

REVIEW ARTICLE

# Plant Breeding Approaches for Enhancing Abiotic Stress Tolerance in Crops: A Review

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## ABSTRACT

Abiotic stresses significantly impact crop productivity, posing a major challenge to global food security, particularly in the context of changing climate. Traditional breeding methods have made progress, but their limitations in addressing complex traits like abiotic stress tolerance highlight the need for innovative approaches. Besides conventional breeding approaches, this paper explores advancements in modern breeding techniques, including marker-assisted selection, tissue culture and genetic engineering, which offer new avenues for developing stress-resilient crop varieties. It also underscores the potential of crop wild relatives and landraces as underutilized reservoirs of genetic diversity for abiotic stress tolerance. The integration of conventional breeding with molecular tools, such as gene pyramiding, QTL mapping and genomic selection, has accelerated the identification and incorporation of desirable traits. Emerging technologies like CRISPR-Cas9 and omics-based approaches further enhance precision and efficiency in breeding programs. This review emphasizes the importance of harnessing diverse genetic resources, optimizing breeding strategies and fostering interdisciplinary collaborations to address current and future challenges. This provides a roadmap for sustainable crop improvement, paving the way for resilient agricultural systems in the face of climate variability.

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## INTRODUCTION

Abiotic stress refers to the adverse impact of non-living environmental factors on the physiology, growth, and productivity of living organisms. Stresses, such as drought, salinity, extreme temperatures, and nutrient imbalances, pose significant challenges to agricultural productivity worldwide. Abiotic stresses are the primary cause of crop losses globally,

reducing average yields of major crops by over 50% (Ezin *et al.*, 2012; Tilman *et al.*, 2002). In some cases, they can decrease the survival and yield of staple crops by up to 70% (Vorasoot *et al.*, 2003; Kaur *et al.*, 2008; Rodriguez *et al.*, 2005; Acquaaah, 2007), severely threatening global food security.

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The increasing frequency and intensity of these stresses, exacerbated by climate change, land degradation, and declining water resources, underscore their growing importance in agriculture (Tester and Langridge, 2010; Kellogg, 2019). In India, for example, 67% of agricultural land is rainfed and often experiences drought. In contrast, the irrigated areas, which constitute 33% of the total cropped area, face environmental challenges such as heat stress. Climate change is expected to exacerbate these conditions, leading to yield further reductions (Bisbis *et al.*, 2018; DaMatta *et al.*, 2019; Leichenko *et al.*, 2014).

Recent climate models predict that crops will increasingly encounter more severe combinations of abiotic stresses in the near future (Chapman *et al.*, 2012; Zandalinas *et al.*, 2018). While research on single stress factors, such as drought or heat, has provided valuable insights, these findings often fail to account for the complex interactions resulting from multiple simultaneous stresses (Mittler, 2006; Suzuki *et al.*, 2014). Encouragingly, significant advancements have been made in understanding the physiological, molecular, and metabolic responses of plants to combined stresses (Perdomo *et al.*, 2015; Barnabás *et al.*, 2008; Zandalinas *et al.*, 2016a, 2016b). Developing crop varieties with enhanced abiotic stress tolerance remains challenging due to the complex genetic and physiological nature of these traits, which often exhibit low heritability (Karavolias *et al.*, 2019). Breeding strategies can focus on modifying the environment or altering the plant genotype. Direct selection under stress conditions or indirect selection in stress-free environments has been employed to improve tolerance (Lewis & Christiansen, 1981).

Classical breeding techniques, such as hybridization, backcrossing, and multiline breeding, have been instrumental in enhancing stress tolerance. However, these methods are often time-consuming and may not keep pace with rapidly changing environmental conditions. The advent of molecular tools, including marker-assisted selection (MAS), genomics, and recombinant DNA technologies, has revolutionized breeding approaches. Techniques such as targeted induced local lesions in genomes (TILLING), a non-transgenic method, and virus-induced gene silencing (VIGS) have shown promise in developing stress-tolerant crop varieties more efficiently (Elmaghrabi, 2018).

This review highlights the advancements in plant breeding approaches, both classical and modern, for improving abiotic stress tolerance in crop plants, emphasizing their applications and limitations in the context of current agricultural challenges.

