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Boron and calcium increase *Pisum sativum* seed germination and seedling development under salt stress

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Abstract

A beneficial effect of B and Ca application on symbiotic interaction between legume and rhizobia under saline conditions has recently been shown, suggesting conventional agricultural practices to increase crop salt tolerance. However, nothing is known about application of both nutrients on early events of legume development under salt stress, prior to the establishment of a symbiotic interaction. Therefore, the effects of different levels of B (from 9.3 to 93 μM B) and Ca (from 0.68 to 5.44 mM Ca) on seed germination, root elongation, plant development, and mineral composition of pea (*Pisum sativum* L. cv. Argona) grown under 0 to 150 mM NaCl, were analysed. Development of plants previously germinated in the presence of salt was more impaired than that of plants put under salt stress once seeds were germinated. A NaCl concentration of 75 mM and 150 mM inhibited pea seed germination and seedling growth. The addition of either extra B or extra Ca to the germination solution prevented the reduction caused by 75 mM NaCl but not that of 150 mM NaCl. However, root elongation and plant development under salt stress (75 mM NaCl) was enhanced only by addition of both B and Ca. When plants were cultivated in the absence of external N, N content in roots and shoots originating from seeds was diminished by salt and enhanced by B and Ca, suggesting a role of these nutrients in remobilisation of seed nutrient stores. Salinity also led to an extremely high concentration of Na^+ ions, and to a decrease of B and Ca concentrations. This can be overcome by addition of both nutrients, increasing salt tolerance of developing pea plants. The necessity of nutritional studies to increase crop production in saline soils is discussed and proposed.

Introduction

High concentrations of salt in soils are one of the factors responsible for decreases in the yield of a wide range of crops in many regions of the world. Approximately one third of irrigated land is significantly affected by salinity (Ghassemi et al., 1995). Irrigated land is estimated to produce more than 30% of the world's food (Munns, 2002), so salinization of this resource is particularly critical.

Plants differ greatly in their response to salinity (Hasegawa et al., 2000), with most legumes classified as salt sensitive species (Greenway and Munns, 1980; Läuchli, 1984). There are three main negative effects of high salt concentrations that influence plant growth and development: water deficit (Munns and Termaat, 1986), ion toxicity associated with excessive Cl^- and Na^+ (Niu et al., 1995), and interference with nutrition, leading to nutrient imbalance (Silberbush and Ben-Asher, 2001). Hence, studying the interaction of salt with nutrients that have key roles in plant development, such as boron and calcium, is important to optimise the growth of legumes under salinity.

Boron and Ca have several common features (i.e. low mobility, high extra-cytoplasmic concentration compared with intra-cytoplasmic content, growth alterations during deficiency. . .). The amount and availability of either of these nutrients influences the distribution (Ramón et al., 1990) and the requirements

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of the other for optimal plant growth (Teasdale and Richards, 1990).

Evidence of a B-Ca interaction for the transport of IAA through the cell membrane has been reported (Tang and De la Fuente, 1986), although most investigations on the topic dealt with the structure and function of the cell wall. In a study of the chemistry of B-Ca intreraction, Van Duin et al. (1987) described how Ca^{2+} is able to form complexes with borate-polyhydroxy-carboxylates through direct interaction with the borate anion. O'Neill et al. (1996, 2001) reported that the rhamnogalacturan II region is stabilized by B through borate-diester bonds with apiosyl residues. Furthermore, Kobayashi et al. (1999) demonstrated that Ca^{2+} promoted *in vitro* formation of dimers of borate-rhamnogalacturan II and proposed that Ca^{2+} stabilizes pectin polysaccharides of the cell wall through ionic and coordinate bonding in the polygalacturonic acid region. This interaction has recently been shown by imnmunolocalization of pectin polysaccharides in cell walls of pea root nodules (Redondo-Nieto et al., 2003). Regarding legume development, Carpena et al. (2000) reported that addition of high Ca during pea growth under B deficiency could mediate mobilisation of B from old to young tissues in pea plants. In agreement with this report, measurements of the concentration of B in nodules indicate that it is higher when B-deficient plants grow with a supplement of extra Ca. This is a sign of a B-Ca interaction in the establishment and maintenance of the symbiosis (Redondo-Nieto et al., 2003).

Mineral analysis in *Pisum sativum* plants nodulated with *Rhizobium leguminosarum*, showed that salt-stress leads to deficiency of potasium, iron, boron and calcium in shoots and nodulated roots, and that a supplement of B and Ca recovered nutrient balance and increased salt-tolerance of the symbiotic nitrogen fixation process (El-Hamdaoui et al., 2003b) by ameliorating nodule development (El-Hamdaoui et al., 2003a). This previous knowledge suggests that using conventional farming practices could increase crop salt tolerance. However, nothing is known about the effects of such practices on early events of legume development under salt stress, prior to the establishment of a symbiotic interaction. Therefore, the aim of this report was to investigate the effects of different B and Ca^{2+} concentrations on the response to salt stress of the early development of *Pisum sativum* plants, including seed germination and seedling growth in the absence of both soil N or symbiotic N_2 fixation.

