss variants. Sample MP contained 47,970 non-synonymous mutations, 657 premature termination variants, and 154 stop codon loss variants.

Similarly, the majority of InDel variants in samples F1-D, F1-ND, FP, and MP were located in intergenic regions, accounting for 55.64%, 55.29%, 55.58%, and 54.95%, respectively. The number of InDel variants in exons was 7,417, 7,092, 6,437, and 6,275, respectively. InDel variants in introns ranged from 13.61% to 13.91%, while those located upstream or downstream of genes ranged from 7.82% to 10.98%. Among the InDel variants located in exons, sample F1-D contained 60 variants causing premature termination, 5 variants causing stop codon loss, and 1,961 variants causing frameshifts. Sample F1-ND had 1,872 frameshift InDel variants, comprising 863 frameshift insertions and 1,009 frameshift deletions, along with 49 premature termination and 4 stop codon loss variants. Sample FP contained 50 variants related to termination, including 46 causing premature termination and 4 causing stop codon loss, alongside 755 InDel sites causing frameshift insertions and 893 causing frameshift deletions. Sample MP exhibited 53 variants causing premature termination, 3 causing stop codon loss, 725 frameshift insertions, and 859 frameshift deletions.

Table 1. Summary of sequencing data quality.

Sample	Raw reads	Raw bases (bp)	Clean reads	Clean bases (bp)	Error rate (%)	Q20%	Q30%	GC%
F1-D	88709024	13306353600	88697312	13156569064	0.0296	95.89	90.49	44.14
F1-ND	85622926	12843438900	85601772	12665149877	0.0300	95.69	90.26	44.08
FP	71473778	10721066700	71464012	10610216227	0.0299	95.77	90.25	44.61
MP	63115140	9467271000	63107628	9411757800	0.0291	96.13	90.87	43.76

Table 2. Sequencing depth and coverage statistics.

Sample	Mapped reads	Mapped rate (%)	Mean depth (x)	Coverage rate (1x) (%)	Coverage rate (5x) (%)	Coverage rate (10x) (%)
F1-D	78035504	98.69	14.89	92.13	86.84	68.48
F1-ND	76143715	98.41	14.22	92.19	86.33	65.55
FP	62700468	98.49	11.81	90.19	81.75	51.7
MP	55134520	98.48	10.74	91.06	79.86	44.54

