



Modern Era of Rice (*Oryza Sativa* L.) Genomics for Precise Genomics-Assisted Drought Breeding

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Abstract

Rice (*Oryza sativa* L.) is an important food crop and requires larger amount of water throughout its life cycle as compared to other crops. Hence, water related stress cause severe threat to rice production. Drought is a major challenge limiting rice production. The screening of rice germplasm under drought and its characterization at the morphological, genetic, and molecular levels revealed the existence of genetic variation for drought tolerance within the rice gene pool. The improvements made in managed drought screening and selection for grain yield under drought have significantly contributed to progress in drought breeding programs. The availability of rice genome sequence information, genome-wide molecular markers, and low-cost genotyping platforms now makes it. Drought possible to routinely apply marker-assisted breeding approaches to improve grain yield under drought. Grain yield QTLs with a large and consistent effect under drought have been identified and successfully Genetics pyramided in popular rice mega-varieties. The transgenic approach Proteomics is successful in generating drought tolerance in rice under controlled conditions, but field-level testing is necessary. Genomics-assisted drought breeding approaches hold great promise, but a well-planned integration Allele mining with standardized phenotyping is highly essential to exploit their full potential.

Keywords: Genomics; Proteomics; Transcriptomics; Drought; Rice and Genetics

Introduction

Water is an important factor in agricultural and food production, yet it is a highly limited resource (Wang et al, 2012). Water deficit stress causes extensive loss to agricultural production worldwide, thus being a severe threat to sustainable agriculture. Feeding continuously increasing population with depleting water supply requires crop varieties that are highly adapted to dry environments [1]. Rice plays a major role as a staple food, supporting more than three billion people and comprising 50% to 80% of their daily calorie intake [2]. Drought stress severely impairs its production. Worldwide, drought affects approximately 23 million hectares of rainfed rice [3].

Rice is highly susceptible to drought stress throughout its life cycle, but huge economic losses or even complete crop failures are observed if stress occurs during flowering [4]. The capacity

of the rice plant to sustain itself and to reproduce in limited water conditions is crucial for rice production in years of drought [5,6]. Thus, it is imperative for rice breeders to develop drought-tolerant high-yielding rice cultivars. Even though several efforts were made to breed for drought tolerance by including tolerant donors in breeding programs, there are few examples of improved rice cultivars that combine acceptable yield potential and drought tolerance. This is mainly because of the genetic complexity of drought tolerance due to its polygenic inheritance, low to medium heritability, significant genotype and environmental interactions, and the confounding effects of other abiotic stresses on drought [7-9]. In view of this, breeders have to screen and select a very large number of genotypes over seasons, years, and locations to successfully develop drought-tolerant varieties. The whole process

is time-consuming, labor-intensive, and expensive. Drought breeding efforts over the past two decades have been successful in standardizing protocols for managed drought screening infield conditions by selecting sites having climatic conditions and soil types similar to those of the drought-prone target environments. Trait phenotyping with grain yield under drought has been proven as an efficient criterion and is currently being used in drought breeding programs [10].

Rice is the first food crop with both of its subspecies, indica and japonica, sequenced [11,12], and also ten wild species of rice belonging to different genomes are being sequenced (www.map.org). Rice has an enormous wealth of genomics and bioinformatics resources that can be used to speed up the breeding process. Recent advances in genomics technologies such as molecular markers and genetic engineering have made rapid strides in the understanding of the molecular basis of drought tolerance and enabled the identification of genes/QTLs for yield and yield-related traits under drought. Now, the stage is set for effective integration of genomics technologies with breeding activities to improve drought tolerance in rice. In our review, we discuss the recent advances in understanding the molecular basis of drought tolerance and the application of genomics technologies in improving drought tolerance in rice.

Genetics and Genomic Prospective of Rice in the Era of Drought Tolerance

Genetic prospective of rice

The knowledge of genetic basis of drought tolerance is vital approach to start a breeding program. It will be more beneficial to evaluate a crop variety under any adverse environmental condition. Genetic of drought is controlled by polygenes [13]. Genetic study

of physical and yield governing traits as well as root traits showed positive interaction with environment followed by polygenic inheritance like additive and dominant type of gene action [13,14-17]. Positive Epistatic gene action were also observed [18,19]. It is estimated that secondary characters of rice cultivar may be beneficial to combat drought tolerance but in field this assumption was not observed yet. Medium to high heritability was observed for grain yield under drought stress breeding on this criteria will be proved enhance rice yield under drought.

