*Review*

# Differential responses to salt-induced oxidative stress in three phylogenetically related plant species: *Arabidopsis thaliana* (glycophyte), *Thellungiella salsuginea* and *Cakile maritima* (halophytes). Involvement of ROS and NO in the control of K$^+$/Na$^+$ homeostasis

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**Abstract:** Salinity, which is usually associated with a nitro-oxidative stress component, is one of the major environmental factors limiting plant growth and development. Plants have thus developed specific ways of dealing with this problem. The compartmentalization of sodium (Na$^+$) ions in vacuoles and the capacity to sharply discriminate between potassium (K$^+$) and Na$^+$ in order to maintain high K$^+$/Na$^+$ ratios are two of the most effective strategies to overcome salt stress. Plants require large amounts of K$^+$ to maximize growth and yields. This macronutrient is involved in physiological processes such as growth, photosynthesis, osmoregulation, enzyme activation, stomatal movement, water and nutrient transport via the xylem and protein synthesis. Resistance to salt stress is mainly related to the capacity of plants to maintain improved K$^+$ uptake despite competition from Na$^+$. The Brassicaceae family includes species such as *Arabidopsis thaliana* (plant model for glycophytes), *Thellungiella salsuginea* and *Cakile maritima* (plant models for halophytes), which exhibit significant variations in response to salt stress. In this review, we provide a comprehensive update with respect to differential responses to salt stress in these three plant species, with particular emphasis on the potential involvement of reactive oxygen species (ROS) and nitric oxide (NO) in maintaining K$^+$/Na$^+$ homeostasis and their contribution to salt tolerance.

**Keywords**: Brassicaceae; differential tolerance; $K^+/Na^+$ homeostasis; nitric oxide; salinity; ROS

**Abbreviations**

| | | | |
|---|---|---|---|
| *HKT* | high-affinity potassium transporter; | *NHX* | sodium-proton exchanger; |
| *NSCC* | non-selective cation channels; | *SOS1* | salt overly sensitive; |
| *RBOH* | respiratory burst oxidase homologue; | *ROS* | reactive oxygen species; |
| *NO* | nitric oxide; | | |

## 1. Introduction

The macronutrient potassium ($K^+$), which is the second most abundant mineral nutrient in plants after nitrogen, needs to be maintained within a range of 100–200 mM in the cytosol for efficient metabolic functioning [1,2]. Table 1 summarizes the most important functions of $K^+$ in higher plants. However, although sodium ($Na^+$) is not an essential nutrient for plants, it can act as a substitute for $K^+$ in relation to some functions, as has been demonstrated under certain $K^+$-limiting conditions. For example, the decrease in nutrient uptake under salt stress conditions could be explained by the osmotic pressure exerted by the soil solution on the plant cell, leading to a reduction in water absorption and consequently nutrient acquisition [3,4]. The maintenance of ionic homeostasis is therefore necessary for plant salt resistance [5,6].To deal with such severe conditions, plants have developed different strategies, in particular, adequate uptake of $K^+$ via discrimination between $K^+$ and $Na^+$ in order to maintain high cytosolic $K^+/Na^+$ ratios. The effect of salt stress on plant $K^+$ nutrition has been the subject of numerous studies of both glycophytes and halophytes. These studies reveal that the presence of $Na^+$ reduces $K^+$ acquisition by inhibiting $K^+$ transport in the aerial parts of the plant. Since $Na^+$ ions are positively charged, they affect $K^+$ absorption and related enzymatic activities in plant cells [7]. Therefore, the survival of plants depends on their ability to maintain homeostasis and transport of $K^+$ across cell membranes [8]. Many studies indicate that relatively closed plant species have an evolutionary stage that enables them to develop adaptive mechanisms to cope with variations in environmental factors. The Brassicaceae family contains several species, the best known being the glycophyte *Arabidopsis thaliana*, the halophyte *Thellungiella salsuginea* (formerly known as *Thellungiella halophila*) and, more recently, *Cakile maritima*. The distinct salt stress responses of these plant species reside in their ability to discriminate between $K^+$ and Na+ and to maintain high $K^+/Na^+$ ratios despite intense competition from $Na^+$. This review highlights the differential responses to salt stress in these three plant species, with particular emphasis on the potential involvement of reactive oxygen species (ROS) and nitric oxide (NO) in maintaining $K^+/Na^+$ homeostasis and their contribution to salt tolerance.