**Principal abiotic stresses impacting agriculture:**

Drought, submergence, salinity, and temperature extremes are among the most significant abiotic stresses limiting plant productivity, shaping the geographical distribution of plant species, and threatening food security. The impact of these stresses is amplified by climate change, which is expected to increase the frequency and severity of extreme weather events (Fedoroff *et al.*, 2010). Globally, abiotic stresses account for an average of 50% of yield losses in crops, with drought, submergence, heat, salinity, and cold being the most significant factors (Ashraf *et al.*, 2008, 2010). Alarmingly, only 9% of the global land area is considered conducive for crop production, while the remaining 91% is affected by various forms of abiotic stress. In India, 120.8 million hectares or 36.5% of the country's geographical area, are degraded due to soil erosion, salinity, alkalinity, acidity, waterlogging, and other edaphic problems (ICAR, 2010). These challenges are expected to intensify under global warming, further exacerbating productivity losses.

Drought is the most critical abiotic stress affecting global food security and is predicted to worsen with climate change (Ebert *et al.*, 2019; Oshunsanya *et al.*, 2019). Drought events, often accompanied by heatwaves, disproportionately damage crops compared to individual stress factors (Hussain *et al.*, 2019; Zandalinas *et al.*, 2016a, 2016b). In South Asia, severe droughts in 1987 and 2002-03 impacted over 50% of India's cropped area and affected nearly 300 million people (Pandey *et al.*, 2015). Similarly, in Southeast Asia, droughts in 2004 devastated 20% of rice lands and displaced millions in Thailand (Pandey *et al.*, 2015).

Submergence stress, often caused by heavy rainfall and flooding, affects agricultural regions worldwide, particularly in low-lying areas. Prolonged submergence deprives plants of oxygen, resulting in reduced photosynthesis, impaired root function, and ultimately, crop loss. Rice is among the most affected crops, with millions of hectares of rice paddies submerged annually, especially in South and Southeast Asia. Developing submergence-tolerant

varieties, such as those carrying the *SUB1* gene, has shown significant promise in improving rice resilience under flood-prone conditions (Xu *et al.*, 2006; Bailey-Serres *et al.*, 2010).

High temperatures are becoming an increasingly global concern due to their adverse effects on plant growth, yield, and quality (Toscano *et al.*, 2019; Chen *et al.*, 2019). Heat stress causes morpho-anatomical, physiological, and biochemical disruptions, ultimately impairing plant development. Breeding for thermo-tolerance using both traditional and advanced genetic approaches can mitigate these challenges (Kjellstrom *et al.*, 2016; Zampieri *et al.*, 2017).

Soil salinity is another critical factor limiting agricultural productivity, particularly in arid and semi-arid regions. Declining irrigation water quality exacerbates this issue, resulting in reduced crop yields (Askari *et al.*, 2017; McFarlane *et al.*, 2016). Globally, over 800 million hectares of land are severely affected by salinity, including 21.5 million hectares in Asia. Projections indicate that by mid-century, up to 50% of fertile land in Asia could become saline, emphasizing the urgent need to develop salinity-tolerant crop varieties (FAO, 2010; Nazar *et al.*, 2011).

Low temperatures are particularly detrimental to plant growth in temperate and high-altitude regions. Cold stress, and reproductive development, often leading to yield losses. Crops such as rice, maize, and wheat are susceptible to chilling and freezing temperatures. Breeding for cold tolerance involves selecting genotypes with enhanced membrane stability, osmotic adjustment, and antioxidant enzyme activity. Advances in molecular markers and genomic tools have accelerated the development of cold-tolerant varieties adapted to challenging environments (Chinnusamy *et al.*, 2007; Sharma *et al.*, 2014).

By 2025, 60–70% more food will be required from the same water resources available today. This necessitates increasing agricultural productivity per unit of land and water, while developing stress-resilient crops tailored for adverse conditions. Integrating conventional breeding approaches with advanced genetic tools offers a promising strategy to address these challenges and ensure sustainable food production.

