Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-6: 653-671

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-6 pp. 653-671**
Journal homepage: http://www.ijcmas.com

Original Research Article

Comparative Physio-Biochemical and Transcriptional Profiling of two Contrasting Accessions of Foxtail Millet (*Setaria italica* L.) in Response to Water Stress Tolerance

Dipti Gawai[1], Mangesh Moharil[1*], Pravin Jadhav[1], Santosh Gahukar[1], Mahendra Dudhare[1] and Suprasanna Penna[2]

[1]Biotechnology Centre, Dr. Panjabrao Deshmukh Agricultural University, Akola, Maharashtra-444104, India
[2]Nuclear Agriculture and Biotechnology Division, BARC, Mumbai, India
**Corresponding author*

A B S T R A C T

Foxtail millet (*Setaria italica* L.) is an important food and fodder grain crop in arid and semi-arid regions of Asia and Africa and is mostly tolerant to abiotic stresses. In an attempt to understand the molecular basis of water-stress response in foxtail millet, physio-biochemical assays and cDNA -based gene expression studies of some drought specific genes was carried out in two contrasting accessions of foxtail millet viz. tolerant IC97189 and susceptible IC97109. The comparative rates of relative water content, leaf water potential, proline, carbohydrates and antioxidant enzymes were found steadily increased, while total chlorophyll content was decreased in leaves of water stressed over unstressed foxtail millet accessions. The comparative transcript responses to water stress were monitored by cDNA-based gene expression profiling. The drought responsible transcriptional factors like DREB1, DREB2, Aquaporins and C_2H_2 showed considerable difference gene expression. Understanding drought tolerance at molecular level particularly identification of relevant genes in foxtail millet is likely to pave the ways for mitigating the drought stress losses in major crops. The study also helps in better understanding of physio-biochemical and molecular mechanisms utilized by plants during adverse growth conditions and define the biomarkers of tolerance against stresses. Further exploiting these traits can enhance the utility of foxtail millet for dry and neglected lands and would also be further useful in development of stress tolerant cultivars.

Keywords

Water stress, cDNA-profiling, Antioxidant analysis, Foxtail millet, Expression analysis, Semiquantitative RT-PCR

Introduction

Worldwide, abiotic stresses adversely affect the crop productivity and quality. Among the various stresses, drought is a major threat to agriculture. Approximately, 70% of yield reduction has been estimated as a direct result of abiotic stresses (Acquaah, 2007). The magnitude of these effects depends on its impact on the plant physiological, biochemical, as well as molecular biological processes and the ability of plant to adapt drought stress (Bulbotko, 1973; Atkinson *et al.*, 2000; Massonnet *et al.*, 2007).

The various adaptive strategies developed by plants to alleviate the adverse effects of these stresses are by altering their molecular functions. Plants also adjust their physiology

and biochemical pathways so as to cope with a period of water deficit (drought). Many features associated with water tolerance are reflected at the cellular level. High relative water content and leaf water potential is reported as the resistant mechanism to drought (Ritchie, 1990). At biochemical level, the improved complex mechanisms for drought adaptation are generally associated with osmoregulation adjustment by using some osmotic regulators such as proline, change in cell membrane permeability, soluble protein content, percent soluble sugar, etc., ultimately affecting photosynthesis and thus the chlorophyll content (Parvaiz and Satyawati, 2008). These responses originate from changes in the gene expression and subsequent action of their gene products. However, the underlying molecular mechanisms for adaptation to water stress in foxtail millet mostly remain unclear (Sakamoto *et al.*, 2008; Lee *et al.*, 2009).

Foxtail millet (*Setaria italica* L.), an elite drought-tolerant crop, is one of the oldest cultivated millet crops serving as food grain in Asia and as forage in America, Australia and Africa ranking second in the world's total production of millets after pearl millet. India ranks second after China in the world in small millet production with Tamilnadu and Andhra Pradesh as the leading producers. The other major attributes of foxtail millet like its small genome (1 C ~ 515 Mb; $2n = 2x = 18$), low amount of repetitive DNA, a highly conserved, inbreeding nature and short life cycle, makes it an excellent experimental model in studying abiotic stress tolerance system (Doust *et al.*, 2009; Li and Brutnell, 2011; Lata *et al.*, 2013). Unfortunately, the crop has remained neglected with little or no importance in today's world and has hence lagged behind in genetic and molecular studies.

The water stress-mediated tolerance in plants is a complex quantitative trait regulated by a large number of up- and down-regulated genes and involves various signalling pathways. The biological differences among the genotypes used, plant growth conditions, stress treatment conditions and their detection methodologies may result in variation in extent of stress adaptive mechanism.

The cDNA-profiling is a cost-efficient technique and does not require specialized expertise to handle data as in other highly technical activities.

Comparison of gene expression profiles between contrasting genotypes can provide much information in understanding the spatial and temporal patterns of gene expression required for abiotic stress tolerance. Accordingly, the objective of this study was to assess the physio-biochemical changes associated with the foxtail millet under water stress, and to identify the key genes that were differentially expressed at early growth stage.

Materials and Methods

Plant material and stress treatments

Induced water stress experiment

Setaria italica L. accessions IC97189 (tolerant) and IC97109 (susceptible) were grown on soil in controlled environment under 16h light at 30˚C/ 8h dark at 25˚C regime and 60% relative humidity. Plants were watered twice a week up to a period of 3 weeks with nutrient solution up to a period of 21 days.

Drought was induced by withholding water in experimental plants while the control (hereafter called as 'unstressed') plants were

regularly watered. The physiological measurements were taken from unstressed and experimental stressed plants after 9th day of drought induction. Figure 1 depicts the illustrations for sample stage selection in foxtail millet under water stress for physiological, biochemical and molecular studies.

Physio-biochemical attributes

Physiological parameters for drought stress viz. relative water content, total chlorophyll content and leaf water potential were analyzed in stressed and unstressed plant samples in 3 replications.

Relative water content (RWC):

Relative water content of flag leaf was calculated as described by Barrs (1968). Dry weight (DW) was measured after oven-drying for 48 hours and RWC was calculated using the equation,

RWC (%) = [(FW-DW) / (TW-DW)] x 100

RWC index was calculated as,

RWC of stressed plant/RWC of control plant x 100

Leaf water potential (Ψ_L)

The leaf water potential was measured using the WP4 Potentio meter. Measurement was taken by taking three fully expanded leaves per plant. LWP index was calculated as,

Mpa of stressed plant/ Mpa of control plant x 100

Estimation of chlorophyll content

Chlorophyll content index (relative chlorophyll value) was measured by using an Opti-Sciences CCM-200 at random points. Prior to each series of measurements, the instrument was calibrated with a blank chamber. Chlorophyll Content Index was expressed as SPMR (SPAD chlorophyll meter reading) and readings are said to correlate positively with Chl *a* content extracted with 90% acetone (Biber, 2007).

