EFFECTS OF DROUGHT STRESS ON SOME OXIDOREDUCTASE ENZYMES AND PROLINE CONTENT IN LEAVES OF SALIX GENOTYPES

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ABSTRACT

The reactive oxygen species are usually generated by normal cellular activities of the aerobic metabolism and play the role of a signal molecule and beneficial growth regulator.

When plants are exposed to stress conditions the concentration of reactive oxygen species increases. Therefore, to neutralize these free radicals and to protect cells from oxidative damage enzymatic and non-enzymatic antioxidant systems are activated. In this paper it was investigated the effect of drought on the activities of antioxidant enzymes and on the proline content in leaves of 10 Sallix clones: RO892, RO1077, RO1082, Cozia1, Fragisal, Pesred, Robisal, Inger, Olof and Jorr.

The studied plantations were Timburesti area on sandy soil, irrigated and non-irrigated version, comparative to the control plantation Radovan area. The samples were collected in July and there were determined the catalase and peroxidase activity 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 the plants subjected to hydric stress there can be observed an increase in peroxidase activity compared to the irrigated plot. The increase in peroxidase activity suggests a state of oxidative stress, the plants activating a defensive system. The results obtained show an increase in catalase activity and in proline content (with few exceptions) in plants grown on sandy soil in comparison with the control plantation.

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

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

INTRODUCTION

Drought is one of the factors that limit plant growth and affected regions are expanding due to climate change. Plants can respond to drought stress by adapting their cellular metabolism and developing various defense mechanisms [1]. One of the earliest responses of plants to drought 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•). [2].

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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.

Another response mechanism of plants is the synthesis and accumulation of osmo-protectants compounds including certain polyols, sugars and amino acids of which the most important is proline [3]. The accumulation of free proline in plant tissues is regarded as a general response to water and other kinds of stresses. 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 [4].

In this paper we have investigated the effect of drought stress on antioxidant enzymes activities and proline content in leaves of ten Salix genotypes grown in sandy soil compared to the control plantation.

MATERIALS AND METHODS

The biological material was represented by ten Sallix clones, seven romanian: RO892, RO1077, RO1082, Cozia1, Fragisal, Pesred, Robisal and three swedish clones: Inger, Olof and Jorr. The behavior of Swedish clones has also been studied in other areas in Romania [5] The studied plantations are located in Timburesti area on sandy soil, irrigated (N 44˚01'08" E 23˚56'25") and non-irrigated (N 44˚01'08" E 23˚56'29") version, comparative to the control plantation Radovan (N 44˚10'05" E 23˚36'13") area [6]. The soil on which was placed the experience is sand, poorly supplied with phosphorus and nitrogen and medium supplied with potassium, with low fertility [7,8]. 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 [9] 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 [9]. 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 μmol proline/ g fw [10].

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

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. **RESULTS AND DISCUSSIONS**

Drought is a multi-dimensional stress, which causes various physiological and biochemical response of plants. The responses to drought stress depend on the species and genotype, the developmental and metabolic state of the plant and the duration and severity of the drought stress [11]. When plants are exposed to drought and water stress the concentration of reactive oxygen species increases. Balance between the production and the scavenging of ROS is critical to the maintenance of growth and metabolism of plants. Therefore, to neutralize these free radicals and to protect cells from oxidative damage enzymatic and non-enzymatic antioxidant systems are activated.

To investigate the effect of drought on the enzymatic antioxidant system in leaves of Salix genotypes, enzymatic activity of catalase and peroxidase was determined. The obtained results show that the activity of antioxidant enzymes varies with the investigated genotype and with the environmental conditions.

Catalase catalyses the dismutation of hydrogen peroxide into water and oxygen and peroxidase decompose H_2O_2 by oxidation of substrates In addition to their role in scavenging systems, peroxidases have been recognized to be involved in several cellular processes, including the control of development, lignification, pathogen defense and the catabolism of growth regulators [12].

The results for peroxidase activity are shown in Figure 1. Peroxidase activity varies with the clone analyzed and the stress level applied. 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).

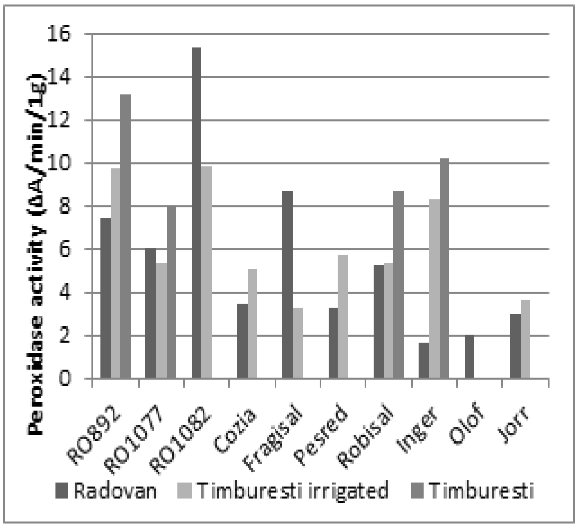
Swedish Salix clones showing the lowest peroxidase activities: Inger (1.62 ΔA/min/1g fw) Olof (2.05 ΔA/min/1g fw) and Jorr (2.96ΔA/min/1g fw) while romanian clones shows higher values, vary as follows: RO1082, Fragisal, RO892, RO1077, Robisal, Cozia and Pesred.

In the case of plants grown on irrigated sandy soil peroxidase activity increases (with few exceptions) compared to control plantation Radovan. The maximum increase in the peroxidase activity was observed at genotype Inger (4.09 fold) while the minimum increase was determined in the genotype Robisal (2.09%).

