REVIEW

Embracing new-generation 'omics' tools to improve drought tolerance in cereal and food-legume crops

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Abstract

Drought stress presents a considerable threat to the global crop production. As a dominant source of vegetarian diet, cereals and grain-legumes remain crucial to meeting the growing dietary demands worldwide. Therefore, breeding cultivars of these staple crops with enhanced drought tolerance stands to be one of the most sustainable solutions to enhance food production in changing climate. Given the context, a more focused survey of environment-defined germplasm sets is imperative to comprehend such adaptive traits. In parallel, uncovering the genetic architecture and the molecular networks that collectively contribute towards drought tolerance is urgently required through rationally combining large-scale genomics, proteomics, and metabolomics data. Also, attention needs to be directed to reasonably quantify the epistatic as well as environmental influences, thereby warranting deployment of analyses like meta-quantitative trait loci (QTL) that encompass multiple environments and diverse genetic backgrounds. Further, innovative techniques like genomic selection (GS) and genome wide association study (GWAS) would help to capture the quantitative variation underlying drought tolerance. Equally importantly, integration of physiological traits-based techniques with ever-evolving 'omics' technologies and the new-generation phenotyping platforms will be of immense importance in advancing our existing knowledge about the genetically-complex and poorly-understood phenomena, such as plant drought response, and a deeper understanding would likely to provide a great impetus to the progress of crop breeding for drought tolerance.

Additional key words: genetic mapping, genomics, metabolomics, molecular markers, phenotyping, proteomics, quantitative trait loci.

Introduction

Most of the crops are vulnerable to drought and experience yield losses up to 50 % or even more (Bray *et al*. 2000). Given the increasing severity of global warming and changing climate, the frequency of global drought is projected to expand beyond 20 % by the end of this century, especially in the regions like South America and Central and Western Europe (Prudhomme *et al.* 2014). Similarly, an alarming proliferation in the water-stressed areas is expected to occur worldwide by 2030, which would influence 50 countries collectively harbouring almost three billion people (Postel 1999, Graham and Vance 2003).

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Drought is a complex phenomenon characterized by an intricate interplay of limiting water availability, lower rainfall, reducing ground water level, and increasing temperature. Measuring and addressing drought stress in crop plants by the conventional breeding efforts is greatly constrained by the low heritability and complex genetic make-up of drought tolerance, and profound environmental interactions (Asfaw et al. 2012, Cabrera-Bosquet et al. 2012, Hill et al. 2013, Jha et al. 2014). In view of the global food security, it is urgently required to appraise the impact of drought and to recognize the significance of climate changes to crop productivity (Mishra and Singh 2010). Furthermore, in order to offer crop-based sustainable answers to the increasing menace of food insecurity and undernourishment, cereals and grain legumes play an increasingly important role as these crops constitute the major proportion of the vegetarian diets, particularly in the developing nations (Graham and Vance 2003, Bohra et al. 2015). Cereals, such as rice, are characterized by extensive water requirement, therefore lower availability of water exerts greater impacts upon the crop yield (O'Toole 1999). Concerning water scarcity that leads to almost 70 % losses in crop yields globally as described by Boyer (1982), millet, however, survive at comparatively less amount of available water (http://www.fao.org/docrep/u3160e/u3160e04.htm).

Similar to the extent of yield-losses recorded in cereal crops, substantial yield reductions were reported in grain legume crops owing to their susceptibility to drought stress. For instance, drought stress was reported to cause up to a 50 % yield loss in several legume crops including soybean (Guttikonda et al. 2014), common bean (Castañeda-Saucedo et al. 2014), chickpea (Varshney et al. 2014), and cowpea (Ahmed and Suliman 2010).

In concern to addressing the drought-stress in crop plants, development of superior genotypes with enhanced stress tolerance and wider adaptability remains the simple, economically-attractive, and climate-friendly strategy (Jha et al. 2014). Here, we review the progress made so far towards resolving the genetic makeup of drought tolerance and implications of new-generation tools/technologies for breeding tolerant cultivars in important cereal and food-legume crops. Eventually, we offer a perspective on the enhanced and efficient use of these rapidly-evolving 'omics' tools and technologies in crop improvement in order to deal with the ever-increasing severity of drought stress worldwide.

Drought tolerance in plants: physiological, biochemical, and molecular insights

The susceptibility of a crop to drought relies largely on the particular growth stage and its water requirement at that time. For instance, flowering and fruit setting stages are generally considered critically vulnerable to drought-stressed conditions. In rice, Pantuwan et al. (2002) reported the reproductive period as the most receptive phase to drought stress, and Ekanayake et al. (1989) concluded that the deterioration in rice yield is due to a marked reduction in fertility as well as in the number of spikelets.

In view of the consideration that drought should be fundamentally measured as moisture stress, Jones (2007) noticed that soil moisture retained in the crop root zone actually supports crop growth. Intrinsically, plants respond to a lower soil moisture or water stress in a very complex fashion that entails various adaptation strategies including a range of molecular, physiological, and biochemical alterations. As an innate capacity to survive under water stressed situations, the expressions of a number of the responsive genes are noticeably altered (Anjum et al. 2011). Water stress in plants is often caused by simultaneously occurring low water availability in soil and a high transpiration rate, and can be ameliorated by increasing water uptake and decreasing stomatal conductance (Jones 1999, Lawlor and Cornic 2002).

