

Emerging Molecular Strategies for Improving Rice Drought Tolerance

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Abstract: Rice occupies a pre-eminent position as a food crop in the world. Its production, however, entails up to 3000 liters of water per kilogram of grain produced. Such high demand makes rice prone to drought easily. Sustainable rice cultivation with limited water resources requires the deployment of a suitable strategy for better water use efficiency and improved drought tolerance. Several drought-related genes have been evaluated in rice for their mode of action in conferring drought tolerance. Manipulation of components of abscisic acid signal transduction, stomatal density, deposition of cuticular wax, and protein modification pathways are emerging as priority targets. Gene reprogramming by microRNAs is also being explored to achieve drought tolerance. Genetically dissected Quantitative Trait Loci (QTLs) and their constituent genes are being deployed to develop drought-tolerant rice varieties. Progressive research and challenges include a better understanding of crucial components of drought response and search for new targets and the deployment of improved varieties in the field.

Keywords: Drought, microRNAs, molecular breeding, rice, transgenics, water-deficit.

1. INTRODUCTION

Climate change is real, and the frequency and intensity of drought are going to increase in the coming decades. This scenario will compel the migration of millions of people due to the lack of sufficient water in their native dwellings. A recent example is from Africa and the Middle East where almost 20 million people faced starvation due to drought. The UN Convention to Combat Desertification (UNCCD), which is a UN body for fighting drought and land degradation, was recently requested to provide enough funding and scientific advisory resources to combat dry periods [1]. In fact, an independent scientific network for land degradation and desertification is missing. It is somewhat ironic that drought has not yet been defined clearly, and it loosely refers to the abnormal scarcity of water [2]. At times, even dry environments are defined as drought. There is an urgent need for a consensus on the definition and methods of drought's impact measurement for synchronization in scientific interventions.

Rice is grown over 150 million hectares area in approximately 100 countries, mainly in Asia. Its demand and production have also increased in African countries over the past 50 years, where drought is more frequent and severe. Therefore, rice is food for humanity, especially in the densely populated areas where poverty is a major issue. The quantum of rice production and the number of people's dependence on it as a major dietary source can give a clear idea of

how devastating drought could be for the human population dependent on rice as a staple. Rice cultivation consumes a vast amount of water, making it vulnerable to drought throughout the growing season [3]. Drought causes approximately 40-90% loss of rice yield. Such a high loss of food crops could potentially feed millions of people. The greater challenge is to sustain successful rice cultivation, which is one of the largest consumers of fresh water in agriculture. Efforts for improving drought tolerance also face challenges with the fact that rice seedlings prefer waterlogged conditions for weed control and efficient iron uptake as rice can absorb only a reduced form of iron prevalent in waterlogged soil. Novel strategies for improving rice productivity in limited water availability are thus critical for next-generation agriculture. Development of precise screening methods for drought tolerance, identification of novel adaptive traits, an early warning system for onset of drought, and cultivation of tolerant varieties can help to sustain rice production in drought-prone areas.

Research on drought stress response in rice has recently been reviewed concerning various enzymatic, structural, and regulatory protein genes capable of conferring abiotic stress tolerance [4-6]. Here we review recent leads obtained on improving rice drought tolerance using the well-proven strategies, namely, transgenics, microRNAs, and molecular breeding (Fig. 1).

2. TRANSGENIC APPROACH FOR IMPROVING RICE DROUGHT TOLERANCE

Availability of high-quality gold standard whole-genome sequence and optimization of methods for raising transgenics in several rice genotypes have revolutionized the function

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nal genomics for drought tolerance in the post-2004 era. A plethora of information is now available on drought/dehydration-responsive transcriptome, epigenome, and metabolome generated using high throughput approaches. Drought results in large-scale alterations in the expression of rice genes, with more than 10,000 genes getting either up- or down-regulated [7]. Additionally, differential methylation in desiccation related genes and their promoter regions correlate well with the degree of drought tolerance in tolerant and sensitive rice cultivars [8]. Hence, both genetic and epigenetic elements contribute towards rice surviving drought. This information is being extensively used to improve drought tolerance using different strategies that can be broadly divided into three categories: enhancing water uptake from the soil, reducing water loss from the plant, and improving internal water-use-efficiency [9]. For this, a variety of genes from rice and other plants, belonging to different pathways, have been tested in rice for imparting drought tolerance using a transgenic approach. Major candidate genes tested for control of drought regulation encode for enzymes for osmoprotectants/ redox status/ protein degradation/ phosphorylation, signaling components, transporters, and transcription factors.

