***Review Article***

**Deciphering molecular breeding approaches to combat against abiotic stress tolerance in Pearl Millet (*Pennisetum glaucum* L*.*)**

**Abstract**

A vital cereal crop grown in arid and semi-arid countries, pearl millet (*Pennisetum glaucu*m L.) is essential to livelihoods and food security in these areas. Subsequently, abiotic stressors including heat, salinity, drought, and nutrient shortages severely limit its output. To enhance stress resilience and yield stability, molecular breeding approaches have emerged as powerful tools for genetic improvement. This review comprehensively explores recent advancements in molecular breeding strategies aimed at improving abiotic stress tolerance in pearl millet. Important techniques are covered in detail, including selection for genomic traits (GS), genome-scale association research (GWAS), quantitative trait loci (QTL) mapping, and marker-assisted selection (MAS). Additionally, the combination of omics technologies—such as transcriptomics, proteomics, and metabolomics—has led to a better understanding of the genes and pathways that are responsive to stress. The role of transgenic approaches and genome editing technologies, such as CRISPR/Cas9, in developing stress-tolerant pearl millet genotypes is also highlighted. Additionally, this review emphasizes the significance of wild relatives and landraces as valuable genetic resources for breeding climate-resilient cultivars. Despite remarkable progress, challenges such as limited genomic resources, genotype-environment interactions, and regulatory constraints remain. Addressing these limitations through interdisciplinary research, coupled with advancements in bioinformatics and high-throughput phenotyping, will accelerate the development of resilient pearl millet varieties. Ultimately, integrating molecular breeding with sustainable agricultural practices will be instrumental in ensuring the long-term adaptability and productivity of pearl millet under changing climatic conditions.

**Keywords:** Pearl millet, abiotic stress, molecular breeding, QTL mapping, genome editing

**Introduction**

A crucial cereal crop, pearl millet (*Pennisetum glaucu*m L.) is especially important in arid and semi-arid areas where it is frequently the only crop that can survive in hostile environments. Abiotic factors like drought, extreme temperatures and salinity are major challenges to pearl millet productivity as climatic variability increases. To enhance its resilience against these stresses, molecular breeding approaches have emerged as promising strategies (Shivhare & Lata, 2017). This draft explores various molecular breeding techniques aimed at improving abiotic stress tolerance in pearl millet (Tara Satyavathi et al., 2021). Even though pearl millet is known for its toughness, it is not immune to abiotic stressors that impair crop growth and output. It’s problems include lodging, extreme temperatures, salt, drought, and poor soil, just like those of other crops. To increase its flexibility, it is essential to recognize and use genetic variants for abiotic stress tolerance (Yadav et al., 2012). The extensive pearl millet germplasm collections in gene banks offer a substantial opportunity to discover and characterize DNA sequences associated with stress tolerance, which can accelerate crop improvement (Wang et al. 2025). Finding and utilizing promising candidate genes to create stress-tolerant cultivars will be made easier by combining precise phenotyping with a better comprehension of the physiological in origin and molecular mechanisms underpinning stress tolerance (Srivastava et al., 2022). Pearl millet's resistance to drought, salt, and high temperatures has been the subject of more research than that of other millets (Shikha et al., 2017; Bollam et al., 2018). The identification and application of pearl millet germplasm for abiotic stress resistance are examined in the sections that follow. A critical cereal crop for millions of people in arid and semi-arid countries, pearl millet (*Pennisetum glaucu*m L.) is essential to maintaining food and nutritional security worldwide. Its exceptional tolerance to drought, high temperatures, and poor soil conditions makes it a staple in marginal environments where other cereals struggle to thrive. Nevertheless, as the effects of the warming environment intensify, pearl millet yield is seriously threatened by abiotic factors as heat, salt, severe drought, and nutrient deficits (Vadez et al., 2012; Bollam et al., 2018). Due to the detrimental effects of these stressors on plant growth, yield, and grain quality, sophisticated breeding techniques are required to create genotypes that are climate resilient. Even while they work well, traditional breeding techniques can involve lengthy selection cycles and are not very accurate in transferring stress-resilient characteristics (Serba & Yadav, 2016). On the other hand, molecular breeding techniques such as genome editing, genome-wide choice (GS), quantitative trait loci (QTL) mapping, and marker-assisted selection (MAS) provide effective means of boosting pearl millet's resistance to abiotic stress and accelerating genetic improvement (Shivhare & Lata, 2017; Kapoor et al., 2022). The diversity of genes available for pearl millet enhancement have been greatly increased by recent developments in genomics and molecular biology. Deeper understanding of the genetic makeup of stress tolerance systems has been made possible by transcriptome analysis, genome-wide association analyses (GWAS), and high-throughput sequencing technologies. Targeted genetic changes now have more options thanks to the discovery of important stress-responsive genes, transcription factors, and regulatory networks. Furthermore, there is great potential for creating stress-resistant pearl millet variants by biotechnological interventions including transgenic techniques and CRISPR-Cas9 genome editing. The effectiveness and accuracy of creating improved genotypes suited to challenging environmental conditions can be increased by combining these molecular breeding techniques with traditional breeding procedures.

