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Physiology of acclimation to salinity stress in pea (*Pisum sativum*)

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ABSTRACT

Pea (*Pisum sativum* L.) seedlings were grown in half strength Hoagland solution and exposed to 0, 10, 25 mM NaCl and 2.5% PEG 6000 for 1 week (pre-treatment). Thereafter plants were exposed to 0 and 80 mM NaCl for 2 weeks (main treatment). The control plants were maintained in half strength Hoagland solution without NaCl. Various physiological parameters were recorded from control, pretreated and non-pretreated plants. There was no negative effect of the pre-treatments on growth (total fresh and dry matter production), and plants pre-treated with 10 mM NaCl had biomass accumulation equal to control plants. The beneficial effect of salt acclimation was also evident in the prevention of K^+ leakage and Na^+ accumulation, primary in roots, suggesting that here the physiological processes play the major role. 2.5% PEG 6000 was not as efficient as salt in enhancing salt tolerance and acclimation appears to be more related to ion-specific rather than osmotic component of stress. We also recorded an increase of the xylem K/Na in the salt acclimated plants. Therefore, the present study reveals that short-term exposure of the glycophyte *P. sativum* species activates a set of physiological adjustments enabling the plants to withstand severe saline conditions, and while acclimation takes place primary in the root tissues, control of xylem ion loading and efficient Na^+ sequestration in mesophyll cells are also important components of this process.

1. Introduction

Salt stress imposes a major environmental threat to agriculture by limiting plant growth and reducing crop yield. The increased salinization of arable land is expected to have global effects, resulting in 30% land loss within the next 25 years (Wang et al., 2003). Therefore, the efforts to increase salt tolerance of crop plants bear remarkable importance for sustainable agriculture.

Salinity affects plant growth and development by imposing osmotic stress on plants, causing specific ion (Na^+) toxicity, affecting activity of major cytosolic enzymes by disturbing intracellular potassium homeostasis, and causing oxidative stress in plant cells (Marschner, 1995; Sairam and Srivastava, 2002; Cuin and Shabala, 2007; Chen et al., 2007). The above effects take place in both root and leaf tissues but operate in different timescales. Specific Na^+ toxicity in leaves becomes critical only after many days (even weeks) after onset of salinity treatment (Munns and Tester, 2008). On the other hand, both massive depletion of cytosolic K^+ in plant roots (Shabala et al., 2006; Shabala and Cuin, 2008) and accumulation of reactive oxygen species in root cells (Demidchik et al., 2003, 2007) operate in a minute timescale; together, these may cause programmed cell death (PCD) in root cells within a couple of hours after exposure to NaCl (reviewed in Shabala, 2009). Given this complexity, it is obvious that plant acclimation to salinity may be also physiologically multifaceted.

In broad terms, acclimation to external environmental changes can occur in plants thanks to internal adjustments within tissues and cells, enabling plant metabolism to proceed under these somewhat altered conditions (Demmig-Adams et al., 2008). In a contrast to adaptation that occurs in plant phylogeny, acclimation occurs during plant ontogeny and describes enhanced stress tolerance of a particular *individual* plant. In the context of salinity, it was widely reported that many plant species increased the ability to tolerate salt stress after being exposed to low level of stress for a certain period of time (Amzallag et al., 1990; Bethke and Drew, 1992; Umezawa et al., 2000; Silveira et al., 2001; Djanaguiraman et al., 2006). The beneficial effects of acclimation included both agronomical (e.g. improved survival rate; higher growth rate; less biomass reduction) and physiological (lower Na^+ accumulation in the shoot; better osmotic adjustment) characteristics (Umezawa et al., 2000; Djanaguiraman et al., 2006). However, the physiological mechanisms beyond this acquired resistance to salinity remain largely a matter of conjecture.

It was suggested that better performance of acclimated plants is a consequence of reduced accumulation of Na^+ in plant leaves

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(Umezawa et al., 2000). However, it remains unclear on weather this phenomenon is related to better Na^+ exclusion from uptake or improved control of Na^+ loading into the xylem (Durand and Lacan, 1994; Lacan and Durand, 1995, 1996), or result from better retrieval of Na^+ from the shoot (Winter, 1982). Beneficial effects of acclimation may be also a consequence of plant improved ability to withstand osmotic stress (Ottow et al., 2005; Saha et al., 2010) and be not related to the specific Na^+ toxicity. Finally, it was suggested that acclimation to salinity may be achieved via enhancing the antioxidant defence system (Saha et al., 2010). Multiple reports suggest that exogenous application of H_2O_2 may lead to plant acclimation to salinity, cold or heat stresses (Prasad et al., 1994a,b; Uchida et al., 2002; De Azevedo Neto et al., 2005), and the cell's ability to scavenge excessive reactive oxygen species (ROS) and protect cell structures is considered to be absolutely essential for salinity tolerance (Polidoros and Scandalios, 1999; Munns and Tester, 2008). It is assumed that plants pre-treatment with moderate levels of H_2O_2 may increase the synthesis of a wide variety of antioxidant compounds and, as such, make plants more prepared for the oncoming oxidative stress imposed by salinity (De Azevedo Neto et al., 2005). This phenomenon is termed "cross-tolerance" and is widely reported in the literature (Bowler et al., 1992; Bowler and Fluhr, 2000; Shabala et al., 2011).