## 2. Na⁺ and K⁺ Transporters

The ionic transport systems operating at the plasmalema and tonoplast play a crucial role in regulating the $K^+/Na^+$ ratio in the cell [9]. Under salt stress conditions, a series of transporters for $Na^+$ and $K^+$, called co-transporters, are activated to ensure high $K^+/Na^+$ ratios [5,10]. The common

transporters for $K^+$ and $Na^+$ include the high affinity $K^+/Na^+$ transporter (HKT) [11], capable of co-transporting $Na^+$ and $K^+$ or only $Na^+$ [12] and KT/HAK/KUP, $K^+(Na^+)/H^+$ cation/proton antiporters (CPAs), and NHX proteins [13]. These transporters can transport 2 $K^+$ ions over 1 $Na^+$ ion. Potassium transporters are classified into two groups: cluster I transporters (AtHAK5, HvHAK1 and OsHAK1) and cluster II transporters (AtKUP4, HvHAK2, OsHAK2, OsHAK7 and OsHAK10). Several KT/HAK/KUP transporters may be involved in plant responses to salt stress [14]. A number of HvHAK1 cluster I transporters mitigate the influx of $Na^+$ [15]. Others transporters, such as KUP-HAK, are known to be highly $K^+$ selective, although the presence of $Na^+$ at certain NaCl concentrations blocks these carriers [15]. For example, a 5 mM concentration of NaCl is sufficient to inhibit cluster II $K^+$ transporters KUP1 responsible for mediating both high- and low-affinity $K^+$ transport [16]. HAK5, a high-affinity K transporter and the largest contributor to $K^+$ acquisition under salt stress and insufficient $K^+$ availability conditions [17], can transport either $K^+$ or $Na^+$ and is often inhibited by $Na^+$ [18], leading to sodium ion accumulation in plant cells [19]. These carriers are therefore important for the balance between $K^+$ and $Na^+$ and are efficiently regulated under salt stress conditions. In this context, many genes and transporters have been identified and shown to contribute significantly to the regulation of $K^+/Na^+$ ratios. As described above, the tolerance of plants to salt stress depends on their ability to regulate the expression of genes encoding several $K^+$ transporters. This can be done by activating the high-affinity $K^+$ transporter, particularly HAK5. According to the literature, many crops, as in the case of wheat, cultivated under saline conditions, are characterized by genetic regulation of $K^+/Na^+$ ratios, resulting in effective discrimination of $K^+$ over $Na^+$ at the xylem loading stage [20]. Recently, a study of tobacco has demonstrated that OsHAK5 expression in plant cells results in an increase in salt tolerance through the accumulation of $K^+$, while $Na^+$ content remains unaltered [21]. Most $K^+$ uptake systems, particularly non-selective cation channels (NSCCs) and high-affinity potassium transporters (HKTs), can be inhibited by $Na^+$ [22].

**Table 1. Summary of the main functions of potassium in higher plants.**

| Function |
| --- |
| • Control of plant water status (stomatal activity) |
| • pH homeostasis, control of membrane electrical potential, and the regulation of cell osmotic pressure |
| • Increasing the rate of photosynthesis. |
| • Enhancing the loading and unloading of photosynthates into and out of the phloem. |
| • Activation of some enzymes |
| • Improve plant stress resistance |

## 3. How does $Na^+$ Affect $K^+$ Uptake?

$Na^+$ enters the cell and plant via non-selective channels located at the plasma membrane. The activity of these channels induces a change in membrane potential and leads to its depolarization. Such modifications in membrane potential makes passive absorption of $K^+$ through inward-rectifying $K^+$ channels thermodynamically impossible, while an activation of outward-rectifying $K^+$ channels occurs simultaneously, leading to a loss of $K^+$. However, an increase in compatible solute synthesis ensures osmo-protection under these conditions, thus sharply reducing the pool of ATP and further

decreasing $K^+$ uptake [23]. Despite the fundamental role played by $K^+$ in, for example, cell turgor pressure regulation [24,25,26], $Na^+$ can substitute for $K^+$ in functions such as osmotic adjustment, enzymatic activity and protein synthesis [27]. In terms of their chemical properties, $Na^+$ and $K^+$ actually share many physicochemical similarities which enable $Na^+$ to compete with $K^+$ at absorption sites and to impair $K^+$ uptake. Thus, NaCl affects $K^+$ status by reducing its uptake as a result of competition between $Na^+$ and $K^+$ for uptake sites as well as high- and low- affinity transporters [6,28,23] or by inducing $K^+$ leakage due to membrane depolarization which activates outward-rectifying $K^+$ channels [29]. According to Shabala and Cuin [23], NaCl can induce $K^+$ deficiency via the two aforementioned mechanisms.

On the other hand, $Na^+$ can have a beneficial impact on the halophytic plants and stimulate plant growth. Through the involvement of $Na^+$ in osmoregulation, the energy used for osmoticum synthesis, such as proline in the form of adenosine triphosphate (ATP), contributes to the maintenance of plant growth. Moreover, large amounts of $Na^+$, at levels similar or equal to those of $K^+$, can be accumulated in the cell [30,31]. The antagonism between these two ions intensifies when the level of $Na^+$ concentrations is excessive, as the accumulation of $Na^+$ in plant tissue leads to a significant loss of $K^+$ [32,33] through $Na^+$, $K^+$ and $H^+$ antiporter activity. As described previously, under severe salinity conditions, the activation of outward-rectifying $K^+$ channels, following the depolarization of the plasma membrane, leads to massive loss of $K^+$ [23,34]. Given that salinity affects many regions around the world and can induce $K^+$ deficiency [35], it is very important for plants to discriminate between $Na^+$ and $K^+$ and to maintain adequate $K^+/Na^+$ ratios.

