DOI: 10.1007/s10535-013-0349-6 BIOLOGIA PLANTARUM 58 (1):179-184, 2014

BRIEF COMMUNICATION

Increased cucumber salt tolerance by grafting on pumpkin rootstock and after application of calcium

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Abstract

Self-grafted and pumpkin rootstock-grafted cucumber plants were subjected to the following four treatments: *1*) aerated nutrient solution alone (control), *2*) nutrient solution with 10 mM $Ca(NO_3)_2$ (Ca), *3*) nutrient solution with 90 mM NaCl (NaCl), and *4*) nutrient solution with 90 mM NaCl + 10 mM $Ca(NO_3)_2$ (NaCl+Ca). The NaCl treatment decreased the plant dry mass and content of Ca^{2+} and K^+ but increased the Na^+ content in roots and shoots. Smaller changes were observed in pumpkin rootstock-grafted plants. Supplementary $Ca(NO_3)_2$ ameliorated the negative effects of NaCl on plant dry mass, relative growth rate (RGR), as well as Ca^{2+}, K^+, and Na^+ content especially for pumpkin rootstock-grafted plants. Supplementary $Ca(NO_3)_2$ distinctly stimulated the plasma membrane (PM) H^+-ATPase activity which supplies the energy to remove excess Na^+ from the cells. The expressions of gene encoding PM H^+-ATPases (*PMA*) and gene encoding a PM Na^+/H^+ antiporter (*SOS1*) were up-regulated when $Ca(NO_3)_2$ was applied. The pumpkin rootstock-grafted plants had higher PM H^+-ATPase activity as well as higher *PMA* and *SOS1* expressions than the self-grafted plants under NaCl + Ca treatment. Therefore, the addition of Ca^{2+} in combination with pumpkin rootstock grafting is a powerful way to increase cucumber salt tolerance.

Additional key words: *Cucumis sativus, Cucurbita moschata,* NaCl, plasma membrane H^+-ATPase, Na^+/H^+ antiport, *SOS1*, salinity.

Salinity is one of the most important abiotic stresses affecting crop yield worldwide (Hasegawa *et al*. 2000). Salinity imposes two constraints on plants: an osmotic effect resulting from the lower soil water potential and ionic effect resulting from the direct ion toxicity and the ion imbalance in the plants (Munns and Tester 2008, Upadhyay *et al.* 2012). The plants usually face calcium deficiency in addition to sodium toxicity under saline conditions, such as in cucumber (Cerda and Martinez 1988) and tomato (Navarro *et al*. 2000). Na^+ impairs the uptake of Ca^{2+} by plants due to ionic interactions in the soil solution (Cramer *et al*. 1986, Suarez and Grieve 1988) or by displacing it from the cell membranes or by some other mechanisms affecting membrane function (Lynch *et al*. 1987).

Calcium is an important factor affecting the maintenance of membrane integrity and ion transport regulation (Läuchli and Epstein 1970). Application of Ca^{2+} has been shown to ameliorate the adverse effects of salinity in a variety of plant species (Caines and Shennan 1999, Shabala *et al*. 2003, Arshi *et al*. 2005, Renault 2005). Elevated Ca^{2+} concentration in the nutrient solution can mitigate the adverse effects of NaCl by inhibiting Na^+ uptake as well as by maintaining K^+/Na^+ selectivity and adequate Ca^{2+} content in roots (Kent and Läuchli 1985). Ca^{2+} can stimulate plasma membrane (PM) H^+-ATPase activity through Ca^{2+}-calmodulin dependent protein kinases (Kłobus and Janicka-Russak

Submitted 23 January 2013, *last revision* 26 March 2013, *accepted* 28 March 2013.

Abbreviations: Pi - inorganic phosphate; PM - plasma membrane; *PMA* - gene encoding plasma membrane H^+-ATPase; RGR - relative growth rate; *SOS1* - gene encoding a plasma membrane Na^+/H^+ antiporter.

Acknowledgements: This work was supported by the 973 Project (2009CB119000), National Natural Science Foundation of China (30871738, 31172000), the Key Project of the Chinese Ministry of Education (109113), and the Natural Science Foundation of Hubei Province (2008CDB081). The first two authors contributed equally to the paper.

