# Bio Technology

# Genetic Engineering for Abiotic Stress Tolerance in Agricultural Crops

[1]Bidhan Roy, [2]S.K. Noren, [3]Asit B. Mandal and [4]Asit K. Basu
[1]Department of Genetics and Plant Breeding, Regional Research Station, Terai Zone,
Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar 736 165, West Bengal, India
[2]College of Post Graduate Studies, Central Agricultural University, Umiam 793 103, Meghalaya, India
[3]Directorate of Seed Research, Khushmaur, P.O. Kathuli, Maunath Bhanjan 275 101, Uttar Pradesh, India
[4]Department of Seed Science and Technology,
Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia 741 252, West Bengal, India

**Abstract:** Abiotic stresses have become an integral part of crop production. One or other persist either in soil or in atmosphere. With the ultimate goal to raise the crop plants with better suitability towards rapidly changing environmental inputs, intense efforts are needed employing physiological, biochemical and molecular tools to improve tolerance ability under abiotic stresses. Attempts have been taken by plant breeders to develop tolerant varieties of different crops for specific abiotic stress. Appreciable improvement also has been done by the molecular biologists regarding to perturbations in gene expression and protein during stress. Employing transgenic technology, functional validation of various target genes involve in diverse processes, such as signaling, transcription, ion homeostasis, antioxidant defense etc. for enhanced abiotic stress tolerance has been attempted in various model system and some of them have been extended to crop plants. The information in the areas of gene and genetic engineering for improvement of crop plants against abiotic stresses are lying unorganized in different articles of journals and edited books. This information has been compiled in this review article in organized way with up-to-date citations, which will provide comprehensive literatures of recent advances.

**Key words:** Abiotic stresses, metal toxicity, genetic engineering, tolerance, crop plants

## INTRODUCTION

Most crops growing under field conditions are often being exposed to various abiotic stresses. The complex field environment with its heterogenic conditions and global climate change are increasing day by day but a few of them challenges facing modern agriculture (Mittler and Blumwald, 2010). A combination of plant breeding approaches will likely be needed to improve significantly the abiotic stress tolerance of crops in the field. A number of abnormal environmental parameters, such as drought, salinity, cold, freezing, high temperature, waterlogging, high light intensity, UV-radiation, nutrient imbalances, metal toxicities, nutrient deficiencies, climate change etc. are collectively termed as abiotic stress. Only 10% of world's arable land may be categorized as free from stress. The rapid change in environmental conditions are likely to override the adaptive potential of plants, these environmental changes mainly originated from anthropogenic activities causing soil and air pollution, thus plants are exposed to natural climatic or edaphic stresses. Among abiotic stresses drought is the main

abiotic factor as it affects 26% of arable area. Water stress is a single most severe, limitation to the productivity of rice in the rainfed ecosystem (Windawsky and O'Toole, 1990). Mineral toxicity/deficiencies are second in importance. Among mineral toxicity, salinity is wide spread and is estimated to affect 10% of the world land surface (Richards, 1995). Increasing salinization of arable land is expected to have devastating global effects, resulting in 30% land losses within the next 25 years and up to 50% by the year 2050 (Wang *et al.*, 2003a). Soil acidity is another major problem common to tropical regions, which constitutes about 3.95 billion ha of land (FAO, 1991). Acid soils caused by combination of aluminium (Al) and manganese (Mn) toxicity are major constraints to soil fertility and crop productivity. Al toxicity problems are of enormous importance in production of rice, maize and sorghum. Low temperature stress accounts about 15% crop area worldwide and such stress mainly prevails in temperate zone.

There is serious concern for food security of developing countries, which demands a conscious effort to improve production from areas commonly exposed to

---

**Corresponding Author:** Bidhan Roy, Department of Genetics and Plant Breeding, Regional Research Station, Terai Zone,
Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar 736 165, West Bengal, India

abiotic stresses. As mindless urbanization and industrialization swallow fertile lands and overuse of pesticides puts the environment jeopardy, researchers are designing crops that could tolerate abiotic stresses. The limitations to increase crop yield on existing cropped areas can be partially overcome with greater production inputs manipulation, which are costly, laborious and beyond the reach of small and marginal farmers. Thus, there is an urgent need to develop varieties that can not only withstand high levels of abiotic stresses but can also maintain optimum yield levels. In conventional breeding programme, genetic markers are being used by plant breeders, which are of morphological traits and controlled by single locus. The morphological markers are not always useful for selection for abiotic stresses. Therefore, molecular breeding (biochemical and/or DNA markers) is advancing as a new chapter for quick improvement in crops suitable for problem soils. Molecular control mechanisms for abiotic stress tolerance are based on the activation and regulation of specific stress-related genes. These genes are involved in the whole sequence of stress response, such as signaling, transcriptional control,

protection of membranes and proteins and free-radical and toxic compound scavenging. The complex plant response to abiotic stress, which involves many genes and biochemical-molecular mechanisms, is schematically represented in Fig. 1. Furthermore, reproductive barriers limit transfer of favourable alleles from interspecific and intergeneric sources. Transgenic development is another straight forward technology to improve crop yield in abiotic stress affected land (Roy and Basu, 2009). The development of tolerant crops by genetic engineering, on the other hand, requires the identification of key genetic determinants underlying stress tolerance in plants and introducing these genes into crops. Introduction of molecular change by genetic engineering takes less time compared to plant breeding methods: only desired gene(s) can be transferred, whereas, in conventional breeding approach is associated with simultaneous transfer of undesired gene(s). For re-cultivation of degraded soils and reclamation of industrial sites, stress tolerant plants are required. The advent of plant transformation may have placed within the grasp of the possibility of engineering greater abiotic stress tolerance in plants.
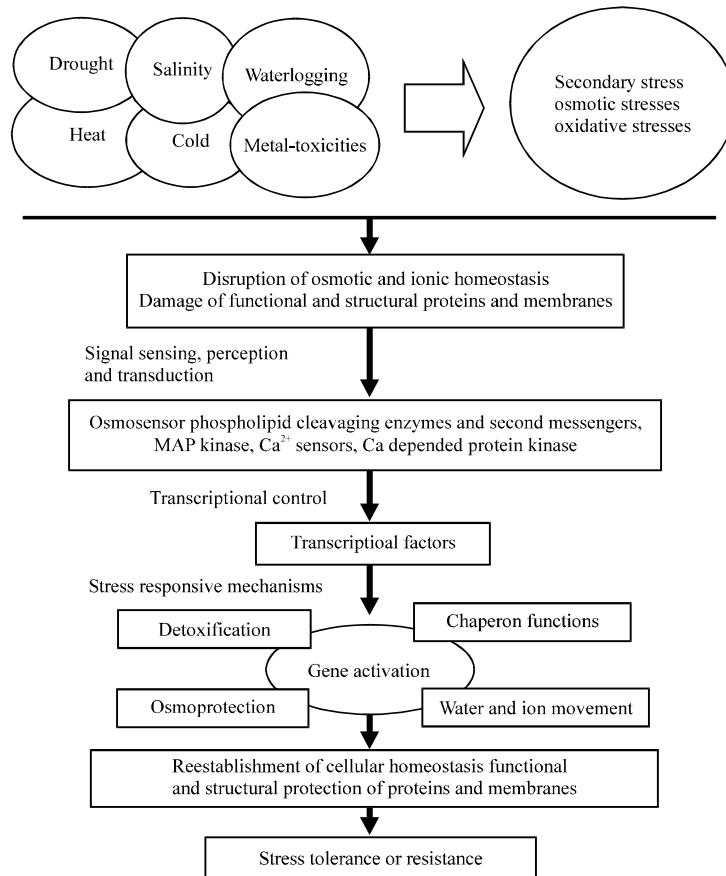


Fig. 1: Plant response to abiotic stress to develop tolerance or resistance

## SALT TOLERANCE

Salt tolerance is an important trait that requires overcoming salinity induced reduction in plant productivity. The genetic response of plants to abiotic stresses is complex involving simultaneous expression of a number of genes. Plant genetic engineering techniques could be effectively utilized to exploit some of the untapped potentials to increase the harvestable crop yield. It involves specific gene manipulation either through over expression or silencing of alien/native genes. A number of genes induced in response to salinity have been identified from a range of organisms adapted to stressful environment. If a salt tolerant gene is identified which can lead to betterment of the crops, it is possible to

transfer that progress in transgenic research for inclusion salinity stress tolerance, which has been presented in Table 1.

Transgenic research has made significant progress in crop genetic improvement with the advent of modern rDNA technologies. A large number of transgenics in diverse crops are on large-scale cultivation. Moreover, multiple genes can be stacked or transformed to a stock of interest through genetic transformation. There are a large number of genes found to be instrumental and there are many functional targets for engineering tolerance to salinity. Few of the genes of importance are briefed below.

Ion transporter and Antiporter genes: A salt concentration of 200 mM is equivalent to 40% of the salt

Table 1: List of transgenic research for salt stress tolerance

| Gene | Gene action | Species | Phenotypic expression by transgenic plant | Reference |
|---|---|---|---|---|
| MtlD | Manitol-1-phosphate dehydrogenase (manitol synthesis) | Tobacco | Increased plant height and fresh weight under salinity stress | Tarezynski *et al.* (1993) |
| p5cs | Pyrroline carboxylase synthetase (proline synthesis) | Tobacco | Enhanced biomass and flowering under salt stress | Kishor *et al.* (1995) |
| betA | Choline dehydrogenase (glycine betaine synthesis) | Tobacco | Increased tolerant to salinity stress | Lilius *et al.* (1996) |
| HVA1 | Group 3 LEA protein gene | *Oryza sativa* | Increased tolerance to drought and salinity | Xu *et al.* (1996) |
| IMT1 | Myo-inositol-O-methyl transferase (D-ononitol synthesis) | Tobacco | Performed better under drought and salinity stress | Shelveleva *et al.* (1997) |
| Nt107 | Glutathione S-transferase | Tobacco | Sustained growth under cold and salinity stress | Roxas *et al.* (1997) |
| p5cs | Pyrroline carboxylate synthetase (proline synthesis) | *Oryza sativa* | Increased biomass production under drought and salinity stress | Zhu *et al.* (1998) |
| DREB | Transcription factor | Arabidopsis | Increased tolerance to cold, drought and salinity | Kasuga *et al.* (1999) |
| NHX1 | - | Arabidopsis | Increased salt tolerance | Apse *et al.* (1999) |
| DnaK | - | Tobacco | Increased salt tolerance | Sugino *et al.* (1999) |
| MsPRP2 | Transcription factor | Alfalfa | Increased salinity tolerance | Winicov and Bastola (1999) |
| Osmotin | - | *Solanum tuberosum* | Increased salt tolerance | Evers *et al.* (1999) |
| proDH | Proline dehydrogenase | Arabidopsis | Tolerant to freezing and high salinity | Nanjo *et al.* (1999) |
| AtHAL3a | Phosphoprotein phosphatase | Arabidopsis | Regulates salinity, osmotic tolerance and plant growth | Espinosa-Ruiz *et al.* (1999) |
| *Glycolase* | - | Tobacco | Increased salt tolerance | Veena Ready and Sopory (1999) |
| AtNHX1 | Over expression of Na$^+$/H$^+$ antiport | Arabidopsis | Transgenic plants grow in 200 mM NaCl | Glenn *et al.* (1999) |
| Mn-SOD | Expression of superoxide dismutase | *Oryza sativa* | Increased salt tolerance | Tanaka *et al.* (1999) |
| GS2 | Chloroplastic glutamine synthetase | *Oryza sativa* | Increased salinity resistance and chilling tolerance | Hoshida *et al.* (2000) |
| HAL1 | - | *Solanum esculentum* | Increased salt tolerance | Gisbert *et al.* (2000) |
| OsCDPK7 | Transcription factor | *Oryza sativa* | Increased tolerance of cold, salinity and drought | Saijo *et al.* (2000) |
| codA | Glycine betaine synthesis | Brassica | Increased salt tolerance | Prasad *et al.* (2000) |
| p5csF | Proline synthesis (feed back inhibition removed) | Tobacco | Survived 200 mM NaCl, reduced oxidative stress induced by osmotic stress | Hong *et al.* (2000) |
| ApoInv | Invertase (Sucrose break down) | Tobacco | Salt tolerance to high osmotic pressure increase in cell sap | Fukushima *et al.* (2001) |
| AtNHX1 | Over expression of Na$^+$/H$^+$ antiport | Tomato | Transgenic plants grow in 200 mM NaCl | Zhang and Bhumwald (2001) |
| DtNHX | Over expression of Na$^+$/H$^+$ antiport | Brassica | Transgenic plants grow in 200 mM NaCl | Zhang *et al.* (2001) |
| *Osmotin* | - | *Oryza sativa* | Increased salt and drought tolerance | Barthakur *et al.* (2001) |
| MtlD | Manitol-1-phosphate dehydrogenase (manitol systhesis) | Rice | Increased salt tolerance | Li *et al.* (2004) |
| Tsase | Trehalose synthetase | Tobacco | Increased tolerance to drought and salt | Zhang *et al.* (2005) |
| pgAHX1 | Over expression of Na$^+$/H$^+$ antiport | *Pennisetum glaucum* | Increased salt tolerance | Rajgopal *et al.* (2007) |
| MnSOD | Expression of superoxide dismutase | Poplar | Increased salt tolerance | Wang *et al.* (2010) |

concentration of sea water and will inhibit growth of almost all crop plants. Ion trans porters selectively transport ions and maintain them at physiologically relevant concentrations while Na$^+$/H$^+$ antiporters also play a crucial role in maintaining cellular ion homeostasis, thus permitting plant survival and growth under saline conditions. The Na$^+$/H$^+$ antiporters catalyze the exchange of Na$^+$ for cytoplasmic pH, sodium levels and cell turgour (Serrano *et al.*, 1998).