Visible symptoms and attributes contributing to drought tolerance include rapid leaf rolling, stay green ability, rapid stomatal closure, and increased root length, water use efficiency (WUE), epicuticular wax content, photochemical quenching, photoinhibition resistance, osmotic adjustment, and membrane stability (Collins et al. 2008, Khazaei et al. 2013). The initial visible symptom and one of prominent survival mechanisms against drought stress is leaf rolling which reduces the transpiration rate and canopy temperature thereby improves WUE (Townley-Smith and Hurd 1979, Clarke 1986). The plants retaining a higher relative water content (RWC) under water deficit conditions tend to be less vulnerable to drought stress, and hence sustain normal growth and yield (Lazacano-Ferrat and Lovat 1999, Costa Franca et al. 2000, Beltrano et al. 2006). Another parameter that merits a careful consideration is the length of roots being in the direct contact with soil, and face immediate effects of reduced soil moisture content. Xiong et al. (2006) noted a significant inhibition in the development of lateral roots after imposing drought stress, and they asserted that the observed changes constitute the adaptive mechanism to the stress. In addition, a series of biochemical changes occur in response to drought stress, which involves an enhanced accumulation of osmolytes, reactive oxygen species (ROS), antioxidants, and anti-oxidative enzymes (Kholová et al. 2011). Moreover, the osmolytes (proline, glutamate, glycine-betaine, and sugar alcohols) play large roles in maintaining membrane stability and enzyme activity under a low water potential (Ashraf and Foolad 2007, Shao et al. 2008, Du et al. 2014, Khattab et al. 2014).

Deciphering drought tolerance using new-generation 'omics' tools and technologies

As a compelling supplement to the recent phenomenal scientific and technological progress in genomics, an ever-advancing comprehension of the physiological mechanisms underlying the drought responses would definitely help to accelerate the progress of genetic improvement of crop drought tolerance. The complexity of drought syndrome can only be tackled with a holistic approach that incorporated a detailed physiological dissection of a crop concerning drought avoidance and tolerance traits. In parallel, optimization of the agronomic practices is also required, which certainly will lead to improved conservation and utilization of soil moisture, and enhanced adaptability of crop genotypes to the changing environment (Harb *et al.* 2010, Seiler *et al.* 2011, Padmalatha *et al.* 2012). The most imperative science materialized in recent years is 'omics' science which is defined as a suite of approaches like genomics, transcriptomics, proteomics, metabolomics, ionomics and plays a vital role in crop improvement by facilitating the identification of genes, proteins, and metabolites associated with drought tolerance and also by characterizing their functions (Zargar *et al.* 2011). An interactive study involving 'omics' allows us to predict the function of genes, proteins and metabolites, and eventually to assess the changes in plants induced by given environmental conditions (Adam 2008, Baginsky *et al.* 2010).

Transcriptomics: Owing to its complex nature, drought involves an intricate network of multiple signalling pathways with a vigorous cross-talk. Therefore, it is necessary to decipher the key genes influencing underlying pathways and to establish their functional connectivity to varying drought severity and also to different growth stages of a crop that undergoes drought stress (Siopongco *et al.* 2006). Gene expression analysis enables access to responsive genes expressed under stress, however, a carefully designed experimental validation is further required to obtain an accurate and stage-specific gene expression (Gimeno *et al.* 2014). In the post-genomics era, functional genomics has been brought to the next level, and massive efforts were undertaken in different crops to reveal differential expression patterns of genes involved in drought stress signalling pathways by means of semi quantitative RT-PCR, real-time PCR, microarray technologies, serial analysis of gene expression (SAGE), massively parallel signature sequencing (MPSS), and more recently by applying advanced genome-wide transcriptome analysis using next generation sequencing (NGS)-based RNA sequencing (RNA-seq) (Kathiresana *et al.* 2006, Neves-Borges *et al.* 2012, Shiriga *et al.* 2014). The limited number of assayed genes set a major limit to semi-quantitative and real-time PCR assays, whereas the use of microarray and whole genome transcriptome analyses permit the construction of a comprehensive landscape of gene expression and also provides a functional genomic framework for a precise and rapid identification of candidates involved. Given its remarkably enhanced capacity in capturing novel transcripts, splicing models, allele-specific expression, and splicing junctions, the more recently introduced RNA-seq technique has widespread applications in genome wide expression profiling (Malone and Oliver 2011). These techniques have been extensively applied for interrogating expression patterns in rice (Minh-Thu *et al.* 2013), maize (Kakumanu *et al.* 2012), sorghum (Dugas *et al.* 2011), and many other important crops with the aim to decipher some novel genes. A recent expression study using quantitative RT-PCR (RT-qPCR) in four rice cultivars showing varying drought tolerance revealed a set of 46 candidate genes strongly influenced by genotype × environment (G × E) interactions (Degenkolbe *et al.* 2013). In a similar manner, the microarray-based analysis of rice panicles of a drought stressed plant showed up-regulation of a series of stress-induced genes, such as GTP-binding protein 3, cellulose synthase-6, heat shock cognate protein, DNA repair protein, zinc finger protein, actin depolymerizing factor, and pectin esterase (Kathiresana *et al.* 2006).