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2004). Ca^{2+} can also activate the salt overly sensitive SOS3-SOS2 protein kinase pathway by activating the Na^+/H^+ exchanger SOS1, a Na^+/H^+ antiporter that ultimately relies on H^+-ATPase activity. Consequently, excess Na^+ is removed from the cell and salt tolerance is enhanced (Halfter *et al*. 2000, Qiu *et al*. 2002, Quintero *et al*. 2002, Kabała and Janicka-Russak 2012).

In recent years, grafting on salt-tolerant rootstock has been demonstrated to be a valid strategy in increasing salt tolerance in vegetable crops, such as tomato (Santa-Cruz *et al.* 2002, Estañ *et al*. 2005), melon (Colla *et al*. 2006, Rouphael *et al.* 2012), and watermelon (Goreta *et al*. 2008). In our previous research, grafting cucumbers onto salt-tolerant rootstock improve their salt tolerance. We found significant differences among the accumulations of Na^+ between self-grafted and pumpkin rootstock-grafted plants under different NaCl treatments. Self-grafted plants mostly accumulate Na^+ in shoots, but Na^+ is mostly accumulated in the roots of pumpkin rootstock-grafted plants (Zhu *et al.* 2008, Huang *et al*. 2011). However, few studies on the effect of Ca^{2+} on the salt tolerance of rootstock grafted plants have been conducted (Bolat *et al.* 2006, Maeda and Nakazawa 2008, Garcia *et al.* 2008, Alizadeh *et al.* 2010). The present study aimed to determine whether the addition of Ca^{2+} in combination with grafting on the pumpkin rootstock can provide an effective way to increase cucumber salt tolerance.

The experiment was carried out from 16 May to 3 July 2010 in a naturally lit glasshouse located in Huazhong Agricultural University, central China (latitude of 30° 27' N; longitude of 114° 20' E; altitude of 22 m). Plants were grown at day temperature of 16 - 34 °C, night temperature of 15 - 24 °C, and relative humidity between 40 - 87 %. A salt-sensitive cucumber (*Cucumis sativus* L.) cv. Jinchun No. 2 was used as a scion or a rootstock and a salt-tolerant pumpkin (*Cucurbita moschata* Duch.) cv. Chaojiquanwang was used as a rootsstock (Zhu *et al*. 2008). Cucumber and pumpkin sown on 16 or 26 May were used as the rootstocks, and the cucumber sown on 1 June was used as the scion. The cucumber plants were grafted onto the pumpkin rootstock or onto themselves on 6 June using the insert grafting procedure described by Lee (1994). After 10 d (16 June), the grafted plants were transferred to 20-dm^3 plastic containers containing full strength Hoagland's solution (Hoagland and Arnon 1950). The nutrient solutions were refreshed at 5-d intervals and continuously aerated. After pre-culturing for 12 d (28 June), plants were subjected to the following four treatments: *1*) aerated nutrient solution alone (control), *2*) nutrient solution with 10 mM $Ca(NO_3)_2$ (Ca), *3*) nutrient solution with 90 mM NaCl (NaCl), and *4*) nutrient solution with 90 mM NaCl + 10 mM $Ca(NO_3)_2$ (NaCl+Ca). To avoid salt shock, NaCl was applied at a rate of 30 mM every 12 h until the final concentration (90 mM) was reached and Ca^{2+} was applied when NaCl reached the final concentration. Each treatment was replicated three times with 12 plants in one replicate and arranged in a completely randomized block design. After 5 d, samples were harvested (3 July) because evident growth differences were observed among the control and the three treatments.

Three plants per treatment were harvested and rinsed with deionized water. The plants were divided into shoots (the part above the graft union) and roots (the part below the graft union), and then dried at 75 °C for 3 d to determine the dry mass. The relative growth rate (RGR) was calculated from the increase in dry mass of plants between the beginning and the end of salinity stress according to Van Hulten *et al*. (2006). The mean initial dry masses of self-grafted and rootstock-grafted plants were 0.33 and 0.36 g $plant^{-1}$ and the shoot dry masses were 1.48 and 2.29 g $plant^{-1}$, respectively. Samples were digested in 98 % (m/m) sulfuric acid and 30 % (v/v) hydrogen peroxide at 120 °C in a *Hot Block* (*Environmental Express,* Mt. Pleasant, SC, USA) for 2.5 h. The content of Na^+, Ca^{2+}, and K^+ was determined by atomic absorption spectrophotometer (*AA 220, Varian,* Palo Alto, CA, USA).