Genomics prospective of rice

Advances in molecular genetics and genomics rice genome is completely sequenced and it is now possible to develop a diverse variation from a single QTL [20-23]. There are several approaches i.e. association mapping, genome-wide selection (GWS). Linkage mapping, Marker assistant selection which can be utilized to develop a drought tolerance rice variety [24]. Genomic diversification can made with such technique's QTL × QTL connections, QTL × environment interactions and QTL introgression. A complete set of these variation is key factor to initiate marking assistant program of drought tolerance in rice [25]. Variability is found in major-effect QTLs for grain yield could be utilized for MAS for drought tolerance in rice.

Rice genomics resources and tools for precise genomics-assisted drought breeding

Advances in molecular genetic lead to high value of genomic diversity, these advance techniques are rapid and very convenient in breeding point of view [26]. As well as improvement in bioinformatics and genomics approach have been proved more beneficial for biotechnologist and molecular researchers

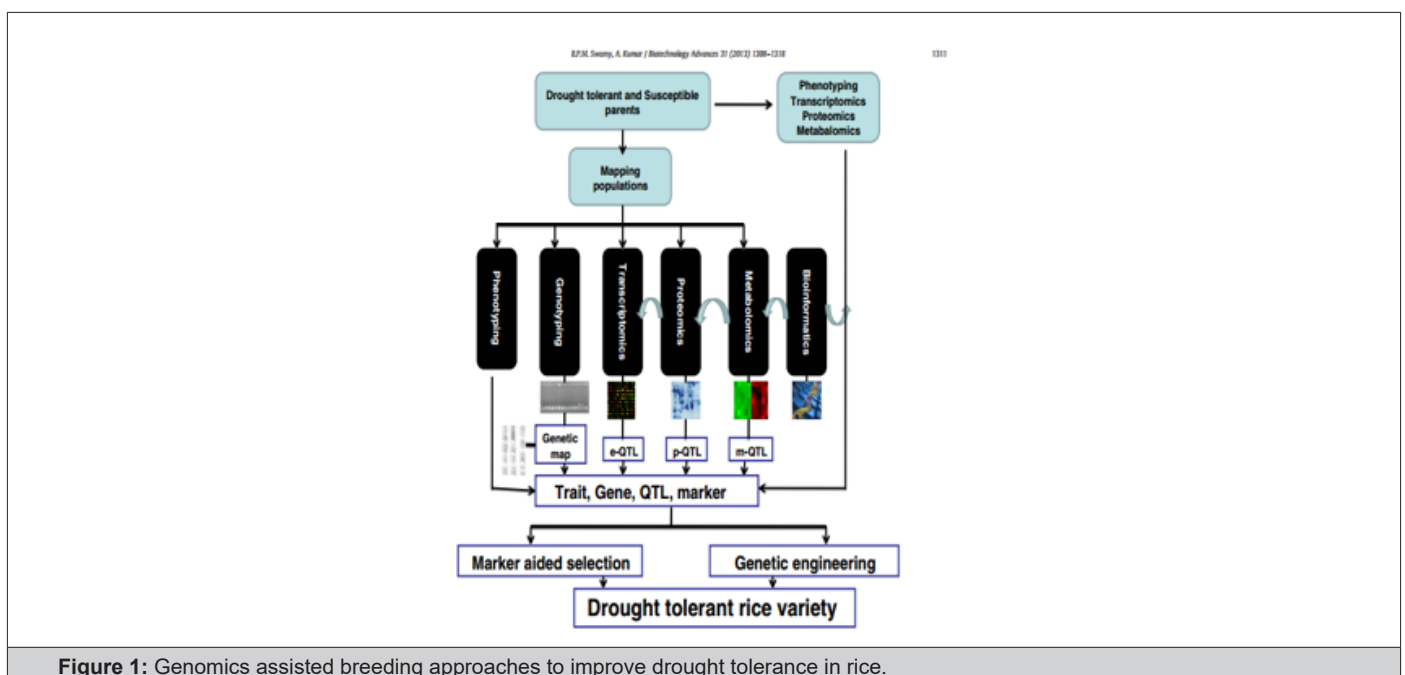


Figure 1: Genomics assisted breeding approaches to improve drought tolerance in rice.