The aim of the study was to investigate the salt-acclimation process in the glycophyte plant *Pisum sativum* by answering two specific questions: (1) which of the two major components – specific ion toxicity or osmotic stress – has a bigger role in plant acclimation to salinity, and (2) whether the acclimation affects take place in plant root or shoot tissues. This was achieved by the whole-plant physiological assessment of plants pre-treated with two levels of NaCl and comparing these results with the data obtained by acclimation in non-ionic (PEG) isotonic media. Our results suggest that exposing *P. sativum* to moderate salinities activates a set of physiological adjustments enabling the plants to withstand severe saline conditions, and that it is the ion toxicity component of salt stress that play a major role in plant acclimation. This acclimation takes place primary in the root tissues, and involves regulating Na^+ loading in the xylem, affecting its following transport to the shoot and a consequent shoot growth.

2. Materials and methods

2.1. Plant material and growth conditions

Pea seeds (*P. sativum* L. var. Onward) were obtained from the Hollander Imports (Hobart, Tasmania). Seeds were sterilized for 10 min with 50% commercial bleach and germinated on a filter paper moistened with distilled water, in darkness at 25 °C. After 3 days of germination, 160 uniform seedlings were selected and transferred in 41 plastic containers containing aerated half strength Hoagland solution (pH 6). The nutrient solution was partially renewed every week so that the nutrient concentration never dropped below 80% of the target value. Eight plants were placed in each container. The experiment was conducted in a temperature-controlled room with 26/19 °C day/night temperatures, 16-h day length (photoperiod), and 300 $\mu mol\ m^{-2}\ s^{-1}$ illumination. The relative humidity was maintained at about 70%.

2.2. Treatments

After 7 days from the transplant to hydroponics, plants were divided into five groups (four containers, with eight plants in each; total 32 plants for each treatment). Three groups were acclimated in half-strength Hoagland containing one of: 10 mM NaCl, 25 mM NaCl, and 2.5% polyethylene glycol (PEG 6000 Sigma Aldrich). The latter treatment was isotonic to 10 mM NaCl treatment. One week later the plants were treated with 80 mM NaCl for 14 days, except one group, which remained as control. Therefore, five treatments altogether were used in this study. These are abbreviated as follows:

- Control – not acclimated and not stressed
- NA – not acclimated, stressed with 80 mM NaCl
- A(10) – acclimated in 10 mM NaCl, stressed with 80 mM NaCl
- A(25) – acclimated in 25 mM NaCl, stressed with 80 mM NaCl
- A(PEG) – acclimated in 2.5% PEG 6000, stressed with 80 mM NaCl.

2.3. Whole-plant physiological assessment

Eight plants were harvested before the start of acclimation (day 10), at the end of acclimation (day 17), and after the salt stress (day 31). Plants were divided into leaves, stems and roots, and their fresh weight (FW) was measured. Samples were then dried at 70 °C for 72 h, and their dry weight (DW) then determined.

2.4. Sap analysis for K^+, Na^+ and osmolality

Eight plants were harvested for each treatment at the end of acclimation and NaCl stress period (day 17 and 31, respectively). For each plant, one of the oldest, fully expanded but not senescing leaves (about 1/3 position from the bottom) was collected. Root samples were also collected by rinsing them thoroughly in 10 mM $CaCl_2$ for 2 min to avoid apoplastic retention of NaCl and blotting them dry with paper towels. Samples were collected in Eppendorf and Falcon tubes, respectively, and stored at −20 °C. Leaf and root sap was extracted using the freeze–thaw method as described before (Chen et al., 2007) and its osmolality was determined using a vapour pressure osmometer (Vapro, Wescor Inc., Logan, UT, USA). For the determination of Na^+ and K^+ contents, samples were diluted 1:50 and measured using a flame photometer as described before (Cuin and Shabala, 2005).

