Modulation of Polyamine Catabolism in Pea Seedlings by Calcium during Salinity Stress

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Abstract

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The relation of polyamine catabolism in the response of *Pisum sativum* to salinity stress was investigated. Pea seedlings were grown in increasing concentrations of Na^+ or K^+ or at different concentration ratios of these ions. We studied the effect of Ca^{2+} supplementation on plants exposed to salinity stress. The parameters measured in the roots and shoots of pea seedlings included biomass production, levels of Na^+, K^+, Ca^{2+} and polyamines and activity of enzymes of polyamine catabolism: diamine oxidase, aminoaldehyde dehydrogenase and peroxidases. Salinity induced increased polyamine levels and higher activity of enzymes participating in polyamine degradation. Supplementation of Ca^{2+} had a positive effect on biomass production and in most cases it stabilised both the polyamine level and the activity of the studied enzymes. Our results confirm the role of aminoaldehyde dehydrogenase and polyamine catabolism in defence mechanisms of pea plants under salinity stress.

Keywords: *Pisum sativum*; calcium ions; potassium ions; enzyme activity; aminoaldehyde dehydrogenase

Abbreviations: ABAL – 4-aminobutyraldehyde; AMADH – aminoaldehyde dehydrogenases; APAL – 3-aminopropionaldehyde; APBAL – *N*-(3-aminopropyl)-4-aminobutyraldehyde; BADH – betaine aldehyde dehydrogenase; DAO – diamine oxidase; DW – dry weight; GABA – γ-aminobutyric acid; PAO – polyamine oxidase; POX – peroxidase; ROS – reactive oxygen species; SD – standard deviation

Water deficiency and high salt concentrations in the soil belong to significant limiting factors in plant growth and development, leading to a pronounced decrease of biomass production and overall negative impact on agricultural productivity. Plants have developed a plethora of molecular and physiological mechanisms to cope with salt stress. Plant strategies towards increased salt tolerance include ion regulation by selective uptake, transport, accumulation or exclusion, compartmentalization of ions on the cellular and whole-plant levels, synthesis of osmoprotectants, changes in photosynthetic pathways and induction of antioxidative defence (Hasegawa *et al*. 2000; Chinnusami & Zhu 2003).

A widespread metabolic adaptation of plants to salinity stress is represented by biosynthesis and accumulation of small organic metabolites that confer the protection of cellular components against osmotic stress but do not interfere with biochemical pathways even in high concentrations (Aziz *et al*. 1999). These organic compounds include sugars, acyclic and cyclic polyols, nitrogen-containing compounds like amino acids, amides, imino acids, polyamines and quaternary ammonium and sulphonium compounds (Bohnert & Jensen 1996). The role of these compounds is to protect cell structures and biomolecules that can be damaged by toxic effect of ions, dehydration or increased production of reactive oxygen species

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(Bohnert & Sheveleva 1998; Serrano *et al.* 1999; Yancey 2005). It was also shown that low molecular organic compounds can contribute to water retention indirectly by controlling the retention of intracellular K^+ in the cell (Cuin & Shabala 2005, 2007).

The response to salinity stress is frequently linked with an increase in the polyamine level and increased activity of polyamine biosynthetic and biodegrading enzymes (Aziz *et al.* 1999; Roy & Wu 2002; Kusano *et al.* 2007). At neutral pH, polyamines are polycations and can bind to polyanions in the cell such as DNA, RNA and phospholipids and stabilise them. The increase in polyamine concentrations in stressed plants can be linked to their positive effect on cells, such as control of pH, maintenance of ion and osmotic balance, and ability to stabilise membranes and detoxify ROS (Bors *et al.* 1989; Flores 1991; Kakkar & Sawhney 2002). Polyamines are also very potent ion channel blockers, both in animal (Lopatin *et al.* 1994; Bowie *et al.* 1998; Lu & Ding 1999) and plant (Brüggemann *et al.* 1998; Liu *et al.* 2000; Pandolfi *et al.* 2010) tissues.

Biogenic polyamines are converted to the corresponding amino aldehydes in the reaction catalysed by amine oxidases. These enzymes include copper-containing amine oxidases (DAO, EC 1.4.3.6.) and flavin-containing polyamine oxidases (PAO, EC 1.5.3.11) (Bouchereau *et al.* 1999). Plant DAOs catalyse the oxidative deamination of di- and polyamine substrates such as putrescine and spermidine, resulting in the formation of 4-aminobutyraldehyde (ABAL) and *N*-(3-aminopropyl)-4-aminobutyraldehyde (APBAL), along with the release of ammonia and hydrogen peroxide (Medda *et al.* 1995). Several plant DAOs have been shown to oxidise propane-1,3-diamine to 3-aminopropionaldehyde (APAL) (Šebela *et al.* 2000b). Plant polyamine oxidases oxidise the polyamines spermidine and spermine producing ABAL and APBAL, respectively, propane-1,3-diamine and hydrogen peroxide (Šebela *et al.* 2001b).

In plants, the degradation products of polyamine catabolism are further metabolised by NAD^+-dependent aminoaldehyde dehydrogenases (AMADHs, EC 1.2.1.19 or EC 1.2.1.54) to the respective amino acids, for example APAL and ABAL to β-alanine and GABA (Awal *et al.* 1997). AMADHs from pea (*Pisum sativum*) and oat (*Avena sativa*) were purified and investigated in detail (Šebela *et al.* 2000a; Livingstone *et al.* 2002; Brauner *et al.* 2003). Pea AMADH is associated with the protoplast and its activity has been localised histochemically in the vascular cambium and pericycle tissue (Šebela *et al.* 2000a, 2001a). Under native conditions the enzyme exists as tetramer (4 × 57 kDa) containing cysteine residue at the active site (Šebela *et al.* 2000a; Brauner *et al.* 2003). AMADH from pea seedlings shows broad substrate specificity, but the best characterised substrates are aliphatic C3-C6 aminoaldehydes (Šebela *et al.* 2000a).

