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ANTIOXIDANT ENZYMES ACTIVITIES AND PROLINE CONTENT IN LEAVES OF SALIX SPECIES GROWN ON FLY ASH DUMPS

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ABSTRACT

For remediation and re-cultivation of polluted soils, including fly ash dumps, stress-tolerant plants are needed, one of the species used for this purpose being the willow tree. A first response of the plant to different types of stress is the activation of the enzymatic and non-enzymatic antioxidant system for the neutralization of excess reactive oxygen species.

The purpose of this paper is to evaluate the activity of catalase and peroxidase and proline content in leaves of 14 clones of Salix (7 Romanian and 7 Swedish) grown on fly ash dumps from Isalnita. The obtained data were compared to those determined for control cultures from Radovan. The samples were collected in July and were determined the catalase and peroxidase activity by colorimetric method. The proline content was determined from sulfosalicylic acid extract by colorimetric method with ninhidric acid as reagent using L proline as standard.

The results obtained show an increase in peroxidase activity and in proline content (with few exceptions) in plants grown on ash dumps in comparison with the control plantation. The increase in antioxidant enzymes activities suggests a state of oxidative stress, the plants activating a defensive system. Oxidoreductase activity and proline content might be used as biomarkers of tolerance/adaptation of Salix specieson degreded soils.

INTRODUCTION

Due to the global energy crisis, renewable energy resources, represented by crop biomass of SRC (Short rotation coppice) are a sustainable option. One of the species used for this purpose is the willow and a particular interest for the energy willow culture is observed (Hernea et al., 2015, Hernea et al., 2016). This interest in obtaining biomass can be connected with the remediation of polluted soils, including fly ash dumps (Soare et al., 2017).

Fly ash characteristic depends on physicochemical properties of coal, coal combustion process storage and climate (Ibrahim, 2015). Numerous scientific studies report that ash deposits contain potentially toxic elements like heavy metals in quantities that for some of them exceed the alert limit (Popa et al., 2013, Soare et al., 2010).These heavy metals are known to induce a state of oxidative stress in plants limiting their survival and growth.

A first response of the plant to this type of stress is the activation of the enzymatic and non-enzymatic antioxidant system for the neutralization of excess reactive oxygen species. 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 (Singh and Tuteja, 2010).

The purpose of this paper is to evaluate the activity of some antioxidant enzymes (catalase and peroxidase) and proline content in leaves of 14 clones of Salix (7 Romanian and 7 Swedish) grown on fly ash dumps from Isalnita.

MATERIALS AND METHODS

The biological material was represented by ten Sallix clones, seven romanian: RO892, RO1077, RO1082, Cozia1, Fragisal, Pesred, Robisal and seven swedish clones: Inger, Tordis, Jorr, Olof, Tora, Torhild and Svem. The behavior of investigated clones has also been studied in other areas in Romania (Babeanu et al., 2017, Hernea et al., 2016, Soare et al., 2015).

The studied plantations are located on fly ash deposits (N 44˚24'41" E 23˚41'07"), resulting from coal burning to Isalnita Power Station, near Craiova, Romania. These deposits are characterized by low fertility and many rehabilitation eco-technologies have been experimented (Babeanu et al., 2004; Popa et al., 2010).

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 (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/1g fresh weight (Babeanu et al., 2010).

Catalase activity(E.C.1.11.1.6) was assayed through the colorimetric method at 570 nm using a Thermo Scientific Evolution 600 UV-Vis spectrophotometer and the results are expressed as mmoles H_2O_2/min/g at 25°C (Babeanu et al., 2010).

Proline content 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 (Matei et al., 2013).

All assays were performed in triplicate and the results presented here are the mean values.

RESULTS AND DISCUSSION

The plants grown on ash deposits are subject to several stress factors: heavy metals, lack of nutrients and water, heat, etc. The responses to these types of stress depend on the species and genotype, the developmental and metabolic state of the plant and the duration and severity of the stress (Singh and Tuteja, 2010).

