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Application of Molecular Tools in Breeding Cereal Crops for Drought Tolerance

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Abstract
Drought tolerance is a quantitative trait, with complex phenotype and genetic control. It is one of the major yield constraints for cereal crops. Drought tolerance in crop plants is not a simple task rather one of the most difficult challenges currently the breeders face. The conventional plant-breeding approach generally used to develop drought-tolerant varieties. It is based on selection for yield and its components under a given drought environment. Modern breeding approaches like Identification of drought related quantitative trait loci (QTLs) joined with marker-assisted recurrent selection and genomic selection are being deployed for enhancing drought tolerance in cereal crops. Some novel mapping populations such as multiparent advanced generation intercross and nested association mapping populations are also being developed for trait mapping at higher resolution, as well as for enhancing the genetic base of cereal crops. Considerable progress can be made in the field of omics, providing valuable information on the structure and behavior of crop genomes, with better understanding of plant responses to environmental stresses. Transgenic and omics based technologies have been shown to be powerful tools holding a tremendous promise for the future.
Keywords: Transgenic, Omics, QTL, Marker-Assisted Recurrent Selection, Novel mapping

Introduction

Drought tolerance is a quantitative trait, with complex phenotype and genetic control (McWilliam, 1989). It is the ability of the plant to survive in water limited conditions (Turner, 1979). However, inducing drought tolerance in crop plants is not a simple task rather one of the most difficult challenges currently the breeders face. This is due to its polygenic nature with low heritability and high G × E interactions (Fleury *etal.,* 2010). This complex nature and also the lack of proper understanding of the underlying mechanisms of drought tolerance explain the slow progress in improving the yield of crops in drought prone environments (Tuberosa, Salvi, 2006; Cattivelli *etal.,* 2008). Understanding the genetic basis of drought tolerance in crop plants is a prerequisite for developing superior genotypes through conventional breeding.

Breeding for drought tolerance is further complicated by the fact that several types of abiotic stress can challenge crop plants simultaneously. High temperatures, high irradiance, scarcity of water, and nutrient deficiencies are commonly encountered under normal growing conditions but may not be amenable to management through traditional farm practices. Certain soil properties such as composition and structure can also affect the balance of these different stresses. Higher plants have evolved multiple, interconnected strategies that enable them to survive unpredictable environmental fluctuations. However, these strategies are not always well developed in the cereal cultivars grown by farmers. At the molecular scale, pathways and gene networks between abiotic stresses overlap; for example, about 40% of drought or high salinity inducible genes are also induced by cold stress in rice (Shinozaki and Yamaguchi-Shinozaki, 2007). Some biochemical mechanisms may have opposing effects under different stresses; therefore tackling tolerance to one stress may lead to sensitivity to another.

In the last century, conventional plant breeding, especially the cereal breeding has played a very vital role in tackling the food productivity issues on sustainable level (Araus *etal.,* 2008; Ashraf, 2010). The Green Revolution, occurring between the early 1940s and the late 1970s, was actually based on conventional breeding leading to development of high yielding cereal crops thus saving millions of people from starvation (Rajaram, 2005). The overall plant response to drought stress is quite complex involving the interaction of different component traits (primary and secondary) with the external environment. Most of the drought related cereal breeding programs concentrate on selection strategies of those cultivars that yield well under drought stress. This selection can be either empirical focusing on primary trait selection such as yield or physiological based on secondary parameters (Araus *etal.,* 2008).

Recent advances in crop physiology, systematic plant phenotyping and genomics have led to new insights in drought tolerance, thus providing crop breeders with greater knowledge of the gene networks and providing new tools for plant improvement to increase crop yield (Tuberosa and Salvi 2006). While plant physiology improves our understanding of the complex network of drought tolerance- related traits thus improving selection efficiency, molecular biology and genomics approaches identify the candidate genes and quantitative trait loci (QTLs) associated with these traits. While QTLs can be deployed in crop improvement

through molecular breeding, candidate genes are the prime targets for generating transgenics using genetic engineering (Varshney *etal.,* 2011). Identification of the ‘‘most appropriate’’ candidate genes along with selection of ‘‘most suitable promoters’’ and generation of a large number of events are critical for the development of desirable transgenics with enhanced drought tolerance using know-how knowledge (Varshney *etal.,* 2011). However, the expensive regulatory process and negative public perceptions of biosafety limit the application of genetic engineering approach, while there is a wider acceptance of products generated through molecular breeding (Vogel 2009; Farre *etal.,* 2010; Varshney *etal.,* 2011) and Targetted Induced Local Lesions in Genome (TILLING).

