THE EFFECTS OF SALT STRESS ON GROWTH AND BIOCHEMICAL PARAMETERS IN TWO MAIZE VARIETIES

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ABSTRACT

The main objective of this study was to examine the influence of salinity on growth and biochemical parameters (NR activity, amino compound accumulation, protein content, some inorganic ion concentrations) of two varieties of maize (Limko and Koka). Salinity (75.0 mol \cdot m^{-3} \text{NaCl} and 37.5 mol \cdot m^{-3} \text{Na}_2\text{SO}_4) significantly reduced fresh and dry weights of the investigated maize varieties. NaCl induced higher reduction in biomass production than Na_2SO_4. Differences in salt tolerance between Limko and Koka were small.

It was found that in both maize varieties the nitrate reductase activity decreased under salt stress (100 mol \cdot m^{-3} \text{NaCl}). This effect was more marked in the Koka variety than Limko. Decrease in nitrate reductase activity had no inhibitory effect on accumulation of protein and free amino compounds. Salinity treatment (100 mol \cdot m^{-3} \text{NaCl}) led to an increase in free amino compound contents in roots and shoots of both investigated maize varieties, but these changes were not very large. The highest increase in amino compound level was observed in roots of Koka and it was 2-fold higher than in control plants. Salt stress did not change soluble protein contents in Limko and Koka varieties with the exception of Limko leaves. There was significant increase in soluble protein content on leaves of Limko and it amounted 128% of the control. Salinity caused a great increase in leaf and root Na⁺ concentration and a decrease in case of Ca²⁺ and K⁺ contents. The declines in Ca²⁺ content in maize roots were 76 and 70% respectively for Koka and Limko roots. The fall in K⁺ concentration was high, but not so drastic as in Ca²⁺. In roots of Koka and Limko the reduction in K⁺ content was approximately 30%. Higher reduction in K⁺ content showed shoots of Limko. In this case K⁺ content lowered to 49% of control.

KEY WORDS: salinity, ion content, nitrate reductase, amino compounds, soluble protein, maize.

INTRODUCTION

Salinization of land is a progressively increasing phenomena. Therefore, the investigations of plant response to salt stress are very important in crop science, plant physiology and agricultural science. Most crop plants are salt-sensitive and salinity induces several morphological, physiological and metabolic changes in plants (Evers et al. 1997; Rawat and Banerjee 1998; Yeo 1998; Alian et al. 2000). Some of the changes are: inhibition of plant growth and development, changes in soluble protein synthesis, accumulation of organic metabolites and altered ion relations (Hasegawa et al. 2000). It has been suggested that these changes are important for increased plant tolerance to salt stress.

Our first aim was to compare the effect of NaCl and Na_2SO_4 on the growth of two maize varieties and to determine which of the salts is more toxic. Since NaCl was more toxic for maize than Na_2SO_4, our second objective was to investigate the influence of NaCl on some biochemical parameters. Because the accumulation of nitrogenous compounds in salt-stressed plants improves their salt tolerance we investigated NR activity, amino compounds and some inorganic ion concentration.

MATERIALS AND METHODS

In the first experiment, maize seedlings (Zea mays L. varieties Koka and Limko) after germination were grown in beakers filled with modified Knop solution (control) or in Knop solution with the addition of different salts 75.0 mol \cdot m^{-3} \text{NaCl} or 37.5 mol \cdot m^{-3} \text{Na}_2\text{SO}_4 (salt treatments). The culture conditions were: 16 h photoperiod (220 \text{μmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}) at 26/20°C day/night temperature, 65-70% relative humidity. Plant growth was measured after 14 days of cultivation. The shoot and root lengths, fresh and dry weights were determined.

