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REVIEW ARTICLE

Strategies of NHX antiporters to deal with salt stress

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Abstract

The adverse effects of salinity on plant growth are generally associated with the low osmotic potential of the soil solution and the high level of sodium toxicity (and chlorine toxicity for some species) which cause multiple perturbations on plant metabolism, growth, and development at the molecular, biochemical and physiological levels. The vacuolar NHX and plasma membrane SOS antiporters mediate cation and proton exchange across the tonoplast and plasma membrane, respectively. The SOS transporters allow the excretion of Na^+ from the cytoplasm to the outside environment and alternatively, NHXs provide Na+ transport from the cytoplasm to the vacuole. Cellular ion homeostasis is an essential phenomenon for all organisms. Most cells manage to maintain a high level of potassium and a low level of sodium in the cytoplasm through the coordination and regulation of different transporters and channels instead of the NHX-type vacuolar antiport. In this article, some important mechanisms in the regulation of ionic ions such as $Na^{+.}$ will be discussed.

Keywords

NHX; salt stress; homeostasis; antiport; tolerance; SOS

Introduction

Salinity stress generally affects plant habitats such as large areas of plant crops of different species of fruit trees designed for national or international production in the world, mainly vegetable crops or horticulture. However, according to the FAO report, more than 6% of the world's land is affected by salinity or sodicity, representing over 800million hectares of land worldwide (1). More than 350 M ha of land is recently affected by salinization, of which more than 50 M ha is in Africa (2). As a result, many species have developed different coping mechanisms. Salt tolerance mechanisms can be classified into three main categories. The first is osmotic stress tolerance, regulated by long-range signals that reduce shoot growth and involve the biosynthesis and accumulation of compatible solutes to maintain water uptake (3). The accumulation of sugars appears to induce the gelling of cellular content by saturating the intracellular environment; this phenomenon, preventing the crystallization of molecules within the cell, limits damage at the level of cellular structures. Stress metabolites, proline, and soluble sugars are naturally accumulated in the leaves of several species (4). At the cellular level, their accumulation in the cytosol is accompanied by a decrease in the concentration of less compatible solutes (salts) and an increase in the cytosol water volume, ensuring the maintenance of osmotic balance (5). Furthermore, soluble sugars are strongly accumulated in vacuoles under

saline stress. A strong correlation has been established between sugar accumulation and the level of salinity tolerance(6). The accumulation of this amino acid, proline, may play a role in cell osmoregulation and osmotolerance during water deficit and serve as an indicator of drought and/or a stress detector(7). Generally, in transgenic plants, it has been demonstrated that the accumulation of mannitol, glycine-betaine, and proline improves their tolerance to saline stress (8).

The second mechanism is ionic exclusion, in which Na^+ transporters reduce the accumulation of toxic Na^+ in roots and leaves. Finally, in plants, an important mechanism for overcoming salt stress is the exclusion of Na^+ from the cytoplasm through the action of Na^+/H^+ antiporters at the plasma membrane or intracellular membranes (9).

This system works by controlling the loading of Na^+ into the xylem and the recovery of Na^+ from the xylem before reaching the photosynthetic tissues in the shoot (10). The early and fundamental events of plant adaptation to salt stress will start with some perceptual and signaling mechanisms via signal and messenger transduction to activate the various physiological and metabolic responses, including the expression of stress response genes. Among the main pathways involved in salt stress signaling are calcium, abscisic acid (ABA), salt overly sensitive (SOS), and also NHX/NHE, which are membrane proteins that mediate cation and proton exchange across the tonoplast to maintain pH regulation and osmoregulation, tolerance, and K^+(11, 12).

Methodology

The search criteria in this article review were centered on cellular transporters, specifically vacuolar, cellular, and endosomal antiporters, primarily in salt-resistant plants. Firstly, we concentrated our efforts on data and results from articles dating back to the eighties, exploring the plant domain's connection to salt resistance through the main cellular transporters NXH, vacuolar, and endosomal. These antiporters contribute to regulating cellular and vacuolar pH, cellular homeostasis, and ultimately salt resistance.

Secondly, we briefly covered membrane cellular transporters like SOS. Conversely, we excluded articles not addressing plant salt resistance or those not focusing on transporters. The keywords used in this article review highlighted antiporters, including vacuolar and endosomal NHX types, membrane SOS, salt tolerance, and the regulation of pH and ion homeostasis.

