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Genetic Enhancement of Plant Lenience to Drought and Salinity

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***Abstract* - Salinity stress limits crop yield affecting plant growth and restricting the use of land. As world population is increasing at alarming rate, agricultural land is shrinking due to industrialization and/or habitat use. Hence, there is a need to utilize salt affected land to meet the food requirement. Although some success has been achieved through conventional breeding, but its use is limited due to reproductive barrier and scarcity of genetic variations among major crops. The genetic engineering has proven a revolutionary technique to generate salt tolerant plants as one can transfer desired gene from any genetic resource and/or alter the expression of existing gene(s). There are examples of improved salinity tolerance in various crop plants through the use of genetic engineering. However, there is a further need of improvement for successful release of salt tolerant cultivars at field level. In this research, we have given a detailed update on production of salt-tolerant plants through genetic engineering. Future prospects and concerns, along with the importance of novel techniques, as well as plant breeding are also discussed.**

***Index Terms* - Helicases; Ion transporter; Lea proteins; Osmoprotectants; Salinity stress; Transcription factors.**

INTRODUCTION

Current world population is about 7.2 × 109 people and is projected to grow by almost 30% over the next 35 years, to reach 9.2 × 109 individuals by 2050 (Figure 1A). FAO estimates indicate that it will be necessary to increase agricultural production by at least 60% over 2005–2007 levels to meet the expected demand for food (Alexandratos and Bruinsma, 2012). Looking back at the recent past this goal, a priori, does not seem so difficult to be achieved. Indeed, in the last 50 years, specifically between 1960 and 2009, world population more than doubled while it was still possible to increase the amount of food per capita, from 2200 Kcal/person/day to an average of more than 2800 Kcal/person/day. This means that today enough food is produced to feed everyone living on this planet. Clearly, this food is not distributed evenly: while food supply in Europe reached in 2009, on average, almost 3400 Kcal/person/day, the mean figures for Africa were below the 2600 Kcal/person/day level (Figure 1B; FAOSTAT, 2015). A fairer distribution of food worldwide is largely a matter of political will and international solidarity. In consequence it is frequently argued that there will be no technical problems to feed the world's population if we are able to share the available food resources. Unfortunately, this reasoning is flawed when taking a look on our present agricultural systems, the challenges posed by the climate change and the need of sustainability.

BEYOND THE GREEN REVOLUTION: PRESENT SITUATION AND CHALLENGES FOR THE NEAR FUTURE

Between the 1940s and 1970s, scientific and technical advances induced a new trend in agricultural practices that enabled growers to increase crop yields dramatically. Such movement was later known as the 'Green Revolution' (GR; Borlaug and Dowswell, 2005). The first major innovation of the GR consisted in the development of new high-yielding, disease-resistant (mainly to stem rust), and semi-dwarf wheat cultivars (Dubin and Brennan, 2009) which then was spread to other crops. Another key point of the GR was the monoculture practice and the replacement of traditional agricultural methods by modern approaches and technologies, including the massive use of agrochemicals (pesticides, herbicides, chemical fertilizers), mechanization of labor, and a large increase in the area of irrigated crop land (Evans,

1998). This new way of conceiving agriculture boosted the food production of primary crops and has resulted in increasing yields every year (Figure 1C).
Despite the undeniable positive effects of the GR, there is also a negative side of the strategies used in the past, which may hamper further increases in food production under the present, quite different circumstances. In many cases, the agricultural systems established during the GR have evolved toward an excessive use of intensive production practices that may not be sustainable. They include, among others: greenhouses for continuous production of certain commodities all around the year, lack of crop rotation, the massive and uncontrolled use of synthetic fertilizers, or the cultivation in semi-arid regions of species with high water requirements, which need large amounts of irrigation water. These practices may cause a series of problems for agricultural production in the near future, such as the appearance of new pathogens and pests and also the depletion, contamination, and salinization of soils and ground waters (Shiva, 1991; Dehaan and Taylor, 2002; McDonald and Linde, 2002). In addition, the use of fertilizers from non-sustainable or non-renewable sources (e.g., synthetic fertilizers, mineral phosphate) may also limit crop yields when their availability decreases in the medium or long term (Shiva, 1991; Stewart et al., 2005).