PM vesicles were isolated according to Kłobus and Buczek (1995). ATPase activity was determined by measuring the release of inorganic phosphate (Pi) (Kabała and Kłobus 2001). PM H^+-ATPase activity was expressed as a difference between the activities measured in the absence and presence of Na_3VO_4. Protein content was estimated by the method of Bradford (1976).

Table 1. Forward (F) and reverse (R) primers used for real time PCR assays.

Gene	Primer pairs
Actin	F: CCAAGCAGCATGAAGATCAA
	R: ATCTGCTGGAAGGTGCTGAG
CsPMA	F: GCAATTTTGGTTCCAGGAGA
	R: CAAGATGTGCTGCTTTTCCA
CsSOS1	F: AGACATTTCCTCAGGTTTTGC
	R: AGAAGCCTTTTCAAGTCGGT

Total RNA was isolated from roots with *Trizol* reagent (*Toyobo*, Osaka, Japan) according to the manufacturer's instructions and then treated with RNase-free DNase to remove contaminating DNA. First-strand cDNA of the total RNA was synthesized using *M-MuLV* reverse transcriptase, and *oligo-(dT)*$_{18}$ was used as a primer following the manufacturer's recommendation (*Fermentas,* Shenzhen, China). Expression of the target genes was measured by real time PCR. The specific primers (Table 1) were designed based on published mRNA [*Genebank* accession Nos. AAZ74666.1, AF289025, and At5g27150] using *Primer Express 3.0* software. *Actin* gene was used as an internal control. The specificity of the PCR reaction was checked with the melting curve analysis. All used primers showed high

specificity for each analyzed gene. Real time PCR was performed using a *LightCycler480 SYBR Green I* master kit (*Roche Diagnostics*, Mannheim, Germany) according to the protocols. PCR amplification included a pre-incubation step at 95 °C for 5 min, followed by 40 cycles at 95 °C for 10 s, 58 °C for 15 s, and 72 °C for 20 s. The PCR products were quantified by the *LightCycler480* RT-PCR detection system with a *SYBR Green I* master kit (*Roche Diagnostics*, Mannheim, Germany). All reactions were run in triplicate. Data were analyzed using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

All determinations were conducted with three replicates. Statistical analysis was carried out using *SAS v. 8.1* software (*SAS Institute*, Cary, NC, USA). Data were presented as the mean ± standard error (SE). Differences between means were established using Duncan's multiple range test at $P < 0.05$.

Salinity is known to reduce the growth rate and dry mass of shoots and roots (Tüzel *et al*. 2003). In the present study, decreased root and shoot dry masses and RGR were observed in grafted cucumber plants under NaCl stress (Table 2). However, the decrements were more pronounced in self-grafted plants indicating that grafting a cucumber on a salt-tolerant rootstock can alleviate the growth inhibition caused by salinity (Table 2). This finding was in accordance with those in tomato (Santa-Cruz *et al.* 2002, Estañ *et al*. 2005), melon (Colla *et al*. 2006), and the other cultivars of cucumber (Zhu *et al*. 2008, Huang *et al*. 2010, Zhen *et al*. 2010). Supplementary $Ca(NO_3)_2$ significantly alleviated the growth inhibition induced by NaCl stress in the pumpkin rootstock-grafted plants. However, the effect was smaller in self-grafted plants demonstrating that the effect of $Ca(NO_3)_2$ in improving the salinity tolerance of grafted cucumber depended on the rootstock species (Table 2). Growth inhibition is also reportedly alleviated by Ca^{2+} application in bean (Lahaye and Epstein 1971), cotton (Cramer *et al*. 1986), wheat (Davenport *et al*. 1997), strawberry (Kaya *et al*. 2003), and tomato (Navarro *et al*. 2000) under NaCl stress. The RGR of self-grafted plants was negative under the NaCl stress since the shoot growth was nearly stopped, some roots were decayed, and the decayed roots were not included in the plant dry mass.

Supplemental $Ca(NO_3)_2$ decreased the Na^+ content in the leaves and the roots of both graft combinations under NaCl treatment, especially for the roots of pumpkin rootstock-grafted plants (Table 3). These findings were consistent with those described by Alam *et al*. (2002),

Table 2. Shoot and root dry masses [g plant^{-1}], RGR [g(d.m.) g^{-1}(d.m.) d^{-1}], activity of PM H^+-ATPase [μmol(Pi) mg^{-1}(protein) min^{-1}], and expression of *PMA* and *SOS1* genes in self-grafted and pumpkin rootstock-grafted plants under control (nutrient solution alone), 10 mM $Ca(NO_3)_2$ (Ca), 90 mM NaCl (NaCl), or NaCl+Ca treatments for 5 d. Values are means ± SE, $n = 3$. Different letters in the same column indicate significant differences ($P < 0.05$) based on the Duncan's multiple range test.