## 4. Importance of $K^+/Na^+$ Ratio for Plant Salinity Tolerance

Plant tolerance to salt stress is related to the maintenance of $K^+$ homeostasis at both the cellular and whole plant levels mainly through high $K^+$ selectivity over $Na^+$ [36]. The maintenance of $K^+/Na^+$ ratios is therefore crucial for plant survival. Maintaining the lowest possible $Na^+/K^+$ ratios in the cytosol, which are regarded as a phytophysiological indicator of salt stress levels, is crucial to preserve vital cell functions [37,38]. Furthermore, the preservation of the intracellular $K^+/Na^+$ ratio, mainly in the cytoplasm, is a crucial factor in maintaining appropriate internal $K^+$ concentrations and plant growth under salt stress conditions [9,39]. Thus, the cytoplasmic $K^+/Na^+$ ratio, considered to be an indicator of salt tolerance [23,40–43], enables plants to minimize $Na^+$ accumulation in the cytosol and to avoid massive loss of $K^+$ [32,44]. Plants tend to promote $Na^+$ exclusion and $K^+$ accumulation in tissues [25,45] in order to maintain a high cell $K^+/Na^+$ ratio [46] and to preserve vital metabolic pathways.

## 5. Role of Transporters in Maintaining $K^+/Na^+$ Homeostasis

$K^+/Na^+$ homeostasis occurs through a series of $Na^+$ and $K^+$ transporters located at the plasma membrane. To regulate cytosolic $Na^+$ concentrations, plants tend to continuously exclude $Na^+$. The principal mechanism of $Na^+$ extrusion is mediated by power pumps located at the plasma membrane $H^+$-ATPases [47].These pumps generate a $H^+$ electrochemical gradient that transports ions and nutrients across the membrane [48]. $H^+$-ATPases provide the plant cell with the energy to promote $Na^+/H^+$ antiporter activity involved in the extrusion of $Na^+$ [48]. Once $Na^+$ enters the plant cell, it needs to be accumulated in vacuoles to preserve the activity of several cytosolic enzymes. At the

tonoplast, these $Na^+/H^+$ antiporters facilitate the sequestration of sodium ions in vacuoles and are encoded by *AtNHX* genes, as described in relation to Arabidopsis [49]. Vacuolar $H^+$-pyrophosphatases, which are responsible for this process, have been identified in plants. Several channels and transporters mediating $Na^+$ transport across the plasma membrane have also been identified [50]. The $Ca^{2+}$-permeable nonselective cation channels (NSCCs) [6] and glutamate-like receptor (GLR) [51] families are among the most well known channels permeable to $Na^+$ [50]. In Arabidopsis plants, 20 non-selective cation channels (NSCC) have been described [52]. $Na^+$ flux is not only regulated at the root but also along its transport pathways to the aerial parts where a series of mainly KORC and NORC outward-rectifying $K^+$ channels [53] and high-affinity $K^+$ transporters (HKTs) play a crucial role in this process. The former are responsible for $K^+$ xylem loading [54], while the latter facilitate the unloading of $Na^+$ from the xylem sap [55,50,52]. In rice, Yang et al [56] found that OsHAK5 mediates high-affinity $K^+$ acquisition and $K^+$-regulated salt tolerance. In *Arabidopsis*, HAK5 expression was observed to increase under salt stress, reaching maximum transcript levels after 6 h of saline treatment [9]. $Na^+$ and $K^+$ transport is due to $Na^+/K^+$-symport activity which controls the transport of both ions. At the molecular level, the mechanisms involved in $K^+/Na^+$ uptake have been very well studied. The best known transporters, which contribute to $K^+$ homeostasis, are the high-affinity HKT and HAK transporters [46,57]. SOS, a genetic locus essential for salt tolerance and potassium acquisition [58] in plants, regulates $Na^+$ efflux across the plasma membrane to the soil solution. Other transporters play a crucial role in salt tolerance by mediating the $K^+/Na^+$ ratio. Some transporters such as OsHKT1;5, TaHKT1;5 and TmHKT;4/5 are able to retrieve $Na^+$ from the xylem in the xylem parenchyma, thus enabling the plant to avoid excessive $Na^+$ accumulation, as in the case of SOS1 detected in *T. salsuginea* [59]. In wild-type *Arabidopsis thaliana*, the application of NaCl for a period of 6 days did not affect HAK5 transporter gene expression [60]. While Bassil et al. [39] observed an increase in HAK5 expression following the application of NaCl, which reached a peak after 6 h of treatment followed by a decrease after 24 h of treatment. In the same species, long term salt stress (16 days) reduced HAK5 expression [18]. Another high-affinity $K^+$ transporter HKT, which is able to transport $Na^+$, has been identified in plants. HKT1;1, which regulates $Na^+$ penetration of plant cell roots and its distribution in the phloem, is crucial for salt stress tolerance in Arabidopsis [61]. Very recent new data indicate that the cation selectivity of HKT1 transporters is convertible by exchanging a single amino acid (Asp or Asn) in the 2nd pore-loop domain. In addition it was demonstrated that the amino acid serine in the 1st pore-loop domain is essential to facilitate $Na^+$ uptake [62]. On the other hand, class II HKT transporters include the high-affinity $Na^+/K^+$ TaHKT2;1 transporter, which acts as a symporter under normal conditions and as a low-affinity $Na^+$ uniporter under salt stress conditions [63]. Recent studies of the model *A. thaliana* glycophyte plant have identified two high-affinity $K^+$ transporters (AtHAK5 and AKT1), which are key elements in plant $K^+$ uptake [64,65]. When $K^+$ concentrations are highly limited [66,67,68] or under salt stress [69,19], AtHAK5 is the only system that ensures adequate $K^+$ uptake, suggesting that $Na^+$ can induce $K^+$ deficiency [70]. In Arabidopsis, AtHAK5 is needed for $K^+$ uptake when this nutrient is limited in the medium under saline conditions, although its expression is affected when $Na^+$ concentrations are considerably higher in this glycophyte species [17]. Sun et al [71] attribute the variations in salinity tolerance between certain *A. thaliana* accessions to their ability to retain $K^+$, with the tolerant accessions actually exhibiting low $Na^+/K^+$ ratios and high tissue $K^+$ content [71]. These findings could be explained by significant up-regulation of genes coding for $K^+$ transporters such as AtHAK5, AtCHX17 and AtKUP1 [71]. Recently,