Materials and methods

Seed germination

Pea (*Pisum sativum* cv. Argona) seeds were surface-sterilized with 70% (v/v) ethanol for 1 min and 10% (v/v) sodium hypochlorite for 20 min and soaked for 4 h in sterile distilled water. 500 seeds per treatment were germinated on trays containing wet Perlite (previously tested to not release B, Ca or salt) at 25 °C. For salinity treatments, NaCl was added at a concentration of 0, 75 mM or 150 mM. Boron was added as H_3BO_3 and Ca as $CaCl_2$. The concentrations of B (from 9.3 μM to 93 μM.) and Ca (up to 5.44 mM) from the different treatments are summarized in Table 1. The pH was always between 6.5–6.7. The percentage of germinated seeds from each treatment was calculated every 24 h. After 6 d, elongation of seedling roots was measured.

Growth of plants

Previously germinated pea seedlings were transferred to plastic growth pots and cultivated on Perlite with FP (Fahraeus for plants) N-free medium (Fahraeus, 1957), as the nutrient solution (0.68 mM $CaCl_2$; 0.5 mM $MgSO_4.7H_2O$; 0.7 mM KH_2PO_4; 0.68 mM $Na_2HPO_4.2H_2O$; 50 μM Fe-EDTA; 9.3 μM H_3BO_3; 10.6 μM $MnSO_4.H_2O$; 0.7 μM $ZnSO_4.7H_2O$; 3.2 μM $CuSO_4.5H_2O$; 1 μM $Na_2MoO_4.2H_2O$). For salinity conditions, NaCl was added to the nutrient solution at a final concentration of 75 mM. Boron and Ca were added as above to establish the different treatments (Table 1). The nutrient solutions were renewed every three days. The pH was always kept between 6.5–6.7. Plants were maintained in a growth cabinet at 22 °C day/18 °C night temperatures with a 16–8 h photoperiod and an irradiance of 190 μmol m^{-2} s^{-1}. Relative humidity was kept between 60% and 70%.

Mineral analysis of plants

Thirty plants of each treatment were randomly selected. Shoots and roots were separated and dried at 80 °C for 24 h to determine dry weight. After milling to ensure homogeneity, 0.5 g of each sample was oven-ashed (2 h at 480 °C arisen after 2 h of gradual increase of temperature) and acid-digested (1 M HCl) at 70 °C for 30 min. Boron was determined on acid-digested samples using Azomethine H at pH 5.1 (Wolf, 1974) and a Technicon Automatic Analytical System. The

Table 1. Boron and calcium treatments used in this study. Enclosed by parenthesis are the effective activities of Ca^{2+} ions (calculated by the Debye-Hückel equation as mmol L^{-1}) in saline solutions with 75 and 150 m*M* NaCl respectively. As boric acid is mostly a non-ionic form (pKa = 9.2), the effective activity of B is the added concentration of H_3BO_3

	0.68 (0.29, 0.24) m*M* Ca^{2+}	1.36 (0.58, 0.48) m*M* Ca^{2+}	2.72 (1.16, 0.96) m*M* Ca^{2+}	5.44 (2.27, 1.90) m*M* Ca^{2+}
9.3 μM				
H_3BO_3	+B+Ca	+B+2Ca	+B+4Ca	+B+8Ca
55.8 μM				
H_3BO_3	+6B+Ca	+6B+2Ca	+6B+4Ca	+6B+8Ca
93 μM				
H_3BO_3	+10B+Ca	+10B+2Ca	+10B+4Ca	+10B+8Ca

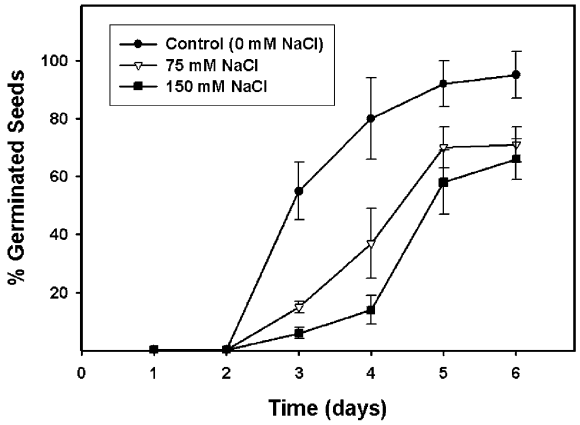

Figure 1. Effects of the presence of salt concentations (0, 75 and 150 m*M* NaCl) on the germination of *Pisum sativum* cv. Argona seeds. 500 seeds per treatment were surface sterilized, plated on trays containing perlite soaked with the different NaCl concentrations. Percentage of germinated seeds was calculated every day over a 6 days period.

concentrations of Ca^{2+}, Na^+ were analysed in a Perkin Elmer Analyst 800 spectrophotometer. To avoid interferences, 0.5% $La(NO_3)_3$, 0.02% CsCl, 5% HCl was added to samples and standards at a 1:10 proportion. Ca^{2+} was measured at 422.7 nm with a PE6017 lamp, Na^+ was measured by emission at 589 nm. The content of N was measured on acid-digested samples by the Kjeldhal method (Bremner, 1965).

Statistical analysis

All the experiments were repeated at least four times and data was statistically analysed by the one-way ANOVA-test. Data in Figures are the means ± standard error.

