

Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars

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ABSTRACT

The effect of osmotic stress on oxidative injury, compatible solutes content and water relations was investigated in two maize cultivars (*Zea mays* L. cv. Ankora – drought-sensitive and cv. Nova – drought-tolerant). Relative water content in leaves of both cultivars decreased after drought treatment, leaf water loss of sensitive cv. Ankora was higher than that of cv. Nova. The 24 h water stress induced by 0.3M sorbitol (–1.4 MPa) resulted in a damage of cell membranes. Lipid peroxidation rose in all studied organs of cv. Ankora and electrolyte leakage in roots of cv. Ankora was much higher than in cv. Nova. Similarly, proline content increased significantly in all studied organs of cv. Ankora. Content of soluble sugars increased in all studied organs of both cultivars, but the mesocotyl of cv. Nova accumulated the highest amount of sugars. The electrolyte leakage was the highest in the roots of both cultivars. Osmotic stress had deep influence predominantly on the roots of both cultivars. It is apparent that stress impact on the drought-sensitive cv. Ankora was deeper than on the drought-tolerant cv. Nova.

Keywords: drought; RWC; electrolyte leakage; lipid peroxidation; proline; sugars

One of the major environmental factors limiting the worldwide productivity and distribution of cereal crops is an osmotic stress resulting from drought. Drought stress triggers various interacting events including the increase of ABA concentration, decrease of xylem pH and conductivity (Bahrum et al. 2002). Under stress conditions, reactive oxygen species (ROS), such as superoxide radicals, singlet oxygen, hydrogen peroxide and hydroxyl radicals can be produced in large amounts. Hydrogen peroxide and superoxide radicals are relatively unreactive, but they can form hydroxyl radicals, which can damage proteins, lipids and DNA (Dat et al. 2000). Peroxidation of lipids, commonly taken as an indicator of oxidative stress, disrupts the membrane integrity of the plant cell. This means that essential solutes leak out from the organelles and from the cell and cause the damage of membrane function and metabolic imbalances (Blokina et al. 2003). In plants, there are many potential places for generation of ROS, such as chloroplasts (Foyer and Noctor 2003), mitochondria and peroxisomes (Del Rio et al. 2002, Liu et al. 2002).

In order to avoid an overproduction of reactive molecules, plants have evolved their antioxidant systems to ensure a control of the cellular redox state (Foyer and Noctor 2003). Osmotic adjustment is also a part of drought avoidance mechanisms. Proline and quaternary ammonium compounds are key osmolytes, which help plants to maintain the cell turgor (Weinberg et al. 1982, Huang et al. 2000). Moreover, there is an additional evidence that these compatible solutes are accumulated in plants at high concentrations to help in alleviating inactivation of the enzymes or loss in membrane integrity due to a water deficiency (Schwab and Gaff 1990). Sucrose, as a member of the sugar family, is thought to function as a typical osmoprotectant, stabilising cellular membranes and maintaining turgor (Mundree et al. 2002).

The effects of drought stress on antioxidative responses have been studied in a number of plant species including wheat, rice, potato, wild grasses, tomato and maize. These studies indicate that the antioxidative response is well correlated with sensitivity and tolerance of the cultivars under

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investigation. Being one of the main food crops cultivated around the world, maize (*Zea mays* L.) is very sensitive to the drought, especially during growth and pollination (Janda 1981). While the responses of antioxidants and their functions in maize to drought have been relatively well studied, almost no information has been collected up to now about the root-shoot differences. Therefore, in order to clarify the root-shoot relationships under the stress condition, we describe the changes of relative water content, leaf water loss as well as the effect of osmotic stress on lipid peroxidation, electrolyte leakage, proline and sugars content of 13-day-old maize plants.

MATERIAL AND METHODS

Seeds of two maize cultivars (drought-sensitive Ankora, drought-tolerant Nova) were obtained from Sempol-Holding, Trnava, Slovakia. After the surface sterilisation with WOLFEN-THIURAM 75W for 30 min, the seeds were rinsed for 1 hour with water and then imbibed for 3 hours in water before being sown on wet filter paper. The seeds were germinated for three days and then were planted into pots containing Hoagland nutrient solution. The seedlings were grown in the growth chamber (Convicon S10/S10H) at 24/18°C (day/night), 70% relative humidity, with a light intensity of 200 $\mu\text{mol}/\text{m}^2/\text{s}$ and a 12 h photoperiod. The plants were cultivated till the stage of the second fully developed leaf. Subsequently, the 24 h osmotic stress induced by 0.3M sorbitol was applied. The water potential of cultivating medium with sorbitol was -1.4 MPa. The two cm apical root segments, mesocotyl, the first and the second leaves were used for the analysis.

