

**An overview of genetic improvement for drought tolerance in rice (*Oryza sativa* L.)**

Syed Ahtisham Masood<sup>1</sup>, Zain Naseem<sup>1</sup>, Madiha Anum,<sup>1,3</sup> Sovaiba Arshad<sup>1</sup>, Anum Jamshaid<sup>1</sup>, Abdul Samad<sup>1,4</sup> and Qurban Ali<sup>2</sup>

<sup>1</sup>. Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan

<sup>2</sup>. Centre of Excellence in Molecular Biology, University of the Punjab Lahore Pakistan

<sup>3</sup>. National Key Laboratory of Crop Genetic Improvement, Huazhong Agriculture University, Wuhan, 430070 P.R. China

<sup>4</sup>. College of Plant Science and Technology, Huazhong Agriculture University, Wuhan, Hubei, P.R. China

Corresponding author: [saim1692@gmail.com](mailto:saim1692@gmail.com), [qurban.ali@cemb.edu.pk](mailto:qurban.ali@cemb.edu.pk)

**Abstract:** Plant growth and productivity of rice (*Oryza sativa*) is significantly affected by numerous biotic and abiotic stress inducing factors. Water deficit is regarded as one of the major abiotic stresses that adversely affect growth and yield of this crop. Drought stress is a restrictive factor for agricultural crops, preventing them from attaining maximum theoretical yield as determined by genetic factors. In plants, development of new varieties having high yield and better performance under water stress conditions demands the proper understanding of their genetic basis. This review describes some major characteristics of water deficit induced effect of drought stress on biological yield and its related traits in indica species of rice.

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**Introduction**

Rice (*Oryza sativa* L.) is one of the vital crops worldwide and many Asian countries directly depend upon it for food and feed purposes. Being important cereal crop in many countries of the world it occupies pivotal role in their economy. Rice having diploid genome with 24 chromosomes and ssp. indica has 466Mb (mega base pairs) genome size (Yu *et al.*, 2002). Agreeing to their genome composition, species of this genus can be categorized into four main groups, *O. ridleyi* group (constituting the HHJJ genome); *Oryza granulate* group (comprising the GG genome); *O. officinalis* group (containing the BB, CC, BBCC, CCDD, and EE genomes) and *Oryza sativa* group (comprising the AA genome). *O. sativa* and *O. glaberrima* cultivated species of rice genus *Oryza*. *Oryza sativa* cultivated in Asia is and important food crops after wheat and serves as major food crop for more than 50% residents of the world (Wang *et al.*, 2014). The BBCC *Oryza* genome (2n=4x=48) is specific of allotetraploid wild species with two homologous genomes, B and C. Three species *O. malampuzhaensis*, endemic to India; *O. minuta*, found in Philippines and Papua New Guinea; and *O. punctata* (tetraploid, 2n=48), broadly dispersed in Africa have this genome (Ge *et al.*, 1999). Only *Oryza punctata* (2n=24) consists of BB genome (Tateoka, 1962), while *Oryza eichingeri*, *Oryza rhizomatis* and *Oryza officinalis* have the CC genome (Amante *et al.*, 1992). The wild species are extensively scattered in the moist tropics and

subtropics of Asia, Central and South America, Africa and Australia. Of the two species, Asian rice (*O. sativa* L.) is extensively grown in 112 states whereas African rice (*O. glaberrima*) is restricted to West Africa (Zhu *et al.*, 2013).

Population pressure has resulted in increase of its demand. It is thence recommended to develop high yielding varieties with resistance to biotic and abiotic stresses in order to meet demands of individuals having rice as staple food. This situation demands the modification of genotypes by harnessing variations present in rice genome. Therefore, yield increasing programs for selection of superior parental genotypes for hybridization are very critical. It contributes 2.7% to value added and 0.6% to GDP. Area under its cultivation decreased by 10.1% in 2012-13 from 2571 M hectares to 2311 M hectares. While, its production increased from 5.54 M tons in 2011-12 to 6.16 million tons in 2012-2013. On hectare basis its yield increased slightly from 2396 to 2398 kg/hectare which isn't satisfactory to cope with increasing demand. So being one of the staple foods it is essential that it must be produced in excess to fulfill the needs of our people and to support our agriculture based economy (Govt. of Pakistan, 2012-13).

