Review Article

Molecular basis of salt stress tolerance in crop plants

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Abstract

Plant growth, survival, biomass production and yield are sensitive to abiotic stress factors. Salt stress is major problem for crop plant growth and yield and thus, salinity regard as a main limiting factor in crop productivity. Modern molecular biology tools if we use it explicate salt stress tolerance mechanism and then specific stress related gene expression is used to generate stress tolerant crops. Identification of genes that play important role in salt tolerance in plants when exposed to high salt levels in soil is very important tool to improve plant tolerance in stress condition. In a high salt concentration expression the genes represent salt tolerance mechanism and that information helpful to recover the plant growth under high salt. In high salt concentration, expressions of many genes encoding proteins increase and improve crop plant growth and productivity. In this review, we sum up the work which has been formerly reported about molecular basis of stress tolerance mechanism of crop plants under high salt levels.

Key words: Salt stress; Wheat; Tolerance Mechanism; Genes.

Introduction:

Agricultural productivity and yield severely decreased due to abiotic stresses such as water stress, salt stress and high temperatures. In arid and semiarid regions, salt stress is one of the central factors which limit plant growth, productivity and yield **[1]**. Almost 800 mha of world land is affected by salts which almost account for 6 % worlds land and 90% of the global cultivated land area affected by abiotic stress factors **[2, 3]**. These abiotic stress factors influence mainly during reproductive stage and it causes significant yield loss. The salinity effects tendency of plants related to its ability to escape or tolerate unfavorable factor and osmotic and ionic adjustment is capacity of plants to overcome salt impact. Tolerance mechanism in crop plants is generally characterized as to avoid or tolerate stress conditions **[4]**. Stress condition effects on plants alleviation practice is not only essential for agricultural improvement, but also play a significant role in increased food production **[5]**. Now it is realized that plant growth and development over a various abitoic stress conditions would allow an increased in yield and productivity. For this purpose high throughput expression analysis of stress specific genes is important. Salt stress tolerance mechanisms are allowed to find genetic level differences within a same plant species by comparing varieties, natural genotypes and germlines **[3]**. In recent years a number of molecular techniques allow isolating, identifying and characterized a

massive number of salinity responsive genes. While an enormous genes have been identified, they are contributing to the overall phenotypic characteristics, but on investigations they revealed a relatively small number of major quantitative trait loci [6]. Genetic character study is a powerful tool to identify traits play main role in salinity tolerance, nevertheless, it is important to use, accurate phenotyping methods that help in the identification of these traits. The prospects of changing the phenotype through manipulation techniques of genetic engineering become much greater if one or few defined regions of chromosomes are of crucial importance. QTL identification consequently and has practical importance it play the main role to enhance stress tolerance [7]. Functional genomics technologies, for example metabolomics, next generation sequencing, proteomics and transcriptomics make it possible to understand the mechanism of plant growth in stress condition. In this review, we will discuss the genes involve in the salinity tolerance mechanism. These studies will lead to a greater understanding of the plant response to salt stress and hence the integration of this data will contribute to improving our ability to generate salt tolerant crops.