Plant peroxidases (POX, EC 1.11.1.7) are monomeric heme-containing enzymes that catalyse a large variety of reactions. Plant peroxidases have been studied for their important role in lignification and suberisation, for their active participation in the formation of diphenyl bridges, cross-linking of hydroxyproline-rich proteins in the cell wall matrix and for their control function of redox state in the apoplast. The involvement of POX in plant stress responses as well as in plant-pathogen interactions has been demonstrated (Siegel 1993).

The aim of this study was to bring more detailed knowledge of the role of polyamine catabolism in plant responses to salinity stress elicited by increasing concentration of Na^+ and K^+ ions and its interaction with Ca^{2+} ions.

MATERIAL AND METHODS

Plant material. Commercially available seeds of pea (*P. sativum* cv. Lantra) were soaked in tap water overnight, germinated for 2 days on moistened filter paper and subsequently transferred to boxes with Perlit EP AGRO layer (Perlit, Šenov u Nového Jičína, Czech Republic) and different salt conditions. Plants were grown in a growth chamber with controlled temperature (21°C during day and 18°C at night), 15 h daylight and the light intensity of 100 μE/m/s. All parameters were determined on three independent sets of grown and salt-treated seedlings. Each parameter value represents the mean value for 3–6 individual plant samples.

Salt treatment. The seedlings were grown under different salt conditions: (A) in water (control experiment) and in 10–80mM NaCl or KCl; (B) in a solution with total 40mM salt concentration and different NaCl/KCl ratio (0:1, 1:0, 1:1, 1:2, 2:1, 1:5, 5:1, 1:10, 10:1); (C) in 10mM and 60mM NaCl or KCl with different concentration of $CaCl_2$ (0–10 mM). The plants were harvested 14 days after their transfer to Perlit. Shoots and roots were

separated immediately after harvesting, frozen in liquid nitrogen and stored at –80°C.

Analysis of fresh weight and length. Ten randomly selected pea plants from each experiment were dried carefully after harvesting by blotting with a filtration paper. Roots and shoots were separated and immediately weighed to determine fresh weight and length.

Enzyme extraction and assays. Frozen shoots or roots (~ 1 g) were ground with a mortar and pestle in 2 ml of ice-cold extraction buffer: 0.1M potassium phosphate buffer, pH 7.0 for DAO and POX; 0.1M potassium phosphate buffer, pH 7.0, containing 15mM 2-mercaptoethanol, 1mM EDTA and 10% (w/v) sucrose for AMADH. Crude homogenates were centrifuged at 10 000× *g* for 10 min (4°C).

AMADH activity was determined spectrophotometrically by monitoring the production of NADH at 340 nm (ε = 6,220 l/mol/cm) (Šebela *et al*. 2000a). The reaction mixture was thermostated at 30°C and contained 0.1M Tris-HCl, pH 8.5, 1mM NAD^+ and pea extract of an appropriate volume. The enzyme reaction was started by the addition of APAL in 1mM final concentration.

POX activity was determined using the guaiacol method (Angelini *et al*. 1990). The reaction mixture was thermostated at 30°C and contained 0.1M K-phosphate buffer, pH 7.0, with 15mM guaiacol and pea extract. The reaction was started by the addition of hydrogen peroxide in 5mM final concentration and monitored by increased absorbance at 436 nm (ε = 4500 l/mol/cm).

DAO activity was assayed as previously reported by the aminoantipyrine method (Cona *et al*. 2003). The reaction mixture was thermostated at 30°C and contained 0.1M K-phosphate buffer, pH 7.0, 2.5 U/ml horseradish peroxidase, 1.7mM 4-aminoantipyrine, and 17mM 3,5-dichloro-2-hydroxybenzenesulphonic acid. To start the reaction, putrescine was added in a final concentration of 2.5mM and the absorbance change at 516 nm was followed (ε = 26 000 l/mol/cm).

All spectrophotometric measurements were carried out using a DU 7500 diode-array spectrophotometer (Beckman, Fullerton, USA) equipped with a water-thermostated cell holder.

Protein assay. Protein content was determined using bovine serum albumin as a standard by the modified Lowry method (Hartree 1972).

Determination of total polyamines. The total amount of polyamines in pea extracts was determined with an amperometric biosensor using immobilised diamine oxidase and polyamine oxidase. A commercial biosensor analyser (M. Jilek Company, Postřelmov, Czech Republic) connected to a PC was used as a potentiostat (Zajoncová *et al*. 2004). Pea amine oxidase and maize polyamine oxidase were purified as described previously (Šebela *et al*. 1998; Federico *et al*. 1989) and the enzymes were immobilised on a cellophane membrane. Six µl of a solution containing 36 µg of amine oxidase (total activity 11 nkat) and 15 µg of polyamine oxidase (total activity 13.2 nkat), 0.1 mg BSA, 0.03 mg gelatin and 0.8 µl of 2% (v/v) glutaraldehyde in a working buffer (10mM K-phosphate, pH 7.0) were pipetted on the cellophane and left to dry in a refrigerator overnight. The cellophane membrane was fixed by 10% gelatin on two Pt screen-printed working electrodes. During measurements, the working buffer was loaded by a peristaltic pump at a flow rate of 1.6 ml/min to the working electrodes maintained at a potential of +650 mV vs. a silver/silver chloride reference electrode. Pea extract samples were injected automatically. Prior to the measurement, hydrogen peroxide was removed from the samples by adding catalase. Solutions containing 1mM putrescine and 1mM spermidine were used as standards for biosensor calibration.

