9 Drought stress tolerance mechanisms in barley and its relevance to cereals

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Abstract

In the changing environment, water is the major limiting factor for crop productivity throughout the world, and there is every need to generate climate resilient crops. Since drought is a complex phenomenon, we need to dissect various mechanisms at the physiological, biochemical and molecular levels in order to generate crop plants with better drought tolerance but without any yield penalties. Accumulated literature points out that improvement at both source and sink levels are needed to elevate final yields under water deficit conditions. Here, we summarize the current status of plant adaptation mechanisms and the strategies that we need to carve for generating drought stress tolerant crops like barley.

9.1 Introduction

Crop plants have been systematically improved for higher yields under irrigated conditions in the last decade. However achieving yield stability under semiarid and drought prone areas remained to be the key target trait to achieve food security. As most of the elite lines among crop plants do not display high resistance to water deficits, there is a need to improve the genetic potential to develop climate resilient lines. In many parts of the world, drought prone areas and desertification is growing due to limited and altered pattern of rain fall. This has a tremendous impact on global agricultural production. Among various abiotic stresses, drought is the single most important factor limiting crop production worldwide in the arid and semi-arid regions (Boyer and Westgate 2004). Mediterranean regions represent a significant area of barley production, also characterized by a long, hot and dry summer.

Barley is an excellent model plant to study the genetics of drought adaptation, as it is not only an economically important crop, but is also known for its high degree of genetic variability for stress tolerance (Sreenivasulu et al. 2010a). Evaluation of barley genotypes for yield stability under drought stress conditions revealed higher genetic plasticity (Khokar et al. 2012, Kalladan et al., 2013). Understanding the molecular mechanisms of plant abiotic stress response is one of the essential areas in plant sciences to derive strategies to develop stress tolerant cultivars. Plant responses to drought stress are complex and evolved different strategies to alleviate the adverse effects of harsh environments by altering their physiological, molecular and cellular functions (Witcombe et al. 2008, Blum 2009, Verslues and Juenger 2011). The characterization of many stress-related genes has been preceded by isolation of candidate genes through traditional way of cloning genes and to extend the approach to large-scale gene expression studies to study drought stress response (Talamè et al. 2007). Major progress in this field has come from the application of different "omics" (transcriptome, metabolite and proteomics) approaches. Application of high-throughput genomics platforms have gained substantial momentum to unravel stress responses. These strategies enabled to identify key stress regulators by deriving regulatory networks (Friedel et al. 2012). Sequence information of complete genomes of model plants and several crop species has significantly enhanced the ability to identify genes associated with drought tolerance. The fundamental molecular mechanisms that underlie the plant abiotic responses are quite conserved among plant species and therefore the knowledge gained in the model plants can be extrapolated to improve stress tolerance in crop species.

In the recent past, emphasis has been laid to molecular dissection of drought tolerance by unraveling the molecular physiological mechanisms at the whole plant level, implementing various phenotyping technologies, attempting towards quantitative trait loci (QTL) cloning through linkage and association mapping or gene discovery by analyzing contrasting lines using transcriptomics and functional genomics strategies (Fleury et al. 2010). Importance of these multifaceted strategies have been reviewed in parts among several crops of cereals such as maize, wheat, rice and pearl millet (Barnabas et al. 2008, Fleury et al. 2010, Sinclair 2011, Mir et al. 2012, Yadav et al. 2011). However, so far we have not clearly understood the holistic mechanisms for improved seed yield *per se* under terminal drought, which will be the major topic of the current review. Drought stress responses have been well studied in vegetative tissues under short-term stress response but little is known about the situation under long-term stress, and its relevance under terminal drought (Govind et al. 2011, Seiler et al. 2011). Due to lack of sufficient knowledge from barley, we have also consulted the knowledge revealed in other cereal species to decipher and summarize the drought tolerance mechanisms.