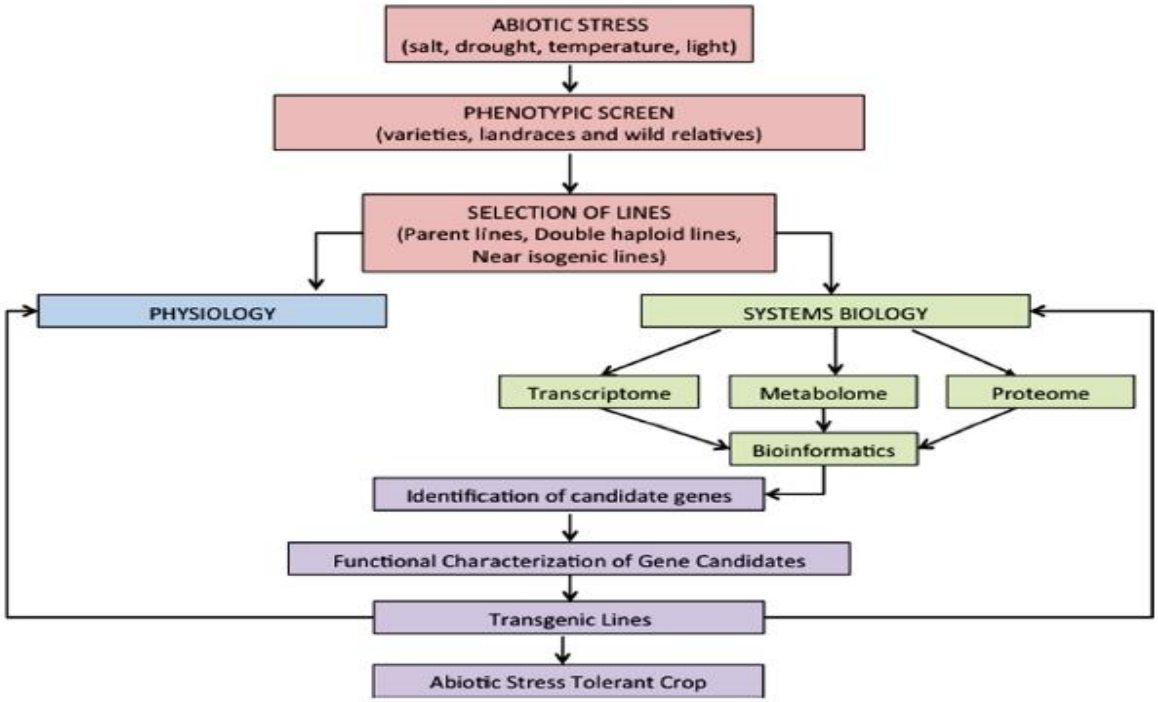

Fig 1. Plant molecular responses under abiotic stress in cereals.

Genetic basis for salt tolerance in crop plants:

Crop plants are considered as glycophytes. Plant species have different capabilities and mechanisms to tolerate salinity, such as *Oryza sativa* is more sensitive to high salt concentration than barley and wheat [1]. In crop plants genetic variation within a species occur and these differences can be identified for breeding of salt tolerant crops [8]. Crop plant having responses to soils high salt concentration occur in two separate phases such as osmotic stress phase and ionic stress phase [9].The

osmotic phase persists for hours to days. Osmotic stress decreases water potential around the roots and considered as an osmotic stress phase. Osmotic stress phase loss of cell turgidity, cell dehydration, reduction in shoot growth, inhibition of photosynthesis and reduction in root elongation of plants **[1]**. On the contrary, ionic stress occurs after weeks or months exposure from high salt concentration and is a result of the accumulation of toxic concentrations of $Na^+$and Cl^- in the cell cytoplasm resulting in decreased growth and yield **[9].** In response to salt stress, crop plants have evolved the following three tolerance mechanisms – (1) osmotic stress tolerance: ability to maintain water uptake and growth, (2) Na^+ exclusion: exclusion of toxic ions from the shoot tissues, and (3) tissue tolerance: compartmentalisation of toxic ions into the vacuole or specific tissues **[1]**.

One approach to identify adaptive traits to abiotic stresses is to screen for genetic diversity in populations. Genetic variation in salt tolerance has been shown inmany cereal crops including barley **[10]** and wheat **[11]**.The focus of most research for enhancing salt tolerance in plants has concentrated on the mechanisms that control Na^+ exclusion **[1]**. The introgression of *Nax2* from the parent line, *Triticum monococcum* , into durum wheat produced a salt tolerant phenotype **[6]**. It was originally thought that there was very little evidence of genetic variation in osmotic stress tolerance **[12]**. Despite a number of recent phenotypic studies, the molecular mechanisms for osmotic stress tolerance remain unknown. Despite a number of recent phenotypic studies, the molecular mechanisms for osmotic stress tolerance remain unknown. Osmotic stress is initially detected by the roots upon exposure to a low water potential (either as a result of water deficit or salinity).