Results

Presentation of NHX-type vacuolar antiport

Intracellular Na^+/H^+ antiport (NHX) are integral membrane proteins residing in the plasma membrane, endosomal compartments, and vacuoles (13). They belong to the CPA1 (cation/proton) antiport family of the cation-proton antiporters (CPA) superfamily (14).In plants, NHX antiporters catalyze the electroneutral exchange of Na^+ and/or K^+ for H^+ using electrochemical gradients generated by the H^+- ATPases, H^+- ATPases and H^+- PPase of the vacuole to direct the movement of Na^+ or K^+ out of the cell or the luminal action of Na^+ or K^+ into vacuoles and intracellular organelles (15).

The vacuolar NHX antiport and the plasma membrane SOS facilitate the exchange of cations and protons across the tonoplast and plasma membrane, respectively. The SOS transporters enable the excretion of Na+ from the cytoplasm to the external environment, while NHXs facilitate the transport of Na+ from the cytoplasm to the vacuole (Table 1).

Table 1. Some biological roles of transporters and their positioning and function in plant cells and organs, respectively

S.No.	Name of the transporters	Plant part in which it is expressed (Root/ leaf/stem etc)	Intracellular Location (cell membrane, tonoplast, etc)	Function	Reference
Stacia vera	NHX1 (Na^+(K+)/H^+ antiporter)	Roots	Root vacuoles	High NHX1 expression is linked to a high Na^+/K^+ ratio during salt stress, enabling Na^+ to be sequestered in vacuoles, thus protecting tissues.	(21)
Punica granatum	PgNHX ($Na^+(K^+)/H^+$ antiporter)	Leaves and roots	Leaf vacuoles, and roots	Overexpression of PgNHX mitigated the effects of salt by sequestering Na^+ in leaf vacuoles, thereby reducing Na^+ accumulation in roots.	(22)
Ipomoea batatas	GmNHX1 ($Na^+(K^+)$/ H^+ antiporter)	Leaves and roots	Vacuole and maintained a higher K^+/Na^+ ratio in roots	Arabidopsis plants expressing GmNHX1 induced Na^+ accumulation in vacuoles, regulating salt-inducible genes to modulate K^+ and Na^+ levels in leaves and roots.	(23)

In addition to SOS1, the SOS pathway consists of two regulatory proteins (SOS2 and SOS3) involved in the response to salt stress. High salt concentrations trigger a calcium signal. The binding of Ca^{2+} to specific sites on the SOS3 protein activates a SOS2 protein kinase (16). The phosphorylated SOS3/SOS2 kinase complex activates the Na^+/H^+ antiporter SOS1, leading to the exclusion of Na+ from the cell (17).

The vacuolar NHX antiport is involved in regulating intracellular potassium balance, salt stress tolerance, and plant growth and development (18). Numerous studies have shown that overexpression of genes associated with Na^+ and K+ transport, through biotechnological or selection techniques, appears as an interesting strategy to enhance salt tolerance and improve crop production (19).

The *Arabidopsis thaliana* NHX gene family comprises a class of six intracellular NHX-like antiporters, which are classified into two groups. Group I contains NHX1 to NHX4 and is located in vacuoles, while group II contains NHX5 and NHX6 and is located in endosomal compartments. Several functions have been associated with plant NHX transporters, including regulating cellular K^+ homeostasis, cell expansion, and salt tolerance (20).

The plant genome, in most cases, contains several isoforms of intracellular NHX. Intracellular NHX transporters are classified in the IC-NHE/NHX family, part of the large family of proton cation antiporters 1 (CPA1) (24). These are further subdivided into vacuolar (Class I) or endosomal (Class II) NHX, based on their sequence similarity and the subcellular localization of representative members (25) (Fig. 1). At this time, most sequenced plant species contain both types of NHX and functional redundancy of vacuolar or endosomal NHX has been reported in Arabidopsis thaliana (25).

Among the first NHX family members to be identified in plants was AtNHX (26), which has been shown to have homology with the Na^+/H^+ antiport, NHE of the plasma membrane of animal cells, and also that of yeast ScNHX1 (encoding vacuolar membrane Na+/H+ antiporter from *Suaeda corniculata*. Moreover, AtNHX1 expression in yeast has been shown to complement the NaCl sensitivity caused by disruption of the ScNHX1 gene (26). A group of researchers proposed grouping human NHE6 and NHE7, yeast ScNHX, and *Arabidopsis* AtNHX1 proteins into a new subfamily of Na^+/H^+ antiports, intracellular NHE. Subsequently, many more intracellular antiport, now called NHX, have been identified in plants, fungi, and animals (27).