Alemán et al [22] have identified the conserved amino acid residue Phe130 in the HAK transporter family, which is required for AtHAK5 transporter selectivity. Moreover, the mutation Phe130Ser in the high-affinity Arabidopsis $K^+$ transporter caused an increase in $K^+$ uptake over $Na^+$. The NHX protein, another $K^+/Na^+$ antiporter, is known to be expressed under salt stress conditions. In *A. thaliana,* overexpression of tomato antiporter LeNHX2 (NHX Class II) resulted in high sensitivity to $K^+$ depletion [72].

Halophytes appear to be more effective in maintaining $K^+$ transporter activity as compared to glycophytes. In *T. salsuginea*, salinity does not reduce $K^+$ uptake via the high-affinity $K^+$ transporter HAK5 to a lesser degree than in *A. thaliana* [69]. In this halophyte, two *HKT* genes, *TsHKT1;1* and *TsHKT1;2*, encoding plasma membrane $Na^+/K^+$ co-transporters have been identified. One of these genes is induced and up-regulated at the transcriptional level under salt stress conditions [42]. This finding highlights the importance of TsHKT1 in the maintenance of $K^+$ uptake and thus in the salt tolerance of this species. With regard to *C. maritima*, molecular studies are required to elucidate the transporters responsible for $K^+$ homeostasis under high salinity stress conditions.

## 6. Differential Behaviors of *Arabidopsis thaliana*, *Thellungiella salsuginea* and *Cakile maritima* in Maintaining Cell $K^+/Na^+$ Homeostasis

To overcome salt stress, plants need to maintain adequate nutrient uptake despite competition from $Na^+$ and $Cl^-$ ions. It is critical for plants to maintain sufficient $K^+$ uptake given the importance of this factor for plant survival. Thus, plants induce high $K^+$-uptake systems such as HAK transporters.

*A. thaliana* is a model plant to investigate the physiology and biochemistry of plants under stressful and non-stressful conditions. *A. thaliana,* known to be sensitive to salt stress, prevents the accumulation of $Na^+$ in its tissues through the extrusion of these ions in the external medium and in vacuoles. SOS1 is a transporter involved in $Na^+$ extrusion at the roots. NHX proteins located at the tonoplast are responsible for $Na^+$ accumulation in the vacuole, thus making Arabidopsis plants salinity-tolerant [73]. On the other hand, *Thellungiella salsuginea* (2n = 14), which is relatively close to *A. thaliana* (2n = 10), presents the characteristics of a halophytic model plant. This plant's tolerance to salinity is due to its high capacity to prevent $Na^+$ accumulation in shoots. This species has thus been found to be more efficient in accumulating less $Na^+$ in its aerial parts as compared to *A. thaliana* [74]. *Cakile maritima* (2n = 18), another plant which is relatively close to *A. thaliana* [75], is an excellent model for investigating salinity tolerance mechanisms in halophytic species. This plant tolerates high levels of salt even during germination and presents a germination rate of approximately 75% at 200 mM NaCl [76]. Unlike *A. thaliana* and *T. salsuginea*, *C. maritima* is able to accumulate $Na^+$ in shoots, which it uses for osmotic adjustments and to maintain growth activity even at high salinity levels [36,77]. Competition from $Na^+$ for $K^+$ uptake is offset by high $K^+$ selectivity and use efficiency levels [78].