Salt tolerance through Transgenes:

Transporter genes for salt tolerance an important strategy for achieving greater tolerance to abiotic stress is to help plants to establish homeostasis under stressful environments, restoring both ionic and osmotic homeostasis. This has been and continues to be a major approach to improve salt tolerance in plants through genetic engineering, where the target is to achieve Na^+ excretion out of the root, or their storage in the vacuole. A number of abiotic stress tolerant transgenic plants have been produced by increasing the cellular levels of proteins (such as vacuolar antiporter proteins) that control the transport functions. A vacuolar chloride channel, AtCLCd gene, which is involved in cation detoxification, and AtNHXI gene which is homologous to NhxI gene of yeast have been cloned and over expressed in Arabidopsis to confer salt tolerance by compartmentalizing Na^+ ions in the vacuoles. Transgenic Arabidopsis and tomato plants that over express AtNHX1 accumulated abundant quantities of the transporter in the tonoplast and exhibited substantially enhanced salt tolerance **[13]**. Salt Overly Sensitive I (SOSI) locus in *A. thaliana*, which is similar to plasma membrane Na^+/H^+ antiporter from bacteria and fungi, was cloned and over expressed using CaMV 35S promoter. The up-regulation of SOSI gene was found to be consistent with its role in Na^+ tolerance, providing a greater proton motive force that is necessary for elevated Na^+/H^+ antiporter activities **[14-16]**. Genes encoding proton pumps, antiporters and ion trans-porters are AtMRP4 (Stomatal guard cell plasma membrane ABCC-type ABC transporter), AtNHX1(Vacuolar Na^+/H^+ antiporter), AtNHX2, AtNHX5 (Vacuolar Na^+/H^+ antiporter), AVP1(AVP1 proton pump overexpression), GmCAX1(Cation/proton antiporter), HKT1(Potassium transporter),

Table 1. Genes encoding proton pumps and ion transporter in salt stress

S.No.	Gene	Gene Action	Plant species	References
1.	AtNHX1	Vacuolar Na^+/H^+ antiporter	Tomato	**[17]**
2.	AtNHX1	Vacuolar Na^+/H^+ antiporter	Canola	**[13]**
3.	AtNHX1 AtNHX2 AtNHX5	Vacuolar Na^+/H^+ antiporter	Arabidopsis	**[18]**
4.	AtNHX1	Vacuolar Na^+/H^+ antiporter	Wheat	**[19]**
5.	HKT1	Potassium transporter	wheat	**[20]**
6.	GmCAX1	Cation/proton antiporter	Arabidopsis	**[21]**
7.	OsSOS1	Plasma membrane Na^+/H^+exchanger	Rice	**[22]**
8.	AtHKT1	Reduce sodium in roots	Arabidopsis	**[23]**
9.	TNHX1 and H+-	Vacuolar Na^+/H^+ antiporter	Arabidopsis	**[24]**
10.	SOD2	Vacuolar Na^+/H^+ antiporter	Arabidopsis	**[25]**
11.	P5CS	Pyrroline carboxylate synthase (proline synthesis)	Potato	**[26]**
12.	mtlD	Mannitol-1-phosphate dehydrogenase (mannitol synthesis)	*Populus tomentosa*	**[27]**
13.	PpDHNA	Dehydrin protein accumulation	Moss	**[28]**
14.	NHX1	proton antiporters	Arabidopsis	**[29]**
15.	AtNHX1	vacuolar Na^+/H^+ antiporter	Arabidopsis	**[30]**
16.	NHX	Na^+/H^+ antiporters	Plants	**[31]**
17.	NHX5 and NHX6	Na^+/H^+ antiporters	Arabidopsis	**[32]**
18.	OPBP1 gene	High resistance in salt stress	Tobacco	**[33]**
19.	SOS	Salt Tolerance	Plants	**[34]**
20.	LeNHX2 and LeNHX4	Regulate NHX activity in salt stress condition	Plants	**[35]**

