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Studies on İdentification of the Drought Tolerant Genes of Wheat

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ABSRACT

Bread wheat (Triticum aestivum L.), is known as the base food staple resource. With increasing the human population, new methods and approaches are needed to gain wheat cultivars with advanced characteristics. Nowadays, the problem is to produce high quality and yielding cultivars. Breeding of tolerant cultivars against biotic and abiotic stresses is desired. Drought stress, appears as water loss from the plant during the definite time, which is higher than the absorbed water by plant from the environment. Drought stress is expected to increase as the most important stress factor in the future due to the climate changes which is being evident from now. Lower cell growth would lead to the deficit in cell wall synthesis and thereby, to the unexpanded leaves and lowering the photosynthetic assimilates. Under drought stress, seed germination potential declines, and chlorophyll and protein synthesis, photosynthesis, and respiration is negatively affected. In winter wheat, resistance against drought stress is controlled by complex morphologic and physiologic mechanisms. In the recent century, despite of using classic breeding methods and gaining the high quality yielding cultivars, desirable tolerance towards environmental abiotic and biotic stresses mainly disease and pests, has not been achieved. In this study, some mechanisms of drought tolerance in wheat were considered.

1. Introduction

Deficient in water content during each season and under unusual conditions is referred to as drought stress. Drought stress appears uncertainly and reduces productivity of living creatures especially plants (Eser et al. 2000). According to Blum (1988), drought tolerance is divided into three categories: 1-escape from drought: ability of plant to get the physiological maturity before onset of the drought period, 2- drought avoidance: resistance against drought stress by reserving water in different growing parts and reducing water loss from the plant, 3- tolerance against drought: plant survives over drought periods by lowering water potential in their different tissues to maintain economic yield.

Selection Features for Drought Tolerance

Many morphological, physiological, and biochemical features have introduced for improving drought tolerant cultivars of winter cereals.

1-Morphological features: these are the oldest and even fastest features used in drought tolerance selection. Having awns, hairy tissues, small and narrow leaves, thick leaves, cuticular layers, deep and numerous roots, raising of root to stem dry weight ratio, number and distribution of stomata, long coleoptiles, twisted leaves, length of being green leaves, and the length of the upper internode, have been related to the drought tolerance from the past.

2- Physiological features: along with the morphological features, include more complicated tests:

Leaf water content: adsorbed water by the roots of the drought tolerant cultivars is retained higher for longer periods rather than drought sensitive ones.

Water potential: water potential is of the most important indices for drought tolerance. Water potential under drought stress varies between -0.2 to 0.6 bars while this range for dry area plants tends to be -2 to -5 bar.

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Plant temperature: plant temperature could be lowered by the water evaporation from the surface, stomatal conductivity and better adaptation efficiency.

3- Biochemical features: some changes might be appeared in metabolic activities under drought conditions like abscisic acid and proline accumulation, as the biochemical events.

Wheat Breeding Methods

Introducing, selection, crossing, mutation, and polyploidy are the wheat breeding methods. Two important topics that might lead to succession appear in breeding efforts during the application of the mentioned methods: these are variation and selection. Variation, which could be introduced spontaneously by small changes over the years, also might be artificially produced by crossing, mutation, and polyploidy. Till now, many deficiencies have been appeared despite of introducing of thousands of improved cultivars. These deficiencies are removed by modern biotechnology and genetic engineering. For example, obligated crossing between species and genus for gene transferring is removed and unfavorable gene transfer to the progeny caused by the linkage, no more tends to be a problem. *In vitro* selections provide cell scale selection rather than the whole plant (Özgen et al. 2005). Despite of achieving many high yielding cultivars by using classic breeding methods in the past century, tolerance against some biotic and abiotic stresses such as pests and diseases, have not yet been gained.

