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THE RESPONSE OF MAIZE SEEDLINGS TO SALT STRESS UNDER INCREASING LEVELS OF PHOSPHORUS

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Abstract

This study was conducted to evaluate the combined effect of increasing doses of phosphate and moderate salinity on the growth and some biochemical parameters of maize seedlings. The experiments were carried out on the maize variety KB 1902 grown in hydroponic cultures under controlled conditions. Salt stress was induced by 50 mmol dm^{-3} NaCl at three levels of KH_2PO_4 (mmol dm^{-3}): 1.0 (standard dose in a nutrient solution), 0.25 (decreased dose), 2.0 (increased dose). After 7 days of cultivation, the plants were harvested, growth parameters were examined and assessments were made of the concentrations of photosynthetic pigments, total protein, soluble carbohydrates and free phosphate. Salt stress caused a significant reduction in the fresh and dry weight of plants, ranging from 33-45% in comparison to the plants grown without NaCl, irrespective of external phosphate concentrations. Concentrations of photosynthetic pigments and total proteins were unaffected by salinity, regardless of the external phosphorus levels. The two higher concentrations of Pi (1.0 and 2.0 mmol dm^{-3}) in the saline nutrient medium resulted in a marked accumulation of phosphorus in the leaves of maize, whereas the lowest level of Pi (1/4 of the standard dose in the nutrient solution) had no effect on the phosphorus content. Both the increased (2 P) and decreased (1/4 P) phosphorus concentration in the external medium induced a significant increase in the content of soluble carbohydrates in the leaves of maize grown under salt stress. In brief, the response of the maize variety KB 1902 to NaCl stress at the initial stage of growth was not found to have been modified by the examined doses of phosphorus, and there was no relationship between NaCl and the increasing doses of phosphate.

Keywords: salinity, phosphorus, carbohydrates, protein, chlorophyll, carotenoids.

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INTRODUCTION

Phosphorus is an important macroelement, whose inadequate supply often limits plant growth. This element is a building block of essential cellular components such as nucleic acids, phospholipids, dinucleotides and adenosine triphosphate, hence its vital role in energy storage and transfer processes, photosynthesis, regulation of enzyme activity as well as metabolism and transport of carbohydrates (Waraich et al. 2011). While many soils around the world contain less P than required by crops, excessive application of P-rich manure and high doses of phosphates may cause accumulation of phosphorus in farmlands and groundwater. Large amounts of phosphates in soils potentially aggrevate the toxicity of some soil pollutants such as NaCl or herbicide residues (Bott et al. 2011). On the other hand, a high concentration of P in soil might contribute to an excessive uptake of this element, simultaneously causing deficiency of some micronutrients in plant tissues (Fageria 2001). The salinization of soil is a grave problem affecting world agriculture. Under saline conditions, plants are subjected to both osmotic stress (reduction in water potential) and an excessive amount of salt (Na^+ and Cl^- toxicity) (Greenway, Munns 1980). Osmotic problems dominate during the first phase of salt stress, when plants are exposed to salinity for a short time. Salt specific effects (ion imbalance and ion toxicity) occur in the second phase, mainly in the older parts of plants after several days of exposure to salinity (Neumann 1997, Munns 2002). It is generally accepted that sodium chloride stress disturbs the ionic balance in plant cells and organs. In many crop plants, NaCl reduces the uptake of such important minerals as K^+, Ca^{2+}, NO_3^- , hence a decrease in the concentrations of these minerals in plant tissues (Grattan, Grieve 1999, Sacała et al. 2002, 2008, Hu, Schmidhalter 2005, Tuna et al. 2008). Literaure data concerning the interaction between P nutrition and salinity are contradictory. There is evidence that salinity may (1) cause an excessive accumulation of P up to a toxic level, (2) induce phosphorus deficiency in plant tissues, or (3) have no effect (Nieman, Clark 1976, Martinez Läuchli 1994, Rogers et al. 2003, Hu, Schmidhalter 2005, Mousavi et al. 2008, Tuna et al. 2008, Guo et al. 2009, Uygur, Yetisir 2009). Bernstein et al. (1974) claimed that some plants (such as maize) were sensitive to a high concentration of P in plant tissue and phosphate supplementation aggravates salt injury. Phang et al. (2009) showed a similar negative effect in soybean plants. They indicated that under salt stress conditions a high external phosphate concentration results in an increased net sodium uptake and reduced cell viability. On the other hand, Madueño-Molina et al. (2008) indicated that an increment of P in a saline solution stimulated higher biomass accumulation and better osmotic adjustment in the wild legume *Rhynchosia minima*. No definitive conclusions have been drawn regarding the relationship between external Pi levels and salt tolerance degree. Thus, the objective of this study was to determine the combined effect of moderate salinity and increasing and relatively high levels of phosphate on the growth and some biochemical parameters in maize seedlings at the initial stage of development.

