

**Genetic Association of transcriptional factors (OsAP2 gene family) to incorporate drought tolerance in rice (*Oryza sativa* L. ssp. indica): An overview**

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**Abstract:** Abiotic stresses like drought, salinity and high temperature are the major problems, which reduce rice crop yield world-wide. Among them drought is most yield limiting factors, which significantly affect its growth, development and lower its yield. Developing drought tolerant rice genotypes is dire need of the time, pre-requisite of which is availability of genetic variation and diversity. The present review describes the methodologies to devise easy, quick and cost effective screening protocols for assessment of genetic variation against drought stress to know stress responsive traits while keeping in view the erratic nature of this crop against abiotic stress drought. The role of various transcriptional factors will also be discussed to understand the mechanism of control against drought.

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### Introduction

Rice (*Oryza sativa* L.) is the most significant crop of world and about more than 50% people of the sphere, mostly in Asia, directly depends upon it for food. Rice is one of the leading cereals in many countries of the world especially those living in developing countries such as Indonesia, China, Vietnam, Bangladesh, Pakistan, Laos and India (Hadiarto and Tran, 2011). The genus *Oryza* includes nearly 22 species with 10 acknowledged genome types out of which six are diploid genome sets (2n=24, AA, BB, CC, EE, FF, and GG) and four are tetraploid (2n=4x=48, BBCC, CCDD, HHJJ, and HHKK). There are two cultured *Oryza* species, mentioning to *O. sativa* and *O. glaberrima*. Asian cultured rice (*Oryza sativa*) is one of the most significant food crops in the sphere, and assists as a chief food basis for more than 50% of the world population (Wang *et al.*, 2014). Rice is grown on all continents except Antarctica, spreading from 50° north latitude to 40° south latitude and from sea level to an altitude of 3000 m. The rice plant also adjusts well to different temperatures. It can grow in temperatures as low as 63°F and it does fine in temperatures up to 97°F (Setter *et al.*, 1995). Rice contribution in value added is 2.7% and in GDP is 0.6%. In 2012-13, rice cultivated area has decreased 10.1% from 2571 million hectares to 2311 million hectares. This is equivalent to 10 % decrease in area from the last year. The production remained 5.54 million tons during the year 2012-13 while 6.16 million tons was the production of last year. Yield has increased from 2396 to 2398 kg/hectare, which is not a great improvement. So for the progress of

country it is essential that rice must be produced in excess because it is our staple need (Govt. of Pakistan, 2012-13).

Requirement of rice is increasing with increasing the population pressure. Thus well designed and dynamic rice breeding programs are desired to build up high yielding genotypes to convene the mounting demand of population. It requires hastening the enhancement of this crop by developing lines pliable to various environmental condition of the country having high yield potential. Therefore instead of maintaining several crosses rice breeder should focus only the minority crosses resulting from parents with high yield potential. For any yield increasing programs selection of better parents for hybridization is tremendously crucial. About 8 million hectares of upland and 34 million hectares of rainfed lowland rice experiences repeated drought stress in Asia (Venuprasad *et al.*, 2009; Ali *et al.*, 2014; Dar *et al.*, 2014; Khan *et al.*, 2014; Abbas *et al.*, 2014; Mustafa *et al.*, 2015). Drought is the major limitation to rice production in the rainfed areas, disturbing 19 million hectare of upland and over 14 million hectare of lowland rice (Pandey *et al.*, 2000). Improving yield of rice in these stressful environments is a vital assignment in view of present global food disaster. This makes the condition disturbing and poses a risk to national food security. AP2 family comprises greatest importance in plants and its members play vital role in response to different abiotic stresses (Todaka *et al.*, 2012; Ali *et al.*, 2013; Ali *et al.*, 2014abc). Drought tolerance in rice can be improved by identification and purposeful studies of stress responsive genes which clarify

molecular mechanisms of stress tolerance and helps in crop improvement. Present review of various researches focused on screening of rice germplasm for drought stress tolerance at the seedling stage.

