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PII: S0098-8472(18)30949-3
DOI: https://doi.org/10.1016/j.envexpbot.2018.08.018
Reference: EEB 3544

To appear in: *Environmental and Experimental Botany*

Received date: 24-6-2018
Revised date: 5-8-2018
Accepted date: 16-8-2018

Please cite this article as: Bosnic P, Bosnic D, Jasnic J, Nikolic M, Silicon mediates sodium transport and partitioning in maize under moderate salt stress, *Environmental and Experimental Botany* (2018), https://doi.org/10.1016/j.envexpbot.2018.08.018

Silicon mediates sodium transport and partitioning in maize under moderate salt stress

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Highlights

- Silicon up-regulates expression of maize *ZmSOS1* Na^+ exporter genes in root tissues
- Silicon increases leaf accumulation of Na^+ due to enhanced xylem loading of Na^+
- Silicon enhances sequestration of Na^+ into the vacuoles of leaf mesophyll cells
- Silicon shifts maize response to salinity towards that typical for halophytes

Abstract

Silicon (Si) is known to alleviate salt stress in various crops; however, the influence of Si on sodium (Na) transport and partitioning at the tissue, cell and organelle levels is poorly understood. Maize (*Zea mays* L.) hybrid sensitive to salt stress was exposed to moderate salt stress (40 mM NaCl; simulating conditions in salinized agricultural soils) without or with supply of 1.5 mM $Si(OH)_4$. We investigated the expression of *SOS* genes encoding Na^+ efflux transporter in various root tissues of maize, paralleled by measurements of tissue Na

concentration. In addition, subcellular localization of Na (using Na fluorescent dye) within the leaf mesophyll cells was also performed. Silicon supplied plants accumulate less Na in both root apex and cortex, but allocate more Na^+ to the leaves via the xylem. This was accompanied by increased expression of *ZmSOS1* and *ZmSOS2* in the root apex and cortex facilitating Na^+ exclusion, and in the root stele for enhanced Na^+ loading into the xylem. Also, Si down-regulated the expression of *ZmHKT1* in the root stele, which further decreased Na^+ unloading from the xylem. Consequently, Si increased accumulation of Na in leaves, but also enhances sequestration of Na^+ into the vacuoles thereby decreasing Na^+ accumulation in the chloroplasts. In response to moderate salt stress in maize, Si shifts the typical glycophyte behavior of this species towards that of halophytes.

Keywords: maize (*Zea mays* L.); salinity; silicon; sodium; SOS1 genes; vacuole.

1. Introduction

Soil salinity is one of the major constraints severely affecting crop yield and food production worldwide. Although NaCl is a major salt present in saline soils, for most crops toxicity of sodium (Na^+) occurs before chloride (Cl^-) ions (White and Broadley, 2001). At higher concentration Na^+ can disrupt osmotic homeostasis, impose imbalance affecting other ions and create oxidative stress as a secondary problem (Zhu, 2001). On the other hand, Na^+ can also be used as a “cheap osmoticum” for maintaining osmotic gradient between the growing cells and salinized surrounding (Kronzucker and Britto, 2011). Many salt tolerant species

(halophytes) accumulate Na to a higher extent than non-tolerant species (glycophytes) (Flowers et al., 1977; Muns and Tester, 2008), which suggests greater ability to manage Na partitioning at the whole plant level. It was argued that protecting photosynthetic apparatus and the other leaf cell organelles from Na^+ toxicity is a mopoe likely mechanism involved in salt tolerance of halophytes, rather than the existence of specific adaptations of enzymes (Munns et al., 1983). Although it appears that both glycophytes and halophytes possess similar mechanisms involved in maintaining Na^+ homeostasis, the halophytes operate more efficiently (Shabala and Mackay, 2011). Furthermore, both groups showed the same set of genes involved in Na homeostasis, but with different expression profiles (Zhu, 2001; Rozema and Schat, 2013).

Although the mechanism by which Na^+ enters the root cell has not been clearly elucidated, it has been assumed to be mainly passive process, which appears to be poorly controlled (Tester and Davenport, 2003). Therefore, plants have developed various mechanisms regarding to the controlled root efflux, fine-tuned xylem loading and unloading, and sequestration of Na^+ at the organ, tissue, cell and organelle levels (e.g. Tester and Davenport, 2003; Muns and Tester, 2008; Shabala and Mackay, 2011). Among these, the transport role of salt overly sensitive 1 (SOS1) and chloride cation co- transporter (CCC) for xylem loading of Na^+, the high-affinity potassium transporter 1 (HKT1) for Na^+ retrieval from the xylem, and the members of tonoplast-localized Na^+/H^+ antiporter (NHX) for vacuolar sequestration of Na^+ are essential (Shi et al., 2002; Munns and Tester, 2008; Kronzucker and Britto, 2011; Zhu et al., 2017).

SOS1 is Na^+ efflux plasma membrane protein primarily expressed in the epidermal cells of root apex and in the xylem parenchyma cells; it has been shown that it is not essential for plant growth and development under optimal growth conditions, but it is important under

saline environment (Shi et al., 2002). SOS1 exports Na^+ accumulated in the root cortex to the soil solution, thus preventing excess xylem sap loading of Na^+ (Shi et al., 2000), whereas SOS1 localized in the xylem parenchyma is involved in active xylem loading of Na^+ (Shi et al., 2002). So far, SOS1-mediated transport of Na^+ has been reported in several plant species such as maize (*Zea mays*) (Estrada et al., 2013), Arabidopsis (*Arabidopsis thaliana*) (Shi et al., 2002; Shabala et al., 2005), tomato (*Lycoperscion esculentum*) (Olias et al., 2009) and the non-crop halophyte *Salicornia dolichostachya* (Katschnig et al., 2015). The salt up-regulated SOS1 transport protein is partially positively regulated by a serine/threonine protein kinase (SOS2) and sensor calcium-binding protein (CBL) (Zhu, 2000; Lin et al., 2009; Zhao et al., 2009), although this regulation has not been confirmed in the study of Shabala et al. (2005) using Arabidopsis *sos* mutants.

HKT1 is primarily localized in the root stele controlling root-to-shoot Na distribution by unloading of Na^+ from the xylem (Davenport et al., 2007). This unloading role of HKT1 has been confirmed in Arabidopsis *hkt1* mutant which, compared to the wild type, accumulated more Na^+ in the xylem sap (Mäser et al., 2002), whereas its overexpression in the root stele markedly decreased shoot Na^+ accumulation (Møller et al., 2009). It also appears that both SOS1 Na^+-loading and HKT1 Na^+-unloading proteins co-localize at the same *Nax* loci of the xylem parenchyma (Zhu et al., 2016).