MATERIAL AND METHODS

Plant materials and treatment

All experiments were conducted on maize seedlings (*Zea mays* L. variety KB 1902; seeds obtained from the Nasiona Kobierzyc Company) grown in hydroponic cultures under controlled conditions: 16 h photoperiod (220 µmol m^{-2} s^{-1}) at 26/20°C day/night, 65-70% relative humidity. The basic nutrient solution was (mmol dm^{-3}): 3 $Ca(NO_3)_2$, 2 KNO_3, 1 $MgSO_4$, 1 KH_2PO_4, 0.2 Fe-EDTA and microelements, pH 6.5 ±0.1. The experiment was designed in a two-factorial arrangement with two levels of NaCl in a nutrient solution (0 and 50 mmol dm^{-3} NaCl) and three levels of phosphate: 0.25 mmol dm^{-3} Pi (1/4Pi), 1 mmol dm^{-3} Pi – the standard dose in a nutrient solution (1Pi), and 2 mmol dm^{-3} Pi (2 Pi). Phosphorus was added as KH_2PO_4. The diminished K concentration in the nutrient solution containing 0.25 mmol dm^{-3} KH_2PO_4 was supplemented by 0.75 mmol dm^{-3} KCl.

Growth parameters and water content (WC)

After 7 days of cultivation, the plants were harvested and separated into roots and shoots. After measuring root lengths, shoot heights and fresh weight, plant organs were dried for 1 h at 105°C and subsequently at 75°C, after which the dry weight was determined. The water content (WC) in roots and shoots was calculated on a fresh weight (FW) basis. The WC was derived from the difference between fresh weight and dry weight divided by fresh weight and multiplied by 100%.

Chemical analyses

Plant nutrients such as proteins, water-soluble carbohydrates, phosphate and photosynthetic pigments were determined in the second and the third leaf of maize seedlings. Photosynthetic pigments were extracted from fresh plant material using 80% acetone. The absorbance of the extracts was recorded at 470, 647, 663 nm, and the concentrations of total chlorophyll (chl *a* + chl *b*) and carotenoids were calculated using Lichtenthaler equations (1987). The other compounds were determined in dry plant material. Pulverized dry leaf material was homogenized with 6% TCA, left for about 20 min at room temperature and then centrifuged (12 000 g, 10 min). After centrifugation, both the supernatant and the precipitate were collected. Inorganic phosphate was measured colorimetrically in the supernatant, previously mixed with H_2SO_4, ammonium molybdate and 1,2,4-aminonaphtholsulfonic acid and incubated at 37°C. The absorbance of the samples was measured at 660 nm. Protein in the precipitate was extracted with NaOH at 100°C for 20 min and then the sample was centrifuged (12 000 g, 10 min). After that, total protein was determined according to the Lowry method (Lowry et al. 1951). Water-soluble carbohydrates were extracted with distilled water at 100°C for 30 min and determined by the Nelson method (Nelson 1944).

Statistical analysis

The experiment was arranged in a randomized complete block design with six replications. The data for all parameters were statistically analysed by the analysis of variance and the differences among mean values were compared by the LSD test (the least significant difference, $p \leq 0.05$).

