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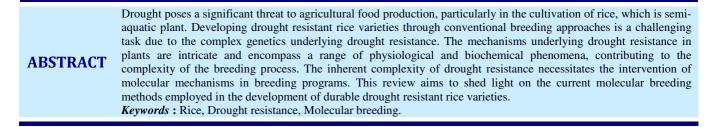
# MOLECULAR BREEDING STRATEGIES FOR ENHANCING DROUGHT RESISTANCE IN RICE : A REVIEW

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## Introduction

Rice is the most important food crop in the world, feeding more than two- third of the world's population. It accounts for over 80 *per cent* of an individual's daily calorie intake, especially in Asian people. (Sahebi *et al.*, 2018). Drought stress severely impairs rice production in rainfed farming, as rice is a semi-aquatic plant with thin cuticular wax as well as associated with speedy stomata closure (Singhal *et al.*, 2016). In the goal of attaining self-sustainability in rice production by 2050, it is a prerequisite to develop a variety of rice tolerant to various abiotic stresses with a special consideration of higher yield and superior quality (Chukwu *et al.*, 2019).

Rice is highly susceptible to drought stress throughout its life cycle, but large economic losses or even complete crop failure is observed when the stress occurs during flowering. Drought stress before flowering may adversely impact yield by delaying the flowering. (Pantuwan *et al.*, 2002). Male sterility as well as embryo abortion is the common effect of drought stress just after pollination at the reproductive stage leads to poor reproductive success (Ozga *et al.*, 2016).

Drought resistance can be defined as the capacity of a plant to produce its highest economic product under a waterdeficit environment relative to water-constraint-free environment. Several factors play an essential role in the response mechanism of a plant to drought stress, such as plant species, age of the plant, growth stage, plants genotype as well as the intensity of drought (Tiwari *et al.*, 2021). Drought tolerance mechanisms include cellular adjustments, physiological acclimation and morphological adaptations which are controlled by genetic factors at different stages. Cellular adjustments for drought tolerance involve increased

chlorophyll content and lower osmotic potential. Physiological acclimation comprises higher stomatal density and conductance, decreased transpiration rates, reduced and early asynchrony between female and male flowering and maturation. and better production, accumulation, assimilation, and seed and biomass yield partitioning. On the other hand, morphological adaptations include increased root thickness and length, waxy and thick leaf coverings, decreased leaf weight and size, smaller epithelial cells, delayed leaf senescence and increased green leaf area (Oladasu et al., 2019).

The ability of rice plants to thrive and produce high yields under limited water conditions is crucial for sustainable rice production, especially in drought-prone years exacerbated by global warming and climate change. Breeding for drought tolerance has become imperative due to these challenges; however, the complex and quantitative nature of the trait has posed significant difficulties in studying drought responses. Consequently, breeders must meticulously screen and select numerous genotypes across multiple seasons, years and locations to successfully develop drought-tolerant varieties. An efficient criterion currently utilized in drought breeding programs is trait phenotyping, particularly focusing on grain yield under drought conditions (Shamsudin *et al.*, 2016).

# Drought stress: Morphological, physiological, biochemical and molecular response

The drought may be defined as the inadequacy of water availability, including precipitation and soil moisture storage capacity in quantity and distribution during the life cycle of a crop to restrict expression of its full genetic yield potential. Drought stress triggers various morphological, physiological, biochemical and molecular responses in rice plants as adaptive mechanisms to cope with water shortage. The mechanisms of drought resistance in plants are indicated in Figure 1. These responses may vary depending on the severity and duration of drought stress. Here are few key responses exhibited by rice crop in response to drought.

#### Morphological response

In rice, morphological response to drought resistance involves increased root length and density, enhanced root hairs, deeper rooting, leaf rolling and delayed flowering, reduced tillering (Kadam *et al.*, 2017; Laza *et al.*, 2019).

#### **Physiological Response**

In rice, one of the primary responses of rice to drought stress is the closure of stomata, reduced photosynthesis, reduced cell expansion and cell division. Furthermore, severe drought conditions can cause significant disruption in photosynthesis and metabolic process, ultimately leading to plant mortality (Anjum *et al.*, 2017). This highlighted that drought stress primarily hampers cell enlargement, while cell divisions are comparatively less affected.

#### **Biochemical response**

In response to drought stress, rice crop exhibits biochemical responses that involves the accumulation of osmolytes such as proline, glycine betaine, and sucrose, as documented by Hien and Jacobs (2002). These osmolytes play a crucial role in maintaining cellular osmotic balance and stabilizing proteins under water deficit conditions. Furthermore, drought stress triggers the production of antioxidants such as superoxide dismutase, catalase, and peroxidase, which act as defence mechanisms against reactive oxygen species (ROS). These antioxidants help scavenge ROS and prevent oxidative damage to cellular components. Additionally, in drought-resistant cultivars, the expression of heat shock proteins (HSPs) is induced to combat the stress, as highlighted by Jha et al. (2012). HSPs function as molecular chaperones, aiding in protein folding, maintaining protein stability, and preventing protein denaturation under stress conditions.