#### **Role of transcriptional factor in drought tolerance**

Lang and Buu (2010) evaluated 290 (Back Crosses) derived from the cross between OM1490 / WAB880-1-38-18-20-P1-HB for drought at flowering (DRF), root dry weight (RDW), and root length (RL). A linkage map was constructed from 12 linkage groups based on the population. Markers associated with drought tolerance were located mostly on chromosomes 2, 3, 4, 8, 9, 10 and 12. QTL mapping was used to determine effects of loci associated with drought tolerance traits. We also mapped QTLs for morphological attributes related to drought tolerance. Chi-square tests ( $\chi^2$ ), single marker analysis (SMA), interval mapping (IM) were combined in the QTL analysis procedure. All approaches used for QTL detection gave similar results. Five QTLs were identified for DT (Drought Tolerance). The proportion of phenotypic variation explained by each QTL ranged from 20.73% to 30.77% for DR, and from 6.23 to 3.39% for morphological characters related to drought at flowering. The study provided detailed information on the relative importance of marker assisted selection of drought tolerance. Bae *et al.* (2011) identified five putative proteins that display a significant sequence identity with CaRmlH1 in the rice genome database and these proteins were named OsRDCPs (*Oryza sativa* RING domain-containing proteins). Among these paralogs, OsRDCP1 was induced by drought stress. OsRDCP1 loss of function knockout mutant and OsRDCP1 overexpressing transgenic rice plants were developed. Phenotypic analysis showed that wild type plants and the homozygous *osrdcp1* G2 mutant line displayed similar phenotypes under normal growth conditions and in response to drought stress. This may be due to complementation by other OsRDCP paralogs. The 35S:OsRDCP1 T2 transgenic rice plants exhibited improved tolerance to severe water deficits. Although the physiological function of OsRDCP1 remains unclear, there are several possible mechanisms for its involvement in a subset of physiological responses to counteract dehydration stress in rice plants.

Cui *et al.* (2011) expressed the rice OsDREB2A gene under the control of a stress inducible promoter (4ABRC) to enlarge the abiotic stress tolerance of rice variety TNG67. T2 and T3 transgenic lines overexpressing OsDREB2A were found to have enhanced survival rates under severe drought stress conditions relative to non-transgenic rice plants. OsDREB2A expression was found to be significantly encouraged by drought and ABA treatment. Results

showed that the prompted overexpression of OsDREB2A driven by the 4ABRC promoter in transgenic rice plants could defend cells during trauma. Gao *et al.* (2011) detected that the expression of OsSDIR1 was up regulated by water stress in rice. OsSDIR1 could supplement the water stress sensitive phenotype of the *sdirl1* mutant, showing that OsSDIR1 gene is a functional ortholog of SDIR1. Transgenic rice with OsSDIR1 indicated solid water stress tolerance compared to control plants when treated with drought stress. Transgenic plants had more closed stomata pores than those of control plants. It was also confirmed by the leaf related water content (RWC) measurements and water loss assay during water stress treatment.

Shen *et al.* (2012) showed that OsWRKY30 interacted with OsMPK3, OsMPK4, OsMPK7, OsMPK14, OsMPK20-4, and OsMPK20-5, and could be phosphorylated by OsMPK3, OsMPK7, and OsMPK14. Overexpression of OsWRKY30 in rice dramatically increased drought tolerance. Overexpression of OsWRKY30AA, in which all SP (serine residue followed by proline residue) sites were replaced by AP (A, alanine), resulted in no improvement in drought tolerance. In addition, the function of transcriptional activation of OsWRKY30 was impaired after SP was replaced by AP. These results proved that the phosphorylation of OsWRKY30 by MAPKs was crucial in order for OsWRKY30 to perform its biological function. Singh *et al.* (2012) conducted an experiment to analyze the effect of potassium iodide (KI) for its suitability as a selection criterion to screen upland rice genotypes. Three different KI concentrations *vis.* 0.25%, 0.50% and 0.75% were applied on eight rice genotypes comprising four tolerant and four susceptible ones. Injuries in young tissues, reduction in yield, panicle length, panicle weight, grain number and grain weight were observed at the KI concentrations of 0.50 and 0.75%. However, 0.25% KI concentration did not show significant injury effects on rice genotypes. The significant correlation of grain yield with apparent translocation rate ( $R = 0.54$ ) was also observed, indicating reduction in current assimilation during reproductive stage, under different KI treatments and tolerant rice genotypes induced an increase in stem reserve mobilization. SDS-PAGE profiling of KI treated rice leaves induced novel protein bands of 30 kDa, 50 kDa and 70 kDa in tolerant rice genotypes. The present study concluded that KI can be used as chemical desiccant for the screening of drought tolerant upland rice varieties at reproductive stage.