Lipid peroxidation was monitored by the spectrophotometric determination of malondialdehyde using thiobarbituric acid (TBA) as described in Hodges et al. (1999) with slight modifications, where 95% ethanol was replaced by 10% trichloroacetic acid (TCA). In the next steps 0.2% TBA and 10% TCA were used. Absorbances were measured at 440 nm, 532 nm, and 600 nm.

Electrolyte leakage (EL) was measured as described by Lutts et al. (1996) with a few modifications. Plant material (0.3 g) was washed with deionized water, placed in tubes with 15 ml of deionized water and incubated for 2 h at 25°C. Subsequently, the electrical conductivity of the solution (L_1) was determined. Samples were then autoclaved at 120°C for 20 min and the final con-

ductivity (L_2) was measured after equilibration at 25°C. The EL was defined as follows:

$$\text{EL (\%)} = (L_1/L_2) \times 100.$$

Proline content was estimated by ninhydrin method of Bates et al. (1975). The chromophore formed was extracted with 3 ml of toluene and the absorbance of resulting organic layer was measured at 520 nm. Calibrations were made with 1mM L-proline as a standard.

Sugar content was determined by the Somogyi-Nelson method using glucose as a standard (Oser 1971).

Relative water content (RWC) was determined for detached maize leaves using the method of Mata and Lamattina (2001). In our experiments, RWC was calculated according to the formula:

$$\text{RWC (\%)} = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100$$

where: fresh weight (FW) was measured at the end of the drought period, and dry weight (DW) was obtained after drying the samples at 80°C for at least 48 h. Turgor weight (TW) was determined by subjecting leaves to rehydration for 2 h after drought treatment.

Leaf water loss was measured according to the method of Xing et al. (2004). After their fresh weight (W_1) was recorded when cut from seedlings, the leaves were left to evaporate under room condition for 2 h and reweighed (W_2).

$$(\text{LWL}) = (W_1 - W_2)/W_1 \times 100$$

All experimental data reported were average means of at least three independent assays with three replicates. Statistical analyses at 5% level ($P < 0.05$) were determined by Student's *t*-test.

RESULTS AND DISCUSSION

One of the early symptoms of water deficiency in plant tissues is the decrease of relative water content (RWC). The reduction of RWC in stressed plants may be associated with the decrease in plant vigour and was observed in many plant species (Halder and Burrage 2003, Lopez et al. 2002). Relative water content in the leaves of plants grown last 24 h at low water potential (-1.4 MPa) decreased significantly in both cultivars compared to their respective controls (Table1). However, the differences between cultivars were very small. On

Table 1. Relative water content (RWC) and leaf water loss (LWL) (in %) in the leaves of two maize cultivars (Nova drought-tolerant and Ankora drought-sensitive) exposed 24 h to 0.3M sorbitol; each value is the mean of at least three independent experiments (\pm SD)

Cultivar	RWC		LWL	
	control	drought	control	drought
Nova	99.56 \pm 0.31	71.39 \pm 1.98**	7.16 \pm 0.43	2.60 \pm 0.13**
Ankora	99.02 \pm 0.44	75.34 \pm 1.69**	12.15 \pm 0.39	3.9 \pm 0.18**

the other hand, there were significant differences between cultivars in leaf water maintenance. Leaf water loss (LWL) of drought-sensitive Ankora plants was much higher than that of drought-tolerant Nova under control conditions (Table 1). Water stress reduced LWL in both cultivars, but this decrease was higher in drought-sensitive Ankora plants. It is apparent, that the Nova plants have higher leaf water maintenance in both control and stress conditions comparing to Ankora.

The occurrence of malondialdehyde (MDA), a secondary end product of the oxidation of poly-

unsaturated fatty acids, is considered a useful index of general lipid peroxidation (Smirnoff 1993). We did not find any changes in MDA content in the leaves and roots of drought-tolerant cv. Nova plants grown 24 h at a low water potential. MDA concentrations significantly increased only in mesocotyl (Figure 1). On the other hand, in drought-sensitive Ankora plants a significant increase of MDA content was measured in all organs studied, the root was however the most affected part of the plant. The higher lipid peroxidation in drought stressed plants was also reported in other stud-