Molecular Markers

In recent years, different marker systems such as Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) or microsatellites, Single Nucleotide Polymorphisms (SNPs) and others have been developed and applied to a range crop species including cereals. AFLPs and SSRs are currently the most popular markers in cereals. An increasing amount of sequence information and the determination of the gene function in cereals will lead in the near future to the preferred use of new marker types, such as SNPs. Application of these markers for genetic studies of cereals have been so much diverse. Main uses include: Assessment of genetic variability and characterization of germplasm; Identification and fingerprinting of genotypes; Estimation of genetic distances between population, inbreeds and breeding material; Detection of monogenic and qualitative trait loci (QTL); Marker-assisted selection; Identification of sequences of useful candidate genes, etc.

Application of Molecular Tools in Breeding Cereal Crops

Addressing the complexity of plant response to drought

The physiological dissection of complex traits like drought is a first step to understand the genetic control of tolerance and will ultimately enhance the efficiency of molecular breeding strategies. Developing and integrating a gene-to-phenotype concept in crop improvement requires particular attention to phenotyping and eco-physiological modelling, as well as the identification of stable candidate genomic regions through novel concepts of ‘genetical genomics’. Knowledge of both the plant physiological response and integrative modelling are needed to tackle the confounding effects associated with environment and gene interaction (Tardieu and Tuberosa, 2010). To maximize the impact of using specific traits, breeding strategies requires a detailed knowledge of the environment where the crop is grown, genotype 9 environment interactions and fine tuning the genotypes suited for local environments. A physiological approach has an advantage over empirical breeding for yield per se because it increases the probability of crosses resulting in additive gene action for stress adaptation, provided that the germplasm is characterized more thoroughly than for yield alone (Reynolds and Trethowan 2007).

Identifying Quantitative Trait Loci (QTLs)

QTLs for drought tolerance have been identified for several major and important crop species like rice, maize, wheat, barley, sorghum, pearl millet, soybean and chickpea. The identification of markers or genes associated with root growth and architecture would be particularly useful for breeding programmes to improve root traits by molecular marker-assisted selection. Few papers have described work on the identification of QTLs for root traits in wheat. Ma *et al*. (2005) found a QTL for root growth rate under Al treatment. QTLs of root traits (primary/lateral root length and number, root dry matter) under control conditions and during nitrogen deficiency were identified in wheat (Laperche *etal.,* 2006). Relative root growth was also used by Jefferies e*t al.* (1999) to map QTL for tolerance to toxic levels of soil boron. However, QTLs corresponding to root architecture in dry environments are yet to be discovered in wheat and barley.

Table 1 QTLs of physiological responses to drought stress identified in wheat and barley

Trait	Species	Drought condition	Chromosome location	Reference
Water-soluble carbohydrate	Wheat	Rainfed field	1A, 1D, 2D, 4A, 6B, 7B, 7D	Yang *et al.*, 2007
Carbon isotope ratio, osmotic potential, chlorophyll content, flag leaf rolling index	Durum wheat	Rainfed field	2B, 4A, 5A, 7B	Peleg *et al.*, 2009
Grain carbon isotope discrimination	Barley	Mediterranean rainfed field	2H, 3H, 6H, 7H	Teulat *et al.*, 2002
Relative water content	Barley	Mediterranean rainfed field	6HL	Teulat *et al.*, 2003
Leaf osmotic potential, osmotic potential at full turgor, osmotic adjustment, carbon isotope discrimination	Barley	Water-deficit in growth chamber	6HL	Teulat *et al.*, 2001; Diab *et al.*, 2004
Water-soluble carbohydrate	Barley	Water-deficit in growth chamber	4H	Diab *et al.*, 2004
Chlorophyll and chlorophyll fluorescence parameters	Barley	Post-flowering drought	2H, 4H, 6H, 7H	Guo *et al.*, 2008
Relative water content	Barley	Water-withholding	1H, 2H, 6H	Chen *et al.*, 2010a

