

At drought conditions, water potential of soil and plant decreases, at advanced stages of plant growth, turgor pressure decreases, stomata close, leaf growth and photosynthesis rate reduce (Ozturk, 1998; Monti, 1986). Drought stress is synonymous with water stress; it happens when water lost as vapor (transpiration) is more than water taken (Sade, 2000). Pearl millet is a drought tolerant crop, and has a little earlier maturity; it also tolerates low soil pH. Pearl millet is the staple cereal in arid and drier semi-arid regions of country. It is the only cereal crop that is capable of producing a reliable yield under the marginal environments and simultaneously responds to high management conditions. Its nutritious grains not only form a crucial component of human diet but also are important for the principal maintenance of ration for ruminant livestock during the dry season.

- **Abiotic Stresses**

**Nicky, J. (2012)** revealed that the plant responses to different stresses are highly complex and involve changes at the transcriptome, cellular, and physiological levels. These responses are exclusive for multiple stresses as compared to the individual ones. Moreover he reported that each kind of stress activate a specific programme of gene expression relating to the exact environmental conditions encountered.

**Krasensky, (2011)** discussed the types of metabolic regulation in response to abiotic stresses along with the signaling events involved in mediating those stress-induced metabolic changes. He emphasized that plants regularly face unfavorable growth conditions, such as drought, salinity, and chilling, freezing, and high temperatures. These stresses can hinder the growth and development of the plants, thereby resulting in the decrease in productivity and sometime cause plant death. Plant stress responses are dynamic and involve complex cross talk between different regulatory levels, including adjustment of metabolism and gene expression for physiological and morphological adaptation.

**Johnsi Rani, R. (2011)** studied salt stress as a major adverse factor that can lower leaf water potential, leading to reduced responses, and ultimately lower crop productivity in arid and semi-arid zone. She found that salt stress reduces the ability of plants to take up water and this quickly causes reduction in growth rate. The initial reduction in shoot growth is probably due to salt effects. If excessive amounts of salt enter into the plants' system, it will eventually rise to toxic levels and reduce the photosynthetic leaf area of the plant that eventually prevents the plant from sustaining its own growth.

**Gill, D.S. (2011)** studied the thermal stress as a major factor in agriculture limiting the growth in different plants. Incessant increase in temperature cause physiological and biochemical changes in crops plants, which has severe consequences on overall growth process affecting different phonological stages of development such as seed germination, photosynthesis, respiration, water relations, membrane stability, hormonal changes as well as primary and secondary metabolites production, ultimately leading to a sharp reduction in grain yield. He highlighted that these detrimental effect of temperature stress can be mitigated by developing crop plants with thermo tolerance using various approaches and stated the role of enhanced expression of various heat shock proteins other stress related proteins and reactive oxygen species in contribute towards major plants responses to high temperature throughout plants ontogeny.

**Yakubu, H. (2010)** studied the effects the varying salt concentration gradient (0.0, 1.4336 and 2.1504 g/kg of soil) on the growth of different varieties of pearl millet in northern Nigeria namely Ex-Borno (Sammil-1), Maiwa, SE-13 (Sammil-6), Yar-Mubi and Buduma. They observed that plant height, shoot and root dry weight were decreased with increasing soil salinization. Maiwa variety was significantly superior to the other varieties in the expression of some parameters. N, P, K, Ca and Mg Concentrations were also significantly decreased with increasing NaCl concentrations, but Na and Cl accumulation increased in the plant tissues.

**Tuteja, N. (2010)** reported that despite of its high stability, DNA might get damaged due to exposure of biotic and abiotic stress factors, and thereby exert genotoxic stress

**FAO, (2009)** estimated that post-harvest losses among food grains in developing countries account for 15–50%. However, the post-harvest losses of fruits and vegetables (such as tomatoes, bananas, and citrus fruit) as well as of sweet potatoes and plantains could be even more alarming, being as high as 50%. It is also estimated that between 20% and 50% of the fish caught in Africa are lost after capture.