Drought-Related Genes and QTLs in Wheat and Barley

Tolerance against drought is a quantitative trait, with a complex phenotype, often confounded by plant phenology. Plant responses to drought are also influenced by the time, intensity, duration, and frequency of the stress as well as by diverse plant–soil–atmosphere interactions (Saint Pierre, 2012). Past efforts to develop drought-resistant crop cultivars by traditional breeding were hampered by low heritability of traits such as yield, particularly under drought, and by large "genotype × environment" interactions (Passioura, 2012; Langridge and Reynolds, 2015).

Drought stress induces a range of physiological and biochemical responses in plants. These responses include stomatal closure, repression of cell growth and photosynthesis, and activation of respiration. Plants also respond and adapt to water deficit at both the cellular and molecular levels, for instance by the accumulation of osmolytes and proteins specifically involved in stress tolerance (Shinozaki and Yamaguchi-Shinozaki, 2007). A research programme for increasing drought tolerance of wheat should remove the problem in a multi-disciplinary approach, considering interaction between multiple stresses and plant phenology, and integrating the physiological dissection of drought-tolerance traits and the genetic and genomics tools, such as quantitative trait loci (QTL), microarrays, and transgenic crops.

However, recent advances in molecular and genomic tools, have enabled the identification of quantitative trait loci (QTLs) and diagnostic DNA markers in a wide range of crops, with the promise of accelerating crop improvement toward future challenges (Salvi and Tuberosa, 2015; Merchuk-Ovnat et al. 2016). Most QTLs for drought tolerance in wheat and its close relative barley, have been identified through yield and yield component measurements under water-limited conditions (Maccaferri et al. 2008; Mathews et al. 2008; von Korff et al. 2008; McIntyre et al. 2009). Agronomic yield under drought-stressed conditions is affected by both constitutive QTLs, i.e. QTLs affecting yield irrespective of environmental conditions, and drought-responsive QTLs, i.e. QTLs affecting yield only under drought conditions (Collins et al. 2008).

QTL mapping is a widely accepted approach to dissect quantitative traits into their single genetic determinants and relating phenotypic differences to their genetic basis (Collins et al. 2008). Recent advances in genome mapping and functional genomics technologies have provided powerful new tools for molecular dissection of drought tolerance (Worch et al. 2011). During the past decade, a large number of studies involving linkage mapping have been conducted in several crops to identify QTLs linked to drought tolerance (Cattivelli et al. 2008; Fleury et al. 2010).

Traditional QTL mapping involves: (1) development of mapping populations segregating for drought tolerance related traits, (2) identification of polymorphic markers, (3) genotyping of the mapping populations with polymorphic markers, (4) construction of genetic maps, (5) precise phenotyping for drought tolerance-related traits, as mentioned above, and (6) QTL mapping using both genotypic and phenotypic data. This process is commonly called linkage mapping/linkage analysis-based QTL mapping (Chamarthi et al. 2011; Rouf Mir et al. 2012).

In summary, 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 (Rouf Mir et al. 2012).

Drought-Related Gene Identification by "Omics"

Emergence of OMICS techniques including transcriptomics, proteomics, metabolomics, and ionomics have helped to identify and characterize the genes, proteins, metabolites, and ions involved in drought signaling pathways (Budak et al. 2015). The tools of genomics offer the means to produce comprehensive data sets on changes in gene expression, protein profiles, and metabolites that result from exposure to drought.

"Omics" studies were also performed to monitor dehydration induced transcripts and proteins of bread and durum wheat cultivars with differing sensitivities to drought, both in stress and non-stress conditions. Methodologies used in transcript profiling studies range from cDNA microarrays to cDNA-AFLP (amplified fragment length polymorphism). For differential protein identification, the common procedures used include 2D (2-Dimensional) gels, various chromatography techniques, and mass spectrometry (Budak et al. 2013). Despite the existence of common regulatory mechanisms across species, the conservation of the molecular response to dehydration across experiments (Mohammadi et al. 2007; Aprile et al. 2009) is low due to variation in stress dynamics, stage of development and tissue analyzed.