In rice cultivated areas there is a major problem of water stress especially in rainfed areas and in approximately 8 M hectares of upland and 34 M hectares of rainfed lowlands face drought stress in Asian countries, the major limitation factor in rice

(Venuprasad *et al.*, 2009). Drought tolerance is a trait with complex heritability. Main reason for less advancement in breeding for water stress tolerance are lack of effective selection criteria and low heritability in case of grain yield under stressful conditions (Ouk *et al.*, 2006; Ali *et al.*, 2013; Ali *et al.*, 2014abc; Khan *et al.*, 2014; Mustafa *et al.*, 2015). Different rice genotypes show variable responses to abiotic stresses. To overwhelm these stress problems, developing stress tolerant rice cultivars is the economical and efficient way to manage with these stresses. It is essential to develop stress tolerant crops especially in the stress stricken parts of the world (Nakashima *et al.*, 2012; Dar *et al.*, 2014; Ali *et al.*, 2014). This makes the condition disturbing and poses a risk to national food security. Present review is about the association of drought stress to major transcriptional factors that are responsible for incorporating drought resistance in rice. When some sort of stress affects a crop, level of several metabolites and proteins increase due to activation of stress responsive genes which provide protection against adverse effects of stress. Therefore, production of crop can be enhanced by utilizing modern molecular techniques that covers improved breeding programs and transgenic methods. Such techniques need introduction of gene of interest into genetic background of crop (Garg *et al.*, 2012). Molecular techniques make it possible to accelerate the desirable gene transfer among varieties and use of wild relatives for novel genes introduction. Use of molecular markers is helpful in analysis of polygenetic characters which were once difficult to study by using traditional methods in plant breeding. By using molecular techniques and the knowledge of bioinformatics it is possible to make genetic relationship between sexually incompatible species too.

#### **Biochemical control for drought tolerance**

Mostajeran and Rahimi (2009) studied the effect of low water supply on the number of heading per hill, number of grain per hill, dry weight of vegetative tissues and panicle and 1000 grain weight in three new cultivars of rice including 216, 829 and Zayandeh Rood were measured under submerged and non-submerged conditions. Simultaneously, the variation in proline and total sugars in sheaths and blades of leaves at different ages was determined. The data indicated that Zayandeh Rood cultivar showed the lowest reduction in shoot dry weight and the number of tillers per hill under non-submerged conditions. Furthermore, the panicle weight and the number of filled grains per spike were higher in Zayandeh Rood cultivar than the other cultivars. In addition, the result of this study show that Zayandeh Rood cultivar in which originated from local

cultivars, have higher ability in solute accumulation such as proline and total carbohydrates than the other new lines. Due to correlation between drought tolerance of Zayandeh Rood and solute accumulation, it may be suggested that the solute accumulation is one of the mechanisms for drought tolerance in rice.

Oh *et al.* (2009) identified 42 genes out of total 139 which were induced by stress conditions like salinity, chilling temperature, drought and abscisic acid. Functional characterization of two genes OsAP37 and OsAP59, from subgroups I and II, respectively, was done. When plants exposed to high-salinity and drought for 2 hours both genes found to be induced. The overexpression of two genes, AP37 and AP59, in rice increased the degree of tolerance of plants to drought and high-salinity at vegetative stage under the control of the constitutive promoter OsCcl1. Results suggested that there is an opportunity to improve drought tolerance in rice using the potential of AP37 gene. Quan *et al.* (2010) reported that the TSRF1 improves the drought and osmotic tolerance of rice seedlings deprived of growth hindrance, firming by physiological analyses of leaf and root growth, leaf transpiration and survival rate under stress. The transgenic rice lines have increased amount of soluble sugars and proline as compared to wild-type plants. TSRF1 activates the expression of SDR, a rice abscisic acid (ABA) synthesis gene causing an enhanced ABA sensitivity in transgenic rice. TSRF1 also increases the expression of proline, MYC and MYB synthesis and photosynthesis related genes, most likely by binding to dehydration responsive element and GCC boxes in promoters of the target genes. Results demonstrated that TSRF1 improves the drought and osmotic tolerance of rice by modulating the increase in stress responsive gene expression.