**Seghatoleslami, M. J. (2008)** studied the effects of drought on different growth stages of Proso millet (*Panicum miliaceum*). They selected four breded genotypes and a local variety of proso millet and plantmed then in a split-plot design with five irrigation treatments. Drought stress caused a great reduction in grain yield and at ear emergence stage. This reduction represented itself in the number of seed per ear and the weight of seeds, but it didn't have any effects on the number of ear per plant. At ear emergence stage, the drought stress increased the floret death and loss of seed size, which resulted in the reduction in the harvest index of both ear and seed per plant. Comparison of genotypes indicated that K-C-M.4 had a greater number of ears and K-C-M.9 had heavier seeds that had higher grain yield. These two genotypes had the highest WUE and their harvest indices were relatively higher.

**Mario houde, (2006)** studied the cold tolerant genes in wheat and identified a large scale ESTs sequencing parameter undertaken by the functional genomics of abiotic stress project (FGAS). They generated 73,521 quality-filtered ESTs from eleven c-DNA libraries from wheat plants exposed to different abiotic stresses and at different development stages. After annotation prepared assembly of the resolved ESTs generated a75488 unique sequences, 31580 contigs and 43908 singletons. Expression analysis indicated that FGAC database is enriched in stress-regulated genes compared to the other public database. Over 43% of unique sequences were classified in to different functional categories according to gene ontology.

**Halli well, (2006)** studies for making up productivity loss in crops due to stress condition, emphases are being given on agriculture sector to generate such varieties of crops that are tailored to provide better stress tolerance and can be grown in fluctuating environmental conditions for increasing plant productivity. Stress causes

changes in normal physiological functions of all plants, including economically important cereals. All these stresses reduce the biosynthetic capacity of plants and might cause damages that would be able to destroy plants. Stress triggers a wide range of plant responses varying from altered cellular metabolism and gene expression to changes in growth rates and crop yields.

**Twid well, E.K. (2002)** revealed soybeans as short-duration industrial crops, having high economic values and important position in the structure of agricultural crops in Vietnam. They are also the ideal crops in the rotation system due to their potential capability for enriching the fertility of the soil. Soybean is a species very sensitive to adverse conditions of the environment such as heat, cold, drought, insect attack, especially drought their poor drought resistance. Drought makes these plants mature about 8 days earlier than usual and prolonged drought limits the process of forming seeds.

**Bray, (2000)** reported the abiotic stresses, such as drought, high salinity, extreme temperature, and flooding as the major causes of crop loss worldwide, reducing average yields for most major crop plants by more than 50%. In particular, salinity is becoming an increasingly global problem and affects approximately 20% of global irrigated agricultural land.

**Yoshiba, (1997)** suggested that genetically engineered crop plants that overproduce proline under temperature stress might acquire osmo tolerance, i.e., the ability to tolerate environmental stresses such as drought and heat stress.

- **Drought Stress**

**Mostafavi, (2011)** studied the water deficit being as one of the most common problems of crop production in the world. The effect of drought stress on germination and early seedling growth of four hybrids of corn (namely KSC704, KSC475, KSC500 and Consor) using five concentrations of PEG 6000 (0, -4, -8, -12 and -16 bar) the experiment were conducted using a completely randomized design with four replications of seed technology in the laboratory at the College of Abuoraihan, University of Tehran. Analysis of variance results for each hybrid were indicated

marked differences for, stress levels and for most investigated traits, which demonstrated high diversity among hybrids. This enabled to screen drought tolerant cultivar to determine the hybrid most tolerant to drought stress conditions. Taking all traits into account it was found that KSC704 was the most resistant and KSC500 was the most sensitive.

**Kumar, R.R. (2011)** proposed that water deficit induced by Polyethylene glycol (PEG) affect physiological and biochemical changes in pigeon pea. The plants were subjected to two progressive stresses: moderate (-0.51 MPa) and severe (-1.22 MPa) respectively. The water stress condition was created by irrigating 14 days old grown seedling pot with PEG nutrient solution and decreasing the osmotic potential -0.04 MPa regularly. Relative water content (RWC) content was significantly reduced under water stress condition along with the increase in the free proline content during water stress condition. Proline is one of the common compatible osmolytes under water stress condition. The genotype exhibited lower accumulation of catalase (CAT) and increased activity of superoxide dismutase (SOD) and Peroxidase (POD) under stressed condition. The data presented a relation between proline content and activated antioxidant defense mechanism during water stress.