Once transported to the leaves via the xylem, Na^+ enters the leaf mesophyll cells. The vacuoles occupy most of the intracellular space of leaf mesophyll cells, so that vacuolar sequestration facilitated by NHX (e.g. Zörb et al., 2005; Bassil et al., 2011) presents an efficient strategy to minimize and retard the detrimental effect of Na^+ toxicity and simultaneously contributes to osmotic adjustment (Flowers et al., 1977).

The beneficial effect of Si under saline conditions has been reported in various plant species including rice (*Oryza staiva*) (Yeo et al., 1999), barley (*Hordeum vulgare*) (Liang, 1999), cucumber (*Cucumis staivus*) (Zhu et al., 2004), tomato (Romero-Aranda et al., 2006), sorghum (Yin et al., 2016), and maize (Moussa, 2006). The addition of Si stimulated synthesis of polyamines (Yin et al., 2016), decreased oxidative damage attributed to Si-increased activity of key antioxidant defence enzymes (Zhu et al., 2004), and modified leaf gas exchange (Soundararajan et al., 2017). Moreover, Si enhanced cell membrane stability and integrity, an important factor in mitigating sodic (Na^+) stress (Liang, 1999). It has been demonstrated that Si regulates transpiration-derived apoplastic Na^+ flow by promoting Casparian band formation in the root endodermis of rice (Yeo et al., 1999). Also, Si enhanced both H^+-ATPase and H^+-PPase activities in root of salt-stressed barley (Liang et al., 2005) creating an electrochemical gradient, thereby providing the driving force for H^+/Na^+ antiport for both plasma membrane and tonoplast. In short-term experiment (30 min) Malagoli et al. (2008) did not find differences in radiolabeled $^{24}Na^+$ efflux to the outer solution from rice roots exposed to Na-silicate (not directly available source for Si uptake) and NaCl, which was further interpreted in the review paper of Coskun et al. (2016) in the way that Si had no effect on putatively SOS1-mediated Na^+ efflux. However, as far as we are aware the direct effect of Si on the expression of *SOS* genes has not been experimentally demonstrated so far.

Soil salinity stress is one of the major threats to sustainable maize production because maize is a glycophyte plant very sensitive to salinity stress (Zhang et al., 2018 and references cited therein). Although maize cultivars differ greatly in their sensitivity to salinity (Zörb et al., 2005) this is not always associated with tissue accumulation of Na^+ (Cramer et al., 1994). Also, maize is a typical Si-accumulating species and transporters (ZmLsi1 and ZmLsi2) for active transport of monosilicic acid [$Si(OH)_4$] have been characterized in maize roots (Mitani et al., 2009a,b), whereas most of the species such as Arabidopsis, tomato and some

halophytes used in the complementary studies so far are not Si accumulators. The main objective of present study was therefore to investigate the role of Si in regulation of Na^+ transport through SOS1 in maize roots grown under moderate salt stress, which is more relevant for agriculture practice and also diminish the osmotic component of salt stress (e.g. Zörb et al., 2009). In addition, in this work we aimed to elucidate the influence of Si on subcellular partitioning of Na^+ in the leaf mesophyll cells.

2. Materials and methods

2.1. Plant material and growth conditions

Maize (*Zea mays* L.) hybrid ZP-560 seeds (Zemun Polje Maize Research Institute, Serbia), characterized as a salt-sensitive genotype (Mandic et al., 2014), were surface sterilized with 10% H_2O_2 for 10 min, soaked in 1 mM $CaSO_4$ overnight and germinated in the rolls of filter paper in dark at 25°C for 4 d. The 4-d-old uniform seedlings were transferred to continuously aerated nutrient solution (3 plants per 3 L-plastic pot) containing (mM): 0.7 K_2SO_4, 0.1 KCl, 2.0 $Ca(NO_3)_2$, 0.5 $MgSO_4$, 0.1 KH_2PO_4, and (in µM): 100 µM $Fe^{III}EDTA$, 0.5 $MnSO_4$, 0.5 $ZnSO_4$, 0.2 $CuSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$, 10 H_3BO_3. Thereafter, 7 d old plants were pre-cultured for 14 d in the nutrient solution without (-Si) or with (+Si) 1.5 mM Si supplied as $Si(OH)_4$, freshly prepared according to Nikolic et al. (2007), and then subjected to 40 mM NaCl (+Na/-Si and +Na/+Si, respectively) for 14 d. In addition, the control plants were grown in a standard nutrient solution without supply of either $Si(OH)_4$ or NaCl (control). Nutrient solutions were renewed every second day and the pH was adjusted to 6 and checked daily. Plants were grown under controlled environmental conditions in a growth chamber with a photoperiod of 16 h : 8 h, temperature regime of 24°C : 20°C (light : dark), photon flux density of 250 µmol m^2 s^{-1} at plant height (provided by led panels; Apollo 8, Cidly Co., Ltd., Shenzhen, China), and relative humidity of about 70%.

The plants were harvested 5 weeks after germination (6 h after light onset) unless mentioned separately in the text. At harvest, plants were divided in two parts, root and shoot, oven dried at 65°C for 48 h, their dry weight was recorded, and the samples were used for Na determination.

2.2. Collection of xylem sap

Xylem sap was collected after plants were decapitated at the stem about 2 cm above the root-shoot interface. To avoid contamination, after 5 min the cut stem was washed with deionized water and soft silicon tube was fixed over decapitated stem; xylem sap was collected for 1h.

2.3. Chlorophyll determination

Chlorophyll content in the fourth topmost fully expended leaves (9 plants per treatment) was approximated nondestructively using a portable Chlorophyll Meter SPAD-502 device (Minolta Camera Co., Osaka, Japan).

2.4. Separation of root tissues

The root tissue quantification of Na was carried out in the three main root segments (i.e. root apex, stele and cortex). Intact roots were washed with deionized water thoroughly, and the root apex (approximately 3 mm length) was cut out. The stele (central cylinder) was mechanically separated from the cortex (the root part approximately 5 cm length located between root apex and lateral root zone) by fine forceps, so that the cortex was undamaged as observed by a light microscope. The tissue material was oven dried at 65°C for 48 h, weighed and used for Na determination.

