ANTIOXIDATIVE RESPONSE OF POPLAR TISSUE CULTURE EXPOSED TO PEG 6000

Popović M. Boris[1], Štajner Dubravka[1], Ždero Pavlović Ružica[1*], Blagojević Bojana[1], Galović Vladislava[2]

[1]Department of Field and Vegetable Crops, Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[2]Institute of Lowland Forestry and Environment, University of Novi Sad, Antona Čehova 13, 21000 Novi Sad, Serbia
e-mail: ruzica.zdero@polj.uns.ac.rs

Abstract
The aim of this work was to investigate the influence of the water deficit in poplar tissue culture (M-1 genotype) through proline content, level of lipid peroxidation and activities of antioxidative enzymes. Plants were exposed to PEG 6000 (100 and 200 mOsm) in *in vitro* conditions for 6 days. Malondialdehyde (MDA) content showed an increase only at 200 mOsm stress. Under the 100 mOsm stress, catalase and guaiacol peroxidase activities were induces indicating their important role in elimination of reactive oxygen species (ROS). Under the 200 mOsm stress, superoxide dismutase and guaiacol peroxidase activities were induced.

Introduction
During drought stress the production of ROS is dramatically elevated. Reactive oxygen species, including superoxide anion, hydroxyl radical and hydrogen peroxide, are derived from oxygen. As a response to ROS accumulation plants activate antioxidative enzymes such as catalase (CAT), non-specific peroxidase and superoxide dismutase (SOD) that cooperate together to mitigate cellular damages like unspecific oxidation of proteins, membrane lipids and nucleic acids [1]. Numerous studies have shown that proline content in higher plants increases under different abiotic stresses [2]. An osmoprotective function as well as antioxidant function of proline has been demonstrated [3].
The aim of this work was to study the variation in proline content, antioxidative enzymes activity (SOD, CAT, and guaoacol peroxidise (GPx)) and level of lipid peroxidation in poplar tissue culture imposed to different levels of PEG 6000.

Experimental
Culture tissue of *Populus canadensis* clone M-1 was obtained from the Institute of Lowland Forestry and Environment (Novi Sad). Calli were cultured on MS medium [4] containing 4 g/L MS, 10g/L sucrose, 3 g/L agar-agar and 0.5 g/L gerlit, pH 5.8 in the absence or presence of 100 and 200 g l^{-1} PEG 6000 (PEG 6000, Aldrich, Germany). After 6 days of treatment extracts were prepared for biochemical analyses.

Enzyme activity assays and soluble proteins
The SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitro-blue tetrazolium [5]. One unit of SOD activity was defined as the amount of enzyme required to produce a 50% inhibition of reduction of NBT at 560 nm. GPx activity was analysed by measuring peroxidation of hydrogen peroxide with guaiacol as an electron donor at 436 nm [6]. Catalase activity was assayed according to Aebi [7]. The decomposition of H_2O_2 was followed spectrophotometrically by the decrease in A_{240}. One unit of

catalase activity corresponded to the amount of enzyme that decomposes 1 μmol of H_2O_2 per minute. The concentration of protein was measured according to Bradford [8].

Determination of proline content

Proline was extracted from frozen tissue with sulphoslicylic acid (3%) and mixture was centrifuged for 15 minutes at 3500 g. Following the method of Bates et al [9] proline was determinate from supernatant after reaction with ninhydrin. The proline concentration is determined from a standard curve using D-Prolin.

Determinations of contents of MDA

Lipid peroxidation (LP) was measured as the amount of MDA [10]. Fresh sample (100 mg) was homogenized using 0.3% (w/v) TBA in 10% (w/v) trichloroacetic acid (TCA), heated at 95°C for 20 min and cooled in an ice bath. After centrifugation (3500 g for 10 min) the absorbance of the supernatant was read at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. As a blank 0.3% TBA in 10% TCA solution was used. The MDA content was calculated according to the molar extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Statistical analysis

Results of the biochemical parameters represent data expressed as means of determinations made in triplicates and tested by ANOVA followed by comparisons of means by the Duncan test ($p<0.05$). Data were analyzed using STATISTICA for Windows version 11.

