Review Article

Towards salinity tolerance in *Brassica*: an overview

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ABSTRACT

Among the various abiotic stresses limiting the crop productivity, salinity stress is a major problem, which needs to be addressed and answered urgently. Since members of Brassicaceae are important contributor to total oilseed production, there is an immediate need being felt to raise Brassica plants which would be more suitable for saline and dry lands in years to come. One of the suggested way to develop salinity tolerant *Brassica* plants is to make use of the broad gene pool available within the family. Efforts of breeders have been successful in such endeavors to a large extent and several salinity tolerant Brassica genotypes have been developed within India and elsewhere. On the other hand, transgenic technology will undoubtedly continue to aid the search for the cellular mechanisms that confer tolerance, but the complexity of the trait is likely to mean that the road to engineer such tolerance into sensitive species will not be easy. However, with increasing number of reports available for suitable genetic transformation for various *Brassica* genotypes, there is a hope that salinity tolerance can be improved in this important crop plant. In this direction, the complete genome sequence of related wild plants such as *Arabidopsis* or crop plants such as rice can also serve as a platform for identification of "candidate genes". Recently, complete genome sequencing of the *Brassica* genomes has also been initiated with the view that availability of such useful information can pave way towards raising *Brassica* with improved tolerance towards these stresses. In the present paper, we discuss the success obtained so far; in raising brassica genotypes with improved salinity tolerance employing both plant breeding and/or genetic engineering tools. **[Physiol. Mol. Biol. Plants 2008; 14(1&2) : 39-49]** *E-mail : ashwanip@mail.jnu.ac.in*

Key words : *abiotic stress; diploid; amphidiploid; mustard; SOS pathway,*

Abbreviations : *SOS - salt overly sensitive; QTL - Quantitative trait loci; PR -pathogenesis-related; LEA - late embryogenesis abundant*

Plants growing under field conditions are exposed to various environmental factors, which constitute their macro and microenvironment. Any deviation in these factors from the optimum levels is deleterious to plants and leads to stress. Stress may be caused due to abiotic factors like high temperature, cold, drought, salinity, or the biotic factors like viruses, insects, nematodes, bacteria, fungi etc. At a given point of time, plant may have to face even a combination of more than one of above mentioned factor. However, amongst these stresses, salinity has emerged as one of the most serious factors limiting productivity of agricultural crops as well as claiming a substantial farmable area. The loss of farmable land due to salinization is in direct conflict with the burgeoning population posing a major challenge for maintaining world food supplies. Thus, there is a deliberate need to raise varieties that can, not only withstand high levels of salt but can also maintain optimum yield levels. However, efforts to improve crop performance under salinity have been elusive owing to its multigenic and quantitative nature. This has given an impetus to follow a combinatorial approach employing both conventional and non-conventional strategies to improve salt tolerance.

Brassica occupy third place among the various oilseed species due to its considerable economic and nutritional value. However, their growth, yield, and oil production are markedly reduced due to salinity. In particular, seed germination and early seedling growth

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have been reported to be relatively more sensitive towards salinity (Ashraf and McNeilly 2004). There is significant inter and intraspecific variation for salt tolerance within *Brassica,* which needs to be exploited through selection and breeding for enhancing salt tolerance. The present text provides a comprehensive account of the studies carried out to decipher inherent variations among the existing cultivars. In addition, the vital role of plant breeding efforts as well as genetic engineering approaches for the improvement of salinity tolerance in *Brassica* has also been emphasized.

Description of *Brassica*

The genus *Brassica* belongs to the mustard family Brassicaceae, a family, of about 435 genera and 3675 species worldwide, with 42 genera (20 of them exotic) and 198 species (37 of them exotic naturalised species) in southern Africa (Willis, 1973; Dreyer and Jordaan, 2000a, b). *Brassica* contains about 100 species, including cabbage, cauliflower, broccoli, brussels sprouts, turnip, various mustards and weeds (Gomez Campo, 1999). This genus is remarkably known for having more important agricultural and horticultural crops than any other genus. In addition to the cultivated species, which are grown worldwide, many of the wild species grow as weeds, especially in North America, South America and Australia. It includes almost 30 wild species, which either belong to wild taxa or have escaped cultivation.

