Plant Environment Development 5(2):28-34, 2016 (July)
©Department of Botany, University of Rajshahi
http://www.botanyru.org/journal

Print ISSN 1994-1501
Online ISSN 2311-3529

Molecular strategies for managing water stress in crop plants

N. K. Paul

Department of Botany, Rajshahi University, Rajshahi, Bangladesh

Abstract

Field crops experience a multitude of environmental stresses and among them water stess is an important one. It is a major limiting factor for crop growth and productivity. Water stress elicits complex cellular responses. Considered once to be an arduous task, genetic engineering for increasing tolerance to abiotic stresses has come due to the progress made in exploring and understanding plant abiotic stress responses at physiological, biochemical, cellular and molecular levels. Plant molecular biology has given several clues in understanding how plants respond under stress. A great deal of success has been achieved in unveiling gene/ protein alterations associated with the preparation of plants against the abiotic stresses. In this paper, molecular mechanisms involved in water stress tolerance in plants have been reviewed.

Keywords: Water stress, Osmotic adjustment, Free radicals, LEA proteins, Molecular biology.

INTRODUCTION

It is commonly observed that field crops experience a multitude of stress conditions which are broadly classified into two categories, namely biotic (viral, bacterial, insect and fungal pathogens) and abiotic stresses (such as those imposed by presence of less or excess water, low and high temperature, excess salts etc.). Paucity of water for long periods due to lack of irrigation, infrequent rains or lowering of water table causes drought stress, whereas excess water through rains, cyclones or frequent irrigation results in flooding or submergence or anaerobic stress. Similarly , cultivation of plants on saline soils or frequent irrigation with groundwater leads to salinity stress and sudden atmospheric heating or cooling due to transient changes in wind patterns, cloud formation or excessive sunlight causes temperature stress. Since most crop plants have not been selected for meeting exigencies caused by such abiotic stress factors, the capacity of these to adjust to such conditions is usually limited.

In the Indo-Bangladesh subcontinent, important crops like rice, wheat, oilseeds and pulses are grown under stress-prone ecosystems, because farming is mostly at the mercy of weather conditions. Therefore, it is important that attempt should be made to reduce the losses in biomass caused by various stresses. However, different crop ecosystems are affected by different abiotic stress factors and to a differential extent (Kabir *et al*., 2015; Grover *et al*., 1998; Khush and Baenziger, 1998) .

Importantly, the degree of susceptibility of different plant species is often varied. There is also some level of variation associated with specific developmental stages of the plant. What adaptations (physiological and biochemical) will allow survival of plants in response to stress regimes? At the heart of all metabolic adaptations are molecular events and it is the molecular event that we mean when we aim at altering genetics of crops. But do we understand how plants face stress in terms of molecular alterations? This is the key issue in plant stress molecular biology studies today.

The overall aim of this paper is to review the current molecular strategies for the improvement of plant water stress resistance.

Molecular Biology of Plant Stress Responses

Although we have extensive knowledge on various aspects of the molecular biology of at least some of plant abiotic stress responses, this knowledge is mostly of phenomenological nature. Uncertainties between causes and effects persist in the study of many stress-tolerance mechanisms. This has been a major reason why manipulation of the abiotic stress resistance of plants lags far behind the engineering of pathogen resistance or fruit quality mechanism. Furthermore, most abiotic stress resistance traits are polygenic and quantitative, thus making them less suitable for genetic engineering than the simple inherited traits.

Several abiotic stresses such as drought, salinity, and extreme temperatures have a common consequence of causing cellular water deficit. Therefore, mechanisms of plant tolerance towards this so-called osmotic stress component and strategies of improving stress tolerance via its manipulation will be discussed simultaneously. Other components of these stresses may have a significant or major impact on overall plant stress resistance, while they are frequently much less studied.

