Current Status of Genomic based Approaches to Enhance Drought Tolerance in Rice (*Oryza sativa* L.), an Over View

Mueen Alam Khan

Department of Plant Breeding & Genetics, College of Agriculture & Environmental Sciences, The Islamia University of Bahawalpur- 63100, Pakistan

Corresponding authors email: mueen_1981@yahoo.com


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Abstract  The availability of ample amount of water is the most limiting factor in rice production especially in drought prone areas. The yield losses due to drought in rice are enormous. Due to quantitative nature of drought tolerance, conventional breeding has met with little success. Recent progress made in the field of genomics enabling us to access genes linked with drought tolerance has enhanced our understanding of this complex phenomenon. Identification of drought related QTLs, and their use in marker assisted breeding, transgenic rice production and evolution of genomic tools provide rice breeders a hope to build high yielding drought tolerant rice cultivars. The objective of this review is therefore to consolidate the current knowledge of molecular breeding and functional genomics which may be influential in integrating breeding and genetic engineering approaches for drought resistance in rice.

Keywords  Drought tolerance; *Oryza sativa* L.; QTLs; Transgenics

1 Importance of rice and severity of drought

Rice (*Oryza sativa* L.) is the second most important cereal crop after wheat of the world utilized exclusively as human staple food. More then 2 billion people worldwide depend on rice for their survival (Datta, 2004). The production of rice must increase in coming years in order to keep pace with increasing population. Rice is a warm season crop, and its cultivation is concentrated in tropical and subtropical climates. Drought is one of the serious abiotic threats to the agriculture. This is due to uneven distribution of rain fall and continued water shortages (Luo and Zhang, 2001). Rice which requires enormous amount of water is extremely sensitive to drought (Lafitte et al., 2004). Very often the rained areas are subjected to drought that can prevail at any growth stage thus greatly reducing the crop yield (Babu et al., 2004). The yield losses due to drought stress at various growth stages of rice are summarized in table 1.

It can be inferred from the table that drought condition can affect the plant at any growth stage; however drought stress during reproductive stage directly results in a loss of grain yields. Keeping in view the increasing threats of water shortages and yield losses due to drought, it is thus imperative for the rice breeders to develop drought tolerant high yielding rice cultivars.

Table 1 Yield reduction in rice due to drought

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Yield reduction (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive</td>
<td>24–84</td>
<td>Venuprasad et al 2007</td>
</tr>
<tr>
<td>Grain filling</td>
<td>30–55</td>
<td>Basnayake et al., 2006</td>
</tr>
<tr>
<td>Grain filling (severe stress)</td>
<td>Up to 60</td>
<td>Basnayake et al., 2006</td>
</tr>
<tr>
<td>Reproductive (severe stress)</td>
<td>48–94</td>
<td>Lafitte et al., 2007</td>
</tr>
</tbody>
</table>
2 The complex nature of drought tolerance and conventional breeding

The drought tolerance is the ability of plant to survive and reproduce in a limited water supply (Turner, 1979; Ashley, 1993). Conventional breeding for drought tolerance in rice has met with little success (Fukai and Cooper, 1995). This is due to the polygenic nature of the phenomenon with low heritability and high G × E interaction (McWilliam, 1989; Ingrams and Bartels, 1996; Zhang, 2004). Furthermore drought stress is accompanied by other biotic stresses like high temperature, and nutrient deficiencies which further complicate the breeding efforts (Fleury et al., 2010). To overcome this, breeder has to screen and select a very large number of genotypes to get the desired ones. This is itself a time consuming and labor intensive operation involving difficulties in field operation and unexpected rainfall.

Several morphological traits affect drought tolerance in rice. Root characteristics such as thickness, depth of rooting, root penetration ability etc., have been associated with drought resistance in rice (Nguyen et al., 1997; Price and Courtois, 1999). Osmotic adjustment is also a reliable parameter making the plant to sustain itself in limited water condition by decreasing osmotic potential and maintaining turgor (Blum, 2005). However, phenotypic selection for these traits is expensive and labor intensive (Boopathi et al., 2002). Furthermore, directly selecting rice cultivars for grain yield under stressed conditions was considered a relatively inefficient method (Fukai and Cooper, 1995). Until recently, there are numerous reports that direct selection for grain yield under stress is effective without reducing the yield potential of rice (as reviewed by Farooq et al., 2009).

