Research review paper

Improving salinity tolerance of plants through conventional breeding and genetic engineering: An analytical comparison

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A B S T R A C T

The last century has witnessed a substantial improvement in yield potential, quality and disease resistance in crops. This was indeed the outcome of conventional breeding, which was achieved with little or no knowledge of underlying physiological and biochemical phenomena related to a trait. Also the resources utilized on programs involving conventional breeding were not of great magnitude. Plant breeders have also been successful during the last century in producing a few salt-tolerant cultivars/lines of some potential crops through conventional breeding, but this again has utilized modest resources. However, this approach seems now inefficient due to a number of reasons, and alternatively, genetic engineering for improving crop salt tolerance is being actively followed these days by the plant scientists, world-over. A large number of transgenic lines with enhanced salt tolerance of different crops can be deciphered from the literature but up to now only a very few field-tested cultivars/lines are known despite the fact that considerable resources have been expended on the sophisticated protocols employed for generating such transgenics. This review analytically compares the achievements made so far in terms of producing salt-tolerant lines/cultivars through conventional breeding or genetic engineering.

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1. Introduction

Although salinity is a major problem for agriculture in arid and semi-arid regions of the world, this menace can be observed in other areas as well. This environmental adversary has caused considerable crop yield losses and hence very low crop productivity. However, to combat this peril, long ago, two key strategies for the utilization of salt-affected lands, i.e., reclamation of salt-affected soils and growing halophytes or salt-tolerant crops/cultivars on such soils, were proposed (Epstein, 1977). The latter approach was referred to as the “biotic approach” (Epstein, 1977; Kingsbury and Epstein, 1984; Ashraf, 1994). The term “biosaline agriculture” is also analogous to this. The biotic approach was considered as the most efficient and economical means of utilizing salt-affected soils by many scientists (Epstein, 1977; Shannon, 1985; Ashraf, 1994) and hence a permanent solution of the problem.

Producing crops tolerant to salinity stress is imperative to run into the growing food demand through sustainable agriculture. This could be achieved either through conventional selection and breeding or through modern molecular biology approaches. Plant breeders have succeeded to some extent in producing salt-tolerant lines/cultivars of some crops through conventional breeding, but the main reason of the limited success through this strategy has been due to the fact that the magnitude of genetic variation in the gene pools of most important crops is very
low. However, alternatively, transgenic approach for improving crop salt tolerance is being actively pursued these days by the plant scientists, world-over, and a number of transgenic lines of different crops can be easily deciphered from the literature.

This review analytically compares the achievements made so far in terms of producing salt-tolerant lines/cultivars through conventional breeding or genetic engineering. It also highlights some of the challenges confronting genetic engineers because one can easily assess that only few lines/cultivars of different crops generated through genetic engineering and molecular biology approaches have thrived well under natural field conditions, though most of the claims of the scientists regarding the good performance of salt-tolerant transgenic lines are based on growth room and glasshouse trials.

2. Selection and breeding for salinity tolerance — A conventional approach

Although considerable progress was made during the 20th century to improve crop yield and quality through conventional breeding, we can see relatively little work on improving the resistance of crops against abiotic stresses, especially salinity stress. Perhaps this has been due to the main reason that plant breeders focused more on improving crop yield and quality than on improving stress tolerance. Nonetheless, plant breeders had been carrying out various breeding programs during the last century wherein they had wisely exploited the genetic variation of crops at intra-specific, inter-specific, and inter-generic levels so as to produce salt-tolerant lines/cultivars. Resultantly, they were able to release a few salt-tolerant lines/cultivars of different potential crops through conventional breeding. It is imperative to note that most of the lines/cultivars produced through conventional breeding were tested under natural field conditions. For example, some lines/cultivars of alfalfa (Medicago sativa L.) such as AZ-Germ Salt II (Dobrenz et al., 1989), AZ-90NDC-ST (Johnson et al., 1991), AZ-97MCE and AZ-97MCE-AT (Al-Doss and Smith, 1998), ZS-9491 and ZS-9592 (Dobrenz, 1999) were tested under natural field conditions. Similarly, two salt-tolerant lines/cultivars of bread wheat (Triticum aestivum L.) such as S24 (Ashraf and O’Leary, 1996) and KRL1-4 (Hollington, 2000) were evaluated on natural salt-affected soils.

These few examples depict that there has been a limited success in producing salt-tolerant cultivars of different potential crops using the conventional breeding approach. The main problem conventional plant breeders have been facing is the low magnitude of genetically based variation in the gene pools of most crop species. Thus, one cannot expect substantial improvements in crop salt tolerance. Under such circumstances, it is advisable to utilize salt-tolerant wild relatives of crop plants as a source of genes for crop improvement for enhanced salt tolerance. But in fact, transferring salt-tolerant genes from wild relatives to domesticated crops is not so easy because of reproductive barriers (Ashraf et al., 2008) and thus very few examples could be tracked down from the literature (Ashraf, 1994; Flowers, 2004) wherein this approach had been effectively utilized. Chinnusamy et al. (2005) have listed some conclusive reasons for limited success in improvement of crop salt tolerance through conventional breeding. The approach as a whole is time-consuming and labor-intensive; undesirable genes are often transferred in combination with desirable ones; and reproductive barriers limit transfer of favorable alleles from inter-specific and inter-generic sources. Due to these reasons genetic engineering, as an alternative strategy to conventional breeding, is being employed emphatically worldwide these days not only for improving stress tolerance but also for improving quality and yield potential of most crops.