Results

Germination of pea (*P. sativum* cv. Argona) seeds was 25% reduced by salt (75 or 150 m*M* NaCl) (Figure 1). Moreover, the presence of 75 and 150 m*M* NaCl in the solution caused a delay of 2–3 d respectively in the commencement of seed germination. When pea seeds were germinated for 3 days in the absence of salt, weights of roots and shoots were not affected by 75 m*M* NaCl after 10 days of growth (Table 2). However, when pea seeds were germinated in 75 m*M* NaCl solution, weights of roots and aerial parts were halved. After 15 days of growth, development of plants was also reduced by 75 m*M* NaCl when seeds were germinated in the absence of salt. These observations suggest that salt severely affected early stages of plant development.

The presence of increasing concentrations of Ca did not affect seed germination in the absence of salt (Figure 2A). In the presence of 75 m*M* NaCl, increasing Ca increased the proportion of germinated seeds and prevented the delay on germination (Figure 2B). However, Ca did not prevent the inhibitory effects of 150 m*M* NaCl on pea seed germination (Figure 2C). Similar to Ca, addition of B up to 55.8 μM (6B treatments) increased germination rate when 75 m*M* NaCl was added (Figure 2E), but not in the presence of 150 m*M* NaCl (Figure 2F). Boron addition as high as 93 μM B (10 B treatments) inhibited germination even in the absence of salt (Figure 2D). The levels of 6B combined with high Ca concentrations (more than 2Ca) delayed and inhibited respectively seed germination in the absence of salt (Figure 3A). In the presence of 75 m*M* NaCl (Figure 3B), the preventive effects of 6B treatment on germination increased as Ca increased. The negative effects of treatment of

Table 2. Effects of salt stress (75 m*M* NaCl) on the early growth of *Pisum sativum* cv. Argona plants. Values in each column followed by the same letter are not significantly different at $P = 0.05$ (Student's *t*-test)

	g (fresh weight) shoot after 10 days	g (fresh weight) root after 10 days	g (fresh weight) root after 15 days	g (fresh weight) shoot after 15 days
Control (0 m*M* NaCl)	0.17 ± 0.05a	0.27 ± 0.08a	0.29 ± 0.09a	0.42 ± 0.13a
75 m*M* NaCl (seeds previously germinated for 3 days without added NaCl)	0.14 ± 0.03a	0.23 ± 0.05a	0.17 ± 0.06b	0.28 ± 0.08b
75 m*M* NaCl (seeds previously germinated for 3 days with 75 m*M* NaCl)	0.07 ± 0.03b	0.14 ± 0.04b	0.09 ± 0.04c	0.18 ± 0.06c