Several isoforms of NHX transporters have been found in plants. The majority of them are unexpressed in the absence of salt stress in all plant tissues but are triggered by salt stress in leaves, roots, stems, or a combination of roots and leaves. Some isoforms are induced by ABA, KCl, dehydration, hyperosmotic stress, or high-temperature stress (28). The expression of the six NHX isoforms in *Arabidopsis* was studied in detail. The predominant isoforms are AtNHX1 and AtNHX2, found in the roots and aerial part. The expression levels of AtNHX3, AtNHX4, and AtNHX6 in these tissues were much lower than those of AtNHX1 and AtNHX2. The expression of Atnhx1 is heightened in leaves but remains unchanged in roots when treated with NaCl or ABC. In seedlings, both AtNHX1 and AtNHX2 are activated by salt stress, hyperosmotic shock, and ABA treatment, while AtNHX5 is exclusively induced by salt stress, and AtNHX4 responds to both salt stress and ABA. AtNHX1 and AtNHX2 are not inducible by NaCl in aba2-1 mutants, indicating that NaCl induction of these transporters is dependent on ABA signaling (29).

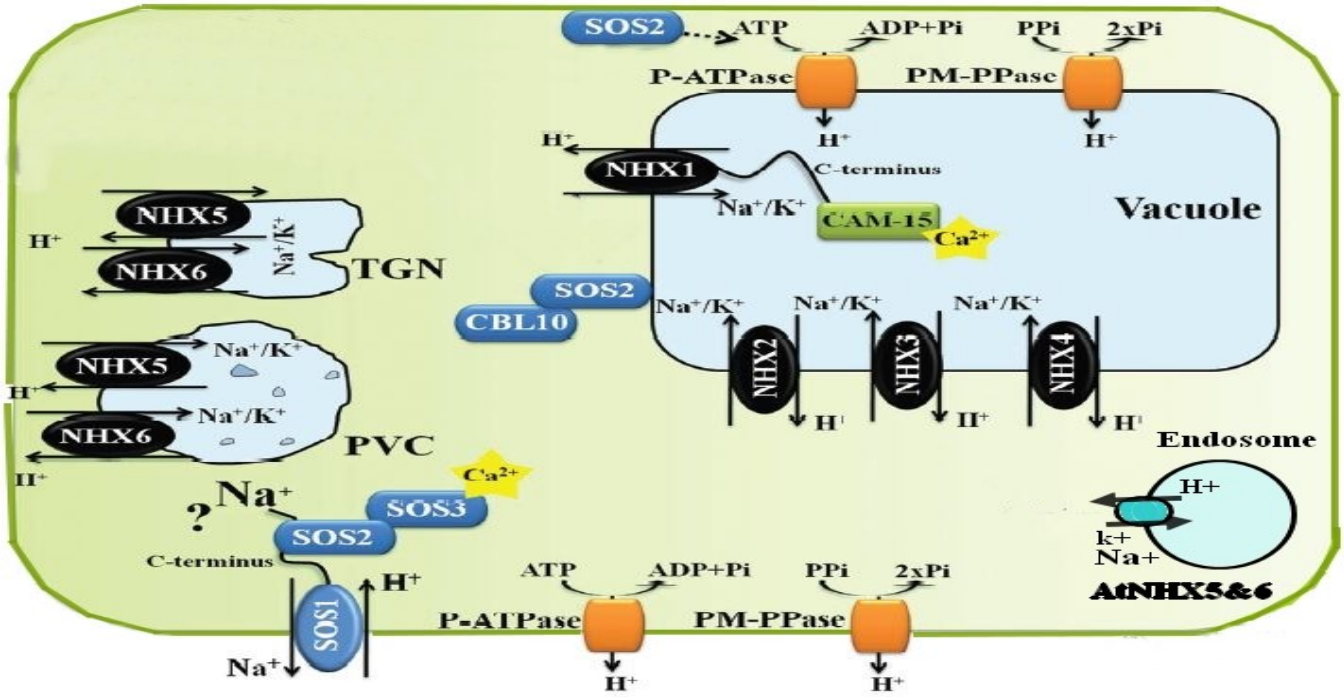

Fig. 1. Schematic representation of the cellular localization, functions, and regulation of the Na^+ type transporters NHX, SOS, and HKT1 in *Arabidopsis* (30). The NHX gene family of *Arabidopsis thaliana* consists of a set of six NHX-type intracellular antiporters, categorized into two groups. Group I include NHX1 to NHX4, which are situated in vacuoles, while group II comprises NHX5 and NHX6, localized to endosomal compartments, trans-Golgi network (TGN), and prevacuolar compartment (PVC).