The goal of this review is to thoroughly go over the most recent developments in molecular breeding techniques for enhancing pearl millet's resistance to abiotic stress. It emphasizes how the creation of climate-resilient cultivars can be accelerated by genomic technologies, marker-assisted breeding, and biotechnology advancements. The paper also discusses the difficulties, gaps in knowledge, and prospects for using molecular breeding to produce pearl millet sustainably. Researchers and breeders can increase pearl millet's adaptability by utilizing state-of-the-art genetic and gene expression technologies, guaranteeing food security and sustainable agriculture in the face of growing climate difficulties.

**Diverse Abiotic Stress in Pearl Millet**

**Types of Abiotic Stress**

**Drought**

The actions and adaptability of pearl millet, a drought-resistant (DR) crop, to water deficits at different phases of growth have been thoroughly investigated (Lata et al. 2015). The crop's development phase has a significant impact on the impact of water stress. For example, poor crop establishment might arise from seedling death caused by water stress across germination or seedling emergence. One of the main causes of inadequate pearl millet yields in semi-arid areas is extreme moisture stress during the seedling stage. However, studies show that following the seedling establishment phase, drought stress has no effect on grain output (Kapoor et al., 2022). The impact of moisture stress has also been studied through various doses of polyethylene glycol (PEG) 6000, and that exhibited substantial impacts on embryonic criteria like germination rate, both shoot and root dimension, and root/shoot ratio. The use of PEG 6000 has been developed as a straightforward: fast, and affordable procedure for assessment pearl millet plant material during sprouting and early seedling growth stages, identifying TNBH 0538, TNBH 0642, and ICMV-221 as superior genotypes under moisture-stressed conditions, while PT6034 showed the least resistance. The influence of drought conditions on seedling germination is related to water availability, with inadequate water. stress significantly affecting leaf the creation and secondary root growth.

Drought exposure throughout the growth stage often has negligible impact on crop development and output, in contrast to post-flowering drought stress. Pearl millet's asynchronously tillering and quick proliferation are responsible for this resilience, which allows for fast healing (Yadav et al., 2024). Furthermore, a plant can minimize stress over the most vulnerable flowering period by delaying blossoming due to vegetative dryness. On the other hand, post-flowering or endowed drought has a detrimental effect on yield stability by drastically decreasing grain and stover production. According to research findings, early-flowering cultivars that have a high harvest index (including panicle harvest index), fewer but productive basal tillers, and lower biomass are more resilient to terminal drought. Two juxtaposing adults collaborates (PRLT2/88-33 X H77/833-2 and 863B-P2 X ICMB 841-P3) and near-isogenic lines (NILs) via severe drought tolerance (DT) QTL transmitted from PRLT2/88-33 (donor) into H77/833-2 (NILS-QTL) under water-deficit were studied in research examining water loss control in pearl millet under non-limiting conditions. Although adequately watered NILS-QTL and drought-tolerant cultivars showed reduced transpiration rates in comparison to hypersensitive genotypes. This data indicate that pearl millet varieties that carry a DT-QTL for conferred resilience to drought, such as tolerant runs down and NIL-QTLs, exhibited lower transpiration frequencies (Tr) and higher ABA levels across several levels of relative the vapor pressure deficits (VPD) even under conditions of adequate hydration (Bollam et al., 2024).