A construct containing the *DtNHX* gene, coding for a vacuolar Na$^+$/H$^+$ antiport from *Arabidopsis thaliana,* was introduced into the genome of *Brassica napus* cv. Westar by Zhang *et al.* (2001). Over expression of the vacuolar Na$^+$/H$^+$ antiport did not affect the growth of transgenic plants since similar growth was observed when wild type and transgenic plants were grown in the presence of 10 mM NaCl. While growth of wild type plants was severely affected by the presence of 200 mM NaCl in the growth solution, transgenic plants grew, flowered and produced seeds. They noted that the transgenic plants grown at 200 mM NaCl produced number of seeds similar to those of wild-type plants grown at low salinity. Moreover, qualitative and quantitative analysis of oil content showed no significant differences between seeds from wild type plants grown at low salinity and transgenic plants grown at high salinity. They also observed that the transgenic plants accumulated up to 6% Na without altering the yield and oil content. Transgenic *Brassica juncia* plants over expressing *pgNHX1* withstand 300 mM salt stress till the seed setting stage and exhibited normal growth phenotype without much loss in seed yield (Rajgopal *et al.*, 2007).

Glenn *et al.* (1999) have engineered transgenic *Arabidopsis* plants that overexpress *AtNHX1*, a vacuolar Na$^+$/H$^+$ antiport, which allowed the plants to grow in 200 mM NaCl. Zhang and Bhumwald (2001) reported the genetic modification of tomato plants to overexpress the *Arabidopsis thaliana AtNHX1* antiport, which likewise allowed those plants to grow in the presence of 200 mM NaCl. Besides providing farmers with a cash crop for salted lands, such, plants may also pull salt out of soils, enabling other crops to thrive again. Wheat productivity is severely affected by soil salinity due to Na$^+$ toxicity to plant cells. Xue *et al.* (2004) generated transgenic wheat expressing a vacuolar Na$^+$/H$^+$ antiport gene *AtNHX1*. The transgenic wheat lines exhibited improved biomass production. The field trial revealed that the transgenic wheat lines produced higher grain yield and heavier and larger grains in the field of saline soil. The transgenic rice accumulated a lower level of Na$^+$ and higher level of K$^+$ in the leaves than the non-transgenic plants under saline environment.

The *Escherchia coli nahA* gene encodes a Na$^+$/H$^+$ antiporter, which plays critical role in ion homeostasis has been transferred into rice (*Oryza sativa* L. sp. *Japonica*) by Wu *et al.* (2005). The transgenic plants showed better germination rate, growth and average yield per plant than control. They also reported higher sodium and proline content in transgenic lines, implying that *nhaA* over-expression enhance osmoregulation by activating the bio-synthesis of proline.

**Compatible solutes:** Compatible solutes accumulate in organisms in response of osmotic stress. The primary function of compatible solute is to maintain cell turgour and thus the driving gradient for water uptake. Studies also indicated that compatible solutes can act as free radical scavengers or chemical chaperon by directly stabilizing membrane and/or proteins (Diamant *et al.*, 2001). The Δ'*adi* (Choline oxidase) gene isolated from the soil bacterium *Arthrobacter globiformis* converts choline to glycine betaine via betain aldehyde (Deshnium *et al.*, 1995). This gene encodes choline oxidase, the enzyme that converts choline to glycinebetaine. Transgenic potato plants expressing the oxalate oxidase enzyme were produced by Turhan (2005) using *Agrobacterium-*mediated genetic transformation. His findings revealed a relatively higher salt tolerance ability of transgenic than the non-transgenic genotypes *in vitro*. However, the glasshouse results were less consistent, but some transgenic genotypes showed superior yield characteristics to the non-transgenic under salinity.

Glycinebetaine is an extremely efficient osmoprotectant widely distributed among plants. It is a quaternary ammonium compound belonging to the organic solutes, which accumulate in higher plants in response to osmotic stress induced by drought, high salinity or low temperature (McNeil *et al.*, 2000). The ability to synthesize and accumulate glycinebetaine contributes to salt and drought tolerance (Grumet and Hanson, 1986). Transformation of *Arabidopsis thaliana* with Δ'*adi* gene resulted in enhanced salt tolerance of transgenic *Arabidopsis* plants (Hayashi *et al.*, 1997). Thus synthesis of glycinebetaine in transgenic plants *in vitro*, as a result of the expression of Δ'*adi* gene, might be very useful in improving the ability of crop plants to tolerate salt stress (Hayashi *et al.*, 2001). Sakamoto *et al.* (1998) reported transgenic rice expressing the Δ'*adi* gene in the chloroplast and the cytosol recovered to normal growth at a faster rate than the wild type after an initial growth inhibition under salt and low temperature stress.

Lilius *et al.* (1996) transformed tobacco plants with Δ'*adi* gene, which encodes for choline dehydrogenase protein (responsible for conversion of choline to betaine

aldehyde) from *E. coli*. The Δ'*adi* gene introduction rendered the transgenic tobacco plants tolerant to high concentration of salt. Betaine aldehyde dehydrogenease (BADH) is the second enzyme in this pathway responsible for conversion of betaine aldehyde to glycinebetaine.

Tobacco has been transformed with the Δ'*adi* and *betB* genes, involved in the glycinebetaine (betaine) pathway of *Escherichia coli* (Holmstrom, 1998). The transgenic plants produced and accumulated osmolyte and exhibited enhanced stress tolerance as shown by enhanced growth under salt stress and improved recovery of PS-II after salt stress. High tolerance to NaCl was also obtained transferring bacterial *beta* gene to cabbage (*Brassica oleracea* var. *capitata*) cultivar Golden Acre through *Agrobacterium*-mediated genetic transformation of hypocotyl explants (Bhattacharya *et al.*, 2004).

**Manitol gene:** Osmotically shocked cells synthesize and accumulate massive amount of osmoprotectory compounds. Such compounds possibly help the cells to lower their osmotic potential and to draw water from the outside medium. Manitol as an osmoprotectory compound is primarily found in microbes. By introducing manitol-1-phosphate dehydrogenease gene (*mt1D*) isolated from *E. coli* (Tarezynski *et al.*, 1993) showed over-expression of manitol in tobacco plants. These transgenic plants showed tolerance to high NaCl levels (250 mM). Seeds of transgenic *Arabidopsis* transformed with *mt1D* gene under control of CaMV 35 promoter over produced manitol and germinated in a medium supplemented with high amount of NaCl (Thomas *et al.*, 1995). Li *et al.* (2004) introduced *mt1D* gene into upland rice (*Oryza sativa* var. *japonica*) by microprojectile bombardment. Growth rate of transgenic plants was significantly higher than the control on MS medium containing 1% NaCl. Non-transgenic plants died after 35 days. They reported less membrane damage and low Na$^+$/K$^+$ ratio than the control under salt stress.

**LEA protein:** Osmotic stresses induce Late-embryogenesis-abundant (LEA) proteins in vegetative tissues of plants. The functions of LEA proteins are largely unknown. Nevertheless, their considerable synthesis during the late embryogenesis, their induction by stress and their structural characteristics permit the prediction of some of their functions. It has been suggested that LEA type proteins act as water binding molecules, in ion sequestration and membrane stabilization. LEA-proteins are encoded by Responsive to Dehydration (RD), Early Responsive to Dehydration (ERD), cold inducible (KIN), cold regulated

(COR) and responsive to ABA (RAB) genes in different plant species. Xu *et al.* (1996) found that the *hva1* gene, which encodes for a specific class of LEA proteins, when overexpressed in rice leads to increased salt tolerance. This study demonstrated that subcellular compartmentalization of the biosynthesis of glycinebetaine was a critical step in attaining enhancement of tolerance for salinity and water stress. Tansformation of Chinese cabagge (*Brassica campestris* ssp. *pekinensis*) by overexpression a *B. napus* Group 3 LEA gene enhanced tolerance to salinity and drought (Park *et al.*, 2005).

**Trehalose:** Trehalose is a non-reducing disaccharide of glucose that functions as a protectant in the stabilization of biological structure and enhances the tolerance of organisms to abiotic stress. Zhang *et al.* (2005) transformed tobacco plants with trehalose synthetase (*Tsase*) gene for manipulating abiotic stress tolerance. They reported higher trehalose accumulation in transgenic plants as compared to non-transgenics. The finding suggested that the transgenic plants transformed with *Tsase* gene can accumulate higher levels of trehalose and have enhanced tolerance to drought and salt stresses.

**H$^+$-pyrophosphatase (H$^+$-Ppase) gene:** An H$^+$-Ppase gene named *TsVP* involved in basic biochemical and physiological mechanisms was cloned from *Thellungiella halophila*. Transgenic tobacco overexpressing *TsVP* had 60% greater dry weight than wild-type tobacco at 300 mM NaCl (Gao *et al.*, 2006). Their findings suggested that over expression of H$^+$-Ppase causes the accumulation of Na$^+$ in vacuoles instead of in the cytoplasm and avoids the toxicity of excess Na$^+$ in plant cells.

## WATERLOGGING TOLERANCE

The major changes in the waterlogged soils are physical, biological and chemical changes. Upon flooding, the pore spaces (filled with air) in the soil become saturated with water, as a result, the soil swells. Since, the exchange of air between the atmosphere and the soil is impeded and since the water particles are held by soil particles and prevent from percolating downward and escaping. The absence of soil air in waterlogged condition causes a change in the varieties of microbes, microscopic, organisms which live in the soil. Anaerobic microbes tend to be much slower; less efficient decomposer of organic matter than the aerobic microorganisms. Consequently, the rate of decay of organic matter tends to be slow in flooded soils. Also the end products produced by
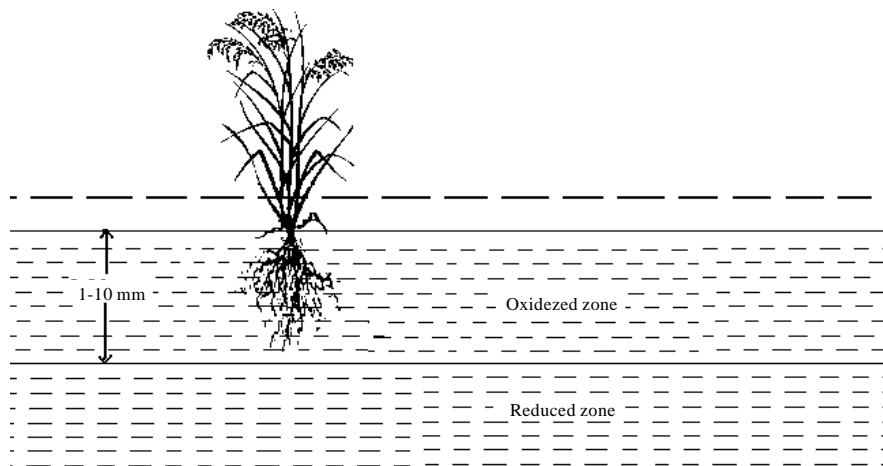
Fig. 2: Chemical zones of flooded soil

Table 2: Transgenics developed in different crop plants against waterlogging tolerance

| Gene | Gene action | Species | Phenotype | Reference |
|------|-------------|---------|-----------|-----------|
| pdc1 | Pyruvate decarboxylase | Rice | Increased submergence tolerance | Qunimio *et al.* (2000) |
| ADH | | Cotton | Increased ethanol fermentation | Dennis *et al.* (2000) |
| ACC | ACC deaminase | Tobacco | Increased flooding (waterlogging) tolerance | Grichko and Glick (2001) |
| pdc1 | Pyruvate decarboxylase | Rice | Rice Over 2-fold greater PDC activity and they had up to 43% greater rate of ethanol synthesis | Rahaman *et al.* (2001) |
| Sub1A | ethylene-response-factor | Rice | Submergence tolerance | Xu *et al.* (2006); Fukao and Bailey-Serres (2008) |
| HRE1 and HRE2 | hypoxia-inducible ethylene response factors | Arabidopsis | Improve the tolerance of the plant to the stress by enhancing anaerobic gene expression and ethanolic fermentation | Licausi *et al.* (2010) |

anaerobic decomposition differ some are toxic to crop plants; particularly those released during the first two weeks after decomposition begins and the toxicity produced during decomposition may stunt the growth of crop plants. Flooded soils develop two distinct chemical zones (Fig. 2); oxidized and reduced zones. The upper oxidized zone (1-10 mm) absorbs oxygen from the water, turns brown in colour and reacts to nitrogen like an unfolded soil. The lower reduced zone, which extends down as far as the water, is extremely low in available oxygen, turns dark blue or gray in colour and takes on chemical properties quite different from those of oxidized layer above.

Genetic engineering provides opportunities for germplasm improvement as well as evaluating the impact of different mechanisms of tolerance to waterlogging without confounding effects of complete changes in the background. Two different approaches have been used to try and identify limiting factors in the response to waterlogging. First is the under-expression of single candidate genes, e.g., for ethanol synthesis, using sense and anti-sense constructs. Second is the over-expression of transcription factors (Dennis *et al.*, 2000). It was

anticipated that both approaches may have a beneficial effect in switching on the longer term adaptation response to low oxygen stress. Few achievements in transgenic development have been presented in Table 2.

Preliminary results of rice transgenic containing the cotton *adh* cDNA suggested that the over expressing pdc do not show increased tolerance to submergence. Qunimio *et al.* (2000) found that Taipei 309 transferred with *pdc1* linked to a constitutive 35S promoter had up to 3-fold higher PDC activities and ethanol synthesis rates when exposed to anoxia compared to non-transformed controls. They also reported that increasing ethanol production up to 6-fold in a range of transgenic lines exposed to anoxia was correlated with an 8-fold increase in percentage survival of lines during submergence under hypoxic conditions. In contrast, Rahaman *et al.* (2001) studied Taipei-309 transformed with pdc1 and found that two transgenic lines had over 2-fold greater PDC activity and they had up to 43% greater rate of ethanol synthesis, however, survival of seed lines exposed to anoxia was even less than that of non-transformed plants. Therefore, the results of rice transgenic need to be repeated for its potential extrapolation.