A more recent study in wheat categorized the entire set of genes and noticed the intricate network involving a number of genes influencing multiple pathways (Reddy *et al.* 2014). In this study, transcriptomes of two different winter wheat cultivars TAM 111 and TAM 112 were investigated under water-deficit conditions, and 474 transcripts are specific to TAM 111, 1 540 to TAM 112, and 1 657 are common to both (Reddy *et al.* 2014). Similarly, Minh-Thu *et al.* (2013) reported a significant change in the expression of 10 537 genes by conducting a transcriptomics study of rice roots and leaves under water stress. More recently, Wu *et al.* (2014) identified a total of 9 298 drought-responsive genes in common bean by employing RNA-seq to drought tolerant and susceptible genotypes and further validated the differentially expressed genes through real-time qPCR. Large-scale RNA-seq data can be effectively combined with linkage mapping and GWAS to finely resolve a quantitative variation underlying the trait-of-interest (Ingvarsson and Street 2011, Majewski and Pastinen 2011, Cubillos *et al.* 2012, Huang and Han 2014). However, it was also noted that the genes expressed under stressed conditions do not necessarily participate in imparting tolerance against drought, but provide an ideological view about the involvement of drought-related genes and relevant signalling components. Like other traits, drought tolerance is also regulated at transcriptional level, and transcription factors have been targeted for their subsequent use in crop improvement strategies.

The prospects of transcriptomics study for crop improvement involves the discovery of causative genes and the associated pathways which further assist for gene cloning and characterization, and the development of large-scale genic markers. For instance, the development

of drought-responsive expressed sequence tags (ESTs) in chickpea and a further analysis revealed a set of 177 novel EST derived simple sequence repeat (SSR) markers for breeding applications (Varshney *et al.* 2009). The gene-based or functional markers thus developed from ESTs and transcriptomic data facilitate direct landing to the gene-of-interest. In wheat, Kadam *et al.* (2012) analyzed transcriptome with the objective to identify drought-responsive genes using the parents and two extreme phenotypic bulks from a RIL population. They noted a differential expression of five candidate genes (within the QTL containing region) between the two extreme bulks as well as the parents.

Proteomics: The application of proteome-wide profiling approaches in characterizing plant phenotypes is gradually coming to focus (Knight and Knight 2001). Major techniques used for expression analysis and sequence analysis in proteomics are polyacrylamide gel electrophoresis (PAGE), two dimensional polyacrylamide gel electrophoresis (2-DE), and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). Also, liquid chromatography (LC)-based proteome analysis is becoming increasingly popular (Komatsu *et al.* 2013). In recent years, a remarkable progress in interrogating proteomes in drought-responsive crop plants has shown a considerable impact on identification and characterization of differential responses of plants facing stresses. Additionally, Ansong *et al.* (2008) has discussed an increasingly important role of proteomics in genome annotation and gene prediction. Despite recent advancements made in the field of protein expression analysis that enabled the examination of changes in protein expression (Koller *et al.* 2002), the number of crop proteome profiles has been limited (Komatsu *et al.* 2013). The examples of proteome studies in major crops under drought stress include rice (Salekdeh *et al.* 2002), maize (Tai *et al.* 2011), wheat (Ford *et al.* 2011, Jiang *et al.* 2012, Ye *et al.* 2013), and barley (Rollins *et al.* 2013). With the introduction of the state-of-the-art tools and techniques, the proteomics is emerging as an appealing research field, however, the full potential of proteomic approach with respect to a drought-induced proteome remains to be explored (Jorrín *et al.* 2007).

As illustrated by Kosová *et al.* (2014a), enabling access to protein-based markers enormously expands the scope of "omics"-assisted breeding for crop improvement. Proteomics is likely to advance the knowledge of plant breeders, thereby assisting them to investigate stress tolerance mechanisms and to develop drought tolerant cultivars (Eldakak *et al.* 2013). Recently, Ghosh and Xu (2014) discovered some specific signatures in a root proteome under different abiotic stresses. Different expressions of proteins under drought stress in different crops and even in different cultivars of the same species suggest different mechanisms of tolerance.

Reports published to date have documented differential expressions of proteins under drought stress.

For instance, by imposing a PEG-induced drought stress to wheat, a set of 21 proteins was reported to undergo changes with 15 proteins being up-regulated and the remaining 6 proteins down-regulated (Ye *et al.* 2013). Likewise, differentially expressed proteins were identified in two drought tolerant wheat cultivars (KDML105 and NSG19), which was related to phytochrome A signalling, DNA repair, and superoxide dismutase in the cv. KDML105. On the other hand, proteins relating to cell/DNA repair and stomatal activities were observed to be over-expressed in the cv. NSG19 (Maksup *et al.* 2012). The differentially expressed proteins in a QTL-pyramided line under drought-stress at seedling stage include peroxiredoxin, ribonuclease, putative chitinase, and positionally, these proteins co-located with the three QTL-containing regions (Xiong *et al.* 2010). A comparative proteomic analysis of drought-responsive proteins of the two wheat genotypes during grain filling led to the detection of a total of 122 protein spots that are considered to be associated with multiple pathways (Jiang *et al.* 2012). In rice, the application of MALDI-MS or ESI-MS/MS facilitated the discovery of 16 drought-responsive proteins of which S-like RNase homolog, an actin-depolymerizing factor and activase of ribulose-bis-phosphate carboxylase/oxygenase (Rubisco) show up-regulation. Conversely, an isoflavone reductase-like protein was noted to be down-regulated (Salekdeh *et al.* 2002). More recently, Hossain and Komatsu (2014) have exhaustively reviewed proteome studies conducted in soybean so far particularly from the context of drought and flood. Similarly, Kosová *et al.* (2014b) have highlighted the role of a late embryogenesis abundant II protein dehydrin under various abiotic stresses in common wheat, durum wheat, and barley.