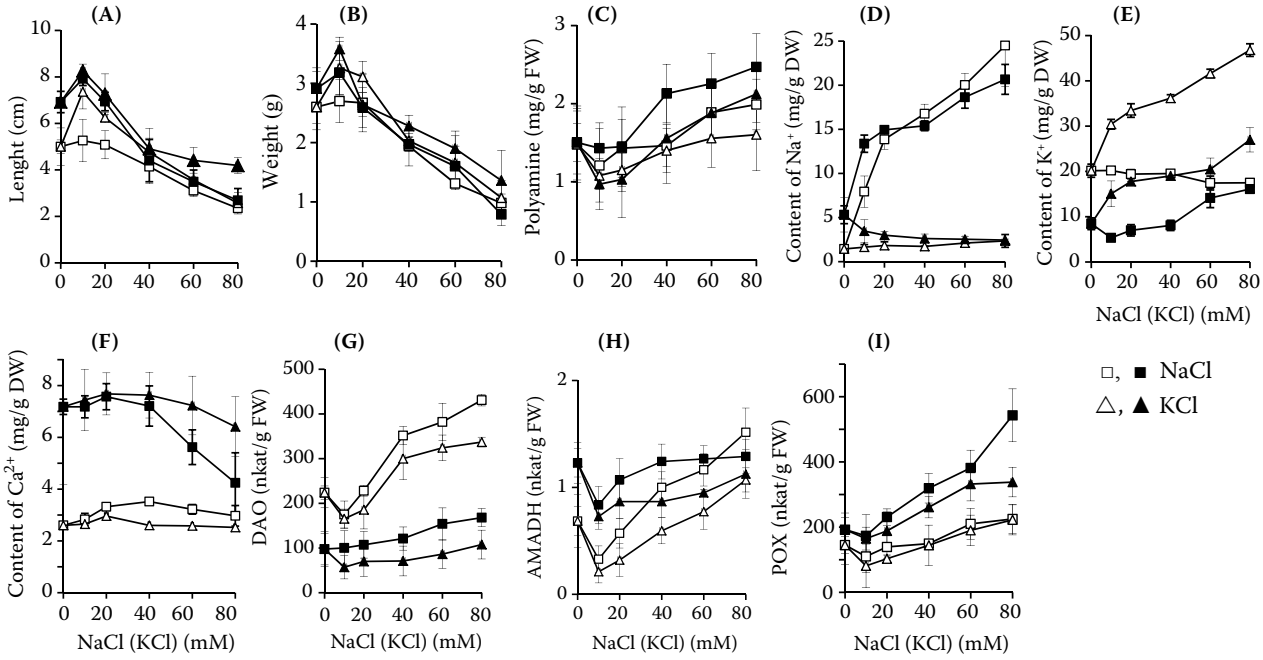
Determination of Na^+, K^+ and Ca^{2+} content. Plant samples were dried at 80°C for 10 hours. Dried ground plant material (0.1 g) was digested with 6 ml of concentrated nitric acid and 2 ml of hydrogen peroxide, and after the sample mineralisation, water was added to the total volume of 50 ml. The Na^+, K^+ and Ca^{2+} content was determined by atomic absorption spectrophotometry on an Avanta Σ spectrometer (GBS Scientific Equipment, Dandenong, Australia).

Statistical analysis. Statistical significance of differences among treatments was evaluated by analysis of variance (ANOVA) ($P < 0.05$), followed by a comparison of means by Bonferroni test, in NCSS 2000 software (Statistical Solutions Ltd., Cork, Ireland).

RESULTS

Effect of Na^+ or K^+ ions

The growth of pea seedlings, estimated as shoot and root length and fresh weight, was highest in 10mM NaCl or KCl, while it was significantly decreased in 20–80mM NaCl or KCl (Figure 1A, 1B).



Seedling length, fresh weight, content of K^+, Na^+ and Ca^{2+} and activity of DAO, AMADH and POX were determined 14 days after the beginning of salt treatment in the shoots (open symbols) or in the roots (closed symbols)

Figure 1. Influence of increasing content of NaCl and KCl on the growth of pea seedlings

Browning of roots and reduction in the length and amount of lateral roots were characteristic of higher salt concentrations (Figure 2). Shoot and root Na^+ and K^+ content in NaCl- and KCl-treated pea seedlings increased with increasing salinity (Figure 1D, 1E). The content of Na^+ did not change after exposure to increasing KCl concentrations, whereas increased K^+ concentrations in NaCl-treated plants seedlings were observed in roots but not in shoots (Figure 1E). The root Ca^{2+} content decreased significantly with increasing NaCl concentration (60–80mM) in the environment (Figure 1F). The amount of total polyamines measured in extracts of pea seedlings generally increased during salt stress (Figure 1C). The lowest level of polyamines was found in the plants grown in 10 mM NaCl or KCl in comparison with pea grown in distilled water or 20–80mM NaCl or KCl. Increased salt concentrations resulted in a gradual increase of DAO, AMADH and POX activities (Figure 1G–1I), with the lowest activities determined in pea seedlings grown in 10mM NaCl or 10mM KCl. AMADH and DAO showed a similar trend of changes in enzyme activities, which were significantly increased mainly in shoots (Figure 1G, 1H). AMADH and POX activities in