Tang *et al.* (2012) found that expression of OsZIP46 was strongly induced by drought, heat, hydrogen peroxide, and abscisic acid (ABA)

treatment; however, it was not induced by salt and cold stresses. Overexpression of the native OsbZIP46 gene increased ABA sensitivity but had no positive effect on drought resistance. The activation domain of OsbZIP46 was defined by a series of deletions, and a region (domain D) was identified as having a negative effect on the activation. We produced a constitutive active form of OsbZIP46 (OsbZIP46CA1) with a deletion of domain D. Overexpression of OsbZIP46CA1 in rice significantly increased tolerance to drought and osmotic stresses. Gene chip analysis of the two over-expressor (native OsbZIP46 and the constitutive active form OsbZIP46CA1) revealed that a large number of stress-related genes, many of them predicted to be downstream genes of ABF/AREBs, were activated in the OsbZIP46CA1 over-expressor but not (even down-regulated) in the OsbZIP46 over-expressor. OsbZIP46 can interact with homologs of SnRK2 protein kinases that phosphorylate ABFs in Arabidopsis. These results suggest that OsbZIP46 is a positive regulator of ABA signaling and drought stress tolerance of rice depending on its activation. The stress-related genes activated by OsbZIP46CA1 are largely different from those activated by the other rice ABF/AREB homologs (such as OsbZIP23), further implying the value of OsbZIP46CA1 in genetic engineering of drought tolerance.

Yaqoob *et al.* (2012) conducted an experiment to evaluate 10 rice genotypes including 8 exotic lines and two well adopted cultivars under drought stress conditions for yield and its components. The mean values for yield and yield components (panicle length, plant height, total tillers per plant, days to heading, productive tillers per plant, 1000 grains weight, maturity days, and grain yield) studied indicated that the cultivar KSK-133 and line HHZ-11-Y-11-Y3-DT1 had high tolerance under drought stress conditions. Results suggested that cultivar KSK-133 may be planted under low water application conditions and line HHZ-11-Y-11-Y3-DT1 may be utilized for breeding drought tolerant rice. Ahadiyat *et al.* (2013) evaluated the characters of drought tolerance, P efficiency and yield of some upland rice lines based on shoot biomass, P accumulation and grain yield. Nine lines of upland rice were tested. The study was arranged in randomized complete block design with three replicates. The result showed that upland rice lines of IR 75885-26-2-3-B-18-B-2-1-B, Unsoed G9 and Unsoed G19 resulted higher in grain yield under drought condition than others. Yet, Unram 1E and Unsoed G13 had potency to drought even low in grain yield. Meanwhile, upland rice lines of IR 75885-25-1-3-B-5-1-2-B-B, IR 75885-26-2-3-B-18-B-21-B and Unsoed G19 resulted higher in grain

yield under low dose of P than others. Unsoed G9 had both characters in efficient and respond to P, and drought tolerance with high in grain yield. Therefore, it needs to evaluate the performance consistency of both characters on those lines through grown under real conditions in the field of rainfed areas.