Similar to other plants, the phytohormone abscisic acid (ABA) is also pivotal to rice drought response. It accumulates preferentially in roots on drought onset and is transported to shoot to regulate stomatal closure and production of ROS and proteins like dehydrins, late embryogenesis abundant (LEA) that are involved in osmotic adjustment [10]. Rice ABA signaling is now understood well and it starts with ABA binding with its receptor (OsPYR1/PYL/RCAR) complex leading to the inhibition of protein phosphatase 2C (PP2C) mediated inactivation of sucrose nonfermenting 1-related protein kinase 2s (SnRK2s). This, in turn, activates ABA-responsive transcription factors like bZIPs to execute a downstream response to stress adaptation, including drought [11, 12]. ABA levels are tightly regulated in response to development and stresses [13]. Thus, ABA biosynthesis, transport, and signaling have a profound impact, making genes of these processes a reasonable choice for manipulation using transgenesis. Rice membrane transporter, OsPM1 serves as an influx carrier facilitating ABA uptake by a cell [14]. *OsPM1* overexpression lines accumulate more ABA resulting in improved drought resistance both at vegetative and reproductive stages. Its knockout lines displayed poor growth and survival rate under drought, cementing ABA transport's vital role in drought adaptation. *OsPM1* is transcriptionally regulated by ABA-responsive transcription factor OsbZIP46, which is a crucial regulator of ABA-responsive transcriptome. OsbZIP46 is, in turn, negatively regulated by its interacting protein MODD, which targets OsbZIP46 for repression under prolonged drought, thereby repressing ABA signaling. Also, OsMODD inhibits transcriptional activation of target genes of OsbZIP46 through chromatin modification. Expectedly, *OsMODD* overexpression plants are drought-sensitive [15, 16]. ABA signaling repression by OsMODD is counteracted by reversible histone monoubiquitination of OsbZIP46 targeted genes by OsHUB2 protein during the initial phase of drought stress. When over-expressed, OsHUB2 renders drought tolerance in transgenic

rice by reducing the OsbZIP46 interaction with OsMODD [16]. Furthermore, knockouts of three ABA receptor genes were generated using the CRISPR/Cas9 approach. The triple mutants in ABA receptors *pyl1/4/6* showed better growth and an impressive increase in seed yield (31%) over wild-type (WT) under well-watered field conditions due to the defective stomata leading to enhanced transpiration [17]. However, these lines performed poorly in drought conditions since ABA signaling is needed for drought response like stomata closer to prevent water loss. Nevertheless, these findings open up the opportunities for optimizing ABA signaling to achieve a balance between growth and drought tolerance. It is noteworthy that spatial/temporal inhibition of gibberellins signaling produced semi-dwarf rice varieties without losing seed yield.