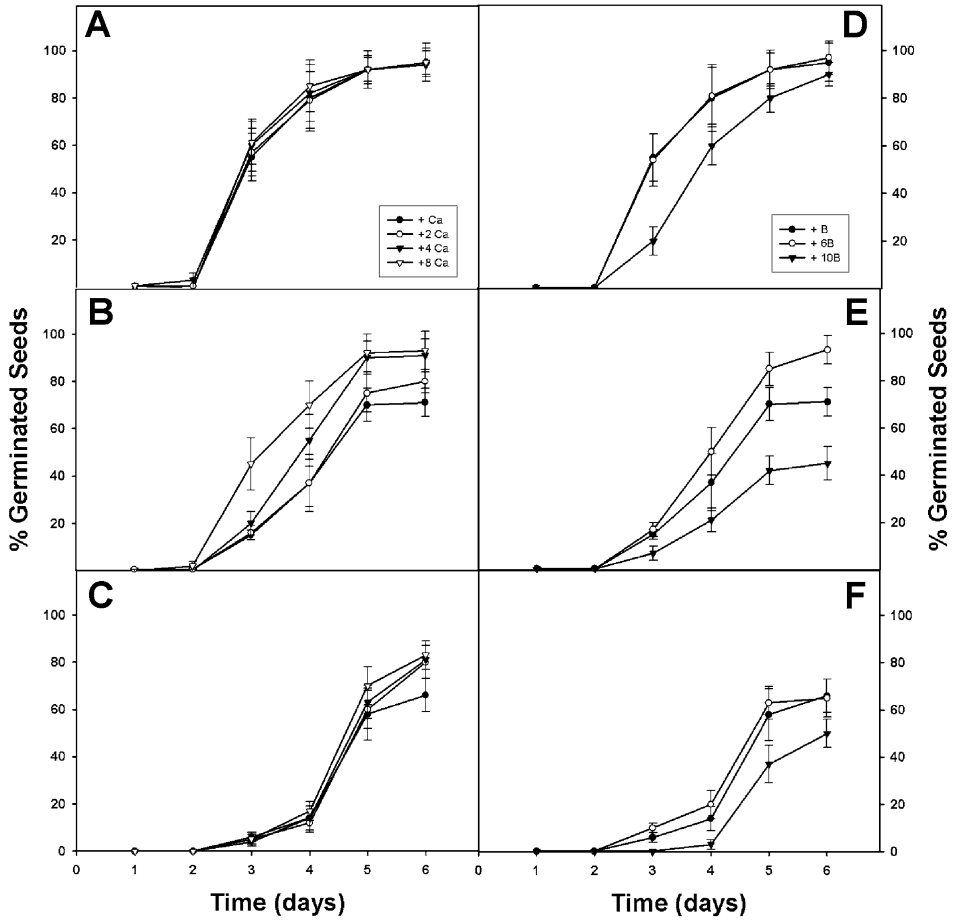

Figure 2. Effects of supplementary Ca^{2+} (+Ca: 0.68 m*M*; +2Ca: 1.36 m*M*; +4Ca: 2.72 m*M*; +8Ca: 5.44 m*M*) (**A**, **B**, **C**) or B (+B: 9.3 μM; +6B: 55.8 μM; +10B: 93 μM) (**D**, **E**, **F**) in solution on the germination of *Pisum sativum* cv. Argona seeds at three levels of NaCl. (**A**, **D**) 0 m*M* NaCl; (**B**, **E**) 75 m*M* NaCl; (**C**, **F**) 150 m*M* NaCl. 500 seeds per treatment were surface sterilized, plated on trays containing perlite soaked with the different NaCl, Ca^{2+} and B concentrations. Percentage of germinated seeds was calculated every day over a 6 days period.

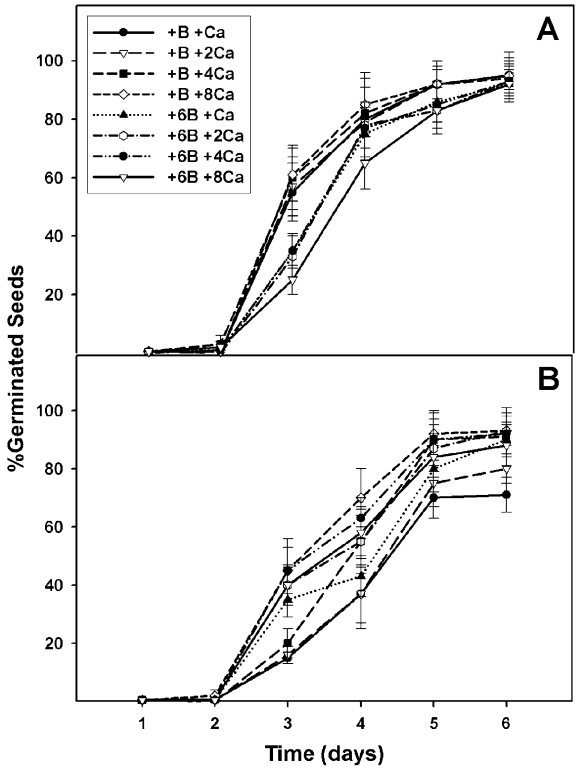

Figure 3. Effects of supplement with combined B (+B: 9.3 μM; +6B: 55.8 μM) and Ca^{2+} (+Ca: 0.68 mM; +2Ca: 1.36 mM; +4Ca: 2.72 mM; +8Ca: 5.44 mM) concentrations on the germination of *Pisum sativum* cv. Argona seeds at three levels of NaCl. (**A**) 0 mM NaCl; (**B**) 75 mM NaCl. 500 seeds per treatment were surface sterilized, plated on trays containing perlite soaked with the different NaCl, Ca^{2+} and B concentrations. Percentage of germinated seeds was calculated every day over a 6 days period.

150 mM NaCl were not clearly prevented at any B-Ca treatment, being 10B treatments always inhibitory (data not shown).

Addition of Ca at the +B level did not have any effects on root elongation at any salt treatment (Table 3 and Figure 4). Levels of 6B and 10B inhibited root elongation in the absence of salt (Table 3 and Figure 4A). However, 6B treatments combined with high Ca levels increased root elongation of seedling treated with 75 mM NaCl (Table 3 and Figure 4B). The highest salt treatment (150 mM NaCl) was always inhibitory for root elongation (Table 3 and Figure 4C).

Since 150 mM NaCl was extremely inhibitory, subsequent experiments were set up with pea plants growing with a concentration of 75 mM in N-free media containing different concentrations of B and/or Ca. Salt stress inhibited plant development, and growth of shoots and roots was increased by applying high