3 Identification of drought related QTLs in rice

Traits showing continuous variation are called quantitative traits and genes which control these traits are referred to as quantitative trait loci (QTLs). QTL mapping in doubled haploid (DH) and recombinant inbred lines (RIL) population is considered to be an efficient methodology to identify genes of agronomic characters (Doebley et al., 2006). The recent progress made in the field of genomics offers new opportunities to dissect the QTLs for drought tolerance. In rice numerous QTLs associated with drought tolerance have been identified and mapped. The cross between upland rice (CT9993) and lowland (IR62266) cultivars has been the center of focus for a number of breeders as it revealed QTLs for morphological and physiological traits. Babu et al (2003) used double haploids (DH) derived from rice lines and subjected them to water stress. The experiment resulted identification of 47 drought related QTLs and phenotypic variation ranged from 5 to 59%. They also identified major QTLs on chromosome 4 with pleiotropic effects on yield under drought stress. Various QTLs for drought tolerance in rice are summarized in table 2.

Courtois et al (2000) also identified 42 QTLs for drought related traits in rice. A QTL for root length and thickness has been mapped on chromosome 9 showing stable expression across different environments (Steel et al., 2006). Obara et al (2010) mapped a major QTL qRL6.1 for root length, on chromosome 6 in rice seedlings grown under hydroponic conditions.

Main problem encountered in these studies were that the QTLs were having a minor affect on the phenotype posing a great challenge for the breeders to discover major QTLs functioning independently to their genetic background (Gowda et al., 2011). Secondly, most of these QTL mapping studies in rice have been conducted using progenies derived from intra specific crosses. Much needed efforts are still required to go for inter specific crosses to explore novel alleles and with their effective incorporation in to the breeding programs for drought tolerance in rice.

4 Marker assisted selection (MAS) for drought tolerance in rice

Selection for drought tolerant rice cultivars is very challenging and tedious operation. Traditionally this selection is based on morphological features that become increasingly difficult for polygenic characters. The selection for polygenic traits can be hastened by using linked DNA markers (William et al., 2007). These markers are very stable and powerful considering the fact that they are unaffected by the external environmental conditions and therefore can be effectively utilized to tag QTLs related...
Table 2 Summary of QTLs associated with drought tolerance in rice

<table>
<thead>
<tr>
<th>Cross</th>
<th>Traits</th>
<th>QTL mapping population</th>
<th>Number of QTLs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO39×Moroberekan</td>
<td>Osmotic adjustment and dehydration</td>
<td>RIL*</td>
<td>1</td>
<td>Lilly et al., 1996</td>
</tr>
<tr>
<td>IR20×63-83</td>
<td>Leaf size and abscisic acid (ABA) accumulation</td>
<td>F2</td>
<td>17</td>
<td>Quarrie et al., 1997</td>
</tr>
<tr>
<td>CT9993×IR62266</td>
<td>Cellular membrane stability (CMS) under drought</td>
<td>DHLs*</td>
<td>9</td>
<td>Tripathy et al., 2000</td>
</tr>
<tr>
<td>CT9993×IR62266</td>
<td>Osmotic adjustment under drought</td>
<td>DHLs</td>
<td>5</td>
<td>Zhang et al., 2001</td>
</tr>
<tr>
<td>Bala×Azucena</td>
<td>Dehydration avoidance</td>
<td>RILs</td>
<td>17</td>
<td>Price et al., 2002</td>
</tr>
<tr>
<td>Bala×Azucena</td>
<td>Yield and its components under drought</td>
<td>RILs</td>
<td>31</td>
<td>Lafitte et al., 2004</td>
</tr>
<tr>
<td>CT9993×IR62266</td>
<td>Yield, yield components under drought</td>
<td>RILs</td>
<td>Many</td>
<td>Jonaliza et al., 2004</td>
</tr>
<tr>
<td>Indica×Upland</td>
<td>Productivity, water status, roots</td>
<td>RILs</td>
<td>39</td>
<td>Yue et al., 2005</td>
</tr>
<tr>
<td>Indica×Japonica</td>
<td>Dehydration avoidance and dehydration tolerance traits</td>
<td>RILs</td>
<td>Many</td>
<td>Yue et al., 2006</td>
</tr>
<tr>
<td>Bala×Azucena</td>
<td>Morphological and physiological traits</td>
<td>RILs</td>
<td>24</td>
<td>Gomez et al., 2007</td>
</tr>
<tr>
<td>IRAT 109×Yuefu</td>
<td>Different root traits</td>
<td>RILs</td>
<td>Many</td>
<td>Qu et al., 2008</td>
</tr>
<tr>
<td>CT9993×IR62266</td>
<td>Physio-morphological traits</td>
<td>RILs</td>
<td>Many</td>
<td>Subashri et al., 2009</td>
</tr>
<tr>
<td>IR 20×Nootripathu</td>
<td>Physio-morphological and yield traits</td>
<td>NILs</td>
<td>22</td>
<td>Gomez et al., 2010</td>
</tr>
<tr>
<td>IR64×Kinandang Patong (KP)</td>
<td>Deep rooting</td>
<td>RILs</td>
<td>1</td>
<td>Uga et al., 2011</td>
</tr>
<tr>
<td>Low land rice cv. Shennong26×Upland rice cv. Haogelao</td>
<td>Photosynthesis parameters</td>
<td>Backcross (ILs)</td>
<td>1~3</td>
<td>Gu et al., 2012</td>
</tr>
</tbody>
</table>