**Houane, L.R. (2007)** took seeds of pearl millet (*Pennisetum glaucum* (L) R. Br.) from six provenances of Tunisia, which were subjected to germination of shoot and root tests on filter paper treated with polyethylene glycol 6000 (PEG 6000) solutions made up to provide osmotic potentials of 0, -1 and -2 MPa. Mean germination percent for all provenances decreased about 73% in -2 MPa compared to control (0 MPa) treatment. Osmotic potential of -1.0 MPa improves the rate of germination but not significantly compared to the control. There were significant differences among the relative germination percent of the provenances in different treatments. Decreases in the external osmotic potential induced decreased shoot growth while a slight increase in root length associate with the -1 MPa treatments was observed for some ecotypes. This reflects an adaptive response involving an increase in root length.

**Sharma, (2005)** found that mild drought stressed plants had higher chloroplastic APX activity than control plants which usually declined at the higher level of drought stress.

**Guilioni, L. (2003)** found that heat stress, singly or in combination with drought, is a common constraint during grain filling stages in many cereal crops of temperate regions. The heat stress lengthened the duration of grain filling with reduction in kernel growth leading to losses in kernel density and weight by up to 7% in spring wheat. Similar reductions were observed in starch, protein and oil contents of the maize kernel and grain quality in other cereals under heat stress.

**Arvind, P. (2003)** demonstrated the presence of APX protein in plants during different stress conditions. Increased leaf APX activity under Cadmium stress has been reported in *Ceratophyllum demersum*. It was reported that pretreatment of *O. sativa* seedlings with H<sub>2</sub>O<sub>2</sub> under non-heat shock conditions resulted in an increase in APX activity and protected rice seedlings from subsequent Cadmium stress. Enhanced activity of APX was also found in salt stressed *A. doliolum*. Significant enhancement in APX activity was noted under water stress in three cultivars of *P. vulgaris* and *P. asperata*.

**Kitchen, (1999)** corroborated that drought, like many other environmental stress (Ex; heat stress, clod stress, salt stress etc.), has severe effect on the crop yield. Low water availability is one of the major causes for crop yield reductions, affecting majority of the farm area.

- **Drought Resistant Genes**

**Linzhou Huang, (2013)** considered Arabidopsis *ERECTA* gene (*ER*) in wheat, *TaER*, as a promising candidate gene for the genetic improvement of water use efficiency (WUE) and drought tolerance in breeding programs. In his study, he isolated two distinct homologues (*TaER1* and *TaER2*) of *TaER* genes in common wheat through *in silico* screening and PCR-based homologous cloning. Sequence analysis revealed that these two genes had a similar intron/exon structure with 27 exons and 26 introns, and each of them encoded a putative Leucine-rich repeat receptor-like serine/threonine

protein kinase in common wheat. The coding sequence (CDS) was 2928 bp for *TaER1*, encoding a protein with 975 amino acid residues, and 2931 bp for *TaER2* deduced to encode 976 amino acid residues, while their corresponding genomic DNA sequences were observed to be 6858 bp and 7114 bp, respectively. Cloning and sequencing of 55 *TaERc* DNA clones revealed five transcript variants of *TaER1* and nine spliced isoforms of *TaER2*, designated as *TaER1.1* to *TaER1.5* and *TaER2.1* to *TaER2.9* respectively. They also designed genome specific primers based on the sequence divergence of the promoter regions between the two homologous genes.