In *A. thaliana* plants, $Na^+$ can enter roots without difficulty due to the presence of various non-selective cation channels [6]. These channels are characterized by very low selectivity for $K^+$ over $Na^+$ [79], resulting in very high $Na^+/K^+$ ratios. Despite their intolerance to salt stress, glycophytes have developed saline resistance mechanisms, especially by maintaining high $K^+/Na^+$ ratios via the activation of ionic transport systems and the establishment of electrochemical gradients across the membrane [80]. Subjecting *A. thaliana* to 400 mM NaCl for 72 h has recently been found to

maintain an ionic balance in roots, which results in high $Na^+/K^+$ ratios [81]. Unlike glycophytes, halophytes are able to support salinity levels of up to 1000 mM NaCl, with, for example, 800 mM NaCl in the case of the halophyte *Sesuvium portulacastrum* [82] and up to 500 mM NaCl in the case of *Cakile maritima* [83]. According to Newell [7], the ability of halophytes to tolerate high salinity levels is due to the compartmentalization of $Na^+$ in vacuoles, the accumulation of osmolytes such as proline and the maintenance of constant $K^+$ tissue concentrations via the induction of several transporters with high $K^+$ selectivity resulting in adequate $K^+/Na^+$ ratios [75]. Given the interaction between $K^+$ and $Na^+$, the halophytes are characterized by high $K^+$ discrimination rates and are able to maintain $K^+$ homeostasis and transport across cellular membranes [8]. *Thellungiella salsuginea* is considered to be a halophytic model plant due to its high tolerance to several abiotic stresses [84,85,86], which explains its status as an extremophile plant, with its tolerance to salt stress being related to its $K^+$ retention capacity [42,87]. *Thellungiella salsuginea* is able to regulate the uptake of $K^+$ which is acquired very efficiently from the medium, thus enabling this species to maintain high $K^+/Na^+$ ratios, which is not the case for *A. thaliana* [88,89,90]. *T. salsuginea* and *A. thaliana* respond differently to salt stress in terms of $K^+$ content, with the highest shoot $K^+$ content being found in the halophytes rather than in the glycophyte. Moreover, although shoot $K^+$ content is decreased by salt stress in *A. thaliana* (10–20%), it is increased by this type of stress in *T. salsuginea* (10–50%). This behavior is explained by the ability of *T. salsuginea* to accumulate less $Na^+$ and more $K^+$ than *A. thaliana* under salt stress conditions due to selective $K^+/Na^+$ channels located at the plasma membrane [91]. With regard to $K^+/Na^+$ selectivity, major differences between *A. thaliana* and *T. salsuginea* plants have been observed. In fact, despite the presence of large $Na^+$ concentrations in the medium, the halophyte species exhibits the largest $K^+$ concentrations and highest $K^+/Na^+$ ratios, as documented by Ghars et al [74] and Bailey et al [75] . This is due to the capacity of the halophyte to prevent $Na^+$ accumulation in its tissues [91] and to maintain high $K^+$-affinity transporter HAK5 activity as compared to the glycophyte species [69].

The voltage-independent channels at the root plasma membrane are extremely potassium-selective and contribute to more efficient $K^+$ uptake in *T. salsuginea* than in *A. thaliana* [91]. In *A. thaliana*, a negative correlation between $Na^+$ and $K^+$ was found, while a positive correlation was detected in *T. salsuginea* [91]. The authors attribute their findings to the fact that both species differ in terms of their $Na^+$ and $K^+$ transport pathways. While intense competition between $Na^+$ and $K^+$ was observed in *A. thaliana* under salt stress conditions, this was not the case for *T. salsuginea*. Following the application of 100 mM NaCl, Volkov et al [91] detected significant differences between *A. thaliana* and *T. salsuginea* in terms of $Na^+$ and $K^+$ uptake and their distribution in shoots in these two species. *T. salsuginea* exhibits a specific behavior pattern following the saline application, and when the medium is deprived of salt, its epidermal cells acquire large amounts of $K^+$. Salt stress treatment significantly modified the distribution of $K^+$ in cells, with the sharp decrease in epidermal $K^+$ concentrations leading to an increase in bulk $K^+$ concentrations. This modification enabled *T. salsuginea* to maintain high $K^+/Na^+$ ratios in mesophyll cells, a pattern not observed in *A. thaliana* which is able to accumulate more $K^+$ than $Na^+$ during the first 25 h of salt treatment. These authors point to the existence of two different pathways for $K^+$ and $Na^+$ permeability to explain this behavior. *T. salsuginea* presents higher selectivity for $K^+$ over $Na^+$ at both the inward- and outward-rectifying K channel $K^+$ uptake sites as compared to *A. thaliana* [91].They point out that the adaptation of *T. salsuginea* to salt stress is linked to high $K^+/Na^+$ selectivity in roots and to the exchange of $K^+$ over $Na^+$ between epidermal and mesophyll cells. More recently, Volkov and

Amtmann [90] demonstrate that *T. salsuginea* maintains $K^+$ uptake despite the presence of salt in the medium due to the slight depolarization generated, thus making the plasma membrane poorly permeable to $Na^+$ under these conditions.