9.2 Plant responses to water deficits

Plants adapt several measures against drought stress. Development of thick cuticle, sunken stomata, development of waxy coating on the leaves and stems, reduction in shoot length, increase in root length that helps in extracting soil moisture, modification of leaves to scales formation of seeds with low water content are some of the important measures that plants adopt under stress. Drought tolerance strategies can be subdivided into escape and avoidance (Chaves et al. 2003). While escape mechanisms are attributed to early maturity by promoting early flowering, characters associated with avoidance include enhanced root system, stomatal and cuticular resistance and leaf rolling and unrolling. During avoidance mechanisms, plants maintain high water status by minimizing water loss due to stomatal closure, reduced leaf area and senescence of older leaves. Drought tolerance represents the ability of the plants to survive and metabolically function to reach the reproductive stage.

Tolerance may involve osmotic adjustments, efficient machinery to quench and scavenge the reactive oxygen species (ROS) formed during stress exposure and appropriate source-sink readjustments (Sairam and Saxena 2000, Sreenivasulu et al. 2007). Though plants have efficient protective mechanisms against drought stress, bleaching of pigments and a loss of quantum yield occurs in many C_3, C_4 and CAM plants. Under drought stress, the photosystem I and II complex, calvin cycle and other primary metabolism genes are known to be down-regulated (Figure 1). However, the response depends on the genetic plasticity (variable among species and genotypes), the stage of plant development, severity and duration of stress (Zhu et al. 2005, Harb et al. 2010). Water deficit in the plants also activates accumulation of chemical antioxidants (ascorbic acid and glutathione), and genes associated with antioxidative enzyme machinery (superoxide dismutase, catalase, glutathione S-transferase), synthesis of osmolytes (proline, glycine betaine, trehalose, mannitol, myo-inositol), ion channels and carriers (Sreenivasulu et al. 2007). ‘Responsive to dehydration’ genes encode hydrophilic polypeptides that play a role in protecting cells against drought or salt stress (Shinozaki and Yamaguchi-Shinozaki 1999). Like-wise, several fatty acid metabolism-related genes also repair the stress-induced damage in cell membranes. Thus, such genes appear to be important to maintain the membrane fluidity under drought and temperature stresses.

Almost all plants respond to water deficits by producing the phytohormone abscisic acid (ABA) especially in the root system (Bray 2002). Such an accumulation in the roots may help the plants in enhanced water uptake, due to increased hydraulic conductivity. ABA transport to the leaves help in closure of stomata for reducing the evapotranspiration. This hormone is also involved in the remobilization of stored reserves from vegetative tissues to the grain, during seed filling under drought stress conditions (Yang et al. 2001, Blum 2005). Liu et al. (2005) also showed that under drought stress, ABA has multiple influences on plant development during the reproductive stage.

The sensing of water loss and its response by different plant organs to mediate drought tolerance is regulated through phytohormonal cross-talk (Kohli et al. 2013). Among the prominent alteration in hormonal imbalances, ABA accumulates to very high levels under drought stress exposure and mediates mainly the osmotic and ionic adjustments at cellular level and subsequently reprogram the transcriptome of a cell under stress exposure (Sreenivasulu et al. 2012). Drought stress response is mediated by both ABA-induced and ABA-independent signal transduction (Shinozaki and Yamaguchi-Shinozaki 1999, Sreenivasulu et al. 2007). With the discovery of ABA receptors (components of PYR/PYL receptors) and the downstream component of a type 2C protein phosphatase (PP2C)–SnRK2 protein kinase complex, the ABA signal transduction mechanisms operated through changes in phosphorylation and dephosphorylation events of channel proteins are noted. These events in conjunction with ABA-induced alteration of cytoplasmic Ca^{2+} and H^+ concentrations, leads to closure of K^+(inward), and opening of the K^+(outward) channels. These changes act as signal for stomatal closure during water deficit and also inhibit H^+-ATPase activity resulting in the reduction of membrane potential. Klimecka and Muszynska (2007) pointed out that Ca^{2+}-dependent protein kinases play a pivotal role in drought stress-related signaling pathways. ABA-regulated gene expression during water deficits is mainly mediated ABI5 and ABA-responsive-element binding factors i.e. AREB/ABFs (belongs to bZIP transcription factor family).