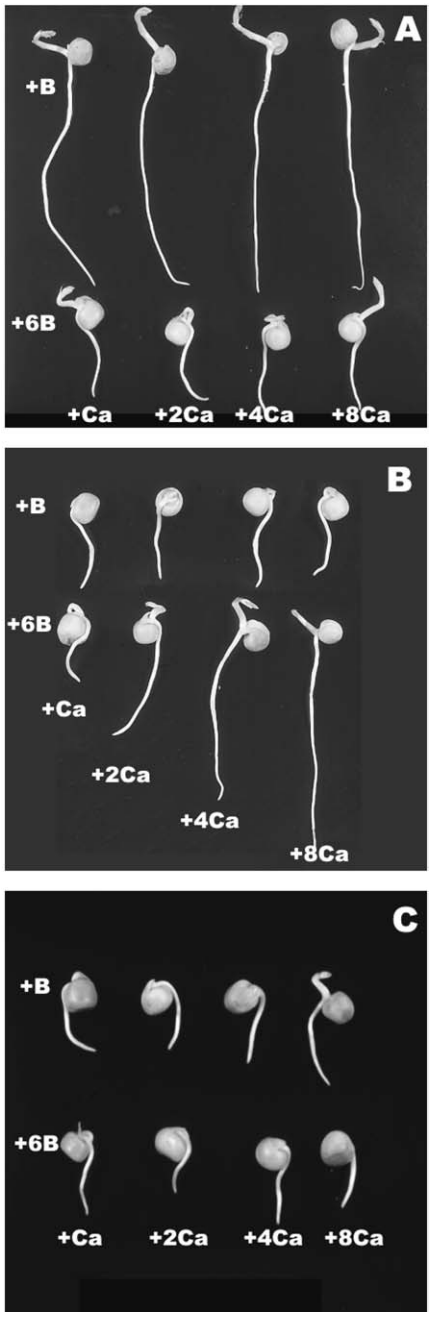

Figure 4. Effects of supplement with combined B (+B: 9.3 μM; +6B: 55.8 μM) and Ca^{2+} (+Ca: 0.68 mM; +2Ca: 1.36 mM; +4Ca: 2.72 mM; +8Ca: 5.44 mM) concentrations on the elongation of roots of *Pisum sativum* cv. Argona seeds 5 days after germination under salt stress. (**A**) 0 mM NaCl; (**B**) 75 mM NaCl; (**C**) 150 mM NaCl.

Table 3. Effects of salt stress and different concentrations of B and Ca on root elongation (mm) of *Pisum sativum* cv. Argona seedlings after 5 days of germination. Values followed by the same letter are not significantly different at $P = 0.05$ (Student's *t*-test)

		Control (0 m*M* NaCl)	75 m*M* NaCl	150 m*M* NaCl
+B	+Ca	61 ± 12a	22 ± 6b	21 ± 6b
	+2Ca	58 ± 14a	19 ± 6b	18 ± 4b
	+4Ca	65 ± 11a	24 ± 7b	21 ± 5b
	+8Ca	63 ± 14a	22 ± 6b	20 ± 5b
+6B	+Ca	24 ± 7b	20 ± 5b	20 ± 4b
	+2Ca	31 ± 7b	32 ± 6c	20 ± 6b
	+4Ca	32 ± 8b	55 ± 10a	19 ± 4b
	+8Ca	25 ± 7b	61 ± 12a	22 ± 5b
+10B	+Ca	21 ± 4b	19 ± 4b	21 ± 4b
	+2Ca	23 ± 5b	22 ± 4b	23 ± 5b
	+4Ca	21 ± 5b	23 ± 5b	19 ± 4b
	+8Ca	24 ± 5b	22 ± 5b	21 ± 4b

B (up to 6B) and high Ca concentrations (Figure 5). The treatment 6B +4Ca produced the highest growth of shoot and root in plants treated with 75 m*M* NaCl (Figure 5, +6B +4Ca treatments).

In 14-day-old pea plants, salt stress decreased N content both in shoot and roots (Figure 6). When plants were grown with concentrations of 6B and 2Ca to 8Ca and under salt stress (75 m*M* NaCl), the decrease in N content was diminished (similar results were obtained after analysis of other nutrients as K+ or root Fe, data not shown).

As typically occurs, exposure of plants to salinity led to a massive entry of toxic Na^+ ions, which is indicated by a high concentration in shoots and roots of 14-day-old plants (Figure 7). Again, the treatments with high B and high Ca levels diminished the concentration of Na^+ in pea shoots (Figure 7A). In roots, treatments of 4Ca and 8Ca at any B concentration led to a decrease of Na^+ (Figure 7B). Besides these toxic levels of Na^+, one of the major constraints for growth of pea plant on saline substrates is B and Ca deficiency (El-Hamdaoui et al., 2003b). Therefore, the content of B and Ca in 14 days old pea plants grown with a saline concentration of 75 m*M* NaCl and with different B and Ca concentrations was analysed (Figure 8). Concentration of B in shoots (Figure 8A) and in roots (Figure 8B) was diminished by salinity. The increase of external B resulted in a recovery of the micronutrient in shoots. However, 10B treatments reached very high B concentrations, which can be toxic both in shoots and roots. The presence of 75 m*M* NaCl also provoked a reduction of the concentration of Ca in pea plants. The addition of extra Ca during the experiments resulted in an increase of the concentration both in shoots (Figure 8C) and in roots (Figure 8D) of salt treated plants. Modifications of the level of B did not have any significant effect on the concentration of Ca. It is noticeable that the effective activity of Ca^{2+} was reduced in saline solutions (Table 1), and concentrations of at least 1.36 m*M* Ca^{2+} (2Ca treatments) had to be added to 75 m*M* NaCl solutions to have a Ca^{2+} activity similar to that of treatments without added NaCl.

Discussion

In previous reports, we showed that a balanced B-Ca nutrition can prevent most of the effects of high salt on the symbiosis between *Rhizobium* and *Pisum sativum* (El-Hamdaoui et al., 2003b). Therefore, B and Ca can increase salt tolerance of nitrogen-fixing pea plants (El-Hamdaoui et al., 2003a). Results presented here show that salt stress also affected early events of plant development (Table 2) and that addition of both nutrients also increased salt tolerance during these first stages of development of non-nodulated pea plants.