Transgenic cotton plants containing the ADH cDNA driven by a constitutive 35S promoter showed 10-30 fold increase in ADH activity and a significant increase in the rate of ethanol fermentation (Dennis *et al.*, 2000). Cotton plants with rice pdc1 cDNA driven by a constitutive 35S promoter produce more pdc protein but had only marginal more PDC activity. They also observed that neither pdc or adh transgenic cotton plants, nor plants containing both constructs showed increased tolerance of hypoxia stress.

## DROUGHT TOLERANCE

Water becomes an increasingly scarce and precious commodity. It is thus essential to improve water use efficiency in agriculture. The development of crop varieties with increase tolerance to drought, both by conventional breeding methods and genetic engineering is important to meet global food demand with less water. Conventional breeding and marker assisted selection are being used to develop varieties more tolerant to water stress. These methods are time taking and resource consuming and germplasm dependent. Genetic engineering is alternative because of its potential to improve abiotic stress tolerance more rapidly. The techniques for gene transformation of crop plants have been applied for identification of genes responsible for drought resistance and their transfer. Genes expressed during stress are anticipated to promote cellular tolerance to dehydration through protective functions in the cytoplasm, cell membrane, alternations of cellular water potential to promote water uptake, control of ion accumulation and further regulation of other gene(s). A comprehensive study on transgenic plants summarized and nature of tolerance has been listed in Table 3.

Table 3: Genes/transgenic plants for drought tolerance

| Gene | Gene action | Species | Phenotypic expression by transgenic plant | Reference |
|---|---|---|---|---|
| Sod | Cu/Z superoxide dismutase | *Solanum tabaccum* | Retained 90% photosynthesis under chilling and heat stress | Sengupta *et al.* (1993) |
| Sod | MnSod | *Medicago sativa* | Increased tolerance to water deficit | McKersie *et al.* (1993, 1999) |
| tstB | Trehalose 6-phosphate synthetase | *Solanum tabaccum* | Increased weight and more efficient photosynthesis under drought stress | Pilon-Smits *et al.* (1995) |
| HVA1 | Group 3 LEA protein gene | *Oryza sativa* | Increased tolerance of drought and salinity stress | Xu *et al.* (1996) |
| HVA1 | Group 3 LEA protein gene | *Oryza sativa* | - | Jain *et al.* (1996) |
| otsA | trehalose-6-phosphate (Trehalose synthesis) | *Solanum tabaccum* | Increased dry weight and photosynthetic activity under drought | Pilon-Smits *et al.* (1996) |
| | Mn Sod | *Medicago sativa* | Increased tolerance to water deficit | McKersie *et al.* (1996) |
| TPS1 | Trehalose 6-phosphate synthetase (trehalose synthesis) | *Solanum tabaccum* | Increased drought tolerance | Romero *et al.* (1997) |
| imt 1 | Myo-inositol o-methyl transferase (D-ononitol synthesis) | *Solanum tabaccum* | Improved performance under drought and salt as shown by better photosynthetic rate | Shelveleva *et al.* (1997) |
| Adc | Arginine decarboxylase | *Oryza sativa* | Reduced chlorophyll loss under stress | Capell *et al.* (1998) |
| Phes | Pyrroline carboxylate synthelase (proline synthesis) | *Oryza sativa* | Increased biomass production under drought and salinity stress | Zhu *et al.* (1998) |
| DREB | Transcription factor | *Arabidopsis thaliana* | Increased tolerance of cold, drought and salinity | Kasuga *et al.* (1999) |
| | Mn superoxide dismutase | *Medicago sativa* | Increased tolerance to water deficit | McKersie *et al.* (1999) |
| OsCDPK7 | Transcription factor | *Oryza sativa* | Increased tolerance of cold salinity and drought | Saijo *et al.* (2000) |
| HVA1 | Group 3 LEA protein gene | *Triticum aestivum* | Increased biomass WUE under stress | Sivamani *et al.* (2000) |
| Tsi1 | Transcription factor | *Solanum tabaccum* | Increased osmotic stress tolerance | Park *et al.* (2001) |
| AhCMO | Chotine monoxygenase | *Solanum tobaccum* | Improved drought tolerance | Shen *et al.* (2002) |
| HVA1 | Group 3 LEA protein gene | Pusa Basmati 1 | Increased stress tolerance in terms of cell integration and growth | Rohila *et al.* (2002) |
| Tsase | Trehalose synthase | Sugarcane | Tolerant to osmotic stress | Wang *et al.* (2003b) |
| TPS1 | Trehalose synthase | *Solanum tabaccum* | High intergrity of chloroplast thylakoid membrane | Lee *et al.* (2003) |
| DREB1A | Transcription factor | *Triticum aestivum* | Increased drought tolerance | Pellegrineschi *et al.* (2003, 2004) |
| AtTPS1 | Trehalose biosynthesis | *Solanum tabaccum* | High germination rates at high levels of manitol | Almeida *et al.* (2004) |
| NPK1 | Expression of nitrogen-activated protein kinase | *Solanum tabaccum* | Enhanced drought tolerance | Shou *et al.* (2004) |
| InsP 5-ptase | Expression inositol polyphosphate 5-phosphatase | *Arabidopsis thaliana* | Enhanced drought tolerance | Perera *et al.* (2008) |
| ABF3 | Transcription factor | *Arabidopsis thaliana* | Increase drought tolerance | Abdeen *et al.* (2010) |

**Trihelose biosynthesis:** Bacterial trehalose-6-phosphate (OtsB) genes introduced in tobacco showed better growth under drought stress (Pilon-Smits *et al.*, 1995). Transgenic plants also showed a better capacity to retain water and preformed more efficient photosynthesis under stress. The gene TPS1 found in yeast encodes for trehalose-6-phosphate synthetase and is involved in biosynthesis of trehalose. The transgenic tobacco plants containing the yeast TPS1 gene exhibited multiple alteration and improved drought tolerance (Romero *et al.*, 1997). By determining the effect of withholding irrigation on the death and damage of leaf (Romero *et al.*, 1997), it has been shown that the transgenic plants have increased drought resistance. Further, Holmstrom (1998) transferred tobacco, resulting in the accumulation of trehalose and improved drought resistance. It is suggested that the improved drought tolerance is due to enhanced water retention capacity. Serrano *et al.* (1998) reported that yeast regulatory gene involved in stress tolerance, TPS1, have been expressed in transgenic plants. Almeida *et al.* (2004) transformed *Nicotiana tabaccum* with *Arbidopsis thaliana* gene (AtTPS1), which is involved in trehalose biosynthesis, by *Agrobacterium*-mediated genetic transformation. They observed high germination rates at higher levels of manitol than did wild type plants.

The trehalose synthetase gene (TSase) gene from *Grifola frondosa* was transferred into sugarcane (Wang *et al.*, 2003b). The high frequency phosphinothricine-resistant plants were obtained from the transformation. Some transgenic plants showed multiple phenotypic alterations and some plants showed improved tolerance to osmotic stress. Another trehalose phosphate synthetase (TPS1) gene from yeast was introduced into tobacco chloroplast (Lee *et al.*, 2003). Stable integration of TPS1 into chloroplast genome of tobacco was confirmed by PCR and Southern blots analysis. Transgenic chloroplast thylakoid membranes showed high integrity under osmotic stress as evidenced by the retention of chlorophyll even when grown in 6% PEG6000, whereas, chloroplasts of untransformed plants were bleached. Thus, they suggested that trehalose functions by protecting biological membranes rather than regulating water potential.

**LEA protein:** Transgenic rice carrying barley HVA1 gene had shown drought resistance (Xu *et al.*, 1996). Gene HVA1 encodes for a group of three LEA proteins which get accumulated in vegetative organs during drought condition. Transgenic rice showed enhanced accumulation of the HVA1 protein, which led to higher growth rates, delayed stress-related damage systems and improved recovery from the removal of stress conditions

(Xu *et al.*, 1996). The transgenic rice plants exhibited constitutive high expression of HVA1 protein ranging from 0.3-2.5 and 0.3-1.0% of the total soluble protein in leaf and root, respectively. Transgenic wheat plants containing the HVA1 gene showed consecutive expression of the transgene resulting in improvement of growth characteristics under water deficient conditions, more biomass and more efficient water use (Sivamani *et al.*, 2000). Rohila *et al.* (2002) transformed Pusa Basmati 1 with HVA1 to increase tolerance against abiotic stresses. They developed transgenic lines which showed increased stress tolerance in terms of cell integrity and growth after imposed salt-and water stresses. Their findings exhibited high levels of LEA3 accumulation in the leaves of transgenic Pusa Basmati 1 rice plants might have conferred the significant increase in tolerance against drought and salt stresses.

**Fructan synthesis:** The bacterial gene sacB found in *Bacillus subtilis* encodes for levan sucrase, which takes part in fructan synthesis. Fructan promotes the process of root branching, thus increasing root surface and water uptake. Pilon-Smits *et al.* (1995) have shown that over-expression of scaB gene from *Bacillus subtilis* leads to high level of fructans in transgenic tobacco cells, leading in higher capacity for osmotic adjustment and this is associated with increased drought tolerance. The additional carbohydrate gained may lead to deeper rooting and greater water-uptake (Pilon-Smits *et al.* 1995; Blum *et al.*, 1996; Schellenbaum *et al.*, 1999). The transgenic tobacco that produces bacterial fructans was produced and examined for growth of the transgenic plants was significantly higher both on fresh weight and dry weight basis under drought stress compared to the wild type tobacco. The transgenic tobacco exhibited significantly more biomass accumulation in roots under drought stress and higher non-structural carbohydrate content under all conditions.

**Proline accumulators:** Proline accumulation has been demonstrated to be associated with abiotic stress (Delauney and Verma, 1993). Some genes related to proline synthesis, transport and accumulation have been identified (Delauney and Verma, 1993). Transgenic tobacco overexpressing P5CS gene transferred from mothbean exhibited a high level of enzyme and produced 10-18 fold more proline than control plant (Kishor *et al.*, 1995). Over-production of proline enhanced root biomass and flower development under drought condition. Zhu *et al.* (1998) also found that transgenic rice with P5CS gene enhanced root biomass and flower development under water stress. Transgenic soybean plants transferred

with antisense *P5CR* (L-Δ'-pyrroline-5-carboxylate reductase) gene showed increased proline accumulation, leading to higher tolerance to water stress (De Ronde *et al.*, 2000).

**Polyamine biosynthesis genes:** In plants, polyamine, accumulate under several abiotic stress stimuli, including drought and salt. It has been suggested that this increase in polyamine concentration could be considered as an indicator of plant stress. With the availability of genes responsible for polyamine biosynthesis such as ADC (encodes for arginine decarboxylase), it is now possible to manipulate polyamine content using sense and antisense constructs of these genes in transgenic plants. Engineering of the plant polyamine biosynthesis pathway has concentrated mostly on two species, tobacco and rice (Kumar and Minocha, 1998; Capell and Christou, 2004). They suggested that further studies are required to understand the tolerance ability of these genes. The increase in putrescine levels in plants under stress might be the cause of stress-induced injury or alternatively a mean of protection against stress. Roy and Wu (2001) expressing oat adc cDNA in rice under control of an ABA-inducible promoter resulted in transgenic rice plants with increased biomass when grown under salt stress. Capell and Christou (2004) have generated a diverse rice germplasm with altered polyamine content. Transgenic rice plant expressing the SAMDC DNA accumulated spermidine and spermine in seed at 2-3 fold higher levels compared to wild type. In another set of experiment, they have obtained ten fold putrescine accumulation in transgenic rice plants laboring oat adc cDNA compared to wild type. Spermidine and spermine de-novo synthesis in transgenic plants under drought stress is corroborated by the activation of the rice SAMDC gene. Transcript levels for rice SAMDC reach their maximum levels at 6 days after stress induction. Such increase in the endogenous spermidine and spermine pools of transgenic plants not only regulates the putrescine response, but also exerts an anti-senescence effect at the whole plant level, resulting in phenotypically normal plants. Wild type plants, however, are not able to raise their spermidine and spermine levels after 6 days of drought stress and consequently exhibit the classical drought stress response (Capell and Christou, 2004).

Choline monooxygenase (CMO) catalyzes the committed step of glycinebetaine (GlyBet) biosynthesis in many flowering plants. Over-expression of AhCMO improved drought tolerance in transgenic tobacco when cultured in medium containing PEG6000 (Shen *et al.*, 2002).

**Dehydration responsive transcription factor:** Plant genomes contain a large number of Transcription Factors (TFs). TFs are key regulatory proteins that enhance or repress the transcriptional rate of their target genes by binding to specific promoter regions upon activation or deactivation of upstream signaling cascade. Most of these transcriptional factors belong to a few large multigene families. Individual members of the same family often respond differently to various stress stimuli, on the other hand, some stress responsive may share the same transcriptional factor (Chen *et al.*, 2002; Kreps *et al.*, 2002). The Dehydration-responsive Element (DRE) and C-repeat binding factors (CBF) was identified as a cis-acting element regulating gene expression in response to dehydration (salt, drought and cold stresses) in *Arabidopsis* (Van Rensburg and Kruger, 1994). The dehydration-responsive transcription factors DREB and C-repeat binding factors (CBF) bind to DRE and CRT cis-acting elements that contain the same motif (CCGAC). Members of the CBF/DREB1 family, such as CBF1, CBF2 and CBF3 (or DREB1B, DREB1C and DREB1A, respectively) are themselves stress-inducible. DREB/CBF proteins are encoded by AP2/EREBP multigene families and mediate the transcription of several genes. DREB1A, a transcription factor that recognizes dehydration response elements, has been shown in *Arbidopsis thaliana* to play a crucial role in promoting the expression of drought tolerant genes (Pellegrineschi *et al.*, 2003, 2004). They have transformed DREB1A gene into wheat. Plant expressing the gene demonstrated substantial resistant to water stress compared with the control under stress condition. Recently, overexpression of CBF4 from barley has been shown to confer salinity, drought and low temperature tolerance in transgenic rice (Oh *et al.*, 2007).

