Plant Signaling & Behavior 5:3, 233-238; March 2010; © 2010 Landes Bioscience

Cytosolic calcium and pH signaling in plants under salinity stress

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Key words: cytosolic calcium, ionic toxicity, osmotic stress, pH, salinity stress, salt tolerance, signaling

Abbreviations: $[Ca^{2+}]$, cytosolic concentration of calcium; ER, endoplasmatic reticulum; pH_{cyt}, cytosolic pH

Calcium is one of the essential nutrients for growth and development of plants. It is an important component of various structures in cell wall and membranes. Besides some fundamental roles under normal condition, calcium functions as a major secondary-messenger molecule in plants under different developmental cues and various stress conditions including salinity stress. Also changes in cytosolic pH, pH_{cyt}, either individually, or in coordination with changes in cytosolic Ca^{2+} concentration, $[Ca^{2+}]_{cyt}$, evoke a wide range of cellular functions in plants including signal transduction in plant-defense responses against stresses. It is believed that salinity stress, like other stresses, is perceived at cell membrane, either extra cellular or intracellular, which then triggers an intracellular-signaling cascade including the generation of secondary messenger molecules like Ca^{2+} and protons. The variety and complexity of Ca^{2+} and pH signaling result from the nature of the stresses as well as the tolerance level of the plant species against that specific stress. The nature of changes in $[Ca^{2+}]_{cyt}$ concentration, in terms of amplitude, frequency and duration, is likely very important for decoding the specific downstream responses for salinity stress tolerance in planta. It has been observed that the signatures of $[Ca^{2+}]_{cyt}$ and pH differ in various studies reported so far depending on the techniques used to measure them, and also depending on the plant organs where they are measured, such as root, shoot tissues or cells. This review describes the recent advances about the changes in $[Ca^{2+}]_{cyt}$ and pH_{cyt} at both cellular and whole-plant levels under salinity stress condition, and in various salinity-tolerant and -sensitive plant species.

Introduction

Soil salinity increases. Soil salinity is a worldwide problem and poses a serious threat to world agriculture, since it reduces the crop yield in the affected areas. There are two groups of plants based on their responses to salt stress: halophytes and glycophytes. Halophytes are native in saline environment and grow well under that condition, whereas glycophytes cannot tolerate salt level to the same degree as halophytes. However, halophytes constitute only 1% of the world's flora.[1] Most of the terrestrial plants are glycophytes with varying level of salt tolerance. Among them most of the crop plants are very sensitive to salt stress. Although the exact information on global extent of salinity-affected areas is varying in different studies, the general perception is that more than 20 per cent of the irrigated land in the world is affected by soil salinity.[2-6] This is a big concern for attaining self-sufficiency in food production for the ever-increasing world population. Moreover, the loss of cultivable land, due to expansion of salinity areas through irrigation practices, as well as sea-level rising, is likely to increase over time and impinge on world food supplies.

Salinity stress in plants. Salinity stress reduces crop growth and yield in different ways. However, NaCl, the dominant salt in nature, elicits two primary effects on plants: osmotic stress and ionic toxicity. Under normal condition the osmotic pressure in plant cells is higher than that in soil solution. Plant cells use this higher osmotic pressure to take up water and essential minerals in root cells from the soil solution. Under salt stress the osmotic pressure in the soil solution exceeds the osmotic pressure in plant cells due to the presence of high salt, and thus, reduces the ability of plants to take up water and minerals like K^+ and Ca^{2+}.[7,8] On the other hand, Na^+ and Cl^- ions can enter into the cells and have direct toxic effects on cell membranes, as well as on metabolic activities in the cytosol.[9-11] These primary effects of salinity stress cause some secondary effects like reduced cell expansion, assimilate production and membrane function, as well as decreased cytosolic metabolism and production of reactive oxygen intermediates (ROSs).