Note: (*) DHL: doubled haploid lines; RIL: Recombinant inbred lines; NILs: Near Isogenic lines; ILs: Introgression lines; a: Similar studies reported in the text have not been included in this table
to drought tolerance. Once these molecular markers are identified to be tagged with QTLs, selection at early segregating generation can be carried out. Close linkage of marker with the QTL and efficient means of screening are the essential requirements for effective MAS (Mohan et al., 1997).

Courtois et al (2003) used MAS to transfer a number of QTLs related to deep rooted character from the japonica upland cultivar “Azucena” to the lowland indica variety “IR64”. MAS selected lines showed a greater root mass and higher yield in drought stress. Similarly, Steele et al (2006) used marker assisted breeding program to improve some root traits related to drought tolerance in an Indian rice cultivar Kalinga III. Cultivar Azucena was used as a donor parent. About 22 near-isogenic lines (NILs) were evaluated and their performance in water limited conditions was markedly improved due to a target segment on chromosome 9 of Azucena. Bernier et al (2007) also screened 436 F3 lines by adopting the same methodology of MAS. They identified a QTL on chromosome 12 having a marked effect on the yield under drought stress conditions.

The advent of molecular markers has revolutionized the screening of complex traits like drought tolerance in crop plants. Molecular markers such as restriction fragment length polymorphism (RFLP) are very reliable and have been extensively used in rice (Mohan et al., 1997). The very first RFLP map for rice was constructed by McCouch et al (1988). Microsatellite markers, also called simple sequence repeats (SSRs), have been widely applied for rice genome mapping for abiotic stress tolerance (Temnykh et al., 2000). Recent developments in DNA marker technology coupled with MAS provide efficient means to plant breeders to carry out selection of rice cultivars under drought prone environments. The only prerequisite requirement for effective MAS program is the stable and continued expression of QTLs under different environments.

5 Transgenic approaches for generating drought tolerant rice

A transgenic approach is one which involves structural modification in traits by transferring desired genes from one species to the other (Ashraf, 2010). This relives the breeder from limitation of using same species for gene transfer. Transgenic approaches are being widely used throughout the world for abiotic and biotic stress tolerance in various crops (Ashraf, 2010). Some of the recent transgenic lines produced in rice for drought stress tolerance are listed in table 3. The important objective of genetic engineers is to incorporate those genes that encode several transcription factors, heat shock and late embryogenesis abundant proteins (LEA), and compatible organic osmolytes.

Transcription factors are basically proteins that are involved in gene regulation. These transcription factors play very important role in almost every stress response. Dehydration-responsive element-binding factors (DREB) are especially important as these regulate genes involved in drought, salinity and freezing (Khan, 2011; Gosal et al., 2009). Much of the work has been done in Arabidopsis with reference to DREB transcription factors. According to Kasuga et al (1999) in transgenic Arabidopsis plants, the over expression of CBF3/DREB 1A when accompanied by constitutive promoter CaMV 35S greatly improved plant’s tolerance to drought, salinity and freezing stresses. Similar DREB genes and promoters have been identified in rice (Dubouzet et al., 2003). Oh et al (2005) successfully engineered the rice with transcription factor CBF3/DREB 1A from Arabidopsis. The stress-responsive NAC (SNAC1) is another class of transcription factors, originally identified as an overexpressed gene induced by drought stress in microarray analysis (Leung, 2008). Over-expression of SNAC1 in transgenic rice showed improved drought tolerance under field conditions (Hu et al., 2006).