In addition, GR caused direct impacts on diet and on diversity. On the one hand, staple crops were improved and the total amount of protein and energy available to people was increased, but not the nutritional value in terms of micronutrients; this, together with the reduction of the variety of products consumed (specially by poor people) led to an increase of the micronutrient malnutrition which is known as ‘hidden hunger’ (Welch and Graham, 2002). On the other hand, modern uniform cultivars with high yields and resistances to many pests and diseases gradually substituted traditional cultivars, heirlooms, and local varieties. This situation was detrimental to the use and conservation of the latter, and many of those cultivars were lost forever (Hammer and Khoshbakht, 2005). That is, GR has favored the global predominance of a narrow range of crop species and cultivars (Figure 1D). Moreover, modern cultivars frequently encompass a low genetic diversity themselves as seed companies usually restrict their breeding materials to a very limited genetic pool. The process of gradual (and irreversible) decline of agricultural diversity, and consequently of their gene pool, i.e., ‘genetic erosion,’ may jeopardize food production in the future (FAO, 2010). Thus, the loss of genetic diversity decreases the opportunities to find new sources of variation to fight future challenges (e.g., new pests and diseases or new races in already known pests and diseases, changing environments) to which modern varieties will not provide resistances or tolerances (Esquinas-Alcázar, 2005).

Nature’s rage influences plants in the form of various biotic and abiotic stresses. Extreme abiotic stress conditions, such as salinity, flooding, heat, drought, and cold, as well as heavy metal toxicity and oxidative stress affect plants in many different ways. Human activities exacerbate these stress conditions to a greater extent. All the abiotic and biotic stresses, including various pathogens, cause havoc to plants eventually limiting their growth and yield potentials. About 50% of crop yields are reduced due to abiotic stresses, making them the major cause of crop failure worldwide [1]. Abiotic stresses are a serious threat to the sustainability of agricultural industry. Naturally, a number of stresses combine with each other and act together; therefore, the negative effects are aggravated to a greater extent when compared to a single stress factor. To combat these stresses, combinations of diverse pathways are triggered [2].

In physical terms, stress is defined as a mechanical force per unit area applied to an object. It is difficult to measure the exact force applied by the stresses because the plants are immotile. This makes it harder to define stress in biological terms. A condition, which may act as a stress for one plant, may be ideal for another plant. Hence, a biological stress can most suitably be defined as a harsh condition or force that impedes the normal functioning of a biological system such as plants [3].

The plasma membrane serves as a barrier that separates a cell from its surrounding environment. Some of the small lipid molecules like steroid hormones are able to pass through this membrane and diffuse into the cytoplasm, whereas the membrane does not allow the water soluble molecules, such as ions, proteins, and other large molecules, to pass through it. Cells start responding when extracellular molecules come in contact with the plasma membrane. This foreign molecule is called an elicitor, and the protein that is present on the cell membrane and interacts with the elicitor is called a receptor. A number of biotic and abiotic stress signals serve as elicitors for the plant cells [4].

SALINITY STRESS AND ITS CAUSES

Total amount of dissolved mineral salts in water and soil is termed as salinity [5]. These salts comprise electrolytes of anions (majorly CO32−, SO42−, Cl−, NO3−, HCO3−) and cations (majorly Ca2+, K+, Mg2+, Na+). Salts that are soluble in water get deposited in the upper layer of soil to a greater extent that hinders the agricultural productivity of that land area [6]. Although fewer salts are present in the rainwater, these salts can be accumulated in the soil over a certain period of time. Salts can also be deposited by soil transported by wind from far off places. Impure irrigation water also contributes to the level of deposited salts in the agricultural lands [7].

Salinity stress is one of the main abiotic stresses and is considered as a restraint to crop yield. Increased salinization of cultivable land has disastrous effects worldwide [8]. Hyperosmotic and hyperionic stresses are caused by increased salinity, which can lead to plant death [9]. A number of factors are responsible for causing salinity in a given area such as the extent of precipitation or evaporation and weathering of rocks. Deserts have high salinity due to the fact that the rate of evaporation is greater than the rate of precipitation. All the key processes within a plant are significantly influenced when the plant is exposed to salt stress [10]. The water stress resulting under salt stress affects the leaf growth and development. Cell division and expansion as well as stomatal opening and closing are negatively influenced by the salinity stress [11]. If the stress condition prevails, then the ionic stress strikes, and eventually, a major decline in photosynthetic rate occurs, and the leaves start to die [12].