Growth inhibition by Na^+ and/or Cl^- toxicity is one of the principal adverse effects of salt stress in plants. However, for Graminaceae crop like rice, Na^+ is the principal reason for causing damage (Tester and Davenport 2003).[12] The sodium ion (Na^+) is very harmful for most plant cells when it is present in the cytosol at concentrations higher than 10 mM. The potassium ion (K^+), on the other hand, is one of the essential and most abundant monovalent cations in cells, and needs to be maintained within 100–200 mM range in the cytosol for efficient metabolic functioning.[13-15] As a co-factor in cytosol, K^+ activates more than 50 enzymes, which are very susceptible to high cytosolic Na^+ and high Na^+/K^+ ratios.[8] Therefore, apart from low cytosolic Na^+,

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Submitted: 11/22/09; Accepted: 11/23/09
Previously published online:
www.landesbioscience.com/journals/psb/article/10740

maintenance of a low cytosolic Na^+/K^+ ratio is also critical for the function of cells.[16,17]

Tolerance mechanisms to salinity stress. Under NaCl-dominated salt stress the key mechanisms against ionic stress include the reduced uptake into the cytosol of the toxic ions, such as Na^+ and Cl^- and also sequestration of these toxic ions either into the apoplast or into the vacuole.[12,18-27] When compartmentalized into the vacuole, Na^+ is no more toxic for cells,[28,29] but also an advantage for growth and osmotic adjustment,[24,30] particularly since the vacuole may occupy more than 95% of the volume of a mature cell. Cytosolic Na^+ also can be compartmentalized in some other sub-cellular organelles like the ER and Golgi bodies.[31]

To combat with osmotic stress imposed by high salinity, plants need to synthesize compatible organic solutes, such as proline, glycine betaine, trehalose, sorbitol, mannitol, pinitol and sucrose in the cytosol.[15,26,32-36] Alternatively, K^+ and Na^+, if compartmentalized into the vacuole, could be the major compatible inorganic solutes used by the plant under salinity stress. For all these defence-response mechanisms to be active during osmotic stress and ionic toxicity, plants firstly need to perceive the stress and then activate the whole signaling cascade, starting by an elevation of $[Ca^{2+}]_{cyt}$, either in coordination with changes in cytosolic pH, pH_{cyt}, or individually.

The measurement of changes in $[Ca^{2+}]_{cyt}$ under high salinity stress has been performed by using different techniques, such as fluorescence microscopy measurements in root hairs,[37] in individual mesophyll protoplast[38,39] and in intact whole plant by using aequorin luminescence.[40-43] A number of studies also reported changes in pH_{cyt} in different plant species under salinity stress, which seem to vary between salinity sensitive and tolerant species. An understanding of the nature of changes in $[Ca^{2+}]_{cyt}$ and pH_{cyt} in salinity-sensitive and -tolerant plant species is very important for practical implication to identify potential future strategy to develop salinity-tolerant crop species. This article reviews the recent advances concerning the $[Ca^{2+}]_{cyt}$ and pH_{cyt} changes in both salinity-tolerant and -sensitive plants under salinity stress and their possible role in the signal transduction to activate stress-response mechanisms.

The role of calcium in plants. Calcium is an essential nutrient for growth and development of plants.[44,45] It plays important structural role in producing plant tissues and enables them to grow better. Calcium increases the plant tissues' resistance under various stress conditions including both biotic and abiotic stresses. Besides these fundamental roles, calcium has been recognized since long time as an important secondary messenger molecule in plants under various developmental cues, as well as under different stresses, including salinity stress. In plant cells the resting cytosolic concentration of calcium, $[Ca^{2+}]_{cyt}$, under normal condition is maintained at nanomolar level, mostly in the range of 10–200 nM, whereas the concentration of Ca^{2+} in cell wall, vacuole, endoplasmic reticulum and mitochondria is 1–10 mM.[46-48] However, specific signals, such as stress can trigger a sudden increase in the $[Ca^{2+}]_{cyt}$ level up to micromolar level that is toxic if it persists for longer time in the cytosol. Therefore, plants have evolved a system to take up excess Ca^{2+} and store it either into the apoplast or into the lumen of intracellular organelles, such as vacuole or endoplasmatic reticulum (ER). The latter stores, together with cell walls, can be used for elevating the $[Ca^{2+}]_{cyt}$ level under stress conditions and transduce the signal to subsequent defense responses.