The NAC gene family members encode one of the largest families of plant specific TFs and are expressed in various developmental stages, tissues and environmental factors. Hu *et al.* (2006) isolated and characterized NAC gene SNAC1. SNAC1 over-expressing transgenic rice plants showed significantly improved drought resistance under field conditions and strong tolerance to salt stress (Hu *et al.*, 2006).

The phosphoinositide pathway and inositol-1,4,5-trisphosphate (InsP$_3$) are implicated in plant responses to stress. To determine the downstream consequences of altered InsP$_3$-mediated signaling, Perera *et al.* (2008) generated transgenic *Arabidopsis thaliana* plants expressing the mammalian type I inositol polyphosphate 5-phosphatase (InsP 5-ptase), which specifically hydrolyzes soluble inositol phosphates and terminates the signal. Rapid transient Ca$^{2+}$ responses to a cold or salt stimulus were reduced by ~30% in these transgenic

plants. Drought stress studies revealed, surprisingly, that the InsP 5-ptase plants lost less water and exhibited increased drought tolerance. The onset of the drought stress was delayed in the transgenic plants and abscisic acid (ABA) levels increased less than in the wild-type plants. Stomatal bioassays showed that transgenic guard cells were less responsive to the inhibition of opening by ABA but showed an increased sensitivity to ABA-induced closure. Transcript profiling revealed that the drought-inducible ABA-independent transcription factor DREB2A and a subset of DREB2A-regulated genes were basally upregulated in the InsP 5-ptase plants, suggesting that *InsP$_3$* is a negative regulator of these DREB2A-regulated genes. These results indicated that the drought tolerance of the InsP 5-ptase plants is mediated in part via a DREB2A-dependent pathway and that constitutive dampening of the InsP$_3$ signal reveals unanticipated interconnections between signaling pathways (Perera *et al.*, 2008).

**Super oxidedismutase:** Within a cell, the SODs constitute the first line of the defense against ROS. It is found in all subcellular locations, like mitochondria, chloroplast microsomes, glyoxysomes, peroxysomes, apoplast and the cytosol. SODs are metaloproteins found in various compartments of plant cells and contain Cu and Zn, Fe or Mn cofactors. Transgenic tobacco plants containing oxidative stress- related genes showed elevated levels of glutathione reductase, superoxide dismutase and ascorbate peroxidase, resulting in enhance drought tolerance (Van Rensburg and Kruger, 1994).

Rice transgenic plants carrying Fe-SOD gene in Annada (Fig. 3a, b) was produced using *Agrobacterium*-mediated transformation system (unpluished data from thesis of Bidhan Roy). This result also confirmed successful *Agrobacterium*-mediated genetic transformation in rice and ample potential of this technique in genetic engineering for crop its improvement. Percent regeneration (%) of transformants as obtained in this study is found to be corroborative with the findings of Lin *et al.* (1995) and Datta *et al.* (1999, 2000).

**Wax production:** The gene designated WXP1, is able to activate wax production and confer drought tolerance in alfalfa (*Medicago sativa*). Overexpression of WXP1 under the control of CaMV355 promoter led to a significant increase in cuticular wax loading on leaves of transgenic alfalfa (Zhang *et al.*, 2005). WXP1 over expression induced a number of wax-related genes. Transgenic leaves showed reduced water loss and chlorophyll leaching-transgenic alfalfa plants with increased cuticular wax showed enhanced drought tolerance demonstrated by
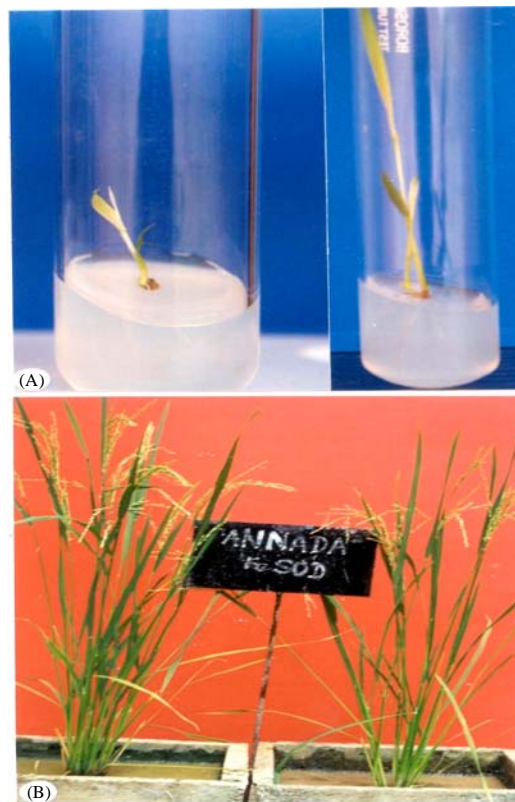


Fig. 3: Regeneration and maintenance of transgenic plants under *in vitro* and *in vivo* containments. (A) Plants turned to be albino on selection medium fortified with 100 mg L$^{-1}$ of kanamaycin and 50 mg L$^{-1}$ cefotaxime, B) Fertile transgenic plant of Annada on cement pots

delayed wilting after watering has been ceased and quicker and better recovery when the dehydrated plants were re-watered.

A full length rDNA of dehydrin BcDh2 from *Boea crassifolia* and its antisense nucleotide sequence was transferred into tobacco, var. NC89 under the control of CMV promoter (Ye *et al.*, 2004). Under progressive water stress, the photosynthetic rate, transpiration rate and stomatal conductance of sense and antisense plants decreased. However, those parameters increased after 24 h of watering and the enhanced was higher in sense and antisense plants than the control.

**Mitogen-activated protein:** Expression of mitogen-activated protein kinase gene (MAPK) genes activates an oxidative signal cascade and lead to the tolerance of freezing, drought, heat and salinity stressing transgenic tobacco. MAPKs perform their function as a part of

kinase modules, which is mainly composed of MAPKs, MAPKKs and MAPKKKs. Shou *et al.* (2004) transformed maize with a tobacco MAPKKK (NPK1). They found that the NPK1 expression enhanced drought tolerance in transgenic maize. Under drought stress, it maintained significantly higher photosynthesis rates than did the non-transgenic control, suggesting that protected photosynthesis machinery is protective from dehydration damage.

**ABA Signaling:** Protecting crop yield under drought stress is a major challenge for modern agriculture. One biotechnological target for improving plant drought tolerance is the genetic manipulation of the stress response to the hormone abscisic acid (ABA). In response of water stress, ABA levels in plant greatly increase resulting closure of stomata, thereby reducing the level of water loss through transpiration from leaves and activate response of genes. ERA1, a gene identified in *Arabidopsis*, encodes β-subunit of a farenesyl-transfarase and involves in ABA signaling. Wang *et al.* (2005) developed transgenic plants which performed significantly better under water stress, with consistently higher yield over conventional varieties of canola, whereas they observed no difference in performance of transgenic and control under sufficient water.

electrons into PS II, maintaining an acceptable NADPH level, preventing further damage.

**Heat shock proteins:** It is important to maintain protein in their functional conformations and preventing aggregation of non-native proteins under stress. Many stress responsive proteins, particularly Heat Shock Proteins (HSPs) have been shown to act as molecular chaperones, which are responsible for protein synthesis, targeting, maturation and degradation in a broad array of normal cellular process. Furthermore, molecular chaperones function in the stabilization of proteins and membranes and in assisting protein folding under stress conditions.

Ahn and Zimmerman (2006) fused DcHSP17.7 gene (a carrot heat shock protein gene encoding HSP17.1) to a 6XHistidine (His) tag to distinguish the engineered protein from endogenous potato proteins and it was introduced into the potato cultivar Desiree under the control of the cauliflower mosaic virus (CaMV) 35S promoter. The integration was confirmed by Western Blot, which showed constitutive integration of DcHSP17.7 in transgenic potato lines before heat stress. They observed improved cellular membrane stability at high temperature, compared with wild type and vector controlled plants. Transgenic potato lines also exhibited enhanced tuberization *in vitro*.

## HEAT TOLERANCE

The successful development of transgenics have been presented in Table 4. The biochemical basis of heat tolerance was investigated by comparing the response of antisense and sense transgenic soybean plant containing L-Delta-1-pyrroline-5-carboxylate reductase gene (De Ronde *et al.*, 2004) with non-transgenic wild-type plant. Upon stress, NADP$^+$ levels decreased in sense plants. Sense plants had the highest ability to metabolize proline after rewatering. They reported dissociation of Oxygen-Evolving Complex (OEC) upon stress. In sense plants, which best resisted the stress, OEC dissociation was by passed by proline feeding

## COLD TOLERANCE

Classical plant breeding has limited success in imparting cold hardiness to crop plants. Biotechnology with its powerful tolls however may provide answer through the isolation of cold fighting genes and thus may help in the development of crop plants that can withstand freezing temperature. Some of the heat tolerant genes/transgenic plants have been listed in Table 5.

Several comprehensive genomic studies based on DNA micro-array performed in the field of plant cold acclimation, identified large number of cold responsive genes (Seki *et al.*, 2001; Fowler and Thomashow, 2002; Kreps *et al.*, 2002; Seki *et al.*, 2002), such as COR, lipid

Table 4: Gene encoding for molecular chaperones and transgenic development

| Gene | Gene action | Species | Phenotypic expression | Reference |
|---|---|---|---|---|
| Hsp70 | Heat-inducible anti-sense HSP70 | *Arabidopsis thaliana* | Increased thermotolerance in transgenic plants | Lee and Schoff (1996) |
| Hsp17.7 | Heat shock protein | *Daccus carota* L. | Increased or decreased thermotolerance | Malik *et al.* (1999) |
| P5CR | Inducible heat shock promoter (IHSP) | *Glycin max* | Increased proline accumulation | De Ronde *et al.* (2000) |
| Hsp101 | Heat shock protein | *Arabidopsis thaliana* | Decreased thermotolerance in Hsp101-deficient (hot1) mutant | Hong and Vierling (2001) |
| Hsp101 | Heat shock protein | *Arabidopsis thaliana* | Manipulated thermotolerance in transgenic plants | Queitsch *et al.* (2000) |
| DcHSP17.7 | Heat shock protein | *Solanum tubersum* L. | Improved cellular membrane stability and enhanced *in vitro* tuberization | Ahn and Zimmerman (2006) |

Table 5: Cold tolerance gens/transgenic plants

| Genes | Gene action | Species | Phenotypic Expression | Reference |
|---|---|---|---|---|
| Sod | Cu/Zn-SOD | Tobacco | Retained 90% photosynthesis under chilling and heat stress | Sengupta *et al.* (1993) |
| Socl | Mn-SOD | Alfalfa | Increased tolerance to freezing stress | McKersie *et al.* (1993) |
| Nt107 | Glutathion Stransferase | Tobacco | Sustained growth under cold and salinity stress | Roxas *et al.* (1997) |
| Wx | Controls amylase synthesis | Rice | Increased amylose content at low temperature | Hirano and Sano (1998) |
| CBF1 | Transcription factor | *Arabidopsis* | Increased cold tolerance | Jaglo-Ottosen *et al.* (1998) |
| *CodA* | Choline oxised (Glycine betaine synthesis) | *Arabidopsis* | Seedling tolerant of salinity stress and increased germination under cold | Hayashi *et al.* (1997) |
| COR15a | Cold induced gene | *Arabidopsis* | Increased freezing tolerance | Steponkus *et al.* (1998) |
| DREB | Transcription factor | *Arabidopsis* | Increased tolerance to cold, drought and salinity | Kasuga *et al.* (1999) |
| AB13 | Transcription factor | *Arabidopsis* | Increased freezing tolerance | Tamminen *et al.* (2001) |
| Gs2 | Chloroplastic glutamine synthetase | Rice | Increased salinity resistance and chilling tolerance | Hoshida *et al.* (2000) |
| OsCDPK7 | Transcription factor | Rice | Increased tolerance to cold, salinity and drought | Saijo *et al.* (2000) |
| SCOF1 | Transcription factor | *Arabidopsis* | Increased winter tolerance | Kim *et al.* (2001) |
| Sod | Cu, Mn, Fe, Zn-SOD | Alfalfa, rye grass | Increased winter hardiness | McKersie (2001) |
| *Osmotin* | induce praline accumulation | Tomato | More tolerant to cold | Sarad *et al.* (2004) |
| atRZ-1a | RNA chaperon protein | Arabidopsis | More tolerant to cold | Kim and Hunseung (2006) |
| AtPCS1 | Phytochilatin synthesis | Tobacco | Cadmium tolerance | Pomponi *et al.* (2006) |

transfer protein and β-amylase. A large number of these genes encode proteins with known enzyme activities, that potentioally contribute to freezing tolerance. Studies revealed that the cold acclimation is controlled by many genes and that cell membranes are particularly vulnerable to cold damage. Lee and Lee (2003) also identified many cold inducible alcohol dehydrogenase, β-amylase and many novel genes.

**Dehydration responsive element:** Many of the known cold regulated genes were under control of a primary master regulator, CBF/DREB1. Dehydration Response Element (DRE) plays an important role in the response to low temperature. The transcription factor DREB1A specifically interacts with DRE and induces the expression of stress tolerance genes in plant.