2.5. Determination of Na

The pulverised dry leaf tissue material (0. 2 g) was digested in 3 mL of conc. HNO_3 + 2 mL of H_2O_2 for 1 h in a microwave oven (Speedwave MWS-3+; Berghof Products + Instruments GmbH, Eningen, Germany). The freshly collected xylem sap was diluted 100 to 500 times (v/v) with deionized water. In all samples, Na was determined by inductively coupled plasma optical emission spectrometry (ICP-OES; SpectroGenesis EOP II, Spectro Analytical Instruments GmbH, Kleve, Germany).

2.6. Determination of Si

Digested leaf samples as described above were diluted with about 15 ml deionized H_2O, transferred into 25 ml-plastic flasks, 1 ml conc. HF was added, and left overnight. After a final dilution of the samples of 1:100 (v/v) concentration of Si was determined by ICP-OES.

2.7. Subcellular localization of Na in leaves

Leaf disks (3 mm in diameter) were infiltrated with 2.5 mM MES (pH 6.5) and 15 µM Na fluorescent dye (Sodium Green, Molecular Probes, USA) in a vacuum-desiccator in several cycles of reducing the pressure, and left to slowly revert to atmospheric pressure until leaflets become completely dark. After incubation for 1.5 h, intact leaf discs were briefly washed with 2.5 mM MES (pH 6.5) solution, placed in oil immersion and analyzed by confocal laser scanning microscopy (Leica SP8, Leica Microsystems GmbH, Wetzlar Germany), using 40 x objective, with the excitation at 488 nm and the emission detection at 510-560 nm. According to the manufacturer the peak excitation and emission wavelengths are in the regions of spectrum where cellular autofluorescence and scattering backgrounds are often low. Quantification of the Sodium Green fluorescence corresponding to Na^+ localized in the organelles (chloroplasts and vacuoles) was performed by Leica LAS AF Software and expressed in arbitrary units.

2.8. Lipid peroxidation assay

Lipid peroxidation level was determined by measuring the production of malondialdehyde (MDA) in the thiobarbituric acid (TBA) reaction according to the method of Hodges et al. (1999). Plant tissue was homogenized in 20% trichloroacetic acid (TCA) and centrifuged at 14,000 g for 20 min at 4ºC. Equal amounts of supernatant and 0.5% TBA in 20 % TCA (w/v) were mixed and heated at 95ºC for 30min. The reaction was stopped by ice-cooling of the tubes. After centrifugation of the reaction mixture at 14,000 g for 15 min, the absorbance was monitored at 532 nm spectrophotometrically, and corrected for unspecific absorption at 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.9. RNA extraction and real-time quantitative PCR analysis

Fresh plant tissue samples (the root tissues separated as described above and the leaf tissue devoid of midrib) were frozen in liquid nitrogen and extracted with 8 M guanidine hydrochloride, 20 mM MES, 20 mM EDTA and 2% β-mercaptoethanol in a mortar. Total RNA was purified using standard phenol-chlorophorm procedure. To remove DNA from the RNA extracts, DNA-free kit (Thermo Fisher Scientific, Waltham, MA, USA) was used according to the manufacturer's protocol.

Reverse transcription reaction was carried out with RevertAid Reverse Transcriptase (Thermo Scientific) using Random Hexamer Primers (100 pmol). Maxima SYBR Green/ROX qPCR master mix (Thermo Scientific) and 400 nM of each primer were used for Real-Time PCR reaction performed on the ABI Prism 7500 Sequence Detection System (Applied Biosystems) and the results were analyzed with 7500 System Software (Applied Biosystems).

The primers used in this study are: for *SOS1* 5'-ACTTGCAGGAGGAATACAAC-3' and 5'-CGAGAAGAGAAGACCACATC-3', for *SOS2* 5'-AAGTTTCCGTTGTTGCTTC-3' and 5'-ACCTCTCTTCAGTTTTGCAC-3', for *HKT1* 5'-TGCTAATGTTTATCGTGCTG-3' and 5'-AGGCTGATCCTCTTCCTAAC-3', for *NHX* (also referred as Na^+/H^+ Exchanger) 5'-CGTGATGTCGCATTACACCT-3' and 5'- CTGGCAAACTCCCACTTCTC-3', and for Ubiquitin *(UBQ)* 5'-GTTGAAGCTGCTGCTGTATCTGG-3' and 5'-GCGGTCGCACGATAGTTTTG-3'. Levels of transcription were calculated with the $2^{-\Delta Ct}$ method using *UBQ* as an internal control. Each PCR was done in triplicate and included no template controls. Sequence data used in this study can be found in the GenBank/EMBL database under the following accession numbers (in parentheses): *ZmSOS1* (NM001176582), *ZmSOS2* (EU907939), *ZmHKT1* (HQ845286), *ZmNHX* (AY270040) and *ZmUBQ* (U29162).

2.10. Statistical analyses

Data were subjected to ANOVA and differences between treatment's means were compared by a posteriori Tukey's test at significance level of 0.05, using the statistical software Statistica 6 (StatSoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Plant growth, leaf chlorophyll content, accumulation of Na and tissue damage

The addition of Si in the form of $Si(OH)_4$ (1.5 mM) to the nutrient solution significantly improved plant growth performance (e.g. dry biomass) of maize plants subjected to moderate salt regime of 40 mM NaCl (Table 1). Both root and shoot dry matter weight was reduced by NaCl stress compared to the control plants. However, Si supply (+Na/+Si) considerably increased shoot but not root dry masses compared to the +Na/-Si treatment. It is important to note here that addition of Si did not affect root and shoot dry biomass and Na concentration

in maize plants grown in the absence of NaCl stress, nor NaCl supply affected Si concentration in both root and leaves (Table 1). On the other hand, Si supply significantly increased the Na concentration in the leaves of NaCl stressed plants (+Na/+Si) in comparison with +Na/-Si plants (Table 1). Exposure of maize plants to a moderate NaCl stress decreased the leaf chlorophyll content measured as SPAD units, and addition of Si significantly increased SPAD readings in NaCl stressed plants (Table 1).

To assess the effect of salt stress on the leaf membrane oxidative damage by ROS, malondialdehyde (MDA) as a product of lipid peroxidation was determined. The lipid peroxidation was significantly increased in the leaves of maize plants exposed to a moderate salinity, whereas supply of Si significantly decreased MDA concentration (Table 1).