The results for peroxidase activity are shown in Figure 1. Peroxidase activity varies between 4.00 ΔA/min/1g f.w. (Pesred) and 10.26 ΔA/min/1g f.w. (Fragisal). Swedish Salix clones showing the lowest average value for peroxidase activities: (6.025 ΔA/min/1g fw) while Romanian clones show higher values (7.53ΔA/min/1g fw).

In a previous research paper the same willow clones were investigated in a control plantation at Radovan area with well-developed plants and a plantation on sandy soil at Tamburesti. For the control plantation Radovan, with plants well hydrated values obtained for peroxidase activity varies between 1.62 ΔA/min/1g f.w.-Inger and 15.38 ΔA/min/1g f.w.-RO1082 (Babeanu et al., 2017).

In the case of plants grown on fly ash the peroxidase activity increases compared to control plantation Radovan (Pesred: 22.32% -Olof: 3.23 fold) with two exception: RO 892 and RO 1082 which had a decrease in enzymatic activity of peroxidase.

Our results are in agreement with other studies reporting the increased peroxidase activity in response to water stress and heavy metal stress in other plants (Babeanu et al., 2010). Increasing peroxidase activity leads to lower H_2O_2 content in the cell and to avoid oxidative stress.

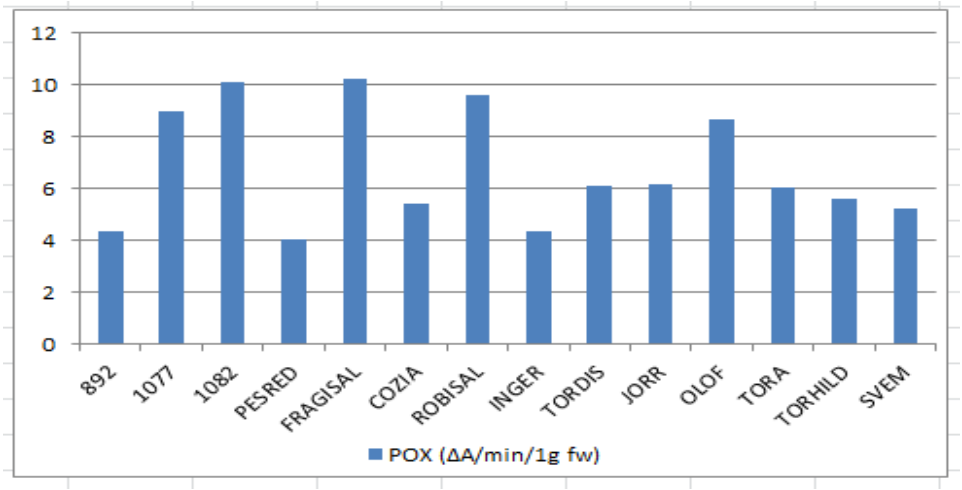
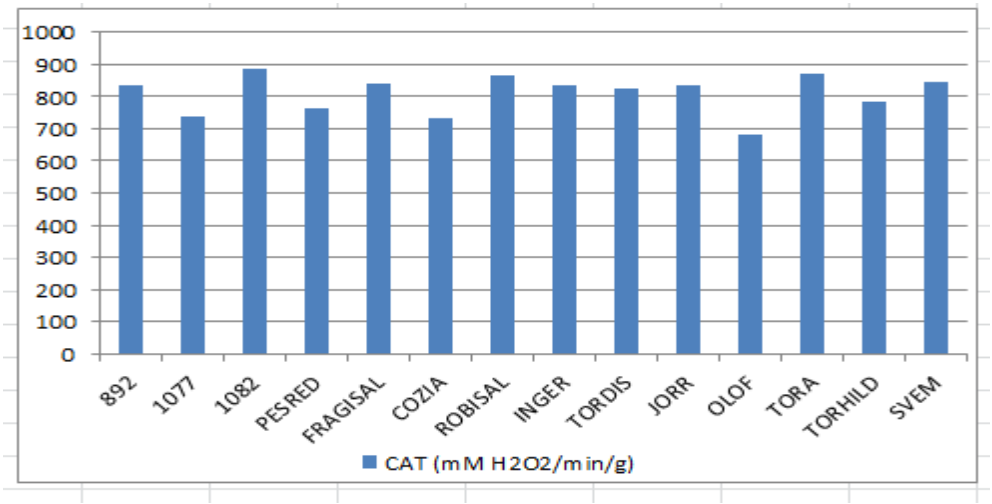