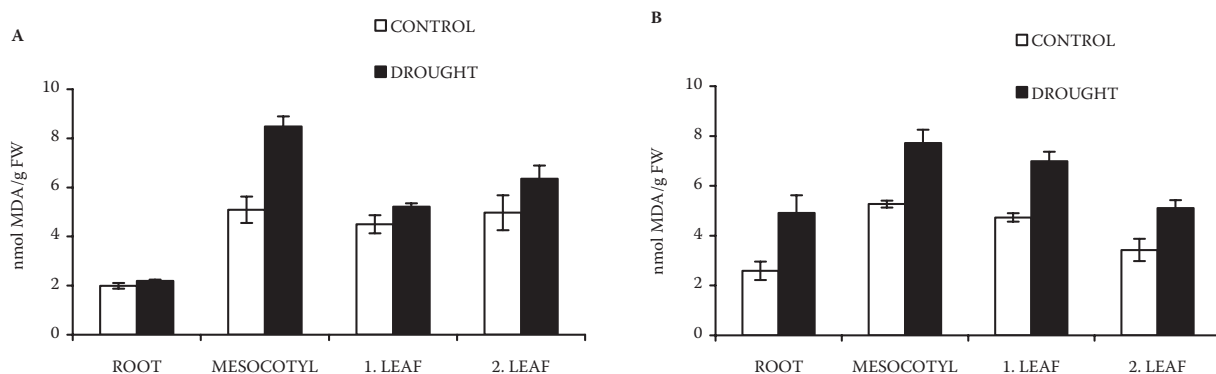


Figure 1. Malondialdehyde content (MDA) in the root, mesocotyl and leaves of two maize cultivars: Nova drought-tolerant (A) and Ankora drought-sensitive (B) exposed 24 h to 0.3M sorbitol; each value is the mean of at least three independent experiments (\pm SD)

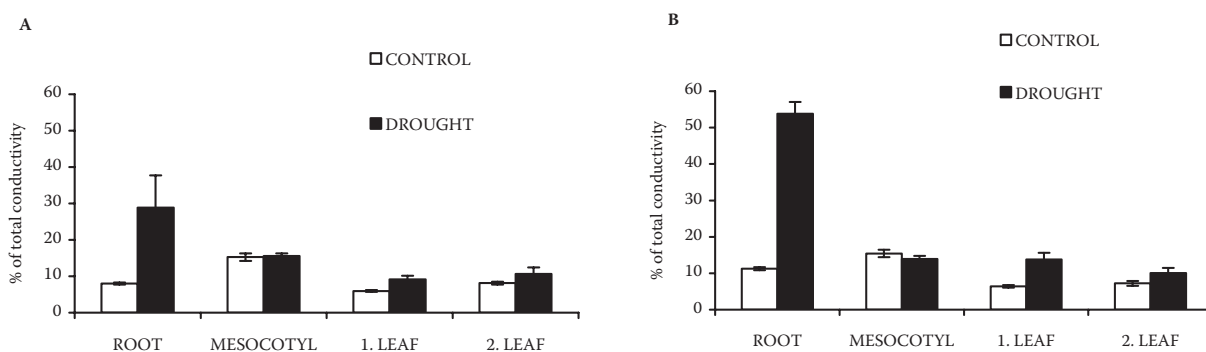


Figure 2. Electrolyte leakage in the root, mesocotyl and leaves of two maize cultivars: Nova drought-tolerant (A) and Ankora drought-sensitive (B) exposed 24 h to 0.3M sorbitol; each value is the mean of at least three independent experiments (\pm SD)

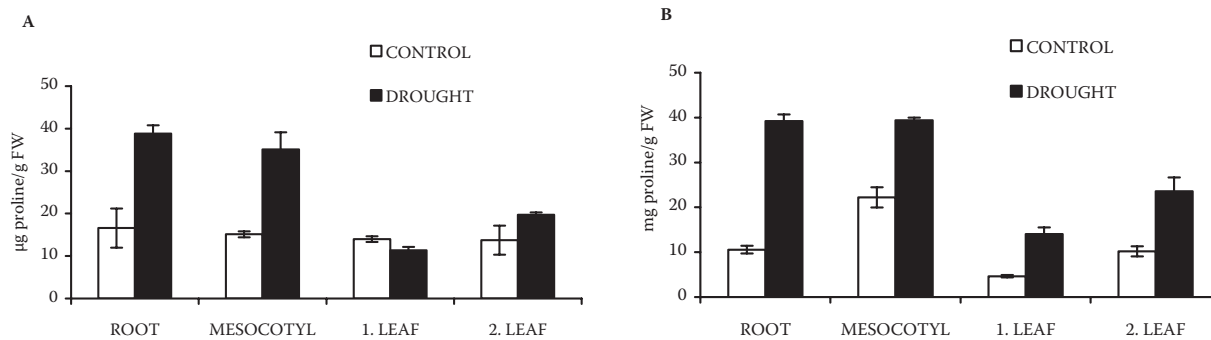


Figure 3. Proline content in the root, mesocotyl and leaves of two maize cultivars: Nova drought-tolerant (A) and Ankora drought-sensitive (B) exposed 24 h to 0.3M sorbitol; each value is the mean of at least three independent experiments (\pm SD)

