

The effects of some osmoprotectant compounds on growth parameters of pea plants (*Pisum sativum*) under saline conditions

A. Balliu, G. Sallaku and T. Nasto

Department of Horticulture and Landscape Architecture, Agricultural University of Tirana, Tirana, Albania.

Abstract

Water solutions of several osmoregulatory/antioxidant compounds; CaCl₂, KNO₃, KH₂PO₄, thiamine and ascorbic acid were tested for their effects on salinity stress alleviation versus tap water as control. The compounds were applied either by seed priming or through rooting medium by irrigation water. Stem length, chlorophyll index and stomata conductance of developed leaves, as well as dry matter of roots and plants were periodically measured and the respective root relative growth rate and plant relative growth rate were computed. The salinity has significantly reduced the growth parameters of just emerged pea plants, as well as their physiological parameters. No significant salinity alleviation effect was achieved by seed priming, but the addition of the osmoregulatory compounds in the rooting medium through the irrigation water has significantly counteracted the salinity stress effects and has significantly improved growth parameters of pea plants. KH₂PO₄ and thiamine seems to be the most effective ones, by restoring the growth parameters of just emerged pea plants up to the level of non-saline conditions. As such, they could be highly effective to improve the stand establishment rate of young plants under adverse soil condition.

Keywords: osmoregulatory compounds, relative growth rate, chlorophyll index, stomata conductance

INTRODUCTION

Earth is a salty planet, with most of its water containing about 30 g of sodium chloride per liter. This salt solution has affected, and continues to affect, the land on which crops are, or might be, grown (Flowers, 2004). Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield (Evelin et al., 2009), which have to cope with two major stresses under high salinity; osmotic stress and ionic stress. The former stress immediately comes over plants in accordance with a rise in salt levels outside the roots, which leads to inhibitions of water uptake, cell expansion and lateral bud development. The latter stress phase develops later when toxic ions such as Na⁺ accumulate in excess in plants particularly in leaves over the threshold, which leads to an increase in leaf mortality with chlorosis and necrosis, and a decrease in the activity of essential cellular metabolisms including photosynthesis (Horie et al., 2012).

The direct effects of salt on plant growth may involve: (a) reduction in the osmotic potential of the soil solution that reduces the amount of water available to the plant causing physiological drought; (b) toxicity of excessive Na and Cl ions towards the cell; and (c) nutrient imbalance in the plant caused by nutrient uptake and/or transport to the shoot leading to ion deficiencies (Evelin et al., 2009). In addition, a secondary aspect of salinity stress in plants is the stress-induced production of reactive oxygen species (ROS). The enhanced production of ROS during salinity stress lead to the progressive oxidative damage and ultimately cell death and growth suppression (Fatemi, 2014).

Plants have evolved biochemical and molecular mechanisms, which may act in a concerted manner and constitute the integrated physiological response to soil salinity. The most important plant strategies are: (i) synthesis and accumulation of compatible solutes; (ii) control of ion uptake by roots, compartmentation, and transport into plant tissues, which constitutes the ion homeostasis strategy; (iii) fine regulation of water uptake and



distribution to plant tissues by the action of aquaporins; and (iv) reduction of oxidative damage through improved antioxidant capacity (Ruiz-Lozano et al., 2012).

In response to abiotic stresses, plants accumulate a number of defense compounds such as ascorbate (vitamin C), glutathione, tocopherols (vitamin E), carotenoids, phenylpropanoids, polyamines, and indoles (Tunc-Ozdemir et al., 2009). Protective effects of some of these metabolites against oxidative stress in plants have been shown by their exogenous application or by measuring altered levels of them in plants with enhanced resistance. There is strong evidence that in many crop plants natural accumulation of osmoprotectants and other organic compounds is very low and this deficiency can be overcome by their exogenous application. Exogenous applications of osmoprotectants, plant growth regulators, fertilizers, and antioxidants have been reported to successfully mitigate the adverse effects of salinity on plants (Khan et al., 2006; Fatemi, 2014).