In the second experiment maize seedlings grew in nutrient solution with the addition of 100 mol \cdot m^{-3} \text{NaCl}. After 7 days of cultivation on salt-containing and control media
the biochemical parameters were measured – NR activity, soluble protein content, free amino compounds and Na⁺, K⁺, Ca²⁺ contents. The soluble protein and free amino compounds were extracted from roots and second leaf of maize according to Weber et al. (1991). Plant material was homogenized in phosphate buffer (50 mol · m⁻³ KH₂PO₄/K₂HPO₄, 1 mol · m⁻³ Fe-EDTA, pH 7.0) and the homogenate was centrifuged 10 minutes at 12 000 g. After centrifugation the soluble protein content was determined by the Bradford’s (1976) method with bovine serum albumin (BSA) as the standard. For the determination of amino compounds the protein was precipitated by 10% trichloroacetic acid, the samples were centrifuged (18 000 g, 3 min) and adjusted to pH 5.5-7.5. Free amino compounds were measured using the ninhydrine reagent (Moore 1968). Nitrate reductase activity was assayed in vivo in roots and second leaf according to the method of Jaworski (1971). The amount of formed nitrite was assayed according Harper and Hageman (1972). The total activity of NR was expressed per g of fresh weight per min. For Ca²⁺, K⁺, Na⁺ analysis the samples of dried plant material (roots and whole shoots) were digested and determined by atomic absorption spectrophotometry.

All experiments were repeated three times and all results are presented as means of three separate experiments. Differences between control and salt treatments were compared by LSD (least significant difference).

RESULTS

Plant growth (Fig. 1)

NaCl and Na₂SO₄, salinity significantly affected growth of both the examined varieties of maize. Growth of plants salinized by NaCl was more inhibited than those salinized by Na₂SO₄. Fresh and dry weights of plants grown under NaCl stress balanced between 22-42% of the control, whereas under Na₂SO₄ these weights lowered to 33-60% of the control. Root length did not change so drastically as accumulation of fresh and dry weights. Shoot length was more reduced than root length. Under NaCl treatment the decrease in dry weight of Limko shoots was more lowered than in roots. Root dry weight decreased to 42% of the control, whereas shoot dry weight was 22% of the control. However under Na₂SO₄ stress root dry weight amounted to 33% of the control and shoot dry weight was 47% of the control. On the contrary, the Koka variety showed a similar reduction in root and shoot growth under saline conditions. The reduction in fresh and dry weights of shoots and roots of Koka grown under NaCl stress was approximately 70%.

It is worth to note that tissue-water content was insignificantly affected by salinity in all the investigated maize varieties (data not shown).

Nitrate reductase activity, protein and amino compound levels (Fig. 2)

Salt stress caused a significant decrease in nitrate reductase activity in roots and leaves of both investigated maize varieties (Fig. 2A). The highest inhibition was observed in roots of Koka – in NaCl stressed plants enzyme activity declined to 255 nmol NO₂⁻ · g⁻¹ FW · min⁻¹ and it was 41% of the control. The lowest decrease in NR activity was found in roots of Limko (75% of control).

Salinity did not change the soluble protein contents in roots of the maize varieties and in leaves of Koka (Fig. 2B). On the contrary, the protein content in leaves of Limko grown under stress conditions was significantly higher than in control plants and it amounted to 128% of the control.

Salinity treatment led to an increase in amino compound contents in roots and shoots of the two examined maize varieties, but these changes were not very high (Fig. 2C). The highest increase in free amino compound level was observed in roots of Koka and it was 2-fold higher than in control plants.