3.2. Root spatial distribution and vascular movement of Na

Measurement of spatial Na concentrations in three main root segments showed different patterns of accumulation. Sodium concentration in the root apex and root cortex decreased significantly in +Na/+Si plants compared to the +Na/-Si plants (Fig. 1). The most prominent difference was in the root apex (Na concentration was decreased by 42% compared to +Na/-Si treatment, Fig. 1A). Similar pattern was also observed in the root cortex (Na concentration decreased by 29% compared to +Na/-Si treatment, Fig. 1B). By contrast, Na concentration in the root stele increased by 8% in +Na/+Si plants compared to +Na/-Si treatment (Fig. 1C). Concomitantly, we measured the xylem concentration of Na^+ in the time course experiment (Fig. 2). The +Na/+Si plants showed no difference in the xylem sap Na^+ concentration after 6 h of NaCl treatment compared to +Na/-Si treatment, while after 24 h the xylem concentration of Na^+ increased by 21% compared to +Na/-Si plants (Fig. 2).

3.3. Expression of Na^+ transporter genes in roots

Expression analyses of the genes coding key Na^+ efflux protein SOS (responsible for Na^+ export to the apoplast) in root apex showed up-regulation pattern in response to NaCl stress (Fig. 3A,B). The changes of expression levels of gens encoding SOS1, and its regulator SOS2 kinase, responsible for Na^+ efflux were more prominent in the Si supplied plants (Fig. 3A,B). Supply of Si also increased the expression of *SOS1* and *SOS2* genes in the root cortex (Fig. 3C,D). Furthermore, expression analyses of *SOS1* and *SOS2* genes in the isolated stele showed a similar expression pattern as in the root apex (Fig. 3E,F). However, the relative expression of *HKT1* gene encoding Na^+ influx transporter responsible for xylem unloading was significantly decreased in the stele of Si-fed plants (Fig. 4).

3.4. Subcellular partitioning of Na^+ and expression of NHX in leaves

Confocal microscopy images of Na^+ specific fluorescence in the leaf mesophyll of maize plants exposed to 40 mM NaCl are shown in Figure 5A. In the non-Si treated plants Na^+ was localized mainly in the chloroplasts, whereas in the Si supplied plants Na^+ was mainly restricted to the vacuolar regions (Fig. 5A,B). In the leaf segments of plants without Si, fluorescence of Na-Green dye was undetectable due to a low Na^+ content (not shown). The stimulating effect of Si on the vacuolar sequestration of Na^+ was further supported by higher relative expression of *NHX* responsible for Na^+ loading into the vacuole (Fig. 5C).

4. Discussion

In general, lowering cytosolic Na^+ pools by decreasing of Na^+ influx from the external solution into the cytosol, and the increase of Na^+ sequestration in the vacuole have been proposed to be major salt-tolerance mechanisms (Munns and Tester, 2008). However, control of Na^+ efflux rather than vacuole compartmentation is considered as a key mechanism in the

root meristem tissue due to the fact that the root apical cells do not possess large vacuoles (Shi et al., 2002).

The SOS pathway is considered to be essential in controlling both Na^+ efflux out of root cortex and long-distance transport within plant tissue via the xylem (Shi et al., 2002; Olias et al., 2009; Munns and Tester, 2008). In Arabidopsis, *sos1* mutant exposed to moderate (but not severe) salinity showed lower leaf accumulation of Na, suggesting that SOS1 is involved in the xylem loading of Na^+ (Ding and Zhu, 1997; Shi et al., 2002). Therefore, increased expression of *ZmSOS1* and *ZmSOS2* (Fig. 3E,F) observed in the mechanically isolated root stele of Si supplied maize plants exposed to 40 mM NaCl is in agreement with the increased Na^+ accumulation in both the xylem sap (Fig. 2) and the leaf tissue (Table 1). Furthermore, addition of Si also increased expression of *ZmSOS1* and *ZmSOS2* involved in Na^+ efflux in both root apex and cortex regions (Fig. 3A-D) which resulting in decreased Na accumulation in the root tissue (Table 1; Fig. 1).

Opposite to SOS1, HKT1 primarily expressed in the xylem parenchyma cells is responsible for retrieving Na^+ from the xylem vessels and hence preventing excessive accumulation of Na^+ in the shoot (Mäser et al., 2002; Davenport et al., 2007). However, very recent study of Zhang et al. (2018) showed that loss of ZmHKT1 function in maize increases xylem sap Na^+ concentration causing increased accumulation of Na^+ in leaves and salt hypersensitivity. Interestingly, the expression of *HKT1* was not detected in the root of halophyte *S. dolichostachya*, which actively accumulates high Na in shoot (Katschnig et al. 2015). Increased expression of *ZmSOS1* and *ZmSOS2* (Fig. 3E,F) and decreased expression of *ZmHKT1* (Fig. 4) observed in the mechanically isolated root stele of Si supplied maize plants exposed to moderate salt stress is in agreement with the increased Na^+ accumulation in both the xylem sap (Fig. 2) and the leaf tissue (Table 1).

Taken together our data suggest that higher Na^+ accumulation in the xylem (Fig. 2) and subsequently leaf tissue (Table 1) of Si feed plants is a consequence of enhanced loading of Na^+ into xylem facilitated by the ZmSOS1 Na^+ efflux transporter (see Fig. 3E) and concomitantly its lower unloading via ZmHKT1 (see Fig. 4). Recently, it has also been demonstrated that Si differently modulates expression of genes encoding root transporters for metals (e.g. Che et al., 2016; Shao et al., 2017) and phosphorus (Kostic et al. 2017; Hu et al. 2018). The exact mechanisms of how Si regulates transcription of various mineral transporter genes are however unclear; one may only speculate that Si somehow indirectly affects transcription factors, as pointed out in the very recent review of Coskun et al. (2018).