Plants	Treatments	Shoot d.m.	Root d.m.	RGR	PM H^+-ATPase	*PMA*	*SOS1*
Self-grafted	control	2.16±0.02f	0.34±0.01de	0.065±0.001bc	4.88±0.63e	1.00±0.05f	1.00±0.35f
	Ca	2.31±0.04e	0.40±0.01bc	0.081±0.004b	11.77±0.72a	2.10±0.13e	2.07±0.11e
	NaCl	1.48±0.03h	0.25±0.01f	-0.010±0.020f	6.81±0.12d	1.80±0.05e	2.01±0.06e
	NaCl+Ca	1.76±0.04g	0.30±0.02e	0.026±0.005e	9.45±0.54c	8.19±0.70c	8.97±0.44c
Pumpkin	control	3.07±0.06b	0.43±0.01b	0.055±0.003cd	7.41±0.52d	0.81±0.01f	0.89±0.02f
rootstock-	Ca	3.87±0.05a	0.54±0.02a	0.100±0.003a	12.02±0.76a	38.98±2.76a	37.35±2.24a
grafted	NaCl	2.53±0.05d	0.37±0.02cd	0.018±0.003e	10.64±0.34b	2.90±0.04d	2.88±0.18d
	NaCl+Ca	2.84±0.03c	0.52±0.02a	0.047±0.001d	11.16±0.42ab	9.79±0.94b	10.54±1.00b

Table 3. Content of Na^+, K^+, and Ca^{2+} [mg g^{-1}(d.m.)] in roots and shoots of self-grafted and pumpkin rootstock-grafted plants under control, Ca, NaCl, or NaCl+Ca treatments for 5 d. Values are means ± SE, $n = 3$. Different letters in the same column indicate significant differences ($P < 0.05$) based on Duncan's multiple range test.

Plants	Treatments	Na^+ shoot	Na^+ root	K^+ shoot	K^+ root	Ca^{2+} shoot	Ca^{2+} root
Self-grafted	control	1.78±0.06de	3.33±0.01e	65.54±1.69a	84.77±2.78a	11.10±1.15e	8.02±0.18c
	Ca	0.52±0.04f	0.66±0.08f	53.52±1.86c	16.65±3.56e	16.09±1.28b	22.30±1.91b
	NaCl	20.51±0.54a	24.12±0.56b	22.95±0.68e	26.28±0.38d	6.61±0.76f	6.97±0.1cd
	NaCl+Ca	9.31±0.39b	11.81±0.81d	49.55±3.74d	25.23±3.94d	10.56±0.76de	7.41±0.45cd
Pumpkin	control	1.43±0.15e	3.12±0.09e	67.14±1.92a	60.47±1.72b	13.64±0.91cd	7.59±0.36cd
rootstock-	Ca	0.26±0.03f	1.00±0.08f	59.13±3.82b	37.73±4.53c	22.80±1.42a	25.32±0.6a
grafted	NaCl	3.81±0.14c	44.15±0.85a	54.70±0.50c	26.18±0.81d	13.46±0.78cd	5.31±0.15d
	NaCl+Ca	1.97±0.05d	18.18±0.93c	58.99±4.98b	10.77±0.38f	15.98±2.58bc	5.24±0.19d

who reported that Ca^{2+} reduced Na^+ accumulation in rice. Ca^{2+} application also reportedly enhances NaCl-induced Na^+ efflux from roots of poplar, and the salt-tolerant *P. euphratica* shows higher Na^+ efflux than the salt-sensitive *P. popularis* (Sun *et al*. 2009). Ca^{2+} inhibitors markedly reduce the Na^+ efflux from *P. euphratica* roots (Sun *et al*. 2010).