2.5. Xylem sap analysis

The same plants used for sap analysis were excised at stem base at the day of acclimation and NaCl stress periods, and xylem sap was collected using the Scholander-type pressure bomb as previously described in Shabala et al. (2010). The pressure values were adjusted individually for each treatment, but in general 10–15 bars were applied to extract xylem from acclimated and not acclimated plants, whereas 15–25 bars were applied for the salt stressed treatments. Potassium and sodium content in the xylem sap were then quantified by using a flame photometer.

2.6. SPAD and Gs measurements

Leaf chlorophyll content was measured on the second topmost fully expanded leaves of all the plants per pot with a chlorophyll meter (SPAD-502 Chlorophyll Meter, Minolta Camera Co. Ltd., Japan) at weekly intervals. At the same time stomatal conductance (Gs) was measured using a Delta-T MK3 porometer (Delta-T devices, Cambridge, UK) from the same leaf.

2.7. Statistical analysis

The experiment consisted of a randomized block of six treatments. Four replications for each treatment were performed. Statistical analysis of data was processed using analysis of variance (one-way ANOVA) and differences between columns were assessed using Tukey's Multiple Comparison Test with the software

Graph-Pad Prism (Ver. 5.0a for MAC OS X). Differences between treatments were considered significant if $P < 0.05$.

3. Results

3.1. Plant growth

At the end of the acclimation week, none of acclimation treatments did show any significant difference with control plants either in fresh and dry weights or in the biomass distribution between root and shoot (data not shown). Also unaffected was the relative water content in either roots and leaves. No statistically significant ($P < 0.05$) differences were also detected by non-destructive SPAD and Gs measurements (data not shown).

One week of acclimation in either 10 mM (defined as A(10) treatment) or 25 mM (A(25) treatment) significantly reduced detrimental effects of 80 mM NaCl stress on plant FW and DW (Table 1). As a result, growth of A(10) and A(25) plants was comparable to controls (Table 1). These beneficial effects were not observed in plants pre-treated in the isotonic non-ionic osmolyte, PEG (A(PEG) treatment). Beneficial effect of acclimation was more pronounced in 10 mM than 25 mM NaCl, both in leaves and roots. The relative water content (RWC) in A(10) and A(25) treatments was also not significantly ($P < 0.05$) different from control (Table 1). The root to shoot ratio in A(10) A(25) and A(PEG) treatments was similar to control plants (Table 1) while in NA treatment there was a dramatic shift toward the shoot biomass.

3.2. Effects on leaf and root ionic relations and osmolality

Acclimation treatment affected both root and shoot ion content (Table 2). A significant decrease in leaf sap K^+ was observed in both A(10) and A(25) treatments, while in roots only A(25) plants showed a small decline in K^+ content. As expected, sodium was higher in plants from both salt-acclimated treatments. Interestingly, this difference in Na^+ and K^+ content after one week of acclimation period was not reflected in the biomass difference compared with control plants (Table 1). PEG-acclimated plants showed no significant ($P < 0.05$) difference in either root or shoot sap K^+ and Na^+ content compared with controls (Table 2).

80 mM NaCl salinity stress significantly enhanced potassium deficiency and a sodium accumulation in the leaves of all the plants without any difference among the treatments (Table 2), showing that acclimation does not prevent Na^+ accumulation in leaves. On the other hand, in roots, potassium contents of A(10) A(25) and A(PEG) were significantly higher than the NA plants. Also lower was root Na^+ content in acclimated plants (Table 2).

The above trend is also reflected in the K^+/Na^+ ratio. Both A(10) and A(25) acclimated plants were able to maintain a higher K^+/Na^+ ratio in their roots compared to NA plants, while in shoots no beneficial effect of acclimation on K^+/Na^+ ratio was found (data not shown).

There was no effect of acclimation on sap osmolality in leaves, while in roots A(25) plants had slightly higher sap osmolality (Fig. 1). Both leaf and root sap osmolality was significantly increased in salt-stressed (80 mM NaCl) samples; A(10) and A(25) had the highest values both in leaves and roots, while the osmolality of A(PEG) did not differ from non-acclimated (NA) plants (Fig. 1).

3.3. Xylem sap analysis

Xylem Na^+ content increased dramatically in A(10) and A(25) treated samples by the end of acclimation period, while no significant ($P < 0.05$) change in the potassium concentration was observed (Fig. 2). No effect of PEG on xylem sap was detected (Fig. 2). Two weeks of salt stress resulted in a significant (five fold) increase in the xylem sap Na^+ concentrations in NA plants compared with control (Fig. 2). At the same time, all acclimation treatments reduced the amount of Na^+ in salt-stressed plants (Fig. 2). No effects of acclimation on xylem K^+ was found in salt-stressed plants (Fig. 2). As a result, the xylem K^+/Na^+ ratio was higher in all acclimated treatments (data not shown).