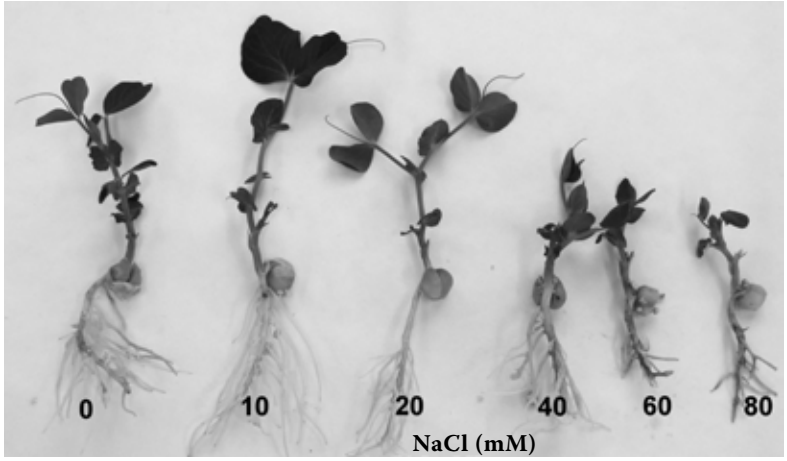
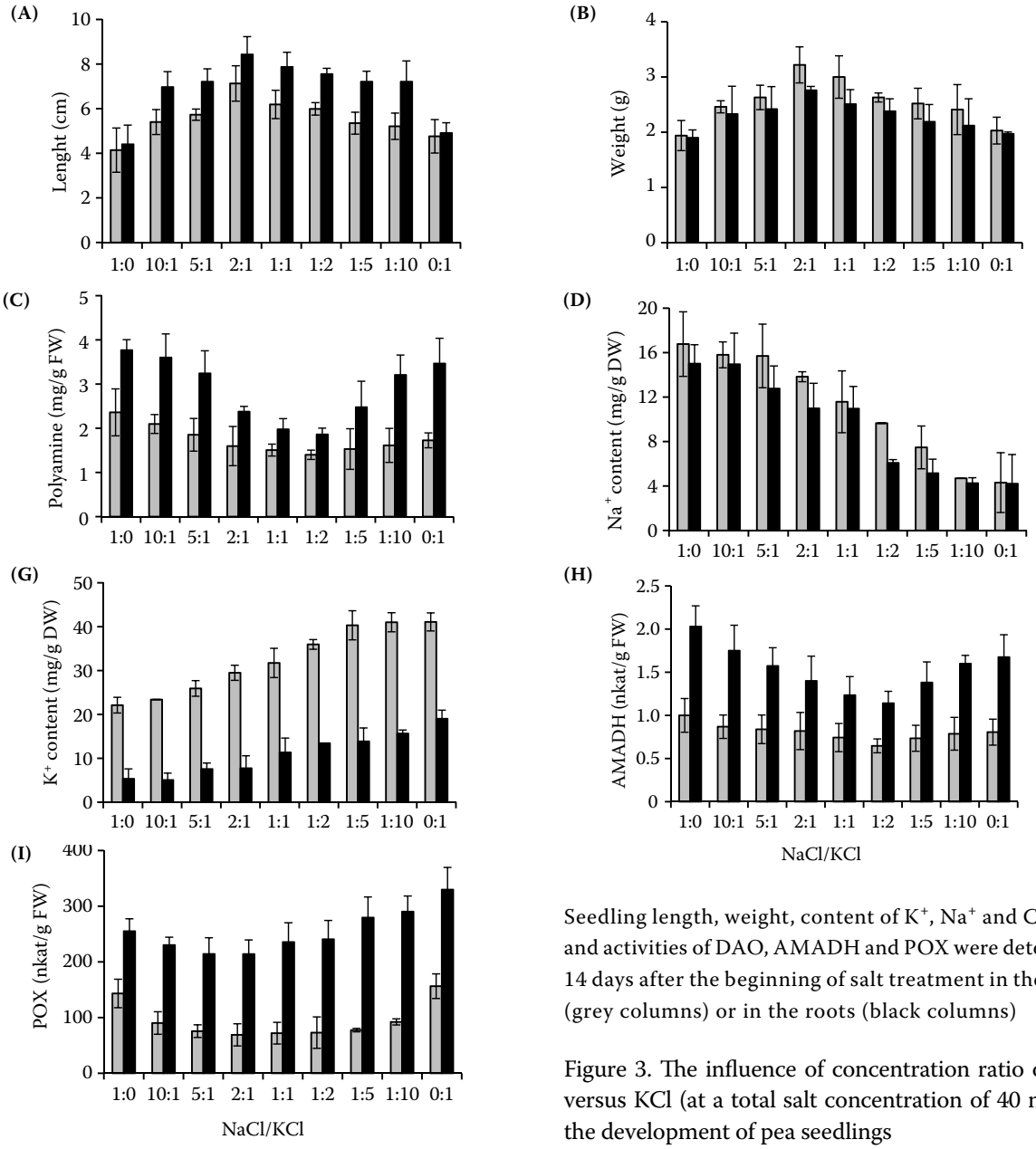


Figure 2. Growth response of pea seedlings to increasing NaCl concentration determined 14 days after the beginning of salt treatment

shoots were higher compared to roots but the opposite was observed for DAO activity.

The influence of different ratios of NaCl and KCl

The same physiological and biochemical parameters were determined in pea seedlings grown using different ratios of Na^+ and K^+ ions, maintaining the same total salt concentration of NaCl plus KCl at a 40mM level in all experiments. The growth of pea seedlings was highest for the 2:1 ratio of Na^+/K^+ ions (Figure 3A, 3B). Substantial differences were observed in the content of K^+ and Ca^{2+} ions in roots and shoots (Figure 3E, 3F). K^+ ions were accumulated mainly in shoots, whereas Ca^{2+} ions appeared mainly in roots. The endogenous content of K^+ and Na^+ changed in dependence on their content in the environment (Figure 3D, 3E), but the concentration of Ca^{2+} was lowest in pea seedlings grown in the medium with the 1:2 ratio of Na^+/K^+ ions. The lowest values of polyamine level, DAO and AMADH activities were detected under the same conditions, i.e. when the 1:2 ratio of Na^+/K^+ was used (Figure 3C, 3G, 3H). On the other hand, the highest decrease in POX activity was detected in plants grown in a medium containing twice more Na^+ than K^+ (Figure 3I).



