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RESEARCH ARTICLE
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Na^+/H^+ ANTIPORTER OVEREXPRESSION FOR DEVELOPING SALT TOLERANCE IN PLANTS

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ABSTRACT
Na^+/H^+ antiporter play a major role in pH and Na^+ homeostasis of cells throughout the biological kingdom, including bacteria, algae, fungi, worms, higher plants, and mammals, including humans. Plant vacuolar Na^+/H^+ antiporter have been shown to play important roles in cellular ion homeostasis, including the sequestration of Na^+ ions into the vacuole, and vacuolar pH regulation. Over-expression of *At*NHX1 and homologues from other plants has been shown to confer significant salt tolerance in a variety of plant species as a result of increased vacuolar sequestration of sodium ions. One possible mechanism by which plants could survive salt stress is to compartmentalize sodium ions away from the cytosol. Both the literature and public databases contain the data of many plant antiporters and their over-expression studies in model and crop plants improved salt tolerance in plants. The current review projects on transgenic plants with salt tolerance reported so far with over-expression of Na^+/H^+ antiporter under constitutive and inducible promoters and it sheds light on various antiporters which developed resistance to salt. Phylogenetic studies of various NHX1 revealed their close relativeness among all the antiporters reported so far. From the current review, scientists can pick up closely related families for overexpression of antiporter genes to generate transgenic plants resistance to salt for improving yield of crop and ornamental plants as well.

Keywords: Antiporter, NHX1, Transgenic Plants, Salt Tolerance, Abiotic Stress.

Introduction

The current global population has reached to 6.786 billion by the end of September 2009 and is expected to reach approximately 9.0 billion by 2025 (US Census 2009). Nearly 1.2 billion people live in a state of absolute poverty and about 800 million people suffer with food insecurity. Low productivity in agriculture is a major cause of poverty, food insecurity and poor nutrition in low-income developing countries, where agriculture is the driving force for broad based economic growth and poverty alleviation. Thus, to meet the increasing needs of the growing human population, it will be necessary to produce 50% more food by 2025. Crop varieties with higher yield and stability are required. During the last few decades, major progress has been made in increasing crop productivity worldwide. Biotechnology has succeeded and will continue to play a major role in the improvement of crop plants against various environmental stresses and offer considerable potentials to the whole world.

Environmental stresses are of two main types; biotic stresses including infection or competition by other organisms due to bacteria, fungi, insects, etc., and abiotic stresses due to light (High intensity and low intensity), Temperature (High, Low-Chilling and Freezing), Water (Deficit-Drought; Excess-Flooding), Radiation (IR, Visible, UV, Ionizing (X-ray and γ-ray) and Chemicals (Salts, ions, gases, herbicides, heavy metals) and Mechanical Factors (Wind and Pressure). When plants face stress conditions because of biotic or abiotic stress they make a balanced system between rate of damage and rate of repair. As plants are sessile organisms, several genes are switched on once they are under stress and tries to overcome the rate of damage, no damage becomes apparent. But once the level of stress increases, the balance tips gradually towards damage. When the rate of repair is slower than the rate of damage, damage becomes apparent. This is probably the situation in plants under stress (Chen and Murata 2002).

Responses of plants to environmental stress:

Use of existing gene pool to produce transgenic plants conferring resistance to abiotic stress conditions:

One important feature distinguishing plants from other complex multicellular organisms is that plants are sessile and thus have to endure environmental challenges such as soil salinity, drought and cold temperatures. Although salt, water and cold stresses are clearly different from each other in their physical nature and each elicits specific plant responses. They also activate some common reactions in plants. The most widely studied common response is the induction of some plant genes by all three stresses (Shinozaki and Shinaozaki, 1997). Plant responses to these stresses involve nearly every aspect of plant physiology and metabolism. Consequently, there exists a complex signaling network underlying plant adaptation to these adverse environmental conditions. (Zhu, 2001).