9.3 Understanding drought stress response using functional genomics

For a given barley genome of 5.1 Gb, genome-wide physical (95% covered) and genetic maps have been developed and predicted 26K genes which shows significant homology to other plant genomes (The International Barley Genome Consortium). Recent insights of barley genome with emphasis of the post-transcriptional processing (alternative splicing, premature termination codons, abundance of repetitive DNA), high-throughput studies on transcriptome, proteome, and metabolome, the availability of genome-wide knockout collections and efficient transformation techniques gave new insights into the structural and functional genomics to understand stress tolerance.

Drought tolerance is a complex trait which involves many molecular, biochemical, physiological, phenological and whole plant responses that enable plants to withstand stress.

From agricultural point of view, drought tolerance essentially means grain yield (Fleury et al. 2010). Because of its complex nature, drought tolerance has to be dissected at different levels to understand the genetic basis of tolerance mechanisms so that we can develop superior genotypes to cope up with the increasing scarcity of water. An upcoming field in plant biology is a systems biology approach which integrates data from different omics such as transcriptomics, metabolomics and proteomics to identify the molecular targets for crop improvement (Kitano 2002, Salekdeh et al. 2009, Sreenivasulu et al. 2013). Such an integrated approach enables to study different processes at the cellular level to unravel cross talk between different signaling components in mediating stress respnses at different depth tissue/organ/organism (Cramer et al. 2011, Jogaiah et al. 2012). Development of drought tolerant varieties in crops remains far behind compared to other traits due to the diverging drought responses of crop plants across different stages of plant life cycle, (Yang et al. 2010). Currently, plant stress responses are studied using either one or a combination of two approaches, mainly transcript and metabolite analysis.

A comparative analysis of barley leaf proteome as affected by drought stress has been carried out recently by Ashoub et al. (2013). They reported the adaptive response of Egyptian barley land races to drought stress using differential gel electrophoresis (DIGE). They observed alterations in proteins related to the energy balance, transcription, protein synthesis, proteins involved in metabolism and chaperones between the drought tolerant and susceptible lines. Although transcriptome analysis is widely used to study various abiotic stress responses in model plants, a poor correlation of transcripts with protein profiles or enzyme activities urged the need to combine the transcriptomics with other approaches such as metabolomics or proteomics. Many studies demonstrated that drought tolerance mechanisms differ substantially between genotypes. Thus, exploring intra-species variation for various tolerant mechanisms through combinatory approaches such as physiology, biochemical responses, genomics and genetics will identify the important source of material for breeding. In barley, most of these studies addressed only either source or sink using physiological or biochemical or molecular and in some cases a combination of aforementioned techniques. Talame et al. (2007) monitored the expression changes in leaves of barley subjected to slow drying conditions. They noticed that ~10% of all transcripts profiled were either up- or down-regulated in short-term shock or long-term drought stress conditions. Some of the examples related to identification of key genes using transcriptome analysis have been used to create transgenic plants and validated under field conditions, which include a stress responsive *NAC* transcription factor (*SNAC*1) (Hu et al. 2006) and late embryogenesis abundant (*LEA*) genes in rice (Xiao et al. 2007). In barley, a transcriptomic approach has been employed to study spike responses to light and drought stresses (Abebe et al. 2010, Mangelsen et al. 2010), as well as near isogenic lines (NILs) differing in nitrogen mobilization during senescence (Jukanti et al. 2008). Homologous genes of different classes participating in LEA biosynthesis , antioxidative pathways and osmolyte synthesis were identified in transcriptomics experiments comparing wheat lines grown under water-stressed conditions (Aprile et al. 2009, Ergen and Budak 2009, Ergen et al. 2009). Few experiments have reported the use of microarrays for gene expression analysis under water deficit conditions or short duration of drought treatments in barley (Walia et al. 2006, Talame et al. 2007). Unfortunately, some of these studies merely focus on a single genotype during drought stress. Hence, separation of genes associated with drought tolerance from that of drought-responsive genes is not easy. Until now, most of the transcriptomic response has been studied in vegetative tissues and recently few attempts were made to reveal the transcriptome alterations in developing grains to understand the yield stability including barley under drought (Worch et al. 2011). However, its full implications in understanding source-sink relationships are yet to emerge.