Source: Fleury *et al*., 2010

QTL cloning for drought tolerance-related traits

In general, QTLs identified through linkage mapping-based approaches have low resolution and have been located in 10–20 cM intervals. The support interval of the QTL may also span several hundreds of genes and identifying the right candidate gene(s) with causal effect on the trait is like finding a 'needle' in the 'genomic haystack'. Therefore, to identify the causal gene(s), positional cloning of QTLs have been undertaken in several crop species (Salvi and Tuberosa 2005; Tuberosa and Salvi 2006). QTL cloning, in general, involves the following steps: Delimiting the QTL region by using a large mapping population (1,500 plants) derived from a cross between two NILs for the target QTL,

- Identifying the contig covering the QTL region by screening the closely linked molecular markers with a large insert library like BAC (bacterial artificial chromosome) library,
- Sequencing the contig and candidate gene identification based on sequence data and
- Validating the effect of candidate gene(s) on phenotype/

Sequence Contigs

A sequence contig is a contiguous, overlapping sequence read resulting from the reassembly of the small DNA fragments generated by bottom-up sequencing strategies. The bottom-up DNA sequencing strategy involves:

- Shearing genomic DNA into many small fragments ("bottom"),
- Sequencing these fragments,
- Reassembling them back into contigs and eventually the entire genome ("up").

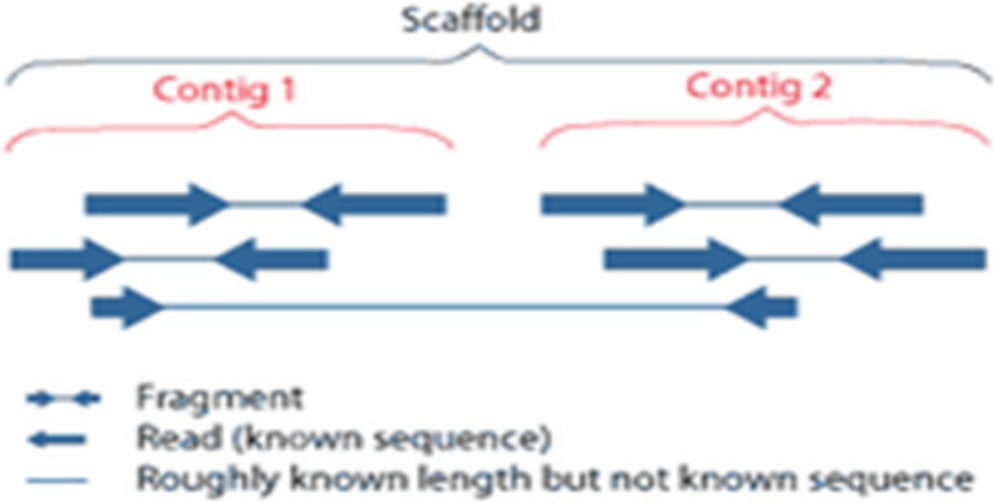

Figure 1: Overlapping reads from paired-end sequencing form contigs; contigs and gaps of known length form scaffolds.

Today, it is common to use paired-end sequencing technology where both ends of consistently sized longer DNA fragments are sequenced. Here, a contig still refers to any contiguous stretch of sequence data

created by read overlap. Because the fragments are of known length, the distance between the two end reads from each fragment is known (Fullwood *etal.,* 2009). This gives additional information about the orientation of contigs constructed from these reads and allows for their assembly into scaffolds. Scaffolds consist of overlapping contigs separated by gaps of known length. The new constraints placed on the orientation of the contigs allows for the placement of highly repeated sequences in the genome. If one end read has a repetitive sequence, as long as its mate pair is located within a contig, its placement is known (Fullwood *etal.,* 2009). The remaining gaps between the contigs in the scaffolds can then be sequenced by a variety of methods, including PCR amplification followed by sequencing (for smaller gaps) and BAC cloning methods followed by sequencing for larger gaps (Gibson *etal.,* 2009).