3.4. Chlorophyll content (SPAD) and stomatal conductance

At the end of the acclimation period, both leaf chlorophyll content and stomatal conductance (Gs) were not significantly ($P < 0.05$) different between treatments (Fig. 3). The 80 mM NaCl stress caused a general decrease in chlorophyll content in all treated plants; this decline was less pronounced in A(10) and A(25) plants (significant at $P < 0.05$). The beneficial effect of acclimation in ionic osmolyte (A(10) and A(25) treatments) was highly pronounced when stress-induced changes in Gs is analyzed (Fig. 3).

4. Discussion

4.1. Acclimation does not impose severe yield penalties

Plants exposure to low level salinity activates an array of processes leading to an improvement of plant stress tolerance. This has already been demonstrated for different herbaceous species such as soybean, rice and sorghum (Amzallag et al., 1990; Umezawa et al., 2000; Djanaguiraman et al., 2006). For example, soybean pretreated for 23 days showed a higher survival rate under severe stress conditions (Umezawa et al., 2000); in rice, one week of pretreatment decreased leaf area and total dry matter production, but improved growth rate and shoot and root length after one week of severe salt treatment (Djanaguiraman et al., 2006); and in sorghum pre-treated plants maintained the same growth rate before and after the exposure to high level of salt, and they could stand a concentration much higher than non acclimated plants (Amzallag et al., 1990).

In our study we did not report any reduction in biomass during the acclimation phase, and after the main salt treatment, the salt-acclimated A(10) plants performed much better compared with the NA (Table 1) and in view of future cultivation in severe environments, such penalty may be accepted by growers. Interestingly, A(10) biomass accumulation was equal to control plants and this was despite plants had lower K^+ content and higher Na^+ content at the end of the pre-treatment (Table 2). This suggests that (1) Na^+ was efficiently sequestered in vacuoles during pre-treatment and main treatment; (2) cytosolic K^+ content did not drop below minimal threshold. The proposed scenario to describe this strategy is that Na^+ is pumped into vacuoles (by NHX exchanger) to prevent cytosolic Na^+ toxicity (Blumwald et al., 2000; Shi et al., 2003); and even if some K^+ may leak from the cytosol, it is quickly replenished from vacuole. As a result, the overall K^+ is lower and overall Na^+ is higher but no yield penalties and no changes in Gs and SPAD are reported (Fig. 3a and b).

4.2. Acclimation to salinity is related to ion-specific rather than osmotic component of stress

Although there are several reports in which the induction of increased salt tolerance by NaCl treatments is investigated, they are mainly focused on the effect of the pre-treatment (Amzallag et al., 1990; Umezawa et al., 2000; Djanaguiraman et al., 2006), and there are no evidences whether this induction is due to ion specific or osmotic component of stress. In order to address this specific issue, we included a pre-treatment with PEG to directly compare its acclimating effect with A(10) treatment. Our data show

Table 1
Root to shoot ratio, relative water contents (RWC), fresh and dry weights after 2 weeks of salt stress. Mean ± SE (n = 8).

Treatment	Root/shoot ratio	RWC (%)	Fresh weight (g)			Dry weight (g)		
			Total	Leaves	Roots	Total	Leaves	Roots
Control	0.76 ± 0.08 b	91.88 ± 0.37 a	35.86 ± 2.66 a	17.70 ± 1.24 a	15.88 ± 1.29 a	2.99 ± 0.19 a	1.92 ± 0.15 a	0.68 ± 0.05 a
NA	1.76 ± 0.19 a	88.65 ± 0.13 c	16.28 ± 3.92 b	4.50 ± 1.81 c	6.90 ± 1.16 b	1.41 ± 0.28 c	0.90 ± 0.18 b	0.33 ± 0.06 b
A(10)	0.80 ± 0.08 b	91.71 ± 0.32 ab	35.55 ± 2.41 a	16.88 ± 0.88 a	15.73 ± 2.00 a	2.92 ± 0.08 ab	1.73 ± 0.04 a	0.69 ± 0.10 a
A(25)	0.74 ± 0.03 b	91.59 ± 0.14 ab	25.78 ± 2.41 ab	13.30 ± 1.06 ab	10.52 ± 0.85 b	2.02 ± 0.19 bc	1.07 ± 0.08 b	0.53 ± 0.05 ab
A(PEG)	0.73 ± 0.03 b	90.63 ± 0.47 b	16.30 ± 1.23 b	7.63 ± 0.87 bc	6.61 ± 0.35 b	1.48 ± 0.05 c	0.93 ± 0.04 b	0.36 ± 0.02 b

Table 2
Na^+ and K^+ content in leaves and roots, measured at the end of acclimation period (acclimation) and after 2 weeks of 80 mM NaCl (2 weeks NaCl stress). Mean ± SE (n = 6).