3. Engineering plants for enhanced salt tolerance: transgenic approach

Transgenic plants are those plants that have been genetically altered by inserting desired genes directly into a plant cell. Such plants are special in a way that they are developed from only a single plant cell. Transgenic plants have been developed through different genetic engineering techniques to contain desirable traits, including resistance to pests, pesticides, diseases or adverse environmental conditions, improved nutritional value, and enhanced product shelf-life. Despite a number of social, political and legal concerns, many countries are now allowing transgenic crop production in conjunction with their conventional crop production. In view of a report by The International Service for the Acquisition of Agro-Biotech Applications (ISAAA) the top five countries growing transgenic crops in 2005, were USA, Argentina, Brazil, Canada, and China (ISAAA, 2006). However, transgenic approach is being effectively pursued by plant scientists these days not only to improve quality and yield but also to increase tolerance to biotic and abiotic stresses in a number of crops. Since the salt tolerance trait involves a number of minor genes (quantitative trait loci) so it is very complex. Thus, improving crop salt tolerance by genetic engineering is not so easy. Nonetheless, for improving salt tolerance trait in various crops through genetic engineering plant biologists have focused much on genes that encode: ion transport proteins, compatible organic solutes, antioxidants, heat-shock and late embryogenesis abundant proteins, and transcription factors for gene regulation (Ashraf et al., 2008). However, in the present review we have discussed only the transgenic lines produced for the overexpression of ion transport proteins, compatible organic solutes, and antioxidants.

4. Transgenic plants for ion transporters

High levels of salt in the growth medium of plants affect their growth and development and ultimately, the yield. To prevent salt damage, plants have developed different mechanisms to reduce Na+ uptake or compartmentalize Na+ into vacuoles. In view of a number of reports it is evident that Na+ moves passively through a general cation channel from the saline growth medium into the cytoplasm of plant cells (Blumwald, 2000; Jacoby, 1999; Mansour et al., 2003; Ashraf et al., 2008), but there is also a sound evidence that Na+ can move actively through Na+/H+ antiporters, which exchange Na+ or Li+ for H+ (Ratner and Jacoby, 1976; Niu et al., 1993; Shi et al., 2003), and the regulation of Na+ uptake and accumulation into plant cells is relied on the regulation of proton pumps and antiporters operating at both plasma membrane and tonoplast (Ashraf, 2004). Na+/H+ antiporters are found in every biological kingdom, from bacteria to humans to higher plants (Padan et al., 2001). There are several families of Na+/H+ antiporters including NhaA, NhaB, NhaC, NhaD, and NapA in prokaryotes, SOD2 and Nha1 in fungi, and AtNHX1 and SOS1 in Arabidopsis (Wu et al., 2005). However, engineering plants for overexpression of genes for different types of antiporters has recently gained a ground as an effective approach for controlling the uptake of toxic ions and hence enhanced plant salt tolerance. In Table 1, transgenic lines of different crops with engineered genes for different types of transporters are listed. Some scientists have shown high resistance of engineered lines to salt stress, but they have not measured growth performance of the lines in terms of biomass production or seed yield (in case of grain crops) under saline conditions. In fact, they measured only germinability or survival of seedlings in saline regime under controlled conditions. For example, Bao-Yan et al. (2008) and Gu et al. (2008) have generated two transgenic lines of Arabidopsis using MsNHX1 from alfalfa (M. sativa) and ZmOPR1 from maize (Zea mays), respectively. They have shown considerable improvement in seed germination of both transgenic lines under saline conditions. Both studies were in fact conducted under controlled growth room conditions, and the plant performance was not appraised as adult or under natural field conditions. Furthermore, other scientists have shown a substantial improvement in growth and biomass production in engineered lines of different crops under saline regimes. For example, Verma et al. (2007) have
### Table 1
Improving plant salt tolerance through engineering genes for ion transporters.