9.4 The effect of drought stress on flower initiation, pollen and ovary development

Barley grown in semi-arid and Mediterranean region is often coincided with terminal drought conditions that affect the yield (Ceccarelli et al. 2007). Passioura (1996) recognized that promoting flower development under stress is the most important trait conferring drought tolerance to achieve yield stability. In barley, late flowering phenotypes have been noticed in temperate environments. Early flowering has identified as an adaptation to short growing seasons and as an escape from the drought stress. Several crops exhibit sensitivity to drought during floral initiation and the pre-meiotic differentiation of floral parts (Winkel et al. 1997). Using microarray technique, Guo et al. (2009) monitored the changes in gene expression at the transcriptional level in barley leaves during the reproductive stage. They used the drought-tolerant genotype Martin as well as the sensitive genotype Moroc9-75. They observed that 17 genes were expressed constitutively in drought tolerant Martin compared with susceptible Moroc9-75 under water deficit and control conditions. Further, they observed that seven annotated genes belong to signaling [calcium dependent protein kinase

(CDPK), and membrane steroid binding protein (MSBP)], anti-senescence and detoxification pathways.

Drought may delay flowering induction or cause total inhibition as pointed out by Winkel et al. (1997). In almost all cereals, meiosis (meiotic stage of plants) is highly sensitive to drought and high temperature (Boyer and Westgate 2004). Due to this, final productivity or yield is reduced up to 75% in wheat (Saini and Aspinall 1981, Saini and Lalonde, 1998) and rice (Sheoran and Saini 1996). Severe water deficit causes pollen sterility in several crops like wheat (Saini et al. 1984, Koonjul et al. 2005) due to abnormalities in microsporogenesis. Sterility of pollen is due to reduced supply of carbohydrates to the anthers and reduced activity of vacuolar and cell wall invertases (Sheoran and Saini 1996, Saini 1997, Koonjul et al. 2005, Oliver et al. 2005). Thus, it appears that the signal for pollen sterility in cereals appears to be lowered carbohydrates and reduction in invertase activity (McLaughlin and Boyer 2004).

Besides causing sterility of pollen grains, drought also delays female organ development in maize and other cereals (Damptey et al. 1976, Blum 2000). The ovary has been found to accumulate ABA under prolonged stress (Boyer and Westgate 2004), but it declines once the plants start flowering. Ash et al. (2001) showed that ABA plays a role in the abortion of female flowers. Yang et al. (2001) also point out that ABA accumulation in the reproductive structures under stress conditions may inhibit cell division, abort female flower structures and thus affect grain development. Since water stress causes serious losses of photosynthates, there would be reduced influx of nutrients including carbohydrates to the female reproductive organs (Figure 1). This would ultimately reduce the final productivity in cereals (Makela et al. 2005). The research results of Zinselmeier et al. (1995) revealed that supply of sucrose to the ovaries is important and sucrose can rescue the ovaries from abortion under water deficit conditions. Under drought stress, sucrose may serve not only as a source of substrate for plant survival, but also acts as a signaling molecule (Boyer and McLaughlin 2007). Since several studies revealed that sucrose and hexose transporters as well sucrose partitioning genes were downregulated in female reproductive organs, associated with ovary abortion. This phenomenon up-regulates the genes for the ribosome-inactivating protein (*RIP2*) and phospholipase D (*PLD1*) (McLaughlin and Boyer 2004) triggering senescence and abortion of ovaries. Therefore, such genes are the natural targets for preventing ovary abortion in cereals (Boyer and McLaughlin 2007).