Treatment	Leaves				Roots			
	Acclimation		2 weeks NaCl stress		Acclimation		2 weeks NaCl stress	
	K^+ (mM)	Na^+ (mM)	K^+ (mM)	Na^+ (mM)	K^+ (mM)	Na^+ (mM)	K^+ (mM)	Na^+ (mM)
Control	127.91 ± 4.17 a	2.4183 ± 0.42 c	105.93 ± 5.49 a	2.55 ± 0.99 b	119.14 ± 5.87 a	5.83 ± 0.21 c	94.83 ± 1.25 a	5.19 ± 0.04 d
NA[a]	–	–	43.06 ± 7.56 b	309.75 ± 12.2 a	–	–	32.57 ± 0.35 c	140.10 ± 6.54 a
A(10)	101.30 ± 3.63 b	39.074 ± 5.48 b	39.92 ± 4.27 b	313.00 ± 4.87 a	113.17 ± 3.29 ab	9.77 ± 0.65 b	44.15 ± 2.45 b	114.96 ± 1.94 bc
A(25)	70.344 ± 3.01 c	89.246 ± 4.78 a	34.84 ± 4.66 b	298.08 ± 18.2 a	99.025 ± 2.68 b	16.69 ± 1.05 a	46.08 ± 2.84 b	123.25 ± 4.33 ab
A(PEG)	132.20 ± 3.66 a	2.4331 ± 0.44 c	58.65 ± 5.17 b	285.21 ± 17.5 a	107.16 ± 5.56 ab	6.53 ± 0.89 c	47.99 ± 1.04 b	99.63 ± 8.15 c

[a] K^+ and Na^+ contents for acclimated NA are not provided because they are the same than control.