Akram *et al.* (2013) determined the effect of drought stress on some physiological and agronomic parameters of three rice cultivars naming Basmati-Super, Shaheen-Basmati and Basmati-385. The field experiment was laid out in split plot design with randomized complete block arrangement, keeping cultivars in main and water stress stages viz; panicle initiation, anthesis and grain filling along with control in sub-plots. Cultivar Shaheen-Basmati exhibited comparatively more tolerance to drought stress at all the three growth stages under study with less reduction in various physiological and agronomic traits. Similarly, less increase in transpiration rate and sterile tillers per hill were observed in Shaheen-Basmati under drought stress at all the three stages. Growth stage panicle initiation was the most sensitive one exhibiting more adverse effects on all the physiological and agronomic parameters under study, PAR, photosynthetic rate, RWC and stomata conductance showed strong and positive correlation with WUE whereas; transpiration rate expressed negative correlation with WUE. All the physiological and yield components under study except transpiration rate and number of sterile grains per panicle had a strong and positive correlation with paddy yield.

Bunnag and Pongthai (2013) selected seven rice cultivars tolerant to water stress at vegetative stage under field conditions. Cultivars were subjected to water stress for 0, 20 and 60 days and the traits recorded were tillers per plant, leaf death, plant height, relative leaf water content, leaf water potential, leaf rolling and proline content. Three groups of rice were made on the basis of drought tolerance level, as drought sensitive, moderately drought tolerant and highly drought tolerant cultivars. Among the seven cultivars, CT9993 was considered as highly drought tolerant and IR58821, IR52561, IR57514, KDML 105 and BT as moderately drought tolerant and IR62266 was considered as sensitive to drought. Degenkolbe *et al.* (2013) sampled leaf blades from the population of cultivars grown under control or long term drought condition and subjected to expression analysis by RT-PCR and metabolite profiling. Under drought stress, metabolite levels correlated mainly negatively with performance parameters, but eight metabolites correlated positively. For 28 genes, a significant correlation between expression level and performance under drought was confirmed. Negative correlations were

predominant. Among those with significant positive correlation was the gene coding for a cytosolic fructose-1,6-bisphosphatase. This enzyme catalyzes a highly regulated step in C-metabolism. The metabolic and transcript marker candidates for drought tolerance were identified in a highly diverse population of cultivars. Thus, these markers may be used to select for tolerance in a wide range of rice germplasm.

Guo *et al.* (2013) explored that *OsDIL* is responsive to different abiotic stresses, like drought, NaCl, cold, and abscisic acid (ABA). The transgenic rice plants with over-expressing *OsDIL* gene were found more tolerant to drought stress compared with wild type during vegetative development stage. The drought responsive genes bZIP46, RD22, SODA1, POD and the ABA synthetic gene ZEP1 were up regulated in the *OsDIL* over-expression lines. Over-expression of *OsDIL* reduced the down regulation of anther developing genes (*OsC4*, *CYP704B2* and *OsCP1*) by drought, providing a tool supporting pollen fertility under drought. Results showed that *OsDIL* is an excellent candidate gene for genetically improving the crop yield in adaptation to hostile situations. Kumari and Pandey (2013) selected eight transcription factors/regulator genes and studied their differential expression pattern in some susceptible and tolerant rice cultivars by imposing different abiotic stresses (drought and submergence). For this experiment growing of plant and stress treatment, RNA isolation and cDNA preparation, expression analysis by RT-PCR and Real Time PCR followed by data analysis was performed. Our result revealed that these TF/regulators are differentially expressed under abiotic stresses as well as plant tissues. Expression of *Arabidopsis* RESPONSEREGULATORS Type-B (*ARR-B*), Vascular plant One Zinc finger protein (*VOZ*) and *GLABROUS1* enhancer-binding protein (*GeBP*) genes families varies during the different stress conditions. This is the first report that indicates the Real-Time PCR based differential expression analysis of abovementioned TFs/regulators. Obtained data might be useful in determining their role in regulating the expression of various genes as well as in co-expression networking analysis. Xiang *et al.* (2013) showed that expression of *OsHsfB2b* was strongly induced by heat, salt, abscisic acid (ABA) and polyethylene glycol (PEG) treatments but was almost not affected by cold stress. Drought and salt tolerances were significantly decreased in *OsHsfB2b* overexpressing transgenic rice, but were enhanced in the *OsHsfB2b* RNAi transgenic rice. Under drought stress, the *OsHsfB2b* overexpressing transgenic rice exhibited increased relative electrical conductivity (REC) and content of malondi aldehyde (MDA) and decreased proline content compared with the wild

