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Maize Roots Responses to Osmotic Stress

- Original scientific paper -

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Abstract: Field testing for drought tolerance was performed on 6,000 accessions from the Maize Research Institute gene bank, under severe drought conditions in Egypt, as well as, under moderate climate conditions in Zemun Polje and Skopje. Five inbred lines, considered as drought tolerant, were chosen for further investigations under controlled experimental conditions. Osmotic stress caused by drought is one of the most important abiotic stresses. In this study osmotic stress was applied with polyethylene glycol (PEG) 10 000. The response to the PEG treatment of these genotypes was analysed in respect to their root morphology, root length, root fresh and dry weight, proline content and peroxidase activity. Results showed that the root development was less in all genotypes under the PEG treatment. The proline content increased, while the peroxidase activity declined in PEG- treated plants.
Key words: Osmotic stress, peroxidase activity, proline

Introduction

One of the most critical challenges crops face is drought, which adversely affects their growth and yield, ***Bartels*** and ***Nelson***, 1994. Under such circumstances, the role of roots in acquiring soil water and nutrients is absolutely essential.

The maize seedling root system consists of the primary root and additional sets of seminal roots emanating from the mesocotyl-radicle junction, ***Cahn et al***., 1989. In addition, nodal roots successively form on the plant stem, some of which emanate above the soil surface. With respect to the root system of mature plants, the primary root is a minor constituent. However, it is a convenient system to study, and although root types can respond differently, ***Volkmar*** 1997), most physiological and molecular features of the primary root may be generally applicable. In several crop species including maize, the growth of roots and shoots is inhibited during water

deficit, but roots continue growing at low water potentials that are completely inhibitory to shoot growth, ***Spollen et al***., 1993.

Water deficiency can induce membrane damages, increase membrane permeability and the accumulation of free radicals in plants, ***Mohammadkhani*** and ***Heidari***, 2007. Reactive oxygen species (ROS) are important signals in the biosynthesis of complex organic molecules, polymerisation of cell wall constituents, and defense against abiotic and biotic stresses, ***Liszkay et al***., 2004. They are produced in both unstressed and especially in stressed plant cells. However, oxidative damage to lipids, proteins and DNA occurs under the excess of ROS. Therefore, it is the balance between the production and the scavenging of ROS that is critical to the maintenance of the active growth and metabolism of the plant and overall stress tolerance, ***Smirnoff***, 1993, ***Foyer*** and ***Noctor***, 2005, ***Carol*** and ***Dolan***, 2006.

To remove the excess of harmful ROS, plant cells possess antioxidative systems consisting of low-molecular mass antioxidants, as well as, antioxidative enzymes, such as superoxide dismutase (SOD), catalase and peroxidase.

In addition to well known antioxidants, such as ascorbate and glutathione, amino acid proline, besides its role in stress protection as an osmolyte, was shown to be a potent scavenger of ROS, ***Matysik et al***., 2002.

The present investigation was designed to identify the changes in the proline content and peroxidase activity in PEG- stressed roots of maize seedlings and their correlation with the root growth, among inbred lines previously chosen as drought tolerant under field conditions.

Material and methods

Plant material and growing conditions. **-** The study was carried out on five maize (*Zea mays* L.) drought tolerant inbred lines maintained at the Maize Research Institute gene bank. They belong to different FAO maturity groups (A_5 - FAO 400 and A_1, A_2, A_3 and A_4 - FAO 500). Seeds were germinated for three days on moistened filter paper and then transferred to plastic pots containing Knopp solution with modified nitrogen content. For the succeeding four days, plants were grown on ¼ strength nutrient solution supplemented with different forms of nitrogen. Nitrogen was supplied in the form of KNO_3, Ca $(NO_3)_2$ and $(NH_4)_2SO_4$. The initial pH of the solution was adjusted to 5.6. Plants were kept in a growth chamber under a 12-h photoperiod at 22/18 ^oC, with the irradiance of 40 Wm^{-2} and relative humidity of 70%. For the terminal 48 h of the growing period, one half of the plants (treatment) were grown on the fresh aerated nutrient solution supplemented with 4% polyethylene glycol (PEG, Mr 10 000), parallelly to control plants grown on the nutrient solution without any addition.