As a result, water is finally saved during grain filling. An examination of F7 recurrent inbred lines (RILs) resulting from a hybrid between 863B X ICMB 841 provided more evidence for this. Four QTLs were linked to elevated Tr under high VPD, with the main QTL mapping to linkage group (LG) 6. These findings reinforce the relationship between water conservation and drought tolerance by lowering Tr under high VPD. West African, Ghanaian, and northwest Indian pearl millet landraces are an important source of genetic variety for abiotic stress tolerance. These landraces are preferred over traditionally produced lines in the arid northwest Indian states of Rajasthan, Gujarat, and Haryana because of their higher grain and combustion yields, especially during drought. For example, CZP9802, a pearl millet variety that comes from landraces in Rajasthan, performs better in terms of grain yield (14–33%) and stover yield (18–36%) than regulate varieties Pusa 266 and ICTP 8203. It also shows remarkable stress adaptation. Because of its rapid blossoming (usually just 48 days) as well as maturity (before 75 days), CZP9802 is able to withstand terminal drought stress, which makes it a good choice for India's dry regions. Namibia also cultivates a lot of Okashana 1, another early-maturing cultivar. Likewise, the development of pearl millet cultivars worldwide, such as ICMV 88904 (issued as ICMV 221), has been greatly aided by the West African landrace Iniadi, which is distinguished by having light period apathy, early maturation, compact panicles, and prominent grains. Downy mildew resistance, the terminal interface drought tolerance, and increased grain yield potential are all displayed by ICMV 221, which is grown in India and various African nations.

**Salinity**

In dry and semi-arid parts of Asia and Africa, salinity is a major abiotic stressor for cereals. Low rain falling, high evaporation rates, and ineffective irrigation techniques make the issue worse by raising water-soluble salt levels, which prevent plants from accessing groundwater. In regions that are prone to dryness and high temperatures, salinization increases, encouraging the upward migration of capillary water and salts that dissolve in water into the root zone of plants. Although pearl millet's natural ability to withstand salinity allows it to be grown in saline environments for grain and fodder, salinity remains a significant barrier in some regions of Africa and India, especially in the West Asia and North Africa (WANA) provinces of the Asian continent. Little is known about how pearl millet reacts to soil salinity in comparison to other crops. Pearl millet's ability to withstand salinity is frequently associated with higher potassium and sodium content and lower shoot nitrogen content (Varshney et al., 2017). Two possible selection criteria for pearl millet germplasm screening at the phase of development are the percentage of shoot biomass ratio and the shoot sodium content. To find breeding materials for pearl millet that can withstand salt, salt-affected field-based pot cultivation processes are employed. In addition to enhanced populations (which comprises open-pollinated varieties), this screening has produced advanced breeding materials, gene pools, composite objects, ancestor lines for possible hybrids, and germplasm accessions that show high grain and forage yields, most likely as a result of improved salt tolerance (Azeem et al., 2025). Some of these materials may be made available for cultivation in the near to medium term following extensive on-farm yield performance assessment. For instance, in 2012, the pearl millet variant "HASHAKI I" was recognized and made available in Uzbekistan as a high-forage variety appropriate for regions afflicted by salt. In order to create regionally transformed, salinity-tolerant genotypes (both open-pollinated types and hybrids), breeding campaigns should utilize these discovered salinity-tolerant pearl millet lines. This will enable farmers to use land that is frequently left fallow to adopt and grow pearl millet in areas afflicted by salt.

**High Temperature**

The ideal temperature for pearl millet growth, which includes the germination of seeds, coleoptile expansion, and the process of photosynthesis is about 35°C. This is because pearl millet is adapted to the hot, dry Sahel regions and many parts of India, where daytime surface temperatures can reach 45°C. Even under hot conditions, pearl millet retains its maximum growth and yield potential, in contrast to the majority of other cereal crops that experience growth-related shortcomings at temperatures exceeding 35°C (Samineni et al., 2025). According to research on how supra-optimal temperatures affect seed germination, germination normally takes place between 35°C and 45°C, but it decreases at 47°C and almost stops at 50°C. Poor plant growth in pearl millet is known to be significantly influenced by high seedbed temperatures, and field research conducted in the Sahel has revealed that seedlings are especially vulnerable to high temperatures in the first 10 days following sowing. Finding genetic variants for excessive-temperature seedbed resilience in pearl millet genotype is essential since it is challenging to regulate high soil temperatures above ground by cultural methods. Despite being sensitive to endowed drought stress, the highly selective hybrid line H 77/833-2, which is widely utilized in northwest India, shows resilience to high temperatures. Near-isogenic lines (NILs), a population for mapping utilized for detecting quantitative trait loci (QTLs) linked to endowed endurance to drought for grain and combustion yield, as well as associated attributes, in pearl millet, have been developed using that variety as a perennial ancestor.