Molecular Mechanisms of Drought

Drought signaling is categorized into ABA-dependent and ABA- independent pathways as ABA is the first line of defense against drought (Budak et al.,2015). ABA-dependent signaling consists of two main gene clusters (regulons) regulated by ABA-responsive element- binding protein/ABA-binding factor (the AREB/ABF regulon) and the MYC/MYB regulon. The ABA-independent regulons include the CBF/DREB (cold-binding factor/dehydration responsive element binding), NAC, and ZF-HD (zinc-finger homeo domain) (Lata and Prasad, 2011). The transcriptional regulatory network based on DREBs is induced by dehydration in wheat. There are two known DREB regulons; DREB1/CBF and DREB2 (Edae et al.2013; Budak et al. 2015).

The wild populations that adapt to drought environments are expected to have genes or alleles for drought and salt tolerance, as well as associated, partly regulatory markers such as AFLPs or SSRs (Nevo and Chen, 2012). Drought tolerance genes and/or QTLs could be cloned and transferred to increase crop tolerance (Araus et al., 2003).

Importance of Triticum dicoccoides in Wheat Domestication and Breeding

Wild emmer wheat (*T. turgidum* ssp. *dicoccoides* (k¨orn.) Thell) is the tetraploid ($2n= 4x= 28$; genome BBAA) progenitor of both domesticated tetraploid durum wheat (*T. turgidum* ssp. *durum* (Desf.) MacKey) and hexaploid ($2n= 6x= 42$; BBAADD) bread wheat (*T. aestivum* L.) (Dong et al. 2009; Budak et al. 2013). Wild emmer wheat, being a potential reservoir of drought-related research, has been the source of several identified candidate drought-related genes with the development of “omics” approaches in the recent decades. The wild emmer gene pool offers a rich allelic repertoire required for wheat improvement of agronomically important traits such as drought tolerance (Nevo and Chen, 2010; Peleg et al. 2005, 2008), grain protein content (Uauy et al. 2006) and grain mineral concentrations (Cakmak et al. 2004).

Marker-assisted selection has been shown to be effective for the introgression of favorable genes/QTLs, conferring primarily disease resistances (reviewed by Peng et al. 2012) from wild emmer wheat to domesticated germplasm. Marker assisted selection has also been used to transfer genes/QTLs conferring several agronomic traits to the domesticated gene pool, including Na^+ exclusion (Munns et al., 2012), plant height (Lanning et al. 2012), tillering (Moeller et al. 2014), spike branching (Zhang et al. 2012), epicuticular wax (Miura et al. 2002), heading time (Tanio and Kato, 2007), and kernel hardness (Lesage et al. 2012). It has been shown that in 58 QTLs out of 110 mapped QTL, the wild emmer allele showed an advantage over the domesticated one (Merchuk-Ovnat et al. 2016).

In recent reports, TdicTMPIT1 (integral transmembrane protein inducible by Tumor Necrosis Factor-α, TNF-α) was cloned from wild emmer root tissue and shown to be a membrane protein which is linked to the response against drought stress, showing increased levels of expression, distinctly in wild emmer wheat during osmotic stress (Lucas et al. 2011a). In another research, it was shown that TdicDRF1 (DRE binding factor 1) which is conserved between crop species, was cloned for the first time from wild emmer wheat. DNA binding domain of this plant, AP2/ERF (APETALA2/ethylene-responsive element binding factor), was shown to bind to drought responsive element (DRE) using an electrophoretic mobility shift assay (EMSA). It was revealed to exhibit cultivar and tissue specific regulation of its expression, through mechanisms involving alternative splicing (Lucas et al. 2011b; Budak et al. 2013). Wide genetic diversity has been found both between and within populations in most variables, under both well watered and water-limited treatments (Peleg et al. 2005). The rich genetic resources are important for gene mapping and gene transfer (Xie and Nevo 2008).