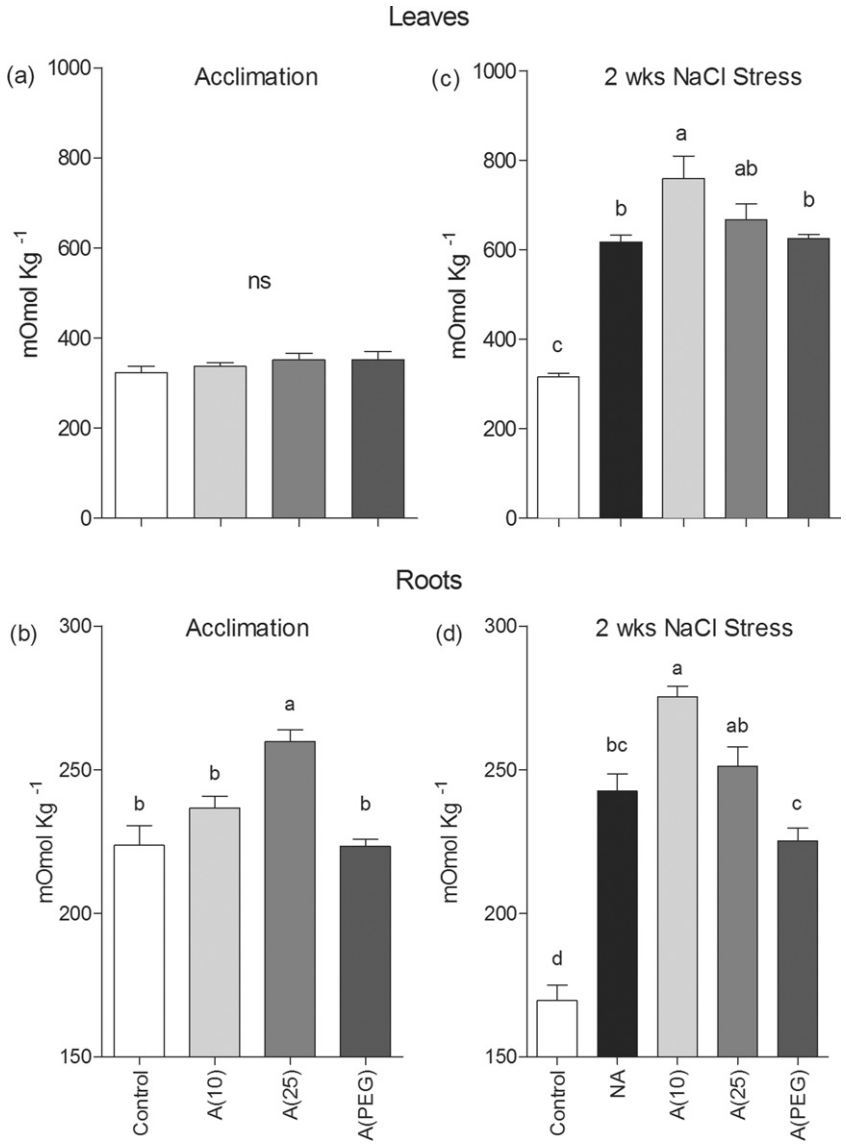

Fig. 1. Osmolality of leaf and root sap measured at the end of acclimation period (a, b) and after 2 weeks of salt stress (c, d). Mean ± SE (n = 6).

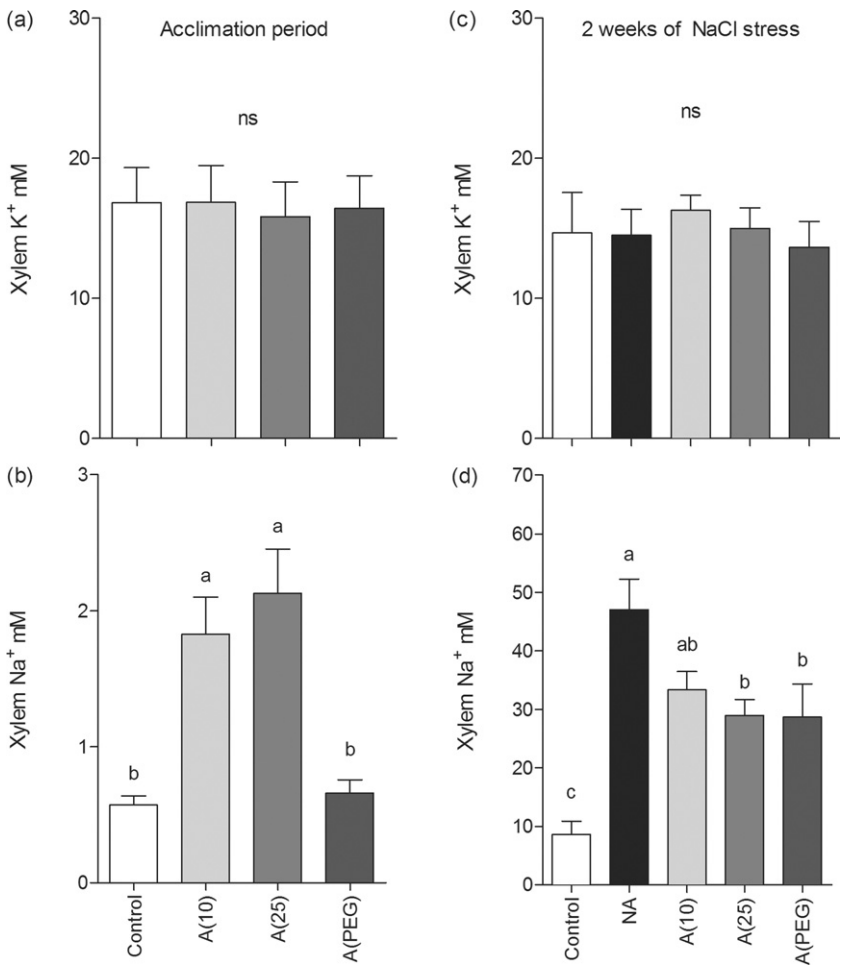

Fig. 2. Xylem sap Na^+ and K^+ content (determined in samples collected by Scholander pressure bomb) measured at the end of acclimation period (a, b) and after 2 weeks of salt stress (c, d). Mean ± SE ($n = 6$).

that PEG was not as efficient as NaCl in triggering acclimation process. In particular, it is not the acclimation to osmotic component of salinity per se, but acclimation in NaCl which does improve plant water relations (e.g. RWC) and osmotic adjustment (e.g. Gs). In this view, it may be suggested that A(10) and A(25) plants use Na^+ as a "cheap" osmoticum and take it to shoot to maintain turgor within the cells, resulting in better water retention and less reduction in Gs. The question which arises is whether this effect is Na^+ specific or not, and further experiments could be done in order to test the effectiveness of other salts (e.g. KCl) in triggering acclimation.