**Impact of Climate Change**

These abiotic stressors are becoming more frequent and intense because to climate change. The creation of robust crop types that can endure these difficulties is required due to changes in the pattern of rainfall and elevated temperatures.

**Molecular Breeding Approaches**

Molecular breeding modifies the genetic composition of crops for increased stress tolerance by fusing conventional breeding techniques with cutting-edge biotechnological instruments. Important strategies consist of:

**1. Marker-Assisted Selection (MAS)**

Breeders can more effectively choose plants with desired qualities by using MAS, which uses molecular markers connected to features of interest, such drought or heat tolerance (Reddy et al., 2015). Quantitative trait loci (QTLs) linked to pearl millet's resilience to environmental stresses have been discovered recently, making it easier to incorporate these qualities into breeding initiatives (Yadav et al., 2002).

**2. Transgenic Approaches**

Through transgenic breeding, particular genes that give pearl millet stress resistance are introduced. This technique makes it possible to directly alter the genetic circuits linked to stress reactions. For example, the genome of pearl millet might be modified to include genomes that encode transcription factors or short RNAs (miRNAs) that control stress tolerance (Anwar et al., 2020).

**Example: CRISPR/Cas9 Technology**

A potent tool for genome editing, the CRISPR/Cas9 system allows for precise alterations to improve abiotic stress tolerance features in crops. Researchers can produce pearl millet variants with increased resilience by focusing on particular genes implicated in stress response pathways (Anwar et al., 2020; Tu et al., 2023).

**3. Genomic Selection**

Utilizing genome-wide markers; additionally, genomic selection forecasts an individual's breeding preferences depending around their genetic composition. By enabling breeders to find superior lines early during the breeding cycle, this method speeds up the process of breeding (Younis et al., 2020).

**4. Transcriptomics and Multi-Omics Approaches**

New developments in transcriptomics shed light on how genes express themselves in response to abiotic stress. By looking at gene expression patterns that vary (DEGs), investigators can identify significant regulatory networks linked to stress responses (Dhawi, 2024, Tu et al., 2023). Multi-omics approaches that include the study of transcriptomics, proteomics, metabolomics, and genomes can provide an extensive grasp of pearl millet's resistance to abiotic stresses.

**Mechanisms of Abiotic Stress Tolerance**

To create resilient pearl millet cultivars, it is essential to comprehend the molecular mechanisms behind abiotic stress tolerance. Table 1 shows important chemical tools along with their associative mechanisms.

**1. Osmoregulation**

To preserve cell turgor under drought, plants produce osmoprotectants such as proline and glycine betaine. Drought tolerance can be increased by upregulating the proliferation of genes that regulate the development of osmoprotectants (Dhawi, 2024).

**2. Antioxidant Defense**

Cellular degeneration results from oxidative stress brought on by abiotic stressors. In order to reduce oxidative damage, antioxidant enzymes like catalase and superoxide dismutase (SOD) must be activated (Tu et al., 2023). Stress tolerance can be improved by transgenic strategies that target these mechanisms.

**3. Heat Shock Proteins (HSPs)**

HSPs are critical for safeguarding cellular activities during heat stress through inhibiting the degradation of proteins and agglomeration. In a number of crops, notably pearl millet, upregulation of HSP genes have been associated with increased heat endurance (Tu et al., 2023).