Drought and salinity stress

Cellular dehydration caused by drought or salinity is a widespread abiotic stress, constituting one of the most stringent factors limiting plant growth and productivity (Boyer, 1982). The responses of the plants to the cellular water deficit are observed at the levels of phenological responses, morphological adaptations, physiological changes and biochemical adaptations. When the water supply is limited, a primary damage to plants results from dehydration of all the cell components. A major secondary damage stems from perturbation of cell electron transport leading to the production of toxic active O_2 radicals (Table 1). According to the current views, drought (dehydration) tolerance includes three major components: Osmotic adjustment, antioxidant capacity and desiccation tolerance *per se* (Bartels, 2005; Zhang *et al*., 1996).

Osmotic adjustment results from the accumulation of compatible solutes within cells, which lowers the osmotic potential and helps maintain turgor. Of all the putative drought resistance traits, the osmotic adjustment has been consistently shown to be associated with sustained plant production and yield under drought stress in several crops such as wheat (Islam et al., 2015; Blum and Pnuel, 1990; Morgan, 1995) and sorghum (Ludlow, 1993). Besides, the accumulated compatible solutes could also protect cell membrane integrity, prevent inactivation of enzymes and alleviate protein denaturation and aggregation (Islam et al., 2015; Bohnert and Jensen, 1996).

Antioxidant capacity is the ability of plants to detoxify active O_2 radicals. Many environmental stresses including drought, chilling, and salt stress impair electron transport system which leads to the formation of activated O_2 (Zhang and Kirkham, 1996). Tolerance to these stresses is correlated with high antioxidant capacity.

Desiccation tolerance is the capacity of cells to survive low water status through the implementation of mechanisms other than osmotic adjustment and antioxidant capacity. One example of such mechanism is the accumulation of specific drought- induced proteins (proteins belonging to the late embryogenesis abundant (LEA) family. These proteins are hypothesized to play a structural role as desiccation protectants (Goyal et al., 2005; Ingram and Barteles, 1996; Bray, 1993). Induction of these proteins was first observed in seeds, which underwent a natural process of desiccation during maturation, and later these and homologous proteins were shown to be induced in plant vegetative tissues subjected to various stresses causing water deficit (Honjoh et al., 2000; Bray, 1993).

Soil salinity is a major constraint to crop growth in the irrigated soils. Many crops are salt sensitive. High soil salinity induces water deficit via its osmotic effect and leads to the production of active O_2 species. Besides osmotic effect, salt stress has a so-called ionic component which is defined as the deleterious effect of high concentration of specific ions (mainly Na, Cl and SO_4 ions) on cell metabolism. Salt tolerance thus encompasses osmotic adjustment and antioxidant capacity, avoidance traits such as root ion exclusion, and the ability to detoxify or tolerate high concentrations of certain ions in the protoplasm and periplasm. Different crop species vary in their ionic tolerance and hence relative importance of osmotic and ionic components in their salt tolerance also differs. This consideration becomes especially important when results of transgenic experiments in one species (e.g. tobacco) are interpreted as universal. Although some success in engineering salt resistant plants has been achieved through manipulation of osmotic component-related genes, ion exclusion (avoidance feature) and ion tolerance mechanism may be of higher significance for overall plant salt tolerance in some species. However, little work has been done towards this end and investigation into this problem is highly warranted.

Table 1. Typical examples of genes (cDNA) induced by osmotic stress in higher plants.

Genes/cDNA	Characteristics/functions	Species
Genes encoding LEA proteins		
Group 1: D19, B19.1	Enhanced water bindings capacity	Cotton, barley
2: D11, rab 16A-D	Structure stabilizer	Cotton, rice
3: D7, HVA 1	Ion sequestration	Cotton, barley
4: D13, le # 25	Preserve membrane structure	Cotton, tomato
5: D29	Ion sequestration	Cotton
6: D34		Cotton
Genes encoding enzymes for osmolyte accumulation		
Proline synthesis related		
pVAB2	Δ'-Pyrroline-5-carboxylate synthetase	Mothbean
pProC1	Δ' -Pyrroline-5-carboxylate reductase	Soybean
Betaine synthesis related		
PBAD	Betaine aldehyde dehydrogenase	Barley
BADH	Betaine aldehyde dehydrogenase	Spinach Sugar # beet
Genes encoding enzymes for removing active O_2		
Superoxide dismutase (SOD) related		
Sd2O	Cytosolic Cu/Zn SOD	Pea
SODCc1	Cytosolic Cu/Zn SOD	Rice
Ascorbate peroxidase (AP) related		
Apx1	Cytosolic AP	Pea
Glutathione reductase (GR) related		
cDNA	Cytosolic GR	Pea
Catalase related		
Cat1-Cat # 3	Catalase	Maize
CAT1-CAT # 3	Catalase	*haliana*
Genes encoding water channel proteins		
7a	Water channel	Pea
RD28	Water channel	*A. thaliana*
Genes whose products have other functions		
PKABA1	Signal transduction (protein kinase)	Wheat
15a	Thiol protease	Pea
Genes whose functions are not clear		
RD20		*halina*
SalT		Rice

