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Review Article

THE ARABIDOPSIS NHX1 GENE: OVEREXPRESSION AND ITS IMPACT ON SALT TOLERANCE IN TRANSGENIC PLANTS

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Abstract: *Abiotic stresses, such as salinity, negatively impact crop growth, development and overall production. The discovery and operational confirmation of new genes will provide the framework for successful genetic engineering techniques to increase crop plants' resistance to salinity stress. The most effective method for maintaining ionic balance in plants under stress due to saltiness is provided by the membrane and vacuolar Na+/H+ counter transporter. The function of AtNHX1, the initially identified vacuolar antiporter from more advanced crops, was extensively researched. In this article, we will review the possible function of the Arabidopsis NHX1 gene that is responsible for salt tolerance and its transformation into Wheat, Barley, Poplar, Fescue, Petunia hybrid, Alfalfa & Soybean. Reason to engineer higher plants' genomes using Arabidopsis NHX1 genes to provide food sustainably in salinity-affected locations.*

Keywords: Abiotic Stress, Salinity, Transgenic Plant, Atnhx1 Antiporter, Gene, Overexpression, Salt Tolerance, Alfalfa, Wheat, Barley, Fescue, Petunia, Soybean, and Ionic Balance

Introduction

The continuing rise in the human population is driving up demand for more products produced by plants. (Li et al.,2010). Globally, abiotic stresses—particularly salinity and shortage of water—are the main reasons for the decline in crop production (Xu *et al*., 2009). One of the main factors that lowers plant productivity is salt stress. (Tester and Davenport, 2003). The soil is saline due to an excess of salts, which is dangerous for both environmental health and agricultural productivity. Salinity in the soil is a widespread issue that affects more than 100 countries and one billion hectares.10% of the land is made up of 950 million hectares of saline soils. An estimated 230 million hectares, or 50%, of land that is watered, are affected by salt (Ruan *et al*., 2010).

Plants experience ion imbalance, hyperosmotic stress, and consequent secondary stresses as a consequence of increased salt concentrations and water deficits (Xu *et al.*, 2009). Plants have evolved a range of adaptive systems, such as ionic balance, osmotic correction, purification, and control of development, to withstand the effects of salt and drought (Bohnert *et al*., 1995; Zhu, 2001). Various investigations have indicated that the combination of saline and alkaline stressors leads to more severe osmotic adjustment capacity loss, trophic ion imbalance, antioxidant system inhibition, and severe suppression of plant development (Amirinejad *et al*., 2017; Chen *et al*., 2017; Wang *et al*., 2017; Wang *et al*., 2020).

The primary effect of salt toxicity is cytoplasmic damage to plant cells that is exclusive to Na+ (Tester and Davenport, 2003). Na+/H+ antiporter activity is generally a major factor in plant salt tolerance. Exchangers or counter transporters are present in eukaryotes and microbes or prokaryotes. Antiporters were discovered that are situated within the outer boundary of plasma (Orlowski and Grinstein, 1997) as well as in several cellular components, such as the multivesicular bodies (*ScNHX1* from Saccharomyces cerevisiae; Nass & Rao, 1998) and tonoplast (*AtNHX1* from Arabidopsis thaliana; Apse *et al*., 1999).

Plants that can withstand salt maintain the ion balance or equilibrium in the cells of plants by implementing effective mechanisms to stop an excessive buildup of Na+ in the cytosol. Among these methods, include limiting the intake of ambient Na+ and boosting the outflow of Na+ outside of the cellular compartment.

Conventional breeding methods such as (interspecies, distant, and intergeneric hybridization) are employed to enhance genetic variation in crop species and their landraces to produce superior high-yielding cultivars but these methods have not yielded much success. (Moghaieb et al., 2014).