Abiotic stress in crop plants can be produced through traditional breeding methods or applying modern genetic engineering methods. However, success in breeding for tolerance has been limited because (a) tolerance to stress is controlled by many genes and their simultaneous selection is difficult; (b) tremendous effort is required to eliminate undesirable genes that are also incorporated during breeding and (c) there is a lack of efficient selection procedure particularly under field conditions (Tialhun *et al.*, 2003). Genetic engineering (GE) offers an alternative approach for developing saline and drought tolerant crops. Unlike classical breeding, GE is a faster and more precise means of technique in achieving improved tolerance (Cushman and Bohnert, 2000) because it avoids the transfer of unwanted chromosomal regions. Moreover through GE, multiple genes can be assembled and simultaneously introduced to the crop of interest (Tialhun *etal*., 2003). To work with GE for salt tolerance in plants there are many functional targets, which are summarized in following Figure 1. The Group I (functional proteins*) genes which express during salt/drought or cold conditions are as follows:

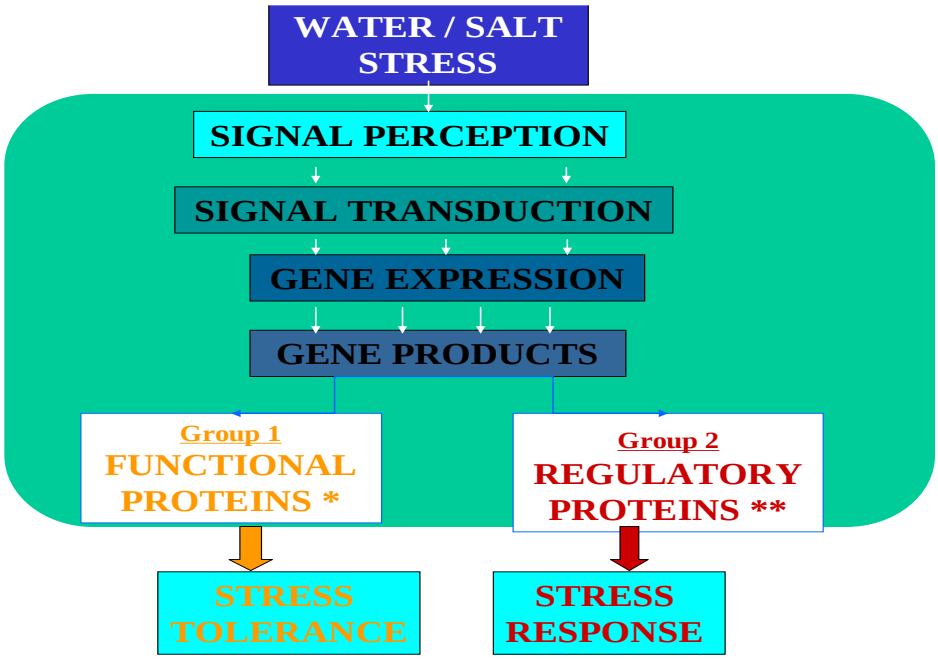

Figure 1. Effect of Water and Salt stress in plants in turn its signal perception with expression of different functional proteins and regulatory proteins

1. Water channel proteins (Aquaporins)- involve in movement of water through membranes
2. Enzymes require for biosynthesis of various osmoprotectants (sugars, proline, glycine-betaine, sorbitol etc.,)
3. Proteins that protect macromolecules and membranes (LEA, osmotin, dehydrins, antifreeze proteins, chaperons, mRNA binding proteins).
4. Proteases for protein turnover (thiol protease, Clp protease, ubiquitin, etc.,)
5. Detoxification enzymes (SOD, Glutathion-S-transferase, soluble epoxide hydroxylase, catalase, ascorbate peroxidase and
6. Transport proteins (Na^+/H^+ transporter)

The Group II genes (regulatory proteins**) function is stress responsive and regulation of signal transduction and gene expression. The products of Group II genes are as follows:

1. Protein Kinases (MAPK, MAPKK, MAPKKK, CDPK etc.,)
2. Transcription factors (DREB1A, DREB1B etc.,)
3. Phospholipase C (PIP turnover)
4. Phosphatases (Calcineurins)
5. Zinc finger motifs (HS1) which are involved in transcriptional activity of many salt responding genes

It is one choice to select any of the genes from Group I or Group II for transferring into glycophytes to obtain salt/drought and cold tolerance through genetic engineering methods. Transformation of plants with the genes involved in the transcriptional control is best choice as it regulates more than one gene which in turn produce transgenic plants more resistant to abiotic stress conditions (Mamidala 2006).