Various *Brassica* species are popular for their considerable nutritional and economic value. They are mainly grown for oil, condiments, vegetables or fodder. *Brassica* species grown predominantly for commercial purposes are rape seeds (*Brassica campestris* L. and *B. napus* L.), mustards (*B. juncea* L. [Czern. & Coss.] and *B. carinata* A.Br.) (Ashraf and McNeilly, 2004). The species *B. napus* and *B. rapa* account for most of the oilseed production in Europe and North America, whereas *B. carinata* is mainly grown in North Africa. The predominant oilseed *Brassica* in India, Nepal and Bangladesh is *B. juncea*. Members of the species *Brassica oleracea* mainly include vegetable crops like Broccoli, Brussels sprouts, cabbage, cauliflower etc. *Brassica* species such as *B. nigra* (L.) Koch and *B. tournifortii* Gouan are grown on a very small scale. Other than the economically important *Brassica* species, various wild relatives belonging to the genera *Diplotaxis, Erucarstrum, Sinapidendron, Hirschfeldia, Trachystoma, Sinapis, Eruca and Hutera* serve as a repository of vast gene pool (Prakash *et al.*, 1999). Wild relatives also possess a number of useful agronomic traits which could be incorporated into breeding programs, including: cytoplasmic and nuclear male sterility; resistance to disease and insect and nematode pests; intermediate C3-C4 photosynthetic activity; and tolerance for cold, salt and drought conditions (Warwick *et al.*, 2000)

Origin and evolution of crop *Brassica*s

Studies suggest that *Brassica* evolved from the genus *Sinapidendron* of the Miocene age through *Diplotaxis-Erucastrum* complex (Gomez-Campo and Tortosa 1974). Furthermore, the cultivated *Brassica* species evolved along two pathways, with *B. nigra* probably derived from the *Sinapis* stock and *B. rapa* and *B. oleracea* derived from *Diplotaxis erucoides* (Song *et al.,* 1990, Warwick and Black 1991, Pradhan *et al*., 1992). The first *Brassica* species to be domesticated was *B. rapa* because its natural area was near the centre of domestication and extended from Mediterranean region to Central Asia in the ancient times. Two centres for the domestication of *B. rapa* have been recognised (Song *et al*., 1988), one of them being Europe represented by the oleiferous turnip, while another is China, represented by the leafy form *pak choi*. The centre of origin for *B. nigra* overlaps with that for *B. rapa*. Both the wild forms of *B. rapa* and *B. nigra* occur together in the Middle East, therefore, it has been proposed to be the first place of origin of *B. juncea.* Regions of China and India are considered as the secondary centres of origin of *B. juncea* (Song *et al.,* 1988). It has been proposed that *B. oleracea* (parent of *B. napus* and *B. carinata*) entered cultivation much later, Atlantic coast being its natural area, far away from the center of domestication (Gomez-Campo 1999).

The cytogenetic structure of crop *Brassicas*

The relationships among the cultivated *Brassica* species were largely clarified by cytological work of Morinaga (1934). According to his hypothesis, the high chromosome number of species *B. napus* (2n = 38, AACC), *B. juncea* (2n = 36, AABB), and *B. carinata* (2n = 34, BBCC) are amphidiploids combining in pairs the chromosome sets of the low chromosome number species *B. nigra* (2n = 16, BB), *B. oleracea* (2n = 18, CC), and *B. rapa* (2n = 20, AA) (Fig. 1). This hypothesis was verified by U (1935) with successful re-synthesis of *B. napus*. Re-synthesis of *B. juncea* and *B. carinata* was accomplished later by Frandsen (1943; 1947). The phenomenon of interspecific hybridization has led to the evolution of the tetraploid *Brassica* species. Natural hybridization is always unidirectional, as nature does not prefer the allopolyploids generated from reciprocal hybridization because of their low fertility. Therefore, the maternal parent of *B. juncea*, *B. napus* and *B. carinata* are always