Osmotic Stress-Induced Genes: Characteristics and Function

An array of water stressed-induced genes has been isolated from plants, and their characteristics are briefly reviewed here. Based on their sequences and predicted functions, these genes can be grouped into six classes: genes encoding late embryogenesis abundant proteins (LEA proteins encoded by lea genes); genes encoding enzymes for synthesis of compatible solutes; genes encoding enzymes for scavenging toxic free radicals of O_2; genes encoding water channel proteins; genes encoding products that have other functions; and genes which do not have matches in sequence databases and whose functions therefore cannot be predicted. Typical examples of genes in each category and their potential functions in plants under water stress are summarized in Table 1.

The multiplicity of water stress-induced genes in plants reflects the complex nature of a plant`s drought response. While the majority of the products of these genes exhibit a known protective activity alleviating the effects of cellular dehydration upon cellular structures, some recently isolated water stress- induced genes have other functions and are related to such pathways as signaling (protein kinases) and protein degradation (proteases) (Table 1). Moreover, other genes which do not exhibit significant sequence similarities to any known sequences have been isolated (Table 1) pointing to the possibility that we are lacking yet more potential important components of the plant drought resistance mechanism. Major groups of water stress-induced genes with potentially significant functions in plant drought resistance are briefly discussed below:

Genes encoding LEA proteins

Based on amino acid sequence similarities among several species, lea genes have been classified into six groups (Table-1). LEA proteins of group one (D19 family) are predicted to have enhanced water-binding capacity, creating a protective aqueous environment for other proteins or structures. These proteins have a high percentage of charged amino acids. LEA proteins of group two (D11 family) also referred to as dehydrins (dhn) and rab (responsive to ABA), have a specific lysine rich region at the C terminus with the core consensus KIKEKLPG and may be structure stabilizers with detergent and chaperone-like properties (Islam et al., 2015; Borovskii et al., 2002; Close, 1996). LEA proteins of group three (D_7 family) contain repeated tracts of eleven amino acids (TAQAAKEKAGE) and are predicted to play a role in the sequestration of ions that are concentrated during cellular dehydration. LEA

proteins of group four (D113 family) have a conserved amino acid sequence at the N-terminus and many replace water to preserve membrane structure. LEA proteins of group five (D 29 family) are similar in chemical properties and functions to LEA proteins of group three, with the difference that residue position in the repeated motifs is not conserved. LEA proteins of group six is distinct from other groups of LEA proteins. They have a balanced hydrophobicity plot and their function is not clear.

Genes encoding enzymes involved in accumulation of compatible solutes

Active accumulation of various compounds defined as a group of compatible solutes` in the cytosol is an important mechanism of water stress tolerance in plants (Islam et al., 2015; Zhang *et al*., 1996). Under osmotic stress, accumulation of compatible solutes increases a plant`s ability to absorb/retain water and therefore to maintain turgor-related metabolic processes. Compatible solutes form a diverse group of compounds encompassing inorganic ions, organic ions, soluble carbohydrates including polyols, amino acids (particularly proline) and quaternary ammonium compounds such as betaine. Several plant genes that code key enzymes of proline, betaine and mannitol biosynthetic pathways have been identified (Table 1).