Seedling length, weight, content of K^+, Na^+ and Ca^{2+} ions and activities of DAO, AMADH and POX were determined 14 days after the beginning of salt treatment in the shoots (grey columns) or in the roots (black columns)

Figure 3. The influence of concentration ratio of NaCl versus KCl (at a total salt concentration of 40 mM) on the development of pea seedlings

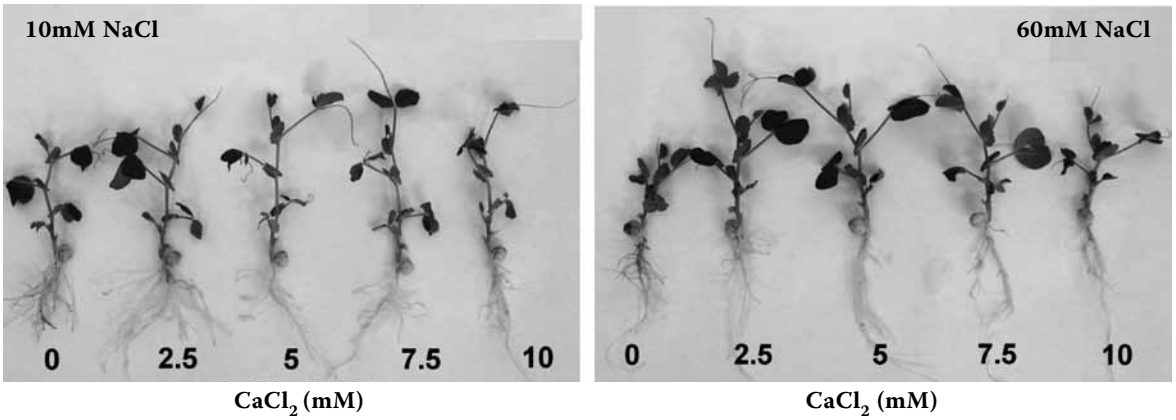


Figure 4. Growth response of pea seedlings to increasing Ca^{2+} concentration in the presence of 10mM and 60mM NaCl determined 14 days after the beginning of salt treatment

Effect of Ca^{2+} ions

Experimental conditions for the study of the influence of Ca^{2+} ions were determined based on previous experiments. The salt concentration of 10mM was chosen because of the highest increase recorded in biomass and plant length, and 60mM when the plant development was suppressed unambiguously (Figure 1). The addition of Ca^{2+} ions to the medium with 10mM and 60mM NaCl or KCl was tested. The optimal concentration of Ca^{2+} ions for the seedling growth was 2.5–5mM $CaCl_2$ (Figure 4, 5A, 5B, 5D, 5E). Higher concentrations of Ca^{2+} ions caused salt stress

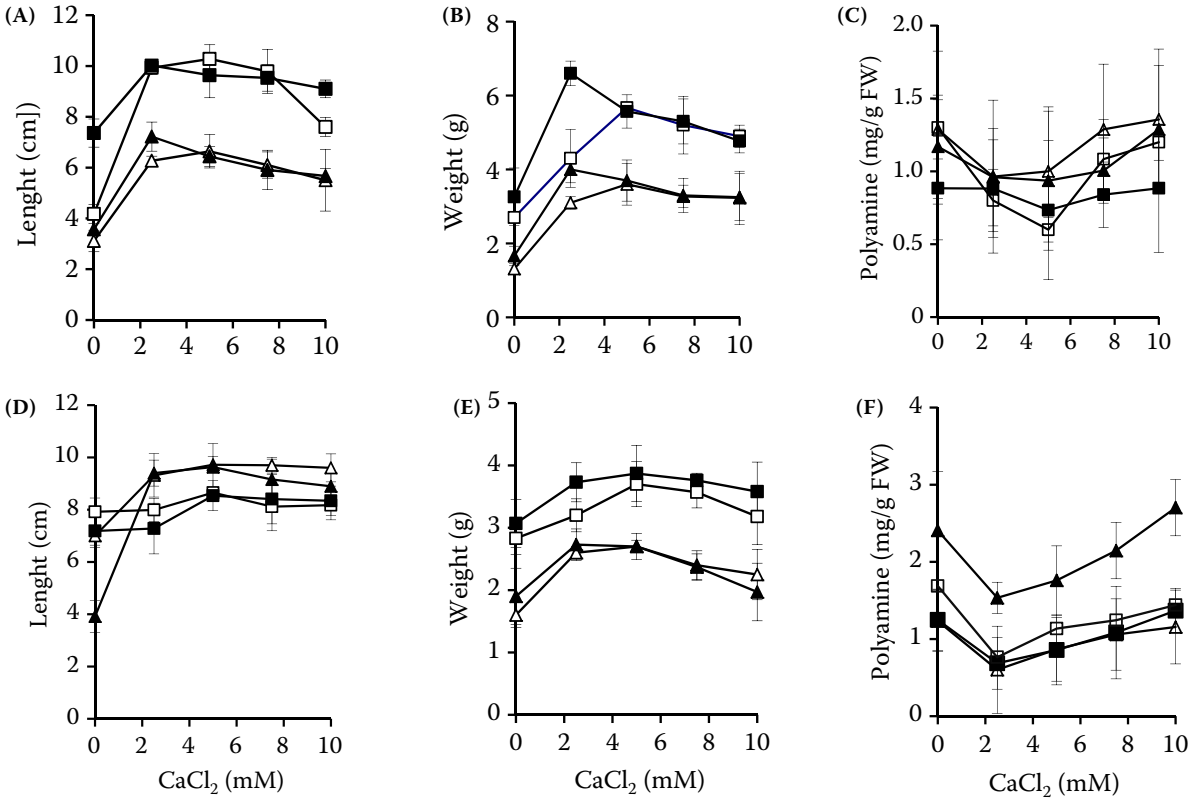


Figure 5. The influence of increasing content of $CaCl_2$ on the length, weight and polyamine content of pea seedlings grown in 1 mM KCl (■), 60mM KCl (▲), 10mM NaCl (□) and 60mM NaCl (△); determined 14 days after the beginning of salt treatment in the shoots (A–C) or in the roots (D–F)

accompanied by an increase in the polyamine level and DAO, AMADH and POX activities (Figure 5C, 5F, 6A–6F). The lowest values of these parameters were observed in the presence of 2.5–5mM $CaCl_2$. The content of K^+ ions in pea seedlings grown in 60mM KCl decreased with increasing concentration of $CaCl_2$. The lowest content of K^+ ions was detected in plants grown in 10mM KCl and 5mM $CaCl_2$ (Figure 7).