Metabolomics is defined as the systematic study of unique chemical fingerprints of a system, and it has recently emerged as a potential tool to reinforce the functional genomics framework (Tyagi *et al.* 2010). To accelerate the trait-based analysis of a complex biochemical process, metabolic profiling along with NGS techniques, transcriptomics and proteomics, help in elaborating our existing knowledge and reveal the underlying cellular biochemical events across diverse conditions (Fernie and Schauer 2008). Wen *et al.* (2014) suggested that examination of metabolite variation in a diversified panel offers a prospective strategy to distinguish genetic factors involved in the manifestation of various metabolic traits. Concurrent with the growing establishment of high-throughput genotyping and sequencing systems, novel large-scale breeding methods like genomic selection (GS) are introduced to harness the unexplored quantitative variation in an unprecedented manner. Given the context, thus it becomes imperative to develop a renewed focus on whole genome metabolome selection (Adamski 2012). An efficient integration of GS with a metabolic selection might offer a more lucid landscape comprising networks of genes/QTLs and

metabolites under drought conditions. Notwithstanding the phenomenal expansions in plant genomics research, the cellular metabolism of plants under stress remains largely unclear. Perhaps, the major reason attributed to this lag is a constant change in the metabolic profile of plants under different stresses experienced at various growth stages. Under drought stress, plant metabolic processes alter in a range of ways including the production of compatible solutes (*e.g.*, proline, glycine betaine, sugar alcohols, and polyamines) that are able to stabilize a hydration shell of proteins, the structure and function of macromolecules, and a pressure potential by osmotic adjustment, to remove the excess of ROS and re-establish cellular redox balance (Bartels and Sunkar 2005, Janska *et al.* 2010). A total of 31 compounds including abscisic acid (ABA), cytokinin, and some phenylpropanoids are produced in maize xylem sap when plants are exposed to drought stress (Alvarez *et al.* 2008). In recent years, attempts have been made to characterize metabolites produced under drought stress both *in vivo* and *in silico*, which included flux-based modelling and analyses of cell metabolism in rice (Lakshmanan *et al.* 2013), grapevine (Hochberg *et al.* 2013), soybean (Silvente *et al.* 2012), pea (Charlton *et al.* 2008), and sorghum (Pavli *et al.* 2013). A comparative metabolite profiling across different species sheds a new light on the fundamental metabolic pathways (Sanchez *et al.* 2012).

The metabolite profiling of a given mapping population can be combined with the genetic linkage maps to obtain greater insights into the genetic makeup of complex traits, thereby rendering metabolomics particularly relevant to crop breeding (Hill *et al.* 2013). For example, Wentzell *et al.* (2007) elucidated the complex regulatory mechanisms of glucosinolates pathway through integrating expression QTL data with metabolic QTLs. Similarly, Hill *et al.* (2013) identified multiple metabolite QTLs in wheat under drought stress and pinpointed some genomic segments that controlled both agronomic traits and specific metabolites. Lisec *et al.* (2011) used a metabolomics-based genetic evaluation system for hybrids through investigating the inheritance pattern of metabolites in hybrids and inbred lines. Metabolomics techniques in conjunction with other "omics" approaches are envisaged to underpin characterization of cultivars and identification of genes, gene function, and functional markers, which will eventually contribute towards increasing the efficiency of the marker assisted breeding programs (Andersen and Lubberstedt 2003, Varshney *et al.* 2005).

Coupling crop breeding and advanced molecular technologies for improving drought tolerance

Given the ever-increasing scientific and technological developments in the field of plant genomics, notable genomic resources were made public not only in major crop species but also in the minor crops of regional importance (Bohra 2013, Bohra *et al.* 2014a,b). In particular, the enormous genomic resources can be accessed through public databases like ESTs for abiotic stress-tolerant species are indexed at the National Center for Biotechnology Information (NCBI) dbEST (http://www.ncbi.nlm.nih.gov/dbEST/). Information pertaining to DNA markers, genetic maps, and transcriptome assemblies is made community-accessible in various crops including rice (http://archive.gramene.org/markers/microsat/), maize (http://www.panzea.org/lit/data_sets.html), soybean (http://soybase.org/), pigeonpea (http://webapp.cabgrid.res.in/pigeonpea/ database1.html; http://webapp.cabgrid.res.in/ pigeonpea/), chickpea (http://cicarmisatdb.icrisat.org/; http://www. nipgr.res.in/ctdb.html), and sorghum (Taramino *et. al.* 1997, Ramu *et al.* 2009, Yonemaru *et al.* 2009 and http://www. gramene.org/). Likewise, single nucleotide polymorphism (SNP) databases (http://www.ncbi.nlm.nih.gov/projects/SNP/) and many other valuable genomic resources were established and noticeably enhance the efficiency of crop improvement programs. These genomic tools/techniques are extensively used in diverse genetic applications including QTL mapping, marker-assisted backcrossing (MABC), and QTL/gene pyramiding, marker assisted recurrent selection (MARS), and GS. However, the most relevant tools are DNA markers that represent multi-utility resources employed in different plant breeding activities like germplasm characterization, phylogenetic analysis, determining seed purity, identification of true F_1s, and more importantly, the MAS and its modern extensions like MABC, MARS, and GS (Collard and Mackil 2008). Assimilating genomics tools to conventional breeding offers new ways to hasten the improvement progress *via* the identification and mapping of quantitative traits (QTs), thus creating unprecedented opportunities for MAS, map-based QTL cloning, and targeted genetic manipulation using reverse genetics technologies. In the following section, we discuss QTL mapping and association genetics that constitute the 'Training or development phase' of crop improvement program (Nakaya and Isobe 2012), accompanied by a brief summary on the introgression of the newly-identified genes/QTLs in crops selected herein.