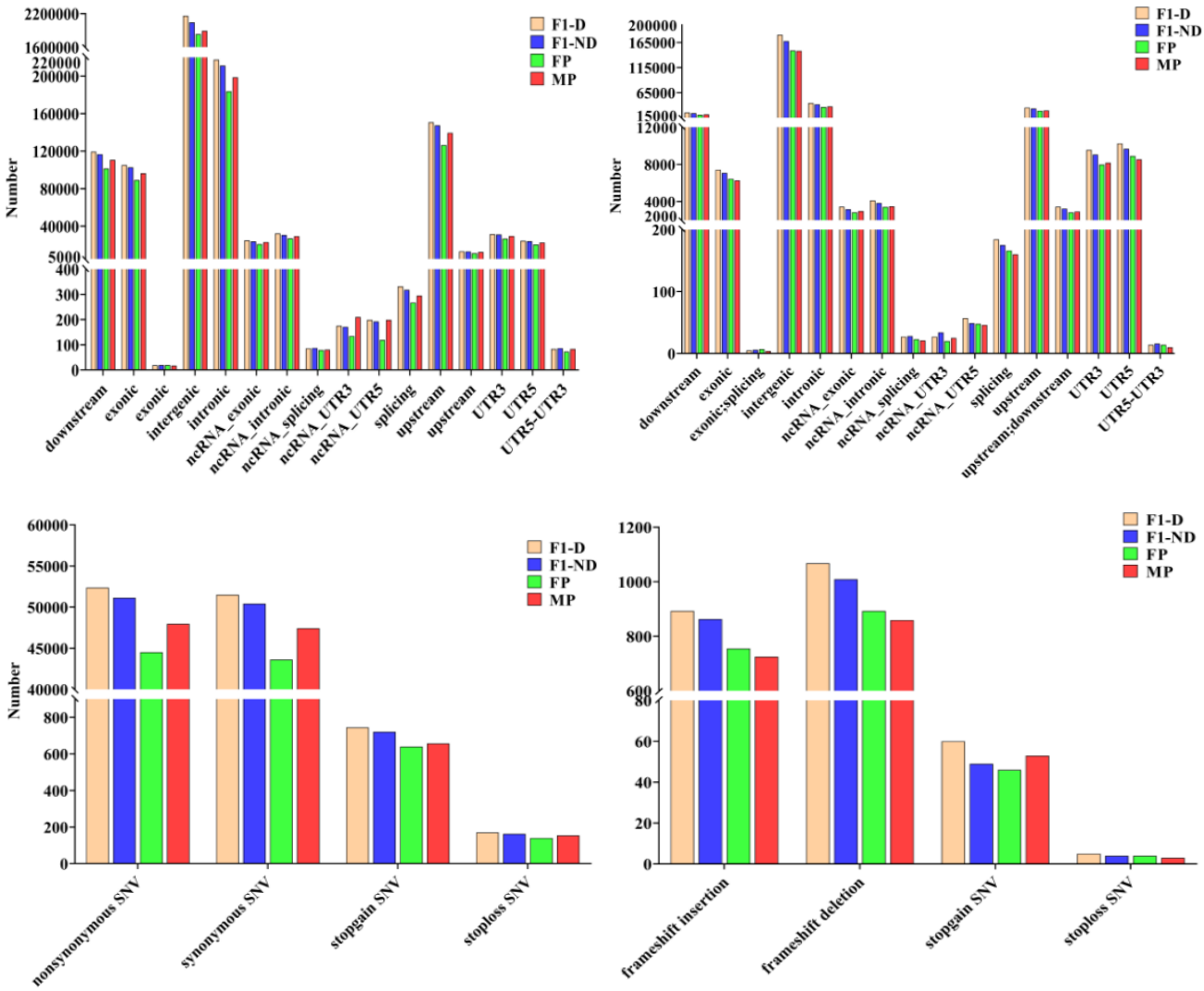

Fig. 1. SNP and InDel variation annotation statistics. (a). SNP detection and annotation results. (b). InDel detection and annotation results. (c). Annotation of SNP variants in exons. (d). Annotation of InDel variants in exons.

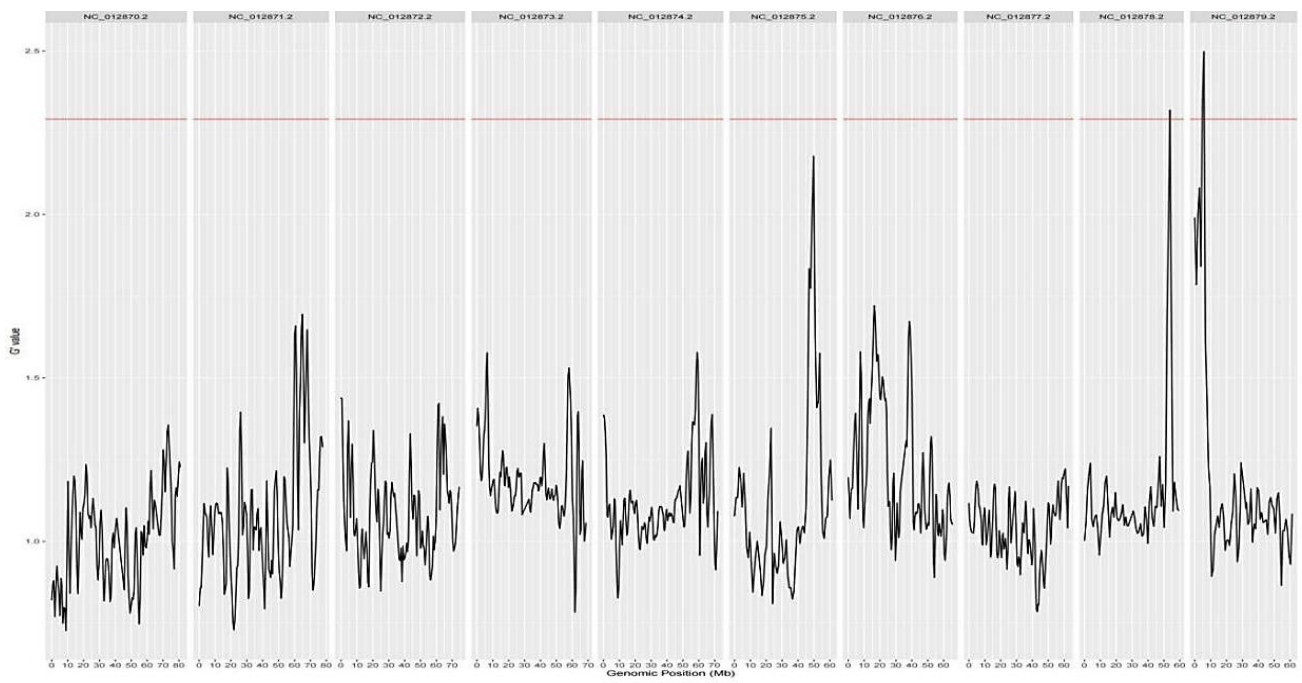

Fig. 2. Distribution of SNP indexes on chromosomes for two subpopulations.