RESULTS AND DISCUSSION

Our objective was to determine the combined effect of increasing but non-limiting concentrations of Pi (0.25, 1.0 and 2.0 mmol dm^{-3}) and moderate salinity on maize seedlings. Soil ions may interact with one another, thus affecting the uptake of essential minerals by plants, disrupting homeostasis in plant tissues and consequently arresting the plant growth. Some research indicated that phosphorus nutrition could modulate plant response to salt stress (Bernstein et al. 1974, Rogers et al. 2003, Tuna et al. 2008, Phang et al. 2009). It was implicated that high Pi level in soil might aggravate plant stress under saline conditions (Bernstein et al. 1974, Nieman, Clark 1976, Cerda et al. 1977, Navarro et al. 2001). Maize (*Zea mays* L.), an important crop in many countries, has high water and nutrient requirements but is considered to be moderately sensitive to salinity. In our experiments, maize seedlings were subjected to salt stress immediately after germination at the stage characterized by high sensitivity to water stress. In the first phase, the negative effect of salinity on plant growth is mostly due to the low osmotic potential of the soil solution (water stress).

Plant growth and phosphate concentration

The results indicated that the maize variety (KB 1902) was sensitive to 50 mmol dm^{-3} NaCl (Figure 1). All the examined growth parameters (except root length) were significantly reduced under salt conditions and the biomass production was significantly lowered. The plants did not display any other visual symptoms of injury (chlorosis, necrosis, wilting). The decline in the growth parameters ranged between 33-45% in comparison to the plants grown without NaCl and the results were similar in the underground and aerial parts of plants. Fresh weight and dry weight were affected similarly. The plant growth parameters (fresh and dry weight of roots and shoots) were