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Transgenic technology is a biotechnological tool used to develop tolerant crops that can survive in unfavorable environmental conditions (Dhankher and Foyer, 2018). Before implementing transgenic crops, it is imperative to identify putative candidate genes that function as critical regulators of several metabolic pathways, such as osmolyte production and ion homeostasis via selective ion absorption (Ahanger *et al*., 2017).

Transgenic plants are those plants whose DNA has been manipulated through genetic engineering. The goal is to infuse the plant with a novel trait not typically present in the species. Transgenic plants carry a set of genes that have been intentionally introduced. Transgene—a gene sequence that has been inserted — may come from another kind of plant or a completely distinct plant/organism. A plant's ability to achieve efficiency and usefulness is intended to be maximized by inserting a variety of genes into it. This method has improved quality, higher yield, and ability to withstand biotic and abiotic stress conditions (Rani and Usha, 2013).

Different genes from the NHX gene family are used to modify crops to withstand more salinity. NHX family of K+, Na+/H+ antiporters are a member of the CPA superfamily, which also contains the CPA1 and CPA2 subgroups and supports pathways for salt stress tolerance (Caldentey, 2019). The role of *AtNHX1*, the first vacuolar antiporter identified from more developed crops, was thoroughly investigated. *AtNHX1* shares elevated levels of similarities in sequences with both the yeast *NHX1* and the mammalian NHX Na+/H+ exchangers (Counillon and Pouyssegur, 2000; Nass *et al*.,1997). Transcript accumulation in leaves following salt treatment is the main way that the Arabidopsis *NHX1* RNA sequence is detected in plant underground parts as well as shoots (Gaxiola *et al*., 1999; Quintero *et al*., 2000). Salt resistance is significantly enhanced by overexpressing the Na+/H+ antiporter, which transports and accumulates salts in the vacuole as in Fig. 1 (Apse *et al*., 1999).

Additionally, under control conditions, transgenic crop plants showing overexpression of a vacuolar Na+/H+ exchanger were able to improve tolerance toward saltiness in rice (Otha *et al*., 2002), cotton (He *et al*., 2005), tomato (Zhang and Blumwald, 2001), and Brassica napus (Zhang *et al*., 2001). Epitope-tagging of heterologously produced *NHX1* revealed that this antiporter contains three "submerged" regions that partially cover the membrane in addition to nine TM domains.

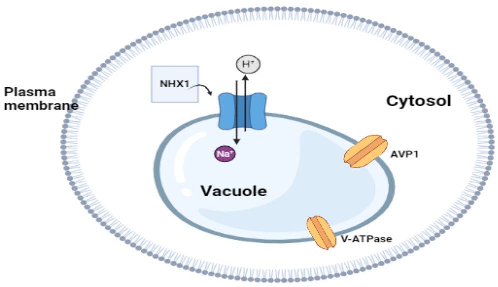

Fig. 1: Diagram illustrating the movement of Na+/H+ across the plasma membrane and tonoplast. *NHX1* allows extra Na+ can be secreted from the vacuole. AVP1 and V-ATPase sustain the proton gradient throughout the tonoplast.

Results

The interaction that occurred between the Arabidopsis *NHX1* the C-end and (AtCaM15) in the interior of the vacuole was dependent on both pH and Ca2+. AtCaM15 is coupled to the *AtNHX1* under typical biological conditions, where the vacuole is acidic (pH 5.5) and Calcium activity is strong. This leads to a larger K+/H+ activity than Na+/H+ activity. AtCaM15 binding to *AtNHX1* was decreased at higher pH values (6.0–7.5), and the Na+/H+ action was greater than the K+/H+ action (Bassil & Blumwald, 2014).

In this article, we will review about *NHX* gene family and the potential involvement of *AtNHX1*, a prospective gene, in increasing tolerance against salinity in a variety of crops, with a focus on Wheat, Barley, Poplar, Fescue, Petunia hybrid, Alfalfa & Soybean.

Genetically engineered plants expressing the Arabidopsis thaliana *NHX1* gene have improved salt tolerance.