Table 3. Functional annotation of key candidate genes in *qKH9*.

Query name	Hit description	Identities of COG/ KOG/ NOG region	Similarities of COG/ KOG/ NOG region
XM_021447574.1	zinc finger C-x8-C-x5-C-x3-H type family protein	49%	59%
XM_021447573.1	zinc finger C-x8-C-x5-C-x3-H type family protein	49%	59%
XM_021447575.1	zinc finger C-x8-C-x5-C-x3-H type family protein	49%	59%
XM_021447576.1	zinc finger C-x8-C-x5-C-x3-H type family protein	49%	59%
XM_021446791.1	zinc finger C_3HC_4 type family protein, putative	76%	83%
XM_021446791.1	zinc finger C_3HC_4 type family protein, putative	67%	80%
XM_002439969.2	ribosomal protein L11 methyltransferase-related, putative	70%	79%
XM_021447413.1	OsDegp8-Putative Deg protease homologue	71%	81%
XM_021447413.1	OsDegp8-Putative Deg protease homologue	82%	88%
XM_002441277.2	OsDegp8-Putative Deg protease homologue	72%	82%
XM_021447412.1	OsDegp8-Putative Deg protease homologue	71%	80%

QTL mapping for drought tolerance and candidate gene analysis: BSA data were analyzed using the QTLseqr package to calculate the sliding-window averages of ΔSNP-index values along each chromosome and to determine 95% confidence intervals based on 1,000 permutation tests. As shown in Fig. 2, two genomic regions significantly associated with drought tolerance at the seedling stage were identified on chromosomes 9 and 10. The region on chromosome 9, designated *qKH9*, spans 29.677 kb, from 53,842,813 bp to 53,872,490 bp, and contains 231 variant sites involving 17 transcripts. The region on chromosome 10, designated *qKH10*, spans 400.118 kb, from 4,996,989 bp to 5,397,107 bp, and harbors 4,039 variant sites involving 144 transcripts.

Within the *qKH9* interval, nucleotide sequences of 161 transcripts were compared against the COG database using BLASTn to obtain functional annotations. Among the 17 transcripts located in this region, 11 were assigned to well-defined functional domains. These domains included zinc finger C-x8-C-x5-C-x3-H type family proteins, zinc finger C_3HC_4 type family proteins, ribosomal protein L11 methyltransferase-related proteins and OsDegp8-like putative Deg protease homologs, among others. Several transcripts were annotated as members of the zinc finger protein family, a class of important transcriptional regulators known to play key roles in plant responses to drought and other abiotic stresses by modulating the expression of stress-responsive genes. In addition, genes related to ribosomal protein methylation and genes encoding Deg protease homologs were identified. Deg proteases are responsible for degrading misfolded or damaged proteins in plant cells and contribute to maintaining protein homeostasis and repairing damaged proteins under environmental stress. Taken together, these functional domains provide theoretical support for the hypothesis that genes within the *qKH9* interval may participate in the regulation of drought tolerance.

In the *qKH10* interval, 144 transcripts were analyzed and 154 functional domains were fully annotated, covering multiple functional categories. These domains included histidine kinases, inorganic H^+ pyrophosphatases, keratin-related proteins, type I cytoskeletal 9 proteins, leucine-rich repeat receptor protein kinase EXS precursors and peroxiredoxins, among others. The genes identified in *qKH10* thus exhibit considerable functional diversity. Histidine kinases are closely involved in signal transduction and regulate plant responses to environmental stresses. Inorganic H^+ pyrophosphatases are related to cellular energy metabolism and may contribute to stress adaptation by regulating proton gradients and energy balance. Keratin-related proteins are implicated in maintaining cell structural stability and protecting plants from mechanical damage. Leucine-rich repeat receptor protein kinase EXS precursors and peroxiredoxins participate in signal perception and transduction and in the scavenging of reactive oxygen species, respectively; the former may activate defense responses by sensing extracellular signals, whereas the latter helps maintain cellular antioxidant capacity under stress conditions. The functional diversity of these domains suggests that the *qKH10* interval may be involved in drought response in sorghum through multiple biological pathways.