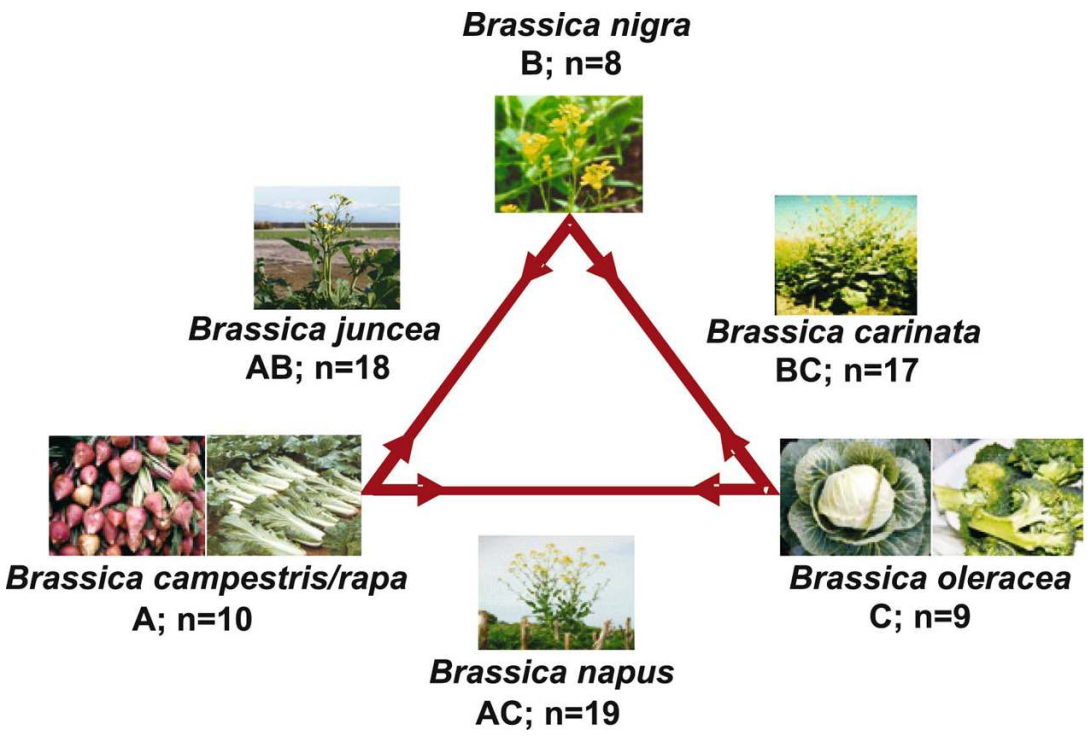

Fig. 1. The U-triangle showing derivation of the high chromosome *Brassica* species from low chromosome species [according to Morinaga (1934) and U (1935)].

B. rapa, *B. oleracea* and *B. nigra,* respectively (Erickson *et al.,* 1983).

Development of salt tolerant *Brassica*

Three basic approaches that are currently being used to obtain stress tolerant *Brassica*, include: (i) screening of preexisting genotypes (ii) conventional breeding for developing salt tolerant lines, and (iii) generation of transgenic plants to introduce novel genes or to alter expression levels of the existing genes to affect the degree of salt stress tolerance.

Screening of preexisting salt-tolerant genotypes of *Brassica*

Salt tolerance is a very complex trait in plant species since it is governed by numerous mechanisms at cellular, tissue, organ, or whole plant levels. Some traits may only be functional at one time in a particular species. In addition, the effect of one mechanism may exclude the effect of the others at certain stages of plant development (Greenway and Munns, 1980; Gorham *et al*., 1991; Ashraf, 1994; Dubey, 1997; Yeo, 1998; Carvajal *et al*., 1999; Makela *et al*., 1999). The cultivated *Brassica* species include both diploid and polyploid species. Most of the *Brassica* species have been classified as moderately salt tolerant. The superiority of the amphidiploid species *B. carinata*,

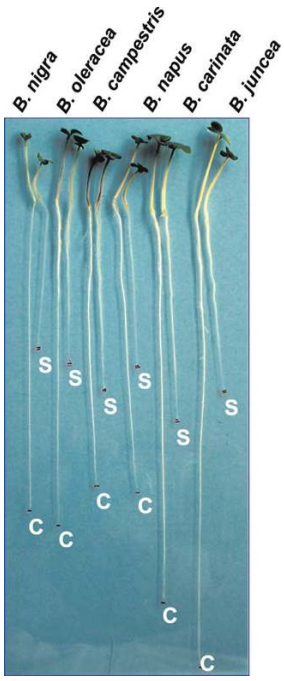

Fig. 2. Interspecific variations in salinity tolerance among various Brassica genotypes which needs to be exploited for raising newer varieties with improved tolerance. Growth response of 4-day-old seedlings of *Brassica* genotypes upon exposure to 200 mM NaCl for 24h which shows decrease in root and shoot length. C: control; S: salinity stress.