Genes encoding enzymes involved in removing toxic free radicals

Overproduction of active O_2 species in plant cells is a general consequence of several abiotic stresses including drought and salt stresses. Hence a plant`s ability to scavenge these toxic O_2 species is considered to be critical for abiotic stress tolerance. Plants have evolved various enzymatic and non-enzymatic protective mechanisms allowing them to scavenge active O_2 species. Among the enzymatic antioxidants, superoxide dismutase (SOD) catalyses the dismutation of superoxide to H_2 and molecular O_2, which maintains a low steady state concentration of superoxide, and therefore, minimizes hydroxyl radical formation by the metal catalysed Haber-Weiss reaction, whereas catalase (CAT) and peroxidase breakdown hydrogen peroxide to water. In plant cells, an alternative and more efficient detoxification mechanism against H_2O_2 is the ascorbate-glutathione cycle where ascorbate peroxidase (AP), monodehydro- ascorbate reductase, dehydroascorbate reductase and glutathione reductase (GR) work together (Islam et al., 2015; Kabir et al., 2015). Genes or cDNAs encoding AP, CAT, SOD and GR from several crops have been isolated (Table 1).

Gene encoding water channel proteins

Recently, a new class of water stress-induced membrane integral proteins has been identified. These proteins possess six putative membrane-spanning domains and a channel-like structure. Members of this family can form water-specific ion or solute channels. Up to date, two genes belonging to this family, 7a from pea and RD-28 from *Arabidopsis*, have been isolated (Table 1). These proteins accumulate in tonoplast membranes during water stress and are hypothesized to maintain the osmotic balance of the cytoplasm by promoting traffic of water and solutes within the cells.

Genes encoding products that have other physiological functions

Products of genes in this category have various functions, mainly including degrading proteins that are denatured during cellular water loss, regulation and signaling, and protection of cellular disruption.

Genes with unknown functions

Many stress-induced genes isolated up to date do not have any significant matches in the gene banks. Therefore, their functions cannot be predicted and need further investigation.

Genetic Transformation For Improving Osmotic Stress Resistance

Large groups of genes induced by water stress are supposed to play a major role in plant water stress tolerance and hence increasing expression of these genes by genetic engineering has been long proposed to improve plant water stress resistance. Recently, genetic modification of selected crop species to overexpress some water stress-induced genes became technically feasible. The main targets for improving plant tolerance to stresses causing water deficit are: (1) engineering increased cellular osmolyte concentrations, (2) expressing antioxidant enzymes, and (3) expressing lea genes. Genes of other classes such as those encoding water channel proteins and ion compartmentation may also serve as genetic targets as suggested by Bohnert and Jensen (1996) and Bohnert *et al*. (1995). However, no reports on their use for plant water stress tolerance improvement are yet available.

Osmolytes

Proline accumulation has been correlated with the tolerance to drought and salinity stresses in plants. Therefore, overproduction of proline in plants has been expected to increase plant water stress tolerance. The major pathway for proline synthesis in plants which takes place in the cytoplasm includes glutamate conversion to

γ- glutamyl phosphate and glutamic γ- semialdehyde (a two-step reaction that is catalysed in plants and animals by a bifunctional enzyme Δ'- pyrroline-5 –carboxylate synthetase, P5CS).

The glutamic-γ-semialdehyde is spontaneously cyclized to Δ'- pyrroline-5- carboxylate, which is then converted to proline by Δ'- pyrroline-5- carboxylate reductase (P5CR)(Barteis and Nelson, 1994; Tylor, 1996). The expression of the gene encoding P5CS is strongly induced during water stress in plants (Iskandar et al., 2011; Yoshida *et al*., 1995) and it is thought that this enzyme catalyses the rate-limiting step in proline synthesis (Delauney and Verma, 1993). Transgenic tobacco plants overexpressing a mothbean P5CS accumulated much more proline than the non-transformed control (Kishor *et al*., 1995). The increase in proline concentration correlated with the enhanced growth under salt and drought conditions. However, the mechanisms through which the increased cytoplasmic proline concentrations may influence water relations at the whole plant level are not immediately obvious (Blum *et al*., 1996).

