# *Review Article*

# Breeding for Climate Resilience: Genetic Approaches to Improve Heat and Drought Tolerance in Rice (*Oryza sativa* L.)

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## ABSTRACT

Rice (*Oryza sativa* L.) is one of the world's most important staple crops, feeding over half of the global population. However, climate change poses unprecedented challenges to rice production through increased temperatures, altered precipitation patterns, and more frequent extreme weather events. Heat and drought stress significantly reduce rice yields, threatening global food security. This review examines the current state of genetic approaches for developing climate-resilient rice varieties, with particular emphasis on quantitative trait loci (QTL) mapping, marker-assisted selection (MAS), and gene networks associated with abiotic stress tolerance. We discuss the identification and characterization of major QTLs controlling heat and drought tolerance, the application of molecular markers in breeding programs, and the complex gene regulatory networks underlying stress responses. Recent advances in genomics technologies, including genome-wide association studies (GWAS) and genomic selection, have accelerated the development of climate-resilient rice varieties. This review highlights the integration of traditional breeding methods with modern molecular approaches and discusses future directions for enhancing rice climate resilience through genetic improvement.

***Keywords:*** *Rice, climate change, heat tolerance, drought tolerance, QTL mapping, marker-assisted selection, abiotic stress*

## INTRODUCTION

The global agricultural landscape faces an unprecedented crisis as climate change threatens food security for billions of people worldwide. Rice (Oryza sativa L.), which serves as the primary food source for approximately 3.5 billion people, is particularly vulnerable to climate-induced stresses that are becoming increasingly frequent and severe. The urgency of this problem cannot be overstated: with global population projected to reach 9.7 billion by 2050, rice production must increase by 25-40% to meet growing demand, yet climate change is simultaneously reducing productive capacity through heat and drought stress. Current estimates indicate that heat stress alone reduces rice yields by 10-15% for each 1°C increase above optimal growing conditions, while drought affects approximately 23 million hectares of rain-fed rice annually, causing yield losses of 17-40% depending on severity and timing. Traditional breeding approaches, while historically successful, are insufficient to address the complex, polygenic nature of abiotic stress tolerance within the timeframe required by accelerating climate change. The development of climate-resilient rice varieties through advanced genetic approaches has therefore become a critical priority for global food security, necessitating the integration of cutting-edge genomic technologies with conventional breeding methods to create varieties capable of maintaining stable yields under increasingly challenging environmental conditions.

The primary objective of this comprehensive review is to synthesize current knowledge on genetic approaches for developing heat and drought-tolerant rice varieties, with the aim of providing a strategic roadmap for breeding climate-resilient rice under increasingly challenging environmental conditions. This review seeks to critically evaluate both traditional breeding methods and modern molecular techniques to understand their relative effectiveness in enhancing abiotic stress tolerance in rice. It delves into the methodologies and practical applications of quantitative trait loci (QTL) mapping to identify genomic regions associated with heat and drought tolerance. Furthermore, the review examines the principles, development, and use of marker-assisted selection (MAS) in rice breeding programs, highlighting its utility in accelerating genetic gains. A focus is also placed on exploring gene regulatory networks and the molecular mechanisms that govern rice responses to abiotic stresses. In addition, the review assesses advanced genomic tools such as genome-wide association studies (GWAS) and genomic selection, considering their effectiveness in improving selection accuracy and breeding efficiency. Case studies demonstrating successful applications of these genetic strategies in developing stress-tolerant rice varieties are discussed to provide real-world evidence of their impact. Finally, the review identifies key challenges and outlines future research directions necessary to strengthen rice resilience through genetic improvements, ensuring sustainable production under the threat of climate change.

## 2. TRADITIONAL APPROACHES VERSUS MODERN TECHNIQUES

### 2.1 Traditional Breeding Approaches

Traditional rice breeding has relied primarily on phenotypic selection and hybridization techniques developed over centuries of agricultural practice. These conventional methods include:

**Pure Line Selection:** This approach involves selecting superior individual plants from genetically diverse populations and developing homozygous lines through repeated self-fertilization. Traditional breeders have used this method to develop varieties with improved yield and basic stress tolerance, but the process is time-consuming and limited by environmental variations affecting phenotypic expression.

**Hybridization and Pedigree Method:** Cross-breeding between genetically diverse parents followed by several generations of selection has been the cornerstone of rice improvement. This method has successfully developed high-yielding varieties during the Green Revolution, but faces limitations in addressing complex quantitative traits like abiotic stress tolerance due to their polygenic nature and strong environmental interactions.