Ion tissue content (Fig. 3)

The presence of 100 mol · m⁻³ NaCl in the nutrient solution resulted in a considerable increase in leaf and root Na⁺ content and a decrease in Ca²⁺ and K⁺ contents. Under stress conditions the Ca²⁺ content decreased to 24 and 67% of the control in roots and shoots of Koka respectively. A similar trend was observed in Limko. The fall in K⁺ concentration was high but not so drastic as Ca²⁺. In roots of Koka and Limko the reduction in K⁺ concentration under salt conditions was approximately 30%. A higher reduction in K⁺ concentration was shown by shoots of Limko. In this case the K⁺ content decreased to 49% of the control. Salinity caused a 9- and 25-fold increase in Na⁺ content in roots and shoots of Koka respectively. However, roots and shoots of Limko accumulated respectively 10- and 53-times more Na⁺ than the control plants. The Na⁺ concentration in shoots of Limko, expressed on tissue-water basis was higher than that in nutrient medium, and it amounted to 113.1 mol (Na⁺) · m⁻³ tissue-water (Table 1). In Limko roots this parameter was 76.6 mol (Na⁺) · m⁻³. The Na⁺ concentrations in Koka roots and shoots, expressed on tissue-water basis, did not exceed that in nutrient medium and they amounted to 94.5 mol (Na⁺) · m⁻³ in roots and 77.5 mol (Na⁺) · m⁻³ in shoots.

DISCUSSION

The results show that salt treatments had a significant impact on the growth of maize. Under salt stress growth of maize roots and shoots was strongly inhibited. Our study showed that NaCl was more toxic for maize than Na₂SO₄. It is worthy of notice, that in both the examined varieties of maize the decrease in fresh and dry weight of roots was very high, whereas length showed little variation. Higher reduction in the root biomass production than the inhibition of root length was also observed for other plants (Kisher 1995). Hamada and Khulaef (1995) found that salinity up to 100 mol · m⁻³ NaCl did not affect significantly shoot and root lengths of wheat. Roots are the first organs affected by salt stress, and it is possible that reduction in root growth is the first consequence of salt presence in root medium. Rodríguez et al. (1997) demonstrated that there was a rapid inhibition in root extension in maize seedlings exposed to NaCl; in addition salt stress caused a considerable decrease in the diameter of the new root tissue. The analysis of maize growth indicated that the varieties Koka and Limko are similarly salt-sensitive.

Salinity can reduce plant growth by osmotic stress, ion toxicity and nutritional imbalance. In short-term experi-
ments, salinity may inhibit plant growth by water stress rather than ion toxicity. However salts present in external medium can interfere with K\(^+\) and Ca\(^{2+}\) nutrition. Our observations indicated that there were insignificant differences in water content between control and salt-stressed maize seedlings therefore growth reduction might be a consequence of changes in ion relations. Ion concentrations in Koka and Limko tissues were significantly affected by 100 mol \(\cdot\) m\(^{-3}\) NaCl. In general, Na\(^+\) accumulation in maize organs was high, but sodium concentration in plants was lower than that in nutrient medium, with exception of Limko shoots. These observations suggest that roots and shoots of Koka and roots of Limko have the abilities to exclude sodium from tissue, whereas shoots of Limko tend to include Na\(^+\). Roots accumulated more sodium than the shoots (on dry weight basis). Alberico and Cramer (1993) examined seven cultivars of maize and indicated that there were large differences in the ability to exclude Na\(^+\). Four of the seven hybrids excluded Na\(^+\) from shoots, but salt tolerance was not positively correlated with Na\(^+\) exclusion. In contrast, Fortmeier and Schubert (1995) compared growth and Na\(^+\) accumulation of two maize cultivars and concluded that Na\(^+\) exclusion contributes to salt tolerance of maize. Similar results were obtained by Cerdà et al. (1995). The results from this report demonstrated that the examined maize varieties are very salt-sensitive in the early stage of growth, and this sensitivity is not simply associated with sodium inclusion. Higher toxicity of NaCl than Na\(_2\)SO\(_4\) can suggest that Cl\(^-\) is the main ion limiting growth of maize. In our study, we did not measure Cl\(^-\) concentration, but many reviewers indicated that its accumulation in plant tissue under NaCl stress is so high as Na\(^+\) concentration or higher (Benes et al. 1996; Jaenicke et al. 1996). Cramer et al. (1994) suggested that the growth-response of maize to

![Graph](image-url)

Fig. 1. The effect of 75.0 mol m\(^{-3}\) NaCl and 37.5 mol m\(^{-3}\) Na\(_2\)SO\(_4\) on growth of roots and shoots of 14-day-old two maize varieties (Koka and Limko).