A concentration of 75 m*M* NaCl caused diminished pea seed germination (Figure 1). This can be overcome by addition of Ca or B singly (Figure 2)

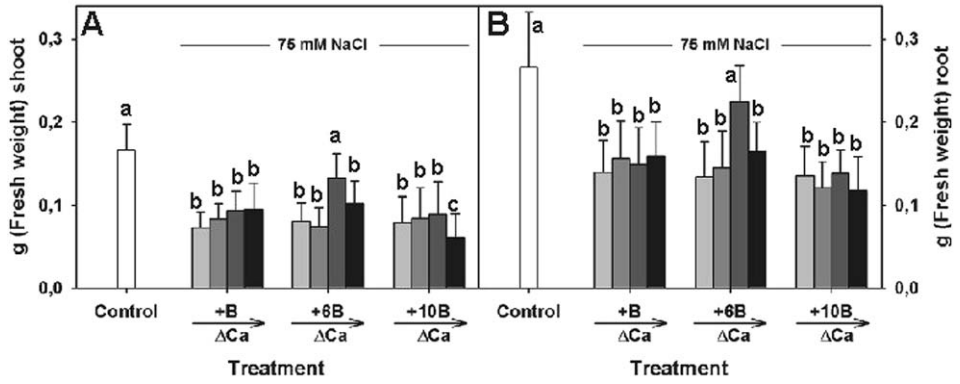

Figure 5. Effects of the different B (+B: 9.3 μM; +6B: 55.8 μM; +10B: 93 μM) and Ca^{2+} (+Ca: 0.68 mM; +2Ca: 1.36 mM; +4Ca: 2.72 mM; +8Ca: 5.44 mM) treatments on the fresh weight of shoots (**A**) and roots (**B**) of *Pisum sativum* cv. Argona plants treated with 75 mM NaCl, after 14 days of growth in media devoid of N. Control (without salt): +B+Ca. : +Ca; : +2Ca ; : +4Ca ; : +8Ca. Values in columns with different letters are significantly different at $P \leqslant 0.05$.

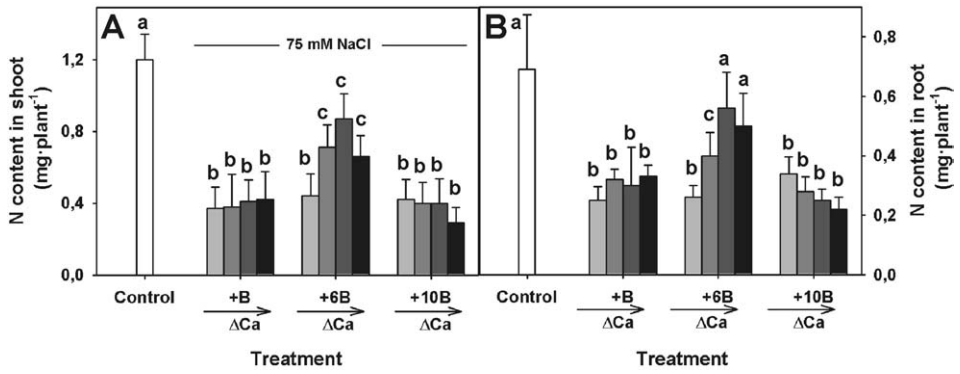

Figure 6. Effects of the different B (+B: 9.3 μM; +6B: 55.8 μM; +10B: 93 μM) and Ca^{2+} (+Ca: 0.68 mM; +2Ca: 1.36 mM; +4Ca: 2.72 mM; +8Ca: 5.44 mM) treatments on the total nitrogen content of shoots (**A**) and roots (**B**) of *Pisum sativum* cv. Argona plants treated with 75 mM NaCl, after 14 days of growth in media devoid of N (therefore, N only came from seed stores). Control (without salt): +B+Ca. : +Ca; : +2Ca ; : +4Ca ; : +8Ca. Values in columns with different letters are significantly different at $P \leqslant 0.05$.

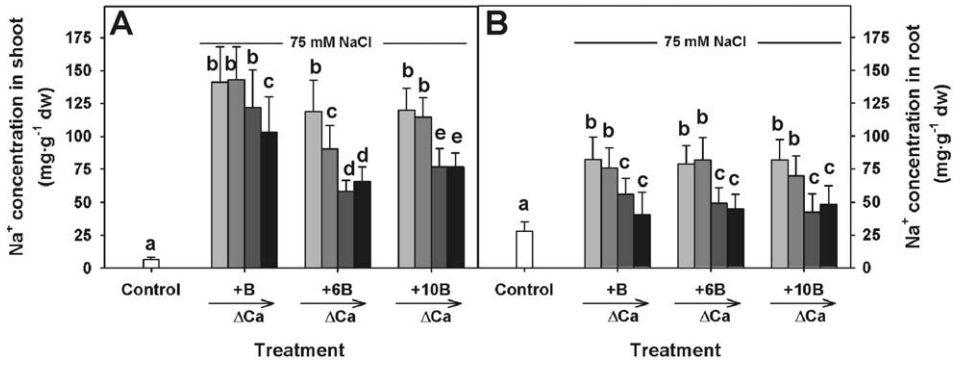

Figure 7. Effects of the different B (+B: 9.3 μM; +6B: 55.8 μM; +10B: 93 μM) and Ca^{2+} (+Ca: 0.68 mM; +2Ca: 1.36 mM; +4Ca: 2.72 mM; +8Ca: 5.44 mM) treatments on the Na^{+} concentration of shoots (**A**) and roots (**B**) of *Pisum sativum* cv. Argona plants treated with 75 mM NaCl, after 14 days of growth in media devoid of N. Control (without salt): +B+Ca. : +Ca; : +2Ca ; : +4Ca ; : +8Ca. Values in columns with different letters are significantly different at $P \leqslant 0.05$.