Deforestation is a leading cause of salt stress. Heavy salt–rich irrigation is the major cause of salinity in agricultural lands. The process of evapotranspiration is responsible for the retention of excessive amounts of salt in the soil every year. This is due to abundant loss of water as a result of both evaporation and transpiration. Almost all of the main agricultural crops are sensitive to salt stress that results in serious damage to the yields of the crops [13]. Soil contents altered by the deposition of large amounts of salt in the soil, and as a result, soil becomes less porous reducing soil aeration and water transport [14]. Salinity stress and drought stress are quite similar in terms of physiology [15].

STRESS SIGNALING PATHWAYS

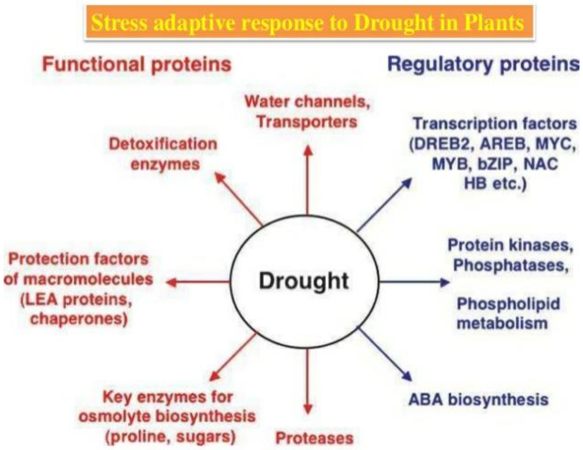
The receptors present on the plant cell surface receive the stress signals and transfer them downstream, resulting in the production of secondary messengers, e.g. reactive oxidative stress (ROS), calcium, and inositol phosphates [16]. Calcium level is further controlled by these messengers within the cell. As a result of this disturbance in the intracellular Ca2+ level, the Ca2+ sensors are triggered, which change their conformation in a calcium-dependent manner [14]. These sensors initiate a phosphorylation cascade by interacting with their respective partners and activate the stress responsive genes or the transcription factors that regulate stress response genes. The products of stress response genes help in plant survival and mitigate the stress conditions. Production of hormones (such as ethylene, salicylic acid, and abscisic acid (ABA) takes place because of changes in gene expression under the stress. Initial signal is amplified by messenger ‘Sensor’ stress response molecules, and a secondary signaling pathway may be induced. Such molecules which do not take part directly in signaling but play a role in alteration of signaling components are called accessory molecules [17].

The stress responsive genes can be divided into two major categories: early- and late-induced genes. Early induced genes are prompted immediately after stress signals are received, and most of the times, they express in shorter period. In this category, a number of transcription factors are included because they do not require synthesis of new proteins for their stimulation. In contrast, late-induced genes are expressed slowly under the stress condition, i.e., express after hours of receiving stress signals, and their expression is persistent [18]. In this gene category, major stress responsive genes, such as (COR cold responsive), KIN (cold induced), or RD (responsive to dehydration), and membrane stabilizing proteins, osmolytes, antioxidants, and LEA (late embryogenesis abundant)-like proteins are included [19].

GENETIC RESPONSES TO SALINITY

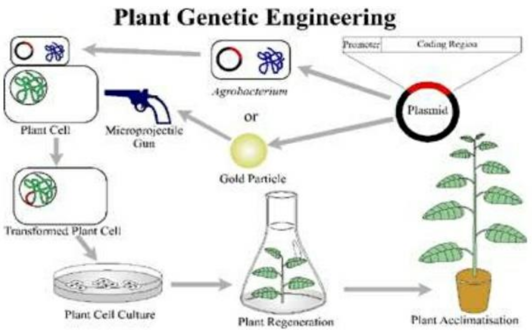
Genetic response in case of salinity stress takes in a complex mechanism that is used by plants to up-regulate or down-regulate (increase or decrease) the production of specific gene products (protein or RNA). These mechanisms have been recognized at different stages of central dogma process like from transcriptional initiation to RNA processing, post-transcriptional modification, and translation, and to the post-translational modification of a protein [32]. Understanding about the transcriptional behavior of plants provides a detailed knowledge about the gene expression at mRNA level. Transcriptional profiling is widely used to screen out candidate genes involved in stress responses. Till now, massive information about the salt responsive genes, transcription factors which either up-regulated or down-regulated, has been identified using transcriptome profiling methodology. Further genomic approaches contribute significant role in encoding, cloning, and characterization of these genes. Gene expression under the certain conditions altered by transcription factors. These factors are considered the most important switches that up-regulate or down-regulate the gene expression. Among them, bZIP, WRKY, AP2, NAC, C2H2 zinc finger gene, MYB and DREB family proteins comprise a large number of stress-responsive members. These transcription factors have the capacity to alter the gene expression by cis-acting specific binding in the promoter region of broad range of genes.