Soil salinity and its stress to plants

Soil salinity is one of the major agriculture problems throughout the world. It is a serious limiting factor in plant growth in general, and in crop productivity in particular. The inhibition of plant growth by salinity tends to be caused by ionic toxicity, disturbance of mineral nutrients uptake coupled with water deficit. More or less, soil contains water-soluble salts. Plants absorb essential nutrients in the form of these salts to grow and develop. However, excessive amount of salt in the soil adversely suppresses plant growth. Soil salinity has now become a serious agricultural problem throughout the

world. Agricultural productivity is severely affected by soil salinity, and the damaging effects of salt accumulation in agricultural soils have influenced ancient and modern civilizations. It is estimated that salinity affects around 20% of world's arable land and more than 40% of irrigated land to various degrees (Rhoades and Loveday, 1990). In extreme cases, productive cultivated land could no longer sustain agricultural production and had to be abandoned. In coastal areas periodic invasions of seawater directly add salts to the soil. In arid or semi-arid regions, ineffective drainage and poor irrigation water mainly lead to soil salinity.

Salinity causes both toxicity and osmotic problem. Most plants have little or no salt tolerance. Traditionally, plants are classified as glycophytes or halophytes based on their capacity to grow on high salt medium (Flowers et al., 1977). Halophytes are tolerant to high concentrations of NaCl, some of which can even withstand salts that are more than twice the concentrations of seawater. Most plants, including the majority of crop species, are glycophytes and salinity is a major stress to them that dramatically limits their growth and productivity. Processes such as seed germination, seedling growth and vigor, vegetable growth, flowering and fruit set are badly affected by high salt concentration, ultimately causing diminished yield or even death.

The chief causes of damage by salinity are: osmotic stress and dehydration; ionic toxicity; secondary stresses such as nutritional disorders and oxidative stress.

Firstly, high salt concentration decreases the osmotic potential of soil solution and thus creates a water stress in plants. As salt concentration in the soil increases, water becomes increasingly difficult for plants to absorb. A plant may actually die from water stress in very moist soil if the salt concentration reaches high enough. The low osmotic potential of saline solutions hampers plant water uptake, making plants "physiological drought".

Meanwhile, ion toxicity due to the excess of NaCl occurs. The accumulation of both Na^+ and Cl^- has injurious effects on plant cells. Sodium toxicity represents the major ionic stress associated with high salinity. Some plant species are also sensitive to chloride. In certain saline soil, alkaline pH may worsen the ion toxicity.

Furthermore, the interaction of salts with mineral nutrition may result in nutrient imbalances and deficiencies. As well, some studies implicated that salt stress could generate reactive oxygen species in plants (Hong et al. 2000), and cause genotoxicity such as DNA damage (Albinsky et al. 1999). The consequence of all these may ultimately bring on plant death as a result of growth arrest and molecular damage (Sairam and Tyagi 2004).

Mechanisms of plant response to salt stress

Plants respond to saline environment in a variety of ways and the mechanisms for plants to avoid salt toxicity are really complicated. Salt tolerance is connected with a lot of characteristics and processes, including morphological, physiological, biochemical, genetical, molecular effects and their interactions. In general, there are three major ways for halophytes to deal with to superfluous salts: salt exclusion, salt secretion and salt compartmentalization.

Some plants, for instance, can absorb water in saline surroundings because of their advanced roots. Their endodermis also restricts transport from root and absorbs ions from xylem flow. Some halophytes have salt glands which can actively secrete salt from the leaves. For these two manners, there exist limitations and little progress of research has been made. Scientists are now most interested in the compatible solutes, key proteins and their genes in salt stress.

Under osmotic stress, an important consideration is to accumulate osmotically active compounds called osmolytes in order to lower the osmotic potential. They are referred to as compatible metabolites because they do not apparently interfere with the normal cellular metabolism. Molecules like glycerol and sucrose were discovered by empirical methods to protect biological macromolecules against the damaging effects of salinity. Later, a systematic examination of the molecules, which accumulate in halophytes and halo-tolerant organisms, led to the identification of a variety of molecules also able to provide protection. Proline and Glycine-betaine are two of the most concerned osmolytes. Characteristically, these molecules are not highly charged, but are polar, highly soluble and have a larger hydration shell. Such molecules will be preferentially solubilized in the bulk water

of the cell where they could interact directly with the macromolecules. The biochemical pathways producing them are now better known. Genes rate-limiting these steps have been cloned and transferred into crop plants to raise the level of osmolytes.