9.5 Carbon partitioning between source – sink tissues under terminal drought

Grain yield in cereals is a result of coordinated activities between source and sink tissues. Under optimal conditions, grain growth or seed yield is generally sink limited (Jenner et al. 1991). Sink strength plays a primary role in grain filling of cereals. Water deficit conditions during terminal drought not only reduced the photosynthesis, but also triggered senescence and shortened grain filling time (Figure 1). Genotypes which possess remobilization capacity, mobilizes stem reserves to the grain filling cite (Yang and Zhang 2006). In cereals like barley, wheat and maize, pre-anthesis stem reserve accumulation affects flower and grain development (Blum 1998, 2000). Cereal crops store excess carbohydrates in the form of soluble sugars or sugar polymers within the vegetative tissues (Davis et al. 2011). They are also capable of storing non-structural carbohydrates in the parenchyma cells of stems surrounding the vascular bundles located within internodes. Stem carbohydrates may be stored as soluble sugars such as sucrose, fructans (as in barley, wheat), or starch which is an insoluble polymers of glucose (Halford et al. 2011). Such a whole-plant carbon partitioning may be necessary to buffer the source-sink interaction which may ultimately help to gain yield stability by providing an alternative source of assimilates, when photosynthetic capacity is limited during the period of drought stress. Accumulation of sugars in the stems may also help the plants to pull water from the soil into the vegetative parts of the plants through adjustment of turgor (Fu et al. 2011). These authors have shown that pre-anthesis accumulation of non-structural carbohydrate reserves in the stem enhances the sink strength of inferior spikelets during grain filling in rice. Reynolds et al. (2011) pointed that optimizing carbon partitioning among vegetative organs (stem) is vital to increase kernel weight. We propose that such a readjustment is based on many interconnecting factors such as photosynthetic efficiency, assimilate competition between organs (newly formed tillers, stem reserve accumulation versus sink strength of developing seeds) and environmental influences such as water and nutrient availability, photoperiod and temperature. The genetic factors controlling partitioning of current assimilates by basipetal and acropetal movement decide the partitioning strategy either to build stem water soluble carbohydrates or to strengthen sink tissues. This eventually decides the fate of seed filling. Newton et al. (2011) also suggested that one way to increase sink strength (developing seed) is through readjustment of non-structural carbohydrates in stems of barley. But, we need to understand in-depth mechanisms about carbohydrate partitioning at the whole plant level, so that we can implement strategies that helps to create better crops.

Nitrogen (N) application at the spikelet differentiation stage improved the pre-anthesis WSC reserves and sink strength in plants. However, under terminal drought, yield losses in cereals are a result of both source and sink limitations. Yield reduction in barley and other crops even with adequate assimilates made available through artificial feeding to developing grain clearly indicates the role of sink activity in determining yield under terminal drought (Westgate 1994). Besides the lower number of endosperm cells being the limiting factor of sink strength, the rate of storage product accumulation and duration of seed filling is also identified as another important target trait to increase grain weight under drought (Sreenivasulu et al. 2012).

9.6 Emphasis of starch metabolism during grain filling under terminal drought

Drought stress affects grain filling in many cereals resulting in reduced grain weight due to impairment in cell division and reduction in starch accumulation (Nicolas et al. 1985). Wallwork et al. (1998) observed changes in endosperm structure and degradation of storage products in the endosperm of barley when exposed to heat stress. Starch being the predominant form of storage product in barley grain, activities of enzymes involved in conversion of sucrose to starch are the major factors determining sink activity and hence crop yield (Duffus 1992). Among various enzymes involved in starch synthesis, sucrose synthase, which catalyses the conversion of sucrose to fructose and UDP-glucose is considered to be one of the important marker enzymes of sink strength in several crops including cereals (Jiang et al. 2012). Its activity was found to be a major determinant of seed filling duration in barley and wheat under both optimal and water deficit conditions (MacLeod and Duffus 1988). A relatively low responsiveness of this enzyme to drought compared to control in maize and in a variety of crops during early grain filling and pollination suggests that its activity may not be a limiting factor for starch synthesis during water limiting conditions (Sheoran and Saini 1996). On the other hand, reduction in the activity of acid invertase, another enzyme involved in the breakdown of sucrose especially during early stages of seed development in barley (Anderson et al. 2002, Sreenivasulu et al. 2004) was pronouncedly inhibited under water limited conditions and also in wheat as well as in maize (Zinselmeier et al. 1995, Dorion et al. 1996). Therefore, fine tuning of different sucrose cleavage pathways as per the requirement in a stage dependent fashion is an important criterion for regulating seed metabolism under drought (Figure 1).