Rice is the first sequenced food crop and has been at the leading position in plant genomics because of its significance in world food 1309 B.P.M. Swamy, A. Kumar / Biotechnology Advances 31

(2013) 1308–1318 security. Rice has small genome size of 390 Mb and known as a model in cereal crops. The genome of have two sub species, japonica (cv. Nipponbare), indica (cv. 93–11),

and organelle genome such as mitochondrial and chloroplast genome has been sequenced and genome of ten different wild rice are being sequenced. All this information is placed in the public domain and is easily accessible for breeding point of view (www.gramene.org, www.msu.edu, www.bgi.org). BAC end sequences, Bacterial artificial chromosome (BAC) libraries, and whole-genome sequence approaches develop various marker systems and led to the construction of physical maps and great-diverse genetic maps [27,28]. Rice contain both functional and non-functional markers which include more than 20,000 SSR markers and over one million SNPs and Indels including useful and non-useful markers [29,30]. For the use of molecular markers in diversity analysis, has proved to be landmarks in molecular genetics, mapping genes/QTLs for various agronomic traits, and their use in marker-assisted breeding (MAB) and also identify candidate genes in positional cloning of QTLs for complex traits studies under various environmental stresses like drought [31]. The accessibility of rice functional genomics include cDNA libraries, transcriptome maps, expressed sequence tags (ESTs), TILLING array networks, insertion mutant genomics libraries and advance detection technique such as real-time PCR, macro array and microarray, serial analysis of genome expression (SAGE), massive parallel signature sequences, genome-

wide association mapping etc. [32], and different proteomics, Transcriptomic studies and metabolomics study have greatly useful for drought tolerance global and local gene expression analyses to evaluate and characterize candidate genes [33-35]. For particular gene expression we must have Transcriptomic information regulatory machinery and housekeeping gene study help us to understand the pathways of gene regulation [36-38]. Table 1.shows rice genomics resources and tools. For genetic engineering, rice crop has modified for tissue culture, direct and indirect gene cloning i.e. agrobacterium mediated and biolistic technique of gene introgression, advance detection method labelled and non-labeled assay for gene confirmation techniques to study copy number of gene at protein or mRNA level [39-41]. For developing, evaluating, and handling transgenic plants there are already international standard biosafety measures. The well-established sources of various types of molecular data and advanced bioinformatics tools have subsidized to searching, querying, depositing, and analyzing all types of information that is being explored by rice scientists [42]. Recent landmarks in rice genome enabled scientist as well as rice breeders to utilized natural and induced diversity for the improvement of Rice crop (Figure 1) [43].

Database Name	Link
Rice genome sequence data	www.msu.edu , www.bgi.org
Full-length cDNA clones	http://cdna01.dna.affrc.go.jp/cDNA/
Rice biological and molecular data	www.gramene.org , www.graingenes.org
	http://www.ncgr.ac.cn/ricd
	http://cdna01.dna.affrc.go.jp/PIPE
Rice annotation	http://rice.plantbiology.msu.edu/
	http://rapdb.dna.affrc.go.jp/
	http://ricegaas.dna.affrc.go.jp/
Transcription factor	http://drtf.cbi.pku.edu.cn/
	http://ricetfdb.bio.uni-potsdam.de/
Rice expression	http://cdna02.dna.affrc.go.jp/RED/
Comparative genomics	http://greenphyl.cirad.fr/
Mining genes from QTLs	http://rice.kps.ku.ac.th:8080/RiceGeneThresher
Rice mutants	
Small RNA	http://rmd.ncpgr.cn/
	http://www.ricefgchina.org/mutant
Rice expression	http://sundarlab.ucdavis.edu/smrnas/
Rice proteome	http://retroryza.fr/
BAC/EST resources	http://mpss.udel.edu/
	http://gene64.dna.affrc.go.jp/RPD/
Phenotype of the mutants	main_en.html
SNP resources	www.genome.arizona.edu
	http://tos.nias.affrc.go.jp/
	http://www.plantgenome.uga.edu/snp
	http://shenghuan.shnu.edu.cn/ricemarke

Recent platform developed for Rice Genomics for drought tolerance

Meanwhile the accomplishment of whole-genome sequencing in rice, numerous well-designed genomics platforms have been recognized in the historical period [44,45]. Stages of metabolomics, proteomics, and phenomics have likewise been progressively well-known and amended, and compatible platforms of bioinformatics investigation and catalogues have also been set up in rice.