Over expression of DREB1A in transgenic *Arabidopsis* plants activated the expression of many of these stress tolerance genes and resulted in tolerance to freezing (Liu *et al.*, 1998; Kasuga *et al.*, 1999; Seki *et al.*, 2001; Urao *et al.*, 2000). Liu *et al.* (1998) and Jaglo-Ottosen *et al.* (1998) activated gene expression by overexpressing a homolog of CBF1, designated DREB1A. Their result indicated that the expression of the CRT/DRE regulation also increase drought tolerance. Liu *et al.* (1998) observed that the overexpression of DREB1A in transgenic *Arabidosis* resulted in dwarf phenotype. Fan *et al.* (2002) cloned an antifreeze gene from carrot and successfully transferred it into tobacco.

**Cold regulated LEA protein:** It was found that the COR15am the mature COR15a polypeptide, acts directly as cryoprotective protein by inhibiting the formation of

hexagonal II phase lipid, a major type of freeze-induced membrane lession in non-acclimated plants (Steponkus *et al.*, 1998). The COR15a gene enhanced the freezing tolerance of chloroplasts in engineered plants by almost 2C, which was nearly one third of the increase seen due to cold acclimation. While, this might not appear as large increase, an improvement of freeze tolerance by 2°C could potentially benefit certain crop plants (Artus *et al.*, 1996). COR85, a group-II LEA protein was shown to be involved in cryoprotection of freezing-sensitive enzymes (Kazuaka and Oeda, 1994).

Overexpression of atRZ-1a did not affect the expression of various cold-responsive genes such as COR6.6, COR15a, COR47, RD29A, RD29B and LTI29 (Kim and Hunseung, 2006). Proteome analyses revealed that overexpression of atRZ-1a modulated the expression of several stress-responsive genes and the transcript levels and RNA stability of these target genes were not affected by atRZ-1a. atRZ-1a successfully complements the cold sensitivity of *Escherichia coli* lacking four cold shock proteins. These results strongly suggest that atRZ-1a plays a role as an RNA chaperone during the cold adaptation process.

**Osmotin:** Sarad *et al.* (2004) developed transgenic tomato with *osmotin* gene. Their preliminary tests revealed that the transgenic plants are more tolerant to cold than wild types.

**Dehydrin:** To elucidate the contribution of dehydrins (DHNs) to freezing stress tolerance, Puhakainen *et al.* (2004) developed transgenic *Arabidopsis* plants over-expressing multiple DHN genes. The transgenic

plants exhibited lower LT50 values and improved survival when express to freezing stress compared to control plants. Thus they concluded that dehydrines contribute to freezing stress tolerance in plants and this could be partially due to dehydrins protective effect on membrane.

**Compatible solutes:** Parvanova *et al.* (2004) transformed tobacco to accumulate different compatible solutes (proline, fructans or glycine-betaine) in order to improve its tolerance to low temperature. The elevated parameters of transgenic plants successfully survived againsed freezing stress. Mukhoopadhyay *et al.* (2004) developed transgenic with OSISAP1 gene confering tolerance to cold, dehydration and salt stress. The gene OSISAP1 was isolated from rice encoding a zinc-finger protein induced under abiotic stresses. Hur *et al.* (2004) also developed transgenic rice with OsP5CS2 gene. This gene encodes for a protein that is highly homologous to δ-1-proline-5-caboxylate synthetase (P5CS), a proline biosynthesis enzyme. Their result indicated that the OsP5CS2 gene is necessary for plant tolerance to salt and cold tolerance.

Potato plants (*Solanum tuberosum* cv. Desiree) transformed with yeast invertase gene acquired a higher tolerance to cold temperature as compared to the control plants, apparently due to the changes in sugar ratio produced by the foreign invertase. The expression of *Osmyb4* in *Arabidopsis thaliana* plants showed a significant increase cold and freezing tolerance, measured as membrane or photosystem-II stability and as a whole plant tolerance (Vannini *et al.*, 2004). They demonstrated by transient expression of Myb4 transactivater the PAL2, ScD9, *SAD* and COR15a cold inducible promoters.

**Heat shock factors:** Various studies showed that plant heat shock proteins are not only express in response to heat shock, but also under water, salt, oxidative stress and at low temperature. Li *et al.* (2003) suggested that Hsf (heat shock factor) gene may play a pivotal role in heat-shock-induced chilling tolerance and constitutive expression of the transcriopton regulated gene in chilling sensitive crops may be useful in improving tolerance against chilling stress. They transferred *Arabidopsis thaliana Hsf1b* (*AtHsf1a*) gene into tomato. The transgenic tomato plants harbouring this gene showed increased chilling tolerance.

## ALUMINIUM TOXICITY TOLERANCE

Optimum growth and productivity and even cultivation of most of the plants is severely restricted in soils with elevated levels of Aluminium (Al). It is the third most abundant element in the earth's crust. Al toxicity mainly targets root apex, resulting in inhibited root growth and function (Roy and Mandal, 2005a). As a result, Al toxicity leads to severe impairment in acquisition of water and nutrients from the soil, which results in a significant reduction in crop yield on acid soils (Delhaize *et al.*, 2007).

In plants with genetic resistance to Al toxicity, the Al-exclusion and uptake from root tips have been found to be correlated to their increased capacity to release organic acids such as citric acid, which chelates $Al^{3+}$ outside the plasma membrane. There are two classes of physiological mechanisms that enable plants to withstand toxic levels of Al in acid soils: exclusion of Al from the root apex and true tolerance to Al in the root and shoot symplasma (Delhaize *et al.*, 2007). Currently, Al-activated exudation of organic acid anions from root apices is the best documented and characterized plant Al tolerance mechanism (Delhaize *et al.*, 2007; Kinraide *et al.*, 2005).

The production of Al-tolerant transgenic plant varieties should be considered an important part of crop management strategies to increase agricultural production on acid soils and to protect forests around strongly acidified industrial regions. At molecular level, identification of tolerant genes to Al-toxicity remains scanty. The first two plant Al tolerant genes are TaALMT1 (Sasaki *et al.*, 2004) from *Triticum aestivum* and SbMATE from sorghum and barley (Furukawa *et al.*, 2007; Magalhaes *et al.*, 2007). Expression of TaALMT1 transgene facilitated Al-activated malate efflux and increased Al tolerance in both tobacco suspension cells (Sasaki *et al.*, 2004) and transgenic barley plants Delhaize *et al.*, 2004). SbMATE underlies the AltSB locus of sorghum, encodes a plasma membrane-localized citrate transporter that belongs to the multi-drug and toxic compound extrusion family (Magalhaes *et al.*, 2007). Magalhaes *et al.* (2007) reported that over-expression of this gene in transgenic *Arbidopsis* plants conferred a significant increase in Al tolerance and root citrate exudation. Liu *et al.* (2009) have utilized an integrated genetical and physiological analysis to identify an member of the *ArbidopsisMATE* family, AtMATE as the functional homolog of SbMATE. AtMATE is expressed primarily in root and is induced by Al.

Development of transgenic tobacco plants, tolerant to Al-toxicity was reported by Pan *et al.* (1994). Mexican scientist also have developed transgenic rice conferring tolerance to high concentration of Al (Conway and Toenniessen, 1999), which could have immense prospects in genetic management of Al-toxicity. Some of other works on Al-tolerant transgenic development have been cited in Table 6.

Table 6: Gene/transgenic plants with gene conferring metal toxicity tolerance

| Gene | Gene action | Species | Phenotypic expression | Reference |
|------|-------------|---------|-----------------------|-----------|
| MsFer | Ferritin (Iron storage) | *Nicotiana tubaccum* | Increased tolerance of oxidative stress caused by excess Fe | Deak *et al.* (1999) |
| parβ | Glutathione S-transferase | *Oryza sativa* | Tolerant to higher concentration of Al-toxicity | Conway and Toenniessen (1999) |
| parβ | Glutathione S-transferase | *Arabidopsis thaliana* | Protects against Al-toxicity and oxidative stress | Ezaki *et al.* (2000) |
| arsC | Overexpression of arsC | *Nicotiana tabacum* | significantly greater Cd tolerance | Dhankher *et al.* (2003) |
| ALMT1 | Aluminum-activated malate transporter | *Barley* | Aluminum tolerance | Delhaize *et al.* (2004) |
| ALS3 | Encodes and ABC transporter-like protein | *Arabidopsis thaliana* | Aluminum tolerance | Larsen *et al.* (2005) |
| ALMT1 | Aluminum-activated malate transporter | *Arabidopsis thaliana* | Aluminum tolerance | Hoekenga *et al.* (2006) |
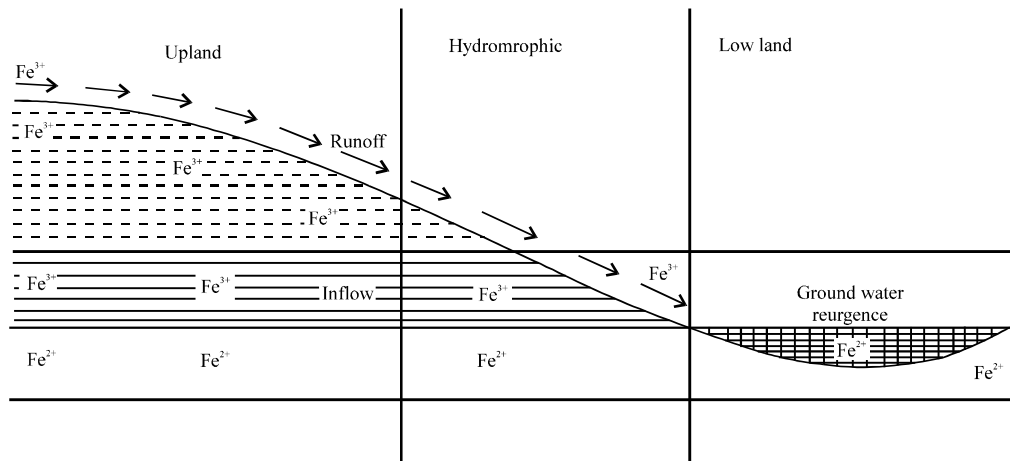


Fig. 4: Cross section of toposequence showing movement of iron and reducing process

## IRON TOXICITY TOLERANCE

Fe-toxicity had been reported as one of the major soil constraints of lowland acid soils, inland valley swamps, costal swamps and irrigated lowlands in utisols and oxisols. Fe is also abundantly found in heavy soils (Tanaka and Yamaguchi, 1973). In the humid forest and moist Savana zones of Africa interflow of ferrous ion occurs from upper slopes (Fig. 4). More than 50% lowland rice is being affected with Fe-toxicity in Siera Leone, Liberia, Guinea, Nigeria, Ivory Coast and Senegal. It was also reported in Sri Lanka, Vietnam, Malaysia, India (Kerala, Orissa, West Bengal and Andaman and Nicobar Islands), Brazil, Colombia, Madagascar and Indonesia (Kalimanta and Sumatra), Philippinese (Sahrawat and Singh, 1995). Young acid sulphate soil in the coastal areas also show rampant symptoms and low lying valleys of Andaman and Nicobar Islands constitute a vast area constrained with Fe-toxicity, which is seriously limiting rice production (Mandal and Roy, 2000, 2003; Roy and Mandal, 2005b). In the people's Republic of China gleyic paddy soils are estimated to cover 7.5-8.0 m ha, where

ferrous iron toxicity in the soil reduce rice yield by 10-20% depending on the intensity of toxicity and tolerance of the rice cultivar (Li *et al.*, 1991; Wan *et al.*, 2003).

**Transgenic development:** The transgenic development study in case of Fe-toxicity tolerance is very scanty. Deak *et al.* (1999) developed transgenic rice with *ferritin* gene to enhance high iron storage and they suggested that the enhanced Fe storage ability can reduce reactive oxygen species. *Ferritin* gene has been also transferred into rice (Khalekuzzaman *et al.*, 2006), which enhanced iron level in the endosperm.

## CADMIUM TOXICITY TOLERANCE

Cadmium (Cd) is a strongly phytotoxic heavy metals in an increasing environmental problem worldwide. It is one of the most dangerous metal due to its high mobility and the small concentration at which its effects on the plants being to appear. It is released into the environment by the power stations, heating systems, metal-working industries or urban traffic. It is recognized as an extremely significant pollutant due to its high toxicity and large

solubility in water (Pinto *et al.*, 2004). Soil solutions which have a Cd concentration ranging from 0.32 to 1.00 mM, can be regarded as polluted to a moderate level (Sanita di Toppi and Garbrielli, 1999). Cd toxicity is highest in acidic environment and decrease as the soil pH is increased (Przybulewska, 2004). Availability of Cd to plants is regulated by pH, redox potential and other physicochemical parameters. The effect of Cd salt on the growth of seedlings was weaker in loamy soil and stronger in sandy soil. The root system was more sensitive to Cd ions than the cotyledons.

**Transgenic development:** Transgenic development is an alternative approach to improve cadmium (Cd) tolerance and its accumulating capacity in crop plants. There are few successful reports on genetically engineered crop plants tolerant to Cd-toxicity (Zhu *et al.*, 1999; Creissen *et al.*, 1999). Over expression of *Escherichia coli gshI* gene in Indian mustard (*Brassica juncia*) increased γ-glutamylcysteine synthetase (γ-ECS) activity leading increased Cd tolerance and accumulation (Yong *et al.*, 1999). Transgenic tobacco (*Nicotiana tabaccum*, var Wisconsin 38) bearing the transgene coding for the polyhistidine cluster, combined with yeast metallothione (HiCUP) showed increasing resistance to the stress response induced by cadmium (Pavlicova *et al.*, 2004). The Cd content of the aerial biomass of transgenic tobacco increased by 45-75% compared with control. Another transgenic tobacco developed by Antosiewicz and Henning (2004) carrying cDNA LTC1, a nonspecific transporter for $Ca^{2+}$, $Cd^{2+}$, $Na^+$ and $K^+$, which displayed substantially higher level of tolerance to Cd and accumulated less Cd in roots. Plants tolerate to heavy metals through sequestration with cysteine-rich peptides, phytochelatins. Cysteine is synthesized by cysteine synthase (CS, E.C. 4.2.99.8) from hydrogen sulfide and O-acetylsrine. Transgenic *Nicotiana tabaccum* var. Xanthi transformed with RCS1, a cytosolic cysteine synthase gene of rice showed higher cyteine synthase activity and Cd toxicity tolerance than the wild type (Harada *et al.*, 2001). Their result suggested that introduction of a cysteine synthase gene into tobacco plants resulted in high level production of sulfur-containing compounds that detoxify the Cd.