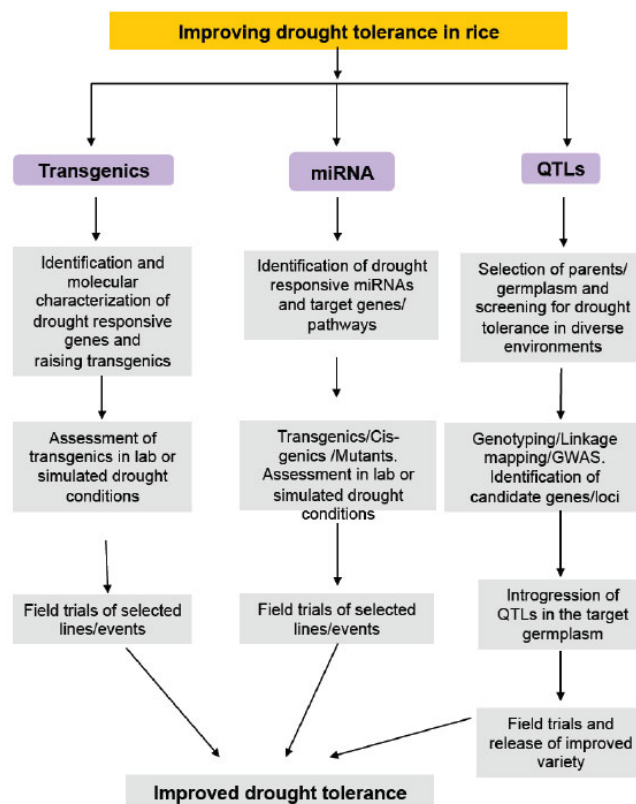


Fig. (1). Emerging molecular strategies for improving rice drought tolerance. Drought related genes, miRNAs, and QTLs have been identified. The genes regulating stomatal closer, cuticular wax deposition, and antioxidant pathways prevent water loss and cellular damage under drought. Transgenics with improved drought tolerance have been raised using such genes. miRNAs regulating drought response pathways and underlying genes provide an alternate strategy. Transgenics with improved drought tolerance should be tested in field conditions to assess the real protection of yield under drought. The classical breeding combined with new molecular tools would help produce new drought-tolerant varieties at a much faster pace.

Reducing stomata density can help restricting water loss; however, it also reduces leaf CO₂ levels by hindering CO₂ exchange between the plant and the environment [18]. A re-

cent study implied developing future-ready water-use efficient rice by targeting stomatal density using epidermal patterning factor OsEPF1. The overexpression transgenics displayed a substantially reduced stomata density and conductance, thus lowering water loss. Consequently, transgenics consumed 40% less water in comparison to WT and exhibited a remarkably improved survival rate under drought stress [19]. Interestingly, when tested under drought with increased ambient CO₂ levels, transgenics showed a higher yield than WT. In an anticipated high CO₂ environment in the future, reducing stomatal density, could be an efficient strategy for developing drought-resilient rice [18]. Optimising stomatal opening can also help improving rice drought tolerance. Transgenic rice overexpressing ABA inducible gene, *OsASR1*, displayed an improved modulation of stomatal closure and consequently enhanced drought tolerance in multigenerational field trials [20].

The cuticular wax deposition is an adaptation to prevent non-stomatal water loss from leaves under drought stress in rice. Cuticular wax is mainly composed of long-chain fatty acids, and over the years, several candidate genes have been reported to enhance the cuticular wax deposition. Zhu & Xiong [21] discovered a novel drought-induced protein with multi-enzyme properties and named this mega enzyme as *Drought-induced Wax Accumulation 1 (DWA1)*. The loss of function *dwa1* plants had impaired wax deposition and increased drought sensitivity in a pot-based experiment. Expectedly, functionally complemented *dwa1* plants expressing *DWA1* under a constitutive promoter showed drought tolerance. Another gene named as *Drought Hypersensitive (DHS)* is a negative regulator of wax deposition as its overexpression hampered wax load and drought sensitivity. *DHS* encodes an E3 ubiquitin ligase and targets a transcription factor *ROC4* for degradation [22]. When overexpressed, *ROC4* transgenics accumulate more wax and exhibit a significantly improved survival rate in comparison to WT under drought stress. Mutating *DHS* binding sites in *ROC4* sequence by precise gene-editing could be an exciting approach for enhancing wax load in leaves and thereby drought tolerance. Drought escape strategy, where plant represses vegetative growth and switches to early flowering to complete its life cycle, is poorly understood at molecular levels. Using transgenesis, it was revealed that both ABA-dependent and -independent pathways integrate to suppress tillering but promote flowering under water scarcity [23]. However, such an approach would not be an ideal target for enhancing yield as reduced tillering often leads to yield loss.

Among the candidate genes holding promise, a gene family encoding A20/AN1 zinc finger domain-containing stress-associated proteins (SAP) has been discovered in rice, and its members are shown to confer drought tolerance in various plants by acting as ubiquitin ligases, redox sensor and regulators of the expression of stress-related genes [24, 25]. A recent *in-silico* study based on publicly available gene expression data found SAP's potential roles in combined stresses (drought and salinity), adding further dynamism to their functions [26]. An unsuspected but novel role of acetic acid was also revealed in drought stress tolerance in plants. Histone deacetylase mutants (*hda6*) exhibited high tolerance to drought, but the known drought-related pathways like ABA