While accumulation of compatible solutes contributes to maintenance of cell growth under conditions of increased ion concentration, many organisms have also developed efficient methods to keep the ion concentration in the cytoplasm at low levels. Membranes, their integral and associated components necessary for the uptake and distribution of ions and solutes, are considered determinants in developing stress-resistant plants. Carriers, channels, symporters and antiporters are mainly concerned with the cellular transport phenomenon in plants. Plant cells need to maintain high K^+ levels, 100-200 mM to maintain normal metabolic reactions. Na^+ levels on the other hand should be less than 1 mM in cytoplasm, and any excess has to be excluded out of the cell or sequestered in the vacuolar compartment.

Sodium transport out of the cell can be accomplished by the operation of plasma membrane-bound Na^+-H^+ antiports. Biochemical evidence for the operation of plasma membrane Na^+-H^+ antiports and the characterization of SOS1, a putative plasma membrane Na^+-H^+ antiport from *Arabidopsis thaliana* (Shi et al. 2003), have been reported. SOS1 gene expression is up-regulated by salt stress. This up-regulation is consistent with the role of SOS1 in salt tolerance. It has been known that NaCl stress also up-regulates the expression of genes encoding plasma membrane H^+-ATPases (Niu *et al.* 1993). Increased H^+-ATPase expression would provide a greater proton motive force that is necessary for elevated Na^+-H^+ antiport activity.

Na^+-H^+ antiports play an important role in pH and ion homeostasis of cells throughout the biological kingdom. At the plant vacuolar membrane, these antiports seem essential for the process of salt tolerance. This kind of protein utilizes the energy available in the electrochemical gradient of protons to regulate the steady state ion distribution between the cytosol and the endomembranal compartments. Therefore, it can prevent the sodium ions from salt from harming the cell and create a balance of ions in the cell that draws water into the plant cell by osmosis. From *Arabidopsis thaliana*, scientists have already cloned and characterized a family of Na^+-H^+ antiports and their encoded gene sequences. Many plants respond to high salt levels by sequestering ions within the vacuole. This process is mediated by a vacuolar Na^+/H^+ antiporter that uses the proton-motif force to concentrate ions against a gradient. By increasing the ion concentration in vacuole, the vacuolar Na^+/H^+ antiporters also function in osmotic homeostasis (Zhu, 2001). Na^+ compartmentation is an economical means of preventing Na^+ toxicity in the cytosol. The compartmentalization of Na^+ (and Cl^-) into the vacuole allows the plants to use NaCl as an osmoticum, maintaining an osmotic potential that drives water into the cells.

Ion Homeostasis

Salt stress disrupts plant ion homeostasis, resulting in excess toxic Na^+ in the cytoplasm and a deficiency of essential ions such as K^+. When salinity results from an excess of NaCl, which is by far the most common type of salt stress, the increased intracellular concentration of Na^+ and Cl^- ions is deleterious to cellular systems (Zhang et al. 2001). In addition, the homeostasis of not only Na^+ and Cl^-, but also K^+ and Ca^{+2} ions is disturbed (Zhang and Blumwald 2001). This disruption of homeostasis occurs at both the cellular and the whole plant levels. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest and even death.

Plant survival and growth under salt stress will depend on adaptations that re-establish ionic homeostasis, thereby reducing the duration of cellular exposure to ionic imbalance. To protect actively growing and metabolizing cells, plants regulate ion movement into tissues (Flowers et al. 1977). Various ion transporters function to limit Na^+ entry into and exit out of plant cells, to regulate Na^+ compartmentation in the vacuole, and to selectively import K^+ over Na^+ into plant cells. Many of

the salt stress induced genes function in ionic homeostasis; these include e.g., plasma membrane Na^+/H^+ antiporters for Na^+ extrusion, vacuolar Na^+/H^+ antiporters for Na^+ compartmentation in the vacuole and high-affinity K^+ transporters for K^+ acquisition.