Discussion

Drought stress is one of the major abiotic factors limiting global crop production, particularly in semi-arid and rain-fed agricultural regions, where it significantly affects crop growth and yield formation. Sorghum, as a typical drought-tolerant crop, shows strong adaptability under drought and water-limited conditions. However, during the seedling stage, the root system is not yet fully developed, making plants more sensitive to water stress, which may lead to inhibited growth or even plant death. Therefore, elucidating the genetic basis of drought tolerance at the seedling stage is of great importance for improving the stability of sorghum production in rain-fed agricultural regions. Previous studies have demonstrated that drought tolerance in crops is generally a complex quantitative trait controlled by multiple genes and strongly influenced by environmental factors (Borrell *et al*., 2014; Amelework *et al*., 2015).

In recent years, with the rapid development of high-throughput sequencing technologies, BSA-seq based on bulked segregant analysis has become an important tool for mapping complex quantitative traits. Compared with traditional linkage analysis methods, BSA-seq has several advantages, including lower population requirements, higher mapping efficiency, and relatively high resolution, and it has been widely used for identifying stress-resistance genes in crops such as rice, maize, and wheat (Takagi *et al*., 2013; Klein *et al*., 2018; Majeed *et al*., 2022). In this study, by constructing bulks from extremely drought-tolerant and drought-sensitive individuals for genome-wide analysis,

two QTL regions significantly associated with drought tolerance at the seedling stage were successfully identified on chromosomes 9 and 10. Among them, the *qKH9* interval is relatively small, with a length of only 29.677 kb, which provides a favorable basis for subsequent fine mapping and candidate gene validation.

Within the *qKH9* region, several candidate genes related to zinc finger proteins were identified in this study. Zinc finger proteins are important transcriptional regulators that play key roles in plant responses to abiotic stresses. Previous studies have shown that C_2H_2-type zinc finger proteins can enhance plant tolerance to drought and salt stress by regulating the expression of stress-responsive genes (Agarwal *et al*., 2007). In addition, some studies have reported that zinc finger proteins participate in the regulation of osmotic adjustment and reactive oxygen species scavenging, thereby enhancing plant adaptation under stress conditions. Therefore, the zinc finger-related genes identified in the *qKH9* interval in this study may regulate the expression of drought-responsive genes and may play important roles in the formation of drought tolerance at the seedling stage in sorghum.

In addition, candidate genes related to Deg proteases were also identified within the *qKH9* region. Deg proteases are important proteins involved in protein quality control in plant cells and mainly function in degrading damaged or misfolded proteins to maintain protein homeostasis within cells. Under stress conditions, cellular proteins are prone to oxidative damage, and Deg proteases can maintain cellular function by removing damaged proteins (Schuhmann and Adamska,2012). Therefore, these genes may potentially contribute to drought stress responses in sorghum by maintaining protein stability under adverse environmental conditions.

Compared with *qKH9*, the *qKH10* interval covers a relatively larger genomic region. It contains multiple types of candidate genes, including histidine kinases, leucine-rich repeat receptor-like kinases, and peroxiredoxin-related proteins. Histidine kinases are important components of plant signal transduction pathways and are involved in environmental signal perception and stress response regulation (Hofmann *et al*., 2020). Leucine-rich repeat receptor-like kinases play key roles in plant immunity and stress signaling pathways. In addition, antioxidant enzymes such as peroxiredoxins can eliminate reactive oxygen species generated during drought stress, thereby reducing oxidative damage to plant cells (Wang *et al*., 2023). The presence of these genes suggests that the *qKH10* region may participate in sorghum drought responses through multiple mechanisms, including signal transduction and antioxidant regulation.

Overall, this study successfully identified two QTL regions associated with drought tolerance at the seedling stage in sorghum using the BSA-seq approach and preliminarily screened several potential candidate genes. These findings not only provide important clues for further understanding the molecular mechanisms of drought tolerance in sorghum but also offer a theoretical basis for the development of drought-resistant molecular markers and marker-assisted breeding. Future studies could combine transcriptomic analysis, gene expression validation, and functional gene editing technologies to further verify the functions of these candidate genes and clarify their roles in the formation of drought tolerance in sorghum.