Up-regulation in the expression of bZIP genes were observed in sensitive wheat cultivar under persistent salinity stress and down-regulation in salt-tolerant variety [33]. It predicts the role of NAC transcription factor in salinity tolerance in both rice and wheat cultivars. In rice, transcriptional regulators, such as DREB1/CBF, DREB2, and AREB/ABF, have been demonstrated to play a significant role in abiotic stress responses [34, 35]. Transcription factors, such as OsNAC5 and ZFP179, show an up-regulation under salinity stress, which may regulate the synthesis and accumulation of proline, sugar, and LEA proteins that in turn play an integral role in stress tolerance [36].



Full-length cDNA is a vital resource for studying the full functional genes in wheat. A group of gene "MYB gene" family analyzed by Zhang et al. [37] that respond to one or more abiotic stress treatments. They isolated 60 full-length cDNA sequences encoding wheat MYB proteins and also construct phylogenetic tree with other wheat, rice, and Arabidopsis MYB proteins to understand their evolutionary relationships and the putative functions of wheat MYB proteins based on Arabidopsis MYB proteins with known functions. Tissue-specific analysis and abiotic stress response expression profiles were also carried out to find potential genes that participate in the stress signal transduction pathway, including the analysis of transgenic Arabidopsis plants expressing the MYB gene, TaMYB32 [38]. In Arabidopsis, salt stress results in up-regulation of AtWRKY8 gene expression, which directly binds with the promoter of RD29A [39]. A large number of genes and transcription factors are up regulated in response to salinity in different plant species, which serve diverse functions [40]. Some of the examples of salt-responsive genes are listed in the Table 1, and these genes are mainly classified into the following functional categories: ion transport or homeostasis (e.g., SOS genes, AtNHX1, and H+ -ATPase), senescence-associated genes (e.g., SAG), molecular chaperones (e.g., HSP genes), and dehydration-related transcription factors (e.g., DREB). Among stress-responsive genes, the SOS transcription gene family is considered to play a very stimulating role in ion homeostasis, thereby conferring salt tolerance [32, 41]. Most of the salinity responsive genes, such as ROS-scavenging and osmotic-regulating genes, are also up-regulated by salinity in salinity tolerant species. Schmidt et al. [42] observed more than 10 extensively up-regulated genes in the halophyte plant species Spartina alterniflora under salt stress. Most of these genes were related to osmotic regulation process.

Genetic engineering



QTL MAPPING IN RELATION TO PLANT SALINITY TOLERANCE

A quantitative trait locus (QTL) is a section of DNA (the locus) that correlates with variation in a phenotype (the quantitative trait). The QTL typically is linked to, or contains, the genes that control that phenotype. Genetic marker is an identifiable fragment of DNA that is linked with a specific point and indicates genetic differences within the genome. Molecular markers act as a 'signs' or 'flags' should not be considered as normal genes. Genetic markers that are tightly linked are referred to as "gene tags" [78]. The DNA markers can be grouped in various categories based on their technical requirements, the number of genetic markers that can be detected throughout the genome, and the amount of genetic variation found at each marker [79]. Restriction fragment length polymorphisms (RFLPs) are one of the earliest types of DNA-based marker system, which detect the variation in restriction fragment length by Southern hybridization, which cause single base changes that led to the creation or removal of a restriction endonuclease recognition site to detect shift in fragment size. Although this technique is an important tool in breeding programs, it has been superseded by microsatellite or simple sequence repeat (SSR) markers and is now rarely used. SSR markers detect variation in the number of short repeat sequences, usually two or three base repeats that allow the detection of multiple alleles. The expressed sequence tag (EST) databases have now opened the opportunity for the identification of single nucleotide polymorphisms (SNPs) that occur at varying frequencies depending on the species and genome region being considered [80].