B. juncea, and *B. napus* over the diploid species, *B. campestris*, *B. nigra*, and *B. oleracea* is evident from different studies (Malik, 1990; He and Cramer, 1992; Kumar, 1995). This is in agreement with the findings of Stebbins (1966), that the polyploid species can generally withstand adverse environmental factors better than their respective diploid ancestors. It has been further suggested that the salt tolerance of amphidiploids has been acquired from the A (*B. campestris)* and C (*B. oleracea* L.) genomes (Ashraf *et al.,* 2001). In our investigation while screening for salinity tolerance, we observed a significant inter- and intra-specific variation within the Brassica genera. The differences included effects of salinity on overall growth (Fig. 2), electrolyte leakage, proline accumulation and the K^+/Na^+ ratio. In response to salinity stress, endogenous Na^+ concentration increased in the various Brassica genotypes whereas K^+ concentration decreased (Fig. 3). However, striking variation was observed between the various genotypes w.r.t. to this physiology. Our results match with the previous reports in that amphidiploid species are relatively more tolerant than the diploid species (Ashraf and McNeilly, 2004). Thus, there is considerable interspecific and intraspecific variation for salt tolerance within brassicas which needs to be exploited through screening and then identification of contrasting genotypes which can serve as promising new varieties or as superior genetic backgrounds for further improvement to raise tolerant varieties.

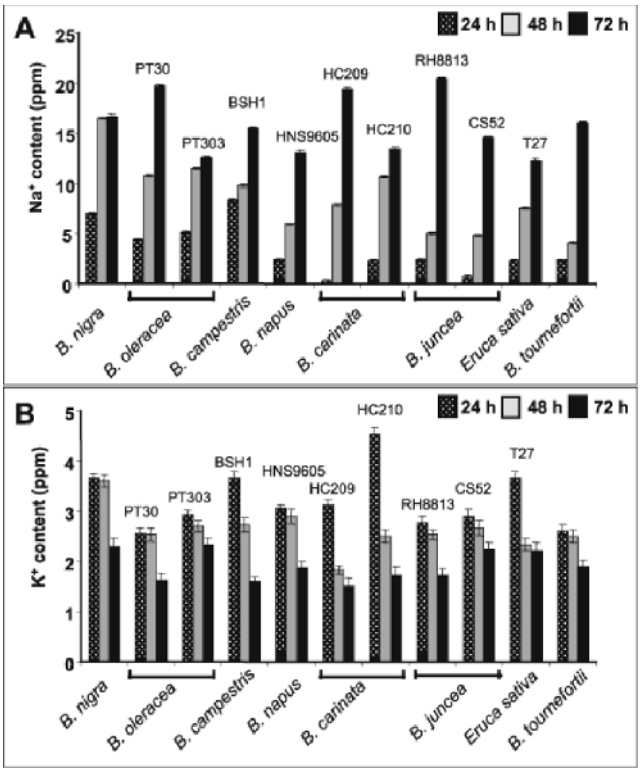

Fig. 3. Various genotypes of Brassica have variable capacity to maintain ion homeostasis under salinity stress. Graph showing endogenous Na^+ and K^+ concentration (ppm) of 4-day-old seedlings of 11 genotypes of *Brassica* treated with 200 mM NaCl for 24, 48 and 72 h. Concentration of Na^+ enhanced (A) and K^+ decreased (B) with duration in all the genotypes with increasing NaCl treatment duration.

Conventional breeding approaches for enhancing salt tolerance in *Brassica*

Breeding for salt tolerance in crop plants is envisaged as one way to combat the worldwide problem of increasing soil salinity in agricultural land. Stresses under adverse soil conditions are highly complex and often compounded with climatic hazards. The stress varies from location to location and even from season to season. Soil stresses are often associated with nutritional imbalance (deficiency/toxicity). The interaction between soil stresses and other environmental factors influence the plant's response to that stress. Such complexities are responsible for the slow adaptability of high yielding crop varieties in adverse edaphic environments. It is, therefore, necessary that crop genotypes must be screened at target sites having adequate stresses in order to identify dependable sources of varietal tolerance. Breeding crop varieties for increased salt tolerance is considered as a promising, energy-efficient and economical approach than major engineering processes and soil amelioration techniques which have gone beyond the limits of marginal farmers (Ashraf and McNeilly, 2004). For a successful breeding program, presence of a great magnitude of heritable variation in the gene pool of a crop is a prerequisite; such genepools are necessary to provide the variability needed. Genetic diversity provides parental material from well-adapted landraces to enhance local adaptation. It helps to overcome susceptibilities to problem soil and also provides the foundation for breeding for novel requirements. If the genetic variability has been totally utilized through continued selection, then variability may be sought through other means such as chemical and radiation mutagens, protoplast fusion, or recombinant DNA techniques.