AtHKT1 (Sodium and Potassium transporter), AtHKT1 (Reduction in Sodium in root), GhNHX1 (Vacuolar Na+/H+ antiporter), HvAACT1(Citrate transporter), HvPIP21 (PIP2 plasma membrane aquaporin Over expression), IRT1 (Divalent cation transporter), NtAQP1 (PIP1 plasma membrane aquaporin), NtPT1 (Phosphate transporter), NRT21 (Nitrate transporter) , OsNHX1 (Vacuolar Na+/H+ antiporter), Os-SOS1 (Plasma membrane Na^+/H^+ exchanger), PcSrp [Serine rich protein (enhancing ion homeostasis)], Pht1 (Phosphate acquisition by roots), PIP (Plasma membrane aquaporin over exression), PgTIP1 (Tonoplast intrinsic protein), PIP2;2 (Plasma membrane aquaporin knockout), PIP1b (Plasma

membrane aquaporin over exression), PIP1bn (Plasma membrane aquaporin over exression), PIP1;4 and PIP2;5 (Plasma membrane aquaporin over exression), RWC3 (Aquaporin overexpression), SOS4 (Involved in the synthesis of pyridoxal-5-phosphate which modulates ion transporters), SOS3(Sodium accumulation in roots), SOS1 (Na+-H+antiporter), SOD2 (Vacuolar Na^+/H^+ antiporter), SsVP-2(Vacuolar Na^+/H^+ antiporter), SsNHX1 (Vacuolar Na^+/H^+antiporter), SULTR1;2 (High affinity root sulfate transporter), TNHX1 and H^+-PPase TVP1(Vacuolar Na+/H+ antiporter), TsVP (Vacuolar Na^+/H^+ antiporter), YCF1(Sequester glutathione-chelates of heavy metals into vacuoles), ZntA(Regulation of Cd, PB and Zn pump).

Ion Homeostasis:

Plants respond to salinity using two different types of responses. Salt-sensitive plants restrict the uptake of salt and adjust their osmotic pressure through the synthesis of compatible solutes. Salt tolerant plants sequester and accumulate salt into the cell and maintaining a high cytosolic K^+/Na^+ ratio in their cells. The maintenance of a high cytosolic K^+/Na^+ ratio and precise regulation of ion transport is critical for salt tolerance **[36]**.The alteration of ion ratios in plants could result from the influx of Na^+ through pathways that also function in the uptake of K^+ **[37]**. This can be achieved by extrusion of Na+ ions from the cell or vacuolar compartmentation of Na^+ ions. Three classes of low-affinity K^+ channels have been identified **[38]**, these are K^+ Inward rectifying channels (K IRC); K^+ outward rectifying channels (KORCs) and Voltage-independent cation channels (VIC). K^+ outward rectifying channels (KORCs) could play a role in mediating the influx of Na+ into plant cells. These channels, which open during the depolarization of the plasma membrane, could mediate the efflux of K+ and the influx of Na^+ ions. Na^+ competes with K^+ uptake through Na^+ / K^+ co-transporters and may also block the K^+ specific transporters of root cells under salinity. This could result in toxic levels of sodium as well as insufficient K^+ concentration for enzymatic reactions and osmotic adjustment. The influx of Na^+ is controlled by AtHKT1, a low affinity Na^+ transporter **[39]**. The knockout mutant (hkt1) from Arabidopsis suppressed Na^+ accumulation and sodium hypersensitivity **[39]**, suggesting that AtHKT1 is a salt tolerance determinant, while the efflux is **[40]** controlled by Salt Overly Sensitive1 (SOS1), a plasma membrane Na+/H+ anti-porter. This antiporter is powered by the operation of H^+ -ATPase. In addition to its role as an antiporter, the plasma membrane Na^+/K^+ SOS1 may act as a Na^+ sensor. The overexpression of SOS1 improved salt tolerance in Arabidopsis **[15]** The compartmentation of Na^+ vacuoles, controlling the salt concentrations in the cytosol ions in vacuoles provides an efficient and cost effective mechanism to prevent the toxic effects of Na+ in the cytosol.