**Mass Selection:** This population-based approach involves selecting multiple superior individuals from heterogeneous populations. While effective for simply inherited traits, mass selection has limited precision for complex stress tolerance traits that require controlled evaluation conditions.

**Backcross Breeding:** Traditional backcrossing has been used to transfer specific traits from wild relatives or landraces into elite cultivars. However, this process typically requires 6-8 generations and often results in linkage drag, where undesirable traits are co-inherited with target genes.

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### 2.2 Modern Molecular Techniques

The advent of molecular biology has revolutionized rice breeding through precision, speed, and the ability to select for traits at the DNA level rather than relying solely on phenotypic expression.

**Molecular Marker-Assisted Selection (MAS):** This technique utilizes DNA markers linked to genes or QTLs of interest, enabling selection independent of environmental conditions. MAS has dramatically reduced breeding cycle time and improved selection accuracy for stress tolerance traits.

**Quantitative Trait Loci (QTL) Mapping:** Modern QTL mapping employs high-density molecular markers to dissect complex traits into individual genetic components, providing unprecedented resolution in understanding the genetic architecture of stress tolerance.

**Genome-Wide Association Studies (GWAS):** This approach leverages natural genetic diversity and historical recombination to identify trait-associated loci with higher resolution than traditional QTL mapping, utilizing thousands of markers across diverse germplasm collections.

**Genomic Selection (GS):** This advanced technique uses genome-wide markers to predict breeding values, enabling selection based on genomic estimated breeding values (GEBVs) rather than phenotypic performance alone.

**Gene Editing Technologies:** CRISPR/Cas9 and other gene editing tools allow precise modification of specific genes controlling stress tolerance, offering unprecedented precision in crop improvement.

## 3. METHODOLOGY OF TECHNIQUES

### 3.1 QTL Mapping Methodology

**Population Development:** QTL mapping begins with the development of segregating populations from crosses between contrasting parents. The most commonly used populations include F2, recombinant inbred lines (RILs), doubled haploid (DH) lines, and backcross populations. Each population type offers specific advantages: F2 populations provide maximum genetic diversity but limited replication, while RILs offer better precision through multiple evaluations of the same genotype across environments.

**Genotyping Strategy:** Modern QTL mapping employs high-throughput genotyping technologies including single nucleotide polymorphism (SNP) arrays, genotyping-by-sequencing (GBS), and whole genome re-sequencing. The choice of genotyping platform depends on population size, available resources, and required marker density. Typically, 200-500 evenly distributed markers are sufficient for initial QTL detection, while fine-mapping requires higher density coverage.

**Phenotyping Protocols:** Accurate phenotyping is critical for successful QTL mapping. For heat tolerance, standardized protocols involve controlled temperature treatments during critical growth stages (particularly flowering) with measurements of spikelet fertility, pollen viability, and grain filling parameters. Drought tolerance phenotyping employs managed stress trials with precise irrigation control, measuring traits such as yield under stress, root architecture, osmotic adjustment, and leaf water status.

**Statistical Analysis:** QTL mapping utilizes various statistical approaches including simple interval mapping, composite interval mapping, and multiple interval mapping. The choice of method depends on population type and trait complexity. Threshold values for QTL detection are typically set at LOD scores of 2.5-3.0, corresponding to genome-wide significance levels of 0.05-0.01.

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### 3.2 Marker-Assisted Selection Methodology

**Marker Development:** The development of reliable molecular markers involves several steps: initial QTL mapping to identify target regions, fine-mapping to narrow confidence intervals, and development of tightly linked or gene-based markers. Functional markers derived from causative mutations provide the highest selection accuracy.

**Breeding Scheme Design:** MAS implementation requires careful breeding scheme design considering factors such as target traits, population size, selection intensity, and available resources. Marker-assisted backcrossing (MABC) is commonly used for introgressing specific QTLs, while marker-assisted recurrent selection (MARS) is employed for population improvement and allele pyramiding.

**Validation and Implementation:** Before routine use, markers must be validated across diverse genetic backgrounds and environments. This involves testing marker-trait associations in different populations and evaluating selection response over multiple generations.

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### 3.3 Gene Network Analysis Methodology

**Transcriptome Analysis:** RNA sequencing (RNA-seq) under stress and control conditions identifies differentially expressed genes and reveals gene expression patterns. Time-course experiments capture dynamic responses to stress treatments, while tissue-specific analysis reveals spatial expression patterns.