BAC contigs

Contig can also refer to the overlapping clones that form a physical map of a chromosome when the top-down or hierarchical sequencing strategy is used (Gregory, 2005). In this sequencing method, a low-resolution map is made prior to sequencing in order to provide a framework to guide the later assembly of the sequence reads of the genome. This map identifies the relative positions and overlap of the clones used for sequencing. Sets of overlapping clones that form a contiguous stretch of DNA are called contigs; the minimum number of clones that form a contig that covers the entire chromosome comprise the tiling path that is used for sequencing. Once a tiling path has been selected, its component BACs are sheared into smaller fragments and sequenced. Contigs therefore provide the framework for hierarchical sequencing (Dear, 2005). The assembly of a contig map involves several steps. First, DNA is sheared into larger (50–200kb) pieces, which are cloned into BACs or PACs to form a BAC library. Since these clones should cover the entire genome/chromosome, it is theoretically possible to assemble a contig of BACs that covers the entire chromosome (Gregory , 2005). Reality, however, is not always ideal. Gaps often remain, and a scaffold consisting of contigs and gaps that covers the map region is often the first result (Gregory , 2005). The gaps between contigs can be closed by various methods outlined below.

Construction of BAC contigs

BAC contigs are constructed by aligning BAC regions of known overlap via a variety of methods. One common strategy is to use sequence-tagged site (STS) content mapping to detect unique DNA sites in common between BACs. The degree of overlap is roughly estimated by the number of STS markers in common between two clones, with more markers in common signifying a greater overlap (Gibson *etal.,* 2009). Because this strategy provides only a very rough estimate of overlap, restriction digest fragment analysis, which provides a more precise measurement of clone overlap, is often used (Gibson *etal.,* 2009). In this strategy, clones are treated with one or two restriction enzymes and the resulting fragments separated by gel electrophoresis. If two clones, they will likely have restriction sites in common, and will thus share several fragments (Dear, 2005). Because the number of fragments in common and the length of these fragments is known (the length is judged by comparison to a size standard), the degree of overlap can be deduced to a high degree of precision.

Gaps between contigs

Gaps often remain after initial BAC contig construction. These gaps occur if the Bacterial Artificial Chromosome (BAC) library screened has low complexity, meaning it does not contain a high number of STS or restriction sites, or if certain regions were less stable in cloning hosts and thus underrepresented in the library (Gregory, 2005). If gaps between contigs remain after STS landmark mapping and restriction fingerprinting have been performed, the sequencing of contig ends can be used to close these gaps. This end-sequencing strategy essentially creates a novel STS with which to screen the other contigs. Alternatively, the end sequence of a contig can be used as a primer to primer walk across the gap (Gibson *etal.,* 2009).

Mapping quantitative trait loci (QTLs) associated with drought tolerance

Traits which show continuous variation (polygenic) are called quantitative traits while genes behind those traits are simply referred to as QTLs. Mapping is putting genes or QTLs in order indicating relative distances among them assigning them to their linkage groups on the basis of their recombination values (Hussain, 2006). Generally the mapping population is derived from crosses between closely related species differing in the traits in question. There is long standing interest in QTL mapping due to the fact that it will ultimately help us to gain insight into very basic architecture of the trait concerned. Five types of populations are generally employed for QTL mapping. These are double haploids, recombinant inbred lines (RILs), backcross populations, near isogenic lines (NILs) and F2 populations. This QTL mapping allows assessing the locations, numbers, magnitude of phenotypic effects, and pattern of gene action (Vinh, Paterson, 2005). Different recent mapping populations used for QTL analysis for drought tolerance in cereals are described in Table below.