Functions of NHX transporters

The primary role attributed to the NHX vacuolar antiport is to effect Na^+ sequestration within the vacuole via a Na^+/H^+ exchange across the tonoplast (31). The movement of protons from the vacuole to the cytoplasm is exergonic due to the pH gradient of 1.5 units between these two compartments. The rate of Na^+ accumulation in the vacuole relative to the cytoplasm can therefore be greater than 30 (101.5 more precisely). The accumulation of Na^+ in the vacuole is achievable against its concentration gradient only 4 to 5 times higher. This transport follows a saturable process depending on the cytoplasmic concentration of Na^+. Biochemical characterization studies of the different NHX-type antiport have shown that they can transport, in addition to Na^+, other monovalent cations such as K^+, Li^+, and Rb^+ (32-15).

The affinity of the NHX antiport for these cations varies depending on the species and analytical conditions (33). The characteristics of this transport in AtNHX1 are generally described as following Michaelis-Menten type saturation kinetics. In addition, modification of either the N- or C-terminal part of the protein alters the affinity (KM, Michaelis constant) and transport kinetics (Vmax, maximum velocity) characteristics. The C-terminal part seems to be crucial for the regulation of transport activity and ion selectivity. An interaction between this part and an essential protein for stress, calmodulin, has been demonstrated. The latter regulates the activity of AtNHX1 in a pH- and vacuolar Ca^{2+}- dependent manner. Thus, under physiological conditions (high concentration of free Ca^{2+} in the vacuole and acidic vacuolar pH), calmodulin would bind to a binding site located in the C-terminal part and give AtNHX1 a better transport affinity for K^+ than for Na^+ (34).

Salinity tolerance

Phenotype analysis of transgenic plants showed that overexpression of NHX genes was responsible for improved salinity tolerance. In this sense, several studies have been done on the salinity tolerance of transgenic plants overexpressing NHX-like genes and revealed that tolerant phenotypes were associated with high leaf K^+ concentrations (35). Since NHX antiporters can exchange K^+ or Na^+ for a proton from the vacuole across the tonoplast, overexpression of these genes could affect cytoplasmic K^+ content by allowing storage of this cation within the vacuole, especially when K^+ is available to the cell (36). In addition to playing a protective and adaptive role under osmotic and salt stress conditions, the decrease in leaf area observed in the nhx1 mutant could be explained by the role NHX1 would have played in K^+ transport and cell turgor required for growth. Activation of Na^+/H^+ antiporters has been observed following the application of salt stress in glycophytes and halophyte species (37).

However, in some sensitive species, such as medium plantain (*Plantago media* L.), no NHX antiport activity could be recorded after the application of salt stress (NaCl, 50 mmol/L) (40). On the other hand, $(Na^+, K^+)/H^+$ exchange activity could be detected in some glycophyte species, such as cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L. Merr), even in the absence of salt stress (38). Furthermore, overexpression of NHX antiport genes has been used to improve salinity tolerance in various plant species. Transformed plants overexpressing NHX antiport genes showed better behavior and growth than wild-type plants under salt stress (39). Transgenic tomatoes overexpressing AtNHX1 and grown in the presence of 200 mmol/L NaCl could grow, flower, and produce fruit. Although the leaves accumulated high amounts of Na^+, the fruits showed low levels. *Arabidopsis thaliana* plants overexpressing AtNHX1 show better growth than wild-type plants and gather Na^+ in their stems under salt stress, confirming the role of this antiport in salinity tolerance (40). On the other hand, nhx1 insertion mutants in *Arabidopsis thaliana* showed a reduced Na^+/H^+ exchange level and greater salinity sensitivity than wild-type plants. Overexpression of AgNHX1 in rice results in better survival in a medium containing 300 mmol/L NaCl, whereas overexpression of OsNHX1 in the same species leads to identical leaf Na^+ and K^+ contents (35).

Regulation of pH

In plants, cytoplasmic pH is determined via primary proton pumps and metabolic processes that produce H^+ or OH-. In this sense, cation/proton antiport will rapidly adjust cytoplasmic pH (41). Different types of stresses can affect cytoplasmic pH, both biotic and abiotic, and these changes can form the basis of many signaling pathways involved in stress responses, developmental processes, hormonal control of stomach movements, gravitational response, growth and elongation (42).