CC index was calculated as,

CC of stressed plant/CC of control plant x 100

Statistical analysis

The mean values worked out from the measurements recorded on five randomly selected plants used for statistical analysis. The data collected was analyzed using analysis of variance (ANOVA) technique. IQ Macros in Excel 2007 software package was used for this purpose.

Biochemical attributes

Sample collection, preparation and enzyme extraction

After the induced water stress experiment, samples were individually collected after 9th day of imposing drought to 21 d old seedling, labelled in plastic bags and immediately frozen using dry ice and later transferred to (minus) -80°C for storage until further use.

For protein and antioxidant enzyme assays, samples were ground to a fine powder with liquid nitrogen and were extracted with Phosphate buffer.

Protein determinations

Protein concentration in the extracts was quantified at 660 nm as described by Lowry

et al., (1951) using bovine serum albumin as a standard (Merck, fraction V).

Non enzymatic assays

Free proline content

Free proline content was determined at 520 nm by ninhydrin method according to the procedure of Bates (1973).

Total carbohydrates

The phenol sulphuric acid method was used to estimate total carbohydrates (Dubois *et al.,* 1956).

Starch

Estimation of Starch was done by using anthrone reagent as described by Hodge and Hofreiter *et al.,* (1962).

Enzyme assays

Activity of Superoxide dismutase

SOD activity was estimated by recording the decrease in absorbance of the enzyme as described by Dhindsa *et al.,* (1980).

Activity of Catalase

The catalase activity was determined according to Luck (1974).

Activity of Peroxidase

The method proposed by Reddy *et al.,* (1995) was adopted for assaying the activity of peroxidase.

Activity of Glutathione reductase

The activity of glutathione reductase was determined according to the procedure described by Mavis and Stellwagen (1968)**.**

Molecular attributes

Plant material, growth conditions and treatments

For studies on expression analysis, samples from the induced water stress experiment, were collected after 9th day of imposing drought to 21 d old seedling and used for RNA extraction.

Sample collection, RNA isolation and cDNA synthesis

Samples from the above experiment collected from stressed and unstressed plants were immediately frozen in liquid nitrogen and stored at -80˚C for RNA isolation. RNA was isolated using TRIzol reagent and cDNA was prepared using Accu Script High Fidelity 1st Strand cDNA Synthesis Kit, Canada. First strand cDNA generated were equalized and used as a template for further amplification studies.

Design of primers and optimization of concentration

Gene specific markers for drought stress studied earlier in various crops were utilized which consisted of aquaporin, DREB1, DREB2 and C_2H_2.

The specificity of all the primers was confirmed by sequence analysis of RT-PCR amplicons derived from *S.italica*. Reference genes for quantifying gene expression and to ensure proper normalization were utilised in the present study which are mentioned in Table 1.

Amplification of cDNA using primers

First strand cDNA generated were equalized and used as a template for further amplification studies. Amplification was carried out by using a Thermal cycler. The

reaction was performed by adding following components in order to sterile thin-walled PCR tubes for each PCR amplification reaction: 12.6µl of RNase-free water, 2µl of 10X PCR Buffer, 1.2µl of 50mM $MgSO_4$, 2.0µl of dNTP mix (2mM each dNTP), 0.5µl of upstream primer (0.1 µg/µl), 0.5µl of downstream primer (0.1 µg/µl), 1µl of experimental first-strand cDNA reaction, 0.2µl of *Taq polymerase* (5U/ul).

PCR programme for cDNA-Drought stress specific genes profiling

Amplifications were performed using thermal cycler (eppendorf) with reaction conditions as 5.00 min at 94°C followed by 39 cycles each of 2 min at 94°C, 2 min at 36-58°C (as per primer) and 3 min at 72°C, and final extension of 15 min at 72°C.

Gel electrophoretic analysis

Separation of amplified fragments was carried out using Bio-rad gel electrophoresis assembly. PCR amplification products were analyzed by agarose gel electrophoresis on 1.5 % agarose gel stained with ethidium bromide solution (0.5 µg/ml).

The computer program AlphaEaseFC (From Alpha Innotech) was used to visualize and analyse the results. All the reactions were repeated thrice, and consistently reproducible bands were scored.

Profile scoring and data analysis

Profile of the amplicons was scored and data analyzed based on the consensus results of three independent runs. Clearly resolved bands (TDFs) of both stressed samples were scored manually on the basis of their presence/absence as well as differences in amplicon intensities to understand differential expression pattern in stressed and unstressed of tolerant and susceptible foxtail millet accessions and assigned up- and down-regulated in comparison with their respective controls.

Gel elution, cloning and sequencing of differentially expressed TDFs

The individual differentially expressed TDF was cut from the gel with a sharp surgical blade, avoiding any contaminating fragment(s). Gel elution of amplicons of interest was performed using Qiagen gel elution kit as per the protocol prescribed in technical bulletin. Sequencing was done through GeneOmBiotech, Pune, India. The sequences are accessed using Bioedit software.

Homology analysis

The sequences of the TDF were analyzed for their homology against the publicly available non redundant genes/ESTs/transcripts in the NCBI Gen-Bank nucleotide and protein database using BLAST algorithms (http://www.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al.*, 1997).

Results and Discussion

Studies of plant responses to abiotic stresses have been of great interest to scientists because of growing water shortages and decreasing soil fertility.

Foxtail millet is an excellent experimental model crop for studying abiotic stress tolerance system.

Hence, foxtail millet (*Setaria italica* L.*)* was selected in the present study to investigate the processes at physiological, biochemical level and to fetch out the molecular mechanism underneath its tolerance.

The effect of water stress on physiological parameters in contrasting foxtail millet accessions under water stress

Water stress represents one of the most important environmental stresses since it limits crop plant production disturbing the normal physiology and creates metabolic imbalance.

Relative water content (RWC)

To understand the physiological changes in water stress condition in plants, changes in relative water content is a less error prone and useful means for determining the physiological water status of plants (Makbul *et al.,* 2011; González and González-vilar, 2001).

The choice of RWC as the best representation of plant water status for assessing genetic differences in dehydration tolerance is supported by genetic association between RWC and plant biomass under dehydration (Blum, 1996; Bhushan *et al.*, 2007). Leaf RWC is an important parameter, which determines the ability of a plant to absorb water under water stress conditions and used as one of the indices to determine drought tolerance ability of the plants. Generally stressed plants have lower RWC than non-stressed plants.

Likewise, in the present study, relative water content was found to be decreased significantly under water stress however, the tolerant cultivars showed the capacity to maintain relatively high RWC (Figure 2). The RWC index of tolerant cultivar IC97189 was 86.28 % which is greater than that of susceptible cultivar IC97109 which was found to be 75.59 %. Hence, it is suggested that the relative water content could help the tolerant cultivar to perform physio-chemical processes more efficiently than sensitive cultivar. The similar results were also obtained by Carter and Patterson (1985) in soybean. This suggested that the relative water content could help the tolerant cultivar to perform physio-chemical processes more efficiently than sensitive cultivars.