<table>
<thead>
<tr>
<th>Gene engineered</th>
<th>Transgenetic host</th>
<th>Source</th>
<th>Trait improved</th>
<th>Growth conditions for testing</th>
<th>Growth improvement in transgenic lines under salt stress</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuolar Na+/H+ antiporter MiNHX1</td>
<td>Arabidopsis thaliana L.</td>
<td>Alfalfa (Medicago sativa L.) A. thaliana L.</td>
<td>Increased osmotic adjustment and MDA content</td>
<td>Laboratory conditions</td>
<td>Germination and seedling growth</td>
<td>Bao-Yan et al. (2008)</td>
</tr>
<tr>
<td>Vacuolar Na+/H+ antiporter AnNHX1</td>
<td>Tall fescue (Festuca arundinacea Schreerb.)</td>
<td>A. thaliana L.</td>
<td>Higher root sodium contents and activity of vacuolar Na+/H+ antiporter</td>
<td>Hydroporons in greenhouse</td>
<td>About 18% improvement in shoot dry weight, and 43% in root dry weight</td>
<td>Zhao et al. (2007)</td>
</tr>
<tr>
<td>Vacuolar Na+/H+ antiporter AgNHX1</td>
<td>Rice (Oryza sativa L.)</td>
<td>Atriplex gmelini</td>
<td>Eight-fold higher activity of the vacuolar-type Na+/H+ antiporter</td>
<td>Hydroporons in greenhouse</td>
<td>50% or 81–100% survival of seedlings</td>
<td>Ohta et al. (2002)</td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter PyNHX1</td>
<td>Rice (O. sativa L.)</td>
<td>Pennisetum glaucum (L.) R. Br. A. thaliana L.</td>
<td>Extensive root system</td>
<td>Hydroporons in greenhouse</td>
<td>About 81% improvement in shoot and root lengths</td>
<td>Verma et al. (2007)</td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter AnNHX1</td>
<td>Wheat (Triticum aestivum L.)</td>
<td>Maize (Zea mays L.), inbred line, Han 21</td>
<td>Improved germination rate, biomass production, grain yield, and leaf K+ accumulation, and reduced leaf Na+</td>
<td>Hydroporons in greenhouse</td>
<td>About 68% increase in shoot dry weight and 26% in root dry weight</td>
<td>Xue et al. (2004)</td>
</tr>
<tr>
<td>OPR1 cDNA designated as ZmOPR1</td>
<td>A. thaliana L.</td>
<td>Improved seed germination and altered transcription of RD22, RD29B and AFR2</td>
<td>Hydroporons in greenhouse</td>
<td>Improved germination</td>
<td>Gu et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter gene AnNHX1</td>
<td>Tall fescue (F. arundinacea Schreerb.)</td>
<td>A. thaliana L.</td>
<td>Better growth at 200 mM NaCl</td>
<td>Growth room and greenhouse</td>
<td>Transgenic plants thrived well at 200 mM NaCl</td>
<td>Tian et al. (2006)</td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter gene OsNHX1</td>
<td>Perennial ryegrass (Lolium perenne L.)</td>
<td>Rice (O. sativa L.)</td>
<td>Higher concentration of Na+, K+ and proline</td>
<td>Growth room</td>
<td>Transgenic plants survived at 350 mM NaCl but all wild type plants died</td>
<td>Wu et al. (2005)</td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter GmNHX1</td>
<td>Tobacco (Nicotiana tabacum L.)</td>
<td>Cotton (Gossypium hirsutum L.) A. thaliana L.</td>
<td>Na+ vacuolar sequestration</td>
<td>Growth room</td>
<td>About 100% increase in dry weight/plant</td>
<td>Wu et al. (2004)</td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter AnNHX1</td>
<td>Tomato (Lycopersicon esculentum L.)</td>
<td>A. thaliana L.</td>
<td>Overproduction of vacuolar Na+/H+ antiporter protein</td>
<td>Hydroporons in greenhouse</td>
<td>Increased growth, and flower and seed production</td>
<td>Zhang and Blumwald (2001)</td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter AvNHX1</td>
<td>Tobacco (Nicotiana tabacum L.) Alfaflia (M. sativa L.)</td>
<td>Araloeus littoralis</td>
<td>Compartmentalized more Na+ in the roots and kept a relative high K+ /Na+ ratio in the leaves High Na+, K+ and Ca2+ in leaves and roots</td>
<td>Greenhouse</td>
<td>About 150% increase in relative dry weight</td>
<td>Zhang et al. (2008)</td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter AvNHX1</td>
<td>Vacular H+ - pyrophosphatase AVPI</td>
<td>Tobacco (Nicotiana tabacum L.) Alfaflia (M. sativa L.)</td>
<td>Growth in transgenic cells or plants and accumulated higher Na+ and lower K+</td>
<td>Hydroporons in growth room</td>
<td>Maintained growth at 0.2 M NaCl while wild type plants died</td>
<td>Bao et al. (2009)</td>
</tr>
<tr>
<td>Yeast (Schizosaccharomyces pombe) sodium 2 SO42-</td>
<td>A. thaliana L.</td>
<td>Yeast (Schizosaccharomyces pombe)</td>
<td>Better in fresh and dry biomass, and accumulated less Na+ and more K+</td>
<td>Greenhouse</td>
<td>Transgenic plants were 87% higher in plant fresh weight and 27.5% in dry weight</td>
<td>Fukuda et al. (2004)</td>
</tr>
<tr>
<td>Plasma membrane Na+ / H+ antiporter SOS1</td>
<td>A. thaliana L.</td>
<td>Wild type (A. thaliana L.)</td>
<td>Improved germination rate, root growth, protein level, chlorophyll contents and plant survival rate, reduced accumulation of shoot Na+</td>
<td>Greenhouse</td>
<td>About 11–15.4% increase in root growth</td>
<td>Shi et al. (2003)</td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter AnNHX1</td>
<td>Brassica napus</td>
<td>A. thaliana L.</td>
<td>Improved plant growth, seed yield and seed oil quality: toxic effect of Na+ was mitigated and increased proline accumulation, and lower cytosolic K+ Germination percentage</td>
<td>Greenhouse</td>
<td>About 2.3% higher in plant fresh weight, and 2.34% in grain yield at 10 mM NaCl</td>
<td>Zhang et al. (2001)</td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter AnNHX1</td>
<td>Maize (Z. mays L.) A. thaliana L.</td>
<td>Improvement in growth, number of flowers and yield</td>
<td>Field</td>
<td>At 0.5% NaCl, germination capacity of transgenic plants was about 80% while that of wild plants was only 13–57%</td>
<td>Xiao-Yan et al. (2004)</td>
<td></td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter BnNHX1</td>
<td>Tobacco (Nicotiana tabacum L.) B. napus L.</td>
<td>Improvement in growth, number of flowers and yield</td>
<td>Greenhouse</td>
<td>Transgenic plants grew better and produced seeds at 200 mM NaCl, while wild type plants died.</td>
<td>Wang et al. (2004)</td>
<td></td>
</tr>
<tr>
<td>Vacular Na+/H+ antiporter cDNA gene, HmNHX1</td>
<td>Tobacco (Nicotiana tabacum L.) Hordeum brevisulatum (Trin.) Link A. thaliana L.</td>
<td>Higher levels of Na+, K+ and Ca2+ in leaves, and relative water contents: protein contents increased about 1.6–2.4-fold, While osmotic potential and stomatal aperture decreased</td>
<td>Greenhouse</td>
<td>Transgenic plants grew well at 250 mM NaCl while wild plants died after 10 days of salt treatment</td>
<td>Lu et al. (2005)</td>
<td></td>
</tr>
</tbody>
</table>