Differences between control and salt treatments were compared by LSD and values marked with the same letter do not differ significantly (5%).
Fig. 2. The influence of 100 mol m$^{-3}$ NaCl on nitrate reductase activity (A), the content of soluble protein (B), and amino compound concentration in roots and leaves of 7-day-old two maize varieties. Differences between control and NaCl stressed plants were compared by LSD and values marked with the same letter do not differ significantly (5%).
salinity was primarily affected by osmotic, not ionic factors. Our data indicate that under NaCl stress K⁺ and Ca²⁺ concentrations significantly decreased and it is possible that the deficiencies in mineral nutrients led to the inhibition of plant growth. Maize roots displayed a very strong deficit in Ca²⁺ content, whereas the disturbances in K⁺ content were lower. It is well known that Ca²⁺ is essential for root elongation, maintenance of integrity and functioning of the plasma membrane and alleviation of mineral toxicity (Rengel 1992; Kimnaide 1998). Rengel (1992) proposed the hypothesis that the primary response to salt stress is disturbance of cell Ca²⁺ homeostasis that affects the cell metabo-

**TABLE 1.** The influence of 100 mol - m⁻³ NaCl on water content and concentration of Na⁺ in roots and shoots of 7-day-old Koka and Limko.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Water content [% fresh weight]</th>
<th>Sodium content [mmol(Na⁺)· kg⁻¹ water in tissue]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Shoots</td>
</tr>
<tr>
<td>Koka</td>
<td>Control</td>
<td>97.3±0.5</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>96.0±0.6</td>
</tr>
<tr>
<td>Limko</td>
<td>Control</td>
<td>97.2±0.6</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>95.6±0.7</td>
</tr>
</tbody>
</table>

Water content (% of fresh weight) was computed as the difference between fresh and dry weights, divided by fresh weight and multiplied by 100%. 

**Fig. 3.** The effect of 100 mol m⁻³ NaCl on ion content of roots and shoots of 7-day-old two maize varieties (Koka and Limko).

Differences between control and salt treatments were compared by LSD and values marked with the same letter do not differ significantly (5%).

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lism. Our data show that a reduced calcium level may be considered as an important factor limiting plant growth.

Salinity stress changes plant metabolism and induces accumulation of various metabolites among which the nitrogen compounds play a significant part. Nitrile reductase, the first enzyme in the nitrile assimilation pathway, is considered to be a limiting factor of plant growth and development (Solomonson and Barber 1990). Moreover NR is known to be very sensitive to environmental stresses. Various workers think that NR activity is inhibited by salinity (Botella et al. 1993; Cramer et al. 1995; Omarov et al. 1998), but there are also contrary observations (Bourgeais-Chaillou et al. 1992; Jaenicke et al. 1996). In our experiments NR activity decreased in all examined tissues. The highest enzyme inhibition was observed in Koka roots and the lowest inhibition was recorded in leaves of Koka. It is possible that the decrease in NR activity is a part of the plant defence mechanisms. Inhibition of the nitrile assimilation pathway reduces the energy requirements during stress and prevents the accumulation of toxic nitrite and ammonia in the cells (Merlo et al. 1994; Ramanjula et al. 1994). On the other hand, a significant decrease in NR activity had no negative effect on synthesis of amino compounds and protein. In stressed maize seedlings the contents of free amino compounds significantly increased, but the concentration of soluble protein did not change in comparison to control plants. Thus inhibition in NR activity did not cause a decrease in accumulation of nitrogen compounds. The decrease in NR activity may be caused by reduction of nitrate concentration in plants grown under NaCl stress. Reduction in both NO$_3^-$ uptake and concentration in plants subjected to salinity is widely reported by many authors (Aslam et al. 1984; Cramer et al. 1995; Jaenicke et al. 1996; Peuke and Jeschke 1999).