ADP-glucose pyrophosphorylase (AGPase), an important rate limiting enzyme of starch synthesis catalyzing the production of ADP-glucose was found to be negatively affected by drought stress in barley (Seiler et al. 2011), wheat and potato (Geigenberger et al. 1997). Similarly, reduction in activity of this enzyme was also noticed under heat stress in wheat and *in vitro* cultured maize (Ahmadi and Baker 2001). Drought stress did not significantly affect the activity of granule bound starch synthase (GBSS) in wheat when occurred during the initial stages of seed development but was negatively affected in maize kernels (Ober et al. 1991). A reduction in soluble starch synthase (SSS) activity in wheat under heat stress was correlated with reduction in starch accumulation (Keeling et al. 1993); however, it was little affected by drought in maize (Dorion et al. 1996). A notable exception to all the above results was reported in a controlled soil drying experiment carried out by Yang et al. (2003, 2004) in rice and wheat during grain filling. These authors found that activities of sucrose synthase, ADP-glucose pyrophosphorylase, soluble starch synthase and starch branching enzyme were significantly enhanced under moderate drought and was positively correlated with increased rate of seed starch accumulation resulting in better seed weight compared to control but with reduced seed filling duration. Enhanced seed filling under mild drying was attributed to accumulation of ABA which enhanced sink strength and remobilization of stem reserves. Similarly, a role of ABA in seed filling under terminal drought was also shown by Seiler et al. (2011) and Govind et al. (2011). But, terminal drought stress had no effect on the germination of barley seeds, but reduced germination was noticed after the accelerated aging test (Samarah and Alqudah 2011). Worch et al. (2011) monitored the expression patterns of drought stress regulated genes in barley during plant ontogeny, mapped and the location of these genes was incorporated into barley SNP linkage map. They pointed out that domestication and breeding have eroded their allelic diversity in the current elite cultivars being used.

9.7 Nitrogen metabolism in developing seeds of barley under terminal drought and its implications on malting

Another important aspect of terminal drought with respect to grain filling in cereals is altered protein metabolism. Among many factors, seed protein content is the most important one determining the end use of barley for malting. Generally, a low protein content which is usually less than 11.5% is preferred for malting, as high protein content was found to negatively affect both malt extract and beer quality (Weston et al. 1993). Terminal drought and heat stress are known to increase seed protein content of barley, rendering it unsuitable for malting (Savin and Nicolas 1996). A major reason for increased seed protein content observed under water deficit conditions is due to the fact that starch deposition is more sensitive to drought than protein. Hence, increase in protein content observed under drought is not an increase in protein deposition *per se* but rather due to the reduction in starch deposition (Brooks et al. 1982). Among seed storage proteins, prolamin (hordein) constitutes more than 50% of the seed nitrogen in barley and is classified into four groups namely B, C, D and γ based on their electrophoretic mobilities. Among hordeins, the major fraction is constituted by B (70-80%) and C (10-12%) while D and γ are considered as minor (Shewry et al. 1985). Studies on the effect of hordein fractions on malting quality revealed that fractions B and D are negatively correlated (Simic et al. 2007); however, no such correlation was found by Shewry et al. (1980) and Riggs et al. (1983). The negative correlation observed between hordeins and malt extract is attributed to a relatively low starch content of the grain compared to protein and also to the fact that starch granules are embedded into a hordein matrix, thus restricting the access for amylolytic enzymes during malting (Molina-Cano et al. 2000). Further, B and D fractions also reduce the yield of malt extract as they have the tendency to form colloidal aggregates and thus reducing malting quality (Smith and Lister 1983). Therefore, seed protein quality is also important in barley, which is seriously affected under water scarcity.