Sensing of salt stress. Salinity stress, like many other abiotic or biotic stresses, has to be perceived before any changes of $[Ca^{2+}]_{cyt}$ and pH_{cyt} occur in the cells. Salinity stress in plants is sensed by both osmotic stress and ionic (Na^+ and/or Cl^-) toxicity and these stresses can be sensed either at the outer or inner surface of the plasma membrane by a trans-membrane protein, or within the cytosol by enzymes.[24] Several osmo-sensors are suggested to be involved in sensing the osmotic stress imposed by high salt, but will not be further discussed here.[49-54]

Concerning Na^+ toxicity in cells, a substantial progress was made in understanding the signal transduction under sodium stress through investigations on the Salt Overly Sensitive (SOS) pathway in Arabidopsis.[34] As proposed by Zhu[34] and also elaborately explained in a recent review by Mahajan et al.[55] the increase in $[Ca^{2+}]_{cyt}$ under salinity stress is read by SOS3, a Ca^{2+} sensor. The SOS3 protein interacts with a SOS2 protein kinase and the SOS3-SOS2 complex then activates the SOS1 protein, a plasma membrane Na^+/H^+ antiporter, thereby re-establishing Na^+ homeostasis in cells. In the SOS pathway, it still remains to clarify how Na^+ toxicity in cell is perceived. However, it has been suggested that the SOS1 protein, which has a long C-terminal tail residing in the cytosol, might sense Na^+.[24,26,56] Kader et al.[38] showed that Na^+ must enter into the cytosol to be sensed in rice protoplasts, which is consistent with the earlier suggestion that the SOS1 protein might sense Na^+ inside the cytosol. On the other hand, for the halophytic plant quince it was shown that Na^+ entry into the cell may not be necessary for cytosolic Ca^{2+} elevation.[39] Therefore, it remains to be clarified, what are the sensors for Na^+ toxicity in planta, and if they are different in different species, such as salinity-sensitive and salinity-tolerant ones.

Changes of $[Ca^{2+}]_{cyt}$ under salinity stress. *The calcium signature.* Within seconds after sensing of salinity stress a transient, stable or oscillating change in $[Ca^{2+}]_{cyt}$ concentration is elicited. This change is required for activating the downstream response mechanisms either through induction or downregulation of the responsive genes. The nature of the $[Ca^{2+}]_{cyt}$ signal in terms of amplitude, frequency and duration of the peak or signal likely has specific role in encoding the particular information for plants under salinity stress. Specific Ca^{2+} signatures are important for plant cells to sense for the subsequent events in the signaling process. Such processes may change with the particular stress,[57] the rate of stress development,[39,43,58] pre-exposure to the stress[40] and the tissue type.[43,57] In some studies with root tissue, or root protoplasts, salt stress was reported to reduce $[Ca^{2+}]_{cyt}$. Within minutes of application of 100 mM NaCl to root cells of Arabidopsis,[37,59] or to corn root protoplast,[60] there was a decrease in $[Ca^{2+}]_{cyt}$. In contrast, many studies revealed an increase in $[Ca^{2+}]_{cyt}$ after salt stress.[11,38-42,57,61-64]

From the results so far, it can be concluded that the change in $[Ca^{2+}]_{cyt}$ is not uniform and varies with species, cell type or tissue type.[38,39,43,57,59] It can also be considered that the specific Ca^{2+} signatures (the increase with different amplitudes and their

duration), as well as the amount of $[Ca^{2+}]_{cyt}$ increased, vary in different studies due to the techniques used and also due to the type of experimental materials used, such as whole plant, root or specific cell types.

Sodium toxicity and osmotic stress may induce different signals in roots and shoots. The results are contrasting in studies whether osmotic stress increases or decreases $[Ca^{2cyt}]$.[38,40,57,59] Moreover, the changes in $[Ca^{2+}]_{cyt}$ differ both in terms of concentration and amplitude depending on the amount of stress given to the cell.[39,43] Tracy et al. found that the osmotic and ionic components of salinity stress induced differential increases in $[Ca^{2+}]_{cyt}$ in Arabidopsis root cells.[43] They reported that the heterogenous $[Ca^{2+}]_{cyt}$ changes under NaCl stress were restricted to the root. Also in experiments with rice protoplasts, as well as with quince protoplasts, different $[Ca^{2+}]_{cyt}$ changes were obtained under sodium stress and under osmotic stress.[38,39] Kiegle et al.[57] reported significant quantitative differences in $[Ca^{2+}]_{cyt}$ elevation in different cell types of Arabidopsis roots. Both under osmotic stress (440 mM mannitol) and salt stress (220 mM NaCl) the endodermis and pericycle cells displayed prolonged oscillation in $[Ca^{2+}]_{cyt}$ that were distinct from the responses of other cell types.