Development phase: generating appropriate molecular tools for breeding applications: The genomic regions associated with the traits showing continuous phenotypic variations are referred to QTLs (Kearsey and Farquhar 1998). Technically, QTL mapping requires a genetic linkage map to establish a connection between the marker genotype and the phenotype-of-interest (single marker analysis, interval mapping, and composite interval mapping) and leads to the detection of genomic regions associated with the QTs (McCouch and Doerge 1995, Mohan *et al.* 1997). Taken the context of drought, the essential prerequisites to map a robust QTL

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are *1*) an accurate and precise phenotyping, *2*) stages of stress imposed, and *3*) the relevant component traits evaluated. Different kinds of segregating populations including F_2, near isogenic line (NIL), recombinant inbred line (RIL), and double haploid (DH) were created to uncover the genomic regions contributing to tolerance against drought. For example, imposing water stress to DH population derived from rice lines (CT9993-5-10-1-M and IR62266-42-6-2), Babu *et al.* (2003) detected 47 QTLs for yield, biomass, phenology, *etc.*, which explains up to 59 % of the variation. Likewise, several researchers have evaluated drought stress tolerance at germination, vegetative, and reproductive stages, and identified QTLs across different crops including rice (Teng *et al.* 2001, Fujino *et al.* 2004, Cheng *et al.* 2008, Ji *et al.* 2009, Wang *et al.* 2011, Mardani *et al.* 2013, Sasaki *et al.* 2013), barley (Mano and Takeda 1997, Guo *et al.* 2008), soybean (Ikeda *et al.* 2009), *etc*. At the seedling stage, five stable QTLs for drought related traits were reported under PEG-induced stress in rice (Srividhya *et al.* 2011). In a similar fashion, a total of 14 QTLs were reported for chlorophyll-related traits in barley (including one common QTL for chlorophyll content) under well-watered and drought-stressed conditions (Guo *et al.* 2008). Several QTLs controlling a range of drought relevant traits were obtained across various crop species like 22 QTLs for leaf, canopy and root related traits in rice (Gomez *et al.* 2010), 33 QTLs for root traits in advanced backcross wheat lines (Ibrahim *et al.* 2012), and 44 QTLs for eleven traits in barley introgression lines (Honsdorf *et al.* 2014). More recently, Barakat *et al*. (2013) reported QTLs that governed up to 73 % of the phenotypic variation in leaf senescence under water-stress. By analyzing a RIL population in cowpea, Muchero *et al.* (2009) mapped 12 QTLs that control drought tolerance both at seedling and maturity stages. In a similar way, Varshney *et al.* (2014) reported occurrence of 45 main-effect QTLs for drought tolerance by analyzing two RIL populations in chickpea. Importantly, a substantial fraction of the entire phenotypic variation was explained by the epistatic QTLs. More recently, the application of genotyping by sequencing (GBS) in a RIL population (ICC 4958 × ICC 1882) led to the integration of 49 SNP markers in the "hot spot" QTL regions that span 14 cM of the chickpea genome. Further, conversion of these SNPs to a cleaved amplified polymorphic sequence (CAPS) and derived CAPS (dCAPS) transforms them into easy-to-use PCR-based markers for a molecular breeding purpose (Jaganathan *et al.* 2015). Some of the recently identified drought relevant QTLs are presented in Table 1 Suppl.

It is important to note here that complex QTs like drought need to be analysed using a meta-QTL approach that rests on the premise of connecting multiple QTL data from numerous populations screened across diverse environments/locations. For example, a meta-QTL analysis in maize enables the localization of seven genomic regions that contain QTLs showing constitutive as well as adaptive expressions (Almeida *et al.* 2013).

Another comprehensive meta-QTL analysis in maize unfolded a set of 68 meta-QTLs that influence grain yield in water-limiting and well watered environments (Semagn *et al.* 2013). However, further attempts to find QTLs effective to multiple populations (up to six) substantially reduce the number of consistent meta-QTLs to four (Semagn *et al.* 2013). Earlier, Hao *et al.* (2010) analyzed QTL data from 12 different maize populations, and importantly, several consensus QTLs of adaptive as well as constitutive nature recovere and are successfully aligned with the candidate genes underlying drought tolerance.

A more recent study undertaken in wheat by Acuña-Galindo *et al.* (2015) revealed 64 meta-QTLs for drought and heat stress in wheat, of which 20 meta-QTLs exclusively controll drought tolerance. Similarly, Almeida *et al.* (2014) observed, through investigating meta-QTLs across three mapping populations, a candidate genomic region in maize that harbours a variety of drought tolerance-relevant traits. Strikingly, these studies deliver defined genomic segments, *i.e.*, the "hot spot" regions, which contain robust QTLs explaining phenotypic variation to a considerably large extent. Similarly, Courtois *et al.* (2009) detected genomic "hot spots" in rice for root architecture by applying a meta-QTL approach, and constructed a community-based web resource allowing access to a large number of root-related QTLs extracted from multiple published studies.