*Saccharomyces cerevisiae* protein YCF1, a member of the ATP-binding cassette pumps $Cd^{2+}$ conjugated to glutathione into vacuoles. Song *et al.* (2003) found improved Cd (II) resistance, enhanced Cd accumulation into vacuoles and elevated Cd content in transgenic plants, which are desirable characteristics for phytoremedition.

In nutshell, the load of abiotic stresses on crop production is being incremented gradually by the directed demands of human beings for their food and luxuries. Thus, abiotic stresses have become an integral part of crop production. The ground water is depleting fast due to both intensive and extensive cultivation during off-monsoon periods as well as supplementation of water through irrigation during monsoon. Poor quality irrigation water and depletion of ground water increased salinity in arid and semi-arid zones. Intensive cultivation and heavy feeder crops lead deficiency of some nutrients and toxicity of others. The industrial growth and increase in vehicles created heavy metal toxicity in many industrial areas. In combination of industrial growth and improvement of transportation systems facilitate aggressive air pollution, which subsequently forcing climate change around the globe. All of these exert greater influence on plant growth and crop productivity.

The productivity of major staple food crops have reached to a plateau. There is very little scope to increase crop production area too. Therefore, more emphasis is required to use problem soils effectively. The soil reclamation is a costly affair and it is temporary. Development of crop genotypes tolerant/resistant to the adverse conditions is the only solution of such problem. To develop tolerant/resistant plant genotypes, the plant breeder and plant biotechnologist should work together and they should have knowledge regarding the injury and tolerance mechanisms in plants for specific stress and plant symptoms to know the nature of abiotic stress, breeding methods and biotechnological approaches. Development of transgenic plants tolerant to specific abiotic stresses is the straight-forward solution to improve crop productivity under unfavourable environments. The promising transgenic can be maintained *in vitro* through repeated subculture (Roy and Mandal, 2006). Those transgenic also can be mass-multiplied using tissue culture technique (Roy and mandal, 2011). Many countries grow transgenic crops under unfavourable biotic and abiotic environments. Transgenic crops yet to be popularize worldwide for cultivation.

## REFERENCES

Abdeen, A., J. Schnell and B. Miki, 2010. Transcriptome analysis reveals absence of unintended effects in drought-tolerant transgenic plants overexpressing the transcription factor ABF3. BMC Genomics, 11: 69-89.

Ahn, Y.J. and L. Zimmerman, 2006. Introduction of carrot HSP17.7 into potato (*Solanum tuberosum* L.) enhances cellular membrane stability and tuberization *in vitro*. Plant Cell Environ., 29: 95-104.

Almeida, A.M., S.S. de Araujo, L.A. Cardoso, M.P. Fevereiro, J.M. Tarne and D.M. dos Santos, 2004. Transformation of tobacco with an *Arabidosis thaliana* gene involved in trehalose biosynthesis as a model to increase drought resistance in crop plants. Proceedings of the 17th EUCARPIA General Congress on Genetic Variation for Plant Breeding, Sep. 8-11, Tullen, Austria, pp: 285-289.

Antosiewicz, D.M. and J. Henning, 2004. Overexpression of LTC1 in tobacco enhances the protective action of calcium against cadmium toxicity. Environ. Pollut., 129: 237-245.

Apse, M.P., G.S. Aharon, W.A. Snedden and E. Blumwald, 1999. Salt tolerance conferred by overexpression of a vacuolar NaC/HC antiport in Arabidopsis. Science, 285: 1256-1258.

Artus, N.N., M. Uemura, P.L. Steponkus, S.J. Gilmour, C.T. Lin and M.F. Thomashow, 1996. Constitutive expression the cold-regulated *Arabidopsis thaliana* COR15a gene effects both chloroplast freezing tolerance. Proc. Nat. Acad. Sci., 93: 13404-13409.

Barthakur, S., V. Babu and K.C. Bansal, 2001. Over-expression of osmotin induces proline accumulation Over-expression of osmotin induces in transgenic tobacco. J. Plant Biochem. Biotechnol., 10: 31-37.

Bhattacharya, R.C., M. Maheswari, V. Dineshkumar, P.B. Kirti, S.R. Bhat and V.L. Chopra, 2004. Transformation of *Brassica oleracea* var. *capitata* with bacterial *bet*A gene enhances tolerance to salt stress. Sci. Hortic., 100: 215-227.

Blum, A., R. Munns, J.B. Passioura and N.C. Turner, 1996. Genetically engineered plants resistant to dry soil and salt stress: How to interpret osmotic relations. Plant Physiol., 110: 1050-1053.

Capell, T., C. Escobar, H. Liu, D. Burtin, O. Lepri and P. Chistou, 1998. Overxpression of oat arginine decaboxylase cDNA in transgenic rice (*Oryza sativa* L.) affects normal development pattern *in vitro* and result in putrecine accumulation in transgenic plants. Theor. Applied Genet., 97: 246-254.

Capell, T. and P. Christou, 2004. Enhanced drought tolerance in transgenic rice. Curr. Opinion Biotchnol., 15: 148-154.

Chen, W., N.J. Provart, J. Glazebrook, F. Katagiri and H.S. Chang *et al.*, 2002. Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stress. Plant Cell, 14: 559-574.

Conway, G. and G. Toenniessen, 1999. Feeding the world in the twenty-first century. Nature, 402: 55-58.

Creissen, G., J. Firmin, M. Fryer, B. Kular and N. Leyland *et al.*, 1999. Elevated glutathione biosynthetic capacity in the chloroplasts of transgenic tobacco plants paradoxically causes increased oxidative stress. Plant Cell, 11: 1277-1292.

Datta, K., R. Velazhahan, N. Oliva, I. Ona and T. Mew *et al.*, 1999. Over expression of cloned rice thaumatin like protein (PR-5) in transgenic rice plant enhance environmental friendly resistance to *Rhizoctonia solani* causing sheath blight disease. Theor. Applied Genet., 98: 1138-1145.

Datta, K., Z. Kouklikova-Nicola, N. Baisakh, N. Oliva and S.K. Datta, 2000. Agrobacterium-mediated engineering for sheath blight resistance of indica rice cultivar from different ecosystem. Theor. Applied Genet., 100: 832-839.

De Ronde, J.A., M.H. Spreeth and W.A. Cress, 2000. Effect of antisense pyroline-5-carboxylate reductase transgenic soybean plants subjected to osmotic and drought stress. Plant Growth Reg., 32: 13-26.

De Ronde, J.A., W.A. Cress, G.H.J. Kruger, R.J. Strasser and J. van Staden, 2004. Photosynthetic response of transgenic soybean plants, containing an *Arabidopsis* P5CR gene, during heat and drought stress. J. Plant Physiol., 161: 1211-1224.

Deak, M., G.V. Horvath, S. Davletova, K. Torok and L. Sass *et al.*, 1999. Plants ectopically expressing the iron-binding protein ferratin are tolerant to oxidative damage and pathogen. Nature Biotechnol., 17: 192-196.

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

Delhaize, E., P.R. Ryan, D.M. Hebb, Y. Yamamoto, T. Sasaki and H. Matsumoto, 2004. Engineering high-level aluminum tolerance in barley with the ALMT1 gene. Proc. Natl. Acad. Sci., 101: 15249-15254.

Delhaize, E., B.D. Gruber and P.R. Ryan, 2007. The roles of organic anion permeases in aluminum resistance and mineral nutrition. FEBS Lett., 581: 2255-2262.

Dennis, E.S., R. Dolferus, M. Ellis, M. Rahaman and Y. Wu *et al.*, 2000. Molecular strategies for improving water logging tolerance in plants. J. Exp. Bot., 51: 89-97.

Deshnium, P., D.A. Los, H. Hayashi, L. Mustard and N. Murata, 1995. Transformation of *Synechocoecus* with a gene for choline oxidase enhances tolerance to salt stress. Plant Mol. Biol., 29: 897-907.

Dhankher, O.P., N.A. Shasti, B.P. Rosen, M. Fuhrmann and R.B. Meagher, 2003. Increased cadmium tolerance and accumulation by plants expressing bacterial arsenate reductase. New Phytol., 159: 431-441.

Diamant, S., N. Eliahu, D. Rosenthal and P. Goloubinoff, 2001. Chemical chaperones regulate molecular chaperones *in vitro* and in cells under combined salt and heat stresses. J. Biol. Chem., 276: 39586-39591.

Espinosa-Ruiz, A., J.M. Belles, R. Serrano and F.A. Culianez-Macia, 1999. *Arabidopsis thaliana* AtHAL3: A flavoprotein related to salt and osmotic tolerance and plant growth. Plant J., 20: 529-539.

Evers, D., S. Overney, P. Simon, H. Greppin and J.F. Hausman, 1999. Salt tolerance of *Solanum tuberosum* L. Over expressing an heterologous osmotin like protein. Biol. Planta., 42: 105-112.

Ezaki, B., R.C. Gardner, Y. Ezaki and H. Matsumoto, 2000. Expression of aluminium-induce gene in transgenic*Arabidopsis* plants can ameliorate aluminium stress and/or oxidative stress. Plant Physiol., 122: 657-665.

FAO, 1991. FAOSTAT agriculture data. Food and Agriculture Organization of the United Nations.

Fan, Y., B. Liu, H. Wang, S. Wan and J. Wang, 2002. Cloning of an antifreeze protein gene from carrot and its influence on cold tolerance in transgenic tobacco plants. Plant Cell Rep., 21: 296-301.

Fowler, S. and M.E. Thomashow, 2002. *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell, 14: 1675-1690.

Fukao, K. and J. Bailey-Serres, 2008. Submergence tolerance conferred by *Sub1A* is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. Proc. Natl. Acad. Sci., 105: 16814-16819.

Fukushima, E., Y. Arata, T. Endo, U. Sonnewald and F. Sato, 2001. Improved salt tolerance of transgenic tobacco expressing apoplastic yeast-derived invertase. Plant Cell Physiol., 42: 245-249.

Furukawa, J., N. Yamaji, H. Wang, N. Mitani and Y. Murata *et al.*, 2007. An aluminum-activated citrus transporter in barley. Plant Cell Physiol., 48: 1081-1091.

Gao, F., Q. Gao, X.G. Duan, G.D. Yue, A.F. Yang and J.R. Zhang, 2006. Cloning of an H$^+$-Ppase gene from *Thellungiella halophila* and its heterologous expression to improve tobacco salt tolerance. J. Exp. Bot., 57: 3259-3270.

Gisbert, C., A.M. Rus, M.C. Bolarin, J.M. Lopez-Coronado and I. Arrillaga *et al.*, 2000. The yeast *HAL1* gene improves salt tolerance of transgenic tomato. Plant Physiol., 123: 393-402.

Glenn, E.P., J.J. Brown and E. Blumwald, 1999. Salt tolerant mechanisms and crop potential of halophytes. Crit. Rev. Plant Sci., 18: 227-255.

Grichko, V.P. and B.R. Glick, 2001. Flooding tolerance of transgenic tomato plants expressing the bacterial enzyme ACC deaminase controlled by the 35S rolD or PRB-1b promoter. Plant Cell Physiol., 42: 245-249.

Grumet, R. and A.D. Hanson, 1986. Genetic evidence for an osmoregulatory function of glycinebetaine accumulation in barley. Austr. J. Plant Physiol., 13: 353-364.

Harada, E, Y.E. Choi, A. Tsuchisaka, H. Obata and H. Sano, 2001. Transgenic tobacco plants expressing a rice synthase gene are tolerant to toxic levels of cadmium. J. Plant Physiol., 158: 655-661.

Hayashi, H., L. Alia-Mustardy, P. Deshnium, M. Ida and N. Murata, 1997. Transformation of *Arabidopsis thaliana* with the coda gene for choline oxidase, accumulation of glycinebetaine and enhanced tolerance to salt and cold stress. J. Plant Physiol., 12: 133-142.

Hayashi, H.A., A. Sakamoto, H. Nonaka, T.H.H. Chen and N. Murata, 2001. Enhance germination under high salt condition of seeds of transgenic *Arabidopsis* with a bacterial gene (cod A) for choline oxilase. J. Plant Res., 111: 357-362.

Hirano, H.Y. and Y. Sano, 1998. Enhancement of *Wx* gene expression and the accumulation of amylase in response to low temperatures during seed development in rice. Plant Cell Physiol., 39: 807-812.

Hoekenga, O.A., L.G. Maron, M.A. Pineros, G.M.A. Cancado and J. Shaff *et al.*, 2006. *AtALMT1*, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in *Arabidopsis*. Proc. Natl. Acad. Sci., 103: 9738-9743.

Holmstrom, K.O., 1998. Engineering plant adaptation to water stress. Acta Univ. Agric. Sueciae Agaria, 84: 1401-6249.

Hong, S.W. and E. Vierling, 2001. Hsp101 is necessary for heat tolerance but dispensible for development and germination in the absence of stress. Plant J., 27: 25-35.

Hong, Z., K. Lakkineni, Z. Zhang and D.P.S. Verma, 2000. Removal of feedback inhibition of Ä1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. Plant Physiol., 122: 1129-1136.