Transcriptomics

Considerate the genes that govern response to drought stress is beneficial in breeding rice with improved drought tolerance. The response to drought stress in rice could also be an advanced development involving several functionally interconnected genes unfold throughout the order. Natural phenomenon Analysis permits the analysis of the expression of thousands of genes in an extremely extra comprehensive and holistic suggests that, providing a world image of changes occurring inside the transcriptome of varied components of the plant beneath stress conditions. Expression analysis results is accustomed establish candidate genes for specific drought tolerance-related traits or physiological mechanisms associated with stress-induced adaptive processes [38,46,47]. In rice, several efforts were created to grasp the differential expression of genes under drought stress by transcription identification pattern RT-PCR and microarray technologies [48] distributed microarray analysis of rice panicles selected from a drought stressed plant and reported up-regulation of the many stress-induced genes like GTP-binding molecule 3, sugar synthase-6, heat shock cognate molecule, deoxyribonucleic acid repair molecule, reductase, zinc finger molecule, protein depolymerizing issue, and polysaccharide esterase [49] far-famed sixty 2 drought-inducible genes in 2-week-old drought-stressed rice seedlings. Widespread rice landrace N22 (Nagina-22) could also be a natural different for lots of molecular characterization work because of its drought tolerance. A drought-induced DNA library was made of N22 and transcription identification was performed, 589 acknowledged stress-related genes were far-famed, and, pattern this knowledge, candidate genes were deduced for drought QTLs [50]. In another study pattern N22, ESTs were generated from drought-stressed rice seedlings; most of the stress-induced genes were related to primary metabolic pathways, transcription, and conjointly the interpretation methodology [51]. Differential expression analysis of upland and lowland rice cultivars victimization cDNA-AFLP unconcealed that quite eight of the entire genes clothed were up-regulated by drought stress in each cultivars.

Fifty-seven and thirty-eight genes were specifically expressed in upland and lowland cultivars, severally. Among the differentially expressed genes, genes for cell rescue, drought defense, signal transduction, nucleotides, organic compound biogenesis, and genes for plant growth and development were specifically expressed in upland rice, whereas genes for macromolecule and ester degradation were specific to lowland rice cultivars [52]. The spatial temporal expression patterns of two-component system (TCS) genes in rice underneath drought stress showed a differential expression pattern in varied organs underneath stress

[53-55]. Reportable genes and signal pathways concerned within the anatomical and morphological developments of rice roots, notably for crown root initiation underneath drought stress [56]. conducted a tissue-specific organic phenomenon study in drought-stressed rice roots, during which sixty-six transcripts were known. Out of those, four transcripts were mapped inside the QTLs for root growth underneath water deficit. In another study, [57] discovered differentially expressed genes in leaves and roots of upland rice and lowland rice. Combining our data of genes concerned in growth and development, reconciling mechanisms with genes that are differentially expressed underneath drought may facilitate determine necessary target traits for drought resistance. The differentially expressed genes also can function candidate genes for additional characterization and for allele-morph mining.

Proteomics

Histone modifications, DNA methylation, and microRNAs are the 3 main epigenetic regulators of drought tolerance. Underneath drought stress, simple protein modifications like acylation and/or methylation pattern among the promoters or writing regions of cistrons cause cistron activation or gene silencing, leading to tolerance of drought stress [58]. Pyrimidine methylation and demethylation by DNA methyltransferases are epigenetic mechanisms adopted by the upper organisms in response to worry. It causes differential regulation of organic phenomenon through either silencing or over [59,60]. There are reports demonstrating that DNA methylation-regulated organic phenomenon could be a response to drought stress. A recent study exploring the genome-wide DNA methylation standing of 2 rice cultivars (DK151 and IR64) with totally different tolerance of drought disclosed important variations within the methylation patterns between the 2 genomes [32]. Methylation and demethylation changes were elicited underneath drought conditions during a biological process and tissue-specific manner and that they accounted for twelve.1% of the entire site-specific methylation variations between the 2 lines. Notably, seventieth of the drought-induced methylation changes were reversed once recovery, and twenty nine remained dateless [32]. Changes in methylation pattern in rice cultivars were reported by [61]. These observations recommend that DNA methylation changes play a task within the response of rice to dehydration conditions in all probability by activating or deactivating stress-responsive genes and resulting in adaptation to drought conditions. The fiber non-coding RNAs like microRNAs (miRNAs) and SiRNAs are concerned within the epigenetic method of drought stress. To date, many drought-responsive microRNAs are known in plants. MicroRNAs play vital roles in post-transcriptional cistron regulation by repression of template RNA translation. In rice additionally, small polymer regulated drought response has been reported [38,62].