Inspired by this theoretical background, the main objective of this study was to examine whether the adverse effects of salt stress on the stand establishment rate of pea plants could be mitigated by exogenous application of several osmoprotectant compounds, either applied through seed priming, or through irrigation water.

MATERIALS AND METHODS

The experiment was conducted in spring 2014, in the experimental facilities of Agricultural University of Tirana, Albania. Graded seeds of a commercial pea cultivar were kept for 24 h in different solutions of several osmoregulatory (CaCl_2 : 100, 200, 500 mg L^{-1} ; KNO_3 : 100, 200, 500 mg L^{-1} and KH_2PO_4 : 100, 200, 500 mg L^{-1}) or antioxidant compounds (thiamine: 10, 20, 50 mg L^{-1} and ascorbic acid: 10, 20, 50 mg L^{-1}), and tap water as control.

60 primed seeds for each respective concentrations of every compound were sown in small plastic pots (80 cm^3 , each) filled with a mixture of peat + vermiculite (1:1 v/v), and kept in a plastic non heated greenhouse for 30 day after sowing. Prior to sowing the substrate was either saturated with tap water (control), or saline tap water (80 mM NaCl). Following, it was kept in full holding water capacity till the end of experiment by sequential addition of equal amounts of tap water, having not allowed the water to drain out. Simultaneously, the same number of (non-primed) seeds were sown in similar peat + vermiculite mixture filled pots. After sowing, the substrate was brought and maintained to full holding water capacity, either by tap water (control), or saline water (80 mM NaCl) the same respective concentrations levels for each osmoregulatory/antioxidant compounds were added as described above.

During the experiment the chlorophyll index (by CM 1000 Chlorophyll Metter, Spectrum Technologies, USA) and the stomata conductance (by SC-1 Leaf Porometer, Decagon Devices, USA) of well-developed leaves were successively measured in 10 randomly selected plants. As well, 10, 20 and 30 day after sowing, 10 plants of each experimental plot were randomly selected, removed from the pots and roots were gently washed free of adhering vermiculite particles. For each plant, stem length was first measured and then plants previously separated in roots and above ground mass, were dried at 60°C for 24 h and individually weighted. The relative growth rate (the increase in dry weight of plants per unit of dry weight, per unit of time; RGR) was individually computed for each experimental plot according to procedure described by Hunt et al. (2002) and Hoffmann and Poorter (2002). Similarly, root relative growth rate (the increase in dry weight of roots per unit of dry weight, per unit of time; RRGR) was also computed and together with root and plant dry matter were used as parameters to assess the effect of different compounds on salinity stress alleviation on pea plants.

Differences for each measured parameter were tested by two way ANOVA, using StatPlus 2009 Professional 5.8.4 (AnalSyst Inc.). LSD test was performed at $p=0.05$ to compare means within and among different treatments.

RESULTS AND DISCUSSIONS

As our data shows, the raise of salinity has dramatically reduced the growth parameters of just emerged pea plants, and significantly deteriorated their physiological

parameters. Compared to control (tap water), saline water has significantly reduced plant height, root and total dry matter of plants, the root relative growth rate and plant relative growth rate. Saline water has also tremendously reduced stomata conductance (thus significantly reduced plant photosynthetic activity), but no significant effects was noticed regarding the chlorophyll index (Table 1).

Table 1. The significance of osmoregulatory compounds effects through seed priming on root dry matter, plant dry matter, stomata conductance and chlorophyll index of pea plants.

Source of variation	p-level			
	Root dry matter (RW)	Plant dry matter (W)	Stomata conductance	Chlorophyll index
Treatment (T)	0.060	0.219	0.119	0.253
Concentration (C)	0.096	0.827	0.416	0.023
T × C	0.006	0.025	0.077	0.577

Seed priming, a controlled hydration technique that enhances seed performance by rapid and uniform germination, is considered one of the most environmentally friendly and pragmatic short-term approaches to combat the effects of environmental stresses on seedling emergence and stand establishment (Cuartero et al., 2006; Khan et al., 2009; Fatemi, 2014). Furthermore, according to some authors (Athar et al., 2008; Gupta and Huang, 2014) seeds performance of various crops can be improved by inclusion of plant growth regulators and hormones during priming and other pre-sowing treatments.