Leaf water potential (Ψ_L)

Many workers have reported that drought tolerant varieties have a smaller water deficit (relative saturation deficit) per unit decrease in water potential of leaf (measured as MPa) than drought sensitive plants (Levitt, 1972; Dedio, 1975; Ashraf et at., 1994a). Leaf water potential also decreased significantly under water stress conditions and the tolerant accession may resist this decrease. The MPa index of tolerant accession i.e. IC97189 was higher (112.4%) than that of susceptible accession i.e. IC97189 which was 107.75 %. The individual LWP of stressed plants for tolerant and susceptible accessions was -6.40 and – 14.17 respectively (not shown in graph). Hence, tolerant accessions showed relatively higher LWP than sensitive accession. Earlier, Gonzalez *et al.,* (2001) has recorded significant decrease in Ψ_L and RWC in barley under drought stress. The significant differences in leaf water potential and RWC that were recorded among the tolerant and susceptible accessions studied here are consistent with Subrahmanyam *et al.*, (2006); Tas and Tas (2007); Siddique *et al.*, (2004); Zhu (2002) and Wahid and Close (2007).

Chlorophyll content

Chlorophyll index is a rapid method and forms one of the indices for estimating resistance to dehydration (Khidse *et al.*, 1982). There are reports about decrease of chlorophyll content in drought stress conditions (Kuroda *et al.*, 1990). The

present study shows that the chlorophyll content decreased with increasing water stress over non stressed conditions. The CC of stressed and unstressed plants for tolerant and susceptible accessions was 12.40/12.50 and 5.70/11.80 respectively (not shown in graph). Hence the CC index of tolerant accession (99.2 %) was found to be higher than that of susceptible accession (48.30%) (Depicted in green bar, Figure 2).

However, the tolerant accession showed very little decrease in CC under stress condition. Ramanjulu and Sudhakar (2000) also reported that lesser decrease in total chlorophyll content in tolerant mulberry cultivar than susceptible cultivar under salt stress.

This decrease is because of reduced photosynthates production under water deficit conditions (Tezara *et al.,* 1999; Anjum *et al.,* 2003; Wahid and Rasul, 2005) as the chlorophyll content is positively associated with photosynthetic rate which increases biomass production and grain yield. Similarly, decrease in total chlorophyll content in the leaves of water stressed plants was reported by several workers earlier (Ramanjulu and Sudhakar, 2000; Gopalakrishna, 2001; Chandraobulreddy, 2005).

The effect of water stress on biochemical parameters in contrasting foxtail millet accessions under water stress

As discussed earlier, one of the widely described plant responses to water stress is osmotic adjustment, which requires accumulation of compatible solutes, such as amino acids, carbohydrates, polyols, tertiary sulfonium and quaternary ammonium compounds which play an important role in maintaining cell turgor, as well as stabilizing proteins and cell membranes.

Accumulation of primary metabolites

Proline accumulation

It has been documented that many amino acids accumulate in plants exposed to various abiotic stresses. Proline is one of the most widely distributed osmolyte, the level of which is elevated in different environmental stresses including drought, salinity and cold stress (Verbruggen and Hermans, 2008; Szabados and Savoure, 2010). Proline is known to accrue widely in higher plants, playing an adaptive role in mediating osmotic adjustment and protecting the sub-cellular structures. Change in proline content has been correlated with its capacity to tolerate and adapt to stress conditions (Balibrea *et al.,* 1997). Accumulation of proline under stress protects the cell by balancing the osmotic strength of cytosol with that of vacuole and external environment (Aspinall and Paleg, 1981). In addition its role as cytosolic osmotica, it is known to interacts with cellular macro-molecules such as enzymes and stabilizes the structure and function of such macromolecules (Jain *et al.*, 2001). In the present investigation, the free proline content was found to be significantly increased in accessions with water stress over unstressed plants. However, a difference in the accumulation of free proline content was observed among the tolerant and susceptible accessions. Higher proline accumulation recorded in drought tolerant accession IC97189, when compared to drought sensitive accession, IC97109 indicates that proline metabolizes slowly as compared to the other antioxidative enzymes and thereby supplies the nitrogen to the cellular metabolic pathways post stress (Bartels and Sunkar, 2005). A direct consequence of higher proline concentration in foxtail millet was relatively higher water retaining capacity as reflected by RWC.

Carbohydrates

It is widely reported that abiotic stresses lead to accumulation of non-structural carbohydrates like sucrose, hexoses and polyhydric alcohols among many plant species. Soluble carbohydrates play an important role in plant metabolism as a source of carbon and energy within a cell. Their level is affected by different stresses, as the carbohydrate content is related to photosynthesis. In the present study, total carbohydrates were found to be increased in the accessions under water stress condition. CHO value recorded in tolerant accession, IC97189 was found to be 111.25 mg $(gFW)^{-1}$ and 81.25 mg $(gFW)^{-1}$ in stressed and unstressed condition respectively with an increase of 26.96% over unstressed condition. Whereas, the CHO content in susceptible accession IC97109 was found to be 108.75 $mg(gFW)^{-1}$ and 67.50 $mg(gFW)^{-1}$ in stressed and unstressed condition respectively with an increase of 37.9% over unstressed condition. CHO content showed a significant increase in susceptible accession under water stress (37.9%), whereas tolerant accession showed a comparatively less increase in CHO content i.e. up to 26.96%. Strong correlation between the carbohydrate accumulation and tolerance to osmotic stresses, such as water deficit or salinity stress has been reported by Bartels and Sunkar (2005).

Similarly, water stress significantly increased starch content in foxtail millet accessions and was found to be lower in unstressed condition in susceptible accessions than in tolerant ones. Starch increased in both the accessions studied, which was recorded as 3.88 and 2.99 mg $(gFW)^{-1}$ in IC97189 in stressed and unstressed condition respectively with 22.93% increase over unstressed condition. The starch content in susceptible accession IC97109 was found to be 3.99 and 1.0 mg $(gFW)^{-1}$ in stressed and unstressed condition respectively with 74.93% increase over unstressed condition. The increase in starch and carbohydrates might be due to their role as protectant during stress, protecting cell membranes (Janska *et al.*, 2010) and their involvement in cell signaling (Hanson and Smeekens, 2009). They also play a role in adaptive mechanisms to stress (Ramel *et al.*, 2009). Soluble sugars function as osmoprotectants during the period of water stress, reducing the detrimental effects of osmotic stress, helping in maintaining turgor, stabilizing cell membranes and protecting plants from degradation (Basu *et al.*, 2007). The increase in sugar content is mostly the effect of starch hydrolysis, which requires enzymes with a hydrolytic activity (Kaplan and Guy, 2004). Figure 3 depicts the effect of water stress on non-enzymatic parameters in foxtail millet accessions under water stress.