Metabolomics

Plants react to drought stress by accumulating various metabolic compounds such as proline, glycine-betain, pinitol, carinitine, mannitol, sorbitol, polyols, trehalose, sucrose, oligosaccharides, and fructans in large quantities [63]. These are chemically dissimilar compounds; they keep the surface of the proteins

hydrated, resulting in decreased water potential and facilitating continuous water movement, which might contribute to sustaining physiological processes such as stomatal opening, photosynthesis, and expansion of cell growth. The role of metabolites in drought tolerance has been thoroughly reviewed in earlier reports [64,65].

Epigenomics

Histone modifications, DNA methylation, and microRNAs are the three main epigenetic regulators of drought tolerance. Under drought stress, histone modifications such as acetylation and/or methylation pattern within the promoters or coding regions of genes cause gene activation or gene silencing, resulting in tolerance of drought stress [59]. Cytosine methylation and demethylation by DNA methyl-transferases are epigenetic mechanisms adopted by the higher organisms in response to stress. It causes differential regulation of gene expression through either silencing or over [60,61]. There are reports demonstrating that DNA methylation-regulated gene expression is a response to drought stress. A recent study exploring the genome-wide DNA methylation status of two rice cultivars (DK151 and IR64) with different tolerance of drought revealed significant differences in the methylation patterns between the two genomes [66]. Methylation and demethylation changes were induced under drought conditions in a developmental and tissue-specific manner and they accounted for 12.1% of the total site-specific methylation differences between the two lines. Notably, 70% of the drought-induced methylation changes were reversed after recovery, and 29% remained unaltered [67]. Changes in methylation pattern in rice cultivars were reported by [32]. These observations suggest that DNA methylation changes play a role in the response of rice to dehydration conditions probably by activating or deactivating stress-responsive genes and leading to adaptation to drought conditions. The single-stranded non-coding RNAs such as microRNAs (miRNAs) and siRNAs are involved in the epigenetic process of drought stress. To date, hundreds of drought-responsive microRNAs have been identified in plants. MicroRNAs play important roles in post-transcriptional gene regulation by repression of mRNA translation. In rice also, micro RNA regulated drought response has been reported [38,39].

Genetic engineering for drought resistance in rice

Drought resistance in rice can be overcome by genetic engineering techniques (Hervé and Serraj, 2009; Mathur et al., 2008). Direct and indirect genetic transformation techniques (e.g. agrobacterium mediated and biolistic) were applied in rice genome for drought resistance. These techniques proved to be very efficient in past few decades. Large numbers of candidate genes were observed against drought stress which was directly involved in post transcriptional modification, signal cascades and metabolite triggering (Yang et al., 2010). Genetic engineering was found efficient technique that allows gene pyramiding for biotic and abiotic stresses. (Cattivelli et al., 2008).

Transcription analysis enables gene expression for specific environmental stress especially for drought at any chronological crop stage. Genetic expression of MAP kinase related genes were found in drought stress (Agrawal et al., 2003); Other heat related proteins like proline, LEA and HSP and other osmolytes (Sato and

Yokoya., 2008; 62; Xu et al., 1996; Zhu et al., 1998); DREB1/CBF is DREB genes (Dubouzet et al., 2003) endo-1,3-glucanase (Akiyama and Pillai, 2001); NAC genes (Hu et al., 2006; Leung, 2008); Ca Dependent protein kinase (Saijo et al., 2000); and trehalose (sugar compounds) (Garg et al., 2002; Jang et al., 2003). Rice genome modified with such gene pyramiding protocol would be better adapted to new environment under drought. Overall efforts interpreted that proper genetic resource and techniques would be very effective for gene expression under stress. Durability and stable performance of genetic assembly of any species is key factor for breeder. Transcriptional factor along with specific genes have been incorporated in rice genome for durable and stable expression of protein under various type of seasonal fluctuations.