Table 2: Summary of most recent quantitative trait loci (QTLs) associated with drought tolerance in cereals

Trait	Cross	Species	QTL mapping population	References
Physio-morphological traits	CT9993 × IR62266	rice	RILs	Subashri et al., 2009
Physio-morphological and yield traits	IR 20 × Nootripathu	rice	NILs	Gomez et al., 2010
Various morpho-physiological traits	Zhenshan 97 × IRAT 109	rice	NILs	Ding et al., 2011
Photosynthesis parameters	Low land rice cv. Shennong 265 × Upland rice cv. Haogelao	rice	backcross (ILs)	Gu et al., 2012
Various morpho-physiological traits	Durum × Wild emmer	wheat	RILs	Peleg et al., 2009
Various productivity and physiological traits	Seri M82 × Babax	wheat	RILs	McIntyre et al., 2010, Suzuky et al., 2010
Leaf growth and ASI	Ac7643 × Ac7729/TZSR W	maize	RILs	Welcker et al., 2007
Root traits	CML444 × SC-Malawi	maize	RILs	Trachsel et al., 2009
Root traits and yield	Lo964 × Lo1016	maize	NILs	Landi et al., 2010
Root traits	Ac7643 × Ac7729/TZSRW	maize	RILs	Ruta et al., 2010
Physiological traits associated with seedling water stress	Zong3 × 87-1	maize	RILs	Liu et al., 2011
Plant senescence, relative leaf chlorophyll contents and root capacitance	CML444 × SC-Malawi	maize	RILs	Messmer et al., 2011
Stay green	Q319 × Mo17	maize	F2	Zheng et al., 2009

NILs= near isogenic lines; RILs= recombinant inbred lines
Source: Mueen Alam KHAN *et al*., 2013

Modern breeding approaches for developing superior germplasm for drought tolerance
Once the candidate genes or markers associated with QTLs for drought tolerance are identified, the next step is their deployment in breeding practices. Some of these approaches are discussed below.

Marker-assisted backcrossing (MABC)
When the QTLs identified for drought tolerance traits contribute higher phenotypic variation, they are considered major QTLs. These QTLs, after validation in desired germplasm, can be used for introgressing drought tolerance from the donor genotypes (generally used for identification of the QTL for the trait) into elite, less drought-tolerant cultivars or breeding lines (recipient parents) without transfer of undesirable or deleterious genes from the donors (linkage drag). The process is commonly referred to as marker-assisted backcrossing (MABC). Superior lines or cultivars are developed that contain only the major gene/ QTL from the donor parent, while retaining the whole genome of the recurrent parent (Hospital 2003; Varshney and Dubey 2009; Gupta *etal.,* 2010). Although MABC has been used extensively for introgressing resistance to biotic stresses, only a few reports are available on the use of MABC to develop the superior lines/varieties for drought tolerance (Table 2). For instance, MABC has been used to introgress root trait QTLs in the elite rice cultivars IR64 and Kalinga III (Shen et al. 2001; Steele et al. 2006). By using these MABC products, a variety namely ‘‘Birsa Vikas Dhan 111 (PY 84)’’ was developed and released in Jharkhand State of India (Steele *etal.,* 2007).

Field evaluation conducted under well-watered and water-stressed conditions in two consecutive seasons indicated that each pair of root-ABA1 backcross-derived near isogenic lines differed significantly and markedly for L-ABA, thus confirming the effectiveness of MAS (Landi *etal.,* 2005). Similarly, a major QTL for improved grain yield in pearl millet under terminal drought stress when transferred into a drought sensitive genotype showed a consistent grain yield advantage (Serraj *etal.,* 2005). Key reports on MABC for drought tolerance have been compiled in Table 3.