**Nguyen Phuong Thao, (2013)** ascertained that plant-specific NAC transcription factors plays important roles in plant response to drought stress. They compared the expression levels of a subset of *GmNAC* genes in drought-tolerant DT51 and drought-sensitive MTD720 under both normal and drought stress conditions aimed at identifying correlation between *GmNAC* expression levels and drought tolerance degree, as well as potential *GmNAC* candidates for genetic engineering. The expression of 23 selected dehydration-responsive *GmNACs* was assessed in both stressed and unstressed root tissues of DT51 and MTD720 using real-time quantitative PCR. The results indicated that expression of *GmNACs* was genotype-dependent. 7 and 13 of 23 tested *GmNACs* showed higher expression levels in roots of DT51 in comparison with MTD720 under normal and drought stress conditions, respectively, whereas none of them displayed lower transcript levels under any conditions. This finding suggests that the higher drought tolerance of DT51 might be positively correlated with the higher induction of the *GmNAC* genes during water deficit.

**Pruthvi, V. (2012)** identified candidate genes from stress responsive crop. He carried out expression analysis of a few drought responsive ESTs from *Arachishypogaea* L. (peanut). The expression patterns of nine AhDR (*Arachis hypogea* drought responsive) clones were analyzed under drought. Quantitative reverse transcription PCR analysis revealed stress responsive nature of the selected genes. The clones AhDR 118 (putative cyclin T-like), AhDR185 (aldehyde reductase-like), AhDR193 (cholin kinase-like) and AhDR 76 (proline amino peptidase-like) showed more than fivefold increase in expression. Highly up regulated genes analyzed for expression

pattern against salinity at seedling level indicated that these genes provide cross protection.

**Rattan S. Yadav, (2011)** validated that quantitative trait locus (QTL) mapping provides a means to dissect complex traits, such as drought tolerance, in their components, each of which is controlled by QTLs. Molecular marker-supported genotypic information at the identified QTLs then enables quick and accurate accumulation of desirable alleles in plant breeding programs. Recent genetic mapping research in pearl millet has mapped several QTLs for grain yield and its components under terminal drought stress conditions. Most importantly, a major QTL associated with grain yield and for the drought tolerance of grain yield in drought stress environments has been identified on linkage group 2 (LG 2), which accounts for up to 32% of the phenotypic variation of grain yield in mapping population testcrosses. The effect of this QTL has been validated in two independent marker-assisted backcrossing programs, where 30% improvement in grain yield general combining ability (GCA) expected of this QTL under terminal drought stress conditions was recovered in the QTL introgression lines. To transfer effectively favorable alleles of this QTL into pearl millet varieties that otherwise are high yielding and adapted to specific agricultural zones, efforts are currently underway to develop closely spaced gene-based markers within this drought tolerance (DT)-QTL.

**Ram Kumar, G. (2010)** reported allele mining as a promising approach to dissect naturally occurring allelic variation for applications in crop improvement programs. This genomic information could be used for isolating novel and superior alleles of agronomically important genes from crop gene pools to suitably deploy for the development of improved cultivars. It also helps in tracing the evolution of alleles, identification of new haplotypes and development of allele-specific markers for use in marker-assisted selection. Realizing the immense potential of allele mining, concerted allele mining efforts are underway in many international crop research institutes.

**Nguyen Thi Lang, (2010)** corroborated that drought is the most important constraint reducing rice yield in rain fed areas. The understanding of molecular basis of genes

tolerance to drought stress helps to breed elite rice cultivars under lower water requirements. To offer some lights on candidate genes response to drought stress, they conducted the experiment in IR64 rice cultivar under artificial drought stressed-condition. Isolating and sequencing of candidate gene tolerant to drought was performed. The analysis of phylogenetic relationship with identified candidate genes in different species was also investigated. The results showed that isolated gene can responses to drought stress; and involves in the regulation of salt stress. It provides an overview and to shorten the list of genes response to drought stress condition.