Conventional Breeding approaches have generated several salt tolerant varieties of *Brassica* (Table 1). In India, Central Soil Salinity Research Institute, Karnal, has been instrumental in developing several promising salt tolerant varieties of *Brassica juncea* which includes CS54, CS52, CS416, CSTR 330-1, CSTR 600-B-10, CSTR 610-10-1-1, and CS12. *B. juncea* var. CS52 has been recommended for cultivation in saline lands having EC

upto 7 to 8 dsm-1 and pH 9.2 – 9.3, average yield 1.1 t ha-1 (www.plantstress.com/files/salt karnal.htm). *B. juncea* var. CS54 has been recently developed which is a high yielding and salinity tolerant variety. Other salt tolerant varieties include Kranti, Varuna and Pusa Bold. Among the other oilseed crops which show promising results under saline soil are *Brassica campestris* var. toria, *Eruca sativa*, *Carthamus tinctorius, Halianthus annus*. Comparative performance of various Brassica varieties which includes Peela raya, SPS-23-1, SPS-23-2, ORI-56-6, P-8-2, RL-18, and brown raya under salinity condition has shown P-8-2 to be most tolerant varieties (Sadiq *et al.*, 2002). Breeding now takes place from a much broader genetic base (greater number of varieties) in many crops. Genetic improvements can be easily adopted by resource-poor farmers for such problem soil environments where there are low-input conditions.

The development of molecular markers for physiological traits has made significant headway in recent years with the advancement of new technologies. Consequently, the use of molecular markers in breeding programs is increasing rapidly as they have been shown to greatly improve the efficiency of the breeding programs. Rapid progress in molecular marker technology over the past few years has led to the development of detailed molecular linkage maps for many plant species (Jain and Selvaraj, 1997). The most important development in this regard is the discovery of DNA-based marker technology referred to as Marker Assisted

Table 1. Salinity tolerant cultivars and lines of Brassica species developed through breeding

Brassica species	Cultivars/lines	Parameter for testing tolerance	References
B. napus	Dunkeld (canola)	Biomass and seed yield	Qasim (2000)
	ST9194	Germination	Puppala *et al.* (1999)
	Rapora, Mytnitskii, Chisayanatane	Seed yield	Pokrovskii (1990)
B. juncea	Common Green	Vegetative stage	Kwon *et al.* (1997)
	Varuna	Germination	Rai (1977)
		Seed yield	Kumar and Malik (1983), Kumar (1984)
	TH 68	Germination	Singh *et al.* (1984)
	RH 30	Seed yield	Dhawan *et al.* (1987), Kumar (1984)
	Pusa Bold, Kranti	Seed yield	Kumar (1995)
	CS4, CS15	Seed yield	Uma *et al.* (1992)
	Pant Rai 2030	Seed yield	Sinha (1991)
	PR 1002	Seed yield	Kumar (1984)
	RH 7818	Seed yield	Dhawan *et al.* (1987)
	DIRA 337	Seed yield	Sinha (1991)
	BM-1, LL-84	Biomass and seed yield	Ashraf (1992)
	P-15, KS-51	Biomass and seed yield	Ashraf *et al.* (1994)
	CS54, CS52, CS416, CSTR 330-1, CSTR 600-B-10, CSTR 610-10-1-1, CS12, Varuna	Biomass and seed yield Germination, Vegetative stage, Biomass and seed yield	www.plantstress.com/files/saltkarnal.htm
B. carinata	C90-1191, P5/80, Yellow Dodella	Germination and seedling growth	Ashraf and Sharif (1997)
	C90-1115, 77-321	Seed yield	Ashraf and Sharif (1998)
B. campestris	BSH1	Germination	Paliwal (1972)
		Seed yield	Kumar (1984)

Selection (MAS). Marker Assisted Selection is non-destructive and can provide information on the genotype of a single plant without exposing the plant to the stress. The technology is capable of handling large numbers of samples. With this technique DNA markers that flank a gene of interest can be easily identified, and any segment of DNA can be used as a marker. Being rapid and economical, this technique provides a powerful tool to accelerate breeding programs for enhancing plant stress tolerance. Salinity being a quantitative character is governed by polygenes. Genes which condition the expression of quantitative characters are referred to as quantitative trait loci (QTL) (Tanksley *et al*., 1996; Foolad *et al*., 2001). The approach of QTL mapping has become crucial to the use of DNA markers in the improvement of crops species (Ramchiary *e al.,* 2007). Several different types of DNA markers such as RFLP, RAPD, AFLP, and SSR are now in vogue in plant breeding, by which molecular maps for different crops can be made (Vos *et al*., 1995; Nguyen *et al*., 1997; Hopkins *et al*., 1999; Saranga *et al*., 2001; Quesada *et al*., 2002; Pradhan *et al.,* 2003). It is hoped that this knowledge generated via molecular maps may as well be extended to target improvement of stress tolerance in plants. For instance, using molecular maps, measurement of plant physiological parameters can be integrated with study of yield and quality, and the specific components of stress tolerance that account for genetic variation in modern crop gene pools can be highlighted. In addition, with the resulting DNA markers, stress tolerance genes in breeding programs can be efficiently manipulated in combination with other genes controlling many other attributes that are necessary to produce an elite cultivar (Foolad and Chen, 1999). The detection of QTLs for stress tolerance also represent an important means towards cloning of stress tolerance genes, an achievement that would be very helpful for use in the analysis of the underlying physiological and biochemical mechanisms.