Transport mechanisms can also actively move ions across the tonoplast into the vacuole, removing the potentially harmful ions from the cytosol. These ions, in turn, act as an osmoticum within the vacuole, which then maintain water flow into the cell (Glenn et al. 1999). Scientists have engineered a single endogenous gene (AtNHX1) encoding a Na^+-H^+ antiport protein (Apse et al.1999) The presence of large, acidic-inside, tonoplast-bound vacuoles in plant cells allows the efficient compartmentation of sodium into the vacuole, through the operation of vacuolar Na^+-H^+ antiports. This Na^+-H^+ antiport transports Na^+ into the vacuole by using the electrochemical gradient of protons generated by the vacuolar H^+-translocating enzymes, H^+-adenosine triphosphatase (ATPase) and H^+-inorganic pyrophosphatase.

Engineering of salt-tolerant plants

Understanding the mechanisms of plant salt tolerance will lead to effective means to breed or engineer salt tolerant crops. To achieve salt-tolerance, the foremost task is either to prevent or alleviate the damage, or to re-establish homeostatic conditions in the new stressful environment. The growth rate must resume, albeit at a reduced rate. Hybridization between salt-tolerant species was also a traditional method for breeding. However, barring a few exceptions, the conventional breeding techniques have been unsuccessful.

The biotechnological development of new salt-tolerant crop plants is largely predicted on the ability of the products of overexpressed salt-stress genes to protect otherwise sensitive plants. As transformation technology evolves, the range of plants amenable to genetic manipulation continues to expand. Genetic engineering opened the door to the genuine success in the salt-tolerant crops.

One strategy for improving the salt tolerance of a plant is to increase the production of small osmolytes or stress proteins that protect or reduce damage caused by salt stress. This strategy was pioneered by the transgenic tobacco plants overexpressing the bacterial mtlD gene. They finally produced mannitol and had enhanced salt tolerance (Tarczynski et al.1993). Since then, a number of osmolytes such as ononitol, proline, glycine-betaine, trehalose, ectoine, and fructan have been engineered in transgenic plants to improve salt tolerance or water-stress tolerance. In another successful collaboration, a Group 3 LEA protein from barley, HVA1 was overexpressed in rice. The transgenic rice seedlings and mature plants had improved resistance to salinity and drought. The experiments of these reports on improving salt tolerance were through the strategy of controlling damage instead of helping plants re-establish homeostasis under stress. They only reached limited extent of salt tolerance and were hard to succeed in crop plants.

Salt response is multigenic trait. One school of thought concluded that salt tolerance would be achieved only after pyramiding several characteristics in a single genotype, whereas each one alone could not confer a significant increase in salt tolerance (Yeo et al. 1988; Cuartero et al. 1999). This viewpoint had been held for a long time until it was challenged by a kind of transgenic tomato overexpressing vacuolar Na^+-H^+ antiports. These tomatoes were able to grow, flower and produce fruit in the presence of 200 mM NaCl (Zhang et al. 2001). Although the leaves accumulated high sodium concentrations, the tomato fruit displayed very low sodium content. Contrary to the notion that multiple traits introduced by breeding into crop plants are needed to obtain salt-tolerant plants, the modification of a single trait significantly improved the salinity tolerance of a crop plant. Very soon after that, transgenic *Brassica* plants that could grow, flower and produce seeds in 200 mM NaCl condition were also cultivated (Zhang et al. 2001).

Information on salt-responsive proteins and genes is crucial for improving salt-tolerance through genetic engineering techniques. But it is only the first step. Further studies using molecular genetics and protein biochemistry techniques are required to identify the specific structural elements of the protein responsible for the stress-mitigating activity. Physiological studies to follow the genetic engineering also are necessary to understand the mechanisms that mediate salt resistance in the

transgenic plants. Finally, bench-top results, however encouraging, do not ensure a marketable product. Once characterized in the laboratory, its ability to promote salt tolerance must be tested in the field.

Soil salinity is one of the major agriculture problems throughout the world. It is a serious limiting factor in plant growth in general, and in crop productivity in particular. The inhibition of plant growth by salinity tends to be caused by ionic toxicity, disturbance of mineral nutrients uptake coupled with water deficit. Many scientists have long been engaged in the research of salt-tolerance mechanisms and the engineering of salt- tolerant plants.