AtNHX1 increases wheat plants' ability to withstand salt.

(Moghaieb *et al*., 2014) Revealed that transgenic wheat lines were able to sustain rapid development under salt stress conditions (about 350 millimolar Salt), whereas the untreated plant was unable to do so. The Agrobacterium tumefacien strain LBA4404 which carries the binary vector pBI-121 containing the At-*NHX1* gene was utilized to create transgenic wheat plants that are salt tolerant. To create a transgenic line that would serve as a control, pBI-121—which carries the genes for β-glucuronidase and not II—was employed. Wheat plants that expressed *AtNHX1* grew more rapidly in high-salinity environments when compared to wild-type controls. For instance, at 300 mM, the plants of the natural variety showed stunted growth. However, by enhancing Na+ build-up and maintaining K+/Na+ balance, gene expression improved salt tolerance. The findings demonstrated that expression of *AtNHX1* improved resistance to salt by maintaining K+/Na+ balance and promoting Na+ accumulation.

AtNHX1 improves salt tolerance in Populus.

Poplar (genus Populus) shows efficient transformability, low size of genome (550 Mb), rapid development, simple growth, and high genetic diversity that made them appealing choices as exemplary methods for researching plant physiology (Bradshaw *et al*., 2000; Taylor, 2002).

Jiang *et al*., 2011, transferred the vacuolar Na+/H+ antiporter gene *AtNHX1* from Arabidopsis thaliana into Populus via Agrobacterium tumefaciens, to increase poplar salinity tolerance). The pBISN1 vector was employed; it has a plant intron that interrupted GUS and a kanamycin resistance gene (nptII) that is controlled by the nopaline synthase (NOS) promoter (Aocs)3AmasPmas.The outcomes verify that poplar trees with overexpression of *AtNHX1* have salt resistance. The transgenic poplar's enhanced salt resistance could be defined via the overexpression of the *AtNHX1* gene, which increases the ability to maintain K+ balance and sequester Na+ into vacuoles. This suggests that the *AtNHX1* gene could be a good choice for creating more plants with salinity tolerance.

AtNHX1 improves salt tolerance in fescue.

Tall fescue, also known as Festuca arundinacea, is a popular perpetual winter grass that is utilized extensively for turf & fodder (Sleper & West, 1996). Self-incompatibility, and hexaploid (2n ¼ 6x ¼ 42) genome of tall fescue permote outcrossing makes traditional breeding challenging (Barnes RF, 1990; Spangenberg G *et al*., 1998).

Zhao *et al*., 2007 reported that tall fescue progenies that have undergone transformation exhibit increased salt tolerance when the *AtNHX1* gene is overexpressed. Agrobacterium tumefaciens strain AGL1 was used to transform embryo-derived calli from four genetic varieties of tall fescue (F. arundinacea). This strain carries the expression vector pROK2U, which contains the (nptII) gene and the ubiquitin promoter. There were no phenotypic alterations or yield reductions in these genetically modified lineages. In contrast to the plants under management, plants with the *AtNHX1* gene exhibited greater resistance to a 200 mM NaCl solution. Because tonoplast vesicles have stronger Na+ /H+ antiporter activity than controls, the transgenic lines' roots showed higher sodium contents and elevated Na+/H+ antiporter activity in vesicles of tonoplasts. The findings unambiguously demonstrated that the buildup of sodium in root cell vacuoles, which is facilitated by vacuolar Na+ /H+ antiporters, lessened tall fescue's sensitivity to the harmful effects of salinity and increased its tolerance to salt.

AtNHX1 improves salt tolerance in Petunia hybrid.

Petunia hybrid is a significant virescence and decorative shrub. A handful of research has been conducted on the use of genome engineering to increase the resistance against salinity & dehydration of Petunia hybrid.