#### Molecular response

Drought stress induces changes in gene expression patterns in rice plants. Specific genes associated with drought tolerance, such as those involved in osmotic adjustment, stress signalling, and protection against oxidative damage, are upregulated to enhance the plant's ability to withstand water deficit conditions (Bray *et al.*, 2002). Various transcription factors, including DREB (dehydrationresponsive element-binding protein) and NAC (NAM, ATAF1/2, CUC2), are activated under drought stress. These transcription factors regulate the expression of downstream stress-responsive genes, enabling the plant to adapt to water scarcity (Nakashima *et al.* 2007).

# **Molecular Breeding Approaches**

The mechanism of drought resistance in rice is highly intricate, involving multiple genetic and physiological processes. Conventional breeding approaches, although valuable, have limitations in addressing the complexity of drought tolerance. Hence, the integration of molecular biology tool has become imperative for successful development of drought resistant rice varieties.

#### **Identification of QTL**

The identification of QTL controlling traits under drought stress involves a sequential process: development of mapping populations segregating for drought resistance traits, identification of polymorphic markers, genotyping mapping populations, accurate phenotyping for drought resistance correlated traits and construction of QTL map using genotypic and phenotypic data (Sahebi et al., 2017). The QTLs linked to different traits under drought stress are shown in Table1. The landmark discovery of qtl1.1 marked the pioneering identification of a QTL linked to drought resistance in rice; located on chromosome 1, qtl1.1 is associated with enhanced drought tolerance and plays a crucial role in influencing grain yield and related agronomic traits under water-limited conditions. This ground breaking finding opened new avenues for understanding the genetic mechanisms underlying drought resistance in rice and has since served as a foundation for further research and breeding endeavours in developing resilient rice varieties.

#### Marker assisted selection

Marker-assisted selection (MAS) plays a pivotal role in breeding for drought tolerance by facilitating the identification of molecular markers associated with key drought tolerance traits in crops. This approach enables breeders to make early and precise selections, resulting in a significant reduction in time and resources required compared to conventional breeding methods. By evaluating specific drought tolerance-related traits such as water-use efficiency, leaf wilting, osmotic adjustment, canopy temperature, and root traits, breeders can effectively identify and prioritize individuals with superior drought tolerance characteristics. Through the integration of MAS, breeders can select individuals or breeding lines that carry the desired drought tolerance alleles based on marker-phenotype associations, accelerating the development of droughttolerant varieties. This efficient and targeted approach enhances breeding efficiency and contributes to the successful development of drought-tolerant crops. Several studies have demonstrated the effectiveness of MAS in enhancing drought tolerance in various crops, including rice (Collard and Mackill, 2008; Kumar et al., 2018; Platten et al., 2019). MAS has proven to be a valuable tool in harnessing the genetic potential for drought tolerance and offers great prospects for sustainable crop improvement in water-limited environments.

#### Marker-assisted backcrossing (MABC)

Marker-assisted backcrossing (MABC) is a powerful tool in breeding drought-resistant crop varieties, as it helps to overcome the problem of linkage drag, commonly associated with traditional breeding methods. Linkage drag refers to the unintended transfer of undesirable traits linked to the target trait during the breeding process. By using molecular markers, breeders can selectively introgress only the desired drought tolerance genes or quantitative trait loci (QTLs) from a drought-tolerant donor parent into an elite recipient variety, while minimizing the transfer of unwanted genetic material. This targeted approach ensures that the improved variety retains the desirable agronomic traits of the recipient parent,

## Molecular-assisted gene pyramiding

Molecular-assisted gene pyramiding is a powerful approach that enables the introgression of multiple genes for drought resistance into a single rice cultivar. This method overcomes the limitations of conventional breeding, where it is challenging to combine different genes in a single variety due to genetic linkage and time-consuming breeding cycles. Using molecular markers, breeders can select and combine multiple genes or quantitative trait loci (QTLs) associated with drought resistance from different parental lines. By strategically designing crosses and utilizing marker-assisted selection, the identified genes or QTLs can be pyramided into a single genetic background (Li et al., 2003). This process allows for the accumulation of beneficial alleles and enhances the overall drought tolerance of the resulting cultivar. Gene pyramiding offers several advantages in drought resistance breeding ; it increases the genetic diversity and stability of the cultivated varieties, leading to improved and more reliable performance under drought stress. Additionally, it reduces the risk of gene breakdown or loss of resistance due to the presence of multiple resistance genes. Furthermore, molecular-assisted gene pyramiding enables breeders to expedite the development of droughtresistant rice varieties by reducing the time required for conventional breeding cycles (Platten et al., 2019). The precise and efficient selection of specific genes or QTLs using molecular markers facilitates the rapid development of improved cultivars with enhanced drought tolerance.