Accumulation of antioxidant enzymes

In the present study, antioxidant enzyme activities changed significantly in response to the water stress. These enzymes operate in different subcellular compartments and respond in concert when cells are exposed to oxidative stress. The results revealed an increase in SOD, POD, CAT and GR activities under drought treatment. Higher SOD activity was seen in IC97189 (0.89 Units min^{-1}) in water stressed condition with a 25.5% increase over unstressed condition.

Accession IC97109 showed a comparatively less SOD activity under water stressed condition i.e. 0.52 Units min^{-1}. Increased activity of SOD is often correlated with increased tolerance of the plant against environmental stresses. Overproduction of SOD has been reported to result in enhanced oxidative stress tolerance in plants (Gupta *et*

al., 1993). It is also suggested that SOD can be used as an indirect selection criterion for screening drought-resistant plant materials (Nayyar and Gupta, 2006).

A significant and time-dependent increase in GR activity has been found in both the accessions exposed to dehydration stress as compared with their respective controls. Higher GR activity was seen in IC97189 (0.24 Units min^{-1}) with an increase of 26.6% over unstressed plants. Whereas, IC97109 (0.09 Units min^{-1}) was recorded with lower activity of GR with 50 % increase over unstressed condition. Adaptation to drought has been reported to involve different protective mechanisms including the capacity to maintain high levels of antioxidants (ascorbate and GSH) and to regenerate them through the induction of GR (Loggini *et al.,* 1999).

Activation of ascorbate–glutathione cycle has earlier been suggested as essential in stressed plants to combat oxidative damage (Alscher *et al.,* 1997). Similar observations were reported in foxtail millet, wheat, rice, sorghum, and pigeon pea (Lata *et al.,* 2011[a]; Loggini *et al.,* 1999; Jogeshwar *et al.,* 2006; Guo *et al.,* 2006; Kumutha *et al.,* 2009) under various abiotic stresses.

The POD activity of 2.03 Units min^{-1} with an increase of 36.14% over unstressed conditions was recorded in tolerant accession, IC97189 under stress. Lower POD activity was seen in IC97109 (0.86 Units min^{-1}) under stress with an increase of 62.86% over unstressed conditions. Some previous studies, as parallel with our results, reported the increased POD activity under drought stress conditions in various plants, like sunflower (Gunes *et al.,* 2008), poplar (Xiao *et al.,* 2008), liquorice (Pan *et al.,* 2006), brassica species (Das and Uprety, 2006), wheat (Csiszar *et al.,* 2005).

Increase in CAT activity under water stress was seen in both the accessions studied here. IC97189 showed the higher CAT activity i.e. 1.11 Units min^{-1} with an increase of 37.50% over unstressed condition. Lower CAT activity was seen in IC97109 (0.79 Units min^{-1}) with a significant 71.89% increase. The results are in support of studies on antioxidative responses to dehydration-induced oxidative stress in core set of foxtail millet cultivars by Lata *et al.,* (2011). Increase in the activity of catalase (CAT) was also reported by Zarei *et al.,* (2012) in tobacco under drought stress.

Hence, the noticed differences in the efficiency of maintaining higher catalase activity could reflect the better adaptability of genotypes to drought-induced oxidative stress. Significant increase in the levels of SOD, POD, GR and CAT were recorded in the tolerant accession, IC97189 suggesting that the higher antioxidant enzymes activity have a role in imparting tolerance against water stress (Mittova *et al.,* 2003; Sharma and Dubey, 2005). The effect of water stress on the activities of antioxidant enzymes participating in the scavenging of ROS is presented in Figure 4. Increased SOD, POD and CAT activities are closely related to stress tolerance of many plants as reported by previous researches (Rahnama and Ebrahimzadeh, 2005; Azevedo Neto *et al.,* 2006; Koca *et al.,* 2007). There are many reports in the literature that underline the intimate relationship between enhanced or constitutive antioxidant enzyme activities and increased resistance to environmental stresses in several plant species, such as rice (Guo *et al.,* 2006), foxtail millet (Sreenivasulu *et al.,* 2000), tomato (Mittova *et al.,* 2003), sugar beet (Bor *et al.,* 2003), oilseed rape (Abedi and Pakniyat, 2010), wheat (Zaefyzadeh *et al.,* 2009; Shahbazi *et al.,* 2010; Ahmadizadeh *et al.,* 2011) and barley (Acar *et al.,* 2001).

Profiling for differentially expressed TDFs in response to drought specific marker genes in contrasting foxtail millet accessions

Accurate normalization of template is an important step in gene expression studies. Hence, to confirm the normalisation of cDNA, appropriate internal control gene like α tubulin and EF1α were used which showed constant expression in unstressed and stressed condition. EF-1 α was used in the present study which was suggested as reliable internal control gene in foxtail millet gene expression studies (Kumar *et al.*, 2013). Semi-quantitative RT-PCR has emerged as a versatile technique in transcriptomics, as it can generate rapid measurement of mRNA levels in minimal tissue samples. Hence, the method was adopted in the present investigation.

Here, the drought stress specific genes/TFs (identified earlier in various crops) were analyzed. Semi-quantitative evaluation of the relative mRNA accumulation of four genes namely, Aquaporin, DREB2, DREB1 and C_2H_2 induced by dehydration stress was performed. Strikingly, the drought stress specific genes were significantly upregulated under stress. Tolerant accessions showed higher expression levels of drought responsive genes/TFs even in unstressed conditions as compared to susceptible accessions. Aquaporin belongs to major intrinsic protein super family which functions as a membrane channel. The designed primer amplified a segment of 145 bp in the selected contrasting accessions in unstressed and stressed conditions. qRT-PCR analysis showed an up-regulation of aquaporin in stressed condition in both the accessions (Figure 5). Based on the IDV values, the relative up-regulation was found to be 27.61 % due to dehydration stress in IC97189. Peng *et al.*, (2007) also reported that aquaporin enhances drought and salt tolerance ability in transgenic Arabidopsis plants (Peng *et al.*, 2007).

Dehydration Responsive Elements have been reported to be involved in various types of abiotic stress responses via ABA-dependent and ABA-independent pathways (Shinozaki and Yamaguchi-Shinozaki, 1997). The DREB transcription factor family is one of the largest and is broadly divided into DREB1 and DREB2 sub families and each sub family contain several paralogs. Among the eight DREB2-type proteins that are reported in Arabidopsis thaliana, DREB2A and DREB2B are thought to be major transcription factors that function under drought and high-salinity stress conditions (Sakuma *et al.*, 2002; Sakuma *et al.*, 2006). In the present study, the designed DREB2 primer amplified a fragment of 215 bp, whereas, DREB1 amplified a fragment of 500 bp in the selected contrasting accessions in unstressed and stressed conditions. qRT-PCR analysis showed an up-regulation of transcript in stressed condition in both the accessions (Figure 5). Based on the IDV values, qRT-PCR analysis of DREB2 showed the relative up-regulation of about 7.86 % due to dehydration stress in IC97189. Whereas, DREB1 showed the relative up-regulation of about 11.80%. Both DREB1 and DREB2 showed higher expression in both unstressed and stressed condition in tolerant accession, IC97189. Several DREB1/DREB2 homologous genes have been isolated from many plants including wheat, rice, barley, rye, sorghum, and oat (Nakashima *et al.*, 2009). The up-regulation of these transcripts in the tolerant cultivar clearly suggests their role in providing dehydration stress tolerance to the selected accession.