Hoshida, H., Y. Tanaka, T. Hibino, Y. Hayashi, A. Tanaka and T. Takabe, 2000. Enhance tolerance to salt stress in transgenic rice over expression chloroplast glutamine synthetase. Plant Mol. Biol., 43: 103-111.

Hu, H., M. Dai, J. Yao, B. Xiao, X. Li, Q. Zhang and L. Xiong, 2006. Overexpressing a NAM, ATAF and CUC (NAC) transcription factor enhancing drought resistance and salt tolerance in rice. Proc. Nat. Acad. Sci., 103: 12987-12992.

Hur, J., K.H. Jung, C.H. Lee and G. An, 2004. Stress-inducible OsP5CS2 gene is essential for salt and cold tolerance in rice. Plant Sci., 167: 417-426.

Jaglo-Ottosen, K.R., S.J. Gilmour, D.G. Zarka, O. Schabenberger and M.F. Thomashow, 1998. *Arabidopsis* CBF1 overexpression induces COR genes and enhances freezing tolerance. Science, 280: 104-106.

Jain, R.K., S. Jain and R. Wu, 1996. Stimulatory effect of water stress on plant regeneration in aromatic Indica rice varieties. Plant Cell Rep., 15: 449-454.

Kasuga, M., Q. Liu, S. Miura, K. Yamaguchi-Shinozaki and K. Shinozaki, 1999. Improving plant drought, salt and freezing tolerance by gene transfer of signal stress-inducible transcription factor. Natl. Biotechnol., 17: 287-291.

Kazuaka, T. and K. Oeda, 1994. Purification and characterization of Cor85-oligomeric complex from cold-acclimated spinach. Plant Cell Physiol., 35: 601-611.

Khalekuzzaman, M, K. Datta, N. Oliva, M.F. Alam, O.I. Jorder and S.K. Datta, 2006. Stable integration, expression and inheritance of ferritin gene in transgenic elite *indica* rice cultivar BR29 with enhanced iron level in the endosperm. Ind. J. Biotechnol., 5: 26-31.

Kim, J.C., S.H. Lee, Y.H. Cheng, C.M. Yoo and S.I. Lee *et al.*, 2001. A novel cold-inducible zinc finger protein from soybean. SCOF-1, enhances cold tolerance in transgenic plants. Plant J., 25: 247-259.

Kim, Y.O. and K. Hunseung, 2006. The role of a zinc finger-containing glycine-rich rna-binding protein during the cold adaptation process in *Arabidopsis thaliana*. Plant Cell Physiol., 47: 793-798.

Kinraide, T.B., D.R. Parker and R.W. Zobel, 2005. Organic acid secretion as a mechanism of aluminum resistance: A model incorporating the root cortex, epidermis and the external unstirred layer. J. Exp. Bot., 56: 1853-1865.

Kishor, P.B.K., Z. Hong, G.H. Miao, C.A.A. Hu and D.P.S. Verma, 1995. Overexpression of delta]-Pyrroline-5-Carboxylate Synthetas increases proline production and confers osmotolerance ion transgenic plants. Plant Physiol., 108: 1387-1394.

Kreps, J.A., Y. Wu, H.S. Chang, T. Zhu, X. Wang and J.F. Harper, 2002. Transcriptome changes for *Arabidopsis* in response to salt, osmotic and cold stress. Plant Physiol., 130: 2129-2141.

Kumar, A. and S.C. Minocha, 1998. Transgenic Manipulation of Polyamine Metabolism. In: Transgenic Plant Research, Lindsey, K. (Ed.). Harwood Academic, London, pp: 187-199.

Larsen, P.B., J.M. Geisler, C.A. Jones, K.M. Williams and J.D. Cance, 2005. ALS3 encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in *Arabidopsis*. Plant J., 41: 353-363.

Lee, J.H. and F. Schoff, 1996. An *Hsp70* antisense gene affects the expression of *HSP70/HSc70*, the regulation of *HSF* and the acquisition of thermotolerance in *Arabidopsis thaliana*. Mol. Gen. Genet., 252: 11-19.

Lee, J.Y. and D.H. Lee, 2003. Use of serial analysis of gene expression technology to reveal changes in gene expression in Arabidopsis pollen undergoing cold stress. Plant Pysiol., 132: 517-529.

Lee, S.B., H.B. Kwon, S.J. Kwon, S.C. Park, M.J. Jeong, S.E. Han, M.O. Byun and H. Daniell, 2003. Accumulation of trehalose within transgenic chloroplast confer drought tolerance. Mol. Breed., 11: 1-13.

Li, D.M., J.J. Tang, J.L. Zhou and S.Q. Li, 1991. The eco-physiological mechanism of rice tolerance for gleyic soil stress and the breeding of varieties tolerance for soil-related stress. Rice Rev. Abstr., 10: 1-4.

Li, H.Y., C.S. Chang, L.S. Lu, C.A. Liu, M.T. Chan and Y.Y. Charng, 2003. Overexpression of *Arabidopsis thaliana* heat shock factor gene (AtHsf1b) enhance chilling tolerance in transgenic tomato. Bot. Bull. Acad. Sin., 44: 129-140.

Li, Z.C., X.C. Zhang, L. Zhang, B.C. Thuang, C.L. Zhang, G.Y. Wang and Y.C. Fu, 2004. Expression of *MtD1* gene in transgenic rice leads to enhanced salt-tolerance. J. China Agric. Univ., 9: 38-43.

Licausi, F., J.T. Van Dongen, B. Giuntoli, G. Novi, A. Santaniello, P. Geigenberger and P. Perata, 2010. *HRE1* and *HRE2*, two hypoxia-inducible ethylene response factors, affect anaerobic responses in *Arabidopsis thaliana*. Plant J., 62: 302-315.

Lilius, G., N. Holmberg and L. Bulow, 1996. Enhanced NaCl stress tolerance in transgenic tobacco expressing bacterial choline dehydrogenase. Bio-Technol., 14: 177-180.

Lin, W., C.S. Anuratha, K. Datta, I. Potrykus, S. Muthukrishnan and S.K. Datta, 1995. Genetic engineering of rice for resistance to sheath blight. Bio-Technol., 13: 686-691.

Liu, J., J.V. Magalhaes, J. Shaff and L.V. Kochian, 2009. Aluminum-activated citrate and malate transporter from the MATE and ALMT families function independently to confer *Arabidopsis* aluminum tolerance. Plant J., 57: 389-399.

Liu, Q., M. Kasuga, Y. Sakuma, H. Abe, S. Miura, K. Yamaguchi-Shinozaki and K. Shinozaki, 1998. Two transcription factors, *DREB1* and *DREB2*, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. Plant Cell, 10: 1391-1406.

Magalhaes, J.V., J. Liu, C. T. Guimaraes, U.G.P. Lana and V.M.C. Alves *et al.*, 2007. A gene in the multidrug and toxic compound extrusion (MATE) familu confers aluminum tolerance in sorghum. Nature Genet., 39: 1156-1161.

Malik, M.K., J.P. Solvin, C.H. Hwang and J.L. Zimmerman, 1999. Modified expression of a carrot small heat shock protein gene, Hsp17.7, results in increased or decreased thermotolerance. Plant J., 20: 89-99.

Mandal, A.B. and B. Roy, 2000. Development of Fe tolerant rice somaclones through *in vitro* screening. Paper Presented in 4th International Rice Genet. Symp., International Rice Research Institute, Philippines.

Mandal, A.B. and B. Roy, 2003. *In vitro* selection of callus tolerant to high levels of Fe-toxicity in rice and their isozyme profiling. J. Genet. Breed., 57: 325-340.

McKersie, B.D., Y. Chen, M. de Bens, S.R. Bowley and C. Bowler *et al.*, 1993. Superoxide dismutase enhances tolerance for freezing stress in transgenic alfalfa (*Medicago sativa* L.). Plant Physiol., 103: 1155-1163.

McKersie, B.D., S.R. Bowley, E. Harjanto and O. Leprince, 1996. Water-deficit tolerance and field performance of transgenic alfalfa over expressing superoxide dismutase. Plant Physiol., 111: 1177-1181.

McKersie, B.D., S.R. Bowley and K.S. Jones, 1999. Winter survival of transgenic alfalfa overexpression superoxide dismutase. Plant Physiol., 199: 839-849.

McKersie, B.D., 2001. Stress tolerance of transgenic plants over expressing superoxide dismutase. J. Exp. Bot., 52: 8-8.

McNeil, S.D., D. Rhodes, B.L. Russel, M.L. Nuccio, Y. Shachar-Hill and A.D. Hanson, 2000. Metabolic modeling identifies key constraints on an engineered glycine betaine synthesis pathway in tobacco. Plant Physiol., 124: 153-162.

Mittler, R. and E. Blumwald, 2010. Genetic engineering for modern agriculture: challenges and perspectives. Annu. Rev. Plant Biol., 61: 443-462.

Mukhoopadhyay, A., S. Vij and A.K. Tyagi, 2004. Overexpression of a zinc-finger protein gene from rice confers tolerance to cold and salt stress in transgenic tobacco. Proc. Nat. Acad. Sci., 101: 6309-6314.

Nanjo, T., T.M. Kobayashi, Y. Yoshida, Y. Kakubari, K. Yamaguchi-Shinozaki and K. Shinozaki, 1999. Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. Febs Lett., 461: 205-210.

Oh, S.J., C.W. Kwon, D.W. Choi, S.I. Song and J.K. Kim, 2007. Expression of barley HvCBF4 enhances tolerance to abiotic stress in transgenic rice. Plant Biotech. J., 5: 646-656.

Pan, A., M. Yang, F. Tie, L. Li, Z. Chen and B. Ru, 1994. Expression of mouse metallothionein-I gene confers cadmium resistance in transgenic tobacco plants. Plant Mol. Biol., 24: 341-351.

Park, J.M., C.J. Park, S.B. Lee, B.K. Ham, R. Shin and K.H. Paek, 2001. Overexpression of the tobacco *Tsi1* gene encoding an EREBP/AP2-type transcription factor enhance resistance against pathogen attach and osmotic stress in tobacco. Plant Cell, 13: 1035-1046.

Park, B.J., Z. Liu, A. Kanno and T. Kameya, 2005. Genetic improvement of Chinese cabbage for salt and drought tolerance by constitutive expression of a *B. napus* LEA gene. Plant Sci., 169: 553-558.

Parvanova, D., A. Povova, I. Zaharivena, P. Lambrev and T. Kostantinova *et al.*, 2004. Low temperature tolerance of tobacco plants transferred to accumulate proline, fructans, or glycine betaine. Variable chlophyll fluorescence evidence. Phytosynthetica, 42: 179-185.

Pavlicova, D., T. Macek, M. Mackova, J. Szakova and J. Balik, 2004. Cadmium tolerance and accumulation in transgenic tobacco plants with a yeast metallothionein combined with a polyhistidin tail. Int. Biodeterioration Biodegradation, 54: 233-237.

Pellegrineschi, A., J.M. Ribaut, R. Tretowan, K. Yamaguchi-Shinozaki, D. Hoisington and I.K. Vasil, 2003. Preliminary characterization of DREB genes in transgenic wheat. Proceedings of the 10th IAPTC and B Congress on Plant Biotechnolgy, June 23-28, Orlando, Florida, USA, pp: 1836-1837.

Pellegrineschi, A., M. Reynolds, M. Pacheco, R.M. Bitro, R. Almeraya, K. Yamaguchi-Shinazaki and D. Hoisington, 2004. Stress-induced expression in wheat of the *Arabidopsis thaliana* DREB1A gene delays water stress symptoms under green house condition. Genome, 47: 493-500.

Perera, I.Y., C.Y. Hung, C.D. Moore, J. Stevenson-Paulik and W.F. Boss, 2008. Transgenic *Arabidopsis* plants expressing the type 1 inositol 5-phosphatase exhibit increased drought tolerance and altered abscisic acid signaling. Plant Cell, 20: 2876-2893.

Pilon-Smits, E.A.H., M.J.M. Ebskamp, M.J. Paul, M.J.W. Jeuken, P.J. Weibeck and S.C.M. Smeekens, 1995. Improved performance of transgenic fructan-accumulating tobacco under drought stress. Plant Physiol., 107: 125-130.

Pilon-Smits, E.A.H., M.J.M. Ebskamp, M.J.W. Jeuken, I.M. van der Meer, P.J. Weisbeck, R.G.F. Visser and S.C.M. Smeekens, 1996. Microbial production in transgenic potato plants and tubers. Indus. Crops Prod., 5: 35-56.

Pinto, A.P., A.M. Mota, A. De Varennes and F.C. Pinto, 2004. Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. Sci. Total Environ., 326: 239-247.

Pomponi, M., V. Censi, D.V. Girolamo, A.D. Paolis, L.D. Toppi, R. Aromolo, P. Costantino, M. Cardarelli, 2006. Overexpression of *Arabidopsis* phytochelatin synthase in tobacco plants enhances $Cd^{2+}$ tolerance and accumulation but not translocation to the shoot. Planta, 223: 180-190.

Prasad, S.R., P.G. Bagali, S. Hittalmani and H.E. Shaahidhar, 2000. Molecular mapping of quantitative trait loci associated with seedling tolerance to salt stress in rice (*Oryza sativa* L.). Curr. Sci., 78: 162-164.

Przybulewska, K., 2004. Influence of the concentration of calcium on growth and development of barley seedlings depending upon soil reactions. J. Elementol., 9: 157-163.

Puhakainen, T., M.W. Hess, P. Makela, J. Svensson, P. Heino and E.T. Palva, 2004. Overexpression of multi-dehydrin gene enhances tolerance to freezing stress in *Arabidopsis*. Plant Mol. Biol., 54: 743-753.