Cha-Um *et al.* (2010) studied that relative water content (RWC) in the leaf of different rice cultivars dropped significantly in relation to reduced soil water content (SWC), especially in PT1 and IR20. In contrast, the proline content in the leaf-blade and leaf-sheath increased when plants were subjected to 7% SWC. The RWC was positively related to chlorophyll degradation. Chlorophyll a, chlorophyll b, total chlorophyll, total carotenoids, maximum quantum yield of PSII, stomata conductance and water use efficiency in rice grown under water-deficit conditions declined significantly in comparison to the control group, leading to a reduction in net-photosynthetic rate. In addition, when exposed to water-deficit, panicle length and fertile grains in KDML105 and NSG19 were stabilized, leading to greater productivity than in PT1 and IR20. These data were utilized as effective criteria for the classification of water-deficit tolerance. From results, KDML105

and NSG19 were identified as water deficit-tolerant, and PT1 and IR20 as water deficit-susceptible. Heinemann *et al.* (2011) conducted two greenhouse experiments to compare the response of normalized transpiration rate (NTR) of three modern (BRS Primavera, BRSMGC uringa and BRS Soberana) and one traditional (Douradão) upland rice cultivars to soil water deficit during the vegetative and reproductive growth stages. This information will support breeding strategies to improve rice yield in a drought-prone target population environments (TPE) in Brazil. NTR and the total fraction of transpirable soil water (FTSW) were calculated, plotted and adjusted according to a sigmoid non-linear model. The  $p$  factor, defined as the fraction of available soil water that can be removed from the root zone before water deficit occurs, was calculated by assuming that it occurs when NTR is equal to 0.95. Modern cultivars had a higher value of  $p$  for the reproductive phase than for the vegetative phase. In addition, these cultivars are better adapted to express their potential yield in regions with low intensity and occurrence of water stress and the traditional cultivar is enabled to better support adverse conditions of water stress. It can be concluded that there is need to precisely characterize drought patterns in TPEs. This information can focus the breeding program to improve drought tolerance in modern upland rice cultivars.

Srividya *et al.* (2011) evaluated a recombinant inbred population in hydroponic culture and phenotyped for diverse responses of seedlings to water shortage forced by poly ethylene glycol (PEG). The ratio between mean value of seedling trait under stress and control conditions was used for assessing drought tolerance. In all 19 putative QTLs relating to five seedling traits like, root length, shoot length, root dry weight, shoot dry weight and root to shoot dry weight ratio under PEG induced stress conditions confirmed that the traditional tall landraces poses hither to unexploited drought tolerant genes and utilization of them as potential donors in breeding for yield enhancement would be rewarding. Wu *et al.* (2011) analyzed some physiological traits which have been closely related to rice yield under the water stress. Some rice backcross lines with drought resistance were discussed, and the effect of water stress after anthesis on rice yield was studied under the artificial control condition. The result indicated the content of malondi-aldehyde (MDA) of rice flag leaf increased obviously, the activity of superoxide dismatase (SOD) of rice flag leaf activity weakened; the various traits of the rice variety with strong drought resistance reduced slightly compared to control and with high yield, while of the rice variety

with weak drought resistance reduced significantly compared to control and with low yield.