Many plants accumulate high levels of free amino acids in response to osmotic stress. These organic solutes may participate in osmoregulation and might function as a protector or stabilizer of macromolecules – enzymes and membranes (Yeo 1998; Hasegawa 2000). Our experiments showed that under salt stress the concentration of free amino compounds was increased, but their content did not exceed 8 mmol · kg$^{-1}$ fresh weight. This value is too low to contribute to osmotic adjustment of maize seedlings. It is possible that this solute acts as a protector of macromolecules. It is well known that salt stress induces changes in soluble protein synthesis (Yeo 1998). A decrease in protein content has often been reported as a biochemical response of plants to salinization (Flowers and Dalmond 1992; Evers and al. 1997; Gilbert et al. 1998). Evers and al. (1997) demonstrated, that in poplar shoots changes in soluble protein content depend on concentrations of NaCl. In our experimental system, the tissue protein contents did not significantly change under salt stress except of Limko leaves. This suggests that salt-induced increase in amino compound level was not related to protein degradation.

In conclusion, we have shown that the two examined varieties of maize (Koka and Limko) are sensitive to salinity, and salt stress alters their physiological status. Plants treated with NaCl modify nitrogen metabolism and change the mineral composition. The drastic decrease in Ca$^{++}$ content may be attributed to salt sensitivity of maize.

LITERATURE CITED

Wpływ stresu solnego na wzrost i niektóre parametry biochemiczne u dwóch odmian kukurydzy

Streszczenie

Celem pracy było zbadanie wpływu zasolenia na wzrost oraz wybrane parametry biochemiczne (aktywność reduktazy azotanowej, zawartość azotu aminowego, białka rozpuszczalnego oraz stężenie jonów) dwu odmian kukurydzy – Limko i Koki. Zasolenie podłoża (75,0 mol · m⁻³ NaCl oraz 37,5 mol · m⁻³ Na₂SO₄) spowodowało znaczną zmniejszenie związane z zasileniem sieciowych i suchej masy badanych roślin. NaCl w większym stopniu hamował wzrost roślin niż Na₂SO₄. Nie stwierdzono wyraźnych różnic w odporności badanych odmian kukurydzy na zasolenie.

NaCl o stężeniu 100 mol · m⁻³ spowodował większą reduktazy azotanowej u obydwu badanych odmian, przy czym ten niekorzystny efekt był bardziej widoczny u Koki. Spadek aktywności NR nie spowodował obniżenia zawartości związanych z aminowych oraz białka rozpuszczalnego. Zasolenie spowodowało niezdecydowaną zmiany zawartości aminowych w korzeniach i pędnikach badanych odmian kukurydzy. Ponad 2-krotne wzrost zawartości związanych z aminowych w stosunku do roślin kontrolnych stwierdzono w korzeniach Koki. U badanych odmian kukurydzy poziom białka rozpuszczalnego nie zmienił się istotnie pod wpływem zasolenia. Wyjątek stanowiła tlenica Limko, w której zawartość białka wzrosła prawie o 30% w stosunku do roślin kontrolnych. Rosliny rosnące w warunkach stresu solnego gromadziły znaczne ilości Na⁺, obniżyło się natomiast stężenie Ca²⁺ i K⁺. Zawartość Ca²⁺ w korzeniach kukurydzy obniżyła się do 76% oraz 70% w porównaniu do roślin kontrolnych odpowiednio dla Koki i Limko. Spadek zawartości K⁺ w korzeniach Koki i Limko nie był aż tak drastyczny, a wynosił około 30%. Większy spadek zawartości K⁺ odnotowano w pędnikach Limko (49% kontroli).

Słowa kluczowe: zasolenie, zawartość jonów, reduktaza azotanowa, związki aminowe, białko rozpuszczalne, kukurydza.