or osmolytes accumulation were unaffected in mutants. Instead, acetic acid levels were elevated in mutants under drought stress [27], resulting in the activation of jasmonic acid (JA) signaling to confer drought tolerance. Interestingly, the exogenous application of acetic acid on rice seedlings significantly improved survival under drought stress. Another such exciting finding involves trehalose-6-phosphate (T6P) where the exogenous application of T6P improved drought tolerance in wheat [28]. T6P role is well-known in plants' adaptive responses under abiotic stresses, including drought. The presence of T6P allows the utilization of sugar for growth, and its absence hampers growth even when sugar is sufficient. Trehalose biosynthesis genes from *E. coli* could enhance the trehalose levels and drought tolerance in rice on overexpression [29]. Moreover, the rice T6P phosphatase gene is essential for seed germination in anaerobic conditions [30]; however, its role in drought tolerance remains yet to be unraveled. It would be exciting to see the manipulation of acetate or T6P levels in rice using conventional transgenesis or gene-editing of endogenous genes for drought resilience. Like trehalose, proline is another osmolyte well known to help plants in mitigating drought stress. Its enhanced levels are often observed in a variety of abiotic stress-tolerant rice transgenics [31]. Proline levels increased significantly in rice overexpressing NAD kinase gene, *OsNADK1*, and transgenics showed an improved drought tolerance [32]. One of the limitations of targeting osmolytes levels is the poor information on the biosynthesis genes of rice, as observed in the case of trehalose or glycine betaine [33]. Future studies should explore the endogenous rice genes for altering their catalytic activities using precise gene-editing.

Transgenics are often tested in a controlled environment for performance under simulated drought conditions; however, drought in nature is more complex and often accompanied by other stresses like heat. Therefore, the assessment of transgenics in natural drought conditions is critical to validate the laboratory-based observations, especially for yield. Only a handful of studies have conducted field trials on transgenics [reviewed in 4] by draining the surface water and holding the irrigation till leaf starts rolling. In field trials, transgenics lines, especially those overexpressing NAC transcription factors and their downstream targets, showed better yield than non-transgenic plants [4, 34]. Remarkably, transgenic rice expressing a galactinol synthase 2 gene (*AtGolS2*) from Arabidopsis showed yield gains of up to 49% in comparison to non-transgenic in multiyear field trials under drought [35]. Similarly, a marked improvement in grain yield was obtained in rice transgenics overexpressing a zinc-finger protein, *OsTZF5*, under an inducible promoter when tested in a field [34]. These results are encouraging; however, transgenics with a constitutive promoter often showed reduced yield in the well-watered field, supporting the use of inducible promoters and the need for further improvements for field application of transgenics [34].

3. MicroRNA GENES PLAY A PIVOTAL ROLE IN REGULATING DROUGHT RESPONSE IN RICE

MicroRNA genes define a novel regulatory dimension regulating various molecular and biochemical processes in

both plants and animals. Initially transcribed as longer transcripts (Pri-microRNA), they undergo several processing steps to generate mature ~20 nt long regulatory RNA molecules. In plants, they primarily act by facilitating the degradation of protein-coding gene transcripts and regulate diverse developmental and biochemical processes, including response to various stress conditions. Since each microRNA gene can target multiple genes, they emerge as major regulatory nodes within plants' entire regulatory schema. Consequently, their impact on plant stress response regulation is also widespread and critically important to understand. Rice has over 600 microRNA genes that can produce over 700 distinct mature microRNA sequences as per the 'miRBase database' (www.mirbase.org). Besides, several independent studies have also identified previously unreported microRNA genes in rice [36]. Gene expression studies at the global level have demonstrated that the rice 'miRnome' is highly dynamic and uniquely responds to various kinds of stress conditions, including drought [37-39]. Up to 10-15% of known microRNA genes are differentially regulated under drought conditions. These drought-responsive microRNA genes target a wide variety of targets, including transcription factors, signaling components/receptors as well as metabolic enzymes. Interestingly, comparative analysis of the 'miRnome' indicated that the drought-responsive expression of some rice microRNA genes is also variety-specific in rice [40, 41]. A group of microRNAs (miR528-5p, miR408-3p, miR2878-5p, miR398b, miR397a, miR1871 and miR159f) termed as 'Cultivar Specific Drought Responsive (CSDR) microRNAs' have a totally inverse drought mediated expression in the flag leaves of drought-tolerant (Nagina 22 and Vandana) and sensitive rice cultivars (Pusa Basmati 1 and IR 64) [42]. More intriguingly, almost all of them were found to be Cu-responsive and shown to target one or other Cu-containing proteins, including Cu-Zn SOD, laccases and plantacyanin genes. This observation led to the identification of differential ROS homeostasis and highlighted the evolution of the variety-specific interplay of ROS and Cu homeostasis mediated by drought-responsive microRNA genes in rice. The connection between Cu/ROS homeostasis and drought remains to be established.