The explanation for Si-enhanced Na accumulation in the leaf tissue and in particular within the vacuoles of mesophyll cells might relate to the use of Na^+ for osmotic adjustment as has already been shown in various plant species including maize (Kronzucker and Britto, 2011; Pandolfi et al., 2016). Utilization of Na^+ as an osmoticum instead of its merely excluding is considered as one of the most distinctive features between halophytes and glycophytes, which could be the reason why plants have not evolved mechanisms for complete Na^+ exclusion from its tissue (Shabala and Mackay, 2011). In contrast to higher Na accumulation in the leaf tissue of Si-treated maize (Table 1) and tomato (Romero-Aranda et al., 2005), decreased leaf Na accumulation has been recorded in some species such as rice and wheat (Liang et al., 2015 and references cited therein). Interestingly, salt-tolerant maize hybrids that translocated more Na^+ from root to shoot and hence accumulated more Na^+ in the leaves showed better growth performance compared to salt-sensitive ones (Pitann et al., 2013). Furthermore, it has recently been demonstrated that Na-acclimated maize accumulated and sequestered more Na^+ in the leaf vacuoles, which were attributed to the osmotic adjustment role of Na^+ (Pandolfi et al., 2016). Present study clearly showed higher vacuolar accumulation of Na^+ in the Si supplied salt-sensitive maize hybrid in comparison with plants not receiving Si which

accumulated more Na^+ in the chloroplasts of mesophyll cells (Fig. 5B). Moreover, increased expression of *ZmNHX* in the salt-sensitive maize hybrid receiving Si (Fig. 5C) corresponded to the expression pattern in the salt-tolerant maize hybrids (Pitann et al., 2013). A higher vacuolar sequestration of Na^+ in regard to Si treatment (Fig. 5A,B) is further supported by decreased lipid peroxidation (an indicator of ROS damage) in the leaves (Table 1).

Conclusion

Silicon up-regulated both *ZmSOS1* and its kinase *ZmSOS2*, hence significantly decreased Na concentration in the root apex, which protects meristem tissue of salt build-up. Subsequently, Si enhanced Na^+ export to the root apoplast by up-regulating *ZmSOS1* and *ZmSOS2* in the root cortex. Also, Si up-regulated expression levels of these genes in the stele involved in an active xylem loading of Na^+, and consequently increased leaf concentrations of Na^+. Increased Na^+ concentration in the xylem sap of Si-supplied plants was also achieved by down-regulation of *ZmHKT1*. Furthermore, Si up-regulated *ZmNHX* responsible for enhanced Na^+ loading into the leaf vacuole and concomitantly decreased Na^+ accumulation in the chloroplasts.

While halophytes accumulate more Na in the shoot and maintain low level in the root tissue, glycophytes, including maize, tend to restrict its accumulation in leaves by controlling xylem loading (Hasegawa et al., 2000) and concomitantly accumulate more Na in the root tissue than in the leaves. However, Si increases Na accumulation in the leaf tissue and concomitantly enhances sequestration of Na^+ into the vacuole of mesophyll cells, thereby shifting typical glycophyte behavior of maize response to moderate salt stress towards that characteristic for halophytes.

Author contribution

P. B. and M. N. conceived the study and designed the experiments. P. B. conducted the experiments, prepared the samples and analyzed data. D. B. conducted the gene expression analyses. J. J. provided technical assistance to P. B. with confocal microscopy and analyzed images. P. B. wrote the first draft and M. N. finalized the manuscript. All the authors read the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work was supported by the Serbian Ministry of Education, Science and Technological Development [OI-173028 to M. N., and in a part OI-173005 and OI-173008]. We thank Dr Ernest A. Kirkby (Faculty of Biology, University of Leeds, UK) for critical reading of the manuscript.

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Figure legends

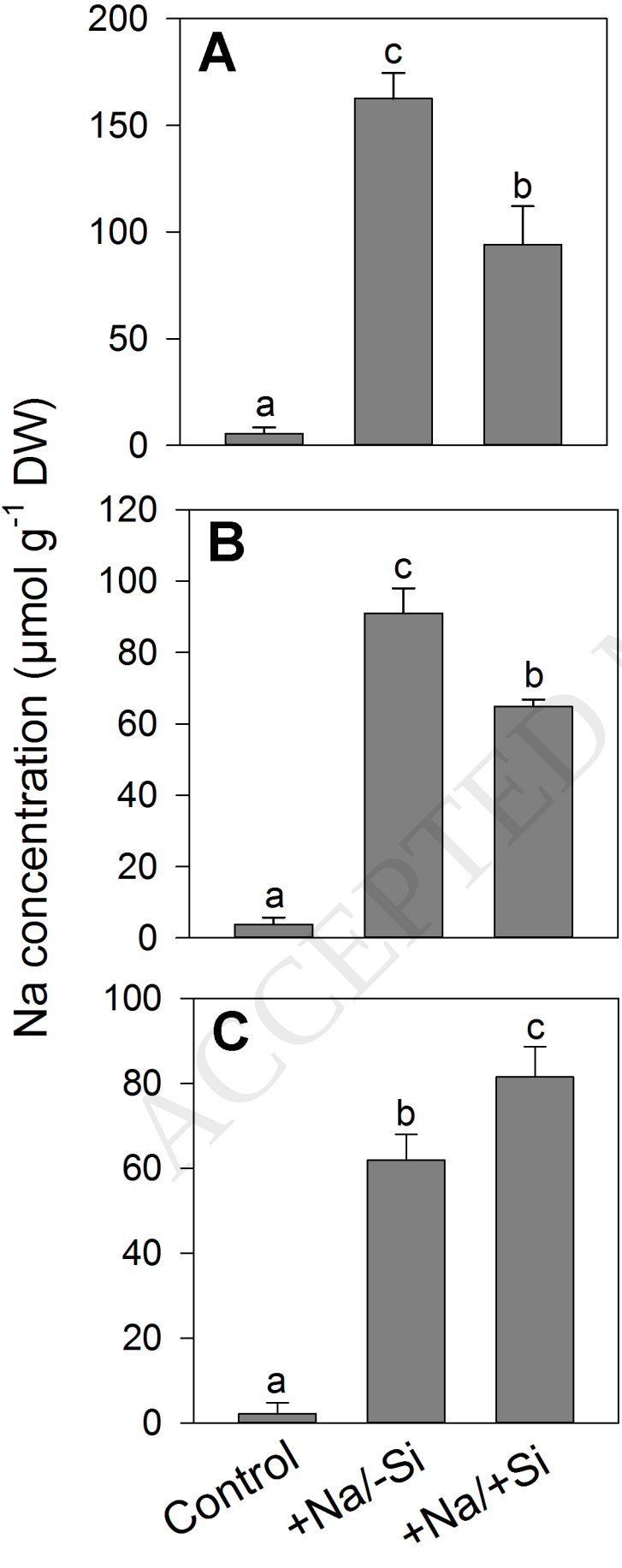
Fig. 1. Spatial distribution of Na in the maize root tissues. Na concentration in the root apex (A), cortex (B) and stele (C). Maize plants were exposed to 40 mM NaCl for 14 d without (+Na/-Si) or with (+Na/+Si) supply of 1.5 mM $Si(OH)_4$; the control plants were grown in the standard nutrient solution. Data are means of 9 plants ± S.D.; different letters denote significant differences ($P < 0.05$).