To gain a greater insight into high $K^+$ selectivity in *T. salsuginea*, Bailey et al [75] studied the effect of low and high salinity levels on $K^+$ content in both *A. thaliana* and *T. salsuginea* and found that treatment with 100 mM NaCl for 72 h during a period of 6 weeks caused a very rapid loss of $K^+$ in the glycophyte due to an increase in $Na^+$ uptake, resulting in high $Na^+/K^+$ ratios. By contrast, the halophyte can maintain the balance between $K^+$ and $Na^+$, which is essential to enable *T. salsuginea* to survive under salt stress conditions. The tolerance of this species to salinity appears to be related to the maintenance of $K^+$ homeostasis via the discrimination between $Na^+$ and $K^+$ which results in low $K^+/Na^+$ ratios. Coskun et al [46] have also studied the responses of *A. thaliana* and *T. salsuginea* to salt stress, with particular emphasis on $K^+/Na^+$ homeostasis. Thus, while salinity significantly reduced $K^+/Na^+$ ratios in both Brassicaceae species, $K^+/Na^+$ values were significantly higher in *T. salsuginea* than in *A. thaliana*, with larger saline concentrations being observed in the medium. The $K^+/Na^+$ ratio was 2-fold higher in *T. salsuginea* following the application of 50 and 75 mM NaCl and 4-fold higher after treatment with 100, 150 and 200 mM NaCl. Coskun et al [46] have also demonstrated that $K^+/Na^+$ levels remain significantly higher in the halophyte species after treatment with 500 mM NaCl as compared to the glycophyte species treated with 200 mM NaCl. They also point out that the application of 50, 100, 150 and 200 mM NaCl did not affect $K^+$ concentration levels in *T. salsuginea,* while a decrease of 40% was observed in *A. thaliana* after treatment with 100 mM NaCl. Under severe salinity conditions (300–500 mM NaCl), $K^+$ content levels in the halophyte species were similar to those in the glycophyte species cultivated under control conditions. These differences between the two species could be explained by the highly $K^+/Na^+$-selective channels found in *T. salsuginea* as compared to *A. thaliana* [91].

*Cakile maritima*, an annual C3 species commonly found on sandy beaches around the world, is of great ecological interest mainly due to its soil fixation capacity. This species is capable of maintaining highly selective uptake of $K^+$ as against $Na^+$ [83] and of increasing $K^+$ use efficiency [78]. These authors attribute this behavior to a $Na^+$ flux regulation mechanism which occurs at the intracellular level and contributes to salt tolerance in this species. In fact, *C. maritima* preserves its $K^+$ content by allocating $Na^+$ to the vacuole and by preventing its accumulation in the cytoplasm [36]. As this behavior is typical of halophyte species, $Na^+$ can be used as an osmoticum to carry out osmotic adjustments [92] and also as a signal to enable the plant to prevent ionic toxicity [93]. $Na^+$ sequestration in *C. maritima* is caused by $Na^+/H^+$ tonoplastic antiport activity which exchanges the $Na^+$ ion over a $H^+$ proton [94]. *C. maritima* is very $K^+$-selective as compared to *A. thaliana* [94]. These authors suggest that efficient transport systems are present in the halophyte species which are responsible for the discrimination of $K^+$ over $Na^+$ to improve ionic status and to preserve crucial metabolic functions.

Indeed, a $Na^+/H^+$ exchange is necessary for the purposes of plant salinity tolerance. Many intracellular NHX transporters, which are responsible for $Na^+$ accumulation in vacuoles, have been identified and are the first family of cation/$H^+$ exchangers to be investigated in plant tissues [95, 96]. Further molecular and genetic studies are required to investigate the capacity of these species to maintain $K^+/Na^+$ homeostasis when subjected to salinity. On the other hand, very recently, a new transporter called KUP7 has been described in Arabidopsis roots; this appears to be crucial for $K^+$

uptake and might also be involved in $K^+$ transport into xylem sap, affecting $K^+$ translocation from root to shoot, especially under $K^+$-limited conditions [97].