9.8 QTL studies: contribution of wild barley for designing drought stress tolerant lines

Wild barley lines are relatively tolerant to both biotic and abiotic stresses. Two QTLs, one located on chromosome 2H and another on 5H were identified which increased the relative yield by 17% on average. These two yield QTLs are also associated with heading date (late heading), thus revealing us about the increase in yield potential *per se* but not by drought escape (Nevo and Chen 2010). They also identified QTLs on chromosome 6H and 7H related to drought tolerance at seedling stage from Israeli wild barley. Current elite crop cultivars are characterised by a limited gene pool due to targeted selection in breeding programs, which reduces their capability to cope with stresses. Crosses of cultivated barley and its progenitor *Hordeum vulgare* ssp. *spontaneum* with favourable effects of the wild barley have the potential to provide alleles with increased drought stress tolerance. To explore intra-specific genetic variation for thousand grain weight (TGW) and seed starch content under terminal drought during the seed filling period, we used a panel of ca. 150 accessions, which included wild relatives, elite lines, several parents of mapping populations and the introgression line population (BC_3) generated from crossings of elite cultivar Brenda with the wild barley

Hordeum vulgare subsp. *spontaneum* accession 584 (HS584) as donor parent. Drought tolerance screening trials were performed in both green house and field conditions in two independent years. From the subset of panel containing breeding lines and gene bank accession, haplotype variation was uncovered in the genes encoding sucrose synthase (types I and II) and starch synthase from 17 candidates of starch biosynthesis/degradation genes. The lines which showed dramatic reduction of starch content under terminal drought possess haplotypes H3 (Hv32), H4 (Hs3, Hs5, Hv10) and H5 (OWB-DOM, Hv29, Hv30) from sucrose synthase II gene and lines possessing haplotype H6 correlate positively to optimum starch accumulation under both control and drought treatments (Worch et al. 2011). We have reported a first step towards the identification of favourable wild barley alleles by constructing a genetic map consisting of drought regulated ESTs as the basis for the creation of a combined linkage map (Worch et al. 2011). A total of 28 major QTLs (LOD score ≥ 3) were detected with hot-spot QTLs for improved yield/TGW and starch under terminal drought on chromosomes 1H ($DTY_{1.1H,}$) and 2H ($DTY_{2.1H}$) from the BC_3 doubled haploid introgression lines (ILs) of *Hordeum spontaneum* 584 (BC_3 DH Hs584) populations (Kalladan et al. 2013). Better performing ILs with Hs584 introgression in a hot spot QTL region (responsible for minimized yield loss under terminal drought) and sensitive ILs (with a severe yield penalty under terminal drought) have been selected to study differential responses to drought stress by transcriptome, metabolite and enzymatic analyses (Sreenivasulu et al. 2010a,b).

9.9 Transgenic approaches for generating drought stress tolerant lines

Various stress responsive genes identified in the past have been subjected to generate transgenic lines in barley and other cereals. Morran et al. (2011) generated transgenic barley plants expressing the *TaDREB2* and *TaDREB3* transcription factors, with both constitutive and drought stress-inducible promoters and they obtained resistance to drought stress. Expression of other *CBF/DREB* genes, together with a large number of stress-responsive late-embryogenesis abundant/cold-regulated/dehydrin genes also displayed tolerance to water stress (Sakuma et al. 2006). Expression of *HvHVA1* in rice confers dehydration tolerance via maintaining cell membrane stability (Babu et al. 2004) while transgenic overexpression of *HvCBF4* in rice resulted in an increase in tolerance to drought, high salinity, and low temperature without stunting growth (Dong et al. 2006). The barley *HvCBF4* gene was overexpressed in transgenic rice which resulted in increased tolerance to low temperature, drought, and high salinity (Oh et al. 2007). In contrast, in a different rice cultivar, the