Association mapping (AM): The AM detects QTLs across a diverse panel by evaluating the patterns of genome-wide linkage disequilibrium (LD), and the association between the genomic variants and the relevant phenotypes. LD is the non-random association of alleles at two loci (Mackay and Powell 2007). The choice of LD mapping in terms of time and space is driven by the fact that it does not rely on creating experimental populations derived from controlled crossing, hence a genetically distinct set of population or exotic germplasm may be used for the purpose (Mitchell-Olds 2010). Additionally, AM bestows several advantages over bi-parental QTL mapping including enhanced genetic resolution, reduced research time, and a greater number of alleles and traits (Yu and Buckler 2006, Rafalski 2010, Huang and Han 2014). In AM, however, remedies proposed to address the issue of "false positives" (arising from inherent genetic structures in natural populations) in turn raise the number of "false negatives" (Bergelson and Roux 2010, Mitchell-Olds 2010, Rafalski 2010, Ingvarsson and Street 2011, Huang and Han 2014). To control spurious associations, individuals are first assigned into defined subgroups, and then kinship (K) is estimated by using a set of unlinked markers having a neutral background and a genome wide distribution (Yu and Buckler 2006). More recently, a high-throughput phenotyping and genome wide SNP markers were combined for detecting significant marker-trait associations (MTAs) in introgressed barley lines (Honsdorf *et al.* 2014) and japonica rice accessions (Courtois *et al.* 2013). A genome-wide association study

performed across 185 cultivated and 38 wild accessions led to the discovery of a few QTLs related to drought tolerance in barley (Varshney *et al.* 2012). By using 98 SSR markers, Sakhi *et al.* (2013) applied AM in sorghum under drought stress conditions and resolved 9 significant QTLs associated with drought-related traits explaining up to 57.5 % of the total phenotypic variation (Table 1). More recently, GWAS as well as candidate gene-based association mapping were conducted across a panel of 300 chickpea accessions using 1 872 markers, and consequently, noteworthy MTAs were detected for drought and related traits (Thudi *et al.* 2014).

Marker-assisted selection: The MAS represents a rapid and precise molecular breeding technique that rests on the premise that superior individuals can be precisely isolated on the basis of the genotype at particular marker loci. In practice, MAS can be exercised in various ways, *e.g.*, the marker-assisted evaluation of breeding material, MABC, pyramiding, early generation selection, and combined MAS (Collard and Mackil 2008). Fortunately, molecular markers have many more applications beyond this scheme. To execute MAS more efficiently, DNA markers must have some key features like a greater reliability, quantity and quality of DNA required, the ease of technical procedure to assay, a high level of DNA polymorphism, and a low cost of assay designing (Mackill and Ni 2001, Mohler and Singrun 2004). Notably, Toenniessen *et al.* (2003) recorded a greater efficacy and accuracy of MAS over conventional plant breeding. Genome wide distributed DNA markers enable exploration of thousands of genomic regions across a comprehensive crop germplasm (Ashraf 2010) and will have profound impacts on breeding temperate and tropical crops (Dwivedi *et al.* 2003).

Marker-assisted backcrossing: Holland (2004) has described three different types of MABC used for foreground selection (Tanksley 1983), recombinant selection, and background selection (Young and Tanksley 1989). In the foreground selection, the genotype of markers linked to a target gene or QTL can be used either in combination with a phenotype or to replace screening for the target gene or QTL, particularly for useful traits that are generally measured through laborious and time-consuming phenotype screening procedures (Hospital and Charcosset 1997). In recombinant selection, back crossed progenies are selected with the target gene and recombination events between the target locus and linked flanking markers. The fundamental idea that underlies recombinant selection is to reduce the size of donor chromosome segment at target locus (Collard and Mackill 2008) and to reduce the chances of continuing linkage drags (Hospital 2005). In background selection, the amount of genome of recurrent parent recovered is identified through markers unlinked to target locus.

MABC has been crucial to address drought tolerance in various crops through remarkably improving the precision of selecting desirable recombinants. A number of QTLs were identified for drought and related traits in most crops, but only a few have been recommended for MABC. MABC is a widely accepted strategy to transfer drought tolerant traits, and a notable empirical evidence is available in important food crops like wheat (Elouafi and Nachit 2004, Ameen *et al.* 2013), rice (Mackill *et al.* 1999, Shen *et al.* 2001, Bernier *et al.* 2007), maize (Bolaños and Edmeades 1996, Ribaut and Ragot 2007), chickpea (Thudi *et al.* 2013, Varshney *et al.* 2014), and pearl millet (Hash *et al.* 2003, Serraj *et al.* 2005). Examples also include four QTLs for deeper roots (Shen *et al.* 2001), four QTLs for root thickness and root length (Steele *et al.* 2006), and QTL (*qtl12.1*) related to yield under drought (Bernier *et al.* 2007) in rice, QTL Deeper Rooting 1 (DRO1) for root growth (Uga *et al.* 2013), and QTL (root-ABA1) for root traits and leaf ABA in maize (Giuliani *et al.* 2005, Landi *et al.* 2005). Demonstrating the fast-track introgression using MABC approach, Varshney *et al.* (2013a) have recently transferred a "hot-spot" genomic region (located on LG 4) from ICC 4958 (donor) to JG 11 (recurrent) in chickpea. The "hot-spot" genomic region encompasses a number of significant QTLs governing a broad range of root and drought relevant traits.