type, while the lower REC and MDA content and increased proline content were found in the *OsHsfB2b*-RNAi transgenic rice. The results suggested that *OsHsfB2b* functions as a negative regulator in response to drought and salt stresses in rice, with its existing B3 repression domain (BRD) that might be necessary for the repressive activity. The experiment revealed the potential value of *OsHsfB2b* in genetic improvement of rice.

Swain *et al.* (2014) evaluated 134 land races that represent diverse geographical regions of India, Indonesia and Philippines, at the vegetative stage under both field and laboratory conditions to identify promising rice genotypes having drought stress tolerance. Thirty day old seedlings were subjected to water stress. Seventy eight genotypes were scored as tolerant. Of the 78 genotypes, 13 had recorded yield over 1.0 t/ha while the tolerant (CR 143-2-2) and susceptible controls (IR 20) recorded 2.70 t/ha and zero yield respectively. Genotypes tolerant to drought stress were also evaluated against poly ethylene glycol-6000 prompted water stress at both germination and seedling stages. Six genotypes i.e. IRGC 45699, IRGC 53989, IRGC 636, IRGC 51231, IRGC 40275 and IRGC 12263 were recognized as drought tolerant with good yield potential (0.7-1.95 t/ha) under drought stress conditions. Zain *et al.* (2014) conducted an experiment to assess the effects of different periodical water stress combined with potassium fertilization regimes on growth, yield, leaf gas exchanges and biochemical changes in rice grown in pots and compare them with standard local rice grower practices. Five treatments including (1) standard local grower's practice (control, 80CF = 80 kg KO/ha + control flooding); (2) 120PW15 = 120 kgK220/ha + periodical water stress for 15 days; (3) 120DS15V = 120 kg KO/ha + drought stress for 15 days during the vegetative stage; (4) 120DS25V = 120 kg KO/ha + drought stress for 25 days and (5) 120DS15R = 120 kg KO/ha + drought stress for 15 days during the reproductive stage, were evaluated in this experiment. Control and 120PW15 treatments were stopped at 100 DAS, and continuously saturated conditions were applied until harvest. It was found that rice under 120PW15 treatment showed tolerance to drought stress evidenced by increased water use efficiency, peroxidase (POX), catalase (CAT) and proline levels, maximum efficiency of photosystem II (fv/f) and lower minimal fluorescence (fom), compared to other treatments. Path coefficient analysis revealed that most of parameters contribute directly rather than indirectly to rice yield. In this experiment, there were four factors that are directly involved with rice yield: grain soluble sugar, photosynthesis, water use efficiency and total chlorophyll content. The residual factors affecting



rice yield are observed to be quite low in the experiment (0.350), confirming that rice yield was mostly influenced by the parameters measured during the study.

### Conclusions

It was concluded from all above discussion that various biochemicals like abscisic acid, malondi aldehyde, proline, peroxidase (POX), serine, catalase (CAT) and superoxide dismutase are produced in the rice plant body through the gene regulation effects. A large number of genes like OsWRKY30, OsMPK3, OsMPK4, OsMPK7, OsMPK14, OsMPK20-4, OsMPK20-5, OsMPK3, OsMPK7, OsMPK14, OsRDCPs, OsRDCP1, OsHsfB2b, OsbZIP46 (OsZIP46CA1) and OsDREB2A are involved in the production of various drought responsive biochemicals to show the gene regulating against drought.

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