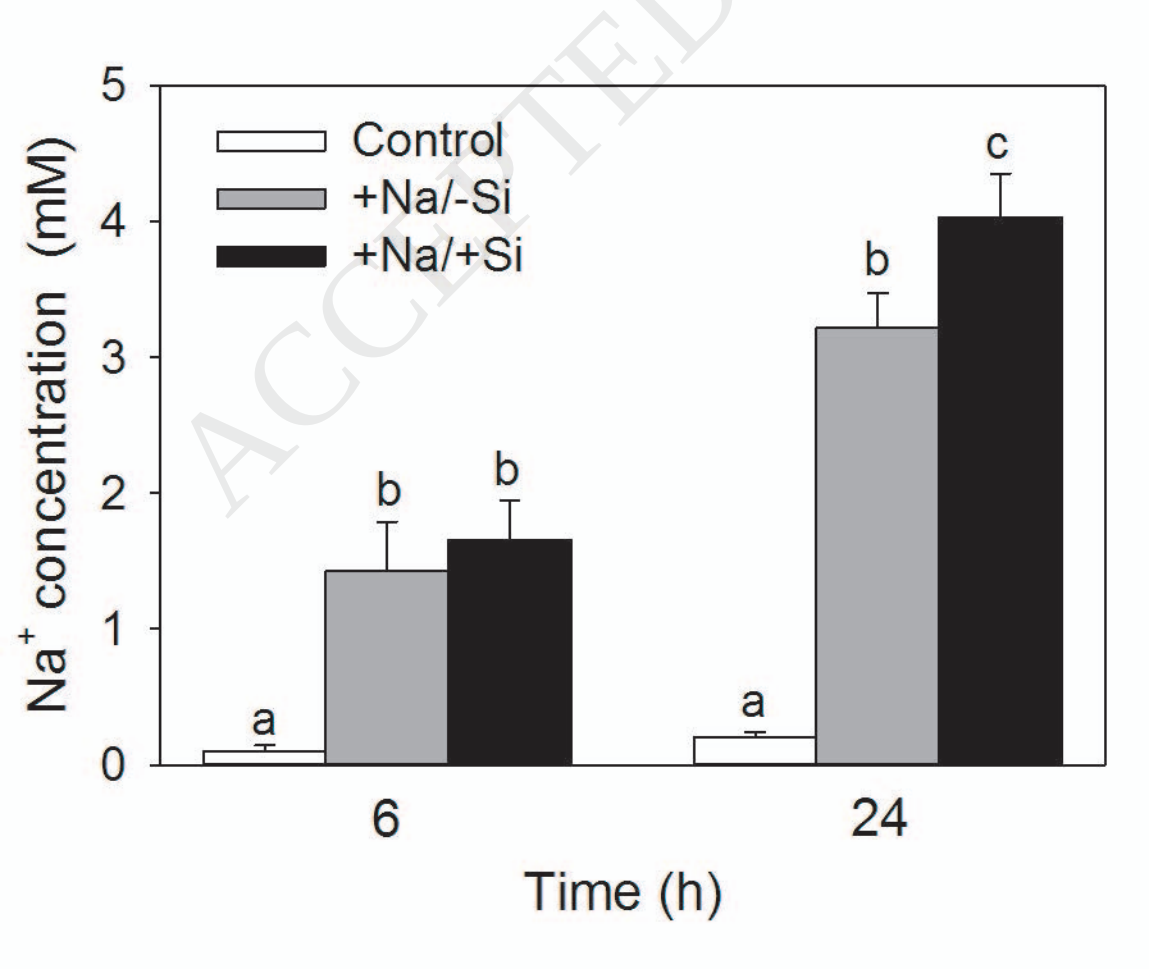
Fig. 2. Concentration of Na^+ in the xylem sap. Two-week old maize plants precultured in a standard nutrient solution were exposed to 40 mM NaCl for 6 h and 24 h without (+Na/-Si) or with (+Na/+Si) supply of 1.5 mM $Si(OH)_4$; the control plants were grown in the standard nutrient solution. Data are means of 9 plants ± S.D.; different letters denote significant differences ($P < 0.05$).

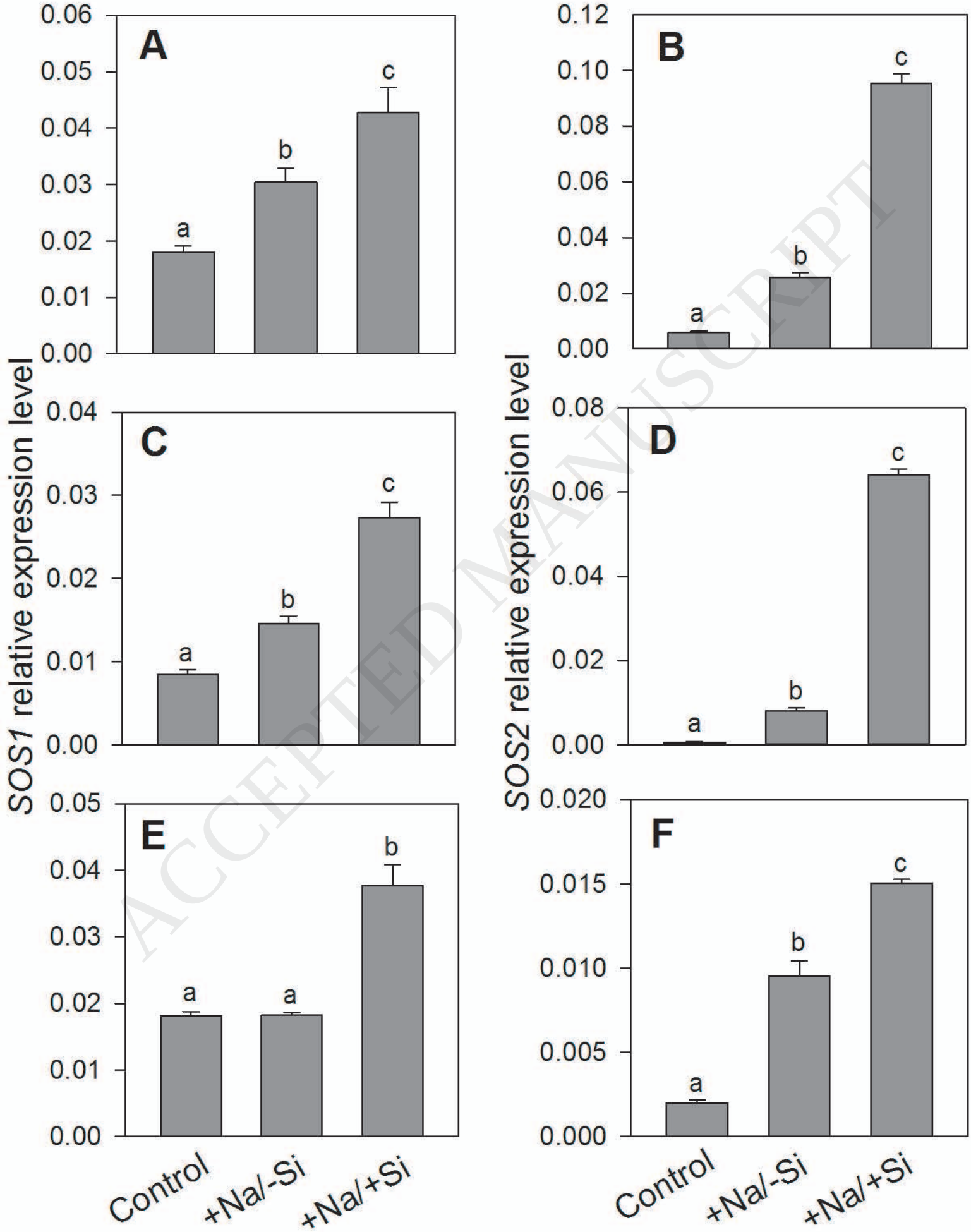
Fig. 3. Effect of Si on the relative expression of *SOS* genes in the root tissues. Expression of *SOS1* (A) and *SOS2* (B) in the root apex. Expression of *SOS1* (C) and *SOS2* (D) in the root cortex. Expression of *SOS1* (E) and *SOS2* (F) in the root stele. Maize plants were exposed to 40 mM NaCl for 7 d without (+Na/-Si) or with (+Na/+Si) supply of 1.5 mM $Si(OH)_4$; the control plants were grown in the standard nutrient solution. Data are means of 3 plants ± S.D.; different letters denote significant differences ($P < 0.05$).