Conclusion

In this study, an F_2 segregating population derived from the drought-tolerant sorghum restorer line LNR-6 and the drought-sensitive restorer line LR-2381 was used to perform genome-wide association analysis of drought tolerance at the seedling stage using BSA-seq technology. Two QTL regions significantly associated with seedling-stage drought tolerance were successfully identified on chromosomes 9 and 10, namely *qKH9* and *qKH10*. The *qKH9* region contained 231 variant sites involving 17 transcripts, while the *qKH10* region contained 4,039 variant sites involving 144 transcripts. Functional annotation indicated that these candidate genes may be mainly involved in biological processes such as zinc finger protein-mediated transcriptional regulation, protein degradation, signal transduction, and antioxidant regulation. These results provide important candidate gene resources for elucidating the genetic basis of drought tolerance at the seedling stage in sorghum and provide useful molecular marker resources for marker-assisted breeding.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author's Contribution: Linlin Yang, Fei Zhang, Yanqiu Wang and Kuangye Zhang designed the study. Jiaxu Wang, Zeyang Zhao and Youhou Duan performed the experiments. Feng Lu, Kai Zhu, Zhipeng Zhang, Han Wu and Fulai Ke performed the data analyses. Linlin Yang wrote the manuscript. Fei Zhang and Kuangye Zhang critically reviewed the manuscript. All authors read and approved the submitted version.

References

Agarwal, P., R. Arora, S. Ray, A.K. Singh, V.P. Singh, Takatsuji, H. Kapoor and S.A.K. Tyagi. 2022. Genome-wide identification of C_2H_2-type zinc finger gene family members and their expression during abiotic stress responses in orchardgrass (*Dactylis glomerata*). *Genome*, 64(4): 189-203.

Amelework, B., H. Shimelis, P. Tongoona and M. Laing. 2015. Physiological mechanisms of drought tolerance in sorghum, genetic basis and breeding methods: A review. *Afr. J. Agri. Res.*, 10(31): 3029-3040.

Belete, T. 2018. Breeding for resistance to drought: A case in sorghum (*Sorghum bicolor* (L.) Moench). *J. Agri. Meteorol.*, 1: 1-10.

Borrell, A.K., J.E. Mullet, B. George-Jaeggli, E.J. van Oosterom, G.L. Hammer, P.E. Klein and D.R. Jordan. 2014. Drought adaptation of stay-green sorghum is associated with canopy development, leaf anatomy, root growth, and water uptake. *J. Exp. Bot.*, 65(21): 6251-6263.

Cattivelli, L., F. Rizza, F.W. Badeck, E. Mazzucotelli, A.M. Mastrangelo, E. Francia, C. Marè, A. Tondelli and A.M. Stanca. 2008. Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. *Field Crops Res.*, 105(1-2): 1-14.

Esposito, S., N. D'Agostino, F. Taranto, F. Fania, S. Pavan, I. Colella, F. Sestili, D Lafiandra and P. De Vita. 2026. Reverse BSA-QTLseq: A new genotype-driven bioinformatics approach for simultaneous trait mapping. *Plant Comm.*, 7(1): 101588.

Habila, S., N. Khunpolwattana, T. Buabooch, I.M. Henry, L. Comai, M. Pongpanich, K. Plaimas, D. Suriya-Arunroj, M. Siangliw, W. Kasettranun, P.R. Purnama and S. Chadchawan. 2025. F_2 bulk segregant analysis reveals salt-tolerant QTLs related to the ubiquitination process. *Sci. Rep.*, 15(1): 38684.

Hassan, M.A., N. Dahu, T. Hongning, Z. Qian, Y. Yueming, L. Yiru and W. Shimei. 2023. Drought stress in rice: morpho-physiological and molecular responses and marker-assisted breeding. *Front. Plant Sci.*, 14: 1215371.

Hofmann A., S. Müller, T. Drechsler, M. Berleth, K. Caesar, L. Rohr, K. Harter and G. Groth. 2020. High-level expression, purification and initial characterization of recombinant Arabidopsis histidine kinase AHK1. *Plants*, 9(3): 304.

Hu, H., L. Yi, D. Wu, L. Zhang, X. Zhou, Y. Wu, H. Shi, Y. Wei and J. Hou. 2024. Identification of candidate genes associating with soybean cyst nematode in soybean (*Glycine max* L.) using BSA-seq. *Peer J.*, 12: e18252.

Hu, W., X. Zhang, D. Zhu, D. Zhao, R. Yong, J. You, Y. Zhang and D. Gao. 2025. Identification of a novel QTL associated with Fusarium head blight resistance on chromosome 3B in common wheat. *Theor. Appl. Gen.*, 138(7): 150.