Figure 1 shows a simplified flowchart model to compare response mechanisms under salinity stress in glycophyte (*Arabidopsis thaliana*) and halophyte (*Thellungiella salsuginea* and *Cakile maritima*) plants to regulate $K^+/Na^+$ ratios.
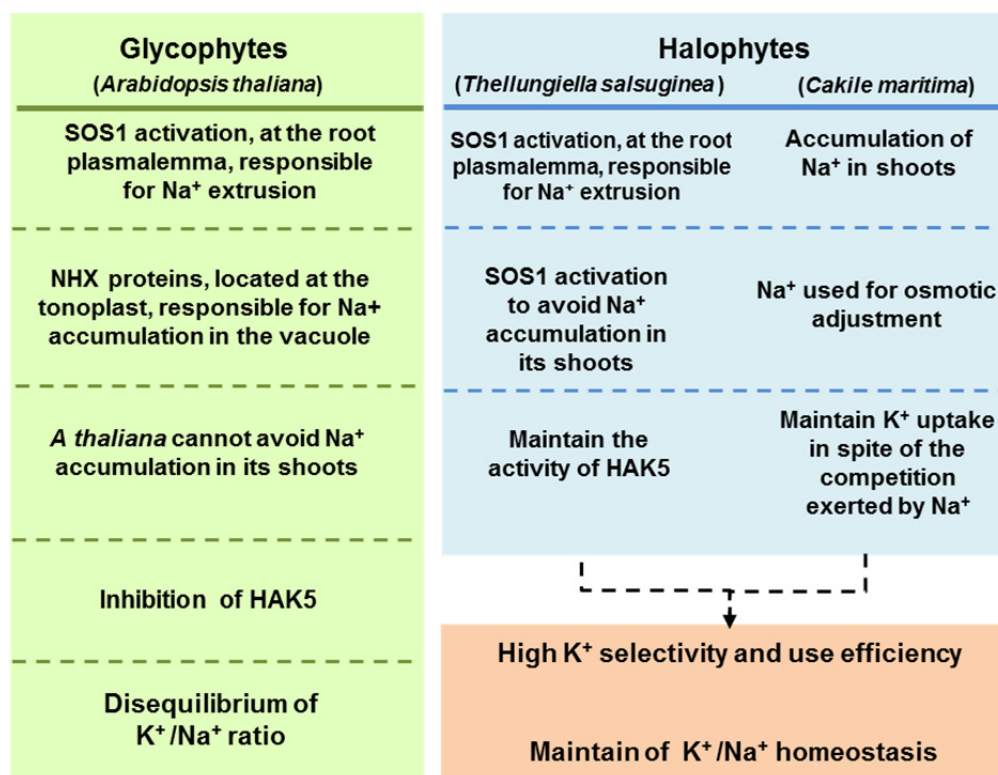


**Figure 1.** Comparative flowchart model of the mechanism of response under salinity stress in glycophyte (Arabidopsis thaliana) and halophyte (*Thellungiella salsuginea* and *Cakile maritima*) plants to regulate $K^+/Na^+$ ratio. HAK5, High Affinity $K^+$ transporter 5. NHX, Sodium-Proton exchanger. SOS1, Salt Overly Sensitive.

## 7. Role of ROS and Nitric Oxide (NO) in Maintaining $K^+/Na^+$ Homeostasis

A strong link between ROS production and the mediation of $K^+/Na^+$ homeostasis has been observed in many plant species [98]. In *Populus euphratica*, NaCl increases the production of hydrogen peroxide ($H_2O_2$), which contributes to the preservation of the $K^+/Na^+$ balance and high antioxidant capacity, enabling this species to regulate its ionic status and antioxidant defense system under long term salt stress conditions [99]. Under salt stress, ROS production increases and acts as a signal to maintain $Na^+/K^+$ homeostasis. ROS actually play an important role in regulating the $Na^+/K^+$ ratio, as ROS content increases significantly under salinity stress [9]. ROS are therefore also involved in regulating HAK5 expression [100,101]. Other studies have demonstrated that ROS also control ionic channel activity [102]. To cope with salinity, plants have developed specific strategies, which include both the regulation of ionic homeostasis and detoxification of ROS under these environmental conditions [10].

The role of ROS in maintaining $K^+/Na^+$ homeostasis is well documented. Ho and Tsay [103] have shown that, under $K^+$ deficiency conditions, ethylene production increases and stimulates the generation of ROS in *A. thaliana*. The increased production of ROS contributes to the regulation of $K^+$ transporters, particularly the high-affinity potassium transporter HAK5 which is induced under these conditions. Thus, ROS are crucial for generating $K^+$ starvation responses at the root level via the regulation of *AtHAK5* gene expression encoding a high-affinity $K^+$ transporter induced under low $K^+$ supply conditions [104].

In this context, a common response to ROS accumulation is the activation of the defense system which includes a diverse range of antioxidant enzymes, especially the superoxide dismutase (SOD), representing the first line of defense against oxidative stress [77]. In *A. thaliana*, a total of seven SOD isozymes has been identified which are differentially regulated in response to oxidative damage [105]. The manipulation of the Arabidopsis genome provides new mutants that are tolerant to salt stress. For example, the *pst1* mutant presents higher levels of SOD activity under extremely stressful saline conditions as compared to the wild-type plant [106]. In *T. salsuginea*, four SOD isozymes have been described [107], and, surprisingly, a total of ten SOD isozymes have been found to be differentially expressed during development and under long term salt stress in the halophyte *C. maritima* [77] (Table 2). The antioxidant α-tocopherol can also contribute to the maintenance of cellular $Na^+/K^+$ homeostasis [108]. Thus, Arabidopsis mutants *vte1* and *vte4*, characterized by tocopherol loss, have been shown to be sensitive to salt stress due to a sharp decrease in their growth and the generation of high oxidative stress levels under saline conditions.