Supplementary $Ca(NO_3)_2$ increased the shoot K^+ content under NaCl treatment and the pumpkin rootstock-grafted plants showed higher K^+ content than the self-grafted cucumber plants (Table 3). Sun *et al.* (2009) reported that K^+ efflux from the root of cold-stressed *P. popularis* is markedly reduced by Ca^{2+} supplementation. *P. euphratica* consistently shows very low K^+ efflux under NaCl treatment. Similarly, salt-tolerant cultivars of barley and wheat exhibit a smaller K^+ efflux than salt-sensitive ones under saline conditions (Chen *et al*. 2005, 2007, Cuin *et al*. 2008). Thus, Ca^{2+} could ameliorate the loss of K^+ induced by NaCl and the pumpkin rootstock-grafted plants might have higher ability to restrict K^+ efflux.

Supplementary Ca^{2+} in both the grafted plants increased the shoot Ca^{2+} content and this phenomenon appeared to be related with the decreased shoot Na^+ content (Table 3). These results agree with the findings of Lopez and Satti (1996), who also found similar responses of tomato plants to salt stress. Thus, supplementary Ca^{2+} could alleviate the toxic effect of NaCl on cucumber plants which could be attributed to stimulated Na^+ exclusion as well as Ca^{2+} and K^+ enhancement in shoots. The pumpkin rootstock-grafted plants showed greater ability to regulate these processes.

For salinity stress tolerance in plants, the activation of PM H^+-ATPases is important for Na^+ exclusion. In many halophytes or glycophytes, PM H^+-ATPase responds to salt stress with increased activity (Matsumoto and Chung 1988, Nakamura *et al*. 1992, Binzel 1995). NaCl stress increased the activities of PM H^+-ATPases in the roots of the both graft combinations (Table 2). Supplementary Ca^{2+} also increased the PM H^+-ATPase activity. Zhao *et al*. (2003) reported that Ca^{2+} can increase PM H^+-ATPase activity. The higher activity of PM H^+-ATPase in the roots of the pumpkin rootstock-grafted plants was coupled with a lower Na^+ content in the shoots indicating the relationship between them. Davenport *et al.* (1997) reported that the ability of Na^+ efflux from plants is higher in salt-tolerant wheat cultivars than in salt-sensitive ones. These results indicate that the higher salt tolerance under supplementary Ca^{2+} could be attributed to the increased Na^+ efflux induced by the increased activity of PM H^+-ATPase, and the effect of supplementary Ca^{2+} was more obvious on the pumpkin rootstock-grafted plants.

Niu *et al*. (1993) reported that the activation of PM H^+-ATPase can be due to the expression of *PMA* induced by NaCl. In this work, we provided evidence that NaCl treatment elevated the *PMA* transcription suggesting that the regulation of PM H^+-ATPase activities by NaCl involved the mRNA level (Table 2). To our knowledge, this report is the first one providing evidence of Ca^{2+} regulating the expression of *PMA* in plants challenged by NaCl. The *PMA* mRNA content in the roots of the pumpkin rootstock-grafted plants evidently increased after $Ca(NO_3)_2$ addition indicating that Ca^{2+} might be involved in the regulation of this gene.

In the current work, the mRNA level of *SOS1* was elevated under NaCl treatment, and the *SOS1* mRNA content in the roots evidently increased under $Ca(NO_3)_2$ supplementation (Table 2). Interestingly, the trend of *SOS1* was highly consistent with that of *PMA* suggesting that they might be closely related. Horie *et al.* (2006) reported that the external Ca^{2+} concentration strongly affects the Na^+ stress response of the SOS pathway. Compared with wild-type plants, *SOS1* mutants accumulate more Na^+ at high external NaCl concentrations (Pardo *et al*. 2006). A hypersensitive signal pathway to Ca^{2+} might exist in the pumpkin rootstock roots and Ca^{2+} affected *SOS1* expression. Then, Na^+/H^+ antiport was stimulated to exclude more Na^+.

In conclusion, supplementary Ca^{2+} significantly increased the salt tolerance of the grafted cucumber, especially in the pumpkin rootstock-grafted plants. The mechanisms included increased PM H^+-ATPase activity and *PMA* expression, better ability of root cells to pump Na^+ from cytosol to external medium regulated by *SOS1*, and increased K^+ and Ca^{2+} content in shoots. The pumpkin rootstock-grafted plants had higher salt tolerance than the self-grafted plants especially under supplementary Ca^{2+} treatment. The reasons can be the higher activity of PM H^+-ATPase, *PMA* expression and *SOS1* expression in the pumpkin rootstock-grafted plants, as well as their better ability to regulate transport of Na^+, K^+, and Ca^{2+} from root to shoot. Consequently, the pumpkin rootstock-grafted plants were able to withstand saline conditions more effectively than the self-grafted cucumber plants.