ies (Fu and Huang 2001, Niedzwiedz-Siegien et al. 2004). Under environmental stresses plant membranes are subject to changes often associated with the increases in permeability and loss of integrity (Blokhina et al. 2003). Therefore, the ability of cell membranes to control the rate of ion movement in and out of cells is used as a test of damage to a great range of tissues. However, no electrolyte leakage was observed in mesocotyl of both cultivars studied despite the fact that the mesocotyl of cv. Nova had also relatively high lipid peroxidation and thus the membrane injury. High level of free proline and soluble sugars in mesocotyl of both cultivars may have a protective effect on cells. In spite of high levels of proline in roots of cv. Ankora the membrane injury of this tissue was relatively high. The electrolyte leakage of the roots of the sensitive cultivar increased from 11 to 54%. The roots of cv. Nova were also attacked by drought, but the increase in ion leakage was not so high (Figure 2). The permeability of leaf membranes showed a lesser increase as compared

to roots, being higher again in sensitive Ankora. Positive correlations between salinity sensitivity and membrane damage in foxtail millet (*Setaria italica*) seedlings were observed by Sreenivasulu et al. (2000). Also Quan et al. (2004) found higher electrolyte leakage in drought stressed maize (*Zea mays* L.) plants than in plants grown under control conditions. Obtained data thus indicate a water stress-induced oxidative injury, especially in drought-sensitive maize plants.

Nevertheless, even if the leaves and root proline contents under control conditions were higher in cv. Nova than in Ankora, the increase under stress conditions was higher in drought-sensitive Ankora. Proline levels increased significantly under stress conditions in all studied tissues of drought-sensitive cv. Ankora being much higher in roots and mesocotyl than in leaves (Figure 3). Higher accumulation of proline in salt-sensitive cultivar of rice (*Oryza sativa* L.), as shown by Lutts et al. (1996), was consistent with results obtained in our experiments. However, higher levels of free proline

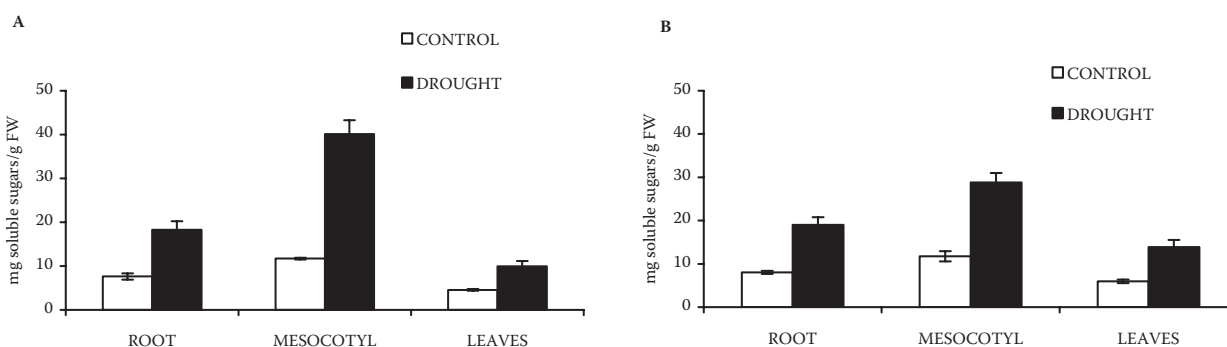


Figure 4. Content of soluble sugars in the root, mesocotyl and leaves of two maize cultivars: Nova drought-tolerant (A) and Ankora drought-sensitive (B) exposed 24 h to 0.3M sorbitol; each value is the mean of at least three independent experiments (\pm SD)

were found in shoots, not in roots. Proline and soluble sugars are the key osmolytes contributing towards osmotic adjustment (Yoshida et al. 1997, Mundree et al. 2002). They can also improve stress tolerance by protecting and stabilizing membranes and enzymes during stress conditions (Rudolph et al. 1986). The exposure of maize plants to 24 h osmotic stress increased sugar accumulation in all studied organs of both cultivars, the mesocotyl of drought-tolerant Nova had however the highest sugar content (Figure 4). An increase in sugar content in drought stressed plant observed also Thomas and James (1999) and Dekánková et al. (2004). This suggested that high levels of proline and soluble sugars in tissues of both cultivars can be caused by high requirement for osmotic adjustment and membrane stabilisation. It seems that the accumulation of osmolytes in organs of Nova was more effective because the membrane stability in this cultivar was higher and the loss of water was lower than in the sensitive one (Figures 3 and 4). Moreover, the free proline content in the first leaf of drought-tolerant plants decreased. As in the cases of lipid peroxidation and ion leakage, the roots seem to be the most sensitive organ to the drought stress caused by sorbitol.

We can conclude that 24 h osmotic stress induced by 0.3M sorbitol had a huge influence predominantly on the roots of 13-days old maize seedlings. It is apparent, that the stress impact on the drought-sensitive cultivar Ankora was higher than on the drought-tolerant cultivar Nova.

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