(continued on next page)
shown about 81% improvement in shoot and root lengths in transgenic rice line containing PnNHX1 from *Pennisetum glaucum*, under salt stress. Similarly, two tobacco (*Nicotiana tabacum*) transgenic lines (*GhNHX1, Wu et al., 2004; AINHX, Zhang et al., 2008*) showed about 100 and 150% higher plant dry weight than the wild type when tested at 300 mM and 400 mM NaCl, respectively. A marked improvement (about 215%) has been recently observed in shoot dry weight of transgenic line of alfalfa overexpressing vacuolar H+-pyrophosphatase gene, AVP1 from *Arabidopsis* when tested under saline conditions (Bao et al., 2009). All these studies and many other listed in Table 1 show remarkable performance of transgenic lines of different crops under saline regimes, but as is evident from the information depicted in Table 1 almost all studies were carried out under growth room or greenhouse conditions and the performance of the transgenic lines was not tested under natural field conditions. However, there is only one study by Xue et al. (2004) in which the performance of a wheat (*T. aestivum*) transgenic line overexpressing vacuolar Na+/H+ antiporter gene, AtNHX1 from *Arabidopsis*, was appraised under both greenhouse and natural field conditions (Table 1).

It is evident that the degree of salt tolerance varies at different phases of life cycle of most plant species (Ashraf et al., 2008), so it is not certain whether most of the transgenic lines that have shown remarkable performance under saline conditions at the initial growth stages (germination and seedling growth) would also maintain their degree of salt tolerance when tested as adult. Also it is not sure whether the transgenic lines tested under controlled growth room or greenhouse conditions would perform similarly under natural field conditions as the latter are exposed to a multitude of environmental factors other than the salt stress.

### 5. Transgenic plants for compatible organic solutes

Of a number of plant responses to salt stress, overproduction of different types of compatible organic solutes is the most common one (Ashraf and Foolad, 2007). The most common organic solutes that play an active role in the mechanism of plant salt tolerance include proline, glycinebetaine, and trehalose etc. All these engineered plants also exhibited increased salt tolerance. For example, the gene for P5CS has been overexpressed in rice (*Su and Wu, 2004*), potato (*Hmidi-Sayari et al., 2005*), wheat (*Sawahel and Hassan, 2002*), and tobacco (*Kishor et al., 1995; Hong et al., 2000*). Indeed, all transgenic lines accumulated proline many fold higher than that in wild type plants and showed a marked increase in growth under saline conditions. Genes encoding key enzymes of glycinebetaine biosynthesis such as choline dehydrogenase, choline oxidase, and choline monoxygenase have been engineered (Hayashi et al., 1997; Nuccio et al., 1998; Bhattacharya et al., 2004). Plants such as arabidopsis (Huang et al., 2000), cabbage (Bhattacharya et al., 2004), rice (Sakamoto et al., 1998), and *Brassica juncea* (Prasad et al., 2000) so transformed showed enhanced salt tolerance. Trehalose, a nonreducing disaccharide, has recently gained a ground as a potential osmoprotectant analogous to proline and glycinebetaine, because its overproduction in plants has been shown correlated with salt tolerance. For example, a transgenic rice line expressing the trehalose gene exhibited enhanced tolerance not only to salinity stress, but also to drought and cold stress (Garg et al., 2002).

### 6. Transgenic plants for enhanced antioxidant production

Despite affecting a multitude of physiological and biochemical processes in plants, salt stress also triggers a number of degenerative

### Table 1 (continued)

<table>
<thead>
<tr>
<th>Gene engineered trait</th>
<th>Transgenic host</th>
<th>Source</th>
<th>Trait improved</th>
<th>Growth conditions for testing</th>
<th>Growth improvement in transgenic lines under salt stress</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma membrane Na+/ H+ antiporter sodium 2 (S022)</td>
<td>Rice (O. sativa L.)</td>
<td>Yeast (S. pombe)</td>
<td>Accumulated higher K+ (29.4%), Ca2+ (22.2%), Mg2+ (53.8%) and lower Na+ (26.6%) contents and enhanced net photosynthetic rate (100%), and decreased MDA level (57.10%)</td>
<td>Greenhouse</td>
<td>32.5% increase in shoot fresh weight at 150 mM and 57.1% at 300 mM NaCl</td>
<td>Zhao et al., (2006)</td>
</tr>
</tbody>
</table>

*Calculated from the original data presented in each study.*
Table 2
Improving plant salt tolerance through engineering genes for osmoprotectants.