The present investigation also showed an up-regulation of C_2H_2 type of zinc finger

transcription factors of about 18.67 % in tolerant accession, IC97189 under water stressed condition. Expression was also found to be higher in unstressed condition in both tolerant and susceptible accession. C_2H_2 type of zinc finger transcription factors (TFs) play crucial roles in plant stress response and hormone signal transduction. Several members of the C_2H_2-type ZF family have been reported to play diverse roles in the plant stress response and the hormone signal transduction. Transcription profiling studies have shown that the transcript level of many C_2H_2-type ZF proteins is elevated under different abiotic stress conditions such as cold, salt, drought, osmotic stress and oxidative stress (Kiełbowicz-Matuk, 2012). A number of stress-responsive C_2H_2-type zinc finger TFs were also reported in response to drought stress (Tian *et al.,* 2010; Sun *et al.,* 2010; Kodaira *et al.,* 2011).

Up-regulated expression due to stress causing notable changes in gene expression particularly those involved in metabolism, proteolysis, and stress signaling has been suggested by many scientists (Zhang *et al.,* 2007; Lata *et al.,* 2010). Hence induction of these transcripts suggests that these genes might impart drought avoidance capacity to the tolerant accession in comparison to the sensitive one, as the expression analysis of up-regulated transcripts between drought tolerant and susceptible cultivars upon dehydration stress suggests their function in dehydration adaptation and tolerance in foxtail millet (Lata *et al.,* 2010). The differential gene expression of drought responsible transcriptional factors like Aquaporins and C_2H_2 obtained could fetch out the molecular mechanism underneath drought tolerance in *Setaria italica.*

Sequence characterisation of the drought responsible genes was done to confirm that the results of amplification are not contaminated with foreign DNA, and we were able to sequence DREB2, Aquaporin and C_2H_2 amplicons form respective tolerant and susceptible accession. However the sequencing results for DREB1 were difficult to reproduce regardless of repetitive experiments. This may be due the differences in genotype used for actual experiments and primer designing. Database analysis of the genes with BLAST revealed that the deduced sequence has high homology to corresponding PREDICTED: *Setaria italica* sequences with an identity of more than 89% and query coverage of more than 68 % for DREB2, AQP and C_2H_2.

Plant growth and productivity are greatly affected by environmental stresses and plants undergo a variety of changes at the molecular level (gene expression) leading to physiological adaptation. Hence, a detailed investigation of stress-responsive genes and their expression kinetics in the tolerant crop is an interesting area of research. It was therefore important to explore stress tolerance mechanism in a relatively abiotic stress tolerant crop like foxtail millet.

The study presented the physio-biochemical and transcriptional changes in foxtail millet under water stress. The results showed that the contrasting accessions of foxtail millet have different responses to water stress, indicating that it is feasible to choose the best accession for resisting drought as some accessions have genetic potential to maintain the higher growth under stress conditions. The various parameters like RWC, LWP and chlorophyll content, some primary metabolites like proline, total carbohydrates and antioxidant enzymes like superoxide dismutase, peroxidase, catalase and glutathione reductase that are involved in osmotic adjustment, especially in conditions of limited water availability, and

are generally found to have elevated concentration level.

Hence, applying different physiological and biochemical tests to appreciate drought tolerance in plant leads to faster selection methods. These findings on biochemical and physiological parameters may serve as *in vitro* selection criteria for drought tolerance in foxtail millet. Also, the study can prove helpful to the farmers in selecting foxtail millet cultivars for unaffected yields in diverse agronomic conditions. Moreover, cDNA-profilling provides a fast, simple and inexpensive approach to compare multiple experimental samples simultaneously for differential expression. The differential gene expression of drought responsible transcriptional factors like DREB1, DREB2, Aquaporins and C_2H_2 that were studied in selected drought tolerant and susceptible accession showed a considerable difference gene expression pattern. This information may provide a foundation for future studies toward determination of functional importance of these drought-responsive genes for developing stress-tolerant plants. TDF-based isolation of full-length genes, their expression and knockout studies will provide insights for managing stress tolerance trait in foxtail millet. These candidate genes can be further utilized as molecular markers for early identification of tolerant progenies in foxtail millet hybridization programs. Hence, this research can provide documentation for breeding/selection of higher drought resistant foxtail millet in arid regions and acquisition of good information for future molecular research.

Table.1 Drought stress specific gene/TFs primers used during the present investigation

Gene	**Sequence**				
Aquaporin	Forward	CCCGTTCAAGAGCAGGTCTTA	C_2H_2	Forward	ACGACACACCAGTGTCCAAA
	Reverse	CCTGTTTGGACTGGCATCTCA		Reverse	GCTGGTTTGTCTGGTGGGAT
DREB2	Forward	GCCTTGTAGTCATTTGGTGGTTT	EF-1α	Forward	TGACTGTGCTGTCCTCATCA
	Reverse	CTCACAACTCCTTTTCTCAAGCT		Reverse	GTTGCAGCAGCAAATCATCT
DREB1	Forward	GGAGCAAGCAGAAACACACA	TUB α	Forward	TACCAGCCACCATCTGTTGT
	Reverse	GCATCGGAAGCCAGAAAAGA		Reverse	GGTCGAACTTGTGGTCAATG

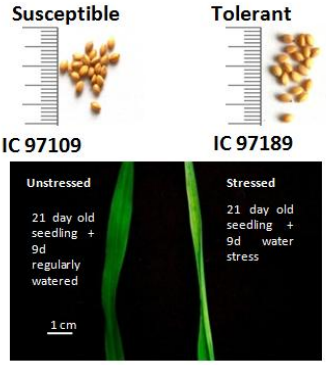
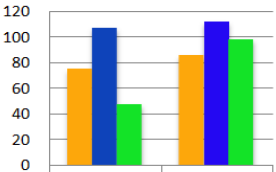


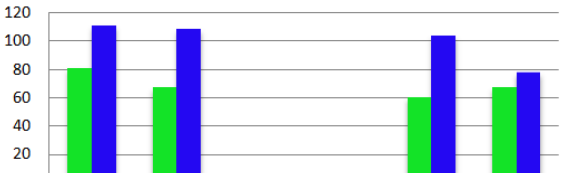
Figure1 Illustrations for sample stage selection in foxtail millet under water stress for physiological, biochemical and molecular studies



	IC97109	IC97189
RWC index	75.59	86.28
LWP index	107.75	112.4
CC index	48.3	99.2

Figure 2 The effect of water stress on physiological parameters in contrasting foxtail millet accessions under water stress

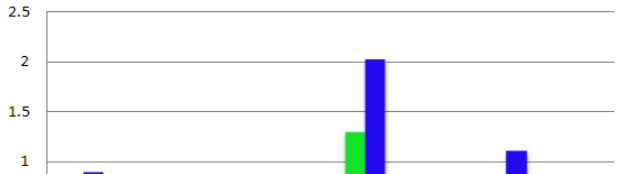
Data for relative water content tolerance index and leaf water potential tolerance index and chlorophyll content tolerance index is presented here.
(Percent values are represented here).