Why genetic modification?

Although, in past, conventional breeding has been a potent source for generating varieties with high yield and quality, very few salt-tolerant cultivars and lines of potential crops are known that have been developed using the conventional strategy. The little success of modern breeding approaches towards the improvement of salt tolerance in crop species is thought to be attributable to the quantitative nature of most of the processes involved, i.e., the salt-tolerance trait is controlled by polygenes (Ashraf 2002; Quesada, 2002). Moreover, the approach is time-consuming and labor-intensive simultaneously allowing the transfer of undesirable genes along with desirable ones. Furthermore, reproductive barriers limit transfer of favorable alleles from interspecific and intergeneric sources. Under such circumstances, genetic engineering has proven to be a powerful means of transferring genes effectively across reproductive barriers. An approach to engineer stress-tolerant crop plant would be to transfer the gene(s) from those organisms adapted to stressful environment to crop plants (Zhang and Blumwald, 2001; Apse and Blumwald, 2002). In the past two decades, forward and reverse genetic approaches have provided great insight into studying abiotic stress tolerance at the molecular level (Zhu 2001a, 2001b, 2002).

Genetic engineering approaches for enhancing salt tolerance in *Brassica*

The work on genetic engineering of tolerance to abiotic stresses began piece meal within a decade of the molecular understanding of pathways induced in response to one or more of the abiotic stresses. In most of the cases the transgenes expressed faithfully but only a limited level of tolerance was provided under stress conditions as compared to the non-transformed wild type plants. The level of many compatible osmolytes responsible for osmotic adjustment was too low to be effective *per se* in providing the required water retention and osmotic adjustment. The use of multiple tolerance mechanisms for one or more of the abiotic stresses through stepwise or co-transformation may help to achieve high levels of tolerance for commercial exploitation.

Understanding the molecular mechanism for providing protection against biotic and abiotic stresses may lead to a generalized master mechanism for stress tolerance. Optimum homeostasis is always a key to living organisms for adjusted environments. Thus, abiotic stress accompanying a number of biological phenomena must be precisely investigated by consideration of plant homeostasis. Bohnert and Jensen (1996), suggested that successful releases of tolerant crops will require large-scale "metabolic engineering" which must include the transfer of many genes. While such an approach was not feasible in the early 1990s (Flowers and Yeo, 1996), this approach is now being widely advocated. It is conceivable that approaches that identify specific genes that are up- regulated or down-regulated either through the analysis of RNA (Kawasaki *et al*., 2001) or proteins (Salekdeh *et al*., 2002) might provide a specific focus for transformation.

Plants can use three strategies for the maintenance of a low cytosolic sodium concentration: sodium exclusion,

compartmentation, and secretion. One of mechanism for sodium transport out of the cell is through operation of plasma membrane-bound Na^+/H^+ antiports, as confirmed by the characterization of *SOS*1, a putative plasma membrane Na^+/H^+ antiport from *Arabidopsis thaliana* (Shi *et al.*, 2000). The efficient compartmentation of sodium is likewise accomplished through operation of vacuolar Na^+/H^+ antiports that move potentially harmful ions from cytosol into large, internally acidic, tonoplast-bound vacuoles. This is accomplished by combined activity of several genes. One such gene, Salt overly sensitive genes (*SOS*) are responsible for maintenance of intracellular Na^+ and K^+ homeostasis in *A. thaliana*. The kinase activity of SOS2 protein is essential for salt tolerance (Liu *et al.,* 2000). Abiotic stresses induce Ca^{+2} signals (Sanders *et al.,* 1999; Knight 2000) that are perceived by SOS3. The SOS3 has been found to interact physically with SOS2 (Guo *et al.,* 2001). The activated SOS2 kinase phosphorylates the SOS1, which is responsible for pumping out Na^+ out of the cytoplasm (Shi *et al.,* 2000). The SOS3-SOS2 complex also regulates the transcript level of *SOS1* and other genes (Fig. 4). Genetic analysis of *A. thaliana* has shown that SOS3, SOS2 and SOS1 are involved in a common pathway of salt tolerance (Zhu *et al.,* 1998).