DISCUSSION

Our results (Figure 1D–1F, 3D–3F) are in accordance with previous observations that increasing concentrations of NaCl or KCl in the medium resulted in increased accumulation of Na^+ or K^+ ions and decreased uptake of other nutrients (Khan *et al.* 2000). In plants, the content of sodium under physiological conditions is lower compared with the content of potassium. In our experiments, we observed in plants grown in 10mM salt medium that the content of sodium was around 2 mg/g DW in the shoot and 5 mg/g DW in the root, whereas the content of potassium was 20 mg/g DW and 8 mg/g DW, respectively (Figure 1D, 1E).

A high Na^+ concentration is toxic to most plants due to the increased Na^+ accumulation in plant tissues with subsequent growth inhibition (Yeo & Flowers 1986). On the contrary, the supplement of a low concentration of Na^+ to the growth medium which is deficient in K^+ can support the growth of some plants (Flowers & Läuchli 1983). The higher salt accumulation observed in pea shoots can be explained by transpiration intake (Hasegawa *et al.* 2000). The translocation of ions between roots and shoots takes place on the interface of the root and xylem (Maathius & Amtmann 1999). A high concentration of ions is often observed in mature and older leaves under salinity stress (Munns 1993).

One of the possible causes of a reduction in nutrient uptake under salinity stress is a high rate of the uptake of ions that are present in high concentrations in the growth solution leading to their excessive accumulation in plant tissues. Although these ions can potentially inhibit the

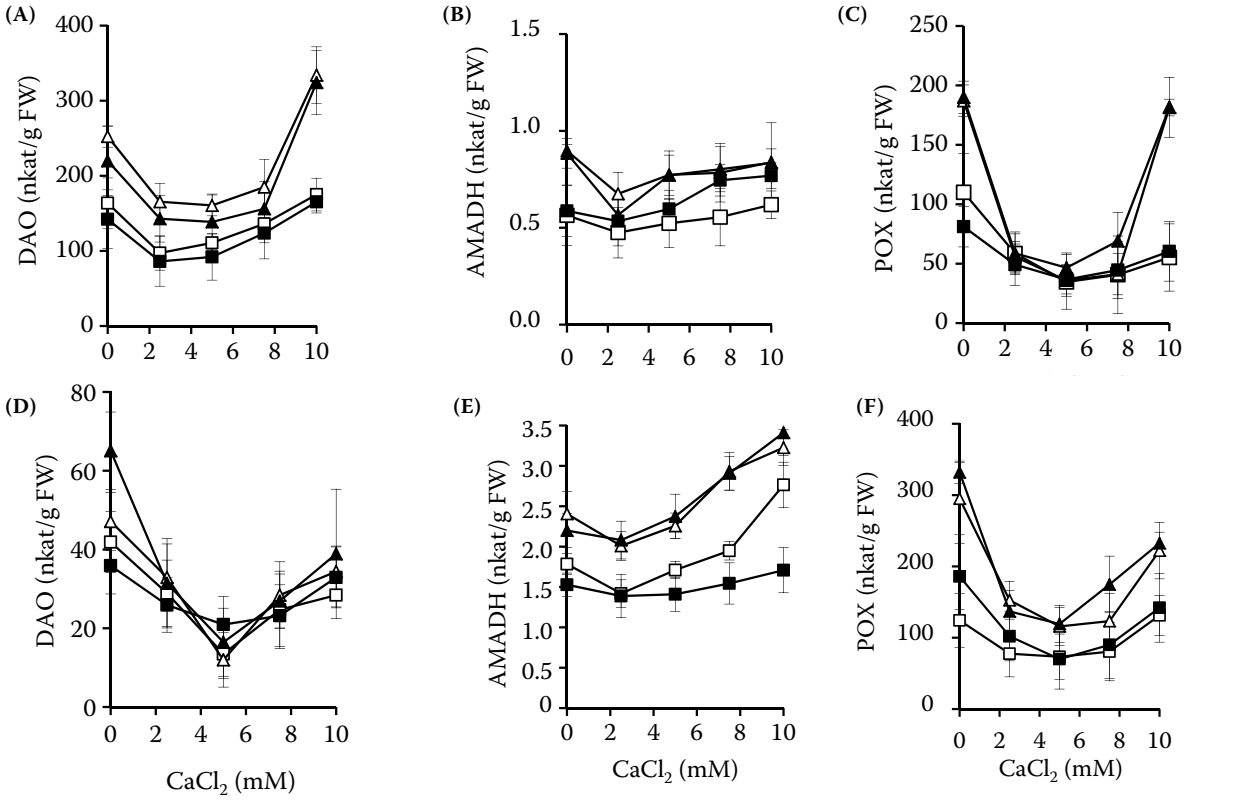


Figure 6. Influence of increasing $CaCl_2$ concentrations on DAO, AMADH and POX activities in pea seedlings grown in 10mM KCl (■), 60mM KCl (▲), 10mM NaCl (□) and 60mM NaCl (△); determined 14 days after the beginning of salt treatment in the shoots (A–C) or in the roots (D–F)