The ability to synthesize and accumulate glycine betaine is widespread among angiosperms and is thought to contribute drought and salt tolerance. In Plants, glycine betaine is synthesized by a two-step oxidation of choline via the intermediate betaine aldehyde, catalysed by choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH). CMO is a specific plant enzyme structurally unrelated to choline dehydrogenases or oxidases which catalyze choline oxidation in other organisms. Osmotic stress induces both enzyme activities of CMO and BADH, suggesting that the pathway is coordinately regulated. Because several crops such as potato, rice, tomato and tobacco do not accumulate glycine betaine and glycine betaine is not actively turned over by these plants, introducing a glycine betaine synthesis pathway may Iead to increased stress tolerance. The cDNA clones for BADH have been obtained from several species such as spinach, sugar beet, barley and sorghum (Table 1). No genes encoding CMO have been yet cloned from plants.

Resistance to osmotic stress and toxic betaine aldehyde resistance in transgenic plants overexpressing enzymes of glycine betaine synthesis was inconsistent in several experiments. Plant BADHs targeted to tobacco chloroplasts conferred tobacco betaine aldehyde resistance. Transgenic tobacco expressing an *E. coli* bet A gene encoding choline dehydrogenase had increased salt tolerance (Khan et al., 2015; Lilius *et al*., 1996), as well as rice and *A thaliana* expressing choline oxidase gene from *Arthrobacter globifomis*. When *E. coli* BADH was expressed in tobacco, less resistance to betaine aldehyde was observed in plants expressing BADH in chloroplasts than in plants expressing BADH in the cytosol. On the contrary, transgenic tobacco plants expressing BADH did not exhibit any increased resistance to osmotic stress induced by polyethylene glycol or by withholding water.
Other compatible solutes which have been overexpressed in plants include mannitol, trehalose and fructan. Mannitol-1- phosphate dehydrogenase from *E. coli* catalyzes the synthesis of mannitol-1- phosphate, resulting in the accumulation of mannitol. Transgenic tobacco plants expressing mtl D gene encoding mannitol-1- phosphate dehydrogenase possessed increased salt tolerance compared with the wild type. Similarly, transgenic tobacco plants with engineered expression of fructan and trehalose exhibited improved drought tolerance.

Antioxidant capacity

What makes the engineering of antioxidant enzymes, particularly attractive is the fact that oxidative damage is a general consequence of environmental adversity. Improving the protection system of plants against oxidative stress could, therefore, be of benefit under various stress conditions. A number of enzymes with antioxidant capacity including SOD, AP and GR were used in plant oxidative stress tolerance.

Different isoforms of SOD exist in the cytosol, chloroplasts and mitochondria of plant cells. Various isoforms of SODs have been engineered into plants to enhance oxidative stress tolerance. These attempts were successful to various degrees. Over expression of chloroplastic Cu/Zn SOD from petunia in tobacco did not increase tolerance to a superoxide-generating herbicide paraquat. The introduction of the same gene in tomato did not increase plant tolerance to chilling –induced photoinhibition. However, over-expressing a mitochondrial MnSOD in chloroplasts conferred tobacco paraquat tolerance. Similarly, potato plants over-expressing tomato chloroplast Cu/Zn SOD and tobacco plants over-expressing pea chloroplastic Cu/Zn SOD or Mn SOD showed improved tolerance to oxidative stress.
Ascorbate peroxidase catalyses the conversion of hydrogen peroxide to oxygen and water through the ascorbate-glutathione cycle. Transgenic tobacco plants overexpressing cytosolic AP, but not chloroplastic AP, are tolerant to paraquat. Similar results are also reported in transgenic tobacco overexpressing a pea cytosolic AP.
Glutathione reductase reduces GSSG to GSH in the ascorbate-glutathione cycle (Kabir et al., 2015). Transgenic tobacco plants with enhanced GR activity

possessed increased resistance to phytooxidative stress, whereas transgenic GR deficient plants were less tolerant to paraquat than the control plants.