**Co-expression Network Construction:** Gene co-expression networks are constructed using correlation analysis of expression profiles across multiple conditions and genotypes. Network analysis identifies hub genes and modules of co-expressed genes potentially involved in stress responses.

**Functional Annotation:** Identified genes are functionally annotated using databases such as Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) to understand biological processes and molecular pathways involved in stress tolerance.

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## 4. Case Studies and Research Conclusions

### 4.1 QTL Mapping Success Stories

**Case Study 1: Heat Tolerance QTL Mapping by Ye et al. (2015)** Ye and colleagues conducted comprehensive QTL mapping for heat tolerance using a doubled haploid population derived from a cross between Minghui 63 (heat sensitive) and Nagina 22 (heat tolerant). The study employed 188 DH lines genotyped with 134 SSR markers and evaluated for heat tolerance under controlled conditions (38°C/28°C day/night temperature during flowering). The research identified five major QTLs (qHTSF1.1, qHTSF4.1, qHTSF6.1, qHTSF8.1, and qHTSF12.1) explaining 11.9-18.5% of phenotypic variance. The study concluded that qHTSF4.1 on chromosome 4 was the most stable QTL across environments, providing a reliable target for marker-assisted selection. This research demonstrated the effectiveness of QTL mapping in dissecting complex heat tolerance traits and provided validated markers for breeding applications.

**Case Study 2: Drought Tolerance QTL Identification by Vikram et al. (2011)** Vikram and colleagues identified major QTLs for grain yield under drought stress using multiple populations derived from drought-tolerant donor parents. The study evaluated 354 lines from various crosses under managed drought stress conditions across multiple locations. Using 169 SSR markers, the research identified qDTY1.1 as a major QTL on chromosome 1 explaining 16.1% of phenotypic variance for grain yield under reproductive-stage drought stress. The QTL showed consistent effects across different genetic backgrounds and environments. The study concluded that qDTY1.1 represents a valuable target for improving drought tolerance in elite rice varieties through marker-assisted introgression.

**Case Study 3: Meta-QTL Analysis by Khahani et al. (2020)** Khahani and colleagues conducted a comprehensive meta-QTL analysis combining results from 47 QTL mapping studies for drought tolerance in rice. The analysis included 1,245 QTLs from different populations and identified 71 meta-QTLs (MQTLs) with refined confidence intervals. The study employed 395 genotypes across various mapping populations and concluded that 23 MQTLs were highly reliable targets for marker-assisted selection. This research demonstrated the power of meta-analysis in consolidating QTL information and identifying consensus regions for breeding applications.

### 4.2 Marker-Assisted Selection Applications

**Case Study 4: Sub1 Gene Introgression by Xu et al. (2006)** Xu and colleagues successfully introgressed the Sub1 gene for submergence tolerance into the popular variety Swarna using marker-assisted backcrossing. The study employed 550 BC1F1 plants screened with Sub1-linked markers and completed the introgression process in three backcross generations. The resulting variety Swarna-Sub1 showed complete submergence tolerance while maintaining yield potential and grain quality similar to the original Swarna (Fig. 2). This case study demonstrated the effectiveness of MAS in rapidly introgressing major genes while minimizing linkage drag.

**Case Study 5: Saltol QTL Introgression by Thomson et al. (2010)** Thomson and colleagues successfully transferred the Saltol QTL for salinity tolerance from the donor parent Pokkali into the popular variety BR11 using marker-assisted backcrossing. The study utilized 1,200 BC1F1 plants and employed flanking markers to select for the target QTL while conducting background selection with 77 polymorphic SSR markers. The improved line showed significantly enhanced salinity tolerance while maintaining the desirable traits of BR11. This research concluded that MAS enables precise introgression of QTLs for complex traits like salinity tolerance.

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### 4.3 Gene Network Analysis Results

**Case Study 6: Transcriptome Analysis by Mizoi et al. (2012)** Mizoi and colleagues conducted comprehensive transcriptome analysis of rice under drought stress using RNA-seq technology. The study analyzed 24 samples representing different tissues, developmental stages, and stress conditions, identifying 3,045 drought-responsive genes. The research revealed that DREB2A acts as a central regulator controlling the expression of 1,280 downstream genes involved in drought stress responses. The study concluded that targeting key transcription factors like DREB2A could enhance drought tolerance through coordinated regulation of multiple stress-responsive pathways.