	IC-97189	IC-97109	IC-97189	IC-97109	IC-97189	IC-97109
	CHO		STARCH		PROLINE	
unstressed	81.25	67.5	2.99	1	60.6	67.53
stressed	111.25	108.75	3.88	3.99	103.89	77.92

Figure 3 The effect of water stress on non-enzymatic parameters in contrasting foxtail millet accessions under water stress.

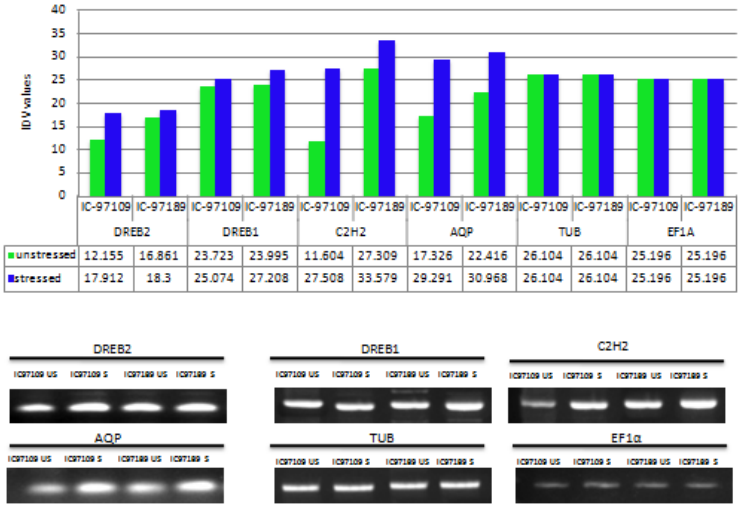
CHO and starch, expressed as mg (g FW) $^{-1}$; Proline expressed as µmoles (gFW)$^{-1}$; US=unstressed; S=stressed



	IC-97189	IC-97109	IC-97189	IC-97109	IC-97189	IC-97109	IC-97189	IC-97109
	SOD		GR		POD		CAT	
unstressed	0.67	0.15	0.18	0.04	1.3	0.32	0.69	0.22
stressed	0.89	0.52	0.24	0.09	2.03	0.86	1.11	0.79

Figure 4 The effect of water stress on enzymatic parameters in contrasting foxtail millet accessions under water stress.

(a) SOD, one unit of enzyme activity defined as inhibiting the rate of reduction of NBT by 50% (b) GR, one unit defined by reduction in 1.0 µmole of oxidized glutathione min^{-1};(c) CAT, one unit of enzyme activity defined as 1 µmol H_2O_2 oxidized min^{-1}; (d) POD, one unit of enzyme activity defined by the formation 1.0 milligram of purpurogall in from pyrogallol.
US-unstressed; S-stressed



	DREB2		DREB1		C2H2		AQP		TUB		EF1A	
	IC-97109	IC-97189	IC-97109	IC-97189	IC-97109	IC-97189	IC-97109	IC-97189	IC-97109	IC-97189	IC-97109	IC-97189
unstressed	12.155	16.861	23.723	23.995	11.604	27.309	17.326	22.416	26.104	26.104	25.196	25.196
stressed	17.912	18.3	25.074	27.208	27.508	33.579	29.291	30.968	26.104	26.104	25.196	25.196

Figure 5 Profiling for differentially expressed TDFs in response to drought specific marker genes under water stress

Samples in response to water stress from two contrasting foxtail millet accessions, IC-97109, IC-97189 were run on 1.5% agarose gel; Graphs show differential expression of Aquaporin, DREB2, DREB1 and C_2H_2; α tubulin and EF1 α used as internal controls; US: unstressed; S: stressed.

References

Abedi T, and Pakniyat H 2010. Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). Czech J Genet Plant Breed 46(1): 27-34

Acar O, Turkan I, and Zdemir FO 2001. Superoxide dismutase and peroxidase activities in drought sensitive and resistant barley (*Hordeum vulgare* L.) varieties. Acta Physiologiae Plantarum 3: 351-356

Acquaah 2007. Principles of plant genetics and breeding. Blackwell, Oxford, UK

Ahmadizadeh M, *et al.*, 2011. Genetic diversity of durum wheat landraces using multivariate analysis under normal irrigation and drought stress conditions. African J Agri Res 6(10): 2294-2302

Alscher RG, Donahue JL, Cramer CL 1997. Reactive oxygen species and antioxidants: relationships in green cells. Physiol Plant 100:224–233

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Anjum F, Yaseen M, Rasul E, Wahid A, Anjum S 2003. Water stress in barley (*Hordeum vulgare* L.). I. Effect on chemical composition and chlorophyll contents, Pakistan J Agr Sci 40:45–49.

Aspinall D and Paleg G 1981. Proline accumulation: physiological aspects. In: The physiology and biochemistry of drought resistance in plants. (Eds.). L, Paleg, D. Aspinall, Academic Press, Sidney, pp 215-228

Atkinson CJ, Policarpo M, Webster AD, Kingswell G 2000. Drought tolerance of clonal Malus determined from measurements of stomatal conductance and leaf water potential. *Tree Physiol* 20(8): 557-563

Azevedo Neto AD, Prico JT, Eneas-Filho J, Braga de Abreu CE, Gomes-Filho E 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of

salt-tolerant and salt-sensitive maize genotypes. Environ Exp Bot 56: 235–241

Balibrea ME, Rus-Alvarez AM, Bolarin MC, Perez-Alfocea F 1997. Fast changes in soluble carbohydrate and proline contents in tomato seedlings in response to ionic and non-ionic iso-osmotic stresses. J Plant Physiol 151:221-22

Barrs HD 1968. Determination of water deficits in plant tissue. In Kozlowski TT (Eds) Water deficits and plant growth. New York, Academic Press,1, pp 235-368

Bartels D and Sunkar R 2005. Drought and salt tolerance in plants. Crit Rev in Plant Sci 24: 23-58

Basu PS, Ali M, Chaturvedi SK 2007. Osmotic adjustment increases water uptake, remobilization of assimilates and maintains photosynthesis in chickpea under drought. Indian J Exp Biol 45:261–267

