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Screening the tolerance of Romanian and Sweeden willow hybrids to saline stress

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Abstract
Willows are deciduous trees and shrubs with more than 450 species worldwide. In Romania, the Danube Delta, the Danube Meadow, and all the other river meadows are areas occupied by willows but no more than 0.5% from the national forest fund. There are protection forests (water protection, stream bank protection, etc.) and production forests. In recent years, the interest in willow short rotation coppice increased, so many hybrids were evaluated for biomass production or phytoremediation. Willow species are known as high-demand water species. In this context, the response of willows to hydric stress is essential for marginal land afforestation. A hydroponic laboratory experiment with two factors, willow hybrids and NaCl concentration level, was initiated. Ten hybrids: seven Romanian hybrids (RO_892, RO_1077, RO_1082, Cozia, Fragisal, Pesred and Robisal) and three Sweden hybrids (Inger, Olof, Tordis) collected from the University of Life Sciences "King Mihai I" from Timisoara and three concentration of NaCl level were tested. The tolerance of studied willow hybrids to saline stress was estimated according to the shoot dry mass index, root dry mass index, the ratio of shoot dry mass to root dry mass. The response of willow genotypes in terms of biomass index showed the tolerance for the first and second levels of NaCl in the case of genotypes RO1077, RO1082, Pesred and Inger. There was no resistance to the third level of NaCl.

Keywords: *Salix*, NaCl tolerance, hydric stress, shoot dry mass index, root dry mass index

Introduction

Willows are deciduous trees and shrubs with more than 450 species worldwide. In Romania, the Danube Delta, the Danube Meadow, and all the other river meadows are areas occupied by willows but no more than 0.5% from the national forest fund. There are protection forests (water protection, stream bank protection, etc.) and production forests [7]. In recent years, the interest in willow short rotation coppice increased, so many hybrids were evaluated for biomass production or phytoremediation. Willow species are known as high-demand water species. Valuable studies made by Keita et al. (2021) [8], that investigated the growth response of three willow species, highlighted the importance of considering species-specific responses to water stress. The success of a willow plantation is dependent by selecting the appropriate willow species based on their tolerance to water stress. In this context, the response of willows to hydric stress is essential for marginal land afforestation.Salix species can restore soil health by improving the structure, microbial life, nutrient density, and carbon levels of the soil, It can be used also to restore lands polluted from fossil fuel extraction and contaminated with heavy metals and radionuclids [1, 5, 12]. Selection of salt-tolerant tree species is considered to be, as Wang et al. (2017), mentioned one of the most economical, effective, ecological and environment-friendly biological measures to solve the soil salinization problem [13].

Material and Method

A hydroponic laboratory experiment with two factors, willow hybrids and NaCl concentration level, was initiated. Ten hybrids: seven Romanian hybrids (RO_892, RO_1077, RO_1082, Cozia, Fragisal, Pesred and Robisal) and three Sweden hybrids (Inger, Olof, Tordis) collected from the University of Life Sciences "King Mihai I" from Timisoara and three concentration of NaCl level were tested. Ro_892, RO_1077 and RO_1082 are hybrids produced by “Marin Drăcea” National Institute for Research and Development in Forestry and the other by The Fruit Growing Research and Development Unit of Vâlcea. To evaluate the tolerance of studied genotypes to saline stress, shoot dry mass index, root dry mass index, and vitality were determinated.

120 cuttings, each of them with aproximately 10 cm length and 1cm diameter were evaluated. Twelve cuttings per hybrid were placed in 150 cmc plastic glasses, in tap water. After nine days the tap water was replaced with Hoagland solution and was daily aerated. The concentration of NaCl solution (NaCl_1=50 mM/l; NaCl_2=100.0 mM/l; NaCl_3=150 mM/l) was added after another five days. Finally, the experiment consist in three cuttings for each experimental variants and the control for each hybrid.

At the end of the saline treatment, green (initial) and dry (final) mass of roots and shoots were evaluated. The green mass was established by weighing. The dry mass was established by weighing after the shoots and leaves were put in metallic boxes and dry for 72 h at 107^0C in a draying oven.Shoot and root dry mass index were calculated by formulas: 100 x shoots dry mass: cutting initial mass and 100 x roots dry mass: cutting initial mass. The ratio S/R was calculated by formulas shoots dry mass: roots dry mass.

Plants metabolic changes were highlighted by proline, guaiacol-peroxidase, and catalase activities.

The activity of catalase (CAT) was determined colorimetrically at λ= 570 nm. [11]. The activity of guaiacol peroxidase (POX) was determined colorimetrically at λ= 470 nm [2]. The proline content (PRO) was determined colorimetrically at λ= 520 nm [3] using L-proline like standard. Because parts of the shoots wilted and some of them died after applied the saline solution, the enzyme activity was developed only on roots system

Statistical analysis (one-way ANOVA) was adopted to investigate the effect of genotype and NaCl treatment. Differences were considered significant at $p < 0.05$.

Results and Discussion

The proportion of resources plants allocate to their shoot or root system is reflected by the shoot dry mass index (SDMI) and root dry mass index (RDMI). The analysis of variance showed a significant influence of genotype for both indexes in terms of genotype [6], (Table 2).