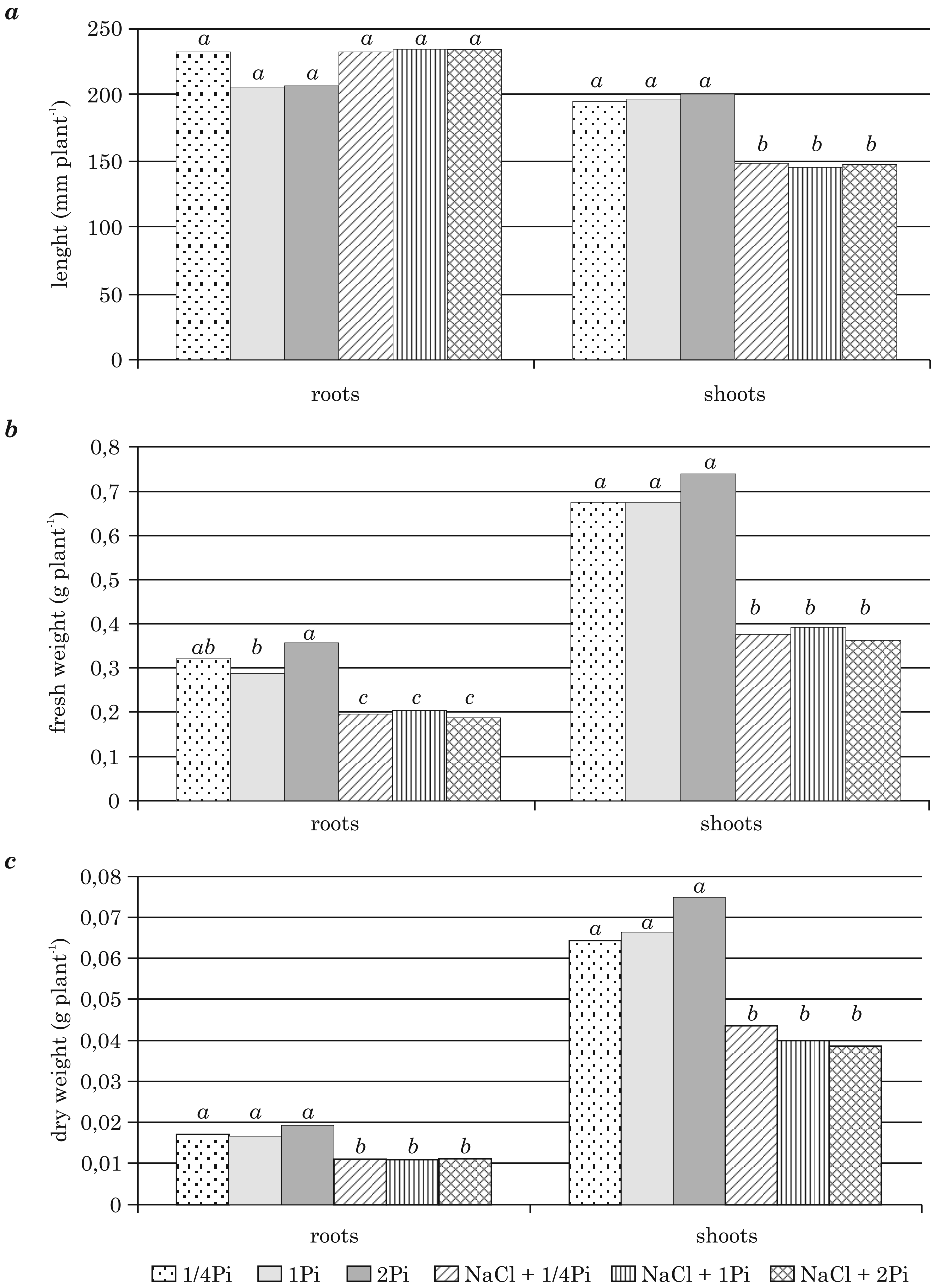

Fig. 1 The influence of NaCl and different doses of phosphorus on: length (*a*), fresh weight (*b*) and dry weight (*c*) of roots and shoots of 7-day old maize seedlings (var. KB 1902). Values with the same letter above the bars do not differ significantly at $P < 0.05$

similar under all the phosphate doses. It is therefore safe to say that no significant response to the Pi level in a saline nutrient medium occurred and that the highest Pi dose (2 mmol dm^{-3}) neither decreased nor improved the salt tolerance of maize seedlings. Our observations are contradictory to the results obtained by Nieman , Clark (1976), who concluded that salinity in combination with a high concentration of orthophosphate (2 mmol dm^{-3}) was more injurious to maize plants. However, Kaya et al. (2003) indicated that supplementary KH_2PO_4 at 272 mg (2 mmol dm^{-3}) kg^{-1} soil could partly mitigate the adverse effects of high salinity on pepper and cucumber plants. Our results coincide with those reported by Rogers et al. (2003) on lucerne grown in hydroponic cultures. These authors did not find any difference in dry matter production between two high Pi treatments (0.5 and 5.0 mmol dm^{-3}), nor any NaCl×P interaction. Moreover, the concentration of P in shoots and in roots did not vary depending on the external NaCl or P conditions. Our results indicate that the accumulation of free phosphate in leaves, regardless of the external phosphorus concentrations, was similar in maize grown without NaCl (Table 1). However, the presence of NaCl in the nutrient solution significantly altered the Pi status in leaves and a tendency to accumulate free

Table 1

Effect of different doses of phosphorus and NaCl on some plant constituents in the leaves of 7-day-old maize seedlings

Doses	Chlorophylls *a* + *b* (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Phosphate (mg g^{-1} DW)	Soluble carbohydrates (mg g^{-1} DW)	Total protein (g g^{-1} DW)
¼ Pi	2.09±0.20	0.339±0.041	4.52±0.30 *b*	16.88±1.06 *b*	0.189±0.009
1 Pi	2.03±0.09	0.346±0.029	4.64±0.32 *b*	13.94±2.56 *b*	0.189±0.016
2 Pi	2.02±0.19	0.336±0.040	4.57±0.25 *b*	15.50±1.30 *b*	0.176±0.011
¼ Pi +NaCl	2.19±0.13	0.401±0.036	4.76±0.36 *b*	21.88±1.19 *a*	0.192±0.021
1Pi + NaCl	1.91±0.20	0.338±0.019	6.24±0.49 *a*	16.31±1.31 *b*	0.180±0.009
2Pi + NaCl	2.02±0.20	0.360±0.040	5.94±0.66 *a*	21.81±2.94 *a*	0.186±0.022
$LSD_{0.05}$	0.32	0.066	0.78	3.00	0.023