HvCBF4 transgene caused enhanced survival to drought, but not to high salinity or cold stress (Lourenço et al. 2011). The AREBs/ABFs can bind to an ABA-responsive (ABRE) *cis*-acting element and *trans*-activate downstream gene expression. AREB/ABF-overexpressing barley plants show ABA hypersensitivity and enhanced tolerance to freezing, drought and salt stress (Furihata et al. 2006). Overexpression of the barley *HVA1* gene in rice led to increased stress tolerance under dehydration stress, compared with the control plants (Rohila et al. 2002). Further, the barley *HVA1* gene under control of a stress-inducible promoter *rd29A* could effectively negate growth retardation under non-stress conditions and confer drought stress tolerance in transgenic mulberry (Checker et al. 2012). From wild barley, the transcription factor *HsDREB1A* has been isolated and incorporated into *Paspalum notatum* using the barley *HVA1* promoter (James et al. 2008). Similarly, *DREB*-like genes were isolated from several species of cereals like rice and wheat and overexpressed in heterologous systems. The results revealed that the transgenics were more drought tolerant compared to their corresponding controls (Hosington and Ortiz 2008, Perera et al. 2008). Another transcription factor identified using transcriptomics under drought is nuclear transcription factor Y subunit B-1 (*NF-YB1*) and the increased drought tolerance contributed by this transcription factor was validated both in *Arabidopsis* and maize through transgenic approach. The transgenic maize line over expressing *ZmNF-YB2* contained higher stomatal conductance and photosynthesis resulting in improved yield due to enhanced drought tolerance (Nelson et al. 2007). Transgenic plants overexpressing these genes were found to have increased drought tolerance under green house conditions. These physiological assessments of transgenic plants for short-term drought survival under glass house experiments are not sufficiently conclusive. Therefore, performance of these wide array of transgenic lines has to be subjected for long term drought stress assessment under field trials to determine yield stability.

9.10 Conclusions

It is of paramount importance to understand the basic physiological and molecular mechanisms underlying drought stress tolerance from the untapped germplasm to develop climate resilient lines. It is essential to identify physiological or biochemical traits that are robust, which can enhance the selection of barley breeding lines, so as to use them in drought prone areas. Terminal drought being a complex game, affects a plethora of genes in source and sink tissues that encode enzymes associated with chlorophyll metabolism, photosynthesis, carbohydrate metabolizing pathways (glycolysis, TCA cycle), besides protein degradation and nitrogen metabolism including amino acid transport (Figure 1). Drought tolerance of a species is ultimately measured in terms of seed yield and thousand grain weight. Grain filling in barley and other cereals depends on the potential of carbon synthesis/storage, partitioning and transport from (1) source to the grain from current photosynthesis and (2) efficient remobilization from reserve pools such as vegetative tissues like stems. Occurrence of drought stress during anthesis and onset of caryopsis development is a very critical factor resulting in impaired grain set, reduced grain weight and yield loss. This is thought to be at least partly due to a decrease in photosynthetic efficiency and changes in remobilization processes. Ovaries of cereals are normally loaded with glucose and starch on the day of pollination under control conditions (McLaughlin and Boyer 2004). The cell wall and soluble invertase enzymes that metabolize sucrose lose their activity when the delivery of photosynthate is curtailed at low water potentials. Under these conditions, previously accumulated starch is consumed through the activation of amylases (Ruan et al. 2010), resulting often in seed abortion. An alternative source of assimilates are stem reserves stored in the form of sugars, starch or fructans, which constitute a buffer in case the source capacities are good alternates. These reserves are readily utilized for grain filling under assimilate reduction, when drought occurs during the peak of seed filling in wheat, rice (Yang and Zhang 2006), and barley (our unpublished results). To create a crop plant that can withstand terminal drought and reduce yield gap, we need to integrate breeding programs, genomics, and systems biology. It is therefore necessary to initiate linking of these complex physiological and biochemical processes to fine tune source-sink relationships.

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Figure 1: Unraveling molecular-physiological consequences of drought stress responses in cereals to design climate resilient yield enhancer lines through target trait improvement.