Yet, we did not found any significant effects of any of compounds were been added at seed priming solutions versus common seed priming in tap water (Table 1), either on plant growth parameters (plant height, root and total plant matter), or their physiological parameters (stomata conductance and chlorophyll index). But, contrary to that we did found significant salinity alleviation effects of the same compounds supplied through rooting medium by irrigation water (Table 2). In our experiment, no effect of concentration of any of compounds used was found (Table 2), much probably that by abundant solutions used as irrigation water quite enough quantities of each compounds were supplied even at the lowest concentrations.

Table 2. The significance of osmoregulatory compounds effects through irrigation water on plant height, root dry matter, plant dry matter, stomata conductance, chlorophyll index, relative growth rate (RGR) and root relative growth rate (RRGR) of pea plants.

Source of variation	p-level						
	Plant height (cm)	Root weight (RW)	Plant weight (W)	Stomata conductance	Chlorophyll content	RGR	RRGR
Treatment (T)	0.000	0.000	0.000	0.000	0.107	0.009	0.000
Concentration (C)	0.071	0.005	0.027	0.100	0.471	0.617	0.340
T × C	0.954	0.572	0.234	0.002	0.136	0.021	0.000

Almost every each of compounds added in the irrigation water (either minerals or vitamins) has restored the growth and physiological parameters up to close to control values, indicating significant salinity stress alleviation effects (Table 3). Thus, it seems that like Khan et al., (2006) have also reported, the effectiveness of exogenously applied osmo-protectant compounds depends on the mode of application. From that, it can be concluded

that a successful application methods have to guarantee that a certain endogenous level of any of the compounds used is reached in the plant.

Table 3. Plant height, root dry matter, plant dry matter, stomata conductance, chlorophyll index, relative growth rate (RGR) and root relative growth rate (RRGR) of pea plants according to osmoregulatory compound effects. Different letters within the same column indicate significant difference at $p < 0.05$.

	Plant height (cm)	Root dry matter (g)	Plant dry matter (g)	Stomata conductance	Chlorophyll index	RGR	RRGR
Control (tap water)	10.04 a	0.14 a	0.46 a	11.53 bc	198.13	0.123 a	0.126 bc
Control-S	6.64 c	0.09 cd	0.24 d	3.83 d	179.13	0.095 b	0.113 c
CaCl ₂	8.17 b	0.12 b	0.38 b	22.92 a	222.25	0.119 a	0.135 b
KH ₂ PO ₄	7.60 bc	0.11 c	0.34 b	15.37 b	228.58	0.122 ab	0.117 c
KNO ₃	7.31 c	0.08 d	0.34 b	20.20 ab	229.96	0.108 ab	0.073 d
Ascorbic acid	6.8 c	0.10 c	0.32 c	7.07 c	195.50	0.116 a	0.106 c
Thiamine	7.70 bc	0.14 b	0.37 b	10.46 c	231.08	0.126 a	0.155 a

One of the most detrimental effects of salinity stress is the accumulation of Na⁺ and Cl⁻ ions in tissues of plants exposed to soils with high NaCl concentrations. High Na⁺ concentration inhibits uptake of K⁺ ions which is an essential element for growth and development that results into lower productivity and may even lead to death (Gupta and Huang, 2014). Therefore, improving K nutrition of plants under salt stress could be essential to minimizing oxidative cell damage, at least in part by reducing ROS formation during photosynthesis and inhibiting activation of O₂-generating NADPH oxidase (Cakmak, 2005). Externally supplied K may also increase endogenous K⁺ content of seeds, required to tolerate salinity at germination, and ameliorate abiotic-stress effects (Umar et al., 2011).