Bates LS, Waldren RP and Teare ID 1973. Rapid determination of free proline for water studies. Plant and Soil 39:205-208

Bhushan D, Pandey A, Choudhary MK, Datta A, Chakraborty S, Chakraborty N 2007. Comparative proteomics analysis of differentially expressed proteins in chickpea extracellular matrix during dehydration stress. Mol and Cellul Proteom 6:1868–1884

Biber PD 2007. Evaluating a chlorophyll content meter on three coastal wetland plant species. J of Agri Food and Env Sci 1:1-10

Blum A 1996. Developing drought and low N-tolerant maize. In: Edmeades GO, Banziger M, Mickelson HR, Pena-Valdivia CB, eds. Proc of a Sympo at the International Maize and Wheat Improvement Centre. El-Batan, Mexico, CIMMYT, 131–135

Bor MF, Ozdemir and Turkan I 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L and wild beet *Beta maritima* L. Plant Sci 164: 77-84

Bulbotko GV 1973. The effect of the physical properties of soils on the development of the root system of apple trees. Soviet Soil Sci USSR 5: 219-224

Carter Jr. TE, Patterson RP 1985. Use of relative water content as a selection tool for drought tolerance in soybean. Fide Agron abstr 77th Annu Meeting, pp 77

Chandraobulreddy P 2005. Molecular cloning and characterization of differentially expressed genes from horsegram (*Macrotyloma uniflorum* (Lam.) Verde.) and groundnut (*Arachis hypogea* L.) under drought stress. Ph.D., thesis, Sri Krishnadeveraya University, Anantapur

Csiszar J, Feher-Juhasz E, Kotai E, IvankovitsKiss O, Horvath GV, Mai A, Galle A, Tari I, Pauk J, Dudits D, Erdei L 2005. Effect of osmotic stress on antioxidant enzyme activities in transgenic wheat calli bearing MsALR gene. Acta Biologica Szegediensis 49: 49–50

Das R, and Uprety DC 2006. Interactive effect of moisture stress and elevated CO_2 on the oxidative stress in Brassica species. J Food Agri and Enviro 4: 298–305

Dedio 1975. Water relations in wheat leaves as screening test for drought resistance. Canadian J Plant Sci 55: 369-378

Dhindsa RS, Dhindsa PP, Thorpe TA 1980. Leaf senescence correlated with increased levels of membrane

permeability and lipid-peroxidation and decreased of superoxide dismutase and catalase. J Exp Bot 32:93–101

Doust AN, Kellogg EA, Devos KM, and Bennetzen JL 2009. Foxtail millet: a sequence-driven grass model system. Plant Physiol. 149:137–141

Dubois M, Gilles KA, Hamilton J, Rebers PA and Smith F 1956. Colorimetric method for determination of sugars and related substances. Anal Chem 28:350

Gonzalez L and Gonzalez-vilar M 2001. Determination of relative water content. In: Reigosa MJ (ed.) Handbook of Plant Ecophysiology Techniques, Dordrecht, Kluwer Academic Publishers, pp 207-212

Gopalakrishna R 2001. Cloning and characterization of moisture stress responsive genes from stress tolerant crop groundnut *(Arachis hypogaea* L.). Ph.D thesis, University of Agricultural Sciences, Bangalore.

Gunes A, Pilbeam D, Inal A, Coban S 2008. Influence of silicon on sunflower cultivars under drought stress, I: Growth, antioxidant mechanisms and lipid peroxidation. Commun Soil Sci & Plant Nutri 39:1885–1903

Guo Z, Ou W, Lu S, Zhong Q 2006. Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. Plant Physiol Biochem 44:828–836

Gupta AS, Heinen JL, Holaday AS, Burke JJ, and Allen RD 1993. Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc Nat Ac Sci USA 90(4):1629–1633

Hanson J, and Smeekens S 2009. Sugar perception and signaling—an update. Curr Opin Plant Biol 12: 1–6

Hodge JE, and Hofreiter BT 1962. *In* Methods in Carbohydrate Chemistry, (Eds. Whistler, R.L. and Be Miller, J.N.), Academic Press, New York.

Jain M, Mathur G, Koul S and Sarin NB 2001. Ameliorative effects of proline on salt stress induced lipid peroxidation in cell lines of groundnut (*Arachis hypogea* L.). Plant Cell Reprod 20: 463-468

Janska A, Marsik P, Zelenkova S, Ovesna J 2010. Cold stress and acclimation: what is important for metabolic adjustment? Plant Biol 12:395-405

Jogeshwar G, Pallela R, Jakka NM, Reddy PS, Rao JV, Sreenivasulu N, Kishor PBK 2006. Antioxidative response in different sorghum species under short-term salinity stress. Acta Physiol Plant 28:465–475

Kaplan F, and Guy CL 2004. β-Amylase induction and the protective role of maltose during temperature shock. Plant Physiol 135:1674–1684

Khidse SR, Bhale NL, and Boriker ST 1982. Proline accumulation and chlorophyll stability index in *Sorghum*. Sorghum News Letters 25: 123- 128

Kiełbowicz-Matuk, A., 2012. Involvement of plant C_2H_2-type zinc finger transcription factors in stress responses. Plant Science 185–186, 78–85

Koca H, Bor M, Özdemir F, Türkan İ 2007. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ Exp Bot 60: 344–351

Kodaira KS, Qin F, Tran LS, Maruyama K, Kidokoro S, Fujita Y, Shinozaki K, Yamaguchi-Shinozaki K. 2011. Arabidopsis Cys2/His2 zinc-finger proteins AZF1 and AZF2 negatively regulate abscisic acid-repressive and auxin-inducible genes under abiotic stress conditions. Plant Physiol 157:

742–756

Kumar K, Muthamilarasan M, Prasad M 2013. Reference genes for quantitative Real-time PCR analysis in the model plant foxtail millet (*Setaria italica* L.) subjected to abiotic stress conditions. Plant Cell Tiss Organ Cult 115:13–22

Kumutha D, Ezhilmathi K, Sairam RK, Srivastava GC, Deshmukh PS, Meena RC 2009. Waterlogging induced oxidative stress and antioxidant activity in pigeonpea genotypes. Biol Plant 53:75–84

Kuroda M, Qzawa T, Imagawa H 1990. Changes in chloroplast peroxidase activities in relation to chlorophyll loss in barley leaf segments. Physiologia plantarum, 80: 555-560

Lata C, Bhutty S, Bahadur RP, Majee M, and Prasad M 2011[a]. Association of an SNP in a novel DREB2-like gene SiDREB2 with stress tolerance in foxtail millet [*Setaria italica* (L.)]. J Exp Bot 62:3387—3401

Lata C, Gupta S, and Prasad M 2013. Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33:328–343

Lata C, Sahu PP, Prasad M 2010. Comparative transcriptome analysis of differentially expressed genes in foxtail millet (*Setaria italica* L.) during dehydration stress. Biochem Biophy Res Commun 393:720–727