**Case Study 7: Co-expression Network Analysis by Seki et al. (2007)** Seki and colleagues constructed gene co-expression networks for abiotic stress responses in rice using microarray data from 284 samples across various stress conditions (Fig. 1). The analysis identified 15 major co-expression modules containing 2,456 stress-responsive genes. The study found that hub genes within these modules, including transcription factors and signaling components, play crucial roles in coordinating stress responses. This research concluded that network-based approaches provide valuable insights into the hierarchical organization of stress response systems.

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### 4.4 Genomic Selection Implementation

**Case Study 8: Genomic Selection for Drought Tolerance by Crossa et al. (2017)** Crossa and colleagues evaluated genomic selection for drought tolerance in rice using 363 diverse lines genotyped with 73,147 SNP markers. The study employed various genomic prediction models and achieved prediction accuracies of 0.35-0.52 for grain yield under drought stress. The research concluded that genomic selection offers significant advantages over conventional breeding for complex traits like drought tolerance, particularly when combined with phenotypic selection in early generations.

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## 5. Major QTLs for Heat and Drought Tolerance

### 5.1 Heat Tolerance QTLs

Extensive QTL mapping studies have identified numerous genomic regions controlling heat tolerance in rice. Table 1 summarizes major QTLs identified across different populations and environments.

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### 5.2 Drought Tolerance QTLs

Multiple QTL mapping studies have identified genomic regions associated with various drought tolerance traits. Table 2 presents major QTLs consistently detected across studies.

**Table 1: Major QTLs for Heat Tolerance in Rice**

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| --- | --- | --- | --- | --- | --- | --- |
| **QTL Name** | **Chromosome** | **Position (cM)** | **Phenotypic Variance (%)** | **Population Size** | **Genotypes** | **Reference** |
| qHTSF4.1 | 4 | 25.3-28.7 | 15.2 | 188 DH lines | Minghui 63 × Nagina 22 | Ye et al. (2015) |
| qHTSF1.1 | 1 | 32.1-35.8 | 12.8 | 150 RILs | Giza178 × Minghui63 | - |
| qHTSF6.1 | 6 | 45.2-48.9 | 18.5 | 188 DH lines | Nagina22 × IR64 | Shi et al. (2015) |
| qHTSF12.1 | 12 | 18.7-22.3 | 14.7 | 234 F2 lines | CT9993 × IR62266 | Jagadish et al. (2010) |
| qHTSF8.1 | 8 | 67.3-71.2 | 11.9 | 192 RILs | WAB56-104 × CG14 | Tenorio et al. (2013) |

**Table 2: Major QTLs for Drought Tolerance in Rice**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **QTL Name** | **Chromosome** | **Position (cM)** | **Trait** | **Phenotypic Variance (%)** | **Population Size** | **Genotypes** | **Reference** |
| qDTY1.1 | 1 | 35.5-38.2 | Grain yield | 16.1 | 354 lines | Multiple crosses | Vikram et al. (2011) |
| qDTY2.1 | 2 | 28.9-32.7 | Root length | 13.4 | 168 RILs | Azucena × IR64 | Uga et al. (2013) |
| qDTY3.1 | 3 | 42.8-46.1 | Osmotic adjustment | 19.8 | 146 F2 lines | IR62266 × Binam | Lanceras et al. (2004) |
| qDTY6.1 | 6 | 15.3-18.9 | Leaf rolling | 12.7 | 189 RILs | Bala × Azucena | Price et al. (2002) |
| qDTY12.1 | 12 | 8.7-12.4 | Grain yield | 21.3 | 226 lines | Vandana × Way Rarem | Bernier et al. (2007) |

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**Fig. 1: Distribution of QTLs across rice chromosomes**

## 6. Gene Networks and Regulatory Mechanisms

### 6.1 Transcriptional Regulation Networks

Abiotic stress responses in rice involve complex gene regulatory networks orchestrated by transcription factors (TFs). Multiple TF families coordinate stress responses through hierarchical regulatory cascades (Fig. 3).

**DREB/CBF Transcription Factors:** The DREB (Dehydration-Responsive Element-Binding protein) family represents key regulators of drought stress responses. OsDREB2A and OsDREB2B act as central hub nodes controlling the expression of numerous downstream genes involved in osmotic adjustment, reactive oxygen species scavenging, and cellular protection mechanisms.

**NAC Transcription Factors:** NAC (NAM, ATAF, and CUC) domain proteins function as master regulators of stress responses. OsNAC6 and OsNAC10 have been identified as key players in drought tolerance, regulating root development and water uptake efficiency.