The amplitude of $[Ca^{2+}]_{cyt}$ differs. In experiments with single cell protoplasts and fluorescence microscopy, the increase in $[Ca^{2+}]_{cyt}$ under different types of stress or upon auxin addition was in nanomolar level.[38,39,65-67] On the other hand, the increase in $[Ca^{2+}]_{cyt}$ concentration was at µmolar level in most of the studies that measured $[Ca^{2+}]_{cyt}$ changes by use of calcium reporting protein, aequorin and luminescence microscopy.[40,42,43,57] Different changes in $[Ca^{2+}]_{cyt}$ could depend on a signal variation between root and shoot cells, as obtained by Tracy et al.[43] Moreover, in experiments with intact plants, or tissues referred above, the increase in $[Ca^{2+}]_{cyt}$ obtained shows a mean value of many cells. The luminescent signal from aequorin expressed in whole plant or tissues may not reflect the Ca^{2+} signal in individual cells as suggested by Dodd et al.[68] It is also likely that cell-wall signaling can take place.[41] In that case the transient spikes in µmolar range found in experiments with tissue or intact plants could depend on cell wall signaling. In a recent study, Tracy et al.[43] found through analysis of spatiotemporal $[Ca^{2+}]_{cyt}$ dynamics, additional levels of complexity in the $[Ca^{2+}]_{cyt}$ signal and suggested that the signals in a cell population might be the result of oscillatory changes of single cells. From the above discussion it is evident that the data on changes of $[Ca^{2+}]_{cyt}$ under salinity stress vary depending on the techniques of measuring the data, duration and intensity of salinity stress imposed, osmotic and ionic components, plant species and type of cells or tissues used.

Sources for $[Ca^{2+}]_{cyt}$ changes. The changes in $[Ca^{2+}]_{cyt}$ appear to be supplied from the the apoplast (influx of Ca^{2+} across the plasma membrane) or from the internal stores like ER, Golgi bodies, mitochondria or vacuole.[69] Calcium-permeable channels in the plasma membrane, which are activated by membrane depolarization, are thought to lead to elevation of $[Ca^{2+}]_{cyt}$ in many species after the perception of a range of stimuli.[38-40,67,69-74] The generation of Ca^{2+} increase in the cytosol further modulates other messengers like inositol phosphate, which induces a further Ca^{2+} elevation in the cytosol through the opening of inositol-(1,4,5)-triphosphate (IP_3)-regulated Ca^{2+} channels.[43,69] There are some studies showing that salinity stress induces a rapid increase in IP_3 concentration in the cytosol.[75,76] By use of the inhibitors verapamil and nifedipine for plasma membrane Ca^{2+}-permeable channels,[38,39,67,77-79] and LiCl for inhibition of Ca^{2+} release from internal stores, like vacuole or ER, the major sources for $[Ca^{2+}]_{cyt}$ dynamics could be suggested.[38,40,80-82] In **Figure 1** a proposed model of salt-stress signaling and tolerance mechanisms is included.

Changes of Ca^{2+} concentration in different parts of the cell. Since long time it has been believed that transient elevation in $[Ca^{2+}]_{cyt}$ concentration plays a vital role in the signaling cascade for the downstream adaptive responses to stress conditions. Different parts of the cell, such as cell wall, ER, Mitochondria, chloroplast and vacuole are thought as sources of $[Ca^{2+}]_{cyt}$ elevation, as mentioned earlier. In a few studies calcium-dependent signaling processes have been proposed to proceed in apoplast and also in some other cell organelles, for example chloroplast[41,83] and mitochondria.[84] More recently, cell nuclei have been suggested to generate their own calcium signals under various stimuli.[85,86] It is still to be clarified whether these cell organelles have their own Ca^{2+}-signaling system under salinity stress and, if so, what are the adaptive responses they induce by the signal. It could be suggested that a Ca^{2+} signal from the cell organelles appears later or even earlier than the cytosolic signal, as they all may have their distinct pathway of activating downstream mechanisms, as suggested by Mazars et al.[86]