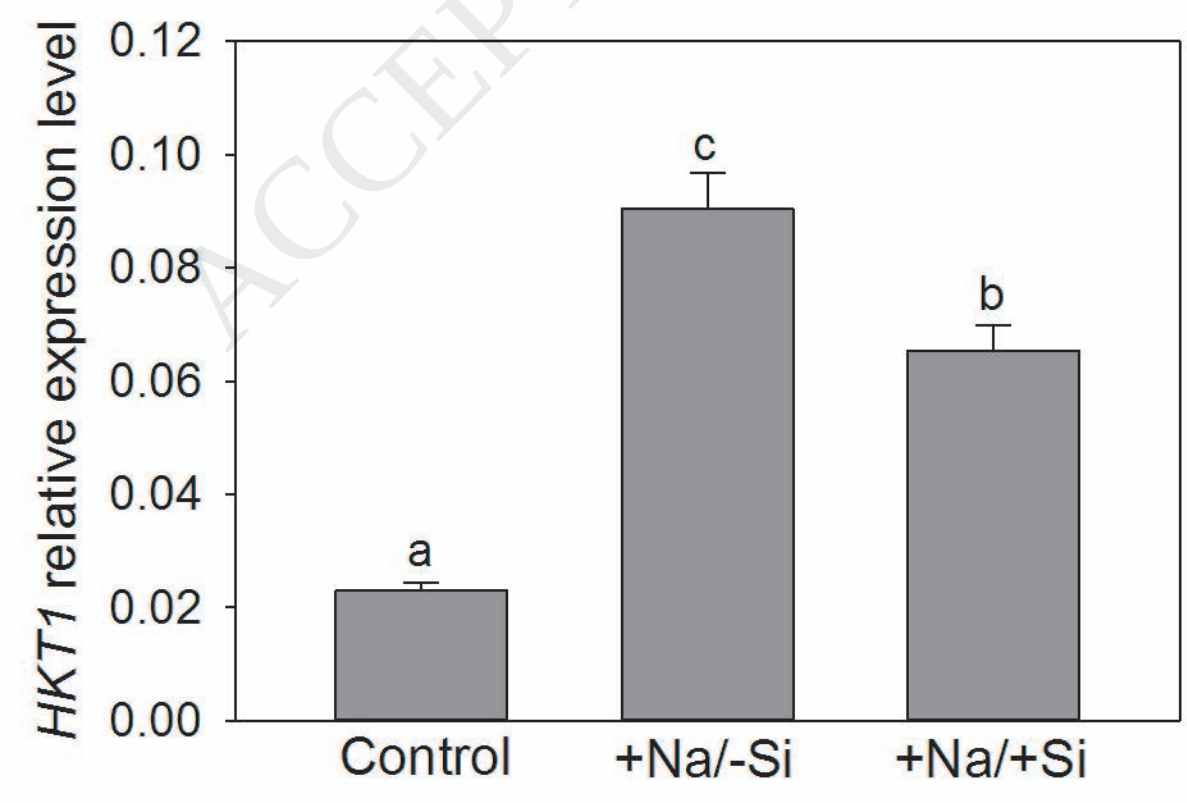
Fig. 4. Effect of Si on the relative expression of *HKT1* genes in the root stele. Maize plants were exposed to 40 mM NaCl for 7 d without (+Na/-Si) or with (+Na/+Si) supply of 1.5 mM $Si(OH)_4$; the control plants were grown in the standard nutrient solution. Data are means of 3 plants ± S.D.; different letters denote significant differences ($P < 0.05$).

Fig. 5. Effect of Si on subcellular distribution of Na^+ and the relative expression of *NHX* in the leaf mesophyll cells. Confocal images of Na^+ (A); quantification of Na^+ content in the vacuoles and chloroplasts in arbitrary units (B); relative expression of *NHX* (C). Maize plants were exposed to 40 mM NaCl for 7 d without (-Si) or with (+Si) supply of 1.5 mM $Si(OH)_4$. Fourth fully developed leaf was used for both subcellular localization of Na^+ and relative expression of *NHX* genes; the leaf discs were incubated with Na^+ indicator Sodium Green for 1.5 h. Data presented in the panels B and C are means of 3 replicates ± S.D.; different letters denote significant differences ($P < 0.05$).