References

Alam, S., Huq, S.M.I., Kawai, S., Islam, A.: Effects of applying calcium salts to coastal saline soils on growth and mineral nutrition of rice varieties. - J. Plant Nutr. **25**: 561-576, 2002.

Alizadeh, M., Singh, S.K., Patel, V.B., Bhattacharya, R.C., Yadav, B.P.: *In vitro* responses of grape rootstocks to NaCl. - Biol. Plant. **54**: 381-385, 2010.

Arshi, A., Abdin, M.Z., Iqbal, M.: Ameliorative effects of $CaCl_2$ on growth, ionic relations, and proline content of senna under salinity stress. - J. Plant Nutr. **28**: 101-125, 2005.

Binzel, M.L.: NaCl-induced accumulation of tonoplast and plasma membrane H^+-ATPase message in tomato. - Physiol. Plant. **94**: 722-728, 1995.

Bolat, I., Kaya, C., Almaca, A., Timucin, S.: Calcium sulfate improves salinity tolerance in rootstocks of plum. - J. Plant Nutr. **29**: 553-564, 2006.

Bradford, M.M.: A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Caines, A.M., Shennan, C.: Interactive effects of Ca^{2+} and NaCl salinity on the growth of two tomato genotypes differing in Ca^{2+} use efficiency. - Plant Physiol. Biochem. **37**: 569-576, 1999.

Cerda, A., Martinez, V.: Nitrogen fertilization under saline conditions in tomato and cucumber plants. - J. hort. Sci. **63**: 451-458, 1988.

Chen, Z., Newman, I., Zhou, M., Mendham, N., Zhang, G., Shabala, S.: Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. - Plant Cell Environ. **28**: 1230-1246, 2005.

Chen, Z., Pottosin, I.I., Cuin, T.A., Fuglsang, A.T., Tester, M., Jha, D., Zepeda-Jazo, I., Zhou, M., Palmgren, M.G., Newman, I.A., Shabala, S.: Root plasma membrane transporters controlling K^+/Na^+ homeostasis in salt-stressed barley. - Plant Physiol. **145**: 1714-1725, 2007.

Colla, G., Rouphael, Y., Cardarelli, M., Massa, D., Salerno, A., Rea, E.: Yield, fruit quality and mineral composition of grafted melon plants grown under saline conditions. - J. hort. Sci. Biotechnol. **81**: 146-152, 2006.

Cramer, G.R., Spurr, A.R.: Responses of lettuce to salinity. I. Effects of NaCl and Na_2SO_4 on growth. - J. Plant Nutr. **9**: 115-130, 1986.

Cuin, T.A., Betts, S.A., Chalmandrier, R., Shabala, S.: A root's ability to retain K^+ correlates with salt tolerance in wheat. - J. exp. Bot. **59**: 2697-2706, 2008.

Davenport, R.J., Reid, R.J., Smith, F.A.: Sodium-calcium interactions in two wheat species differing in salinity tolerance. - Physiol. Plant. **99**: 323-327, 1997.

Estañ, M.T., Martinez-Rodriguez, M.M., Perez-Alfocea, F., Flowers, T.J., Bolarin, M.C.: Grafting raises the salt tolerance of tomato through limiting the transport of sodium and chloride to the shoot. - J. exp. Bot. **56**: 703-712, 2005.

Garcia-Legaz, M.F., Lopez-Gomez, E., Beneyto, J.M., Navarro, A., Sanchez-Blanco, M.J.: Physiological behaviour of loquat and anger rootstocks in relation to salinity and calcium addition. - J. Plant Physiol. **165**: 1049-1060, 2008.

Goreta, S., Bucevic-Popovic, V., Selak, G.V., Pavela-Vrancic, M., Perica, S.: Vegetative growth, superoxide dismutase activity and ion concentration of salt-stressed watermelon as influenced by rootstock. - J. agr. Sci. **146**: 695-704, 2008.

Halfter, U., Ishitani, M., Zhu, J.K.: The *Arabidopsis* SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. - Proc. nat Acad. Sci. USA. **97**: 3735-3740, 2000.

Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J.: Plant cellular and molecular responses to high salinity. - Annu. Rev. Plant Physiol. Plant mol. Biol. **51**: 463-499, 2000.