(Xu *et al*., 2009) generated transgenic Petunia hybrida with enhanced salt tolerance via expressing the *AtNHX1* gene. The binary Ti vector pCAMBIA1301-35SN was used for Agrobacterium tumefaciens transformation. Hygromycin phosphotransferase (HYG) gene resisting hygromycin B as an identifiable marker is present in the binary Ti vector pCAMBIA1301-35SN, which was created by incorporating the cauliflower mosaic virus (CaMV) 35S regulator and nopaline synthase terminator sequence. Compared to Normal Petunia plants, the transgene plants' foliage tissue contained increased ratios of Na+, K+, and the amino acid proline Additionally, the plants with transgenic genes maintained higher water levels and a higher K+/Na+ ratio. These findings showed that the transgene Petunia plants were able to withstand dryness and salt owing to the upregulation of the vacuolar sodium /hydrogen+ exchanger. According to this study, inducing *AtNHX1* may have a significant impact on a plant's ability to withstand dryness in addition to its involvement in resistance to salt. Expressing *AtNHX1* excessively may have a significant impact on a plant's ability to withstand drought in addition to its involvement in salt tolerance.

AtNHX1 improves resistance against salinity in Soybean

The vegetable soybean, or Glycine max (L.) Merrill is a legume vegetable with a distinct flavor and taste that is strong in proteins, minerals, and vitamins. It is native to China, especially the southern Yangtze River region. Nonetheless, the majority of vegetable soybean plants encounter numerous biotic and abiotic challenges (Chen *et al*., 2011).

(Li *et al*., 2009) produced over six generations of GMO soybeans that can withstand salt. The kanamycin resistance gene (nptII) and the *AtNHX1* gene, which is regulated by the cauliflower mosaic virus 35S promoter were introduced into the Agrobacterium tumefaciens strain LBA4404 by transformation. For more than six generations, the salt resistance and reliability of *AtNHX1* expression were assessed in the soybean mutants. For more than six generations, the transgenic soybean with *AtNHX1* expression showed enhanced resistance to salt, not altering the fatty acid composition. This indicates that NHX-like antiporters have a significant deal of promise for use in building salt-tolerant oleaginous crops. Transgenic soybean plants accumulated a high concentration of Na after being treated with a salt concentration of 300 mmol/L NaCl. This finding implies that, in situations of stress due to salt, Arabidopsis*NHX1* is involved in controlling Na+ and other ion balances.

AtNHX1 improves salt tolerance in Alfalfa.

(Stritzler *et al*., 2018 established Alfalfa-*AtNHX1* with a low nitrogen concentration and a strong salinity tolerance. Devised a highly effective method for employing the binary vector pPZP200BAR to quickly and affordably produce transgenic alfalfa libraries and change the highly regenerative alfalfa clone C23.This is the first account of the development of high-quality bean fodder in saline environments. Transgenic plants (*AtNHX1*) expressing *AtNHX1* that were injected with 1021 exhibited a 38% increase in biomass accumulation. The results showed that salt-tolerant transgenic plants combined with a normally stress-resistant bacterium that fixes nitrogen may be considered an efficient method for producing excellent feedstock at the right amount in salinized environments.

AtNHX1 improves salt tolerance in Barley.

(Adem *et al*., 2015) introduced Arabidopsis *AtNHX1* gene encoding vacuolar Na+/H+ Exchanger in barley. Through Agrobacterium-mediated transformation, modified barley (cv. Golden Promise) expressing *AtNHX1* utilizing the CaMV 35S promoter was effectively produced. For the transformation, the pMDC32 expression vector was utilized. It is not beneficial to just express Arabidopsis *AtNHX1* in barley to improve overall plant performance under salinized environments. The findings demonstrated that there is no benefit to Comparative hydration levels and enhanced exchange of gas from expressing *AtNHX1* in barley. Moreover, there is no increase in chlorophyll content from expressing *AtNHX1* in barley. Plants that express *AtNHX1* exhibited high-shoot Na1 and K1 build-up. Barley plants expressing *AtNHX1* showed a build-up of potassium and sodium in their leaves.