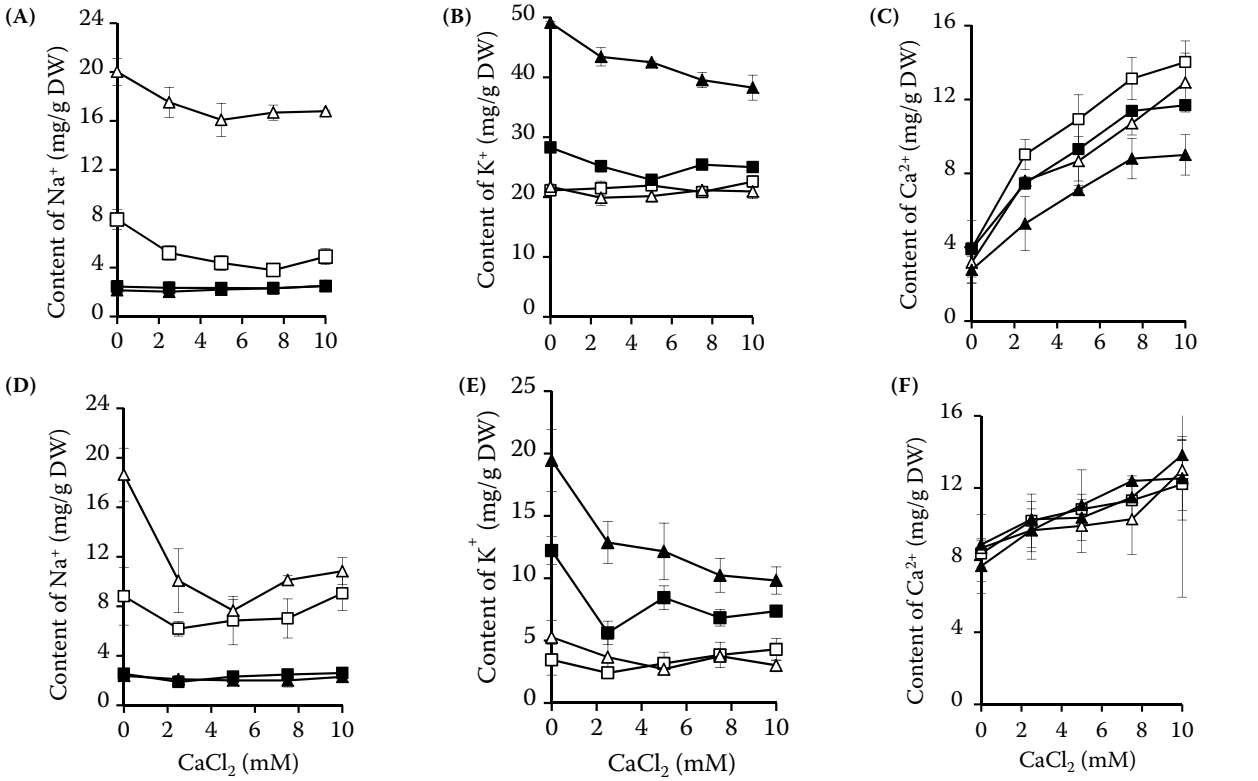


Figure 7. Influence of increasing $CaCl_2$ concentrations on the endogenous K^+, Na^+ and Ca^{2+}content in pea seedlings grown in 10mM KCl (■), 60mM KCl (▲), 10mM NaCl (□) and 60mM NaCl (△); determined 14 days after the beginning of salt treatment in the shoots (A–C) or in the roots (D–F)

uptake of other ions through the roots and their transport into the shoots, it was repeatedly shown that an increase in NaCl-induced K^+ leak rather than a reduction in K^+ uptake is the major cause of salinity-induced K^+ deficiency in plants (Shabala & Cuin 2008).

The decreased content of K^+ ions in plants as a consequence of salt stress can cause growth reduction, because potassium belongs to the essential elements for plant life. Moreover, it is the main cationic inorganic nutrient for the majority of land plants. The role of potassium in plants is well documented and includes balancing the overall charge in the cytoplasm, activation of important enzymes and contribution to the total cell turgor maintaining the structural stability of non-lignified plants (Maathius & Sanders 1996; Maathius & Amtmann 1999).

During the study of the effect of various Na^+/K^+ ratios in the medium, we observed the steepest decrease of biomass production in pea seedlings grown in a solution containing either NaCl or KCl. The results indicate that pea plants prosper better in the presence of both cations. The lowest content of polyamines and the lowest AMADH and DAO activities were detected in pea seedlings grown at the Na^+/K^+ ratio ranging from 1:1 to 1:2, in accordance with previous data on usual $Na^+$and K^+ levels in plants (Jaworska & Kmiecik 1999).

The salinity-induced polyamine accumulation in pea seedlings is likely to be connected with an increase in the activities of enzymes involved directly or indirectly in polyamine degradation. In this study we aimed to explore changes in AMADH, DAO and POX activity in relation to their involvement in the plant defence response to salt stress. These enzymes and products of their reactions participate in important physiological processes in plants (Martin-Tanguy 1997; Šebela *et al.* 2001a, b). For example, DAO and POX are localised in the cell wall where they cooperate significantly. DAO catalyses the oxidative deamination of biogenic amines to the respective aldehydes and ammonia accompanied by the production of hydrogen peroxide (Bouchereau *et al.* 1999). DAO-based production of hydrogen peroxide is implicated in oxidative burst, cell death and POX-mediated lignification, suberisation and cell wall stiffening (Angelini *et al.* 1993; Wisniewski *et al.* 2000; Walters 2003). The amino aldehydes