Na^+-H^+ antiports play an important role in pH and ion homeostasis of cells throughout the biological kingdom and closely related across the plant kingdom (Figure 2). At the plant vacuolar membrane, these antiports seem essential for the process of salt tolerance. This kind of protein utilizes the energy available in the electrochemical gradient of protons to regulate the steady state ion distribution between the cytosol and the endomembranal compartments. Therefore, it can prevent the sodium ions from salt from harming the cell and create a balance of ions in the cell that draws water into the plant cell by osmosis. From *Arabidopsis thaliana*, scientists have already cloned and characterized a family of Na^+-H^+ antiports and their encoded gene sequences.

Transgenic techniques have proved feasible for introducing foreign genes into plants to improve their salt tolerance. AtNHX1 codes for a vacuolar Na^+-H^+ antiport from *Arabidopsis thaliana*, the overexpression of which in *Arabidopsis*, tomato and *Brassica* plant allowed the transgenic plants to grow in 200mM NaCl. So far, in spite of these successful examples, there is no evidence of the role of AtNHX1 in stomatal conductance and drought tolerance in plants transformed with the Na^+-H^+ antiport genes.

Table 1 lists out the various crop plants engineered with antiporter gene. Apse etal (1999) first reported the importance of AtNHX1 overexpression in *A.thaliana* followed by overexpression of its homologues in many crop and model plants. As salinity stress is a continuing and increasingly deleterious obstacle to the growth and yield of crop plants, owing to irrigation practices and increasing demands on fresh water supply, the engineering of salt tolerant crop plants has been a long-held and intensively sought objective (Apse and Blumwald, 2002). Since salt tolerance is a multigenic trait, the acquisition of a salt tolerant crop may depend on the transfer of several genes (Bohnert *et al.,* 2001). Identification of genes associated with an increase in salt tolerance would be a first step in engineering halotolerance. The transfer of individual genes provides a valuable tool to achieve some improvement in salt tolerance, and to identify key genes actually involved on the process. In a different approach, the identification of genes in model organisms whose expression is functionally related to salt tolerance by genetic analysis could be of great value for transformation of crop plants.

Acknowledgements

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Table 1: An overview of transgenic plants conferring resistance to salt tolerance due to over expression of antiporter NHX1:

S.No	Gene/Source and Promoter used	Trait improved in host transgenic plants under salt stress	Reference
1	AtNHX1/*A thaliana* SCP	Transgenic *A thaliana* survived at 200mM NaCl	Apse *et al.,* 1999
2	AtNHX1/*A thaliana* SCP	Transgenic *tomato* survived at 200mM NaCl	Zhang *et al.*, 2001
3	AtNHX1/*A thaliana* SCP	Transgenic *Brassica napus* survived at 200mM NaCl	Zhang *et al.*, 2001

4	AgNHX1/*A gmelini* CaMV35S	Rice with Eight-fold higher activity of the vacuolar-type Na+/H+ antiporter	Ohta *et al.,* 2002
5	AtNHX1/*A thaliana* CaMV35S	Transgenic rice survived at 200mM NaCl	Fukuda *et al.,* 2004
6	AtNHX1/*A thaliana* CaMV35S	Transgenic Wheat has shown improved germination rate, biomass production, grain yield, and leaf K^+ accumulation, and reduced leaf Na^+ and about 68% increase in shoot dry weight and 26% in root dry weight was observed	Xue *et al.,* 2004
7	BnNHX1/*B napus* CaMV35S	Transgenic Tobacco plants could grow, flower and produce seeds normally under the treatment of 200 mM NaCl	Wang *et al.,* 2004
8	AtNHX1/*A thaliana* CaMV35S	Transgenic *Maize* survived at 200mM NaCl and shown efficient seedling growth	Yin *et al.,* 2004
9	AtNHX1/*A thaliana* CaMV35S	Transgenic *Beet root* tolerated additional 171–256 mM NaCl	Yang *et al.,* 2005
10	HbNHX1/*H brevisubulatum* SCP	Transgenic tobacco showed Improved growth, leaf Na+ and K+ contents, Na+/K+ ratio and proline contents. Dry weight of transgenic seedlings increased by 1.8-fold	Lu *et al.* 2005
11	OsNHX1/*O sativa* CaMV35S	Transgenic rye grass plants survived at 350 mM NaCl but all wild type plants died	Wu *et al.,* 2005
12	AtNHX1/*A thaliana* CaMV35S	Transgenic Tall fescue has survived at 200mM NaCl	Tian *et al.,* 2006
13	SsNHX1/*S salsa* CaMV35S	Transgenic rice survived at 300mM NaCl	Zhao *et al.,* 2006
14	AtNHX1/*A thaliana* CaMV35S	Transgenic *Beta vulgaris* survived at 200mM NaCl	Yang *et al.,* 2005
15	GmNHX1/*G max* CaMV35S	Transgenic Lotus survived at 200mM NaCl	Sun *et al*., 2006
16	AtNHX1/*A thaliana* CaMV35S	Transgenic Tomato survived at 250mM NaCl	Praveen 2006
17	*PgNHX1/P glaucum* ABRP/MARS	Transgenic Rice flowered and produced seeds at 150mM NaCl	Verma *et al.,* 2007
18	TaNHX1/*T aestivum* P2 X 35S	Transgenic *A.thaliana* plants survived at 200mM NaCl	Brini *et al*., 2007
19	AeNHX1/*A elongatum* CaMV35S	Transgenic Arabidopsis and Festuca plants survived at 200mM NaCl	Qiao *et al* 2007
20	SsNHX1/*S salsa* CaMV35S	Transgenic *A thaliana* survived at 200mM NaCl and seedlings could	Li *et al.,* 2007