Transgenic rice germplasm has been widely evaluated in vitro condition for better result it is needed to be evaluated in environmental condition. Hence transgenic approaches are preferred over traditional breeding program which is very laborious and time consuming. Genetic engineering is novel breeding technique widely used along the world to combat the global warming specially drought. It is needed that genetic engineering in rice would be very selective and appropriate method especially for drought stress (Bhatnagar-Mathur et al., 2008; Yang et al., 2010).

Water is important component for Food security yet it is scarce resource in the world [67]. Water scarcity limit the agricultural growth worldwide. It is urgent need to develop a drought tolerant variety to produce quality food for the growing population [1]. Rice is staple food along the worldwide which feed three billion population, depending upon daily dietary needs [2]. Rice crop is severely affected by water deficiency which significantly reduces per hectare production. Rainfed rice yield is reduced by drought stress approximately 23 million hectare worldwide [3].

Rice crop is highly sensitive to water scarcity but during flowering stage, it was observed that the water stress cause economic loss on large scale. [4]. The ability of rice plant to stand in water scarcity condition is important for production of rice during drought season [5,6]. Breeder must develop rice varieties suitable for water deficit conditions. Recently several research programs have been introduced to develop drought tolerance rice cultivars utilizing multidiscipline approaches. Polygenic traits were introgressed in rice germplasm for better adaptation of rice variety under adverse biotic and abiotic stresses. Thus, it is imperious for breeders to develop cultivars of high yielding and drought tolerant. [7-9]. Genetic variability and durability of drought tolerance genes are novel approach for the stable production of rice against drought. Screening of these lines and introgressed to cultivated germplasm is highly demanding objective for development of drought tolerance rice variety. But this process required time, cost and human capital. Research has been accomplished to develop drought tolerance rice variety in past few decades. Maximum production with sufficient adaptability of cultivar under drought condition is prerequisites for the initiation of breeding program [10].

Cultivated rice has two wild species named as *O. indica* and *O. japonica*, both have completely sequenced [12- 13], as well as more than 10 rice wild progenitor have been sequenced (www.map.org).

Breeding of rice crop can be modified by utilizing bioinformatics and genomic tools which had been widely used in rice genome. Genetic engineering and MAS proved to be latest and innovative breeding strategy to evaluate genetic architecture of drought tolerance genes in rice. By utilizing these strategies rice production would be increased under drought conditions. Yield related genes/QTLs expressed under drought condition have been successfully identified. Now, it is highly demanding objective to incorporate these genes into rice by using Genetic engineering approach. This review will benefit the scientist to develop drought tolerance rice cultivar by understanding the genetic and genomics application of innovative research tools in breeding programs.

An integrated approach is the way forward

For drought tolerance, breeding efforts in rice have clearly revealed that a multidisciplinary approach is necessary. Utilizing Standard approach to study the physiological basis of drought tolerance helps to determine the specific anatomical and morphological diversity, and pathways. Selection for donor parents above mentioned variation and approaches proved to be useful in molecular and traditional breeding program for drought tolerance [7]. With the help of diverse genomics technologies such as molecular markers, proteomics, Transcriptomics, epigenomics, metabolomics and, together with conventional breeding approaches facilitated to identification and characterization of genes/QTLs that is responsible for specific gene regulation under drought (Ashraf, 2010) [68]. Marker assisted breeding is proved to be very rapid and efficient technique for the introgression of drought tolerance QTLs/genes in rice [10,69]. A transgenic method allows the manipulation of drought-tolerant genes from different sources [70-75]. Through breeding procedures after the evaluation of transgenic plants and gene incorporation, these genes can be transferred to other genetic database [76-80]. Functional genomic study may be very crucial in future crop improvement. Breeder can only utilize these technologies when reasonable variation and integration is observed in rice germplasm.

Conclusions

Drought tolerance trait is very complex genetic character which is main cause of drought tolerance rice germplasm. However drought stress and its mitigation strategies were explored in last few years. Genetic variability for specific trait is key factor to start a breeding program. Genetically and physiological screening of rice germplasm for grain yield, QTLs, genomic marker and MAS would be very effective to develop drought resistant rice cultivar. There are several Quantitative trait loci for Rice yield under drought have been transformed and would be utilized in future research. Advancement in functional genomics significantly helps to evaluate various molecular pathways and genetic resource participated in drought tolerance. Rice genome can be edited for drought tolerance and genetic pyramiding of drought responsive genes would be revolutionize rice productivity. Drought tolerance variety of rice can be developed through genetic engineering techniques.

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