Results and discussion

Reactive oxygen species (ROS) are generated under stress conditions and antioxidant enzymes protect the cell structures against ROS. As shown in Figure 1, activity of GPx increased under both treatments, while SOD activity was significantly increased only at 200 mOsm stress conditions. CAT activity increased significantly at 100 mOsm stress, but at the highest level of stress it was at control level. Similar results were obtained by Turkan et al [11]where was observed high and significant induction of plant peroxidases to PEG 6000 induced osmotic stress, and lower sensitivity of CAT activity.

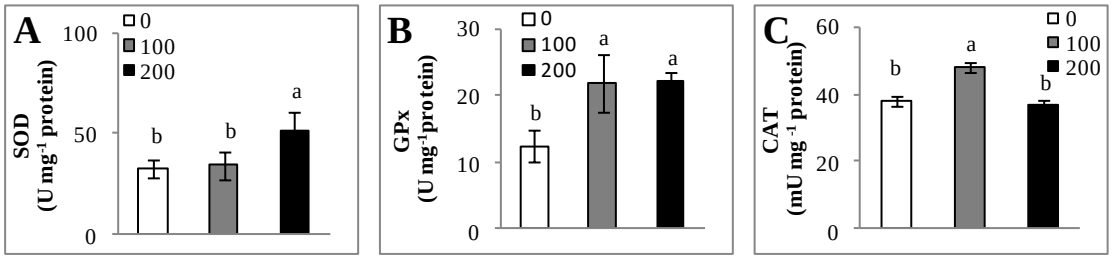

Figure 1. The effect of PEG 6000-induced stress (100 and 200 mOsm) on SOD (A), GPx (B) and CAT (C) activity in poplar tissue culture. Different letters (a, b, c) indicate statistically significant differences at ($P < 0.05$) according to Duncan test.

Abiotic stresses induced accumulation of many compounds such as ascorbate, glutathione, betaine, polyamines and proline in affected plant. In Table 1 are presented the results concerning proline accumulation in investigated tissue. The highest and significant increase of free proline was recorded by the highest stress (200 mOsm PEG 6000), by 199.34%. It is well known that proline metabolism is responsive to various environmental stresses such as drought, osmotic pressure, or ultraviolent irradiation leading to proline accumulation as a survival mechanism [3].

Results of our study agree with former finding that under NaCl treatment proline accumulation in *Populuseuphratica* callus was induced and was 5.6 times higher than in control [12].
The occurrence of MDA, a secondary end product of the oxidation of polyunsaturated fatty acids, is considered a useful index of general lipid peroxidation [13].As can be seen in Table 1 only the highest PEG 6000 concentration (200 mOsm) provoked the significant increase of MDA content (for 52.38%). Previous investigation on *Populuskangdingensis* drought affected many processes including increases in free proline, MDA and SOD activity [14].

Table 1. The effect of PEG 6000-induced stress (100 and 200 mOsm) on proline content and MDA levels in poplar tissue culture

	Proline content (µmol g^{-1} FW)	**MDA content (nmol g^{-1} FW)**
control	$422.37^{b} \pm 19.3$	$46.37^{b} \pm 4.7$
100 mOsm	$425.87^{b} \pm 34.7$	$50.00^{b} \pm 4.2$
200 mOsm	$1265.34^{a} \pm 35.4$	$70.66^{a} \pm 9.5$

*Values marked with same letter do not differ significantly at $p< 0.05$ (Duncan test)

Conclusion

Poplar tissue culture responded to 100 mOsm stress by enhanced CAT and GPX activities which could explain why stress did not cause increased level of lipid peroxidation. Under 200 mOsm conditions, stress provoked higher SOD and GPx enzyme activities and proline accumulation, but it was not enough to mitigate lipid peroxidation.

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