The importance of the action of the NHX antiport in regulating vacuolar pH is well illustrated by the evolution and change of flower coloration in Ipomoea nil. The color of the flowers varies from reddish-purple at the flower bud stage to blue after the buds have opened. The color of the petals is due to anthocyanin pigments accumulated in the vacuole of the cells. These anthocyanins give a blue color under alkaline conditions and a red color under acidic conditions. Thus, in flowers, the transition from red to blue is accompanied by an increase in the vacuolar pH of the cells from 6.6 to 7.7, or by 1.1 units, making the medium relatively essential (43). The high vacuolar pH suggests that the vacuoles are alkaline to the cytosol. Achieving such an alkaline pH by an electro neutral K^+/H^+ antiport mechanism would require higher concentrations of K^+ in the cytoplasm than in the vacuole, as is the case under conditions of K^+ depletion in cortical and epidermal cells of barley roots (32).

Thus, it turns out that, under normal conditions, pH regulation is carried out by an exchange of K^+ rather than Na^+ for a proton. Indeed, the vacuole occupies 80 to 90 percent of the cell volume and is the preferred storage site for K^+ (44). Furthermore, the ability of NHX antiporters to perform K^+/H^+ exchanges allows the plant, under normal growth conditions, to sequester K^+ (mainly) within the vacuole, thus contributing to osmotic regulation and control of the turgor pressure necessary for cell expansion and growth (45).

K^+ homeostasis

The activity of the vacuolar antiport NHX results in acidification of the vacuole, which protects enzymatic reactions from Na^+ toxicity and controls turgor pressure (46). In addition, it is involved in K^+ homeostasis under normal growth conditions (20). In most cases, cellular K^+ ions accumulate in the vacuole, where they are involved in the maintenance of cell turgor and expansion. However, cytoplasmic K^+ ions have both osmotic and biochemical functions. K^+ is actively included in the vacuole under normal growth conditions; otherwise, it is transported from the vacuole to the cytoplasm in situations of severe K^+ deficiency to maintain adequate cytosolic concentrations. Under these conditions, cytoplasmic acidification occurs, which could represent a signal to induce a supply of high-affinity K^+ or a drain of K^+ from the vacuole (32).

A decreased pH gradient between the vacuole and the cytoplasm may also decrease the driving force that uses the K^+/H^+ antiport mechanism for K^+ accumulation. In Thermodynamics, active entry of K^+ into the vacuole, under conditions of K^+ sufficiency, can be mediated by a K^+/H^+ antiport. Still, active flux requires a K^+/H^+ symport system, assuming the vacuole is more acidic than the cytoplasm, a condition that does not always occur (36). Overexpression of AtNHX1 in tomatoes causes deficiency symptoms despite increased K^+ supply and content (47). In this case, the decrease in K^+ cytosolic concentration could cause a K^+ deficiency signal that would increase the cation supply. *Arabidopsis* mutants that do not express NHX1 have less leaf area and smaller epidermal cells. This may be related to a vacuolar deficiency of K^+, which is required for turgor generation and cell expansion. In this regard, it has been observed that these mutants show a lower K^+ supply in the roots and a lower K^+ content in the aerial part (42). The high level of expression of some NHX proteins in the guard cells of stomata also suggests that these proteins are essential for accumulating vacuolar K^+ and the rapid turgor changes in these cells (37-28).

Conclusion

Plants have evolved highly sophisticated mechanisms to maintain cellular homeostasis under conditions such as salinity, drought, cold, freezing, or intense heat. The multitude and complexity of the mechanisms responsible for the ionic homeostasis of plants, as well as the intricacy of their regulation and interconnections, at both the cellular and whole-plant levels, are remarkable. The involvement of sophisticated and tightly regulated stress detection mechanisms, effective signal transduction pathways, efflux or compartmentalization of toxic ion systems, and key detoxification strategies, such as the accumulation of osmoprotectants, highlights how fascinating the history of plant ionic homeostasis under environmental stress is.

Nearly two decades of in-depth molecular studies have established the involvement of transporters NHX, SOS1, and HKT in plant salt tolerance. Genes encoding some of these transporters have since been successfully used as genetic tools to enhance salt tolerance in model and cultivated plants. It is therefore conceivable that improving salt tolerance through the overexpression of one of the mentioned genes may reach its limits due to excessive disruption of related cellular and physiological processes.

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Authors' contributions

MM. Mooted the idea and topic of this research. AM, AS, HA, GH, HE, JZ. Prepared the revised version of this manuscript. MP, KV, MB provide structure.

Compliance with ethical standards

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