Salt secretion is considered as an adaptive strategy to regulate plant tissue ion concentration (Liphschitz and Waisel, 1982). Salt tolerance is not exclusively associated with cellular Na^+ homeostasis, but also involves adaptations to secondary effects of salinity such as oxidative damage and changes in the levels and composition of fatty acids of the major glycerolipids in roots and leaves of a wide range of plants. Changes in the level of fatty acid saturation/unsaturation have been reported as a response to salt stress, and a reduction in the levels of triacylglycerols containing unsaturated fatty acids has been reported in seed oil from cotton under salt stress.

Recently studies of salinity tolerance in plants have ranged from genetic mapping to molecular characterization of salt induced genes. Increasing understanding of biochemical pathways and mechanisms that participate in plant stress responses has made it evident that many of these responses are common protective mechanisms that can be activated by salt, drought and cold, even if sometimes through different signaling pathways. Currently, transgenic plants have been used to test the effect of overexpression of specific prokaryotic or plant genes, known to be up-regulated by salinity stress. Attempts have been made to raise transgenic *Brassica* with candidate gene approach only,

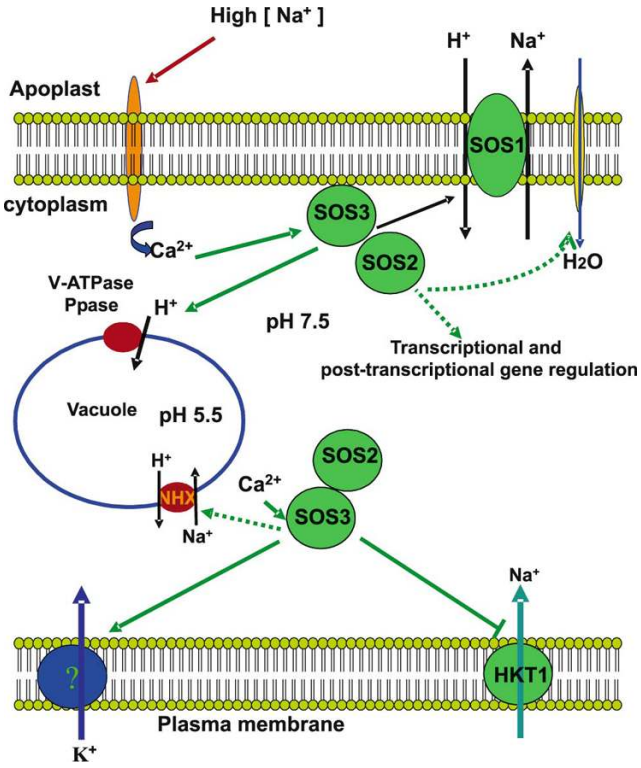

Fig. 4. Cartoon depicting the ion homeostasis in plant cell under salt stress as mediated via SOS pathway. SOS3 is a Ca^{+2} sensor, SOS2 is a kinase, SOS1 and NHX are Na^+/H^+ antiporter at plasma membrane and vacuolar membrane respectively. HKT1 is Na^+ transporter (modified from Zhu, 2002).

making use of the genes having proven role in ion homeostasis, osmolytes accumulation etc., to make them more tolerant to salinity stress (Table 2). Details of these reports are presented in the following text.

Engineering glycinebetaine pathway

Glycine betaine 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). It is one of the so-called 'compatible solutes' reported to be involved in osmotic adjustment and protecting the cells against osmotic stress. It is synthesized in the chloroplasts from choline via betaine aldehyde, through a short pathway involving two enzymes, choline monooxygenase and betaine aldehyde dehydrogenase, respectively. However, in bacteria, biosynthesis of glycine betaine occurs via a two-step oxidative reaction. Choline dehydrogenase first catalyzes the conversion of choline into the intermediate glycine betaine aldehyde, with subsequent oxidization by betaine

Table 2. Salinity tolerant transgenic Brassica developed through genetic modification