### **Breeding methodologies:**

When adopting any breeding method for crop improvement, it is crucial to consider the species'

mode of reproduction, whether it is self-pollinating, cross-pollinating, or asexual. The choice of breeding approach is closely tied to the type of cultivar and the genetic control of the target traits. Breeding for abiotic stress tolerance is particularly challenging because individual plants often respond differently to similar stress factors. This complexity makes it challenging to improve multiple resilient traits simultaneously, yet achieving this is the primary goal of plant breeders. The multifaceted nature of abiotic stress tolerance in plants complicates efforts to enhance stress resilience genetically. Despite these challenges, breeding for abiotic stress tolerance is a critical strategy to mitigate yield losses. Scientists globally are working to develop crop varieties with improved performance in stress-prone environments. Developing crop varieties with inherent tolerance to salinity, drought, and heat is recognized as a promising, resource-efficient, cost-effective, and socially acceptable solution to ensure agricultural sustainability and food security.

### **Traditional breeding approaches**

Breeding for abiotic stress tolerance begins with assembling genetic variation through the collection and evaluation of available germplasm. If the desirable variability is absent within a locality or species, exotic germplasm introductions are employed. This classical approach remains a cornerstone of all breeding strategies. Several salinity-tolerant rice varieties have been developed worldwide through selection and introduction methods. The salt-tolerant rice varieties Damodar (CSR-1), Dasal (CSR-2), and Getu (CSR-3) are pure-line selections from local traditional cultivars originating from the saline-affected Sunderbans delta in West Bengal, India (Meena *et al.*, 2016). Likewise, Jhona-349, SR-26B, Bhura Rata 4-10, Patnai-23, Hamilton, and Vytilla-1 were site-specific selections from landraces. Additionally, varieties such as Jaladhi-1 (a selection from Kalakhersail), Jaladhi-2 (a selection from Baku), Jalaprabha (a composite selection), Neeraja (a selection from a landrace), Dinesh (Jaladhi-2/Pankaj), and Hangseswari (a pure line selection) were developed through selection for deep-water tolerance (Collard *et al.*, 2013).

The pedigree selection method, one of the oldest and most widely used breeding methods, is highly effective for developing resistance in crop varieties, mainly when major genes govern traits. This method achieves a combination of multiple genes controlling

biotic and abiotic stress tolerance (Khush, 1984). Due to the complexity of abiotic stress traits, grain yield is often used as a selection criterion under stress conditions, despite its low heritability and genotype-by-environment ( $G \times E$ ) interactions. This method has proven effective in developing superior genotypes for grain yield in cultivar development programs (Oladosu *et al.*, 2019; Reddy *et al.*, 2019). Pedigree breeding involves crossing two lines, each contributing desirable genes, and selecting superior genotypes in subsequent generations. Lineage is maintained until genetic stabilization, typically in F7 or F8 generations. This method provides opportunities for iterative selection across generations, allowing breeders to achieve uniformity and superior trait combinations. However, the method is labor-intensive and resource-demanding, leading breeders to adapt and simplify it.

The modified bulk-pedigree method is another approach that combines the strengths of pedigree and bulk breeding methods, while requiring fewer resources (Collard *et al.*, 2017; Souleymane *et al.*, 2017; Meena *et al.*, 2016; Fischer *et al.*, 2018). It is useful for less heritable traits, where individual F2 plants are bulk-harvested up to F4 or F5 generations. Selection for superior genotypes resumes in later generations, similar to the pedigree method. For highly heritable traits, early-generation selection (F2 or F3) is followed by bulk propagation and panicle selection in F5 or F6 generations (Júnior *et al.*, 2018). This approach is advantageous when resources, such as land, laboratory facilities, or labor, are limited, or when the selection environment cannot distinguish between desirable and undesirable genotypes. For instance, drought and submergence-tolerant genotypes are bulked during early generations, with elite lines selected in later stages for yield and other agronomic traits. The modified bulk-pedigree method simplifies procedures, reduces labor, and minimizes expenses during early segregating generations.