4.3. Physiological process in roots are involved in plant acclimation

In the literature, rice pre-treated with low level of NaCl showed a general increase in the accumulation of K^+ over non pre-treated plants in roots and shoots, and promoted root length (Djanaguiraman et al., 2006); whereas in soybean acclimation resulted in a higher accumulation of Na in leaves. This is consistent with our results, in fact the A(salt) treatments prevent K^+ leakage and Na^+ accumulation in roots compared with NA (Table 2). This suggests that acclimation in salt involves modifications to ion channels involved in maintenance of optimal cytosolic K/Na ratio, primary in roots. The maintenance of such equilibrium is very important for the ability of a plant to survive in saline environments (Boursier and Läuchli, 1990; Cuin et al., 2003; Colmer et al., 2006; Shabala and Cuin, 2008) K^+ is essential for key metabolic processes in the cytoplasm, such as enzymatic reactions, protein synthesis and ribosome; and Na^+ can dramatically compete with it thanks to the similarity in physicochemical properties (Marschner, 1995).

The control of Na^+ uptake and distribution within the plant is very important for salt tolerance, and several mechanisms may reduce specific Na^+ toxicity in roots. The latter could restrict Na^+ uptake, actively exclude Na^+ back to the soil solution and/or enhance compartmentation of excessive Na^+ in root's vacuole. In particular, the plasma membrane salt overly sensitive (SOS1) activity is fundamental for Na^+ extrusion from root (Shi et al., 2002; Zhu, 2003), and its over expression could be responsible for a lower net uptake of Na in roots. Also, an efficient accumulation of Na^+ in the vacuoles has a dual benefit in saline condition: enhanced vacuolar sequestration results in avoidance of the toxic Na^+ accumulation in cytosol, as well as contributes to the turgor adjustment (Shabala and Lew, 2002). This sequestration is mediated by tonoplast Na^+/H^+ exchangers of the NHX family (Zhang et al., 2001; Yokoi et al., 2002). As far as unidirectional sodium uptake may concern, there appears to be no major difference in unidirectional Na^+ uptake between varieties contrasting in their salinity tolerance, as reported for several species (Davenport et al., 1997; Chen et al., 2007). This does not exclude, however, a possibility that acclimation may affect unidirectional Na^+ uptake; future experiments with $^{22}Na^+$ radiotracers may shed a light on this issue.