Heat shock and late embryogenesis abundant proteins (LEA) are among the class of those proteins that accumulate when drought conditions ensue. Thus, protecting the plant from adverse effects of drought was urgent (Wang et al., 2004; Gosal et al., 2009; Hussain et al., 2011). Over expression of OsWRKY11 allele under the control of the heat shock protein 101 (HSP101) promoter enhanced heat and drought tolerance in rice (Wu et al., 2009). Working on the same path, Tao et al (2011) identified two alleles OsWRKY45-1 and OsWRKY45-2 showing differential
<table>
<thead>
<tr>
<th>Transgene</th>
<th>Source organism</th>
<th>Transformation method</th>
<th>Trait improved</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVA1</td>
<td>Barley (Hordeum vulgare L.)</td>
<td>Particle gun</td>
<td>Transgenic plants showed improved tolerance to drought conditions</td>
<td>Xu et al., 1996; Babu et al., 2004</td>
</tr>
<tr>
<td>CBF3/ DREB 1A</td>
<td>A. thaliana</td>
<td>Agrobacterium</td>
<td>Drought and salinity tolerance</td>
<td>Oh et al., 2005</td>
</tr>
<tr>
<td>SNAC 1</td>
<td>Oryza sativa L.</td>
<td>Agrobacterium</td>
<td>Transgenic plants showed improved tolerance to drought conditions</td>
<td>Hu et al., 2006</td>
</tr>
<tr>
<td>HvCBF4</td>
<td>Hordeum vulgare L.</td>
<td>Agrobacterium</td>
<td>Improved drought and salinity tolerance</td>
<td>Oh et al., 2007</td>
</tr>
<tr>
<td>Os LEA-3–1</td>
<td>Oryza sativa L.</td>
<td>Agrobacterium</td>
<td>Transgenic plants showed increased growth under drought conditions</td>
<td>Xiao et al., 2007</td>
</tr>
<tr>
<td>Transcription factor (AP37) encoding Oryza sativa cytochrome c gene (OsCc1)</td>
<td>Oryza sativa L.</td>
<td>Agrobacterium</td>
<td>Transgenic plants showed increased growth under drought conditions</td>
<td>Oh et al., 2009</td>
</tr>
<tr>
<td>Triticum aestivum salt tolerance-related gene (TaSTRG)</td>
<td>Triticum aestivum L.</td>
<td>Agrobacterium</td>
<td>Transgenic rice plants over expressing TaSTRG gene showed higher salt and drought tolerance</td>
<td>Zhou et al., 2009</td>
</tr>
<tr>
<td>Tomato ethylene response factor (ERF) protein TSRF1</td>
<td>Tomato (Lycopersicon esculentum L.)</td>
<td>Agrobacterium</td>
<td>TSRF1 improved the osmotic and drought tolerance of rice seedlings without growth retardation</td>
<td>Quan et al., 2010</td>
</tr>
<tr>
<td>Tomato ethylene response factor (ERF) protein JERF1</td>
<td>Tomato (Lycopersicon esculentum L.)</td>
<td>Agrobacterium</td>
<td>Over expression of JERF1 significantly enhanced drought tolerance of transgenic rice</td>
<td>Zhang et al., 2010</td>
</tr>
<tr>
<td>Tomato ethylene response factor (ERF) protein JERF3</td>
<td>Tomato (Lycopersicon esculentum L.)</td>
<td>Agrobacterium</td>
<td>Over expression of JERF3 significantly enhanced drought tolerance of transgenic rice</td>
<td>Zhang et al., 2010</td>
</tr>
<tr>
<td>Rice OsDREB2A gene with stress-inducible promoter (4ABRC)</td>
<td>Rice (Oryza sativa L.)</td>
<td>Agrobacterium</td>
<td>Over expression of OsDREB2A significantly enhanced drought and salt tolerance of transgenic rice</td>
<td>Cui et al., 2011</td>
</tr>
<tr>
<td>Rice OsDREB2A gene with stress-inducible promoter rd29A</td>
<td>Rice (Oryza sativa L.)</td>
<td>Agrobacterium</td>
<td>Over expression of OsDREB2A significantly enhanced drought and salt tolerance of transgenic rice</td>
<td>Mallikarjuna et al., 2011</td>
</tr>
<tr>
<td>Sorghum ShDREB gene with stress induced promoter CaMV3S or rd29A</td>
<td>Sorghum bicolor L</td>
<td>Agrobacterium</td>
<td>Over expression of ShDREB2 significantly enhanced drought tolerance and yield improvement in transgenic rice</td>
<td>Bihani et al., 2011</td>
</tr>
<tr>
<td>Rice OsSDIR1 gene</td>
<td>Rice (Oryza sativa L.)</td>
<td>Agrobacterium</td>
<td>Over expression of OsSDIR1 gene significantly enhanced drought and salt tolerance</td>
<td>Gao et al., 2011</td>
</tr>
</tbody>
</table>
expression in transgenic plants regarding salt stress and similar expression for drought and cold tolerance.