Figure 1Peroxidase activity in leaves of Salix clones

The obtained results for catalase activity are shown in Figure 2. In the case of plants grown on fly ash deposit catalase activity varies between 683.5μM H_2O_2/min/g (Olof) and 885.3 μM H_2O_2/min/g (RO1082). In this case catalase activity increases compared to control plantation Radovan (from 2.72 % -Olof to 73.84 % -RO 1077) except for the clones Pesred, Cozia,Tordis, Jorr and Torhild which had a decrease in catalase activity (8.83%, 12.09%, 11.22 %, 11.56% respectively 3.28%).

For the control plantation Radovan, catalase activity ranges from 423.53 μM H_2O_2/min/g - RO1077 to 947.26 μM H_2O_2/min/g –Jorr (Babeanu et al., 2017). .

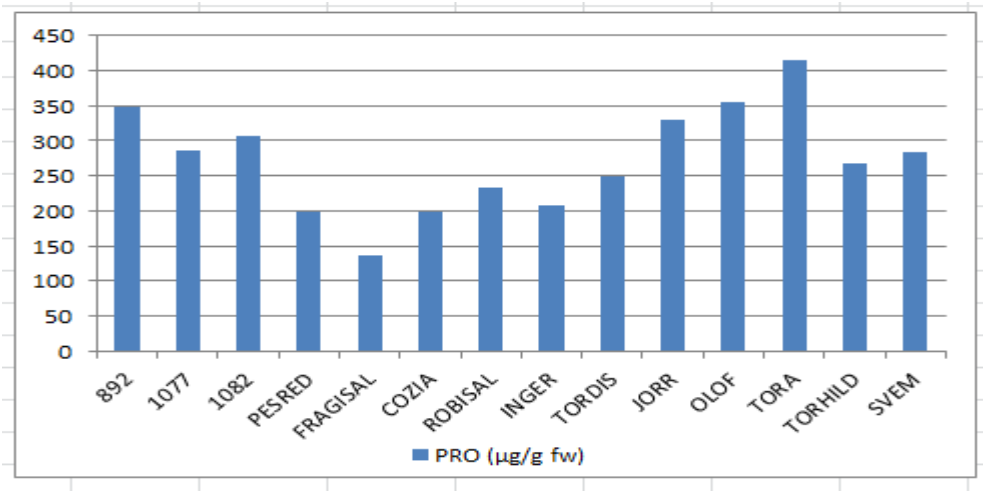
Figure 2 Catalase activity in leaves of Salix clones



The proline content varies with analyzed clone (figure 3). In the case of plants grown on fly ash the proline content varies between 198.16 μg/1g fw (Cozia) and 414.75 μg/1g fw (Tora).

The proline content for all investigated clones is higher in plants grown on fly ash compared to reported data for well-developed plants grown in the Radovan plantation.

Figure 3Proline content in leaves of studied Salix clones



For the control plantation Radovan, with plants well hydrated, the results obtainedfor proline content varies between 95.61 µg/1g fw-RO1077 and 210 µg/1g fw-RO1082 (Babeanu et al., 2017). Accumulation of proline as a signal for adaptive plant responses to stress has been reported for many species (Babeanu et al., 2012, Storaska et al., 2008).

CONCLUSIONS

The analyzed biochemical indices show a dependency with the investigated genotypes

Fly ash conditions may disturb the redox homeostasis and lead to oxidative stress, increasing production of reactive oxygen species. Balance between the production and the scavenging of reactive oxygen species is critical to the maintenance of growth and metabolism of plants.

The high levels of peroxidase and catalase activity and proline content suggests a state of oxidative stress, the plants activating a defensive system.

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