Hoagland, D.R., Arnon, D.S.: The water culture method for growing plants without soil. - Calif. agr. exp. Stat. Circ. **347**: 1-32, 1950.

Horie, T., Horie, R., Chan, W.Y., Leung, H.Y., Schroeder, J.I.: Calcium regulation of sodium hypersensitivities of *sos3* and *athkt1* mutants. - Plant Cell Physiol. **47**: 622-633, 2006.

Huang, Y., Bie, Z., He, S., Hua, B., Zhen, A., Liu, Z.: Improving cucumber tolerance to major nutrients induced salinity by grafting onto *Cucurbita ficifolia*. - Environ. exp. Bot. **69**: 32-38, 2010.

Huang, Y., Bie, Z., Liu, Z., Zhen, A., Jiao, X.: Improving cucumber photosynthetic capacity under NaCl stress by grafting onto two salt-tolerant pumpkin rootstocks. - Biol. Plant. **55**: 285-290, 2011.

Kabała, K., Janicka-Russak, M.: Na^+/H^+ antiport activity in plasma membrane and tonoplast vesicles isolated from NaCl-treated cucumber roots. - Biol. Plant **56**: 377-382, 2012.

Kabała, K., Kłobus, G.: Characterization of the tonoplast proton pumps in *Cucumis sativus* L. root cells. - Acta Physiol. Plant. **23**: 55-63, 2001.

Kaya, C., Ak, B.E., Higgs, D.: Response of salt-stressed strawberry plants to supplementary calcium nitrate and/or potassium nitrate. - J. Plant Nutr. **26**: 543-560, 2003.

Kent, L.M., Läuchli, A.: Germination and seedling growth of cotton: salinity-calcium interactions. - Plant Cell Environ. **8**: 155-159, 1985.

Kłobus, G., Buczek, J.: The role of plasma membrane oxidoreductase activity in proton transport. - J. Plant Physiol. **146**: 103-107, 1995.

Kłobus, G., Janicka-Russak, M.: Modulation by cytosolic components of proton pump activities in plasma membrane and tonoplast from *Cucumis sativus* roots during salt stress. - Physiol. Plant. **121**: 84-92, 2004.

Lahaye, P.A., Epstein, E.: Calcium and salt toleration by bean plants. - Physiol. Plant. **25**: 213-218, 1971.

Läuchli, A., Epstein, E.: Transport of potassium and rubidium in plant roots: the significance of calcium. - Plant Physiol. **45**: 639-641, 1970.

Lee, J.M.: Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. - HortScience **29**: 235-239, 1994.

Livak, K.J., Schmittgen, T.D.: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. - Methods **25**: 402-408, 2001.

Lopez, M.V., Satti, S.M.E.: Calcium and potassium-enhanced growth and yield of tomato under sodium chloride stress. - Plant Sci. **114**:19-27, 1996.

Lynch, J., Cramer, G., Läuchli, A.: Salinity reduces membrane-associated calcium in corn root protoplasts. - Plant Physiol. **83**: 390-394, 1987.

Maeda, Y., Nakazawa, R.: Effects of the timing of calcium application on the alleviation of salt stress in the maize, tall fescue, and reed canarygrass seedlings. - Biol. Plant. **52**: 153-156, 2008.

Matsumoto, H., Chung, G.C.: Increase in proton-transport activity of tonoplast vesicles as an adaptive response of barley roots to NaCl stress. - Plant Cell Physiol. **29**: 1133-1140, 1988.

Munns, R., Tester, M.: Mechanisms of salinity tolerance. - Annu. Rev. Plant Biol. **59**: 651-681, 2008.

Nakamura, Y., Kasamo, K., Sakata, M., Ohta, E.: Stimulation of the extrusion of protons and H^+-ATPase activities with the decline in pyrophosphatase activity of the tonoplast in intact mung bean roots under high-NaCl stress and its relation to external levels of Ca^{2+} ions. - Plant Cell Physiol. **33**: 139-149, 1992.

Navarro, J.M., Martínez, V., Carvajal, M.: Ammonium, bicarbonate and calcium effects on tomato plants grown

under saline conditions. - Plant Sci. **157**: 89-96, 2000.

Niu, X.M., Narasimhan, M., Salzman, R.A., Bressan, R.A., Hasegawa, P.M.: NaCl regulation of plasma membrane H^+-ATPase gene expression in a glycophyte and a halophyte. - Plant Physiol. **103**: 713-718, 1993.