<table>
<thead>
<tr>
<th>Gene engineered</th>
<th>Transgenic host</th>
<th>Source organism</th>
<th>Trait improved</th>
<th>Growth conditions for testing</th>
<th>Growth improvement in transgenic lines under salt stress</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A novel DREB (dehydration responsive element binding protein) gene, designated as BjDREB1B</td>
<td>Tobacco (Nicotiana tabacum L. cv. NC89)</td>
<td>Brassica juncea L.</td>
<td>Transgenic plants accumulated 3.2–4.4-fold higher proline under normal conditions and 2.5–2.9-fold at 150 mM NaCl and 46.6% higher chlorophyll under normal conditions while, 171.4% at 600 mM NaCl as compared to those in wild types</td>
<td>Controlled conditions</td>
<td>Biomass or yield was not measured</td>
<td>Cong et al. (2008)</td>
</tr>
<tr>
<td>Δ1-Pyrroline-5-carboxylate reductase (Tps1)</td>
<td>Arabidopsis thaliana L.</td>
<td>Triticum aestivum L.</td>
<td>Transgenic seedlings produced 3-fold more proline under normal conditions, while 2.5–4 times higher at 150 mM NaCl than those in wild types. In contrast, MDA contents decreased 16.6% more under normal, while 24% under saline conditions as compared to those in wild types</td>
<td>Growth room</td>
<td>About 25.3% improvement in relative root length at 50 mM NaCl, while 31.1% at 100 mM NaCl</td>
<td>Ma et al. (2008)</td>
</tr>
<tr>
<td>Δ1-pyrroline-5-carboxylate synthetase (p5cs)</td>
<td>Rice (Oryza sativa L.) cv. Kenfong</td>
<td>Moth bean (Vigna aconitifolia)</td>
<td>Transgenic plants accumulated higher concentration of p5cs mRNA and showed faster growth rate of transgenic plants. About 120–315% more proline content in transgenic plants under control and 163–216% at 300 mM NaCl as compared to that in wild type</td>
<td>Greenhouse and growth room</td>
<td>About 30–93% improvement in shoot fresh weight and 37–74% in root fresh weight at 200 mM NaCl</td>
<td>Su and Wu (2004)</td>
</tr>
<tr>
<td>Mannitol-1-phosphate dehydrogenase (M1PD) and glucitol-6-phosphate dehydrogenase (GthD)</td>
<td>Loblolly pine (Pinus taeda L.)</td>
<td>Agrobacterium tumefaciens strain LBA4404</td>
<td>Improved survival rate, synthesis and accumulation of mannitol and glucitol, and enhanced ability to tolerate high salinity in transgenic plants</td>
<td>Greenhouse</td>
<td>Plant survival rate of transgenic plants was about 78.1% at 85 mM and 62.8% at 120 mM NaCl after 15 weeks of salt treatment, while wild plants did not survive even after 9 week growth</td>
<td>Tang et al. (2005)</td>
</tr>
<tr>
<td>NADP-dependent sorbitol-6-phosphate dehydrogenase (SOPDH)</td>
<td>Japanese persimmon (Diospyros kaki Thunb.)</td>
<td>Apple (Malus x domestica Borkh.)</td>
<td>Higher sorbitol contents (14.5–61.5 µmol g⁻¹ f.wt) at 50 mM NaCl in transgenic plants while absent in wild type. Fv/Fm was also improved in transgenic plants</td>
<td>Growth room</td>
<td>Improved growth was observed at 50 mM NaCl</td>
<td>Gao et al. (2001)</td>
</tr>
<tr>
<td>Trehalose-6-phosphate synthase (TP51)</td>
<td>Tomato (Lycopersicon esculentum L.) cv. UC82B</td>
<td>Yeast (Saccharomyces cerevisiae)</td>
<td>Higher trehalose contents (0.15 mg/g), improved plant growth and higher chlorophyll (25–35%) and starch (35–65%) contents in transgenic plants at 100 mM NaCl as compared to wild types</td>
<td>Greenhouse</td>
<td>Improved seed dry weight (about 23.2%)</td>
<td>Cortina and Culianez-Macia (2005)</td>
</tr>
<tr>
<td>Δ1-pyrroline-5-carboxylate synthetase (P5CS)</td>
<td>Potato (Solanum tuberosum L. cv. Nicola)</td>
<td>A. thaliana L.</td>
<td>At 100 mM NaCl transgenic plants showed 3–10 times increase in proline level as compared to that in wild type plants. Transgenic plants also showed an improved tolerance to salinity through a much less altered tuber yield and weight compared to non-transgenic ones</td>
<td>Greenhouse</td>
<td>Improved plant growth. Reduction in tuber yield was only 29–42% in transgenic plants, while in wild type it was 63% under saline conditions</td>
<td>Himida-Sayari et al. (2005)</td>
</tr>
<tr>
<td>Choline dehydrogenase (betA)</td>
<td>Cabbage (Brassica oleracea var. capitata) cultivar ‘Golden Acre’</td>
<td>Escherichia coli</td>
<td>Improvement in plant biomass, chlorophyll contents, and relative water contents, and less negative water potential and osmotic potential of transgenic plants as compared to those of wild type plants at 150 and 300 mM NaCl</td>
<td>Greenhouse</td>
<td>Improved plant biomass about 21.3% at 150 mM and 20% at 300 mM NaCl</td>
<td>Bhattacharya et al. (2004)</td>
</tr>
<tr>
<td>Δ1-pyrroline-5-carboxylate synthetase (P5CS)</td>
<td>Wheat (T. aestivum L.)</td>
<td>Moth bean (V. aconitifolia)</td>
<td>Transgenic plants expressed higher level of P5CS mRNA, and P5CS protein, and accumulated 2.5-fold higher proline than that in wild type plants</td>
<td>Greenhouse</td>
<td>Transgenic plants were unaffected up to 200 mM NaCl and a small growth reduction was observed at 250 mM NaCl, while wild type plants died or showed stunted growth at 100 mM NaCl</td>
<td>Sawahel and Hassan (2002)</td>
</tr>
<tr>
<td>bet gene cluster i.e., betA (choline dehydrogenase), betB (betaine aldehyde dehydrogenase), betI (putative regulatory protein), and betT (choline transport system)</td>
<td>Fresh water cyanobacterium (Synechococcus sp. PCC7942)</td>
<td>E. coli</td>
<td>Transgenic plants showed improved cell growth even at 0.4 M NaCl and exhibited higher activities of PSI and PSII at 200 mM NaCl than those in wild type plants</td>
<td>Culture medium</td>
<td>Cell number increased 125.5% at 0.4 M NaCl</td>
<td>Nomura et al. (1999)</td>
</tr>
</tbody>
</table>
Table 2 (continued)