On the other hand, the Arabidopsis double mutants of plant membrane-bound NADPH oxidases (NOXs), also known as respiratory burst oxidase homologues (RBOHs), specifically *atrbohD1/F1* and *atrbohD2/F2*, in which the generation of the superoxide radical ($O_2^-$) is considerably inhibited, have been found to be much more sensitive to NaCl treatments than wild-type plants. These two Arabidopsis double mutants also have much higher $Na^+$ content, lower $K^+$ content and consequently higher $Na^+/K^+$ ratios than WT mutants under salt stress conditions [109]. These results strongly suggest that ROS generated by both AtrbohD and AtrbohF act as signal molecules to regulate $Na^+/K^+$ homeostasis, thus improving the salt tolerance of Arabidopsis plants [109]. Studies of $H_2O_2$ signaling in halophytes and glycophytes demonstrate that the kinetics of this process are faster in halophytes than in glycophytes [110]. In the halophyte *Cakile maritima*, $H_2O_2$ reached a peak in leaves 4 hours after salt stress treatment and was maintained after 16 h and 24 h of treatment before decreasing after 72 h. On the other hand, in *A. thaliana*, the $H_2O_2$ levels detected in leaves continued to be higher even after 72 h of treatment [94].

Nitric oxide (NO) is a free radical molecule involved in a wide spectrum of plant processes [111]. It contributes to salt stress tolerance by acting as a signal molecule when produced in small quantities; even when accumulated in the plant cell, NO enhances anti-oxidative responses by stimulating its special scavengers [112,113]. Moreover, some studies show that NO helps to maintain the $K^+/Na^+$ equilibrium in *Populus euphratica* under long-term salt stress [99]. It has also been found to contribute to $K^+$ homeostasis under salt stress by regulating both the $Na^+/K^+$ ratio and $H^+$-ATPases located at the plasma membrane [114] through its involvement in establishing a proton gradient generated by $H^+$-ATPase ($H^+$-PPase) activity, thus contributing to $K^+/Na^+$ homeostasis [115].

Up to now, little information has been available on how NO regulates $K^+$ homeostasis under salt stress in Brassicaceae species, especially in relation to the model plant *A. thaliana*. However, a recent study [116] has pointed out that NO is involved in regulating $K^+$ absorption and $Na^+/K^+$ ratios

under salt stress in *A. thaliana*. These authors have demonstrated that NO lowers *A. thaliana* $K^+$ absorption by repressing the AKT1 channel function. Thus, in *A. thaliana* exposed to salt stress, NO up-regulates $K^+$ absorption and the $Na^+$ to $K^+$ ratio through its impact on the gene encoded for vitamin B6; this leads to an excessive accumulation of vitamin B6 (PLP) which is responsible for the repression of $K^+$ channel AKT1 activity, resulting in a reduction in $K^+$ uptake by roots [116]. Arabidopsis mutant (*Atnoa1*) plants, with reduced endogenous levels of NO, display higher $Na^+$ to $K^+$ ratios in shoots than wild-type plants due to enhanced $Na^+$ accumulation and reduced $K^+$ accumulation when plants are exposed to NaCl, suggesting that NO is involved in maintaining $K^+/Na^+$ homeostasis [117].

The molecular connections, which explain how plants exposed to salinity rapidly accumulate NO and reorganize $Na^+$ and $K^+$ content, have begun to be elucidated. Thus, using an *A. thalina* mutant called *sno1* (sensitive to nitric oxide 1), which is allelic to the well-known mutant *sos4* (salt overly sensitive 4), NO has been shown to be capable of reducing $K^+$ channel AKT1-mediated plant $K^+$ uptake by modulating vitamin B6 biosynthesis [116]. However, in the halophyte *Kandelia obovata,* exposed to exogenous applications of NO, NO has been demonstrated to greatly contribute to the $K^+/Na^+$ balance under high salinity conditions by activating the AKT1-type $K^+$ channel and $Na^+/H^+$ antiporter, which are critical components in the $K^+/Na^+$ transport system [118].

**Table 2.** Type and number of superoxide dismutase (SOD) isozymes present in *C. maritima*, *T. salsuginea* and *A. thaliana*.

| Plant species | MnSOD | FeSOD | CuZnSOD | Ref. |
|---|---|---|---|---|
| *C. maritima* | 2 | 1 | 7 | 77 |
| *T. salsuginea* | 1 | 1 | 2 | 107 |
| *A. thaliana* | 1 | 3 | 3 | 120 |

## 8. Conclusion

The tolerance of plants to salt stress resides in their ability to maintain ion homeostasis despite competition from sodium ions. Plants need to preserve $K^+$ uptake under saline conditions in order to maintain their biological activity. Salt-tolerant plants are capable of maintaining high $K^+/Na^+$ ratios, which are later up-regulated at different levels through hormonal control, nitric oxide, ROS production, the antioxidant system and gene expression. A comparative study of the *A. thaliana*, *T. salsuginea* and *C. maritima* Brassicaceae species reveals that halophytes are more efficient in terms of salt resistance due to their ability to maintain $K^+$ uptake and high $K^+/Na^+$ ratios.

## Acknowledgements

**Conflict of Interest**

The authors declare that there are no conflicts of interest related to this study.

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