produced by DAO reaction are further metabolised by AMADH (Šebela *et al*. 2000a). One of the best AMADH substrates, 3-aminopropionaldehyde, is the precursor of β-alanine, which can provide the osmoprotectant β-alanine betaine in complete methylation. 4-aminobutyric acid (GABA) is another important product of polyamine degradation via 4-aminobutyraldehyde, although it is formed predominantly by cytosolic glutamate decarboxylase (EC 4.1.1.15) (Ramputh & Bown 1996). GABA is involved in various physiological processes in plants including the regulation of cytosolic pH, carbon influx into the tricarboxylic acid cycle, protection against oxidative stress and cellular signalling (Kinnersley & Turano 2000; Bouché & Fromm 2004). Recently, a significant increase in the GABA level was demonstrated in pea seedlings following mechanical damage (Petřivalský *et al*. 2007). The measured levels of DAO, AMADH, and POX activity in pea shoots and roots are in accordance with the previously published results (Angelini *et al*. 1990; Petřivalský *et al*. 2007). The lowest polyamine content and activities of the studied enzymes were detected in plants grown in 10mM NaCl or KCl. This would confirm the polyamine participation in the metabolism of plants exposed to stress caused by mineral nutrient deficiency in addition to salt stress (Bouchereau *et al*. 1999). More pronounced changes in the studied parameters were observed after treatment with the increased NaCl content in comparison with the effect of increased KCl concentration. This is related to the less harmful effect of K^+ ions in plants and correlates well with previous data on the function of Na^+ and K^+ ions in vital plant processes (Maathius & Amtman 1999).

As previously reported, plants reduce the effects of salinity stress by increased production of polyamines (Flores 1991; Aziz *et al*. 1999; Bouchereau *et al*. 1999). The increased enzyme activities concerned with polyamine degradation, which were observed in plants exposed to salinity stress and which conversely decreased after the addition of Ca^{2+} ions, confirm their participation in the defence mechanisms of pea seedlings. Far from being only a means of regulating the polyamine levels, plant amine oxidases participate through their reaction products in important processes under physiological and stress conditions (Bouchereau *et al*. 1999; Šebela *et al*. 2001a, b; Walters 2003). The fact that pea does not accumulate the effective osmoprotectant glycine betaine (Weigel *et al*. 1986) indicates that AMADH, instead of betaine aldehyde dehydrogenase, may play a major role in defence responses of pea plants to salinity stress due to its participation in the biosynthesis of another compatible osmoprotectant GABA (Bouché & Fromm 2004). Although pea AMADH does not oxidise betaine aldehyde at all, its N-terminal amino acid sequence surprisingly resembles those of various BADHs (Šebela *et al*. 2000a). The participation of pea AMADH in adaptation to stress caused by mechanical damage, which involves polyamine catabolism, GABA generation and lignification, has already been demonstrated in pea plants (Petřivalský *et al*. 2007).

One of the possible solutions to a reduction of the negative salinity effect on plants is the addition of calcium. Ca^{2+} ions play a significant role in plant physiology and metabolism as they influence the permeability of the cell wall and membranes, exert a stabilising effect on protein conformation and affect the enzyme activity (Cramer *et al*. 1986). Calcium ions play an important role of versatile cell messengers in plant cells and mediate the cellular responses to stress signals (Reddy & Reddy 2004). The release of Ca^{2+} from the intracellular stores and a consequent increase in cytosolic Ca^{2+} concentrations have been described in previous studies on salinity stress (Sultana *et al*. 2001; Kaya *et al*. 2002). It was shown that supplemental Ca^{2+} had a beneficial impact on plant performance under saline conditions by reducing the net Na^+ influx into plant cells due to the Ca^{2+} blockage of non-selective cation channels (Demidchik *et al*. 2002) and as a result of the blockage of depolarisation-activated K^+ channels (Shabala *et al*. 2006) enabling efficient K^+ retention in the cytosol.

In our experiments, the addition of Ca^{2+} ions in a concentration of 2.5–5mM to the growth medium had a positive effect on the biomass production of pea seedlings. The calcium-mediated reduction of the negative effects of NaCl or KCl can be explained by the improvement of the K^+/Na^+ selectivity of plasma membrane channels (Murata *et al*. 1998) and by the protection of cell membranes against the harmful effects of salinity (Busch 1995). The deficit of Ca^{2+} ions can cause disturbances of the root system, which is more sensitive in comparison with the shoots. The root disturbance includes browning of the epidermis and cortical cells, decreased formation of root hairs, inhibition of lateral root formation and cell death at the root tip. These symptoms were observed in roots of plants

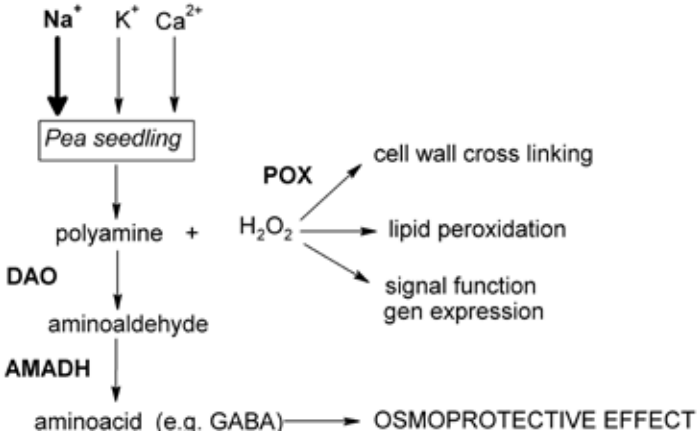


Figure 8. Involvement of polyamine catabolism in plant defense mechanisms under salinity stress

exposed to 60mM and 80mM NaCl (Figure 2), where we also measured a significant decrease in calcium content. The addition of 2.5–5mM Ca^{2+} to the growth medium alleviated the negative effect of salinity stress on plants (Figures 5A–5F), and it also decreased polyamine content and DAO, AMADH and POX activities increased by high salt concentrations (Figures 6A–6F).

In summary, our results show the important role of polyamine catabolism as a component of the refined plant defence systems under salinity stress (Figure 8). The obtained results also clearly demonstrate the participation of aminoaldehyde dehydrogenase in the pea response to salinity stress, which is the main outcome of the present study.

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