Efforts have been made to engineer LEA genes in rice which resulted in improved tolerance to drought (Xu et al., 1996; Cheng et al., 2002; Xiao et al., 2007). However deciphering the role of LEA proteins still require a lot of work which is to be done.

Among organic osmolytes, trehalose is a non reducing sugar present in naturally drought tolerant plants. There are reports of improved drought stress tolerance due to engineering trehalose genes in rice (Garg et al., 2002; Jang et al., 2003).

Most of these transgenic lines have been tested in laboratory conditions. There full scale evaluation in field would provide important information for the further exploitation of transgenic work in breeding programs. The ultimate aim of transgenic technology is to identify and manipulate the genes in plants to improve their performance in drought without jeopardizing their physiological responses. Nevertheless transgenic approaches offer new opportunities to drought tolerance in rice by engineering genes from different sources (Cattivelli et al., 2008).

6 Approaching drought tolerance through functional genomics

The goal of functional genomics is to understand how the genome functions to make a whole plant and also deciphering the information conserved in genes making up the genome. Second, utilizing that information for crop genetic improvement is good (Jiang et al., In Press). Full genome sequence of rice (International Rice Genome Sequencing Project 2005; Goff et al., 2002; Yu et al., 2002; Sasaki et al., 2002; Feng et al., 2002) provides a basis for functional genomic technologies (microarray, express sequence tags (EST) etc.) by knowing the expression and sequence of thousands of genes. These genes are then screened by using RNA expression profiling to identify potential candidate genes with their putative functions related to drought stress. Using this expression profiling, Kawasaki et al, 2001, identified some putative genes in rice.

Rice genomics has greatly progressed in recent years. Several functional genomic approaches like macro and micro array have been applied in rice (Kawasaki et al., 2001; Rabbani et al., 2003). Gorantla et al (2005) used functional genomics and generated a large number of ESTs from cDNA libraries and identified 589 genes involved in drought stress. These ESTs were also helpful to dissect drought QTLs and candidate genes. The availability of large number of ESTs, microarrays in combination of bioinformatics, will reveal the function of rice candidate genes involved in drought tolerance (Shimamoto and Kyoza, 2002; Langridge et al., 2006).

Proteomics is a large and emerging field pertaining to the study of proteins especially with reference to their structure and function. Fortunately our knowledge of rice proteome is relatively advanced as compared to other crops (Komatsu and Tanaka, 2005). Even then proteomics studies related to drought are at their infancy (Ansuman et al., 2011). Salekdeh et al (2002) identified more then 2000 proteins involved in drought stress. After this they were able to identify 42 proteins in relation to their affect on drought to infer their functions in detail. Another study conducted on rice by Ali and Komatsu (2006) focused on a protein actin depolymerizing factor (ADF). They observed a marked increase in concentration of ADF in drought tolerant plants suggesting the importance of this protein with reference to drought stress. Rabello et al 2008 identified 22 proteins putatively associated with drought tolerance using mass spectroscopy.

Micro RNAs are a newly identified class of small single stranded non-coding RNAs playing their role in post transcriptional gene regulation targeting mRNAs for cleavage or translational repression (Zhao et al., 2007). They used the oligonucleotide microarray in rice to see the expression profile of micro RNA in drought stress and identified two micro RNAs being induced in drought stress.

7 Conclusion and future directions

Drought tolerance improvement is probably one of the challenging tasks of rice breeders. This is due to its complex and unpredictable nature. In past few years, the world has witnessed a substantial progress in the field of genomics making us to understand the
underlying mechanisms of drought tolerance. The future contribution of genomics will depend on our ability to map QTLs and their effective incorporation in to marker assisted breeding programs. With its far reaching implications, transgenic approach will have its role in future as far as engineering drought tolerant genes is concerned. However this approach can be used to its full potential only when the transgenic plants are tested in field conditions. This will allow us to evaluate the impact of introduced genes under stress conditions. Functional genomics also hold a tremendous promise for the future. Yet many efforts are still needed to further develop these approaches for making them readily usable by the rice breeders. On a more realistic note an interdisciplinary and comprehensive breeding strategy is what required for successful exploitation of genomics to drought prone environments.

**Authors’ Contribution**

MAK planned and conducted experiments, analyzed the data and wrote the the manuscript. The author has read and approved the final manuscript.

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