Marker assisted gene pyramiding: Pyramiding aims to assemble multiples genes or QTLs into a single genotype (Ashikari and Matsuoka 2006). Pyramiding and introgression of multiple genes/QTLs affecting the same phenotypic trait remains a daunting challenge due to complexity in phenotypic selection methods, and more often, condition is exacerbated owing to epistatic interactions. Large scale genotyping facilities revolutionized MAS in breeding system by considerably reducing the span of breeding cycles and facilitating gene pyramiding (Xu and Crouch 2008). Gene/QTLs pyramiding can be achieved through either of the following schemes: multiple-parent crossing or complex crossing, backcrossing, and recurrent selection. Marker-assisted pyramiding multiple QTLs will be a promising approach to enhance the stability of crops under stress (Richardson *et al.* 2006). The success of marker assisted gene pyramiding depends on multiple factors like the number of gene/QTLs involved, the distance between the QTLs, the number of parents involved or required, MTAs, and the relative cost. The effects of multiple-QTLs have been manifested in a number of crops like wheat, barley, soybean, groundnut, and chickpea (Richardson *et al.* 2006, Li *et al.* 2010, Ravi *et al.* 2011, Wang *et al.* 2012, Varshney *et al.* 2014). To the best of our knowledge, only limited reports were published to date pertaining to pyramiding drought and drought-related QTLs.

Marker assisted recurrent selection: Given the complex inheritance, inter-loci interactions and a strong genotype × environment (G × E) influence, the MABC program might not stand very productive for developing crops tolerant to abiotic stresses (Ribaut and Ragot 2007).

To overcome the limitations routinely faced in the MABC scheme, MARS approach that identifies individuals carrying the most significant marker-effects in each selection cycle was invented (Bernardo 2008). Introduced as a marker-aided modification to the conventional recurrent selection method, MARS represents a molecular breeding scheme that enables a substantial increase in the frequency of several beneficial alleles with additive and small individual effects (Bernardo and Charcosset 2006, Bohra 2013). Simulation and empirical examples of MARS are available for many crops including maize (Semagn *et al.* 2015, http://www.generationcp.org/research/research-initiatives/maize), wheat (Charmet *et al.* 2001), sorghum (Abdallah *et al.* 2009), rice (Grenier *et al.* 2012), soybean (Eathington *et al.* 2007), and chickpea (Varshney *et al.* 2013b). As a logical extension of the bi-allelic MARS, a more sophisticated multi-allelic MARS scheme was proposed by Ribaut and Ragot (2007) to efficiently gather multiple alleles into a single inbred line.

Genomic-wide predictions (GWPs): GS is a new-generation molecular breeding method in which genome wide markers are required so that almost all favourable QTLs, particularly small-effect QTLs influencing the trait, could be efficiently captured (Meuwissen *et al.* 2001, Goddard and Hayes 2007, Cabrera-Bosquet *et al.* 2012). As a prerequisite, high-density genotyping assays are to be in place to exercise GS for crop improvement. According to Nakaya and Isobe (2012), GS is executed in two phases: a training phase that aims to train GS models using individuals scored both genotypically and phenotypically, followed by a breeding phase involving selection from the breeding population. The breeding population does not require any phenotypic recording, nevertheless, the population is assayed with high-density DNA markers (Heffner *et al.* 2009, Cabrera-Bosquet *et al.* 2012). The estimated genomic breeding values (GEBVs) constitute the basis for the selection of superior individuals from the breeding population (Goddard and Hayes 2007, Nakaya and Isobe 2012). GS holds a greater relevance in cases where the phenotyping of a trait-of-

interest (like stress adaptation) demands extensive field screening, cost, and labour (Cabrera-Bosquet *et al.* 2012). Though GS is a reliable and powerful tool, it remains computationally challenging and also entails large-scale and accurate phenotypic screens and an appropriate choice of GEBV prediction methods so that the GEBV estimates can be achieved with the utmost accuracy (Heffner *et al.* 2009). Practical implications of GS for improving drought related traits were discussed in several crops like maize (Ziyomo and Bernardo 2012, Zhao *et al.* 2012), soybean (Shu *et al.* 2013), *etc.* More recently, Semagn *et al.* (2015) have reviewed the progress in developing stress-resilient maize using novel breeding techniques including MARS and GS. Concerning grain yield under drought stress, Beyene *et al.* (2015) recently performed a GWP-based selection across eight bi-parental populations in maize and reported 2- to 4-fold superiority of GS over the traditional (phenotype-based) pedigree method. Evident from an increasing number of simulation and experimental results obtained across different crops, the substantially increased genetic gain per unit time in GS offers compelling opportunities for crop breeders to adopt such genome wide improvement schemes to expeditiously develop stress resilient and high yielding genotypes (Beyene *et al.* 2015, Semagn *et al.* 2015). An elaborated scheme aiming at the quicker availability of resilient cultivars by combining crop breeding with modern "omics" technologies is presented in Fig. 1 Suppl.