Free proline determination. **-** Free proline was extracted from maize roots and its concentration determined by the method of ***Bates et al***., 1973. Roots were homogenised (1:10 w/v) with 3% sulfosalicylic acid. The filtered homogenate was mixed with acid ninhydrin solution and boiled for 15 min. After extraction with

toluol, the absorbance of the reaction product was determined at 520 nm. The proline content was calculated from the calibration curve made with different concentrations of the proline solution and expressed as μg g^{-1}FW.

Enzyme assays and protein determination. **-** For the peroxidase (POD) assay the root tissue was homogenised (1:10 w/v) with 100 mM K-phosphate buffer, pH 7.5. The homogenate was centrifuged at 20 000 g for 15 min and the supernatant used for the enzyme analysis. To determine phenolic peroxidase activity, ferulic acid (A_{286}; ε = 16.8 $mM^{-1}cm^{-1}$) was used as a hydrogen donor. The reaction mixture consisted of 0.1 mM phenolic, 1mM H_2O_2 in 50 mM K-phosphate buffer, pH 6.5 and about 3.3 μg of protein. The calculation of specific enzyme activity was done on the protein basis. The protein content was measured by the method of ***Lowry et al***., 1951, with bovine serum albumin as a standard. All the assays were performed at 30°C.

Results and Discussion

At low water potential caused by PEG, the maize primary root growth is inhibited, causing shorter, thinner roots compared with roots grown under normal conditions, ***Sharp et al***., 1988. In our investigations, the inhibition of the root length ranged from 10.7 up to 40% in all PEG-treated inbred lines over the respective controls (Figure 1). Also, the accumulation of fresh and dry matter is reduced in all analysed genotypes under PEG treatment. The reduction ranged from 21.5 up to 62% and from 4.4 up to 45.7% of root fresh and dry weight, respectively (Figures 2A and 2B).

In order to look into drought stress induced biochemical changes and to elucidate adaptive mechanisms at the cellular level, we used PEG as an osmoticum

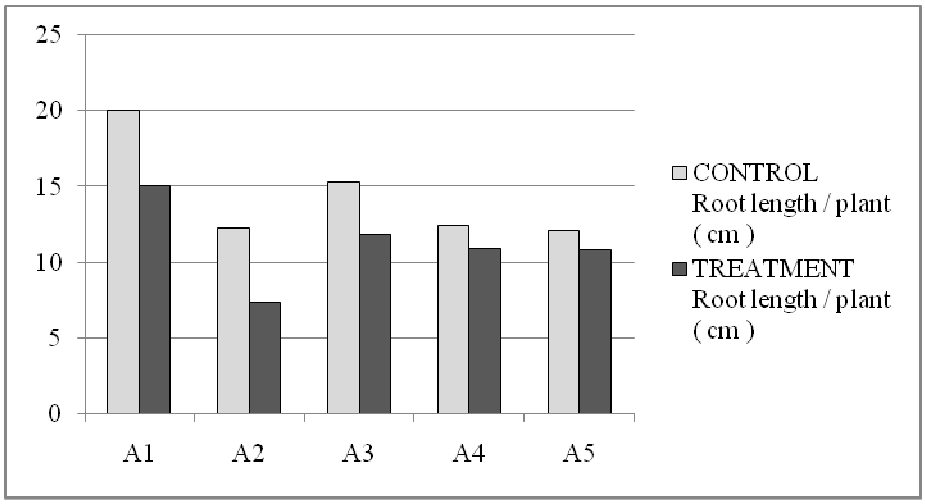

Figure 1. Average values of root length (cm) - Prosečna vrednost dužine korena (cm)