Attempts to improve oxidative stress tolerance by the manipulation of a single antioxidant enzyme have not yet always been successful presumably because of the need for a balanced interaction of protective enzymes. A simultaneous increase in several components of the antioxidant defense system (such as SOD, AP and GR) may be necessary in order to obtain a substantial increase in the stress tolerance. In accord with this, transgenic tobacco plants with enhanced cytosolic activities of both GR and SOD exhibited further increased tolerance to paraquat compared with transgenic plants with GR or SOD alone.

Late embryogenesis abundant (LEA) proteins

Because of their unique properties, proteins belonging to LEA family are another target for manipulation of water stress tolerance. Bartels (2005) transformed a few drought responsive genes from the resurrection plant *Craterostigma plantagineum* to tobacco. Although these plants overexpressed introduced genes at both mRNAs and protein levels, their drought stress tolerance did not significantly increase as measured by ion leakage assay. However, Xu *et al*. (1996) found that transgenic rice plants, which expressed a barley lea gene HVAI maintained higher growth rates than the non-transformed plants under both water-deficit and salt-stress conditions. The extent of stress tolerance correlated with the level of HVAI protein accumulation.

The foregoing account has dealt with the overall progress made in molecular biology of abiotic stress responses especially water stress. It is beyond any doubt that basic stress molecular biology has played the important background role in production of water stress-tolerant transgenics. There is need to not only continue but to encourage work with added zeal on this important area of plant molecular biology in the days when the new areas of genomics and proteomics are expected to lead to major discoveries.

References

Bartels , D. and D. Nelsen. 1994. Approaches to improve stress tolerance using molecular genetics. Plant Cell Environment 17: 659-667.

Bartels D. 2005. Desiccation Tolerance Studied in the Resurrection Plant *Craterostigma plantagineum*. Integr. Comp. Biol. 45 (5): 696-701.

Blum, A. and Y. Pnuel. 1990. Physiological attributes associated with drought resistance of wheat cultivars in a Mediterranean environment. Australian J. Agric. Res. 41: 799–810.

Blum, A., J.W. Johnson, E. L. Ramseur and E.W. Tollener. 1996. The effect of a drying top soil and a possible non-hydraulic root signal on wheat growth and yield. Expl. Bot. 42: 1225–1231.

Bohnert, H. J. and R. G. Jensen. 1996. Metabolic engineering for increased salt tolerance — the next step. Aust. J. Plant Physiol. 23:661-667.

Bohnert, H. J. and R. G. Jensen. 1996. Strategies for engineering water stress tolerance in plants. Trends in Biotechnology. 14: 89-97.

Bohnert, H.J., D.E. Nelson and R.G. Jensen. 1995. Adaptations to environmental stresses. Plant Cell 7: 1099-1111.

Borovskii GB1, Stupnikova IV, Antipina AI, Vladimirova SV, Voinikov VK. 2002. Accumulation of dehydrin-like proteins in the mitochondria of cereals in response to cold, freezing, drought and ABA treatment. BMC Plant Biol. 2, 5.

Boyer J. S. 1982. Plant productivity and environment. Science 218: 443–448.

Bray, E. A. 1993. Molecular responses to water deficit. J. Plant Physiol. 103: 1035–1040.

Close, T. J. 1996. Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins. Physiol. Plant. 97: 795–803.

Delauney, A. J. and D. P. S. Verma. 1993. Proline biosynthesis and osmoregulation in plants. Plant J. 4: 215-223.

Goyal K, Walton LJ, Tunnacliffe A. 2005. LEA proteins prevent protein aggregation due to water stress. Biochem Journal, 15, 151-157.

Grover, A., A. Pareek, S.L. Singla, D. Minhas, S. Katiyar and S. Ghawana, 1998. Engineering crops for tolerance against abiotic stresses through gene manipulation. Curr. Sci 75: 689–696.

Honjoh K., Matsumoto H., Shimizu H., Ooyama K., Tanaka K., Oda Y., Takata R., Joh T., Suga K., Miyamoto T., et al. Cryoprotective activities of group 3 late embryogenesis abundant proteins from Chlorella vulgaris C-27. Biosci. Biotechnol. Biochem. 2000;64:1656–1663.