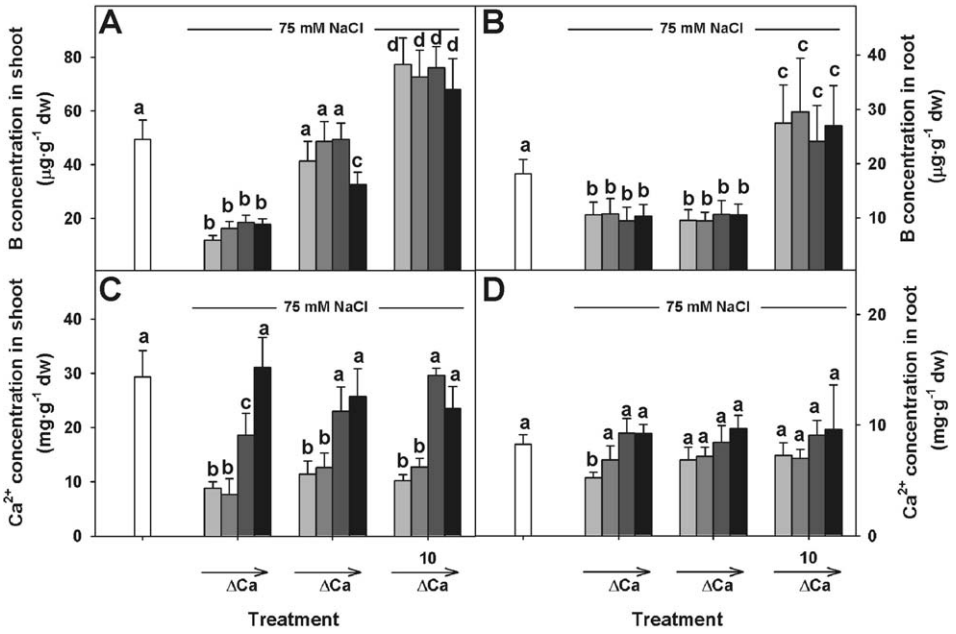

Figure 8. Effects of the different B (+B: 9.3 μM; +6B: 55.8 μM; +10B: 93 μM) and Ca^{2+} (+Ca: 0.68 mM; +2Ca: 1.36 mM; +4Ca: 2.72 mM; +8Ca: 5.44 mM) treatments on the B (**A**, **B**) and Ca^{2+} (**C**, **D**) content of shoots (**A**, **C**) and roots (**B**, **D**) of *Pisum sativum* cv. Argona plants treated with 75 mM NaCl, after 14 days of growth in media devoid of N.14 days old *Pisum sativum* cv. Argona plants treated with 75 mM NaCl. Control (without salt): +B+Ca. ▨: +Ca; ▩: +2Ca ; ■: +4Ca ; ■: +8Ca. Values in columns with different letters are significantly different at $P \leqslant 0.05$.

or together (Figure 3), provided excessive B was avoided. However, B and Ca did not increase tolerance at 150 mM NaCl. Based on Greenway and Munns (1980), legumes are classified as salt-sensitive plants (Katerji et al., 2000; Maas and Hoffman, 1977), therefore, 150 mM salt can be considered as an extremely high salt stress.

Other authors also reported a negative effect of salinity on seed germination (Kord and Khalil, 1995; Schmidhalter and Oertli, 1991). The effects of salinity can be due to osmotic problems, as available water for seed germination diminishes (Bernstein and Hayward, 1985). Additionally, alfalfa seed germination was more inhibited by salt than by organic solutes (Uhvits, 1946), indicating a specific toxic effect of ions. Results shown in Figure 2 suggest that inhibitory effect on seed germination is more due to ion toxicity, as increasing Ca^{2+} (increasing therefore solution ionic strength), had no effect on germination in the absence of salt or ameliorated it under salt stress. Most toxic effects of NaCl can be attributed to Na^+ toxicity (for a review see Tester and Davenport, 2003). Figure 7 indicates that the concentration of Na^+ in salt treated plants is extremely high after 14 days of growth, therefore, Na^+ toxicity could occur. High concentrations of Na^+ can cause a range of osmotic and metabolic problems for plants. Moreover, some effects of high salt are the deficiency of other nutrients, as indicated for B and Ca in Figure 8, due to interference of salt ions with nutrient uptake (Silberbush and Ben-Asher, 2001). Effective activities of ions such as Ca^{2+} are also reduced in saline solutions (Table 1), and this effect can also explain the reduced concentration of Ca^{2+} in salt treated plants.