Indeed, in our experiment by the addition of K⁺, as potassium nitrate (KNO₃) or two hydrogen potassium phosphate (KH₂PO₄), the plants were been able to restore their relative growth rate (RGR) up to the level of control (tap water). Highly significant effects were also recorded regarding stomata conductance (Table 3). Comparing KNO₃ and KH₂PO₄, clearly two hydrogen potassium phosphate was been more effective. As other authors have reported (Porcel et al., 2012), salinity cause not only K deficiency but also P deficiency and thus, the supply of KH₂PO₄ was effective in correcting both nutrient deficiencies. Similar results were also reported by Cakmak (2005).

The role of calcium to alleviate the adverse effect of NaCl and other salts on plant species is well known. Three separate mechanisms of Ca²⁺ alleviation were identified; the displacement of cell-surface toxicant, the restoration of Ca²⁺ at the cell surface if the surface Ca²⁺ has been reduced by the toxicant to growth-limiting levels, and the collective ameliorative effect beyond two first mechanisms (Kinraide, 1998). In our case also, calcium chloride (CaCl₂) was one of the most effective compounds. Its stress alleviation effects were especially effective regarding plant weight, plant and root relative growth rates and stomata conductance (Table 3). As it is also previously reported by Liu and Zhu (1998), externally supplied Ca²⁺ have reduced the toxic effects of NaCl, presumably by facilitating higher K⁺/Na⁺ selectivity, and thus enhancing overall plant metabolic activity.

Of the various non-enzymatic antioxidants, such as tocopherols, carotenoids, and phenols, ascorbic acid occurs ubiquitously in plants and has been reported to play a vital role in alleviating the adverse effects of salt on plant growth and metabolism in many crop plants (Khan et al., 2006). In general, effects of ascorbic acid in mitigating the adverse effects of salt stress have been ascribed to activation of some of the enzymatic reactions, accelerated cell division and/or cell enlargement (Sadak et al., 2010), activation or

suppression of key enzymatic reactions, induction of stress responsive proteins synthesis, and the production of various chemical defense compounds (Athar et al., 2008).

Thiamin, as well, has been reported to alleviate the effects of several environmental stresses in plants. The exogenous application of thiamin was shown to counteract the harmful effects of salinity on growth and to confer resistance to fungal, bacterial, and viral infections in rice, arabidopsis, and specific vegetable crop species (Horie et al., 2012). It is a necessary ingredient for the biosynthesis of co-enzymes, and is the most effective vitamin for production of IAA (Ghosh et al., 2008).

Both, ascorbic acid and thiamine, have contributed to alleviate the salinity stress of just emerged pea plants in our experiment (Table 3), but thiamine seems to be much more effective. It has restored the relative growth rate of plants (RGR), root dry matter and stomata conductance up to the level of control (tap water). Since by thiamine application a full restoration of root dry matter was achieved, while was even over passed the root relative growth rate of control plants, it is clear that thiamine application could be highly effective to improve the stand establishment rate of young plants under saline soil condition.

CONCLUSIONS

Salinity has significantly deteriorated the growth and physiological parameters of pea plants. Thus, in saline conditions the stand establishment rate of just emerged pea plants were significantly smaller compared to non-saline conditions.

The use of several osmoregulatory or antioxidant compounds could restore the growth parameters of pea plants under saline conditions up to the level of non-saline conditions. Ca and K compounds and thiamine seems to be the most effective one regarding the salinity effects alleviation on the stand establishment rate of pea plants. Yet, the effect of osmoregulatory or anti oxidative compounds depends on the method of application. The application through root medium seems to be much more effective compared to seed priming.

ACKNOWLEDGEMENT

This results are part of the work conducted in the framework of EUROLEGUME Project (Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed), founded by 7th Research Framework Programme of the European Union (FP7 Research Project No. 61378). This publication was made possible, also, by the support of Albanian Agency of Research, Technology and Innovation (ARTI).