<table>
<thead>
<tr>
<th>Gene engineered</th>
<th>Transgenic host</th>
<th>Source organism</th>
<th>Trait improved</th>
<th>Growth conditions for testing</th>
<th>Growth improvement in transgenic lines under salt stress*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol 1-phosphate dehydrogenase (mTID)</td>
<td>A. thaliana L.</td>
<td>E. coli</td>
<td>Mannitol (0.05–12.00 μmol g⁻¹ fresh weight) was synthesized in transgenic plants but not in wild type plants. Germination percentage, fructose, inositol, and proline contents of transgenic plants were improved under 200 mM NaCl</td>
<td>Growth room</td>
<td>Transgenic seeds containing mannitol germinated up to 400 mM NaCl while in wild plants germination ceased at 100 mM NaCl</td>
<td>Thomas et al. (1995)</td>
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<td>Δ1-pyrroline-5-carboxylate synthetase (PCS)</td>
<td>Tobacco (N. tabacum L.)</td>
<td>Moth bean (V. aconitifolia)</td>
<td>Transgenic plants synthesized 10–18-fold more proline and showed improved tolerance with respect to root length, root dry weight, capsule number and number of seeds per capsule at 500 mM NaCl as compared to wild type</td>
<td>Greenhouse and growth room</td>
<td>Transgenic plants had 30% higher root length, 170% root dry weight, 254% capsule number and 42.1% seed number per capsule at 500 mM NaCl as compared to those of wild type</td>
<td>Kishor et al. (1995)</td>
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<tr>
<td>-myo-inositol 1-phosphate synthase from P. coreanum (Roxb.) designated as PtINO1</td>
<td>Tobacco (N. tabacum L.)</td>
<td>Halophytic rice (Porteresia coarctata) (Roxb.)</td>
<td>Transgenic plants had improved growth, about 2–7-fold increase in the inositol contents and about 2-fold higher photosynthetic competence at 300 mM NaCl as compared to the non-transformed plants</td>
<td>Greenhouse and tissue culture room</td>
<td>Growth improvement was observed in transgenic plants at 300 mM NaCl but biomass was not recorded</td>
<td>Majee et al. (2004)</td>
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<td>Choline oxidase (COX)</td>
<td>A. thaliana L., (N. tabacum L. and Brassica napus L.</td>
<td>Arthrobacter pascens</td>
<td>Improvement in growth, photosynthetic rate and glycinebetaine synthesis in transgenic plants</td>
<td>Growth chamber</td>
<td>37.5% increase in shoot dry weight of transgenic &amp; napus plants at 300 mM, 90.6% in A. thaliana at 100 mM and 200% in tobacco at 150 mM NaCl</td>
<td>Huang et al. (2000)</td>
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<tr>
<td>Δ1-pyrroline-5-carboxylate synthetase (PCS129A)</td>
<td>Tobacco (N. tabacum L.)</td>
<td>Moth bean (V. aconitifolia)</td>
<td>Proline content was 2-fold higher than that in wild type. Transgenic plants also had improved seedling growth, and enhanced malondialdehyde production at 200 mM NaCl</td>
<td>Tissue culture and growth room</td>
<td>Improved germination rate (67%) and seedling fresh weight (33.3%) at 200 mM NaCl</td>
<td>Hong et al. (2000)</td>
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<tr>
<td>-2,4-diaminobutyric acid acetyltransferase (ectA), -2,4-diaminobutyric acid transaminase (ectB) and -ectoine synthase (ectC)</td>
<td>Tobacco (N. tabacum L.)</td>
<td>Halomonas elongata</td>
<td>Improved accumulation of ectoine, increased tolerance to hyperosmotic stress, and better growth in transgenic plants</td>
<td>Tissue culture room</td>
<td>Transgenic plants had normal growth pattern at 500 mM NaCl while in wild type plants it was inhibited</td>
<td>Nakayama et al. (2000)</td>
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<td>Myo-inositol O-methyltransferase (Imt)</td>
<td>Tobacco (N. tabacum L.)</td>
<td>Mesembryanthemum crystallinum</td>
<td>Improvement in methylated inositol O-mononitol content, photosynthetic CO₂ fixation and stable solute accumulation in transgenic plants at 250 mM NaCl</td>
<td>Hydroponics in greenhouse</td>
<td>Transgenic growth at 250 mM NaCl but data was not presented</td>
<td>Sheveleva et al. (1997)</td>
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</table>