The role of pH in stress signaling. It is likely that not only Ca^{2+}, but also protons, function as second messengers in plant cells, since there are steep differences in both calcium concentration and pH within a plant cell. Under normal condition the cell cytosol pH is around 7.5, while the apoplast and vacuolar lumen have a pH around 5.5.[15] Moreover, intracellular pH can be dramatically modulated for transferring the signal to downstream responses.[87,88] Changes in intracellular pH are reported for many developmental issues in plants, such as root tip growth,[89-91] nodulation,[92,93] elicitation of benzophenanthridine alkaloids,[94] response to hormone activity such as gibberellic acid[95] and abscisic acid.[96] Changes in pH are also found during plant defence responses against various stresses.[38,97,98] An influx of H^+ from the apoplast into the cytosol was reported for many plants during elicitation of the hypersensitive response,[98] whereas an efflux of H^+ from the cytosol into the apoplast or vacuole was obtained in case of some other plants species.[38,41]

Changes of pH_{cyt} under salinity stress. Change in intracellular pH also acts as secondary messenger in response to different stress conditions including salinity stress.[87,88,98] The $[Ca^{2+}]_{cyt}$ and pH homeostasis in cells are closely linked.[99] Upon shifting of $[Ca^{2+}]_{cyt}$ under salinity stress, cells are challenged with the excess of other monovalent ions in the cytosol like H^+.[41,58,100] Transient shifts in intracellular and apoplastic pH are reported as essential steps in several signal-transduction processes, and pH is involved in cell signaling, either directly, or in cross talk with plant hormones, or Ca^{2+}.[41,87,101-104] However, the nature of cytosolic pH change also differs depending on osmotic and ionic components of salinity stress and plant species.[6,38,41] Gao et al.[41] reported that osmotic stress imposed by mannitol did not alter pH_{cyt} in intact