Table 1: Transgenic plants developed with Salt Tolerance

Technique of transformation	Explants	Transgene	Crop	Results	Reference
Agrobacterium tumefaciens (LBA4404)	Seed	*AtNHX1*	Wheat	Under salt exposure (350 mM NaCl), the modified wheat lines were able to sustain growth.	Moghaieb *et al*., 2014

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Agrobacterium tumefaciens n LBA 4404	Seedlings	*AtNHX1*	Poplar	Recombinant poplar displayed greater tolerance to NaCl than the wild-type (WT) variety.	Jiang *et al.*, 2011
Agrobacterium tumefaciens (AGL1)	Embryo-derived calli	*AtNHX1*	Fescue	Plants with the *AtNHX1* gene exhibited greater tolerance to a 200 mM NaCl solution.	Junsheng Zhao *et al.*, 2007
Agrobacterium tumefaciens (LBA4404)	Leaves	*AtNHX1*	Petunia hybrid	Transgenic petunia hybrid plants demonstrated greater tolerance to severe levels of sodium chloride and drought.	Xu *et al.*, 2009
Agrobacterium tumefaciens (LBA4404)	Cotyledonary node	*AtNHX1*	Soybean cv. Liaodou	In modified soybeans, *AtNHX1* expression improved salt tolerance without affecting the amount of fatty acids present.	Li *et al.*, 2009
Agrobacterium tumefaciens	Cotelydons	*AtNHX1*	Alfalfa	When coupled with strain B401, *AtNHX1* exhibited high nitrogen fixation rates under saline conditions.	Stritzler *et al.*, 2018
Agrobacterium tumefaciens	Seeds	*AtNHX1*	Golden Promise barley (Horduem vulgare L.)	Null segregants have no difference, indicating that there is no advantage of incorporating the *AtNHX1* gene in barley.	Adem *et al.*, 2015

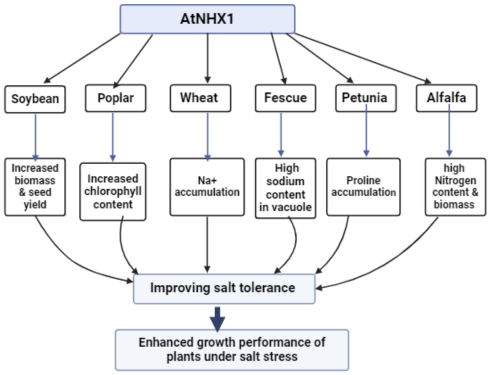

Fig. 2. An illustration of the pathways behind salt tolerance in transgenic plants that express *AtNHX*

Conclusion and Future Prospects

Plant genetic engineering has recently placed a lot of emphasis on the expression of vacuolar Na+/H+ exchanger genes (*NHX*) to create crops that can withstand salt, in particular, the creation of transgenic plants.

Arabidopsis *NHX1* transgenes incorporated into other crops such as (Wheat, Barley, Soybean, Poplar, Fescue, Petunia hybrid, and Alfalfa) are shown to have the potential to mitigate salinity (Table 1). The transgenic plants mentioned above demonstrated normal growth performance under salt stress conditions, with no flaws, due to their enhanced tolerance to salinity stress. It has demonstrated a successful strategy for increasing the output of vegetable and fodder legume crops in salinity-prone locations. Transgenic plants expressing *AtNHX1* showed considerable improvements in ion homeostasis, ROS scavenging ability, antioxidant enzyme activities, and osmolyte production with unhindered photosynthetic machinery (Fig. 2). These findings suggest that this gene regulates plant defense mechanisms under stressful conditions.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department concerned.

Consent for publication

Approved

Funding

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Conflict of interest

The authors declared the absence of a conflict of interest.

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