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Oxidoreductase enzymes activities and proline content in leaves of four Salix genotypes☆

Cristina Băbeanu[1], Mihaela Corneanu[2], Cornelia Buzatu-Goanță[2], Viorica Balica (Piturca)[3]*

[1] University of Craiova, Faculty of Sciences, Department of Chemistry, Calea București 107 I, Craiova, Romania

[2] Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael Ist of Romania" from Timisoara, 119 Aradului Street, Timisoara, Romania

[3] University of Craiova, Faculty of Agriculture, Master student Environmental Protection in Agriculture, 19 Libertatii Street, Craiova,
* E-mail: cbabeanu@yahoo.com

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Abstract:
In this paper it was investigated the effect of environment conditions on the activities of antioxidant enzymes and on the proline content in leaves of four Salix clones. The studied plantations were Radovan (Dolj) and Ghilad (Timis). The catalase and peroxidase activities were determined by colorimetric method. The proline content was determined from sulfosalicilc acid extract by colorimetric method with ninhidric acid as reagent using L proline as standard. The obtained results show that the activity of antioxidant enzymes varies with the investigated genotype and with the environmental conditions. In the case of plants subjected to water and salt stress, an increase in antioxidant enzyme activity and proline content can be observed. This increase in studied biochemical indices suggest a state of oxidative stress, the plants activating a defensive system. Measurement of catalase and peroxidase activity and proline content might be used as biomarkers to assess the tolerance of willows for environmental stress.

Keywords: catalase, environmental stress, peroxidase, proline, Salix

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1. INTRODUCTION

Willows have recently attracted great interest due to their energy biomass and important pharmaceutical role [1,2]. They are a very popular species that grows and develops well on soils unsuitable for agriculture and are widely used in phytoremediation and restoration of degraded soils [3].

It is well known that environmental stresses affect plant growth and development. Among the abiotic stresses in a natural environment we list: extreme temperatures, salt stress, drought and heavy metals. Plants can respond to stress by adapting their cellular metabolism and developing various defense mechanisms [4]. One of the earliest responses of plants to abiotic stress is the accumulation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO\cdot$) [3, 5]. If they are not neutralized, reactive oxygen species can cause lipid peroxidation, membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands. In order to limit oxidative damage under stress conditions plants have developed a series of detoxification system that scavenge the reactive oxygen species. The plant antioxidant system is composed of both enzymatic and non-enzymatic components such as: superoxide dismutase (SOD) (E.C 1.15.1.1), ascorbate peroxidase (APX) (E.C 1.11.1.11), glutathione reductase (GR) (E.C 1.6.4.2) catalase (CAT) (EC 1.11.1.6), peroxidases (POX) (EC 1.11.1.7) reduced glutathione, ascorbic acid, α-tocopherol and carotenoids [6-8].

Another response to various types of stress is the accumulation of osmolytes, low-molecular-weight organic compounds, highly soluble such as sugars, sugar alcohols, polyamine and amino acids of which the most important is proline [9]. These compounds stabilize macromolecular structures, scavenge reactive oxygen species (ROS), and maintain membrane integrity. Proline has several functions during stress: osmotic adjustment, osmo-protection, free radical scavenger and antioxidant, protection of subcellular structures and

proteins from denaturation, regulation of cytosolic acidity, regulation of cellular redox potential, preservation of enzyme structure and activity and nitrogen reserve [10,11].

In this paper it was investigated the effect of environment conditions on the activities of antioxidant enzymes and on the proline content in leaves of four Salix clones grown in three different areas.

2. MATERIALS AND METHODS

2.1. Materials

The biological material was represented by leaves of four Salix clones: Tordis, Tora, Torhild and Sven. The studied plantations are located in Radovan (Dolj) area on phaeozem soil (N 44°10′05″ E 23°36′13″), Ghilad (Timis) area on alluvial soil (N 45°28′719″ E 21°02′199″) and Ghilad (Timis) on saline soil (N 45°27′116″ E 21°10′261″). The samples were collected in July and analized fresh.

2.2. Analysis methods

Enzyme assays: Fresh tissue was homogenated with 0.1 M phosphate buffer, pH 7.0 (1:20 w:v) containing 0.1 mM EDTA. Homogenates were centrifuged for 20 min at 10,000 r.p.m. and the supernatants were used for enzyme assay.

Total soluble peroxidase activity, POX (guaiacol-type E.C.1.11.1.7) was assayed by measuring the increase in A_{436} due to the guaiacol oxidation and their activity was expressed as ΔA/min/g fresh weight [12].

Catalase activity, CAT (E.C.1.11.1.6) was assayed through the colorimetric method of Sinha (1972) at 570 nm using a H_2O_2 as standard and the results are expressed as mM H_2O_2/min/g at 25°C [13].

Proline content (PRO) was determined in 3% aqueous sulfosalicylic acid extract by spectrophotometry at 520 nm following the ninhidrin method, using L-proline as a standard. The results are expressed as μg proline/ g fw [14].

The spectrophotometric measurements were performed with a Thermo Scientific Evolution 600 UV-Vis spectrophotometer with VISION PRO software. All determinations were performed in triplicate, and all results were calculated as mean.

3. RESULTS AND DISCUSSION

3.1. Results

The analyzed biochemical indices show a dependency with the investigated genotypes and the environmental conditions.