Shuttle breeding is another approach that evaluates pre-breeding or advanced materials at diverse locations to identify adaptable genotypes. Initially developed by CIMMYT for wheat improvement, this method accelerates breeding cycles by advancing generations across multiple locations and seasons. Nobel laureate Dr. Norman Borlaug popularized it and later adapted for rice breeding in 1982 (Mackill *et al.*, 2013). In rice, logistical challenges such as seed transport across international borders, intellectual property rights, and germplasm protection have limited

its widespread adoption. Despite these hurdles, the private sector, especially in temperate regions, continues to employ off-season nurseries for shuttle breeding.

Backcrossing is a recurrent hybridization technique to incorporate specific traits into elite cultivars. It is highly effective for traits such as drought, submergence, and salinity tolerance (Oladosu *et al.*, 2014). For example, IRRI utilized this method to improve drought tolerance in rice. A large-scale backcross breeding program involving three recurrent parents and 203 donor lines has developed numerous introgression lines with enhanced abiotic stress tolerance (Ali *et al.*, 2006). The number of backcross generations depends on the relative performance of donor and recurrent parents.

Induced mutagenesis is used when desired genes are absent in the existing germplasm. For instance, the barley cultivar IZ Bori (Kt3026) was developed using sodium azide mutagenesis and exhibits high cold tolerance (Tomlekova, 2012).

Recurrent selection improves complex traits such as photosynthetic efficiency and drought tolerance. In maize, varieties like ZM621 and ZM303 were developed through this approach. For instance, ZM301 C1 was derived using S1 recurrent selection in Botswana from CIMMYT parental material (Lekgari *et al.*, 2004).

Traditional approaches to breeding crop plants with improved tolerance to abiotic stress have achieved limited success (Watson *et al.*, 2019; Pandey *et al.*, 2019). This limited progress can be attributed to several factors. Breeding efforts have predominantly focused on grain yield as the primary selection criterion, often at the expense of specific physiological and adaptive traits that directly contribute to stress tolerance. This yield-centric approach has limited the capacity to address the multifaceted challenges posed by abiotic stresses. Breeding for tolerance traits is further complicated by the intricacies of genotype-by-environment ( $G \times E$ ) interactions, which obscure the genetic basis of tolerance and hinder effective selection. These interactions make it difficult to develop cultivars with consistent performance across varying environments. Additionally, simple physiological traits, which could serve as reliable proxies for tolerance and are less influenced by  $G \times E$  interactions, have

been underutilized in breeding programs. Another significant constraint arises from the limited genetic variability available for introducing novel traits. The reliance on closely related species as sources of desirable traits restricts the genetic pool, narrowing opportunities for the development of cultivars with enhanced resilience to abiotic stresses. Overall, progress in developing high-yielding, drought-tolerant cultivars through conventional breeding has been slow. This is mainly due to challenges in accurately defining target environments, the complex interplay of drought tolerance traits with environmental conditions, and the lack of robust and widely applicable screening methodologies (Oladosu *et al.*, 2019; Taunk *et al.*, 2019; Soumya *et al.*, 2023).

### **Modernization in plant breeding approaches**

Traditional plant breeding methods have served as a foundation for crop improvement for decades. Still, they often fall short in addressing complex traits such as abiotic stress tolerance, nutrient use efficiency, and biotic stress resistance (Sarkar *et al.*, 2021). These methods are constrained by factors such as the extensive time required for backcrossing to eliminate undesirable traits, reliance on observable phenotypes, and the limited genetic diversity within the accessible gene pool. To overcome these limitations, modern plant breeding approaches incorporate advanced techniques, including marker-assisted selection (MAS), tissue culture, genomic tools, genetic engineering, and precision breeding.

Marker-assisted selection (MAS) has revolutionized the breeding process by enabling the precise incorporation of desirable traits, particularly in cases where environmental factors significantly affect phenotypic expression. MAS is especially effective for traits with low heritability, complex environmental interactions, or traits difficult to evaluate in the field. For example, in rice, MAS has been used extensively to introgress QTLs for salinity tolerance into high-yielding mega-varieties (Semikhodskii *et al.*, 1997; Meena *et al.*, 2016). The ability to pyramid multiple traits, such as resistance to abiotic stresses, makes MAS an indispensable tool in modern breeding programs.