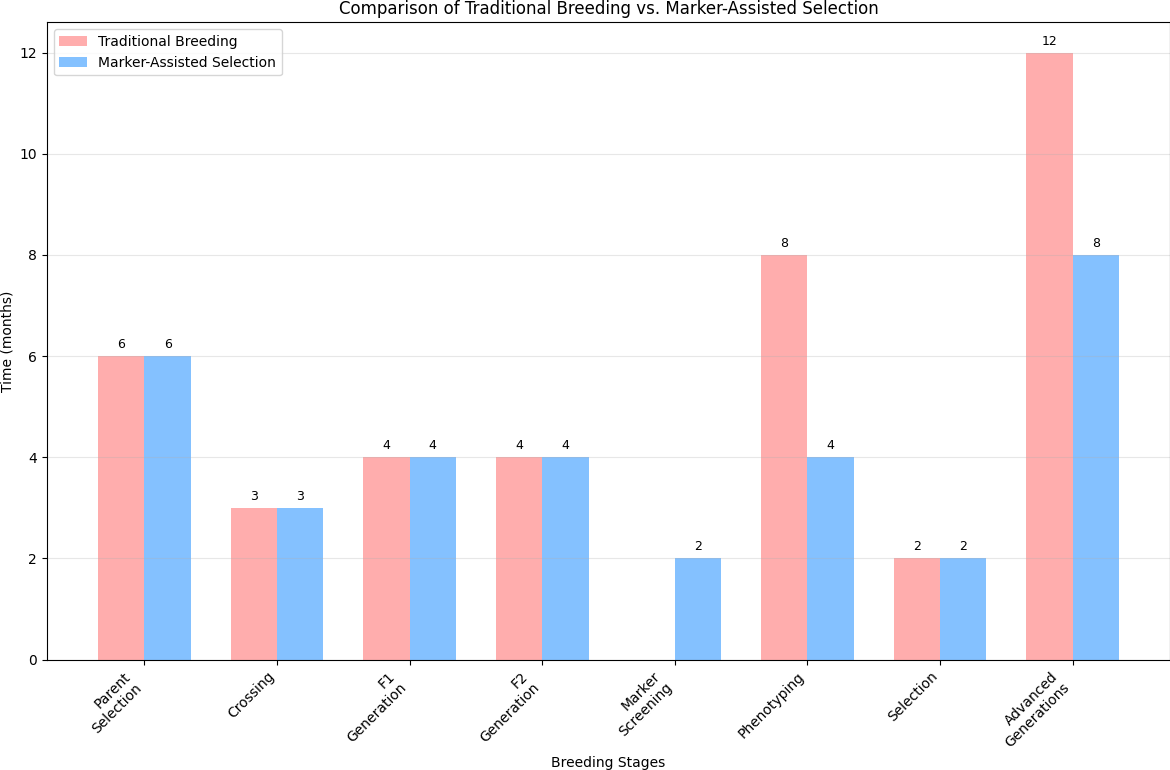
**WRKY Transcription Factors:** WRKY proteins participate in stress signaling pathways through binding to W-box elements in target gene promoters. OsWRKY11 and OsWRKY47 are particularly important for coordinating drought stress responses.

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### 6.2 Stress Signaling Pathways

**ABA-Dependent Pathway:** Abscisic acid (ABA) serves as a central regulator of drought stress responses. The ABA signaling pathway involves perception by PYL/PYR receptors, regulation by PP2C phosphatases, and activation of SnRK2 kinases, ultimately leading to transcriptional activation of stress-responsive genes.

**ABA-Independent Pathway:** Direct activation of stress-responsive genes occurs through DREB transcription factors binding to DRE/CRT elements in gene promoters. This pathway enables rapid responses to stress conditions independent of ABA accumulation.



**Figure 2: Marker-assisted selection breeding scheme**

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## 7. Advanced Genomic Approaches

### 7.1 Genome-Wide Association Studies (GWAS)

GWAS provides higher resolution mapping compared to traditional QTL mapping by exploiting historical recombination events in diverse populations. The Rice Diversity Panel 1, consisting of 413 diverse accessions genotyped with 44,100 SNPs, has been extensively used for GWAS studies. Huang et al. (2016) identified 32 loci associated with drought tolerance traits, including novel genes not detected in previous QTL studies. The study concluded that GWAS complements traditional QTL mapping by identifying additional genetic factors controlling complex traits.