#### **Gene Expression and molecular markers**

Expression of stress responsive genes is regulated by a number of transcription factors. DRE-binding proteins (DREBs) belong to the AP2/ERF transcription factor family and the expression of the genes encoding these proteins is activated under abiotic stress conditions. The role of transcription factors of AP2/ERF family in plant abiotic stress responses was considered with distinct importance on the regulations and purposes of two main categories of DREBs, DREB1 and DREB2 (Mizoi *et al.*, 2012). Two types of transcription factors, OsDREB1s and OsDREB2s have been recognized as responsive to abiotic stress. OsAREBs for instance OsAREB1 have been recognized as vital constituents in ABA reliant transcriptional complexes in rice. OsNAC/SNACs comprising OsNAC6 have been categorized as a factor which control expression of different genes significant against abiotic stresses in rice (Todaka *et al.*, 2012). Baker (2012) performed random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) and inter simple sequence repeats (ISSRs) markers to detect the genetic diversity among 6 new rice lines and 4 cultivars with different responses to drought tolerance and establish specific DNA markers associated with drought tolerance. Among 16 RAPD primers tested, only 5 produced bands polymorphic between lines with an average of 5.2 bands per primer (ranging from approximately 252 to 1232 bp) and 73.02 % were polymorphic. Among the tested ISSR primers, only five amplified polymorphic ISSR loci with an average number of 4.4 bands per primer (ranging from approximately 80 to 813 bp) and the mean percentage of ISSR polymorphism was 90.91. Based on band polymorphisms generated by RAPD-PCR and ISSR after using the primers, the highest similarity value (0.93) was found between P-5-3-b line and P-5-3-a line and the lowest value (0.44) was found between P-5-3-b line and Giza 172. The dendrogram separated all cultivars and new lines into two clusters and indicated that the cross of tolerant line (P-5-3-b) and susceptible cultivar (Giza 172) is suggested as the most suitable cross for drought tolerance analysis studies as they have the lowest similarity value (0.44) and also grouped in distinct cluster. Since two fragments of about (315 and 505 bp) were visualized using HP15 primer in the genomic DNA of the drought tolerant lines while were absent in the sensitive cultivars, they can be considered as positive drought tolerant markers.

Gomez *et al.* (2012) conducted three experiments for screening drought tolerant rice genotypes at seedling stage. Genotype KHS7 showed

the maximum resistance, while TS2 exhibited the minimum. A strong negative correlation was found between percentage of soil water content and leaf drying. In vitro evaluation of three genotypes under polyethylene glycol induced stress revealed TCS17 with a higher tolerance to drought over TNGS14 and CSY112. Thus, a rapid, simple, and feasible technique was established to select water stress tolerant rice genotypes at seedling stage under controlled conditions. Ji *et al.* (2012) studied the drought-responsive mechanisms at the physiological and molecular levels in two rice genotypes with contrasting susceptibility to drought stress stage. After 20 d of drought treatment, the osmotic potential of leaves reduced 78% and 8% in drought susceptible rice cultivar Zhenshan97B and tolerant rice cultivar IRAT109, respectively. The panicle lengths had no obvious changes in drought stressed Zhenshan97B and IRAT109, suggesting that drought stress impose less effect on assimilate translocation from leaf to vegetative growth of panicles showed more extensive deeper root growth that could be considered a second line of defense against drought stress. The Ci/Ca ratio exhibited enhancement over reduction of gin both cultivars, reflecting the non-stomatal limitation to photosynthesis occurred during drought stress. Orthophosphate dikinase, glycine dehydrogenase, ribulose biphosphate carboxylase (Rubisco), glycine at a reproductive IRAT109 hydroxy methyl transferase ATP synthase were down-regulated for Zhenshan97B in response to drought stress, suggesting the reduction of capacity of carbon assimilation in this rice cultivar. In drought-stressed IRAT109, transketolase, Rubisco were down-regulated, however, Rubisco activase and peptidyl-prolylcis-trans isomerase, which might alleviate the damage on Rubisco by drought stress, were up-regulated. The increased of chloroplastic superoxide dismutase [Cu-Zn] and dehydro ascorbate reductase might provide antioxidant protection. Rice-growing areas span the tropics, subtropics, semi-arid tropics and temperate regions of the world. The predominantly rice-growing areas in Asia are often threatened by severe abiotic stresses, of which the most common is drought. Drought stress suppresses leaf expansion, tillering and midday photosynthesis and reduces photosynthetic rate and leaf area due to early senescence. All of these factors are responsible for and of against tonesin damage by and abundances dehydration. Najafabadi (2012) studied a rice NF-YA transcription factor gene was partially characterized following dehydration. Disrupting the gene via a T-DNA insertion resulted in drought tolerant plants and a high rate of recovery after water resupply. It was demonstrated that the improved drought tolerance of the mutant is primarily due to non-stomata

mechanisms such as free radical scavenging, which might be related to changes in metabolism of carbohydrates.