Lee SH, Lee KW, Kim KY, Choi GJ, Yoon SH, Ji HC, Seo S, Lim YC, Ahsan N 2009. Identification of salt-stress induced differentially expressed genes in barley leaves using the annealing control-primer-based gene fishing technique. Afr J Biotechnol 8:1326–1331

Levitt J., 1972. Response of plants to stresses. Academic Press. New York and London. p.697

Li P, and Brutnell TP 2011. Setaria viridis and *Setaria italica*, model genetic systems for the Panicoid grasses. J Exp Bot 62: 3031–3037

Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F 1999. Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol 119:1091–1099

Luck H 1974. Methods in enzymatic analysis. Academic Press, New York, 885

Makbul S, Saruhan-guler N, Durmus N and Guven S 2011. Changes in anatomical and physiological parameters of soybean under drought stress. Turk J Bot 35:369-377

Massonnet EC, Serge R, Erwin D, Regnard JL 2007. Stomatal Regulation of Photosynthesis in Apple Leaves: Evidence for Different Water-use Strategies between Two Cultivars Catherine. Ann Bot 100(6):1347-1356

Mavis RD, and Stellwagen E 1968. Purification and subunit structure of glutathione reductase from bakers' yeast. J Biol Chem 243:809–814

Mittova V, Tal M, Volokita M, and Guy M 2003. Up−regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt−induced oxidative stress in the wild salt−tolerant tomato species *Lycopersicon pennellii*. Plant Cell Environ 26:845–856

Nakashinia K, Ito Y, Yamaguchi-Shinozaki K 2009. Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. Plant Physiol 149: 88-95

Nayyar H, and Gupta D 2006. Differential sensitivity of C3 and C4 plants to water deficit stress: Association with oxidative stress and antioxidants. Environ Exp Bot 58:106-113

Pan Y, Wu LJ, Yu ZL 2006. Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (*Glycyrrhiza uralensis* Fisch), Plant Growth Regul 49:157–165

Parvaiz A, Satyawati S 2008. Salt stress and phyto-biochemical responses of plants: a review. Plant soil environ 54:89–99

Peng Y, Lin W, Cai W, Arora R 2007. Overexpression of a Panax ginseng tonoplast aquaporin alters salt tolerance, drought tolerance and cold acclimation ability in transgenic Arabidopsis plants. Planta 226 (2007) 729–740

Rahnama H, and Ebrahimzadeh H 2005. The effect of NaCl on antioxidant enzyme activities in potato seedlings. Biol Plant 49:93–97

Ramanjulu S, and Sudhakar C 2000. Proline metabolism during dehydration in two mulberry genotypes with contrasting drought tolerance. J Plant Physiol 157: 81-85

Ramel F, Sulmon C, Gouesbet G, Coue´e I 2009. Natural variation reveals relationships between pre-stress carbohydrate nutritional status and subsequent responses to xenobiotic and oxidative stress in Arabidopsis thaliana. Ann Bot 104:1323–1337

Reddy KP, Subhani SM, Khan PA, Kumar KB 1995. Effect of light and benzyl adenine on dark treated growing rice (Oryza sativa) leaves-changes in peroxidase activity. Plant Cell Physiol 26: 987-994

Ritchie SW, Nguyan HT, Holaday AS 1990. Leaf water content and gas exchange parameters of two wheat genotypes differing in drought resistance. Crop Sci. 30: 105-111

Sakamoto H, Matsuda O, Iba K 2008. ITN1, a novel gene encoding an ankyrin-repeat protein that affects the ABA-mediated production of reactive oxygen species and is involved in saltstress tolerance in Arabidopsis thaliana. Plant J 56:411–422

Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K 2002. DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and coldinducible gene expression. Bioch Bioph Res Co 290: 998–1009

Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki 2006. Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. The Plant Cell 18:1292-309

Shahbazi H, Taeb M, Bihamta MR and Darvish F 2010. Inheritance of antioxidant activity of bread wheat under terminal drought stress. American- Eurasian J Agric and Environ Sci 8(6): 680-684

Sharma P, and Dubey RS 2005. Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regul 46:209–221

Shinozaki, K. and Yamaguchi-Shinozaki, K. 1997. Gene expression and signal transduction in water-stress response. Plant Physiol 115: 327-334

Siddique MRB, Hamid A, and Islam MS 2004. Drought stress effects on water relations of wheat. Botanical Bulletin of Academia Sinica 41:35-39

Sreenivasulu N, Grimm B, Wobus U, Weschke W 2000. Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). Physiol.Plant 109: 435–442.

Subrahmanyam D, Subash N, Haris A,

Sikka AK 2006. Influence of water stress on leaf photosynthetic characteristics in wheat cultivars differing in their susceptibility to drought. Photosynthetica 44:125-129

Sun SJ, Guo SQ, Yang X, Bao YM, Tang HJ, Sun H, Huang J, Zhang HS. 2010. Functional analysis of a novel Cys2/His2-type zinc finger protein involved in salt tolerance in rice. J Exp Bot 61: 2807–281

Szabados L, and Savoure A 2010. Proline: a multifunctional amino acid. Trends in Plant Science 15: 89–97

Tas S, and Tas B 2007. Som physiological responses of drought stress in wheat genotypes with different poliodity in turkiye. World J Agri Sci 3(2): 178-183

Tezara W, Mitchell VJ, Driscoll SD and Lawlor DW 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401: 914-917

Tian ZD, Zhang Y, Liu J, Xie CH 2010. Novel potato C_2H_2-type zinc finger protein gene, StZFP1, which responds to biotic and abiotic stress, plays a role in salt tolerance. Plant Biol (Stuttg) 12: 689–697

Verbruggen N, and Hermans C 2008. Proline accumulation in plants: a review. Amino Acids 35: 753–759

Wahid A, and Close TJ 2007. Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves, Biol Plantarum 51:104–109

Wahid A, and Rasul E 2005. Photosynthesis in leaf, stem, flower and fruit, in: Pessarakli M. (Ed.), Handbook of Photosynthesis, 2nd ed., CRC Press, Florida, pp479–497

Xiao X, Xu X, Yang F 2008. Adaptive responses to progressive drought stress in two Populus cathayana populations. Silva Fennica 42:705–719

Zaefyzadeh M, Quliyev RA, Babayeva SM, Abbasov MA 2009. The effect of the interaction between genotypes and drought stress on the superoxide dismutase and chlorophyll content in durum wheat landraces. Turk J Biol 33:1–7

Zhang J, Liu T, Fu J, Zhu Y, Jia J, Zheng J, Zhao Y, Zhang Y, Wang G 2007. Construction and application of EST library from *Setaria italica* in response to dehydration stress. Genomics 90:121–131

Zhu JK 2002. Salt and drought stress signal transduction in plants. Annu Rev Plant Physiol and Plant Mol Biol 53: 247–273