Literature cited

- Athar, H.R., Khan, A., and Ashraf, M. (2008). Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environ. Exp. Bot.* 63, 224–231.
- Cakmak, I. (2005). The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *J. Plant Nutr. Soil Sci.* 168, 521–530.
- Cuartero, J., Bolarín, M.C., Asíns, M.J., and Moreno, V. (2006). Increasing salt tolerance in the tomato. *J. Exp. Bot.* 57 (5), 1045–1058. PubMed
- Evelin, H., Kapoor, R., and Giri, B. (2009). Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Ann. Bot.* 104 (7), 1263–1280. PubMed
- Fatemi, S.N. (2014). Ascorbic acid and its effects on alleviation of salt stress in sunflower. *Annu. Res. Rev. Biol.* 4 (24), 3656–3665.
- Flowers, T.J. (2004). Improving crop salt tolerance. *J. Exp. Bot.* 55 (396), 307–319. PubMed
- Ghosh, S., Sengupta, C., Maiti, T.K., and Basu, P.S. (2008). Production of 3-indolylacetic acid in root nodules and culture by a *Rhizobium* species isolated from root nodules of the leguminous pulse *Phaseolus mungo*. *Folia Microbiol. (Praha)* 53 (4), 351–355. PubMed
- Gupta, B., and Huang, B. (2014). Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *International Journal of Genomics 2014 (Article ID 701596)*: 18 <http://dx.doi.org/10.1155/2014/701596>.
- Hoffmann, W.A., and Poorter, H. (2002). Avoiding bias in calculations of relative growth rate. *Ann. Bot.* 90 (1), 37–



42. PubMed

Horie, T., Karahara, I., and Katsuhara, M. (2012). Salinity tolerance mechanisms in glycophytes: an overview with the central focus on rice plants. *Rice* 5 (1), 11. PubMed

Hunt, R., Causton, D.R., Shipley, B., and Askew, A.P. (2002). A modern tool for classical plant growth analysis. *Ann. Bot.* 90 (4), 485–488. PubMed

Khan, A., Ahmad, M.S.A., Athar, A.U., and Ashraf, M. (2006). Interactive effects of foliary applied ascorbic acid and salt stress on wheat (*Triticum aestivum* L.). *Pak. J. Bot.* 38 (5), 1407–1414.

Khan, H.A., Pervez, M.A., Ayub, C.M., Ziaf, K., Balal, R.M., Shahid, M.A., and Akhtar, N. (2009). Hormonal priming alleviates salt stress in hot Pepper (*Capsicum annuum* L.). *Soil and Environment* 28 (2), 130–135.

Kinraide, T.B. (1998). Three mechanisms for the calcium alleviation of mineral toxicities. *Plant Physiol.* 118 (2), 513–520. PubMed

Liu, J., and Zhu, J.K. (1998). A calcium sensor homolog required for plant salt tolerance. *Science* 280 (5371), 1943–1945. PubMed

Porcel, R., Aroca, R., and Ruiz-Lozano, J.M. (2012). Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. *Agron. Sustain. Dev.* 32, 181–200.

Ruiz-Lozano, J.M., Porcel, R., Azcón, C., and Aroca, R. (2012). Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. *J. Exp. Bot.* 63 (11), 4033–4044. PubMed

Sadak, M.Sh., Abdelhamid, M.T., and El-Saady, A.-K.M. (2010). Physiological responses of faba bean plant to ascorbic acid grown under salinity stress. *Egyptian Journal of Agronomy* 32 (1), 89–106.

Tunc-Ozdemir, M., Miller, G., Song, L., Kim, J., Sodek, A., Koussevitzky, S., Misra, A.N., Mittler, R., and Shintani, D. (2009). Thiamin confers enhanced tolerance to oxidative stress in Arabidopsis. *Plant Physiol.* 151 (1), 421–432. PubMed

Umar, S., Diva, I., Anjum, N.A., Iqbal, M., Ahmad, I., and Pereira, E. (2011). Potassium-induced alleviation of salinity stress in *Brassica campestris* L. *Cent. Eur. J. Biol.* 6 (6), 1054–1063.