Species	Genes	Encoding protein	Phenotype	Reference
B. napus	*codA*	Choline oxidase	Moderate salinity tolerance and enhanced shoot growth	Huang, *et al*., 2000
	AtNHX1	Vacuolar Na^+/H^+ antiporter	Salt tolerance, growth, seed yield and seed oil quality	Zhang, *et al*., 2001
	PR10	Pathogenesis related (PR)	Enhances germination and growth in the presence of NaCl	Srivastava *et al*., 2004
B. juncea	*codA*	Choline oxidase	Tolerance to salinity stress, enhanced growth	Prasad *et al*., 2000
	PgNHX1	Vacuolar Na^+/H^+ antiporter	Tolerance to salinity stress, exhibited normal growth	Rajagopal *et al.*, 2007
B. oleracea	*betA*	Betaine aldehyde	Salinity tolerance	Bhattacharya, *et al*., 2004
B. campestris	*Lea*	Group 3 Late embryo-genesis abundant	Salinity and drought tolerance	Park *et al*., 2005

aldehyde dehydrogenase into the osmoprotectant glycine betaine (Landfald and Strom, 1986). Introduction of *codA* gene from bacteria for glycinebetaine into *B. napus* and *B. juncea* significantly enhanced their salt tolerance (Huang, *et al*., 2000; Prasad *et al*., 2000). The seeds of transgenic lines showed enhanced capacity to germinate under salt stress as compared to that of the wild type. The seedlings of transgenic plants that expressed *codA* gene also showed significantly higher growth than that of the wild type under salt stress conditions.

High tolerance to NaCl was also obtained transferring bacterial *betA* gene to cabbage (*Brassica oleracea* var. capitata) cultivar 'Golden Acre' through *Agrobacterium*-mediated transformation of hypocotyl explants (Bhattacharya, *et al.,* 2004). The transformants exhibited higher tolerance to NaCl stress compared to untransformed parent plants. Assessment of salinity tolerance of transgenic plants through physiological parameters revealed better growth response and greater stability in maintaining plant water relations at increasing levels of salinity.

Engineering ion transpoters

The overexpression of *AtNHX1,* a vacuolar Na^+/H^+ antiporter from *A. thaliana*, in *Arabidopsis* and tomato plants allowed the transgenic plants to grow in 200 mM NaCl, suggesting the possibility of engineering crop plants with improved salt tolerance. Zhang *et al*., (2001) transformed *Brassica napus* cv Westar with construct containing the *AtNHX* gene, coding for a vacuolar Na^+/H^+ antiporter from *Arabidopsis thaliana*. Overexpression of the vacuolar Na^+/H^+ antiporter 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 100 mM NaCl. While growth of wild-type plants was severely affected by the presence of 200 mM NaCl in the growth solution, transgenic plants were able to grow, flower, and produce seeds. Recently, *NHX1* from *Pennisetum glaucum* has been reported to confer high level of salinity tolerance when overexpressed in *B. juncea*. Transgenic *B. juncea* plants over expressing *PgNHX1* withstand 300mM salt stress till the seed setting stage and exhibited normal growth phenotype without much loss of seed yield (Rajagopal *et al.*, 2007). Taken together these reports establish an important role played by the single gene i.e., NHX in controlling ion homeostasis under salinity stress in plants.

Engineering pathogenesis related protein

Pathogenesis-related (PR)-10 family are induced in many plants by phytopathogens and environmental stresses (Liu and Ekramoddoullah, 2004). Genes encoding the class ten pathogenesis-related (PR10) proteins have been cloned and characterized in many angiosperm species and a few gymnosperm species. Constitutive expression of pathogenesis related gene, PR10, from pea, in

Brassica napus enhances germination and growth in the presence of NaCl (Srivastava *et. al*. 2004). The role of PR10 protein has been indicated in protecting the seedlings from salinity stress.

Engineering late embryogenesis abundant protein

Transformation of Chinese cabbage (*Brassica campestris* ssp. *pekinensis*) by over expressing a *B. napus* Group 3 *LEA* gene enhanced tolerance to salinity and drought (Park *et. al*. 2005). The plants were transformed with *Agrobacterium* strain LBA4404 containing the binary vector pIG121-LEA, which carried LEA protein gene linked to CaMV promoter and terminator sequences, and the neomycin phosphotransferase II *(NPT*II) gene as selectable marker. Transgenic Chinese cabbage plants demonstrated enhanced growth ability under salt and drought stress conditions. The increased tolerance was reflected by delayed development of damage symptoms caused by stress. The increased tolerance also showed improved recovery upon the removal of stress condition.

With the availability of several transgenic Brassica plants over-expressing one or other salinity related gene, it is hoped that transgenic plants with better adaptability towards salinity are not far away. Of course, the support from plant breeding is also required in further improving these genotypes for other desirable traits. More importantly, the suitability of these genetically engineered crops for public consumption and acceptance also needs to be addressed before these crops really make their way to the end user.

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