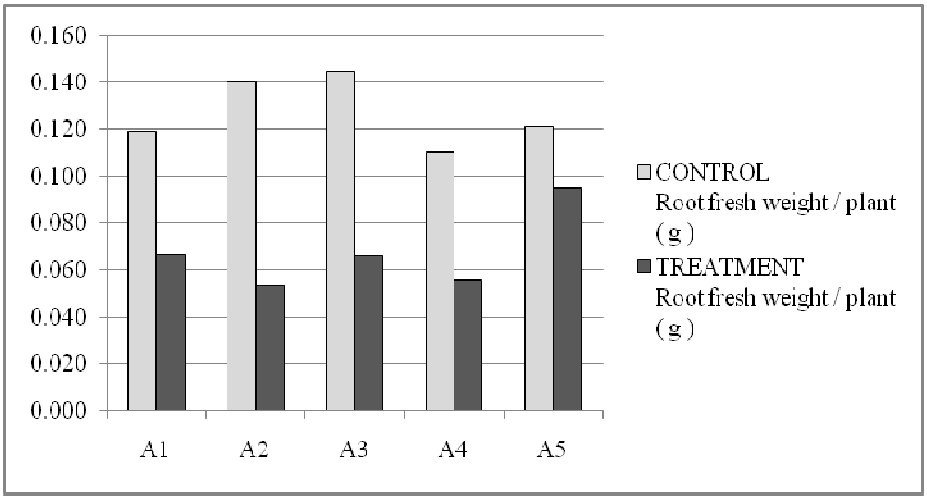

Figure 2A. Average values of root fresh weight (g) - Prosečna vrednost sveže mase korena

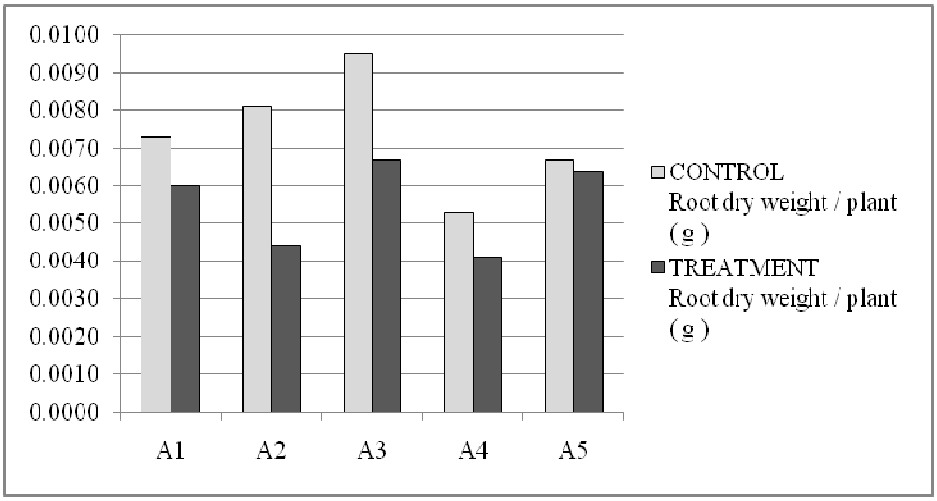

Figure 2B. Average values of root dry weight (g) - Prosečna vrednost suve mase korena

to investigate the status of proline pool in maize seedlings grown under normal and stress conditions. This experiment was undertaken to identify differences in the proline content among inbred lines under osmotic stress.

Among amino acids, the accumulation of proline is frequently reported in many plants or tissues in response to a variety of abiotic stresses, ***Hare*** and ***Cress***, 1997. However, the precise role of proline accumulation is still elusive: act as an osmo - regulator, ***Delauney*** and ***Verma***, 1993, an osmo - protector, ***Csonka***, 1989, or

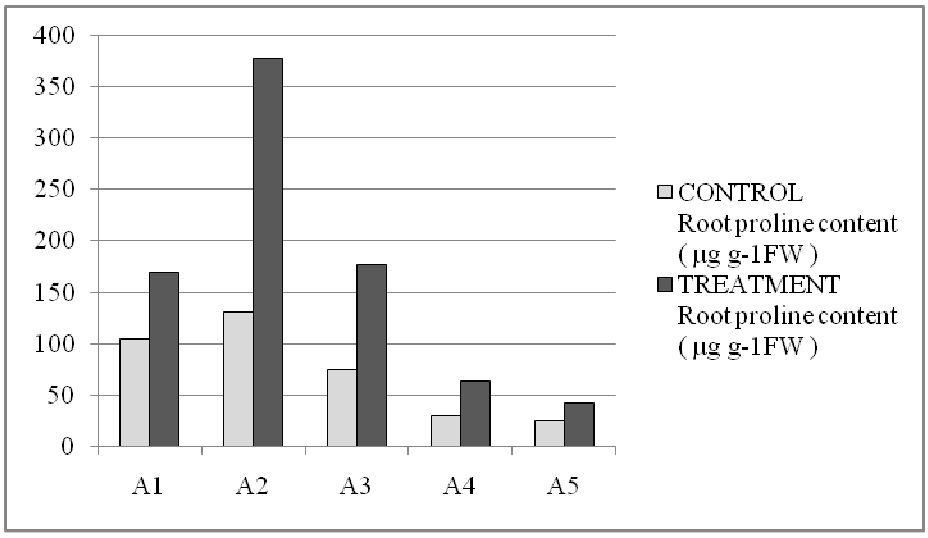

Figure 3. Root proline content (µg g^{-1}FW) - Sadržaj prilina u korenu (µg g^{-1}SM)

a regulator of the redox potential of cells, ***Bellinger*** and ***Larher***, 1987. The proline content ranged from 25 up to 380 µg g^{-1} FW and was higher in all PEG treated genotypes (Figure 3), which is confirmed in previous experiments in maize roots, ***Valentovič et al***., 2006; ***Vuletić et al***., 2010.