Raman *et al.* (2012) evaluated 129 advanced rice breeding lines under rainfed drought-prone situations at three locations in eastern India ARB 8 and IR55419-04 recorded the highest drought yield index (DYI) and are identified as the best drought tolerant lines. The proposed DYI provides a more effective assessment as it is calculated after accounting for a significant genotype x stress-level interaction across environments. For rainfed areas with variable frequency of drought occurrence, Mean yield index (MYI) along with deviation in performance of genotypes from currently cultivated popular varieties in all situations helps to select genotypes with a superior performance across irrigated, moderate and severe reproductive stage drought situations. IR74371-70-1-1 and DGI 75 are the two genotypes identified to have shown a superior performance over IR64 and MTU1010 under all situations. For highly drought prone areas, a combination of DYI with deviation in performance of genotypes under irrigated situations can enable breeders to select genotypes with no reduction in yield under favorable environments compared with currently cultivated varieties. For rainfed areas with variable frequency of drought stress, use of MYI together with deviation in performance of genotypes under different situations as compared to presently cultivated varieties will help breeders to select genotypes with superior performance under all situations.

Sharoni *et al.* (2012) investigated the gene expression responses in the root, leaf, and panicle of three rice genotypes under two drought stress conditions. They identified common and specific genes in all tissues from two near isogenic lines, IR77298-14-1-2-B-10 (drought tolerant) and IR7729814-1-2-B-13 (drought susceptible), under drought stress conditions. The majority of the genes that were activated in the IR77298-14-1-2-B-10 were members of the AP2/EREBP Gene family. Non-redundant Genes (sixteen) were found in the drought-tolerant line, and four genes were selected as candidate novel reference genes because of their higher expression levels in IR77298-14-1-2-B-10. Most of the genes in the AP2, B3, and B5 subgroups were involved in the panicle under severe stress conditions, but genes from the B1 and B2 Subgroups were down-regulated in the root. Of The four subfamilies, RAV exhibited the highest number of up-regulated genes (80%) In the panicle under severe stress conditions in the drought-tolerant line compared to Minghui 63 under normal conditions, and the gene structures of the RAV subfamily may be involved in the response to drought stress in the

flowering stage. The results provided a useful reference for the cloning of candidate genes from the specific subgroup for further functional analysis.

Yu *et al.* (2012) evaluated 95 diverse rice landraces or varieties within a field screen facility based on the 'line-source soil moisture gradient', and their resistance varied from extremely resistant to sensitive. The method of Ecotype Targeting Induced Local Lesions in Genomes (EcoTilling) was used to analyze the diversity in the promoters of 24 transcription factor families. The bands separated by electrophoresis using Eco tilling were converted into molecular markers. STRUCTURE analysis revealed a value of  $K = 2$ , namely, the population with two subgroups (i.e., indica and japonica), which coincided very well with the UPGMA clusters (NTSYS-pc software) using distance-based analysis and In Del markers. Then the association analysis between the promoter diversity of these transcription factors and the DT index/level of each variety was performed. The results showed that three genes were associated with the DT index and that five genes were associated with the DT level. The sequences of these associated genes are complex and variable, especially at approximately 1000bp upstream of the transcription initiation sites. The study illuminated that association analysis aimed at Eco tilling diversity of natural groups could facilitate the isolation of rice genes related to complex quantitative traits.

Yun *et al.* (2012) identified quantitative trait loci (QTL) for drought tolerance (DT) at reproductive stage were identified using 77 BC2F2 advanced backcross introgression lines (ILs) derived from a cross of Teqing  $\times$  Binam in Teqing background. 23 QTL were identified under reproductive stage drought and irrigated conditions for panicle number per plant (PN), thousand grain weight (TGW), seed fertility (SF), plant height (PH), and grain yield per plant (GYP). Most QTL detected at reproductive stage showed obvious differential expression to drought stress. In addition, another eight QTLs. Jin *et al.* (2013) reported that OsAP21 is a transcriptional factor gene that belongs to the AP2/ERF family CBF/DREB subfamily of rice. They expressed OsAP21 in *Arabidopsis thaliana* plants and found that the transgenic *Arabidopsis* plants with OsAP21 exhibit better growth than the wild type plants under drought and salinity stress conditions. Analysis of RT-PCR for RD29B gene showed that the overexpressed plants with OsAP21 had high expression level of RD29B gene than the wild type plants and these differences could be increased by the application of salt and drought stress treatments. Results showed that the OsAP21 gene may play a vital role in improving the response of transgenic *Arabidopsis* plants against drought and salt stress.