The overexpression of AtNHX1, resulted in the generation of transgenic Arabidopsis **[17]**, tomato **[13]**, rice **[41]**, maize **[42]**, tall fescue plants that were not only able to grow in significantly higher salt Concentration (200 mM NaCl) but could also flower and set fruit. The cellular response of salt-tolerant organisms to both long- and short-term salinity stresses includes the synthesis and accumulation of a class of osmoprotective compounds known as compatible solutes. These relatively small organic molecules are not toxic to metabolism and include proline, glycinebetaine, polyols, sugar alcohols, and soluble sugars. These osmolytes stabilize proteins and cellular structures and can increase the osmotic pressure of the cell **[43]**. This response is homeostatic for cell water status, which is perturbed in the face of soil solutions containing higher amounts of NaCl and the consequent loss of water from the

cell. Glycinebetaine and trehalose act as stabilizers of quartenary structure of proteins and highly ordered states of membranes. Mannitol serves as a free radical scavenger. It also stabilizes sub cellular structures (membranes and proteins), and buffers cellular redox potential under stress. Hence these organic osmolytes are also known as osmoprotectants **[33]**. Genes involved in osmoprotectant biosynthesis are upregulated under salt stress and concentrations of accumulated osmoprotectants correlate with osmotic stress tolerance. Although enhanced synthesis and accumulation of compatible solutes under osmotic stress is well known, little is known about the signaling cascades that regulate compatible solute biosynthesis in higher plants. Salt tolerance of transgenic tobacco engineered to over accumulate mannitol was first demonstrated by **[44]**. The other examples of compatible solute genetic engineering includes the transformation of genes for Ectoine synthesis with enzymes from the halophilic bacterium *Halomonas elongata* **[45]** and sorbitol synthesis in *plantago* spp **[46]**. Initial strategies aimed at engineering higher concentrations of proline began with the overexpression of genes encoding the enzymes pyrroline-5-carboxylate (P5C) synthetase (P5CS) and P5C reductase (P5CR), which catalyze the two steps between the substrate (glutamic acid) and the product (proline). P5CS overexpression in transgenic tobacco dramatically elevated free proline. However there is strong evidence that free proline inhibits P5CS. **[47]** achieved a two-fold increase in free proline in tobacco plants by using a P5CS modified by site directed mutagenesis. The procedure alleviated the feedback inhibition of P5CS activity by proline and resulted in improved germination and growth of seedlings under salt stress. In spinach and sugar beet which naturally accumulate glycinebetaine, the synthesis of this compound occurs in the chloroplast. The first oxidation to betaine aldehyde is catalyzed by choline mono-oxygenase (CMO). Betaine aldehyde oxidation to glycinebetaine is catalyzed by betaine aldehyde dehydrogenase (BADH) **[48]**. In Arthrobacter globiformis, the two oxidation steps are catalyzed by one enzyme, choline oxidase (COD), which is encoded by the codA locus **[49, 50]** used choline oxidase of A. globiformis to engineer glycinebetaine synthesis in Arabidopsis and subsequently tolerance to salinity during germination and seedling establishment was improved markedly in the transgenic lines. **[51]** used COX from A. panescens, which is homologous to the *A. globiformis* COD, to transform arabidopsis, *B. napus* and tobacco. In this set of experiments COX protein was directed to the cytoplasm and not to the chloroplast. Improvements in tolerance to salinity, drought and freezing were observed in some transgenics from all three species, but the tolerance was variable. The results offered the possibility that the protection offered by glycinebetaine is not only osmotic but also function as scavengers of oxygen radicals. The level of glycinebetaine production in transgenics could be limited by choline. A dramatic increase in glycinebetaine levels (to 580 mmol/g dry weight in Arabidopsis thaliana) was achieved when the growth medium was supplemented with choline **[51]**. The enhancement of glycinebetaine syntheses in target plants has received much attention **[52,53]**.

Conclusion

In this review we summarizes the stress related genes and transcription factors that help plant to survive in stress conditions. Through recent research and findings it is a clear to introduce stress tolerance genes into crop plants, additionally to establishing gene stacking or gene pyramiding. The use of transgenes to improve the tolerance of crops to abiotic stresses remains an attractive option. Although progress in improving

stress tolerance has been slow, there are a number of reasons for optimism. An important issue to address is how the tolerance to specific abiotic stress is assessed, and whether the achieved tolerance compares to existing tolerance.

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