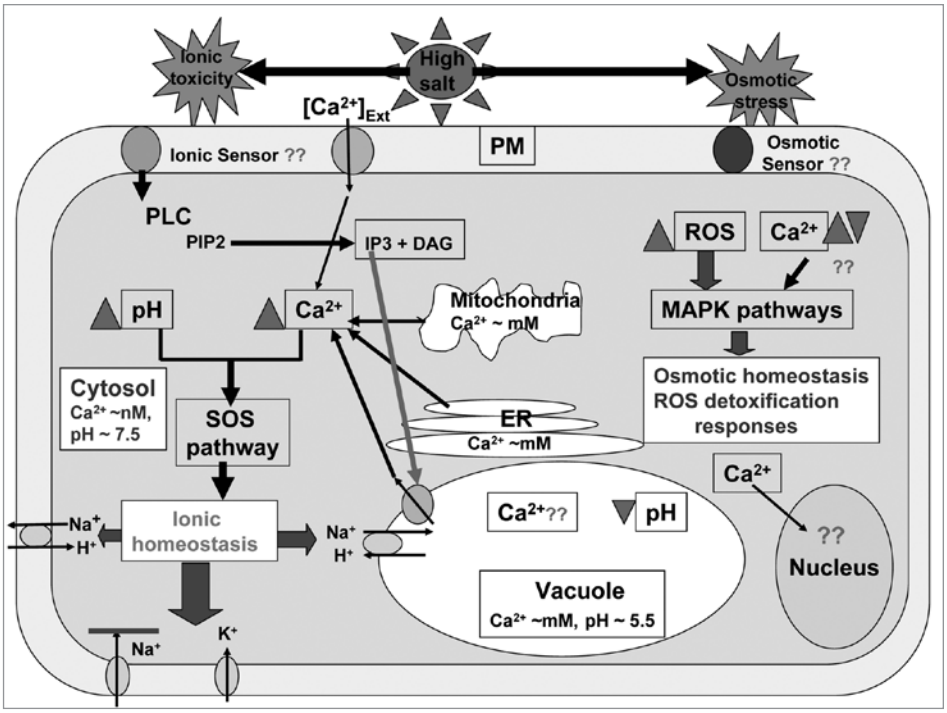

Figure 1. A proposed model of salt-stress tolerance in plants at cellular level. The sensing of *ionic stress* induces a transient elevation of cytosolic Ca^{2+} and pH.[6,38,39,43] Cytosolic-Ca^{2+} elevation is attributed with the influx of Ca^{2+} from cell wall, as well as from the vacuole. ER and mitochondria may also contribute to cytosolic-Ca^{2+} elevation. The cytosolic-pH increase seems to be linked with vacuolar-pH decreases.[38] Cytosolic Ca^{2+} elevation and pH increase, either independently or in coordination, induces the SOS pathway for ionic homeostasis through inhibition of Na^+ entry, enhancing K^+ uptake into the cell, and sequestration of cytosolic Na^+ either into the apoplast or vacuole.[24,34,55] Cell organelles, such as nucleus, mitochondria, ER etc., might also have their own Ca^{2+} elevation and subsequent signaling cascade for downstream responses.[85,86] *Osmotic stress*, in contrast to ionic toxicity, may either decrease or increase cytosolic-Ca^{2+} level, which might be linked with the production of reactive oxygen species (ROS).[52] ROS then activates the MAPK pathways for osmotic homeostasis and detoxification responses.

Arabidopsis roots, whereas addition of NaCl to the same roots caused a decline in pH_{cyt}. Also osmotic stress imposed by sorbitol did not change pH_{cyt} in rice or quince.[38,39] On the other hand, the ionic component induced a transient cytosolic acidification under salinity stress in salinity-sensitive rice.[38] A rise in vacuolar pH, which could be attributed to cytosolic acidification, was obtained in salt-sensitive plants upon exposure to salinity stress.[105,106] In contrast, Halperin et al.[37] did not find any change in cytosolic pH in Arabidopsis roots upon salt stress.

In case of salt-tolerant species reported so far, it has been found that pH_{cyt} increased under salinity stress and that the increase was attributed to the ionic component of salinity stress, not the osmotic stress.[38,39] This is consistent with the study of Caracuel et al.[107] that shows that salinity tolerance in *Fusarium oxysporum* is correlated with the activation of PacC, a transcription factor that activates Na1-ATPase, by alkaline pH in the cytosol. By contrast, cytosolic acidification in *Saccharomyces cerevisiae* confers salt tolerance by activating NHA, a plasma membrane Na^+/H^+ antiporter.[108,109] To our knowledge there is no report showing cytosolic acidification in salinity-tolerant plant species. Therefore, we believe that cytosolic alkalinization under the ionic component of salinity stress could be a unique trait of salinity-tolerant plant species. Shifting in cytosolic pH is likely related to a change in pH either in apoplast (if H^+ movement occurs between cytosol and apoplast) or in vacuole (if H^+ movement occurs between cytosol and vacuole). Kader et al.[38] showed a vacuolar acidification in combination with cytosolic alkalinization in salt-tolerant rice cultivar Pokkali. It is quite interesting that the regulatory C-terminal domain of the tonoplast Na^+/H^+ antiporter resides in the vacuole,[110] and the antiporter is suggested to be regulated by changes of pH in the vacuole.[111] Indeed, vacuolar compartmentalization of cytosolic Na^+ is the dominant tolerance mechanism in salt-tolerant rice cultivar Pokkali under NaCl-dominated salt stress.[6,27,112]

Conclusion

Salinity-tolerance in plants is a multigenic trait with many quantitative trait loci (QTLs) associated with ion transport and tolerance.[5] Therefore, plants need to possess a wide range of adaptation mechanisms for osmotic stress as well as ionic toxicity to be tolerant under high salinity. For the development of salinity-tolerant crop species it is imperative to have very clear understanding of the tolerance mechanisms available in plants. Generation of messenger molecules like changes in cellular Ca^{2+} and pH with specific amplitude and duration, and also in specific organelles, seems to play very vital role for activating the defence-response mechanisms for salinity tolerance. Until recently, studies mostly concentrated on changes of cytosolic Ca^{2+} and pH signaling in plants under salinity stress. However, recent reports are suggesting that changes in Ca^{2+} concentration, as well as changes in pH

in a cell, could occur in other parts as well, such as apoplast, mitochondria, nucleus, vacuole etc. It is likely that salinity stress elicits differential Ca^{2+} and pH signatures in different parts of the cell to activate specific tolerance mechanisms and these signatures are likely to vary between salinity tolerant and sensitive plant species. There are few reports showing difference in cellular Ca^{2+} and pH signatures between salinity sensitive and tolerant plant species and, therefore, more attention to this area is needed.

Acknowledgements

This work was supported by Wallenberg foundation and Sida, Sweden.

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