**Yiwei Jiang, (2010)** described Prairie junegrass (*Koeleria macrantha*) as a native cool-season C3 grass having vast potential low water requirement. They characterized the antioxidative responses and candidate gene expression in prairie junegrass subjected to drought stress. Two drought-tolerant (TOL-1 and TOL-2) and two drought-susceptible (SUS-1 and SUS-2) genotypes were subjected to 7 days of drought stress. Leaf relative water content (RWC) of SUS-1 and SUS-2 was 72.1% and 73.8% and RWC of TOL-1 and TOL-2 was 90.1% and 85.4% in drought-stressed plants, respectively. Drought stress did not affect chlorophyll fluorescence, lipid peroxidation, and antioxidative enzyme activities of superoxide dismutase (SOD), catalase (CAT), peroxidase, ascorbate peroxidase (APX), or glutathione reductase for tolerant or susceptible genotypes. The TOL-2 and SUS-2 genotypes were further examined for candidate gene expression. Drought stress did not alter expression levels of CAT and chloroplastic copper/zinc SOD (Cu/ZnSOD), but increased levels of APX in either genotype, compared with their relative controls. Expression of P5CS encoding D1-pyrroline-5-carboxylate synthetase and P5CR encoding D1-pyrroline-5 carboxylate reductase for proline biosynthesis were up-regulated under drought stress for both genotypes; however, expression of P5CR was more strongly induced under drought stress for TOL-2, compared with its control.

**Bernhard J. (2009)** identified novel alleles of *eukaryotic translation initiation factor 4E (eIF4E)* gene, which is required for resistance to agronomically important bymoviruses, in barley germplasm. This method involves scanning for sequence variations in cDNA-derived PCR amplicons using High-resolution melting (HRM) followed by direct Sanger sequencing of only those amplicons which were predicted

to carry nucleotide changes. The method described by them involved investigation of total cDNA rather than genomic DNA, thus permitting the analyses of shorter (up to 300-bp) and fewer overlapping amplicons to cover the coding sequence. This strategy further reduced the allele mining costs. The sensitivity and accuracy of HRM for predicting genotypes carrying a wide range of nucleotide polymorphisms in *eIF4E* approached 100%.

**Xiao-Yun Jia, (2007)** reported the possible association of Calreticulin (CRT) gene with drought and heat stress, the main abiotic stresses limiting cereal crop production worldwide. A full-length cDNA encoding calreticulin protein namely TaCRT was isolated from wheat (*Triticum aestivum* L.), followed by analysis of gene expression, drought response and physiological responses RWC by PEG6000 in wheat.

**Agarwal, P. (2007)** observed the regulation of some stress responsive gene, abiotic stress-mediated gene expression via the DNA transcription factors of which drought-responsive element-binding (DREB) proteins play an important role. There are two types of DREBs. Presently, the function of DREB1 type protein is well studied; however, limited information is available for DREB2. In their study, a cDNA with an open reading frame of 332 amino acids, encoding the transcription activation factor *DREB2A*, was cloned from *Pennisetum glaucum*, a stress tolerant food grain crop. Phylogenetic tree revealed that *PgDREB2A* is more close to DREBs isolated from monocots, though it forms an independent branch. The *PgDREB2A* transcript was up regulated in response to drought within 1 h of the treatment, whereas the induction was delayed in response to cold and salinity stress. However, during cold stress, the transcript was found to be induced more as compared to drought and salinity.

**Santiago, C. (2006)** carried out genetic association studies, which are rapidly becoming the experimental approach of choice to dissect complex traits, including tolerance to drought stress, that is the most common cause of mortality and yield losses in forest trees. In their study the pattern of polymorphism of 18 candidate genes for drought-stress response in *Pinus taeda* L., an important tree crop. Data analyses based on a set of 21 putatively neutral nuclear micro satellites did not show population genetic structure or genome wide departures from neutrality.

**Miyake C. (1994)** described APX gene as a class I hemeperoxidase, found in *Chlamydomonas*, *Euglena* (Cyanobacteria) and in a red alga *Galdieria partita*. APX has also been found in the protozoan *Trypanosoma cruzi* and insects. It has never been reported from animals, except for one case from bovine eye APX, which is the most efficient system or enzyme for scavenging H<sub>2</sub>O<sub>2</sub>. Nitric oxide (NO) can bind to the heme group of APX, thereby inhibiting it. Although APX and GPX both help in scavenging ROS but APX is distinct from Guatathione peroxidase (GPX). APX shows higher specificity for ascorbate, glycoprotein and is inactivated under electron-donor depleted conditions.