In the case of catalase activity results are shown in Figure 1. For the plantation Ghilad catalase activity ranges from 306,74 mM H_2O_2/min/g (Svem) to 532,3 mM H_2O_2/min/g (Tora). For the plantation Radovan, with high temperatures and hydric stress, catalase activity ranges from 668,37 mM H_2O_2/min/g (Svem) to 930 mM H_2O_2/min/g (Tordis). In the case of salt stress, the activity of catalase increases from 1.81 to 2, 36 times compared to the values obtained in the case of same climate.

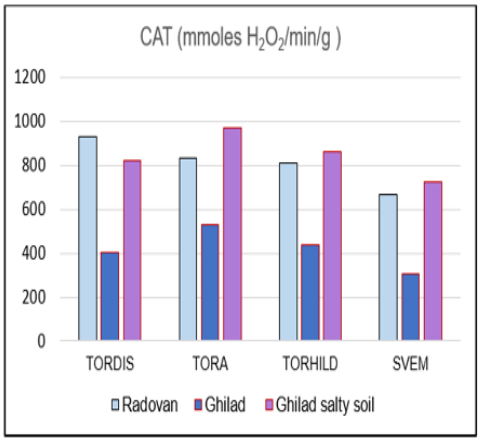

Figure 1. Catalase activity in leaves of Salix genotypes.

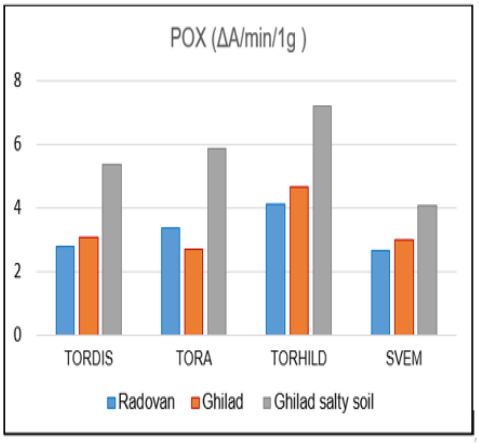

Figure 2. Peroxidase activity in leaves of Salix genotypes.

The results for peroxidase activity (POX) are shown in Figure 2. POX varies between 2,65 ΔA/min/1g (Svem) and 4,12 ΔA/min/1g (Torhild) for Radovan; between 2,7 ΔA/min/1g (Tora) and 4,66 ΔA/min/1g (Torhild) for Ghilad and between 4,08 ΔA/min/1g (Svem)

and 7,23 ΔA/min/1g (Torhild) for Ghilad salty soil. The increase in the case of salt stress is from 1.35 times (Svem) to 2.17 times (Tora). For willow leaves, the increase in peroxidase enzymatic activity in case of salt stress is also reported in other studies [15].

The results for proline content (PRO) are shown in Figure 2. For the Ghilad plantation, with plants well hydrated, the results obtained for proline content varies between 106,68 μg/g fw (Tordis) and 250 μg/g fw (Tora). In the case of salt stress, the activity increases from 1.81 to 2,36 times compared to the values obtained in the case of Ghilad with the same climate. For Radovaan plantation the proline content varies between 89,05 μg/g fw (Tora) and 199,51 μg/g fw (Tordis).

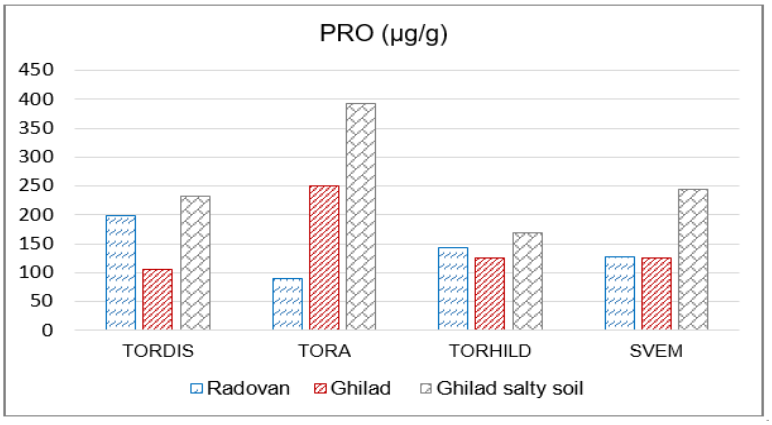

Figure 3. Proline content in leaves of studied Salix genotypes.

In this work, we comparatively studied well-hydrated plants from the Ghilad plantation, plants exposed to salt stress from the Ghilad salty soil plantation, and plants exposed to water stress and drought from the Radovan plantation.

Salt stress is one of the most devastating abiotic stresses that affects agricultural productivity in several ways. High concentrations of salt in soil cause water stress by decreasing osmotic potential, causing ionic toxicity, nutrient deficiencies and imbalances, membrane disorganization, oxidative stress, perturbing important physiological and biochemical processes such as inhibition of photosynthesis [16]. The results obtained show a first response to

stress factors by activating the antioxidant enzymatic system and increasing proline content.

4. CONCLUSION

The analyzed biochemical indices show a dependency with the investigated genotypes and the investigated areas. The different types of stress can disturb the redox homeostasis and lead to oxidative stress, increasing the production of reactive oxygen species.

In the case of plants exposed to salt stress and drought, catalase activity, peroxidase activity and proline content (with few exceptions) increase. This increase suggests a state of oxidative stress, the plants activating a defensive system.

Measurement of catalase and peroxidase activity and proline content might be used as biomarkers to assess the tolerance of willows for environmental stress.

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