These DNA marker types could be associated with quantitative traits, which are known as quantitative trait loci (QTLs). Mapping of QTLs for salt tolerance have been a slow process due to the complexity of this trait and poor understanding about it. Ren et al. [81] discovered a gene locus named as QTL SKC1, which codes for a transporter that removes Na+ from the xylem [82]. Several QTLs have been identified in different crop plants. QTLs for yield and physiological characteristics were identified at a late stage of growth of barley under salinity stress [83]. A total of 10 traits were considered for which 30 QTLs were identified under salt stress and nonstress conditions. Of these 30, 13 QTLs were discovered under salt stress [83]. In white clover, QTLs for salt tolerance were identified at the vegetative stage of plants and the results showed that, in white clover, multiple QTLs are responsible for controlling the salt tolerance [84]. However, QTLs for salt tolerance in tomato were detected at the seedling stage of Solanum pennellii and Solanum lycopersicoides plants. In S. pennellii, four major QTLs were detected, for salt tolerance, on chromosomes 6, 7, and 11, whereas in Solanum lycopersicoides six major QTLs were identified under salt stress on chromosomes 4, 6, 9, and 12 [85]. QTLs for salt tolerance in soybean were identified on chromosome 3 [86]. QTLs identified by SSR markers in various plants are given.

RESULT/CONCLUSION

Agriculture is immensely affected by salinity worldwide and is predicted to be a larger problem in near future. The damaging effects of high salinity can be seen in plants at organismic level, leading to immature death or decreased productivity. Some plant species are more tolerant to these detrimental effects than others. Salt stress leads to high yield losses worldwide. Therefore, the changes aimed at overcoming these issues need to be fully implemented as soon as possible. Information related to the biochemical indicators at the cellular level may act as selection criteria for salt tolerance in different crops. There are many transgenic plants with high stress tolerance towards abiotic stress, yet stress tolerance has complex mechanism that includes multiple physiological and biochemical changes and multiple genes. Transgenic plants, which are commercially valuable, should have relatively high productivity and other traits important for their yield. Genetic modification, moreover, should be combined with marker-assisted breeding along with stress-related genes and QTLs. These strategies must be integrated, and such approaches should be combined to effectively increase plant stress tolerance.

It is clear that agriculture and food production faces at the moment one of its biggest challenges in history. Feeding 9.2 × 109 people is not going to be an easy task. Up to now, apart from a few exceptions, neither traditional plant breeding nor genetic engineering has delivered widely used commercial stress-tolerant varieties. Nevertheless, research lines in progress are providing promising results and we should be confident that in the coming years the combination of both approaches will allow the improvement of AST for our major crops. Moreover, the complementary approach of promoting ‘saline agriculture’ dependent on highly salt-tolerant halophytes, could be used to reclaim salinized crop land already lost for agriculture, and also naturally saline, marginal soils, using brackish, or sea water for irrigation. This will add the advantage that they will not compete with standard crops for limited resources (i.e., good-quality irrigation water and fertile crop land).

REFERENCES

[1] Abebe, T., Guenzi, A. C., Martin, B., and Cushman, J. C. (2003). Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. Plant Physiol. 131, 1748–1755. doi: 10.1104/pp.102.003616

[2] Abideen, Z., Ansari, R., Gul, B., and Khan, M. A. (2012). The place of halophytes in Pakistan’s biofuel industry. Biofuels 3, 211–220. doi: 10.4155/bfs.11.158

[3] Alexandratos, N., and Bruinsma, J. (2012). World Agriculture Towards 2030/2050. The 2012 Revision. ESA Working Paper No. 12-03. Rome: Food and Agriculture Organization, Agricultural Development, Economics Division.

[4] Anbessa, Y., and Bejiga, G. (2002). Evaluation of Ethiopian chickpea landraces for tolerance to drought. Genet. Res. Crop Evol. 49, 557–564. doi: 10.1023/A:1021210601480

[5] Apel, K., and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373–399. doi: 10.1146/annurev.arplant.55.031903.141701

[6] Apse, M. P., Aharon, G. S., Snedden, W. A., and Blumwald, E. (1999). Salt tolerance conferred by overexpression of a vacuolar Na+/H+ antiport in Arabidopsis. Science 285, 1256–1258. doi: 10.1126/science.285.5431.1256

[7] Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnol. Adv. 27, 84–93. doi: 10.1016/j.biotechadv.2008.09.003

[8] Ashraf, M. (2010). Inducing drought tolerance in plants: recent advances. Biotechnol. Adv. 28, 169–183. doi: 10.1016/j.biotechadv.2009.11.005

[9] Ashraf, M., and Foolad, M. R. (2007). Improving plant abiotic-stress resistance by exogenous application of osmoprotectants glycinebetaine and proline. Environ. Exp. Bot. 59, 206–216. doi: 10.1016/j.envexpbot.2005.12.006