Table 3: Some examples of marker-assisted selection (MAS) for drought tolerance in crop plants

Crop	Trait improved	No. of genes/QTL transferred	Reference
Rice	Yield and grain quality under drought	Multiple QTL	Steele et al. (2006, 2007)
Cotton	Drought tolerance-related traits	7 QTLs	Levi et al. (2009)
Common bean	Drought tolerance-related traits	Multiple QTL (9 RAPD markers)	Schneider et al. (1997b)

Source: Mueen Alam KHAN *et al*., 2013

Marker-assisted recurrent selection (MARS)
To overcome the limitations of MABC, particularly when multiple QTLs control the expression of a complex trait, the MARS approach, which involves intermating selected individuals in each selection cycle, has been

recommended (Eathington *etal.,* 2007; Ribaut and Ragot 2007; Bernardo 2010). It generally involves the use of an F2 base population, and can be used in self-pollinated crops like wheat, barley and chickpea for developing pure lines with superior per se performance. MARS has the additional advantage of overcoming the limitation of inadequate improvement in the frequency of superior alleles in F2 enrichment, since MAS is practiced in each cycle following intermating to improve the frequency of favourable alleles (Eathington *etal.,* 2007). The successful use of MARS has been reported in sweet corn (Edwards and Jonson 1994), sunflower and soybean (Eathington *etal.,* 2007). Similar MARS breeding programmes are being conducted at several other international institutes including ICRISAT, the French Centre for International Agricultural Research (CIRAD) and University of California-Riverside, USA for improving drought tolerance in chickpea, sorghum and cowpea, respectively (Kulwal *etal.,* 2011).

Genome-wide selection (GWS)

Genome-wide selection (GWS) or genomic selection (GS) is another important approach to develop superior germplasm lines with overall excellent performance in a target environment. Genome-wide marker genotyping is used for GWS rather than selected markers showing significant associations (as in case of MARS) with the traits of interest. In summary, individuals in a phenotyped population (generally referred to as the 'training population') are genotyped using genome-wide markers and breeding values of alternative alleles of all the markers are fitted as random effects in a linear model. Individuals in subsequent recurrent selection generations are then selected based purely on the sum of those breeding values [genomic estimated breeding value (GEBV)]. Therefore, GWS reduces the frequency of phenotyping and similarly also increases annual gains from selection by reducing cycle time (Rutkoski *etal.,* 2010). Several groups have recently started exploring the GWS approach in both self- and cross-pollinated crops with some modifications for both types of crops (Bernardo, 2010). The success of the GWS approach is dependent on the availability of a diverse and representative training population. Furthermore, the phenotyping of the training population is crucial and additional lines should be integrated over time to increase the effectiveness and relevance of the gene effect estimates.

This approach has been recently used to improve durable stem rust resistance in wheat (Rutkoski *etal.,* 2010) and eventually could be systematically explored to bring different components of mutagenic drought tolerance using the GWS approach.

Application of Omics Technology

The applications of omics type technologies are beginning to have an impact in enhancing our understanding of plant's responses towards external environmental stimuli. The term "omics" is a blend of high throughput genomics, proteomics (analysis of protein complement) and metabolomics approaches. The generation of expressed sequence tags (ESTs) from cDNA libraries and complete genome sequence information in *Arabidopsis* and rice provide valuable information about gene discovery (Sreenivasulu *etal.,* 2007). Houde *et al*. (2006) reported that the digital expression analysis of in the identification of several pathways associated with abiotic stress tolerance in wheat. With the advancement of DNA microarray technology, several hundred stress induced genes have been identified in plants (Umezawa *etal.,* 2006). cDNA and oligonucleotide microarrays have been widely used in plants, such as *Arabidopsis,* rice, maize (Vij, Tyagi, 2007). Seki *etal.* (2001) constructed *Arabidopsis* full-length cDNA micro arrays using about 1300 full-length cDNAs. Forty-four genes were identified as drought inducible. Kawasaki *etal.* (2001) first reported the use of microarray to study global gene expression profiling in response to abiotic stress in rice. Later 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. Wang *et al.* (2007) compared gene expression between upland and lowland rice cultivars under drought stress using cDNA microarray. Compared with rice, the genomes of other cereals are large and complex (Paterson, 2006). Even then the projects to sequence the genomes of some cereals have been undertaken like in maize, sorghum (Bedell *etal.,* 2005) and wheat (Varshney *etal.,* 2006).

Apart from ESTs, other techniques like serial analysis of gene expression (SAGE), array-based transcript profiling technologies and quantitative real time PCR (qRT-PCR) allow us to assess the high throughput expression of thousands of genes involved in drought tolerance (Sreenivasulu *etal.,* 2007). Investigating the effects of drought on the protein composition may also provide a clue towards understanding a link between external environmental stress and plant development (Barnabás *etal.,* 2008).