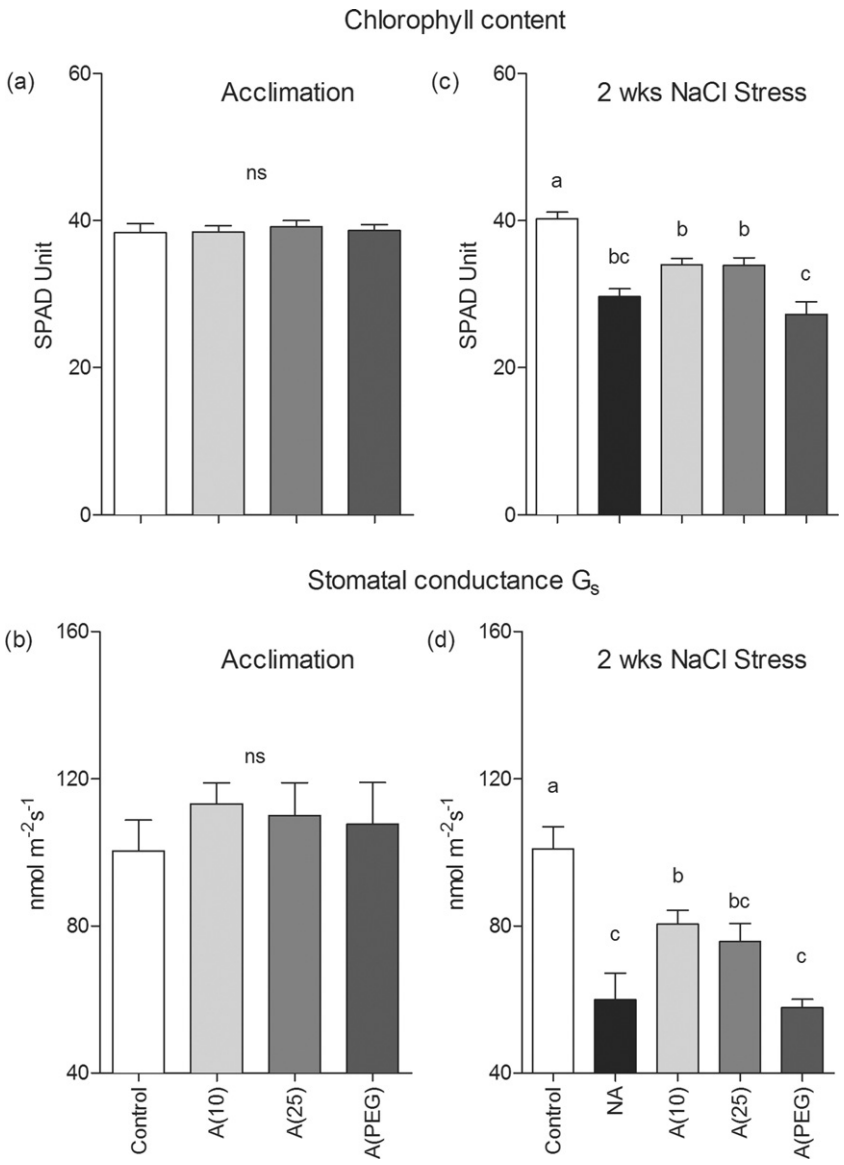

Fig. 3. Chlorophyll content (measured by chlorophyll meter SPAD), and stomatal conductance (Gs) measured at the end of acclimation period and after 2 weeks of salt stress. Mean ± SE ($n = 6$).

4.4. Regulation of xylem ion loading is essential for acclimation

Exclusion of Na^+ from the shoots is often named as the most essential feature of salinity tolerance in plants (Garthwaite et al., 2005; Munns and Tester, 2008). Lower accumulation of Na^+ in leaves was reported in salt-acclimated soybean plants (Umezawa et al., 2000), in our work no significant difference in total leaf Na^+ was found between acclimated and non-acclimated plants (Table 2). However, much better overall performance of acclimated plants (Table 1 and Fig. 3) suggests a substantial difference in Na^+ sequestration between various leaf tissues and/or intracellular compartments. Indeed, high Gs in salt-acclimated plants is indicative that a larger fraction of accumulated Na^+ was excluded from the cytosol, and can be explained as follow.

In order to reduce Na^+ in the shoots plant can either minimize the entry from the root symplast (Gorham et al., 1990; Davenport et al., 2005), to reduce loading or maximize Na^+ retrieval from the xylem (Davenport et al., 2007), or export Na^+ from the leaf into the phloem (Berthomieu et al., 2003). Our results on xylem ionic content (Fig. 2) show that A(salt) plants had lower xylem Na^+ content, and this seems to contradict the fact that shoot Na^+ concentrations were not significantly different between A(salt) and NA (Table 2). This suggests that the process of Na^+ loading into the xylem is highly dynamic process. It appears that immediately after stress onset, the rate of Na^+ loading into the xylem of acclimated plants may be even higher than in non-acclimated ones. In this way, acclimated plants can quickly get Na^+ to the shoot, sequester them in vacuoles and use it to maintain shoot turgor, optimal Gs (Fig. 3) and, hence, preserve the growth (Table 1). However, continuous Na^+ loading into the xylem is a dangerous option, as the overall amount of Na^+ coming into the mesophyll tissue may exceed the vacuolar sequestration ability in this tissue. Thus, once the osmotic adjustment in the shoot is achieved, plants reduce the rate of Na^+ loading into the xylem. As the data reported in Fig. 2 represents only one "snapshot" taken 2 weeks after stress onset, it appears that by that time acclimated plants have already reduced the rate of Na^+ loading into the xylem. More detailed kinetic studies (e.g. daily xylem sap sampling) are needed to validate this hypothesis.

An additional support for the proposed scenario comes from the literature. Chen et al. (2007) have compared kinetics of Na^+

accumulation in leaves of barley varieties contrasting in their salinity tolerance. Salt tolerant varieties had much higher Na^+ content in the leaves after 1 week of salt stress; this content remained rather stable over the next few weeks. On the contrary, sensitive varieties had initially lower Na^+ content in leaves but accumulated it progressively over the time and had much more shoot Na^+ few weeks after NaCl treatment (Chen et al., 2007). It was also found that plant's ability to maintaining higher xylem K/Na ratio was also one of the key attributes of salinity tolerance in barley (Shabala et al., 2010). This is consistent with our current data for peas, showing a reduction of Na^+ in the xylem of salt acclimated plants without any reduction of K^+ and resulting in the overall increase of the xylem K/Na ratio at the end of the experiment.

5. Conclusions

Plant salinity tolerance is a multifaceted physiological trait. In this study we show short-term exposure of the glycophyte *P. sativum* species activates a set of physiological adjustments enabling the plants to withstand severe saline conditions. Our data suggest that ion-specific component of salt stress plays a major role in plant acclimation. While acclimation takes place primary in the root tissues, control of xylem ion loading and efficient Na^+ sequestration in mesophyll cells are also important components of this process.

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