		resume and keep normal growth after treatment at 78^0C for 5 h	
21	PgNHX1/*P glaucum* CaMV35S	Transgenic *B. juncea* plants over expressing PgNHX1survive, set flowers and normal seeds in presence of up to 300 Mm NaCl. The transgenic plants accumulated more Na+ in the leaves than in seeds.	Rajagopal *et al.,* 2007
22	OsNHX1/*O sativa* CaMV35S	Transgenic rice plants displayed a little accelerated growth during seedling stage but showed delayed flowering time and a slight growth retardation phenotype during late vegetative stage, suggesting that the OsNHX1 has a novel function in plant development.	Chen *et al* 2007
23	AtNHX1/*A thaliana* CaMV35S	Transgenic cotton has shown enhancement of photosynthesis and contributed to better growth under salt conditions in addition to their improvement such as proline accumulation to protect cells against NaCl stress.	He *et al.,* 2007
24	AtNHX1/*A thaliana* CaMV35S	Transgenic buckwheat plants were able to grow, flower and accumulate more rooting in the presence of 200 mM sodium chloride. Moreover, the content of important nutrients was not affected by the high salinity of the soil	Chen *et al.,* 2008
25	AtNHX1/*A thaliana* CaMV35S	Transgenic tobacco survived at 300mM NaCl	Soliman *et al.,* 2008
26	AtNHX1/*A thaliana* CaMV35S	Transgenic potato survived at 200mM NaCl	Zhang *et al.,* 2007
27	SeNHX1/*S.europaea* CaMV35S	Transgenic tobacco survived at 200mM NaCl	Zhou *et al*., 2008
28	AtNHX1/*A thaliana* CaMV35S	Transgenic *A meliltoides* survived at 200mM NaCl and shown reduced cell membrane damage under salinity	Zhao etal., 2008
28	AtNHX1/*A thaliana* CaMV35S	Transgenic *F arundinacea* has shown resistant to salt at 200mM NaCl	Tian *et al*., 2008
29	AtNHX1/*A thaliana* beta/ *E.coli* CaMV35S	Transgenic tobacco harboring both beta and AtNHX1 exhibited higher percentage of seed germination, better seedling growth and higher fresh weight than lines that had betA or AtNHX1 alone.	Duan *et al*., 2009

30	AtNHX1/*A thaliana* CaMV35S	Transgenic *Petunia hybrida* has shown enhanced drought and salt tolerance.	Xu *et al.*, 2009
31	MsNHX1/*M sativus* CaMV35S	Transgenic lines of Arabidopsis suggested that MsNHX1 might function in promoting the osmotic adjustment capacity and alleviating the oxidation of membranes under salt stress	An *et al*., 2008