Thus proteome analysis is applied to study the alterations in gene expression in relation to drought (Hu *etal.,* 2010). Salekdeh *et al*. (2009) working on the proteome analysis identified more than 1000 proteins in rice. Out of these, 42 were differentially expressed in drought stress. Ali and Komatsu (2006) performed a proteomic analysis on rice leaf sheaths and identified a protein actin depolymerizing factor (ADF). The increased level of ADF in drought tolerant plants suggested that ADF is one of the target proteins induced in drought stress. Recently Yang *et al.* (2011) performed a proteome analysis of rice roots to identify water deficit responsive proteins among two cultivars IR64 and 'Azucena'. Out of 700 proteins detected, only 15 showed different

responses to water stress between two ecotypes.

Similar proteome analysis has also been started in other cereal crops as well. Riccardi *et al.* (2004) identified 46 proteins in maize leaves. They found an increase in quantity of these proteins in leaves of plants subjected to water stress. Hu *et al*. (2011) found a differential expression of 22 proteins in maize roots in response to drought stress.

Metabolomics is one of the omics used to acquire comprehensive information about the metabolites in plants (Okazaki, Saito, 2012). The metabolite changes in plants in response to environmental stresses suggest that complete metabolite profiling may provide valuable insights into stress tolerant mechanisms of plants (Langridge *etal.,* 2006). Metabolomics is a relatively new area of research and it is expected that when combined with genomics, transcriptomics and proteomics, it will help us to understand and interpret many complex biological processes (Langridge *et al*., 2006; Okazaki, Saito, 2012).

From the above discussion, it can be inferred that considerable progress has been made in the field of omics, providing valuable information on the structure and behaviour of crop genomes, with better understanding of plant responses to environmental stresses (Langridge, Fleury, 2011). However, there are challenges and issues that need to be tackled and considered for successful exploitation of the omics technologies. Some of these are regulatory variations, precise phenotying, technical and cost related issues (Varshney *et al.,* 2006).

Transgenics

The identification of candidate genes is critical for our understanding of molecular and physiological mechanisms of drought tolerance in cereals, as it will enable us to use transgenic approaches in breeding for abiotic stress tolerance (Dolferus *etal.*, 2011). A transgenic approach is one that involves some structural modifications in traits through gene transfers from one species to the other (Ashraf, 2010). As the regulatory networks underlying the abiotic stress responses are being fully understood, more and more candidate genes will be identified to be used in development of transgenic plants (Barnabás *et al*., 2008).

A detailed description of drought tolerance genes can be found in the review of Hadiarto and Tran (2011). A number of such genes associated with drought tolerance have been identified. Like transcription factors that upregulate and downregulate the expression of other genes. Some of the other identified stress-responsive genes are functional genes which encode metabolic components, such as late embryogenesis abundant (LEA) proteins and osmoprotectant-synthesizing enzymes. (Yang *etal*., 2010 as reviewed by Hadiarto and Tran, 2011). Most important and well-studied class of transcription factors is drought responsive element binding (DREB) factors especially *DREB1A* and *DREB2A* identified in *Arabidopsis* as well as in cereal crops (Hussain *etal*., 2011). Initial studies with DREB started with *Arabidopsis*. Over-expression of *DREB1*/*CBF* in *Arabidopsis* resulted in the activation of expression of many stress-tolerance genes and the tolerance of the plant to abiotic stresses was greatly improved (as reviewed by Gosal *etal.* 2009). In most of the cases the over expression of *DREB1A* is obtained by using constitutive (CaMV 35S) promoter or the dehydration inducible (rd29A) promoter. In transgenic *Arabidopsis* plants Kasuga *etal*. (1999) found that overexpression of *CBF3*/*DREB*1A accompanied by constitutive promoter CaMV 35S greatly improved plant's tolerance to abiotic stresses including drought stress. Similarly, the use of the stress inducible promoter rd29A in conjunction with *DREB1* has been found to enhance drought tolerance in tobacco (Kasuga *etal*. 2004) and wheat (Pellegrineschi *etal.,* 2004). RD29 genes are induced by desiccation, cold and salt stresses thus endowing plants to tolerate these stresses (Jia *etal.,* 2012). A list of some of the recent transgenic lines produced in cereal crops is given in Table 4.