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| Table 1: Widely used molecular tools to combat with abiotic stresses | | | | |
| Molecular Breeding Approach | **Description** | **Molecular Mechanisms Involved** | **Stress Type Targeted** | **Outcome/Benefits** |
| Marker-Assisted Selection (MAS) | Uses DNA markers linked to genes controlling abiotic stress tolerance to select superior plants in breeding programs. | QTLs (Quantitative Trait Loci) associated with stress tolerance traits are identified and used for marker development. Markers help track favorable alleles during breeding[2](https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2016.02069/full). | Drought, salinity, heat. | Accelerates breeding by selecting plants with desired stress tolerance genes. Increases efficiency and precision compared to traditional phenotypic selection. |
| Marker-Assisted Backcrossing (MABC) | Uses markers to accelerate the recovery of the recurrent parent genome in backcrossing programs while introgressing target genes. | Foreground selection uses markers linked to the target gene. Background selection uses markers across the genome to select individuals with a high percentage of the recurrent parent genome. | Drought, downy mildew. | Faster recovery of the recurrent parent phenotype. Reduces linkage drag (transfer of undesirable genes linked to the target gene). |
| Gene editing (CRISPR-Cas9) | Precise modification of specific genes related to abiotic stress tolerance. | Targets specific DNA sequences in the pearl millet genome, creating mutations or inserting desired genes. This can enhance the expression of stress tolerance genes or knock out negative regulators. | Heat, salt, drought. | Precise and efficient way to improve stress tolerance. Can introduce novel traits or enhance existing ones. |
| Transcriptomics Analysis | Study of the transcriptome (the complete set of RNA transcripts) to identify genes and pathways involved in stress response. | RNA sequencing (RNA-Seq) is used to compare gene expression levels between stressed and non-stressed plants. Differentially expressed genes (DEGs) are identified and can be used as targets for molecular breeding. | Heat and salt. | Provides insights into the molecular mechanisms underlying stress tolerance. Identifies key genes and pathways that can be manipulated to improve stress tolerance. |
| Allele Mining | Exploitation of natural genetic variation within pearl millet germplasm to identify favorable alleles for abiotic stress tolerance. | Screening of diverse pearl millet accessions for stress tolerance traits. Identification of superior alleles using association mapping or genome-wide association studies (GWAS). | Drought, salinity, heat. | Utilizes existing genetic resources to improve stress tolerance. Can identify novel genes and alleles that are not present in elite cultivars. |
| QTL Mapping | Identifies genomic regions (QTLs) associated with abiotic stress tolerance traits. | Uses bi-parental mapping populations and molecular markers to map QTLs. Statistical analysis is used to determine the association between markers and traits. | Drought, salinity. | Provides information on the genetic architecture of stress tolerance traits. Helps in the development of markers for MAS and MABC. |

**Future Perspectives**

Pearl millet's ability to withstand abiotic stressors could be greatly improved by incorporating molecular breeding techniques. As research advances, a number of crucial issues need to be addressed:

1. Functional Validation: Continued efforts are needed to validate candidate genes identified through genomic studies and assess their roles in abiotic stress responses.

2. Field Trials: Implementing field trials for newly developed varieties will provide insights into their performance under real-world conditions.

3. Collaboration: Collaborative efforts among researchers, breeders, and farmers are essential to ensure that molecular breeding advancements translate into practical solutions for enhancing pearl millet productivity.

**Conclusion**

To summarize up, exploring molecular breeding techniques to improve Pearl Millet's (*Pennisetum glaucu*m L.) resistance to abiotic stress is a critical step in ensuring sustainability of agriculture in the face of environmental difficulties and climate change. The incorporation of cutting-edge genomic technologies, including CRISPR-Cas9 gene editing and marker-assisted selection, has created new opportunities for finding and modifying important features linked to resilience to heat stress, salinity, and drought. Researchers can create cultivars that not only endure harsh environments but also preserve high nutrient-rich nutritional value and yield stability by utilizing the genetic diversity present in pearl millet. Additionally, the collaborative efforts between molecular biologists, agronomists, and breeders are essential for translating these scientific advancements into practical applications. Field trials and multi-environment testing will be crucial to validate the performance of these new cultivars under varying climatic conditions. Additionally, engaging with local farming communities will ensure that the developed varieties meet the specific needs of farmers while promoting sustainable agricultural practices. Ultimately, by fostering innovation and collaboration within the agricultural sector, we can pave the way for a more sustainable future where Pearl Millet thrives even in the most challenging environments. Embracing these molecular breeding strategies will not only benefit farmers but also ensure that Pearl Millet continues to play a crucial role in addressing nutritional needs across diverse populations worldwide.

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