Peroxidases are the enzymes involved in growth regulation by producing polymeric products such as lignin and suberin or by cross-linking cell-wall polymers. H_2O_2 is removed during these reactions, thus make peroxidases to be considered as antioxidative enzymes. In our experiments the PEG treatment induced a decline in the peroxidase activity in all genotypes. The activity decline ranged from 0.5 up to 28.9% over the respective controls (Table 1). Peroxidase activity changed significantly and differentlly as drought progressed. In *C. setosa,* ***Terzi*** and ***Kadioglu***, 2006, POD activity strongly increased in leaf and petiole, but decreased in root during stress.

For many crops water restriction can contribute to substantial variation in free amino acids levels and antioxidative enzymes, that may have implications for their compositional analyses.

Specifically, it implies that these analyses should focus on a target physiological trait as broad profiling is unlikely to yield data that can be solely correlated to important agronomic traits. In other words, recorded differences in physiological parametars can be attributed to many factors, particularly environmental and climatic.

Table 1. Effect of 4% PEG Treatment on Peroxidase Activity
Efekat 4% PEG-a na peroksidaznu aktivnost

Inbred line Inbred linija	FAO maturity group FAO grupa zrenja	PEG	Peroxidase specific activity $\mu mol_{ferulic\ acid}\ mg^{-1}_{prot}\ min^{-1}$ Specifična aktivnost peroksidaze $\mu mol_{ferulic\ acid}\ mg^{-1}_{prot}\ min^{-1}$
A1	500	+	8.03
		-	9.08
A2	500	+	8.14
		-	8.18
A3	500	+	2.39
		-	2.88
A4	500	+	5.05
		-	7.13
A5	400	+	4.85
		-	6.83

Conclusion

The response mechanisms of a reactive oxygen scavenging system and lipid peroxidation to drought stress are very complex, as they are not only dependent on a plant genotype and stress intensity, but there are also different modes of enzyme reactions.

Our investigation shows that the proline accumulation is closely associated with the growth inhibition induced by PEG in respect to root fresh and dry weight.

Osmotic stress induced by PEG caused a decline in peroxidase activity, which could be supposed as diminishing root ability in water up-take, as well as in metabolic changes expressed as an increase in the proline content.

In view of considerable variations in the protective mechanisms against ROS in different plants, further work is planned to establish a more detailed analisys of drought tolerance in selected maize genotypes.

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References

Bartels, D. and ***D.E. Nelson*** (1994): Approaches to improve stress using molecular genetics. Plant Cell Environ. 17: 659-667.

Bates, L.S., S.P. Walden and ***I.D. Teare*** (1973): Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207.

Bellinger, Y. and ***F. Larher*** (1987): Proline accumulation in higher plants: A redox buffer? Plant Physiol. 6: 23-27.

Cahn, M.D., R.W. Zobel and ***D.R. Bouldin*** (1989): Relationship between root elongation rate and diameter and duration of growth of lateral roots of maize. Plant Soil 119: 271-279.

Carol, R.J. and ***L. Dolan*** (2006): The role of reactive oxygen species in cell growth: lessons from root hairs. J. Exp. Bot. 57: 1829-1834.

Csonka, L.N. (1989): Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev. 53: 121-147.

Delauney, A.J. and ***D.P.S. Verma*** (1993): Proline biosynthesis and osmoregulation in plants. Plant J. 4: 215-223.

Foyer, C.H. and ***G. Noctor*** (2005): Redox homeostasis and antioxidant signaling: a metabolic interface between perception and physiological responses. Plant Cell 17: 1866-1875.

Hare, P.D. and ***W.A. Cress*** (1997): Metabolic implication of stress-induced proline accumulation in plants. Plant Growth Regul. 21: 79-102.