Tissue culture techniques offer a complementary approach to crop improvement. These methods operate under controlled environmental conditions, reducing time and space requirements for developing stress-tolerant crops. Somaclonal variation, arising from cell culture, has been a key innovation for introducing

genetic diversity. For example, Pokkali, a traditional salt-tolerant rice variety, was subjected to somaclonal variation, resulting in improved lines such as TCCP 266-2-49-B-B-3, which retained its salinity tolerance while exhibiting superior agronomic traits (Gregorio *et al.*, 2002). Similar *in vitro* approaches have led to the development of salt-tolerant potato lines and hybrids, which have been identified through rigorous screening (Ochatt *et al.*, 1999; Zapata & Abrigo, 1986).

F1 anther culture is another significant advancement in reducing the breeding cycle. Through this approach, doubled haploid (DH) lines can be developed rapidly, allowing for the quicker isolation of stress-tolerant genotypes. At IRRI, the use of another culture led to the development of high-yielding, salinity-tolerant lines, such as IR51500-AC11-1 (PSBRc50), which became the first indica rice variety released for cultivation in adverse environments (Senadhira *et al.*, 2002). This technique has also been applied to other crops, yielding promising results in enhancing stress tolerance and agronomic performance.

Genetic engineering has expanded the horizons of plant breeding by allowing the direct manipulation of genes associated with complex traits. Transgenic approaches have been employed to introduce genes that enhance stress tolerance. For instance, the incorporation of the HVA1 gene from barley into wheat improved drought tolerance in Egyptian cultivars (Chauhan & Khurana, 2010, Khatun *et al.*, 2021). Genetic modifications, such as the insertion of Na<sup>+</sup>/H<sup>+</sup> antiporters and H<sup>+</sup> pyrophosphatases in Arabidopsis, have demonstrated improved tolerance to salinity and drought stresses (Brini *et al.*, 2007).

The advent of genomic tools, including quantitative trait loci (QTL) mapping, CRISPR-Cas9-based gene editing, and genome-wide association studies (GWAS) has further accelerated breeding progress. QTL mapping has successfully identified loci for abiotic stress tolerance, including aluminum toxicity in rice (Xue *et al.*, 2007) and salinity tolerance in barley and wheat (Ellis *et al.*, 1997; Wang *et al.*, 2007). CRISPR-Cas9 offers unprecedented precision in editing target genes, enabling the development of crops with enhanced yield and stress tolerance. The integration of genomic selection with MAS has also improved the efficiency of breeding programs by enabling the selection of desirable traits at an early stage of crop development.

Modern plant breeding continues to evolve with innovations in phenotyping, data integration, and artificial intelligence. High-throughput phenotyping platforms, combined with machine learning, have improved trait prediction and selection efficiency, particularly under complex environmental conditions. These advancements underscore the shift towards a data-driven, systems biology approach to crop improvement, enabling breeders to address global challenges such as food security, climate change, and sustainable agriculture.

## CONCLUSION

Recent advancements in biotechnology have provided plant breeders with innovative tools to incorporate desirable traits more effectively through techniques like gene pyramiding. However, the success of these approaches hinges on the integration of traditional breeding methods with physiological and genomic insights. Breeding for abiotic stress tolerance is becoming increasingly crucial, not only to address current challenges but also to mitigate the impacts of future climate scenarios characterized by more frequent and severe stress events. The untapped potential of crop wild relatives and landraces as sources of abiotic stress tolerance offers a significant opportunity for crop improvement. Despite their rich genetic diversity, these resources remain underutilized in breeding programs and are often underrepresented in germplasm collections. Advances in molecular technologies, such as genome sequencing, gene editing, and genomic selection, are now enabling faster and more precise identification of beneficial traits, paving the way for the development of stress-resilient varieties. To address the challenges posed by changing climates and ensure food security, it is essential to rapidly discover, conserve, and utilize genetic variability from all available resources. Collaborative efforts that combine advanced molecular tools with traditional breeding practices will play a pivotal role in harnessing this genetic diversity, enabling the development of high-yielding, stress-tolerant crop varieties that are better equipped to thrive in diverse environmental conditions.

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