Table 4: List of transgenic lines produced in cereal crops for drought tolerance

Transgene	Crop	Trait improved	Reference
HVA1	rice	transgenic plants showed improved tolerance to drought conditions	Xiao et al., 2007
HVA1	wheat	transgenic plants showed improved tolerance to drought conditions	Sivamani et al., 2000
CBF3/DREB1A	rice	drought and salinity tolerance	Oh et al., 2005
SNAC1	rice	transgenic plants showed improved tolerance to drought conditions	Hu et al., 2006
OsNAC10	rice	transgenic plants showed improved grain yield and tolerance to drought	Jeong et al., 2010
Os LEA-3-1	rice	transgenic plants showed increased growth under drought conditions	Xiao et al., 2007
Tomato ethylene response factor (ERF) protein *TSRF1*	rice	*TSRF1* improved the osmotic and drought tolerance of rice seedlings without growth retardation	Quan et al., 2010
Tomato ethylene response factor (ERF) protein *JERF3*	rice	over expression of *JERF3* significantly enhanced drought tolerance of transgenic rice	Zhang et al., 2010
Sorghum *SbDREB2* gene with stress-induced promoter CaMV35S or rd29A	rice	over expression of *SbDREB2* significantly enhanced drought tolerance and yield improvement in transgenic rice	Bihani et al., 2011
Rice *OsDREB2A* gene with stress-inducible promoter rd29A	rice	over expression of *OsDREB2A* significantly enhanced drought and salt tolerance of transgenic rice	Mallikarjuna et al., 2011
Rice *OsSDIR1* gene	rice	over expression of *OsSDIR1* gene significantly enhanced drought and salt tolerance	Gao et al., 2011
mtlD (osmoprotectant)	wheat	improved fresh and dry weights, plant height, and flag leaf length in transgenic plants	Abebe et al., 2003
Asr1 (*A* putative transcription factors)	maize	transgenic maize lines showed improved tolerance to drought	Jeanneau et al., 2002
ZmNF-YB2 (an orthologous maize transcription factor from the nuclear factor *Y(NF-Y)* family)	maize	transgenic maize lines showed improved tolerance to drought	Castiglioni et al., 2008
ZmNF-YB2	maize	transgenic maize plants showed 50% increased yield under drought conditions	Nelson et al., 2007
OsPFA-DSP1 (a rice protein tyrosine phosphatase)	rice	transgenic rice and *Tobacco* plants showed sensitivity to drought stress	Liu et al., 2012

Source: Mueen Alam Khan *et al*., 2013

Summary

Analysis of the response to drought has been further complicated by the absence of a genome sequence and the generally poor genomics resources have been limiting. New developments in sequencing, marker development, and genome analysis have created the opportunity to revisit the way in which we structure populations for analysis and tackle specific components of drought tolerance. Phenotyping has now become the major cost and rate-limiting step in the genetic analysis of drought tolerance and many other traits, and the development of rapid and cheap procedures to characterize components of the drought response will be critical in improving genetic resolution.

It is essential to integrate crop physiology, genomics and breeding approaches to dissect complex drought tolerance traits, understand the molecular basis of drought tolerance and develop the next-generation crops for our changing climate. Considerable progress can be made in the field of omics, providing valuable information on the structure and behavior of crop genomes, with better understanding of plant responses to environmental stresses. Identification of traits and genotypes associated with drought tolerance is absolutely necessary. Concerted efforts are required to fully understand the physiological and genetic basis of drought tolerance. Focus should be on screening resistant germplasm and discovering potential candidate genes. Characterization and mapping of such genes at the physiological and molecular level will be key factors in the application of molecular marker technology to the development of more drought tolerant cultivars. Transgenic and omics based technologies have been shown to be powerful tools holding a tremendous promise for the future

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