Yooyongwech *et al.* (2013) subjected rice varieties including Thai jasmine rice, M401 mutant line derived from KDML105, and Pathumthani 1 to water withholding for 14 days (water deficit stress) and then represented by 5.28% soil water content (SWC) and well watering (control; 31.9% SWC) in the pot culture until booting stage (inflorescence formation). Water use efficiency (WUE), net photosynthetic rate (P), transpiration rate (E), stomatal conductance (g) and expression of *PIP1:2* and *PIP2:1* were examined in flag leaf tissues of rice grown under control and water deficit stress. WUE in MT401 mutant plant subjected to water deficit stress was increased, whereas Pin both MT401 and KDML105 was maintained. Transcriptional levels of *OsPIP1:2* and *OsPIP2:1* in the MT401 grown under water deficit stress was up-regulated by 2.0-2.5 folds higher than those in KDML105 and PT1 genotype. The expression of *OsPIP2:1* in MT401 mutant plant was maintained when plants were exposed to water deficit condition, resulting in stabilization of WUE at the cellular levels. In addition, panicle length and number of spikelet per panicle in MT401 mutant were retained well under water deficit, suggesting MT401 as water deficit tolerant type.

Zhuang *et al.* (2013) isolated the rice OsAP23 gene, which encodes an AP2/ERF transcription factor from rice using a yeast one hybrid system. The OsAP23 transcription factor belongs to the B3 group of the ERF subfamily. Germination results for transgenic *Arabidopsis* plants hosting the OsAP23 gene revealed greater inhibition at the germination and seedling stages with increasing concentration of NaCl compared to the wild-type plants. Exogenous ABA also reduced the rate of germination of transgenic *Arabidopsis* seeds that overexpress OsAP23 compared with wild-type *Arabidopsis* seeds. In addition, following exposure to high salt concentrations, several stress-responsive regulatory genes were induced to a significantly greater extent in the wild-type lines compared with lines that overexpress OsAP23. The results suggested that OsAP23 might be a negative regulator of the salt stress response in higher plants. Rachmat *et al.* (2014) introduced pCambia 1305 harboring *OsNAC6* chimaeric gene with CaMV 35S promoter into rice zygotic embryo using *Agrobacterium tumefaciens* mediated transformation to regenerate transgenic rice overexpressing the transgene. As many as 39 putative transgenic lines, in which 21 lines positively harbored *hpt* gene, have been regenerated. The positive identification of *hpt* in the regenerated transgenic rice indirectly indicated integration of the targeted *OsNAC6* since both transgenes were part of the same T-DNA. Further analysis indicated the presence of 1-3 copies of transgene integration in the

genome. The expression of *OsNAC6* transgene in the transgenic rice line C.73, C.83 and C.91 were higher than wild type non-transgenic one. Further analysis indicated those three transgenic lines carrying *OsNAC6* transgene exhibited higher tolerance against drought and salinity stresses. Moreover, three known stress-associated regulatory genes (AP2, Zinc finger protein and MYB) were up-regulated in those three transgenic lines. The findings demonstrated that *OsNAC6* might be a candidate of stress-responsive NAC regulatory gene that can be used to develop either drought or salt tolerant transgenic plants.

### Conclusions

It was concluded from all above discussion that the improvement in the drought tolerance in crop plants can be made through the use of modern molecular techniques that covers improved breeding programs and transgenic methods. Molecular techniques make it possible to accelerate the desirable gene transfer among varieties and use of wild relatives for novel genes introduction. Use of molecular markers is helpful in analysis of polygenetic characters which were once difficult to study by using traditional methods in plant breeding. By using molecular techniques and the knowledge of bioinformatics it is possible to make genetic relationship between sexually incompatible species too.

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