As emphasized by Jonas and Koning (2013), technical requirements of GS including the introduction of G × E modeling are to be carefully attended to grasp the full potential of GS scheme in crop breeding. Comparing the prediction accuracy of GE and non GE models in maize bi-parental populations, Zhang *et al.* (2015) observed greater accuracies with GE models, particularly in the case of complex traits. Similarly, bearing in mind the representative environment that encompasses the relevant limiting factors (biotic as well as abiotic), a cautiously created target population of environment (TPE) might greatly aid in measuring the genotype stability (Heslot *et al.* 2015).

Transgenic approaches for genetically manipulating drought tolerance

Diverse sets of genes and metabolites controlling drought related traits have been identified through genomic approaches. As a result of a complex signalling network operating under drought stress, plants overproduce various proteins [transcription factors (TFs), enzymes, molecular chaperones, and other functional proteins] and other metabolites (Song *et al.* 2014). Transgenic strategies to reduce the impact of drought stress involve the over-expression of proteins (TFs and enzymes) and/or over-production of some metabolites. Variety of genes has been genetically manipulated to date in order to validate their functions and roles under drought stress in crops using over-expression or suppression through transgenic

technologies. Multiple families of TFs are considered to be involved in imparting drought tolerance. The role of TF family members, such as dehydration responsive element-binding factor (DREB), basic leucine zipper (bZIP), zinc-finger proteins, and NAM-ATAF-CUC2 (NAC) have already been identified and validated through transgenic technology. These are being used as promising candidates for incorporating drought tolerance in crops, hence are used for the development of transgenic drought tolerant crops (Ariel *et al.* 2007, Anbazhagan *et al.* 2015). The over-expression of bZIP family TFs, ABA-responsive element-binding proteins/factors (AREBs/ABFs) AREB1/ABF2, ABF3, or

AREB2/ABF4 enhances ABA sensitivity and drought tolerance in *Arabidopsis* (Fujita *et al.* 2005, Oh *et al.* 2009). Likewise, the over-expression of CBF3/DREB1A and ABF3 of *Arabidopsis* under the maize ubiquitin promoter (Ubi1P) in transgenic rice increases drought tolerance in rice seedlings showing delayed leaf rolling and wilting under stress (Oh *et al.* 2005).

Concluding remarks and future outlook

Keeping in view the relevance of enhancing drought tolerance in crop plants, a noteworthy progress has been achieved, and an increasing focus is being placed towards accelerating the pace of breeding drought-tolerant cultivars. In the context, large-scale genomics tools particularly based on NGS technologies have emerged as potential additions to conventional breeding (see Bohra *et al.* 2014 a,b). To this end, an increasing number of sequenced crop genomes offer a set of computationally-predicted drought responsive genes to which follow-up studies could be specifically concentrated. For instance, pigeonpea and soybean genomes are predicted to harbour 111 and 109 drought-responsive genes, respectively (see Varshney *et al.* 2012). In addition, ever-accumulating whole genome sequencing and re-sequencing data are likely to shed a new light into the complex and poorly-understood genetic architecture of drought tolerance. Recent examples include a resequencing attempt in maize that intends to reveal SNPs and candidate genes associated with drought tolerance through resequencing whole genomes of 15 inbreds (Xu *et al.* 2014).

Applied recently in barley by Honsdorf *et al.* (2014), the next generation phenotyping assays, such as "The Plant Accelerator", would assist greatly in the precise genetic dissection of drought tolerance. With dramatically evolving genotyping and phenotyping systems and concurrent developments in analytical softwares, refined QTL techniques like meta-QTL mapping that encompasses multiple environments and diverse genetic backgrounds are urgently required to be undertaken (Swamy *et al*. 2011, Almeida *et al.* 2014). Additionally, as it has been demonstrated recently in faba bean, new ways are being provided to enable a more focused interrogation of the environment-defined germplasm sets accompanied by an expeditious incorporation of the newly discovered resilient donors/alleles in genetic improvement schemes (Khazaei *et al.* 2013). It is a well-established fact that even after discovering tolerant genotypes, it remains a daunting task to precisely introgress these beneficial traits into diverse genetic backgrounds; consequently, integrated genomics, physiological, and breeding approaches are imperative to breed stress resilient crops in today's changing climate and shifting agricultural scenario (Mir *et al.* 2013).

Furthermore, the success of network-oriented crop improvement projects also hinges on the regular deposition of the genotyping and phenotypic information/data arising from multiple investigations to readily-accessible global platforms (Ingvarsson and Street 2011, Fridman and Zamir 2012). The new insights thus gained will certainly guide the research community in choosing and optimizing the appropriate breeding protocols (Fridman and Zamir 2012). We hope that with the help of enabling technologies and methodological improvements, tolerant genotypes could be bred with an improved precision and a greater efficiency. And subsequently, the desirable segregants/recombinants could be increasingly recovered at a rate that would not otherwise be possible to attain through routine conventional means.

Combining "omics" tools with breeding activities incorporates drought tolerance in crop genotypes. The Fig. 1 Suppl. illustrates the rational integration of a range of "omics" assays to efficiently examine and utilize the trait variation. The trait-linked DNA markers/QTLs or GWPs can be used to select the drought tolerant candidates. Once the tolerant genotype is identified, it can either be used directly for a large-scale commercial cultivation or re-introduced as donor to the next cycle.

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