Improved Drought Stress Tolerance in Plants via Gene Transfer

While QTLs can be deployed in crop improvement through molecular breeding, candidate genes are the prime targets for generating transgenics using genetic engineering (Varshney et al. 2011). Realistic experimental protocols to screen for drought adaptation in controlled conditions are crucial if high throughput phenotyping is to be used for the identification of high performance lines, and is especially important in evaluation of the transgenes where stringent biosecurity measures restrict the frequency of open field trials. Dehydration-responsive element binding (DREB) transcription factors have been reported to enhance drought resistance in transgenic plants including tomato, peanuts, rice, barley, and wheat (Pellegrineschi et al. 2004; Oh et al. 2005; Bhatnagar-Mathur et al. 2007; Wang et al. 2008; Xiao et al. 2009; Morran et al. 2010). While many reports have demonstrated increased drought resistance in DREB transgenic plants under laboratory and greenhouse conditions in several crops (Dubouzet et al. 2003), very few studies have tested the performance and productivity of transgenic lines in the field (Xiao et al. 2009; Yang et al. 2010).

Field performance of 14 transgenic wheat lines previously selected under greenhouse conditions for survival to severe drought (SURV) and high water use efficiency (WUE) was evaluated by Saint pierre et al. (2012). The objective of the study was to assess biomass production (BM) and yield performance (YLD) of transgenic events relative to control lines under different water regimes in field conditions. Selected transgenic wheat lines have shown drought stress signs later than control ones. Greenhouse experiments after severe drought stress have confirmed transgenic plants superiority in terms of surviving under these conditions. In addition, selected lines for water use efficiency (WUE), have been identified as being acceptable for combination of yield gaining (and high yielding in case of sufficient irrigation) and keeping stable performance.

Abscisic acid biosynthesis, catabolism, and signaling plays an important role over stress period (Krannich et al. 2015). In addition, ABA acts as adaptive responses and improves seed maturation, dormancy, and senescence during environmental stresses like drought (Finkelstein 2013; Miyakawa et al. 2013). It has been shown that barley originated ABA-sensitive gene (*HVA1*) transferred to wheat, has improved growth under limitations of soil water content. Comparing to the non-treated control plants, higher seed yield and plant biomass in transgenic ones has been achieved.

Ethylene, as a gaseous plant hormone has been identified for improving many growth stages along with being in close relations with biotic and/or abiotic stress responses such as stomata closure mechanism. Ethylene biosynthesis and its signaling ways are considered as plant responses to different abiotic stresses like salinity and water deficit. Identification of genes relating to the stress responses is useful for drought tolerant plants breeding (Krannich et al. 2015). Cellular functions such as osmotic active solutes (osmolytes), proteins, and enzyme activities are required for drought responses. Proline, glycine, betaine, and trehalose are among different osmolytes which have been found to be in relation with salinity and drought stress events. Different enzymes involving the metabolism of these osmolytes, seems to be potential candidates for breeding drought tolerant plants (Burg and Ferraris 2008). Oxygen radicals and H_2O_2 form reactive oxygen species (ROS) are produced during drought conditions and results in cell damage and/or membrane injuries (Krannich et al. 2015) and enzymatic activities like catalase and superoxide dismutase could alleviate their negative effects facing environmental stresses mainly in case water deficit.

The accumulation of osmolytes during stress is well documented. Recent studies have demonstrated that the manipulation of genes involved in the biosynthesis of low–molecular-weight metabolites, such as proline, have improved plant tolerance to drought and salinity in a number of crops (Zhu et al. 2005). Initial attempts to obtain transgenic plants over expressing proline employed vector constructs with the *P5CS* gene linked to a constitutive promoter, such as CaMV 35S (Zhang et al. 1995; Sawahel and Hassan, 2002). In an experiment, Zhu et al. (1998) used a 49 bp ABA-responsive element from barley *HVA22* gene fused to a 180 bp rice actin 1 minimal promoter and the hva22i element to obtain a stress-inducible promoter (AIPC–ABA-inducible promoter complex). This promoter was used to increase the level of P5CS in transgenic rice plants, which led to a drought and salt-induced accumulation of the proline content and increased tolerance to both stresses (Zhu et al. 1998; Su and Wu, 2004).