Queitsch, C., S.W. Hong, E. Vierling and S. Lindquist, 2000. Heat shock protein 101 plays a crucial role in thermotolerance in *Arabidopsis*. Plant Cell, 12: 479-492.

Qunimio, C.A., L.B. Torrizo, T.L. Setter, M. Elllis and A. Grover *et al.*, 2000. Enhancement of submergence tolerance in transgenic rice overproducing pyruvate decarboxylase. J. Plant Physiol., 156: 516-521.

Rahaman, M., A. Grover, W.J. Peacock, E.S. Dennis and M.H. Ellis, 2001. Effects of manipulation of pyruvate decarboxylase and alcohol dehydrogenase levels on the submergence tolerance of rice. Austr. J. Plant Physiol., 28: 1231-1241.

Rajgopal, D., P. Agarwal, W. Tyagi, S.L. Singla-Pareek, M.K. Reddy and S.K. Sopory, 2007. *Pennisetum glauca* $Na^{+}/H^{+}$ antiporter confers high level of salinity tolerance in transgenic *Brassica juncia*. Mol. Breed., 19: 137-151.

Richards, R.A., 1995. Improving crop production on salt-affected soils: By breeding or management?. Exp. Agric., 31: 395-408.

Rohila, J.S., R.K. Jain and R. Wu, 2002. Genetic improvement of Basmati rice for salt and drought tolerance by regulated expression of a barley *Hva1* cDNA. Plant Sci., 163: 525-532.

Romero, C., J.M. Belles, J.L. Vaiya, R. Serrano and F.A.C. Macia, 1997. Expression of the yeast trechalose-6-phosphate synthetase gene in transgenic tobacco plants: Pleiotropic phenotypes include drought tolerance. Planta, 201: 293-297.

Roxas, V.P., R.K. Jr. Smith, E.R. Allen and R.D. Allen, 1997. Over expression of glutathione-S-transferase/ glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress. Nature Biotechnol., 15: 988-991.

Roy, M. and R. Wu, 2001. Arginine decarboxylase transgene expressing and analysis of environmental stress tolerance in transgenic rice. Plant Sci., 160: 869-875.

Roy, B. and A.B. Mandal, 2005a. Anther culture in *indica* rice and variation in major agronomic characters in androclones of Karnal local. Afr. J. Biotechnol., 4: 235-240.

Roy, B. and A.B. Mandal, 2005b. Development of aluminium tolerant line through *in vitro* selection. Euphytica, 145: 221-227.

Roy, B. and A.B. Mandal, 2006. Rapid and recurrent mass-multiplication of androgenic embryos in indica rice. Indian J. Biotechnol., 5: 239-242.

Roy, B. and A.K. Basu, 2009. Abiotic Stresses in Crop Plants-Breeding and Biotechnology. New India Publishing Agency, New Delhi, ISBN 10: 81-89422-94-4, pp: 1-544.

Roy, B. and A.B. Mandal, 2011. Profuse microtillering of androgenic plantlets of elite indica rice variety IR 72. Asian J. Biotechnol., 30: 165-176.

Sahrawat, K.L. and B.N. Singh, 1995. Management of iron toxic soil for lowland rice cultivation in West Africa. Proceedings of the 3rd African Soil Science Society Conference, (ASSSC'95), Africa, pp: 617-624.

Saijo, Y., S. Hata, J. Kyozuka, K. Shimamoto and K. Izui, 2000. Over-expression of a single $Ca^{2+}$-dependent proline kinase confers both cold and salt/drought on rice plants. Plant J., 23: 319-327.

Sakamoto, A., Alia and N. Murata, 1998. Metabolic engineering of rice leading to biosynthesis of glycine betaine and tolerance to salt and cold. Plant Mol. Biol., 38: 1011-1019.

Sanita di Toppi, L. and R. Gabbrielli, 1999. Response to cadmium in higher plants. Environ. Exp. Bot., 41: 105-130.

Sarad, N., M. Rathore, N.K. Singh and N. Kumar, 2004. Genetically engineered tomatoes: New vista for sustainable agriculture in high altitude regions. New Directions of Diverse Planet: Proceedings of 4th International Crop Science Congress, Brisbane, Australia, 26 Sep-1 Oct, 2004.

Sasaki, T., Y. Yamamoto, B. Ezaki, M. Katsuhara and S.J. Ahn *et al.*, 2004. A wheat gene encoding an aluminum-activated malate transporter. Plant J., 37: 645-653.

Schellenbaum, L., N. Sprenger, H. Schuepp, A. Wiemken and T. Boller, 1999. Effects of drought, transgenic expression of a fructan synthesizing enzyme and of mycorrhizal symbiosis on growth and soluble carbohydrate pools in tobacco plants. New Phytol., 142: 67-77.

Seki, M., M. Narusaka, H. Abe, M. Kasuga and K. Yamaguchi-Shinozaki *et al.*, 2001. Monitoring the expression pattern of 1300 Arabidopsis genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell, 13: 61-72.

Seki, M., M. Narusaka, J. Ishida, T. Nanji and M. Fujita *et al.*, 2002. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J., 31: 279-292.

Sengupta, A., J.L. Heinen, A.S. Haladay, J.J. Barke and R.D. Allen, 1993. Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc. Natl. Acad. Sci., 90: 1629-1633.

Serrano, R., F.A. Culianz-Macia and V. Moreno, 1998. Genetic engineering of slat and drought tolerance with yeast regulatory genes. Sci. Horticulturae, 78: 261-269.

Shelveleva, E., W. Chamara, H.J. Bohnert and R.G. Jensen, 1997. Increased salt and drought tolerance by D-ononitol production in transgenic *Nicotiana tabacum* L. Plant Physiol., 115: 1211-1219.

Shen, Y.G., B.X. Du, W.K. Zhang, J.S. Zhang and S.Y. Chen, 2002. AhCMO, regulated by stresses in *Atriplex hortensis*, can improve drought tolerance in transgenic tobacco. Theor. Applied Genet., 105: 815-821.

Shou, H.X., P. Bordallo and K. Wang, 2004. Expression of *Nicotiana* protein kinase (NPK1) enhance drought tolerance in transgenic maize. J. Exp. Bot., 55: 1013-1019.

Sivamani, E., A. Bahieldin, J.M. Wraith, T. Al-Niemi, W.E. Dyer, T.H.D. Ho and R.D. Qu, 2000. Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley *HVA1* gene. Plant Sci., 155: 1-9.

Song, W.Y., E.J. Sohn, E. Martino, Y.J. Lee and Y.Y. Yang *et al.*, 2003. Engineering tolerance accumulation of lead and cadmium in transgenic plants. Nature Biotechnol., 21: 914-919.

Steponkus, P.I., M. Uemura, R.A. Joseph, S. Gilmour and M.F. Thomashow, 1998. Mode of action of the *COR15a* gene on the freezing tolerance of *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA, 95: 14570-14575.

Sugino, M., T. Hibino, Y. Tanaka, N. Nii and T. Takabe, 1999. Overexpression of DnaK from a halotolerant cyanobacterium *Aphanothece halophytica* acquires resistance to salt stres in transgenic tobacco plants. Plant Sci., 146: 81-88.

Tamminen, I., P. Makela, P. Heino and E.T. Palva, 2001. Ectopic expression of AB13 gene enhances freezing tolerance in response to abscisic acid and low temperature in *Arabidopsis thaliana*. Plant J., 25: 1-8.

Tanaka, A. and K. Yamaguchi, 1973. A role on the nutritional status of the rice plant in Italy, Portugal and Spain. Siol Sci. Plant Nutr., 19: 161-171.

Tanaka, Y., T. Hibino, Y. Hayasi, A. Tanaka and S. Kishitani *et al.*, 1999. Salt tolerance of transgenic rice overexpressing yeast mitrochondrial Mn-SOD in chloroplasts. Plant Sci., 148: 131-138.

Tarezynski, M.C., R.G. Tensen and H.J. Bohnert, 1993. Stress protection of transgenic tobacco by production of the osmolyte mannitol. Science, 259: 508-510.

Thomas, J.C., M. Sepathi, B. Arendall and H.J. Bohnert, 1995. Enhancement of seed germination in high salinity by engineering manitol expression in *Arabidopsis thaliana*. Plant Cell Environ., 18: 801-806.

Turhan, H., 2005. Salinity response of transgenic potato genotypes expressing the oxalate oxidase gene. Turk. J. Agric. For., 29: 187-195.

Urao, T., K. Yamagnchi-Shinozaki and K. Shinozak, 2000. Two component systems in plant signal transduction. Trends Plant Sci., 5: 67-74.

Van Rensburg, L. and G.H.J. Kruger, 1994. Applicability of abscisic acid and (or) proline accumulation as selection criteria for drought tolerance in *Nicotiana tabacum*. Can. J. Bot., 72: 1535-1540.

Vannini, C., F. Locatelli, M. Bracale, E. Magnani and M. Marsoni *et al.*, 2004. Overesxpression of the rice *Osnmyb4* gene increase chilling and freezing tolerance of *Arabidopsis thaliana* plants. Plant J., 37: 115-127.

Veena Reddy, V.S. and S.K. Sopory, 1999. Glyoxalase I from *Brassica juncea*: Molecular cloning, regulation and its over-expression confer tolerance in transgenic tobacco under stress. Plant J., 17: 385-395.

Wan, J.L., H.Q. Zhai, J.M. Wan and H. Ikehashi, 2003. Detection and analysis of QTLs for ferrous iron toxicity tolerance in rice (*Oryza sativa* L.). Euphytica, 13: 201-206.

Wang, W., B. Vinocur and A. Altman, 2003a. Plant responses to drought salinity and extreme temperatures: Towards genetic engineering for stress tolerance. Planta, 218: 1-14.

Wang, Y.P., Q.C. Liu, A.X. Li, H. Zhai, S.S. Zhang and B.L. Liu, 2003b. *In vitro* selection and identification of drought-tolerant mutants in sweet potato. Agric. Sci. China, 2: 1314-1320.

Wang, Y., J. Ying, M. Kuzma, M. Chalifoux and A. Sample *et al.*, 2005. Molecular tailoring of farnesylation for plant drought tolerance and yield protection. Plant J., 43: 413-424.

Wang, Y.C., G.Z. Qu, H.Y. Li, Y.J. Wu, C. Wang, G.F. Liu and C.P. Yang, 2010. Enhanced salt tolerance of transgenic poplar plants expressing a manganese superoxide dismutase from *Tamarix androssowii*. Mol. Bio. Rep., 37: 1119-1124.

Windawsky, D.A. and J.C. O'Toole, 1990. Prioritizing Rice Biotechnology Research Agenda for Eastern India. The Rockfeller Foundation, New York, USA., pp: 86.

Winicov, I. and D.R. Bastola, 1999. Transgenic over expression of the transcription factor *Alfin1* enhances expression of the endogenous *MSPRP2* gene alfalfa and improve salinity tolerance of the plant. Plant Physiol., 120: 473-480.

Wu, L., Z. Fan, L. Guo, Y. Li, Z.L. Chen and L.J. Qu, 2005. Over-expression of the bacterial *nhaA* gene in rice enhances salt and drought tolerance. Plant Sci., 168: 297-302.

Xu, D., X. Duan, B. Wang, B. Hong, T.D. Ho and R. Wu, 1996. Expression of a late embryogenesis abundant protein gene, *HVA1*, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol., 110: 249-257.

Xu, K., X. Xu, T. Fukao, P. Canlas and R. Maghirang-Rodriguez *et al.*, 2006. *Sub1A* is: An ethylene-response-factor-like gene that confers submergence tolerance to rice. Nature, 442: 705-708.

Xue, Z.Y., D.V. Zhi, G.P. Xue, H. Zhang, Y.X. Zhao and G.M. Xia, 2004. Enhance salt tolerance of transgenic wheat (*Triticum aestivuns* L.) expressing a vacuolar $Na^+/H^+$ antiporter gone with improved grain yields in saline soils in the field and a reduced level of leaf $Na^+$. Plant Sci., 167: 849-859.

Ye, S., W. Jia, Y. Zhang, Y. Hu, Q. Wu and Z. Lin, 2004. Improvement of drought tolerance in transgenic tobacco plants by a dehydrin-like gene transfer. Agric. Sci. China, 3: 575-583.

Yong, L.Z., E.H. Pilon-Smits, A.S. Tarun, S.U. Weber, L. Jouanin and N. Terry, 1999. Cadmium tolerance and accumulation in Indian mustard is enhanced by overexpressing ã-glutamyl cysteine synthetase. Plant Physiol., 121: 1169-1177.

Zhang, H.X. and E. Blumwald, 2001. Transgenic salt tolerant tomato plants accumulate salt in the foliage but not in the fruits. Nat. Biotechnol., 19: 765-768.

Zhang, H.X., J.N. Hodson, J.P. Williams and E. Blumwald, 2001. Engineering salt tolerant Brassica plants: Characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. Proc. Nat. Acad. Sci. USA., 48: 12832-12836.

Zhang, S.Z., B.P. Yang, C.L. Feng and H.L. Tang, 2005. Genetic transformation of tobacco with the trehalose synthase gene from *Grifola forndosa* Fr. enhances the resistance to drought and salt in tobacco. J. Intensive Plant Biol., 47: 579-587.

Zhu, B., J. Su, M.C. Chang, D.P.S. Verma, Y.L. Fan and R. Wu, 1998. Over expression of a Ä1-pyrroline5-carboxylate synthetase gene and analysis of tolerance to water and salt stress in transgenic rice. Plant Sci., 139: 41-48.

Zhu, Y.L., E.A.H. Pilon-Smits, L. Jounin and T. Terry, 1999. Overexpression of glutathione synthetase in *Brassica juncia* enhances cadmium accumulation and tolerance. Plant Physiol., 199: 73-79.