Khan, M.A., F. Tong, W. Wang, J. He, T. Zhao and J. Gai. 2018. Analysis of QTL–allele system conferring drought tolerance at seedling stage in a nested association mapping population of soybean [*Glycine max* (L.) Merr.] using a novel GWAS procedure. *Planta*, 248(4): 947-962.

Khan, M.A., M. Iqbal, M. Akram, M. Ahmad and M. Jamil. 2013. Recent advances in molecular tool development for drought tolerance breeding in cereal crops: a review. *Zemdirbyste*, 100(3): 325-334.

Kiriga, W.J., Q. Yu and R. Bill. 2016. Breeding and genetic engineering of drought-resistant crops. *Int. J. Agri. Crop Sci.*, 9(1): 7.

Klein, H., Y. Xiao, P.A. Conklin, R. Govindarajulu, J.A. Kelly, M.J. Scanlon, C.J. Whipple and M. Bartlett. 2018. Bulked-segregant analysis coupled to whole genome sequencing (BSA-Seq) for rapid gene cloning in maize. *G3-Genes Genom. Gen.*, 8(11): 3583-3592.

Liu, S., H. Wang and F. Qin. 2023. Genetic dissection of drought resistance for trait improvement in crops. *The Crop J.*, 11(4): 975-985.

Majeed, A., P. Johar, A. Raina, R.K. Salgotra, X. Feng and J.A. Bhat. 2022. Harnessing the potential of bulk segregant analysis sequencing and its related approaches in crop breeding. *Front. Gen.*, 13: 944501.

Min, H., K. Wang, T. Wang, X. Cheng, E. Habyarimana, Y. Wang, D. Hu, Y.H. Wang and L. Wang. 2025. Association mapping and candidate gene identification for drought tolerance in sorghum. *Front. Plant Sci.*, 16: 1629615.

Pinto, D.R., C.B. Menezes, A.J. Carvalho, A.F. Portugal, P.C. Magalhães, K.J. Silva, C.V. Santos, A.F. Campos, S.M. Oliveira and I. Aspiazú. 2023. Seedling selection of sorghum for drought tolerance based on root morphology. *Int. J. Maize & Sorghum*, 22: e1310.

Schuhmann, H. and I. Adamska. 2012. Deg proteases and their role in protein quality control and processing in different subcellular compartments of the plant cell. *Physiologia Plantarum*, 145(1): 224-234.

Takagi, H., A. Abe, K. Yoshida, S. Kosugi, S. Natsume, C. Mitsuoka, A. Uemura, H. Utsushi, M. Tamiru, S. Takuno, H. Innan, L.M. Cano, S. Kamoun and R. Terauchi. 2013. QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *The Plant J.*, 74(1): 174-183.

Tuberosa, R., S. Salvi, S. Giuliani, M.C. Sanguineti, M. Bellotti, S. Conti and P. Landi. 2007. Genome-wide approaches to investigate and improve maize response to drought. *Crop Sci.*, 47: S-120-S-141.

Wang M., I. Ahmad, B. Qin, L. Chen, W. Bu, G. Zhu and G. Zhou. 2025a. Identification and comprehensive evaluation of drought tolerance in sorghum during germination and seedling stages. *Plants*, 14(12): 1793.

Wang, J., J. Song, H. Qi, H. Zhang, L. Wang, H. Zhang, C. Cui, G. Ji, S. Muhammad, G. Sun, Z. Xu and H. Zhang. 2023. Overexpression of 2-Cys Peroxiredoxin alleviates the $NaHCO_3$ stress-induced photoinhibition and reactive oxygen species damage of tobacco. *Plant Physiol. Biochem.*, 201: 107876.

Wang, M., I. Ahmad, M.E. Hussien Ibrahim, B. Qin, H. Zhu, G. Zhu and G. Zhou. 2025b. Differential responses of two sorghum genotypes to drought stress at seedling stage revealed by integrated physiological and transcriptional analysis. *Agriculture*, 15(16): 1780.

Zheng, L.Y., X.S. Guo, B. He, L.J. Sun, Y. Peng, S.S. Dong, T.F. Liu, S. Jiang, S. Ramachandran, C.M. Liu and H.C. Jing. 2011. Genome-wide patterns of genetic variation in sweet and grain sorghum (*Sorghum bicolor*). *Genom. Biol.*, 12(11): R114.