Liszkay, A., E. van der Zalm and ***P. Schopher*** (2004): Production of reactive oxygen intermediates (O^{-2}, H_2O_2, and ·OH) by maize roots and their role in wall loosening and elongation growth. Plant Physiol. 136: 3114-4123.

Lowry, O.H., N.J. Rosebrogh, A.L. Farr and ***R.J. Randal*** (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Matysik, J., Alia, B. Bhalu and ***P. Mohanty*** (2002): Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Curr. Sci. 82: 525-532.

Mohammadkhani, N. and ***R. Heidari*** (2007): Effects of drought stress on protective enzyme activities and lipid peroxidation in two maize cultivars. Pak. J. Biol. Sci. 10: 3835-3840.

Sharp, R.E., W.K. Silk and ***T.C. Hsiao*** (1988): Growth of the maize primary root at low water potentials. I. Spatial distribution of expansive growth. Plant Physiol. 87: 50-57.

Smirnoff, N. (1993): The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol. 125: 27-58.

Spollen, W.G., R.E. Sharp, I.N. Saab and ***Y. Wu*** (1993): Regulation of Cell Expansion in Roots and Shoots at Low Water Potentials. In: Water Deficits. Plant Responses from the Cell to the Community, eds JAC Smith, H Griffiths, Bios Sci Publ, Oxford, U.K., pp. 37-52.

Terzi, R. and ***A. Kadioglu*** (2006): Drought stress tolerance and antioxidant enzyme system in *Ctenanthe setosa*. Acta Biologica Crasoviensia *Series Botanica* **48** (2): 89-96.

Valentovič, P., M. Luxová, L. Kolarovič and ***O. Gašparíková*** (2006): Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. Plant Soil Environ. **52** (4): 186-191.

Volkmar, K.M. (1997): Water stressed nodal roots of wheat: effects on leaf growth. Aust J Plant physiol. 24: 49-56.

Vuletić, M., V. Hadži-Tašković Šukalović, K. Marković **a**nd ***J. Dragišić Maksimović*** (2010): Antioxidative system in maize roots as affected by osmotic stress and different nitrogen sources. Brief communication. Biol. Plant. **54** (3): 530-534.

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Odgovor korenovog sistema klijanaca kukuruza na osmotski stres

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R e z i m e

Suša je jedan od najznačajnijih abiotičkih faktora, koji značajno utiče na smanjenje konačnog prinosa zrna kod kukuruza. Predviđanja globalnih klimatskih promena za XXI vek idu u pravcu povećanja temperature vazduha, veće evapotranspiracije i učestalije pojave suše. Za selekciju kukuruza danas, kao i u doglednoj budućnosti, izvori poželjnih svojstava koji se nalaze u bankama gena biće od velikog značaja. Materijal koji se čuva u banci gena Instituta za kukuruz, po obimu i sadržaju, pruža velike mogućnosti. Na osnovu ispitivanja tolerantnosti na sušu kod 6.000 uzoraka iz banke gena Instituta za kukuruz u poljskim uslovima umerenog (Zemun Polje i Skoplje) i aridnog klimata (Egipat), odabrano je pet inbred linija, okarakterisanih kao tolerantne, za dalja ispitivanja u kontrolisanim laboratorijskim uslovima. Linije pripadaju različitim FAO grupama zrenja (A_5 - FAO 400 i A_1, A_2, A_3 i A_4 - FAO 500). Osmotski stres izazvan sušom je jedan od najznačajnijih abiotičkih faktora. U ovom radu je ispitivan efekat polietilen glikola (PEG) 10 000, kao osmotikuma, na rast korena klijanaca. Merena je dužina, sveža i suva masa korena, određivan je sadržaj prolina u korenu i merena je aktivnost antioksidativnih enzima. Rezultati ispitivanja pokazuju smanjenje porasta korenovog sistema, povećanje sadržaja prolina, kao i smanjenje specifične aktivnosti peroksidaza kod svih tretmana u odnosu na kontrolnu varijantu, kao odgovor na primenjeni osmotski stres.

Naredna istraživanja vezana za odgovor biljke na osmotski stres bi uključila i nadzemni deo klijanaca, kao i molekularnu karakterizaciju genotipova tolerantnih prema suši. Odabrani genotipovi poslužiće za formiranje *core* kolekcije koja će u sebi sadržati ovo svojstvo i biti osnova u programima oplemenjivanja kukuruza.

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