(Gruszka et al. 2007) evaluated transgenic wheat plants expressing a heterologous *P5CS* gene controlled by the stress-inducible promoter AIPC under water deficit. They reported that the effects of water deficit on wheat plants transformed with the D1-pyrroline-5-carboxylate synthetase (P5CS) cDNA of *Vigna aconitifolia*, encodes the key regulatory enzyme in proline biosynthesis, under the control of a stress-induced promoter complex—AIPC. It was cleared that drought stress, resulted in the accumulation of proline and the tolerance to water deficit observed in transgenic plants was mainly due to protection mechanisms against oxidative stress and not caused by osmotic adjustment.

Previous works with model transgenic plants has shown that cellular accumulation of mannitol can alleviate abiotic stress. Abebe et al. (2003) found that ectopic expression of the *mtlD* gene for the biosynthesis of mannitol in wheat improves tolerance to water stress and salinity. They evaluated tolerance to water stress and salinity using calli and T2 plants transformed with (+mtlD) or without *mtlD* (-mtlD). They showed that fresh weight of -mtlD calli was reduced by 40% in the presence of polyethylene glycol and 37% under NaCl stress. Furthermore, they found that growth of +mtlD calli was not affected by stress. In their experiment, it was identified that the amount of mannitol accumulated in the callus and mature fifth leaf (1.7–3.7 µmol/g fresh weight in the callus and 0.6–2.0 µmol/g fresh weight in the leaf) was too small to protect against stress through osmotic adjustment. In other words, the improved growth performance of mannitol-accumulating calli and mature leaves is due to other stress-protective functions of mannitol.

MYB type proteins are produced during the different plant growth stages and expressed as stress responses. Zhang et al. (2012) have identified *TaMYB30* gene which encodes R2R3-type MYB protein expressed under polyethylene glycol (PEG) stress in wheat. Three homologous sequences (*TaMYB30-A*, *TaMYB30-B*, and *TaMYB30-D*) of *TaMYB30* gene expressed under PEG stress conditions in haploid barley, have been distinguished with equal expression levels from which, *TaMYB30-B* gene was selected for the investigation. Over expression and increased tolerance to drought stress in germination and seedling periods, have been studied in transgenic *Arabidopsis with TaMYB30-B gene*, in details. Furthermore, over expression of this gene has changed the levels of the expression and physiologic responses of other stress-related genes. All of which increase plant tolerance against stress. Also improvement in wheat drought signaling and tolerance by transgenic approaches were done by Bahieldin et al.,2005; Wang et al. 2006; Gao et al. 2009; Saad et al. 2013 and Fehér-Juhász et al. 2014 (Budak et al. 2015).

2. Results and Discussion

Some QTL's for Morpho-physiologic features and yield under drought conditions have been found using parental lines different in responses to drought stress and preparing linkage maps.

In recent years, high-throughput screening applications, through "omics" strategies, were found several candidate genes using Triticum species with different ability to resist drought.

Drought response analysis is complex and difficult process in the absence of wheat genomic sequence data.

With advances in sequencing technology in recent years, the bread wheat genome sequence is almost complete, whereby determination of the drought tolerance alleles of wild germplasms is possible. "OMICS" strategy is particularly involved in drought research because the response to osmotic stress has not only genetic base, also known to be arranged in post-transcriptional and post-translational stages. Furthermore, recent developments in genetic technology will make possibility of the regulation and manipulation of drought-resistance.

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