An exciting aspect of microRNA biology is its capacity to coordinate the developmental process with the plant stress response. Several miRNA genes have been analyzed in detail to unravel their role in defining plant drought tolerance (Table 1). One of the most intensely studied cases is that of the highly conserved microRNA gene family, OsmiR156. The miR156 gene family members target SQUAMOSA promoter-binding protein-like (SPL) transcription factors in rice and are known to play a critical role in regulating the juvenile's timing to an adult phase transition. A high level of miRNA156 expression favors maintenance of the juvenile phase by inhibiting the expression of OsmiR172 *via* SPL transcription factor. Studies have shown that various stress conditions, including drought conditions, positively regulate OsmiR156 expression, thereby delaying the phase transition [43]. Thus, miR156 defines a critical regulatory node that links and coordinates plant development to the prevailing

stress conditions, thereby playing a critical role in defining plant survival under stress conditions. Another interesting microRNA gene that is implicated in both development and drought tolerance is OsmiR166, which targets members of the HD-ZIP III gene family members in rice. This microRNA has been shown to regulate the development of leaf in rice, including the leaf vasculature development. The OsmiR166 knock-down plants exhibited rolled leaves, probably due to smaller bulliform cells. The transgenic plants also exhibited elevated drought tolerance, which was likely due to a reduced rate of transpiration [44]. Similarly, OsmiR164, which is also a highly conserved microRNA family in plants, has six known members in rice. This microRNA family targets several members of the NAC transcription factor family. The transgenic analysis revealed that overexpression of these NAC genes leads to sensitivity towards drought conditions in rice [45]. However, overexpression of a mutated *OsNAC2* which inhibits miR164b resulted in improved drought tolerance [46].

Besides transcription factors, microRNAs also target signaling components. For example, OsmiR393 is known to target auxin receptor genes and regulate multiple developmental processes such as tillering, early flowering, root, and seed development. Rice plants overexpressing OsmiR393 also showed increased sensitivity to both salinity and drought stress [47]. It has also been shown that OsmiR393 regulates lateral root development under stress conditions by regulating another rice microRNA, OsmiR390 [48]. Interestingly, OsmiR390 is also involved in auxin signaling since it targets the auxin response factor *via* a tasiRNA.

Thus, microRNA genes play a pivotal role in modulating the developmental and biochemical processes in response to drought stress conditions in rice. More large-scale functional studies and precise information of the target genes are required to understand the complete dimension of microRNA activity in rice. The presence of functionally active miRNA variants (5' isomiRs) further complicates miRNAs' regulatory spectrum [49]. Also relevant is the molecular schema that regulates the expression of microRNA genes themselves. Simple differential regulation of microRNA expression can have a cascading effect on plant development and metabolism. Evolution seems to have developed simple regulatory tweaks that change expression patterns to adapt rice varieties to different environmental conditions better. Using such circuits for genetic manipulation of drought would be a rewarding endeavour (Fig. 1).

4. GENETIC DISSECTION OF DROUGHT TOLERANCE IN RICE

The efforts to develop drought-tolerant rice by breeding face challenges because most of the relevant traits have complex quantitative genetic inheritance patterns and are primarily governed by multiple genes/QTLs (quantitative trait loci). Dissection of the complex genetic architecture of drought tolerance traits using diverse genomics-assisted breeding strategies is thus a prerequisite for rapid genetic improvement of rice. In this perspective, high-resolution genetic mapping

Table 1. Selected drought-related miRNAs and their target genes in rice.