Similarly to other studies (Bliss et al., 1986; Marcar, 1986), Ca addition increased germination under salt stress (Figure 2B). It is postulated that this Ca effect can be due to a protection against membrane damage due to high salt, and the subsequent invasion by microorganisms that inhibits seed germination. Moreover, it is known that toxic effects of Na^+ can be ameliorated by addition of Ca^{2+} to the external solution (LaHaye and Epstein, 1971). Although the effects of Ca^{2+} are likely to be complex (Cramer, 2002), its protection against Na^+ is due, at least in part, to an inhibition of the accumulation of Na^+ into the roots and shoots of plants (as shown in Figure 7) and an enhancement of K^+ transport (Cramer et al., 1987; Liu and Zhu, 1997). NaCl apparently has competitive effects on Ca^{2+} binding to proteins, cell

membranes or cell walls (Cramer and Läuchli, 1986), which can also explain some toxic effects of salinity that can be offset by addition of Ca^{2+}. The effect of extracellular Ca^{2+} on Na^+ and K^+ transport has been attributed to the activity of the SOS signalling pathway (Liu and Zhu, 1998). High salt results in a rise of cytosolic Ca^{2+}. The amplitude of this rise of Ca^{2+} is thought to depend on the concentration of extracellular Ca^{2+}. Increasing cytosolic Ca^{2+} activates SOS3, leading to changes in expression and activity of Na^+ and K^+ transporters. Another possibility is that extracellular Ca^{2+} first alters Na^+ influx directly, and it is cytosolic Na^+ levels that activate the SOS pathway via SOS3. SOS3 interacts directly with SOS2, a serine/threonine protein kinase (Halfter et al., 2000), which increases transcription and activity of SOS1, an Na^+/H^+ antiporter localized to the plasma membrane (Shi et al., 2002). It has also been suggested that the pathway for Ca^{2+} effects on Na^+ influx is through inhibition of non-selective cation channels not requiring cytosolic signalling (Davenport and Tester, 2000; Demidchik et al., 2002). Therefore, it is likely that several pathways for Ca^{2+}-sensitive Na^+ influx exists.

Besides, the known effects of Ca addition, results indicate that addition of B up to a level of 6 times normal (6B treatments) (Figure 2E) without or with Ca (Figure 3B) also ameliorated seed germination under moderate salt stress (75 m*M* NaCl). High salt lead to a decrease of B (Figure 8A, B), and a continuous supply of B is required for the development of apical root meristem (Bohnsack and Albert, 1977; Krueger et al., 1987). It is well known that viability of seeds containing low B is impaired (Bell et al., 1989; Rerkasem et al., 1997). Therefore, addition of B can help germination under salt stress. Nevertheless, let very high concentrations of B, toxic effects appeared both in the presence and in the absence of salt (Figure 2D–F and Figure 3).

As shown in Table 3 and Figure 4, salt stress also inhibited pea root elongation, which affected subsequent plant development. Salt diminished the concentration of B and Ca (Figure 8). Due to the importance of both nutrients for the development of apical meristems (Blevins and Lukaszewski, 1998; Leonard and Hepler, 1990), the presence of a supplement of both B and Ca is needed to stimulate root elongation under salt stress (Table 3 and Figure 4). Furthermore, despite nutrients coming from cotyledons, fast root growth requires a continuous new supply of low mobile nutrients as B and Ca (see Marschner, 1995 and refs. therein). Root elongation also depends on cell turgor pressure and hence, on the stability of the cell wall. Both B and Ca are required for the stability of the pectin fraction in cell walls (Kobayashi et al., 1999). In a previous report (Bolaños et al., 2003), we have shown accumulation of pectin polysaccharides in cytosol instead of cell walls in pea nodules developed under salt stress. This typical effect of loss of pectin organisation in the absence of B (Matoh and Kobayashi, 2002), could not be reversed by B but only after addition of both B and Ca to salt stressed plants. Although both nutrients can play complementary roles so that Ca could prevent some effects of B deficiency, both nutrients are absolutely required for the maintenance of cell wall structure (Redondo-Nieto et al., 2003). Therefore, although Ca increased germination under salt stress (Figure 2B), the low availability of B provoked by 75 m*M* NaCl (Figure 8A–B), made necessary a continuous supply of extra B for root elongation (Figure 4B). However, the increase of B supply up to 10B in the presence of salt or up to 6B in the absence of salt had inhibitory effects of root elongation. Some typical effects of B toxicity, as its capacity of complexing intracellular pyridine nucleotide coenzymes (Loomis and Durst, 1992), or its adverse effects on cell division (Liu et al., 2000) could explain those results.

Finally, a supplement of B and Ca was needed for development of pea plants (Figure 5). Besides the relationship stated above between both nutrients, Figure 6 indicates that plants developed under salt stress could mobilize low N from seed storages. Again B and Ca enhanced N uptake from cotyledons, indicating a possible role of these nutrients for mobilisation of storages during the early events of plant development.

Previous reports suggest that a proper B and Ca nutrition can facilitate salt tolerance in the highly salt sensitive *Pisum sativum-Rhizobium leguminosarum* symbiosis by enhancing the interaction between plant and bacteria (El Hamdaoui et al., 2003a). Overall results presented here indicate that a balanced B-Ca relationship also increases salt tolerance during the early stages of plant development and growth prior to the interaction with *Rhizobium*. Extrapolation of these results to soil conditions is obviously premature, since saline soils have a more complex behaviour than controlled laboratory experiments. Nevertheless, besides genetic approaches searching for tolerant cultivars, studying the nutritional relationship between B and Ca for other crops can lead to simple farming practices to increase salt-tolerance and crop production.

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