Control
+Na/-Si
+Na/+Si
Na+ concentration (mM)
0
1
2
3
4
5
a
b
b
a
b
c
6
24
Time (h)




HKT1 relative expression level
0.12
0.10
0.08
0.06
0.04
0.02
0.00
a
c
b
Control
+Na/-Si
+Na/+Si

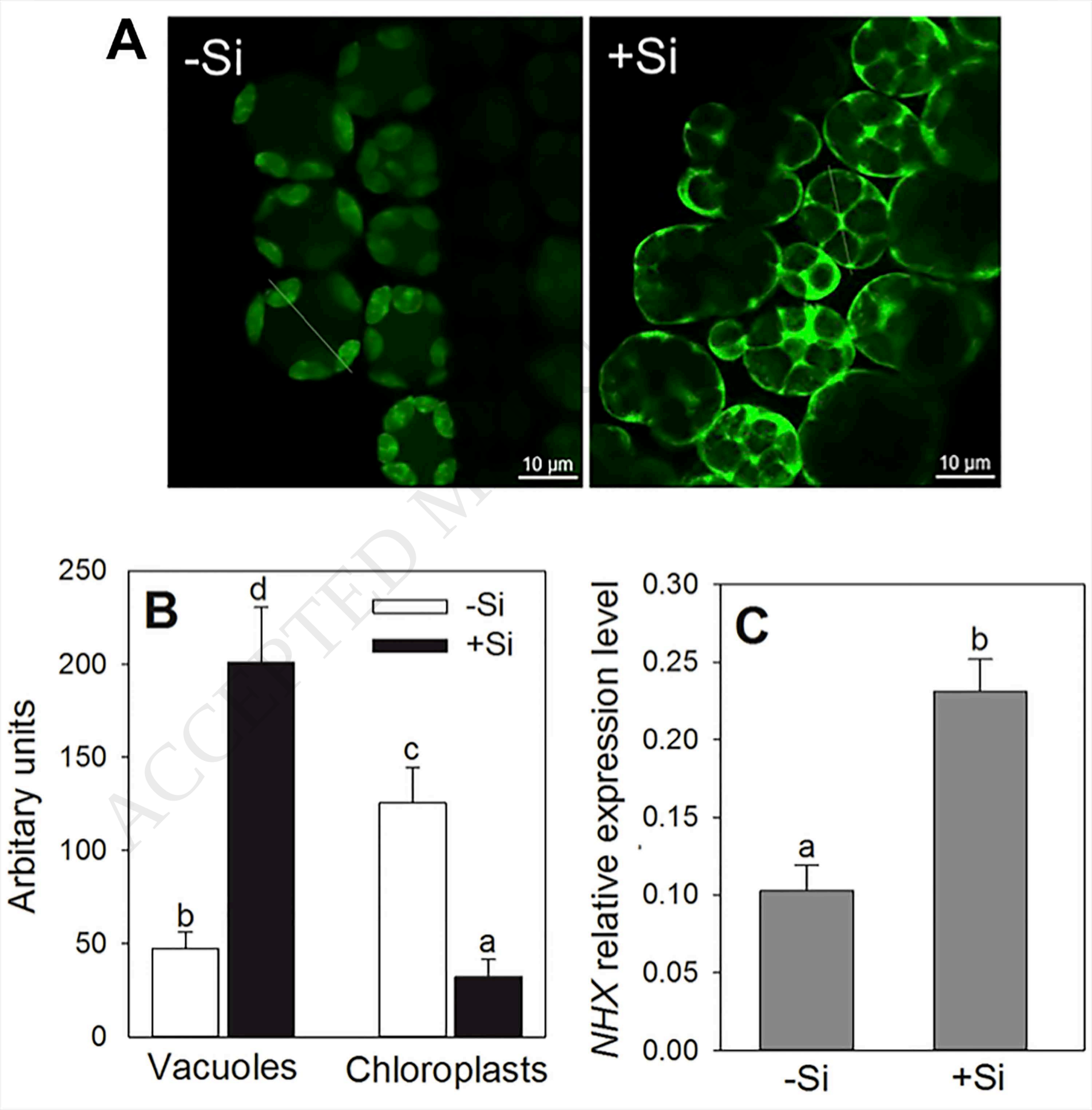

A
-Si
+Si
10 μm
10 μm
B
-Si
+Si
Arbitary units
250
200
150
100
50
0
d
c
b
a
Vacuoles
Chloroplasts
C
NHX relative expression level
0.30
0.25
0.20
0.15
0.10
0.05
0.00
a
b
-Si
+Si

Table 1 Plant biomass, tissue concentrations of Na and Si, chlorophyll content and leaf malondialdehyde (MDA) concentration.

Parameters	Control	+Si	+Na/-Si	+Na/+Si
Root dry weight (g)	0.29 ± 0.03^{b}	0.27 ± 0.03^{b}	0.15 ± 0.02^{a}	0.18 ± 0.02^{a}
Shoot dry weight (g)	2.65 ± 0.37^{b}	2.69 ± 0.18^{b}	1.12 ± 0.14^{a}	2.44 ± 0.16^{b}
Root Na concentration (µmol g^{-1} DW)	1.6 ± 0.2^{a}	1.7 ± 0.4^{a}	84 ± 7^{c}	60 ± 5^{b}
Leaf Na concentration (µmol g^{-1} DW)	2.4 ± 0.2^{a}	2.5 ± 0.4^{a}	153 ± 19^{b}	185 ± 2^{c}
Root Si concentration (mg g^{-1} DW)	4.0 ± 0.3^{a}	11.2 ± 1.3^{b}	4.2 ± 0.2^{a}	11.5 ± 1.7^{b}
Leaf Si concentration (mg g^{-1} DW)	5.8 ± 1.2^{a}	18.7 ± 2.1^{b}	5.5 ± 0.4^{a}	17.9 ± 2.4^{b}
Leaf chlorophyll content (SPAD-units)	36.4 ± 1.9^{c}	38.1 ± 3.1^{c}	22.3 ± 1.2^{a}	27.1 ± 2.4^{b}
Leaf MDA concentration (nmol g^{-1} FW)	5.7 ± 0.4^{a}	6.5 ± 0.8^{a}	14.9 ± 0.7^{c}	9.6 ± 0.5^{b}

Maize plants were exposed to 40 mM NaCl for 14 d without (+Na/-Si) or with (+Na/+Si) supply of 1.5 mM $Si(OH)_4$; the control plants were grown in the standard nutrient solution. Data are means of 9 plants ± S.D; different letters denote significant differences ($P < 0.05$).