In severe drought, in the case of non-irrigated plantation Tamburesti have survived only clone RO892, RO1077, Robisal and Inger. In this case of non-irrigated plantation Tamburesti the peroxidase activity increases both compared to control plantation Radovan (RO 1077: 34.84% -Inger: 5.28 fold) and to irrigated plantation on sandy soil (Inger: 23.24%-Robisal: 61.94%).

Our results are in agreement with other studies reporting the increased peroxidase activity in response to drought stress in other plants [9, 10, 13]. Increasing peroxidase activity leads to lower H2O2 content in the cell and to avoid oxidative stress.

In the case of catalase activity results are shown in Figure 2. 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).



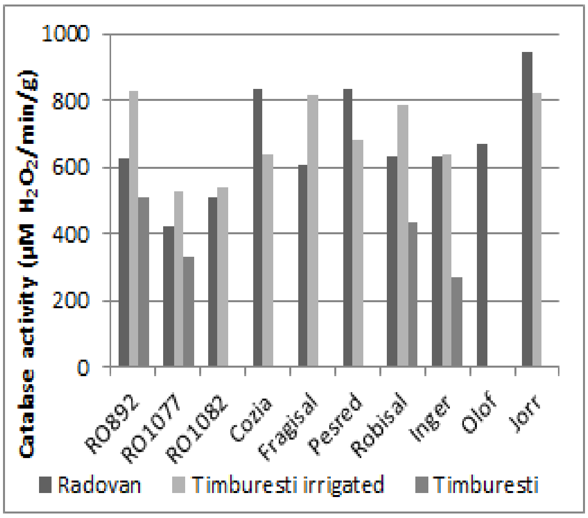


Figure 1. Peroxidase activity in leaves of Salix clones

Figure 2. Catalase activity in leaves of Salix clones

In the case of irrigated plantation Tamburesti catalase activity varies between 527.16 µM H_2O_2/min/g (RO1077) and 825.13 µM H_2O_2/min/g (RO892). In this case catalase activity increases compared to control plantation Radovan (from 1.67 % -Inger to 34.11 % -Fragisal) except for the clones Cozia, Pesred and Jorr which had a decrease in catalase activity (23.51%, 18.39% respectively 13.10%). .

In the case of non-irrigated plantation Tamburesti, under severe drought the catalase activity decreases both compared to control plantation Radovan and to irrigated plantation on sandy soil.

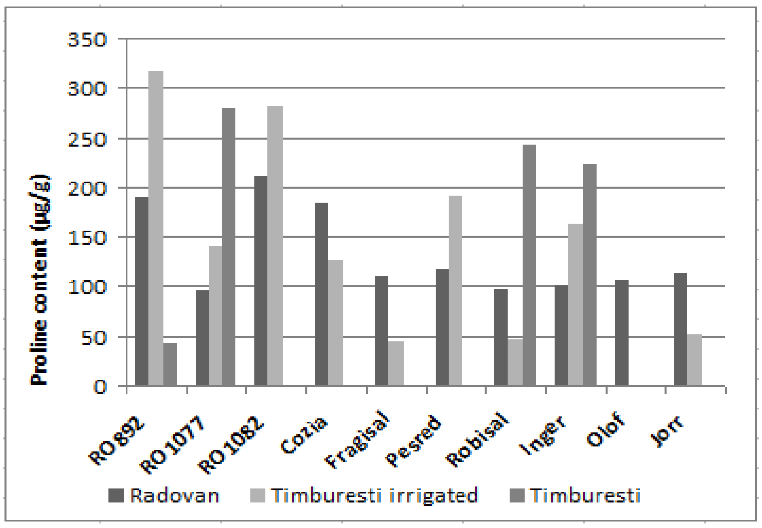


Figure 3. Proline content in leaves of studied Salix clones

Another parameter that has been studied is proline content. The proline content is a good indicator for screening drought tolerant varieties in water stress condition [9, 10, 13]. The proline content varies both with analyzed clone and with the applied stress (figure 3). For the control plantation, with plants well hydrated, the results obtained for proline content varies between 95.61 µg/1g fw (RO1077) and 210 µg/1g fw (RO1082)

. In the case of irrigated plantation Tamburesti proline content increases compared to control plantation Radovan except for the clones Cozia, Fragisal, Robisal and Jorr which had a decrease in proline content.

In the case of non- irrigated plantation Tamburesti the proline content increases both compared to control plantation Radovan (from 33.49% RO1082 to 67.78% RO892) and to irrigated plantation on sandy soil (from 36.61 % Inger to 4.22 fold Robisal). Accumulation of proline as a signal for adaptive plant responses to stress has been reported for many species [9, 10, 14].

CONCLUSION

The analyzed biochemical indices show a dependency with the investigated genotypes and the water stress levels. Drought 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.

For the control plantation Radovan, with plants well hydrated mean values obtained for peroxidase activity and proline content for Romanian Salix clone are the highest while Swedish Salix clones shows the lowest values.

In the case of plants grown on irrigated sandy soil peroxidase activity increases (with few exceptions) compared to control plantation Radovan. In severe drought, in the case of non-irrigated plantation the peroxidase activity increases both compared to control plantation Radovan and to irrigated plantation on sandy soil. The increase in peroxidase activity suggests a state of oxidative stress, the plants activating a defensive system.

The results obtained show an increase in catalase activity and in proline content (with few exceptions) in plants grown on sandy soil in comparison with the control plantation. Romanian Salix clones shows the lowest values of catalase activity.
The use of proline accumulation in plants suffering from droughts will be of major practical importance: it would provide an easy screening criterion for field performance of genotypes.

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

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