* Calculated from the original data presented in each study.

reactions mediated by reactive oxygen species (ROS) such as superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (•OH), and singlet oxygen (¹O₂). Although ROS in plants are generated under optimal growth conditions and their concentration is usually found low (Polle, 2001), most environmental adversaries trigger enhanced production of ROS (Karpinski et al., 2003; Laloi et al., 2004). When a plant experiences a stress, ROS are produced through pathways such as photorespiration, mitochondrial respiration, and from the photosynthetic apparatus (Ashraf, 2009). One of the most prominent effects of salt stress on plants is that it limits the supply of CO₂ to the leaf by closing the stomata, which in turn causes overreduction of photosynthetic electron transport chain, thereby resulting in the generation of reactive oxygen species (Halliwell and Gutteridge, 1985; Thompson et al., 1987). These ROS are cytotoxic and can adversely affect normal metabolism by causing a substantial oxidative damage to biomolecules such as membrane lipids, proteins, and nucleic acids (Mckersie and Lesher, 1994). Despite being toxic, ROS play a vital role in various important physiological phenomena such as cell signaling, gene regulation, senescence, apoptosis, pathogen defense, etc. (Dat et al., 2000; 2003; Laloi et al., 2004; Gechev et al., 2006). However, plants have their innate systems for scavenging/detoxifying the ROS, which are commonly referred to as antioxidants (Asada, 1999). The most common antioxidants found in plants are ascorbate, glutathione, α-tocopherol, and carotenoids, whereas antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and enzymes of ascorbate–glutathione cycle (Ashraf, 2009). Most of the antioxidant enzymes can substantially lower the levels of superoxide and hydrogen peroxide in plants (Ali and Alqurainy, 2006).

A number of studies that appeared in the literature during the last few decades have revealed that improvement in the salt tolerance of different plant species is possible through engineering the genes for specific enzymes which can effectively scavenge reactive oxygen species (Van Camp et al., 1994; Foyer et al., 1994, 1995; Poldoros and Scandalios, 1999). However, some studies listed in Ashraf (2009) provide sound evidence that overexpression of genes for antioxidant enzymes causes enhanced salt tolerance in different crops. For example, dehydroascorbate reductase (DHAR), an enzyme that reduces the oxidized ascorbate in plant cells, is vital for recycling ascorbate. In a recent study, Ushimaru et al. (2006) have shown that the expression of rice DHAR in transgenic arabidopsis enhanced the degree of salt tolerance measured as germinability under salt stress. While engineering genes for different types of superoxide dismutase in plants such as rice (Tanaka et al., 1999), tobacco (Badawi et al., 2004), Chinese cabbage (Tseng et al., 2007), and arabidopsis (Wang et al., 2004) a marked improvement in salt tolerance of these crops was achieved. However, in these studies salt tolerance was appraised
either by the activities of specific enzymes involved in oxidative stress tolerance or just measured germination or seedling growth. Similarly, transgenic plants overexpressing glyoxalase pathway enzymes that disallow an increase in accumulation of methylglyoxal (MG) have been developed in different plants such as tobacco (Singla-Pareek et al., 2003; Yadav et al., 2005), Japonica and indica rice (Verma et al., 2005), and Escherichia coli (Yadav et al., 2007). All transgenic plants/organisms showed enhanced tolerance to salt stress, although in most cases early stage growth performance was measured. Only Singla-Pareek et al. (2003) grew the transgenic tobacco plants till maturity and recorded seed yield, but this study, like the other studies, was carried out under controlled conditions. Genes for glutathione S-transferase (Roxas et al., 2000) and catalase (Al-Taweel et al., 2007) were also engineered in tobacco. The transgenic lines excelled the wild types in salt tolerance (Ashraf, 2009).

Although substantial increases in the activities of different antioxidant enzymes in response to salt stress have been reported, some controversies have been reported while drawing correlations of salt tolerance with antioxidant enzyme levels (Gosset et al., 1994; Van Camp et al., 1996; Fadzilla et al., 1997). Recently, while assessing the performance of transgenic arabidopsis plants expressing the rice dehydroascorbate reductase gene Ushimaru et al. (2006) reported that excessive enhancement of the DHAR activity in transgenic plants resulted in decreased tolerance to stress, and that an appropriate quantity of DHAR expression is necessary for improvement of stress tolerance. Thus, it is necessary to draw parallels between the degree of salt tolerance and the levels of antioxidant enzymes in engineered plants so as to ascertain whether or not there is a progressive relationship between the two variables.

As is evident from Ashraf (2009) only a single gene manipulation has been done in most cases, but as a result of this a marked improvement in salt tolerance has been shown. There is a consensus from some researchers that not only one antioxidant enzyme activity needs to be enhanced to counteract oxidative stress, but in fact more than one related enzymes need to be enhanced for better performance. For example, overexpression of SOD is only effective when the other related enzymes such as APX or catalase or both are also overexpressed (Badawi et al., 2004).

It is not surprising to note here once again that the transgenic lines of different plants so produced for enhanced activities of antioxidant enzymes were tested under controlled growth room or greenhouse conditions. Not a single line has been exposed to natural field conditions wherein a number of edaphic and climatic factors have interactive effects on plant growth.