Pardo, J.M., Cubero, B., Leidi, E.O., Quintero, F.J.: Alkali cation exchangers: roles in cellular homeostasis and stress tolerance. - J. exp. Bot. **57**: 1181-1199, 2006.

Qiu, Q.S., Guo, Y., Dietrich, M.A., Schumaker, K.S., Zhu, J.K.: Regulation of SOS1, a plasma membrane Na^+/H^+ exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. - Proc. nat Acad. Sci. USA. **99**: 8436-8441, 2002.

Quintero, F.J., Ohta, M., Shi, H., Zhu, J.K., Pardo, J.M.: Reconstitution in yeast of the *Arabidopsis* SOS signaling pathway for Na^+ homeostasis. - Proc. nat Acad. Sci. USA. **99**: 9061-9066, 2002.

Renault, S.: Response of red-osier dogwood (*Cornus stolonifera*) seedlings to sodium sulphate salinity: effects of supplemental calcium. - Physiol. Plant. **123**: 75-81, 2005.

Rouphael, Y., Cardarelli, M., Rea, E., Colla, G.: Improving melon and cucumber photosynthetic activity, mineral composition, and growth performance under salinity stress by grafting onto *Cucurbita* hybrid rootstocks. - Photosynthetica **50**: 180-188, 2012.

Santa-Cruz, A., Martínez-Rodríguez, M.M., Pérez-Alfocea, F., Romero-Aranda, R. and Bolarín, M.C.: The rootstock effect on the tomato salinity response depends on the shoot genotype. - Plant Sci. **162**: 825-831, 2002.

Shabala, S., Shabala, L., Volkenburgh, E.V.: Effect of calcium on root development and root ion fluxes in salinised barley seedlings. - Funct. Plant Biol. **30**: 507-514, 2003.

Suarez, D.L., Grieve, C.M.: Predicting cation ratios in corn from saline solution composition. - J. exp. Bot. **39**: 605-612, 1988.

Sun, J., Dai, S., Wang, R., Chen, S., Li, N., Zhou, X., Lu, C., Shen, X., Zheng, X., Hu, Z., Zhang, Z., Song, J., Xu, Y.: Calcium mediates root K^+/Na^+ homeostasis in poplar species differing in salt tolerance. - Tree Physiol. **29**: 1175-1186, 2009.

Sun, J., Wang, M.J., Ding, M.Q., Deng, S.R., Liu, M.Q., Lu, C.F., Zhou, X.Y., Shen, X., Zheng, X.J., Zhang, Z.K., Song, J., Hu, Z.M., Xu, Y., Chen, S.L.: H_2O_2 and cytosolic Ca^{2+} signals triggered by the PM H^+-coupled transport system mediate K^+/Na^+ homeostasis in NaCl-stressed *Populus euphratica* cells. - Plant Cell Environ. **33**: 943-958, 2010.

Tüzel, Y., Tüzel, I.H., Üçer, F.: Effects of salinity on tomato growing in substrate culture. - Acta Hort. **609**: 329-335, 2003.

Upadhyay, A., Upadhyay, A.K., Bhirangi, R.A.: Expression of Na^+/H^+ antiporter gene in response to water and salinity stress in grapevine rootstocks. - Biol. Plant. **56**: 762-766, 2012.

Van Hulten, M., Pelser, M., van Loon, L.C., Pieterse, C.M.J., Ton, J.: Costs and benefits of priming for defense in *Arabidopsis*. - Proc. nat Acad. Sci. USA. 103: 5602-5607, 2006.

Zhao, H.C., Zhu, T., Wu, J., Xi, B.S.: Effect of Ca^{2+} on H^+-ATPase activity of plasma membrane in wheat root. - Colloid Surface B **28**: 147-151, 2003.

Zhen, A., Bie, Z., Huang, Y., Liu, Z., Li, Q.: Effects of scion and rootstock genotypes on the anti-oxidant defense systems of grafted cucumber seedlings under NaCl stress. - Soil Sci. Plant Nutr. **56**: 263-271, 2010.

Zhu, J., Bie, Z.L., Huang, Y., Han, X.Y.: Effect of grafting on the growth and ion concentrations of cucumber seedlings under NaCl stress. - Soil Sci. Plant Nutr. **54**: 895-902, 2008.