Values in the same column followed by different letters are significantly different at $P \leq 0.05$).

phosphate was observed. The concentration was approximately 30% higher in plants grown without NaCl than in plants grown under two higher Pi levels (1 and 2 mmol dm^{-3}). Hu et al. (2006) indicated that salinity increased the total P concentration in leaves of wheat 26 days after sowing. In our short-term experiment, the increased concentration of Pi in maize leaves could be a positive effect that promoted osmotic adjustment under salt stress. Treeby and van Steveninck (1988) also suggested that moderate Pi concentrations played a beneficial role in the osmotic adjustment of cells, whereas a high Pi supply did not contribute to osmoregulation and Pi accumulated in cell walls. It is conceivable that over a longer period of time, Pi accumulating in plants could reach a toxic level, as suggested by Nieman, Clark (1976).

Other plant constituents

There were relatively small differences in the concentration of the other plant constituents (Table 1). Concentrations of photosynthetic pigments and total proteins were unaffected by salinity regardless of the external phosphorus levels. The concentration of soluble carbohydrates in the leaves of maize grown without NaCl was similar at all examined Pi levels. However, under salt stress, a tendency to accumulate these compounds in leaf tissues was observed (Table 1). The increase in the concentrations of soluble carbohydrates under salt conditions at different Pi levels ranged between 17-41% in comparison to plants grown without salt. The highest sugar accumulation occurred both under excessive (2 P) and lowered (1/4 P) phosphorus levels in an external medium, and it reached *ca* 21.8 mg g^{-1} DW. The accumulation of soluble carbohydrates may be an important component of the osmotic adjustment of cells under salt stress. Some reports show that salt stress induces accumulation of soluble sugars (Cheeseman 1988, Zheng et al. 2008, Mousavi et al. 2008). Gibson (1988) maintains that salt tolerance mechanisms in plants involve carbohydrate metabolism, which is influenced by phosphorus nutrition. Our results also showed that phosphorus nutrition could modify the level of accumulated soluble sugars under saline conditions. Ashraf and Harris (2004) conclude that information regarding the role of carbohydrates in the adaptation of plants to salinity is not sufficient to definitively acknowledge that sugar accumulation is universally associated with salt tolerance. These compounds may contribute to osmotic adjustment and osmoprotection, but their major function is to fuel metabolism. Under stress conditions, plant growth and also sugar utilization are reduced whereas export from leaves is decreased, stimulating the accumulation of water-soluble carbohydrates (Khelil et al. 2007).

Salinity treatment did not significantly reduce the water content in maize roots but caused its slight decrease in shoots independent of the external levels of Pi (Table 2).

Table 2

The influence of different doses of Pi and NaCl on water content in the roots and shoots of 7-day-old KB 1902 maize seedlings

Doses	Water content (% of fresh weight)	
	Roots	Shoots
¼ Pi	95.2±0.4 *a*	91.6±0.2 *a*
1 Pi	94.8±0.6 *ab*	91.6±0.3 *a*
2 Pi	94.4±0.4 *b*	91.6±0.2 *a*
¼ Pi +NaCl	94.8±0.5 *ab*	91.2±0.2 *b*
1Pi + NaCl	94.8±0.6 *ab*	90.9±0.3 *b*
2Pi + NaCl	94.5±0.3 *b*	91.0±0.3 *b*
$LSD_{0.05}$	0.7	0.4

Values in the same column followed by different letters are significantly different at $P \leq 0.05$).

CONCLUSIONS

1. Salinity caused a significant reduction in the growth of maize seedlings and the examined doses of phosphorus did not modify this growth response. Regardless of the phosphorus dose, NaCl was a dominant factor in suppressing the growth of young maize seedlings.

2. Among the examined constituents, photosynthetic pigments and total protein did not change significantly under the exprimental conditions, whereas free phosphate and water-soluble carbohydrates were significantly altered.

3. Increasing the level of phosphorus in the external environment did not affect the early growth of maize seedlings under saline conditions.

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