miRNA	Target ID	Target Description	Association with Drought Tolerance
osa-miR156	LOC_Os06g45310	OsSPL11 - SBP-box gene family member	-ve
	LOC_Os06g49010	OsSPL12 - SBP-box gene family member	
	LOC_Os07g32170	OsSPL13 - SBP-box gene family member	
	LOC_Os08g39890	OsSPL14 - SBP-box gene family member	
	LOC_Os11g30370	OsSPL19 - SBP-box gene family member	
	LOC_Os01g69830	OsSPL2 - SBP-box gene family member	
	LOC_Os02g04680	OsSPL3 - SBP-box gene family member	
	LOC_Os02g07780	OsSPL4 - SBP-box gene family member	
osa-miR166	LOC_Os03g01890	START domain containing protein	-ve
	LOC_Os12g41860		
	LOC_Os10g33960		
	LOC_Os03g43930		
	LOC_Os01g10450	Protein kinase domain containing protein	
	LOC_Os06g01304	Spotted leaf 11	
	LOC_Os05g23290	Retrotransposon protein Ty3-gypsy subclass	
	LOC_Os03g44820	Glutamyl-tRNA	
osa-miR164	LOC_Os04g20164	Amine oxidase precursor	+ve
	LOC_Os03g38550	Retrotransposon protein unclassified	
	LOC_Os07g12510	AP2 domain containing protein, expressed	
	LOC_Os03g47940	GDSL-like lipase/acylhydrolase, putative, expressed	
	LOC_Os06g46270	No apical meristem protein, putative, expressed	
	LOC_Os02g36880		
	LOC_Os08g10080		
	LOC_Os12g41680		
	LOC_Os06g23650		
	LOC_Os04g41540	OsCML22 - Calmodulin-related calcium sensor protein, expressed	
	LOC_Os03g50040	Phytanoyl-CoA dioxygenase, putative, expressed	
	LOC_Os05g39650		
	LOC_Os12g18650	Regulator of chromosome condensation domain containing protein, expressed	
	LOC_Os10g02630	Transposon protein, putative, CACTA, En/Spm sub-class, expressed	
	LOC_Os12g08810	VTC2, putative, expressed	
osa-miR393	LOC_Os12g28270	Amidohydrolase, putative, expressed	-ve
	LOC_Os04g58734	Expressed protein	
	LOC_Os04g32460	OsFBL16 - F-box domain and LRR containing protein, expressed	
	LOC_Os05g05800	OsFBL21 - F-box domain and LRR containing protein, expressed	
	LOC_Os06g06880	Ser/Thr protein phosphatase family protein, putative, expressed	

such as QTL and expression QTL (eQTL) mapping as well as trait association analysis to delineate functionally relevant genomic loci governing drought tolerance are found much expedient. To accomplish these, significant efforts have been made for the phenotypic and genetic characterization of natural and mapping/mutant populations for diverse drought tolerance traits at a global scale to drive the marker-assisted breeding and generation of genetically tailored, drought tolerant, and high-yielding rice cultivars.

4.1. Phenotypic and Genotypic Characterization of Rice Genetic Resources for Drought Tolerance

Rice is grown in a wide range of eco-geographical regions globally, and its available genetic resources are rich in trait diversity. The rice germplasm resources (core/mini-core collections) include landraces, wild species accessions, cultivated varieties and breeding lines representing various agro-climatic regions of the world and are conserved efficiently in different International and National GeneBanks [50, 51]. Multi-environment replicated phenotyping of available core/mini-core germplasm accessions of rice for diverse drought stress tolerance traits has been performed using platforms for automated modern high-throughput phenotyping and E (environmental)-typing. Wide phenotypic variations for diverse physiological, agro-morphological, biochemical and anatomical features defining the drought tolerance traits in these rice accessions are apparent. Contrasting accessions for major drought tolerance traits are further utilized as parents to generate mapping populations such as recombinant inbred lines (RILs), near-isogenic lines (NILs) and double haploids (DHs) in rice. Some variability is also induced with different chemical and radiation mutagens to generate the mutant population for their phenotypic and genetic characterization for drought tolerance (<http://tilling.ucdavis.edu>; <http://www.iris.irri.org>). For instance, an ethyl methanesulfonate (EMS)-induced mutant population generated in an upland *indica* rice accession Nagina 22 is phenotypically and genotypically characterized for drought tolerance for their effective use in genetic improvement of rice [52, 53]. In this perspective, speed breeding, which emerged to be a powerful strategy for rapid generation advancement of mapping and mutant population and their precise large-scale phenotyping for diverse agronomic traits, including drought tolerance, will be useful for accelerated genetic enhancement of rice.