7. Challenges and prospects

Although substantial efforts have been made during the last century to develop salt-tolerant lines/cultivars of various crops using conventional plant breeding methods, there has been limited success in achieving the desired goal. The reasons for the limited success through conventional breeding have been enumerated in a number of already published reviews (Flowers, 2004; Yamaguchi and Blumwald, 2005; Ashraf et al., 2008). With the advent of molecular biology techniques it was presumed that developing stress-tolerant cultivars would be convenient and relatively less time consuming. However, while critically reviewing the transgenic lines of different crops produced so far for different physiological pathways (Tables 1 and 2), it is amply clear that again there has been a limited success in producing salt-tolerant cultivars through genetic transformation. The ‘hyperbolic claims’ (Flowers, 2004) made in the literature in developing transgenic plants with increased stress tolerance are based simply on the performance of transgenic lines produced and tested under controlled conditions of growth room or greenhouse, and one cannot find any conclusive reports except the one listed in Table 1 (wheat transgenic line tested in a field trial by Xue et al., 2004) in the literature reflecting the testing of the performance of transgenic cultivars under natural field conditions.

The main reason for the limited success of producing salt-tolerant cultivars through genetic engineering is that in most cases only a single gene has been transformed, although it is now widely known that salt tolerance trait is multigenic in nature and a multitude of physiological, biochemical and molecular processes are involved in the mechanism of salt tolerance (Zhu, 2002). Thus, the results of a number of studies wherein improvement in salt tolerance has been shown many fold in terms of plant survival, germinability, biomass production or accumulation of a specific metabolite under saline stress, should be used with some caution, unless the lines so produced are tested under natural stressful environments. It is highly likely that the performance of controlled conditions (laboratory or glasshouse) tested transformed lines may differ under natural stress environments due to the fact that the interplay of a number of edaphic and climatic factors may influence overall plant growth and development (Ashraf et al., 2008).

Furthermore, in majority of the studies, salt tolerance of transgenic lines has been appraised using only sodium chloride, although it is now well established that most soils contain significant amounts of salts other than sodium chloride (Buckman and Brady, 1967). For example, in Pakistan depending on the nature of a dominant salt in a soil, salinity is mainly of two types, i.e. chloride salinity and sulfate salinity (Musahtaq and Rafiq, 1977). Thus, performance of a transgenic line evaluated in sodium chloride may not show such performance when tested on a natural salt-affected soil containing a mixture of salts or a predominant salt other than NaCl. In fact, it is evident from a number of earlier studies that the response a crop or a crop cultivar to chloride or sulfate varies to a great extent (Curtin et al., 1993; Rogers et al., 1998; Inal, 2002). Under the situation when salt tolerance of a crop varies with growth stage, there is a need to appraise salt tolerance at each growth stage so as to assess overall salt tolerance of the crop.

While critically looking at all the reports cited in Tables 1 and 2 and many other such in the literature, it is evident that most of transformation experiments have been carried out with the model plants such as arabidopsis and tobacco, and there have been astounding successes in terms of enhanced salt tolerance. There is now a dire need to introduce these salt tolerance genes into crop plants. However, it is not sure what will happen when the genes transferred to the model plants are expressed in cultivated species (Flowers, 2004). Thus, the results regarding the salt tolerance of transgenic arabidopsis or tobacco plants cannot be predictive of crop species. Under such situation the sensible approach is to apply transformation technology directly to a particular crop of interest so as to achieve a meaningful outcome.

Many of the reports cited in the present review or others in the literature show that while breeding or engineering for enhanced salt tolerance the key objective of almost all plant breeders and bioengineers has been to produce crop cultivars/lines which will produce high biomass or seed yield on salt-affected soils. Appraisal of performance of such salt-tolerant cultivars on normal soils (non-saline) is usually ignored, although farmers always desire to choose a cultivar that produces high yield on both normal and salt-affected soils. The vital issue to be addressed is whether the manipulation of a single or of many genes is necessary to modify complex traits like salt tolerance. Supposedly, if a single gene transformation can substantially lead to enhanced salt tolerance, this suggests either that altering the
levels of some fundamental components has an ample effect on a number of other processes or that salt tolerance is not as complex as it is in the real sense (Flowers, 2004).

Another key issue is that many of the stress-related metabolic phenomena are not well understood yet. This is indeed one of the major impediments of achieving highly salt-tolerant plants. Thus, an inclusive knowledge of all metabolic phenomena related directly or indirectly to mechanism of salt tolerance is vital for generating salt-tolerant plants through molecular breeding. After the acquisition of complete knowledge of all stress-related metabolic phenomena and identification of target genes and their expression under salt stress, transfer of multigenes to transgenic plants as advocated by Bohnert and Shen (1999) and Flowers (2004) is now possible. Protocols and expertise are now available by which small fragments of DNA each containing a large number of genes could be transferred to plants (Hamilton et al., 1996; Cherian et al., 2006). However, further advances in genomics and proteomics research would make this multigene transfer strategy more effective and precise in future.

In conclusion, if achievements made to date in developing salt-tolerant plants through conventional breeding or genetic engineering have been relatively successful in improving the yield potential, disease resistance, and abiotic stress tolerance of most crops, but undoubtedly, at the expense of little resources and with little or no knowledge of underlying biochemical pathways of stress tolerance. In contrast, genetic engineering has resulted into relatively little success particularly in terms of enhanced crop stress tolerance despite the fact that a large sums of resources have been utilized in generating transgenic plants. Crop Sci 2006;56:481–95.