**Grover, A. (2008)** studied the heat shock proteins, heat shock factors, protein metabolism, thermo tolerance, and transgenic plants. High temperature-induced gene expression system is one of the best-studied model systems for analyzing induced gene expression. The molecular basis of heat shock (HS) response to high temperature tolerance has been genetically engineered in plants mainly by over-expressing the heat shock protein genes or indirectly by altering levels of heat shock transcription factor proteins. Apart from heat shock proteins, thermo tolerance has also been altered by elevating levels of osmolytes, increasing levels of cell detoxification enzymes and through altering membrane fluidity.

- **Methods to Identify Drought Resistance**

**Moody, D. E. (2014)** reported a variety of methods to identify differences in gene expression, including subtractive hybridization, differential display, serial analysis of gene expression and micro array hybridization. Some of these techniques have been used to investigate changes in gene expression in livestock, and important resources are being developed that will facilitate their further use for evaluating genes.

**Vincent Vadez, (2012)** proposed that pearl millet is highly resilient to some of the driest areas of the world, like the Sahel area or fringes of the desert in India. Despite this, there is a wealth of variation in pearl millet genotypes for their adaptation to drought, which has been used to breed better adapted cultivars. Pearl millet has also some development plasticity thanks to a high tillering, ability, which allows compensating for possible drought related failure of the main culm under intermittent

drought. The development of molecular tools for breeding has made great progress in the last 10 to 15 years and markers, maps, ESTs libraries, BACs are now available and a number of quantitative trait loci (QTLs) for different traits, including drought, have been identified. Most of the work on drought has focused on the drought tolerance index (DTI), an index that reflects the genetic differences in drought adaptation that are independent of flowering time and yield potential.

**Gazendam, I. (2007)** recognized and isolated the genes conferring drought tolerance in cowpea. A cDNA library enriched for cowpea genes expressed specifically during responses to drought was constructed using suppression subtractive hybridization (SSH) resulting in the construction of library consisting of 4160 individual clones. Preliminary sequencing results identified two clones to be known in stress-related plant genes (GST (glutathione-S-transferase) and PR-1 (pathogenesis-related protein-1)).

**Bo Ouyang, (2007)** used suppression subtractive hybridization (SSH) and micro array approaches to understand the molecular basis of plant responses to salt stress and identify the potential important candidate genes involved in the early stage of tomato responses to severe salt stress. Firstly, SSH libraries were constructed for the root tissue of two cultivated tomato (*Solanum lycopersicum*) genotypes name LA2711, a salt-tolerant cultivar, and ZS-5, a salt-sensitive cultivar, to compare salt treatment and non-treatment plants. Then a subset of clones from these SSH libraries were used to construct a tomato cDNA array and micro array analysis was carried out to verify the expression changes of this set of clones upon a high concentration of salt treatment at various time points compared to the corresponding non-treatment controls. A total of 201 non-redundant genes that were differentially expressed upon 30 min of severe salt stress either in LA2711 or ZS-5 were identified from micro array analysis most of these genes have not previously been reported to be associated with salt stress.

**Denis, V. (2000)** studied that Supression subtractive hybridization (SSH) is one of most powerful and popular method for isolating differentially expressed transcripts. SSH generated libraries typically contain some background clones representing non-

differentially expressed transcripts. We developed a simple procedure that substantially decreases the number of background clones. This method is based on the following differences between target and background cDNAs. Background molecule has only one orientation with the respect to the two different flanking adapter sequences orientations.

**Adams, M.D. (1990)** used technological advances facilitating high-throughput sequencing leading to the conception of expressed sequence tag (EST) libraries .The EST sequences, generated by randomly picking clones from a cDNA library, performed a single sequencing reaction to produce 300 to 500 bp of sequence per clone. Differences in gene expression may be identified by counting the number of times a particular sequence appears in EST libraries of genes from different sources. However, EST sequences are often generated from cDNA libraries that have been normalized to equalize the abundance of clones representing different transcripts.