Ingram, J. and D. Bartels. 1996. The molecular basis of dehydration tolerance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47: 377-403.

Iskandar HM1, Casu RE, Fletcher AT, Schmidt S, Xu J, Maclean DJ, Manners JM, Bonnett GD. 2011. Identification of drought-response genes and a study of their expression during sucrose accumulation and water deficit in sugarcane culms. BMC Plant Biol. 2011 Jan 13;11:12.

Islam M, Begum MC, Kabir AH, Alam MF. 2015. Molecular and biochemical mechanisms associated with differential responses to drought tolerance in wheat (Triticum aestivum L.). Journal of Plant Interactions 10(1), 195-201.

Kabir AH, Rahman MM, Haider SA Paul NK. 2015. Mechanisms associated with differential tolerance to Fe deficiency in okra (Abelmoschus esculentus Moench). Environmental and Experimental Botany 112, 16–26.

Kishor, P.B., S. Sangam, R. N. Amrutha, P. Sri-Laxmi, K. R. Naidu, K. R. S. S. Rao, S. Rao, K. J. Reddy, P. Theriappan and N. Sreenivasulu. 1995. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Curr. Sci. 88: 3.

Khan MS, Ahmad D, Khan MA. 2015. Utilization of genes encoding osmoprotectants in transgenic plants for enhanced abiotic stress tolerance. Electronic Journal of Biotechnology, 18(4), 257-266.

Kush, G. S. and P. S. Baenziger. 1998. Crop improvement: emerging trends in rice and wheat. In: Crop Productivity and Sustainability-Shaping the Future. (Eds.). V. L. Chopra, R. B. Singh, and A. Varma. New Delhi: Oxford and IBH Publishing Co. Pvt. Ltd. pp. 113-125.

Lilius, G., N. Holmberg and L. Bülow. 1996. Enhanced NaCl stress tolerance in transgenic tobacco expressing bacterial choline dehydrogenase. Biotechnology14:177–180.

Ludlow, M. M. 1993. Physiological mechanism of drought resistance. In: Biotechnology for arid land plants. (Eds.) T. J. Mabry, H. T. Nguyen, R. A. Dixon and M. S. Bonness, pp. 11-34. Austin, Texas, USA: IC2 Institute, University of Texas.

Morgan, J.M. 1995. Growth and yield of wheat lines with differing osmoregulative capacity at high soil water deficit in a range of field environments. Aust. J. Agric. Res. 37: 449–457.

Taylor, C. B. 1996. Proline and water deficit: ups, downs, ins and outs. Plant Cell. 8:1221–1224.

Xu, H. N., K. Z. Li, F. J. Yang, Q. H. Shi and X. F. Wang. 1996. Overexpression of CsNMAPK in tobacco enhanced seed germination under salt and osmotic stresses. Molecular Biology Reports 37(1): 3157–3163.

Yoshiba, Y., T. Kiyosue, T. Katagiri, H. Ueda, T. Mizoguchi, K. Yamaguchi-Shinozaki, K. Wada, Y. Harada and K. Shinozaki. 1995. Correlation between the induction of a gene for delta1-pyrroline-5-carboxylate synthetase and the accumulation of proline in *Arabidopsis thaliana* under osmotic stress. Plant J. 7: 751-760.

Zhang, A., M. Jiang, J. Zhang, M. Tan and X. Hu. 1996. Mitogen-activated protein kinase is involved in abscisic acid-induced antioxidant defense and acts downstream of reactive oxygen species production in leaves of maize plants. Plant Physiol. 141:475-487.

Zhang, H., J. Wang, R.D. Allen, U. Nickel and H.M. Goodman. 1996. Cloning and expression of an *Arabidopsis* gene encoding a putative peroxisomal ascorbate peroxidase. Plant Mol. Biol. 34: 967–971.

Zhang, J. and M.B. Kirkham. 1996. Enzymatic responses of the ascorbateglutathione cycle to drought in sorghum and sunflower plants. Plant Sci. 113: 139-147.