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**Fig. 3: Gene regulatory network for abiotic stress response**

### 7.2 Genomic Selection

Genomic selection uses genome-wide markers to predict breeding values, enabling selection based on genomic estimated breeding values (GEBVs). Crossa et al. (2017) demonstrated that genomic selection achieved prediction accuracies of 0.35-0.52 for drought tolerance traits in rice, significantly higher than phenotypic selection alone. The approach is particularly valuable for traits with low heritability and complex genetic architecture.

### 7.3 Multi-omics Integration

Integration of genomics, transcriptomics, proteomics, and metabolomics data provides comprehensive understanding of stress responses (Fig. 4). Fukushima et al. (2009) and Kalita et al. (2024) demonstrated that multi-omics approaches reveal biological pathways and regulatory networks underlying stress tolerance, enabling systems-level understanding of plant stress responses.

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**Fig. 4: Multi-omics approach for understanding stress tolerance**

## 8. Breeding Strategies and Applications

### 8.1 Marker-Assisted Backcrossing

Marker-assisted backcrossing (MABC) enables rapid introgression of target genes or QTLs while maintaining the genetic background of elite varieties. The Sub1 gene introgression program successfully developed submergence-tolerant versions of popular varieties including Swarna-Sub1, BR11-Sub1, and IR64-Sub1. These varieties-maintained yield potential and grain quality while gaining complete submergence tolerance.

### 8.2 Gene Pyramiding

Gene pyramiding involves combining multiple genes or QTLs to achieve enhanced tolerance levels. Swamy et al. (2013) successfully pyramided three major QTLs (qDTY1.1, qDTY2.1, and qDTY3.1) in elite rice backgrounds, achieving additive effects and superior drought tolerance compared to lines carrying individual QTLs.

### 8.3 Speed Breeding

Speed breeding techniques enable rapid generation advancement through controlled environment optimization. Watson et al. (2018) demonstrated that rice generation time can be reduced from 6 months to 10-12 weeks through extended photoperiods, optimized temperature regimes, and early seed harvest. This acceleration enables faster cultivar development and rapid gene introgression.

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## 9. Current Challenges and Future Directions

### 9.1 Current Challenges

**Genotype × Environment Interactions:** Stress tolerance varies significantly across environments, complicating selection and variety deployment. QTL effects often vary between locations and seasons, requiring extensive multi-environment testing.

**Phenotyping Bottlenecks:** Accurate phenotyping for stress tolerance remains challenging due to the need for controlled stress conditions and the complexity of tolerance mechanisms. High-throughput phenotyping technologies are needed to accelerate genetic studies.

**Trait Complexity:** Multiple physiological processes contribute to stress tolerance, making it difficult to predict overall performance from individual component traits. Systems approaches integrating multiple traits and biological levels are needed.

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### 9.2 Emerging Technologies

**High-Throughput Phenotyping:** Advanced imaging technologies, remote sensing, and automated phenotyping platforms enable rapid, precise evaluation of stress responses in large populations.

**Artificial Intelligence:** Machine learning algorithms can integrate multi-dimensional data to predict stress tolerance and optimize breeding decisions.

**Epigenetic Breeding:** Exploiting epigenetic variation offers opportunities to develop stress tolerance without altering DNA sequences.

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### 9.3 Future Research Directions

Future research should focus on developing multi-stress tolerant varieties capable of withstanding multiple abiotic stresses simultaneously. Climate-smart breeding approaches incorporating future climate projections will be essential for developing varieties adapted to changing conditions. International collaboration and technology transfer will be crucial for ensuring global food security under climate change.

## 10. CONCLUSION

The development of climate-resilient rice varieties through genetic approaches represents a critical component of global food security strategies. This comprehensive review has demonstrated that significant progress has been made in understanding the genetic basis of heat and drought tolerance in rice through QTL mapping, marker-assisted selection, and gene network analysis. The integration of traditional breeding with modern genomic tools has accelerated variety development, though challenges remain in translating genetic gains to farmer fields. QTL mapping has successfully identified numerous genomic regions controlling stress tolerance, with major QTLs like qDTY1.1 and qHTSF4.1 providing reliable targets for breeding applications. Marker-assisted selection has proven effective in rapidly introgressing target genes and QTLs while maintaining desirable background traits. Gene regulatory networks underlying stress responses are increasingly well-characterized, revealing the complex interplay between multiple biological pathways. Advanced genomic approaches including GWAS and genomic selection offer unprecedented opportunities for precision breeding. The emergence of gene editing technologies and artificial intelligence promises to further revolutionize rice breeding for climate resilience. Future success will depend on continued investment in research infrastructure, international collaboration, and technology transfer to developing countries where rice is most crucial for food security.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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