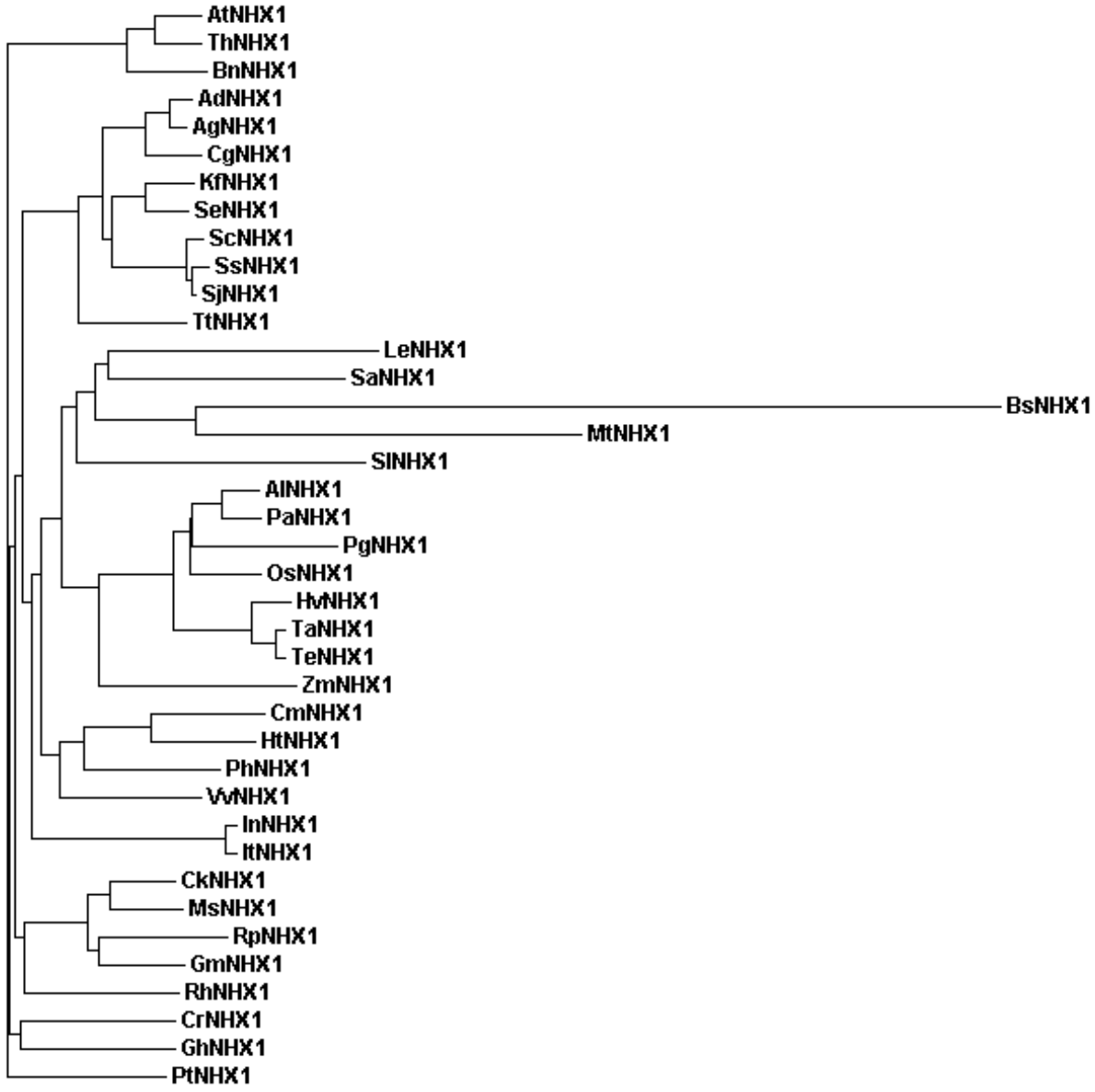

Figure 2: Phylogenetic Tree of Na+/H+ antiporter protein sequences. Software packages, ClustalX and JalView, were used to do multiple sequence alignment and generate phylogenetic trees. The accession numbers of Na+/H+ antiporters are: AF510074, AY211397, AB038492, AAQ07963.1, AAV80466, AAO38856.1, CAJ26377, AY371319, AY607026, EF396235, DQ812099, AF515632, AAR27596, EF159151, AAS17948, BAB16381, AB054979, AY825250, DQ304690, CAC84522, AY513732, ABO84538, OsNHX1 AB021878, AY660749, AB051817, AAY90136, AB211145,

EF675631, AB199912, DQ512716, AY131235, AY261806, AAX45242, AB198178), AY261806, AF527625, AY040246, ABJ97691, AAR19752, CAN61480, AY270036

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