The reference genome sequence of rice [54] has driven the genome resequencing and global transcriptome sequencing of diverse accessions using various next-generation sequencing (NGS) strategies. These efforts overall enabled the fast generation of vast genetic marker resources such as simple sequence repeat (SSR), single nucleotide polymorphism (SNP) and insertion-deletion (InDel) markers at a genome-wide scale in rice [54]. The resequencing of genome of drought-tolerant (Nagina 22) and sensitive (IR 64) rice accessions detected about 660000 SNPs at a genome-wide scale for their broader use in marker-assisted breeding of drought tolerance in rice [55]. The genome resequencing of 3000 rice accessions, including contrasting drought tolerant and

sensitive accessions, has generated about 20 million SNPs for their efficient use in rice genetic enhancement for drought stress tolerance [56]. The efficacy of genome-wide 700K and gene-based 50K SNP chips in expediting the genomics-assisted breeding and genetic improvement of rice for drought tolerance has been well-demonstrated in the recent past [57, 58]. The diverse mutant population and natural core and mini-core germplasm accessions are also being assayed through targeting induced local lesions in genomes (TILLING) and EcoTILLING to mine useful functional alleles in the genes associated with rice drought tolerance. Novel allelic variants from cloned genes such as *OsDREB1F* as well as nine candidate abiotic stress-responsive genes governing drought tolerance in rice [57, 59] have been unraveled.

4.2. Delineation of Molecular Signatures Governing Rice Drought Tolerance

The genetic (QTL/eQTL mapping) and association mapping are found most useful genomics-assisted breeding strategies to scan molecular tags (QTLs, genes, novel alleles) modulating drought tolerance traits for genetic enhancement of rice. QTL mapping studies identified many important QTLs governing various drought-responsive root system architecture traits, including root length, root biomass, root number, root diameter, *etc.* One of these major root length regulating QTLs, *DRO1* (*DEEPER ROOTING 1*) has successfully been fine mapped to a major single effect gene. *DRO1* was found to promote root length in rice by regulating cell elongation in the root tips. Introgression of *DRO1* superior allele into shallow-rooting rice cultivar enabled it to avoid drought by increasing root length ultimately leading to higher yield under drought stress [60]. Apart from root system architecture, other drought-related anatomical and morphological traits like leaf rolling, stomatal density, and stomatal aperture size, *etc.* and physiological traits such as osmolyte concentration, cell membrane stability, and reactive oxygen species scavengers accumulation, *etc.* have been targeted in QTL mapping to identify major QTLs/genes associated with drought tolerance in rice [61]. Grain yield under drought stress is the most widely studied trait for identifying QTLs regulating drought tolerance in rice. A number of factors affect grain yield under drought stress, spikelet fertility being the most important one. To date, many QTLs regulating grain yield under drought stress have been identified. Some of these QTLs like *qDTY1.1*, *qDTY2.2*, *qDTY2.3*, *qDTY3.1*, *qDTY3.2*, *qDTY6.1*, and *qDTY12.1* have been found to be effective across different genetic backgrounds/environments and therefore are of immense importance for breeding drought-tolerant rice varieties [62-64]. Using the traditional QTL mapping, eQTL mapping, NGS-led whole-genome population resequencing and QTL-seq strategies, about 100 QTLs/eQTLs governing drought stress tolerance traits have been identified and mapped in rice [65, 66]. Further efforts have identified about 10 genes harboring major QTLs governing drought tolerance in rice. Utilizing the NGS-led genotyping-by-sequencing strategy for QTL mapping in five multiple mapping populations, major four stable and novel

QTLs governing grain yield under reproductive stage drought stress are detected in rice [67]. A major QTL *qDTY1.1* of which validated in multiple genetic backgrounds and showing undesirable linkages with plant height, is found much robust and thus has been utilized efficiently in marker-assisted genetic enhancement of rice for drought tolerance.