# **Genomic selection**

Genomic selection has played a transformative role in breeding for drought tolerance, enabling breeders to efficiently select and develop crop varieties with enhanced water stress resilience. By leveraging statistical models and large-scale genomic data, genomic selection goes beyond traditional breeding methods, offering a more comprehensive assessment of an individual's genetic potential. In a study by spindel *et al.* (2015) and Kumar *et al.* (2018) genomic selection was used to enhance yield.

The significant advantages of genomic selection are its reliance on genomic estimated breeding values (GEBVs) rather than relying solely on time-consuming and resourceintensive field evaluations (Chen et al., 2017). GEBVs are calculated based on the intricate relationships between genetic markers and target traits, enabling breeders to predict the performance of individuals for drought tolerance at an early stage. This early selection facilitates the identification of superior individuals, accelerating the breeding process by reducing the need for extensive phenotypic evaluations. In the context of drought tolerance, genomic selection has been particularly effective. (Gowda et al., 2014). By utilizing GEBVs generated through genomic selection, breeders can strategically select and prioritize individuals with superior drought resistance-related genetic profiles for further breeding. This approach allows for the accumulation of multiple favourable alleles associated with drought resistance, enhancing the overall resilience of crop varieties to water stress conditions.

# Conclusion

The susceptibility of rice to drought stress, coupled with its significance as a staple crop for a large portion of the global population, highlights the critical need to prioritize breeding for drought resistance. However, conventional breeding methods face limitations such as slow progress and the challenge of linkage drag, necessitating the integration of molecular approaches. QTL mapping, marker-assisted selection (MAS), marker-assisted gene pyramiding, and genomic selection have emerged as powerful tools in rice breeding for drought resistance. These molecular approaches offer advantages in terms of precision, efficiency, and speed, allowing breeders to target specific genomic regions, select individuals with desired traits, combine multiple genes for drought resistance, and predict genetic potential based on genomic estimated breeding values (GEBV). By harnessing the power of genomics, breeders can expedite the development of improved rice varieties with enhanced drought resistance, thereby ensuring global food security.

Table 1: List of QTLs linked to the traits associated with the drought resistance

QTL	Associated trait	References
<i>qDTY1.1</i>	Grain yield	Vikram et al., 2011, Ghimire et al., 2012
<i>qDTY1.2</i>	Grain yield	Sandhu et al., 2014
<i>qDTY1.3</i>	Grain yield	Sandhu et al., 2014
qDTY2.1	Grain yield	Venuprasad et al., 2009
qDTY2.2	Grain yield	Swamy et al., 2013
qDTY2.3	Grain yield	Palanog et al., 2014, Sandhu et al., 2014
<i>qDTY3.1</i>	Grain yield	Dixit <i>et al.</i> , 2014
qDTY4.1	Grain yield	Swamy et al., 2013
qDTY6.1	Grain yield	Dixit <i>et al.</i> , 2014
<i>qDTY6.2</i>	Grain yield	Dixit <i>et al.</i> , 2014
<i>qDTY9.1</i>	Grain yield	Swamy et al., 2013
<i>qDTY10.1</i>	Grain yield	Vikram <i>et al.</i> , 2011
<i>qDTY12.1</i>	Grain yield	Bernier et al., 2007
qlr8.1	Leaf rolling	Lin et al., 2007
qLR9.1	Leaf rolling	Barik et al., 2019
qDLR8.1	Leaf rolling	Dixit <i>et al.</i> , 2012
qLD9.1	Leaf drying	Barik et al., 2019

Source: Modified from Panda *et al.* (2021)

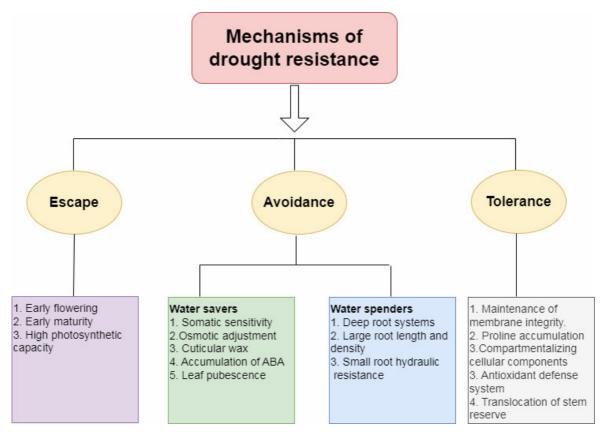


Fig. 1 : Mechanisms of drought resistance in plants

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