The genome-wide association study (GWAS) based on 529 natural accessions identified 110 genomic loci associated significantly with 21 diverse drought-responsive root traits like root length, root volume and root dry weight under both normal and water stressed conditions. This study further identified two candidate genes, *Nall* and *OsJAZ1* associated strongly with deep root dry weight under drought conditions and shallow root dry weight under normal conditions, respectively, in rice [60]. Like-wise, other drought-related traits like root morphology and relative water content have also been targeted in GWAS of drought tolerance in rice [68, 69]. Candidate gene-based association analysis identified natural allelic variants in an ERF (ethylene response factor) transcription factor, *OsLG3*, that enhances drought stress tolerance by inducing reactive oxygen species (ROS) scavenging [70].

4.3. Genomics-assisted Breeding for Genetic Improvement of Rice for Drought Tolerance

The functionally relevant molecular tags exhibiting major effects on drought tolerance have been introgressed and pyramided into the various contrasting rice cultivars successfully through marker-assisted breeding to develop high-yielding drought-tolerant rice varieties. Using the marker-assisted foreground and background selection, a diverse array of known drought-responsive major QTLs (*DTY1.1*, *DTY2.1*, *DTY2.2*, *DTY3.1*, *DTY3.2*, *DTY9.1*, and *DTY12.1*) are introgressed into multiple high-yielding Indian mega rice varieties for their genetic enhancement for drought tolerance [71]. Adopting the similar marker-assisted selection (MAS) strategy, two drought-responsive major root architecture-related genes/QTLs, *DROI* and *QTL-9* have also been introgressed into shallow-rooted cultivars like IR 64 and Birsa Vikas Dhan 111, which significantly increased the root length both under drought and well-watered condition [60]. Apart from this, different drought-related major QTLs, *qDTY12.1*, *qDTY2.3* and *qDTY3.2* have been pyramided together that significantly improved yield under drought stress in rice [64]. Efforts have also been made to pyramid major QTLs governing both flood and drought tolerance in rice [72]. This overall signifies the potential utility of drought-responsive major genes/QTLs in developing drought-tolerant rice cultivars with enhanced yield. The complex genetic inheritance pattern (pleiotropic/epistasis) and complications in genetic background effects on drought tolerance traits as well as linkage drag of drought-responsive QTLs suggest the essentiality of using multiple advanced genomics-assisted breeding approaches for the genetic enhancement of rice for drought tolerance (Fig. 1).

CONCLUSION AND FUTURE PERSPECTIVES

New genes, microRNAs, allelic targets and knowledge-based approaches are needed for the improvement of drought tolerance in rice. The emerging areas like drought memory and optimized roots growth offer exciting avenues [73]. Encounter with drought develops “memory” in plants, which increases their tolerance to future drought episodes. A plethora of studies in model plants like *Arabidopsis* has revealed that accumulation of signaling proteins and transcription factors, especially those related to plant hormones and epigenetic modifications, are involved in the formation of short-term memory in response to drought. However, in the case of rice, limited information is available on a mechanism of drought memory. Nonetheless, recent studies implicate that rice genes also show distinctive drought memory patterns. Future efforts should be directed towards understanding the mechanisms of drought-induced memory in rice as it may increase its chances of survival in an ever-changing environment. Further, it was recently discovered in barley and maize that root growth is inhibited towards large air spaces in soil, mimicking water absence, by an auxin-mediated response [74]. Such a strategy rationalizes the plant’s investment of carbon in root growth towards water-rich areas. Such an understanding of rice root growth patterns would help optimize root architecture for the local environment using conventional or transgenesis approaches. Sadly, transgenics or GM technology suffered from a strong protest by not so well-informed activists forcing the government(s) to enact over-regulations. Despite that, GM growing areas/crops are increasing worldwide due to apparent gains in crop productivity. Gene editing offers an exciting alternative that could potentially allay the fear associated with GM. However, the recent views in Europe on classifying gene-edits and GM in the regulated category could be a setback for research application. Transgene-free precisely gene-edited plants are not distinguishable from random mutants generated through well-accepted chemical or radiation approaches; and, therefore, could have been deregulated. Until regulatory regimes are rationalized, the rapid increase in the availability of genomic resources and the advancement of breeding technologies like “speed-breeding” will make molecular breeding an appropriate choice for improving drought tolerance in rice.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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