The highest value for SDMI was registered for RO_1077 Control (2.826574). Other higher values were registered for RO_1082 (2.272650), Pesred (2.033427) and Olof (2.198809), (Fig.3). For genotype Inger, higher values were registered not only for Control (2.094643) but also for the first concentration of saline treatment (2.368969). In the case of RDMI, higher values were registered for different treatments, the first concentration of NaCl in case of RO_1082 (0.639940), the third treatment concentration in case of RO_1082 (0.649216), Fragisal (0.556961) and Pesred (0.798882) and Control in case of Inger (0.605342) (Table 1). As the salt treatment concentration gradually increased, the average root number, length, and rooting index decreased significantly, as observed by Ran et al. (2021), in a similar experiment on *Salix alba* L [10]. The germination and elongation of roots are promoted by low salt concentration, but root growth is comprehensively inhibited under increasing salt stress [10].

In terms of ratio S/R only the Control of Inger and Tordis are significantly different from the most other genotype and experimental treatment.

The effect of NaCl on the catalase and peroxidase activity is presented in Fig.1. Under salinity and environmental stress, the plants react and modify the activity levels of antioxidant enzymes like catalase (CAT), and peroxidase (POX) by increasing the production of reactive oxygen species (ROS) and, for ROS scavenging, [9].

In this experiment, there is no pattern in the activity of catalase. A decrease was highlighted in the activity of peroxidase. The same pattern was observed for proline activity (Fig. 2). Similar results were reported by Buzatu et al. (2021) for other willow genotypes [4].

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Table 1. Shoots dry mass: cutting initial mass (SDMI), roots dry mass: cutting initial mass (RDMI), shoots dry mass: roots dry mass (S/R) and vitality in hydroponic saline experiment

Genotype	NaCl treatment	SDMI		RDMI		Ratio S/R	
		Mean	TD	Mean	TD	Mean	TD
RO_892	NaCl_1	1.296905	bc	0.277031	b	0.213667	b
	NaCl_2	1.308456	bc	0.203871	b	0.156333	b
	NaCl_3	1.134547	bc	0.405478	b	0.379333	b
	C	1.669521	bc	0.281065	b	0.183000	b
RO_1077	NaCl_1	1.447433	bc	0.369414	b	0.252667	b
	NaCl_2	1.819249	bc	0.416907	b	0.225667	b
	NaCl_3	1.074124	bc	0.367279	b	0.364667	b
	C	2.826574	a	0.502200	b	0.170667	b
RO_892	NaCl_1	1.721242	bc	0.639940	ab	0.371000	b
	NaCl_2	1.529107	bc	0.490533	b	0.321667	b
	NaCl_3	0.919168	c	0.649216	ab	0.690333	ab
	C	2.272650	ab	0.885293	a	0.394333	b
Cozia	NaCl_1	0.831307	c	0.401521	b	0.482333	ab
	NaCl_2	1.133932	bc	0.286953	b	0.252333	b
	NaCl_3	1.156363	bc	0.402616	b	0.449000	b
	C	1.281934	bc	0.389155	b	0.297333	b
Fragisal	NaCl_1	1.675858	bc	0.581208	a	0.348667	b
	NaCl_2	1.464259	bc	0.247902	b	0.179667	b
	NaCl_3	1.393922	bc	0.556961	ab	0.394000	b
	C	1.233412	bc	0.399430	b	0.335333	b
Persed	NaCl_1	1.874960	b	0.487007	b	0.259333	b
	NaCl_2	1.347600	bc	0.375873	b	0.285333	b
	NaCl_3	0.906264	c	0.798882	ab	0.905000	a
	C	2.033427	ab	0.425870	b	0.210000	b
Robisal	NaCl_1	0.879355	c	0.177831	b	0.200333	b
	NaCl_2	1.441180	bc	0.230376	b	0.078333	b
	NaCl_3	1.408335	bc	0.310992	b	0.216333	b
	C	1.715877	bc	0.315382	b	0.177000	b
Inger	NaCl_1	2.368969	ab	0.461316	b	0.185333	b
	NaCl_2	1.432984	bc	0.362435	b	0.254667	b
	NaCl_3	1.739935	bc	0.230636	b	0.136667	b
	C	2.094643	ab	0.605342	ab	0.918333	a
Olof	NaCl_1	1.619469	bc	0.303254	b	0.187333	b
	NaCl_2	1.441383	bc	0.380327	b	0.261333	b
	NaCl_3	0.963754	c	0.192818	b	0.203667	b
	C	2.198809	ab	0.423654	b	0.193000	b
Tordis	NaCl_1	1.091706	bc	0.341143	b	0.310333	b
	NaCl_2	0.952268	c	0.331155	b	0.356667	b
	NaCl_3	1.016312	bc	0.320653	b	0.315667	b
	C	0.840888	c	0.477399	b	0.654000	a

Table 2. Analysis of Variance for hydroponic saline experiment. Effect of genotype and saline treatment on shoots/roots dry mass: cutting initial mass

Character	Marked effects are significant at p < .05000. Factors: 1- Genotype; 2- NaCl treatment					
	1		2		1 x2	
	F	P	F	p	F	p
SDMI	3.417703	0.000968	7.401722	0.000140	3.427403	0.000002
RDMI	4.868682	0.000017	2.259047	0.085247	2.318302	0.000760
S/R	1.589310	0.127123	2.335212	0.077473	1.755131	0.017256

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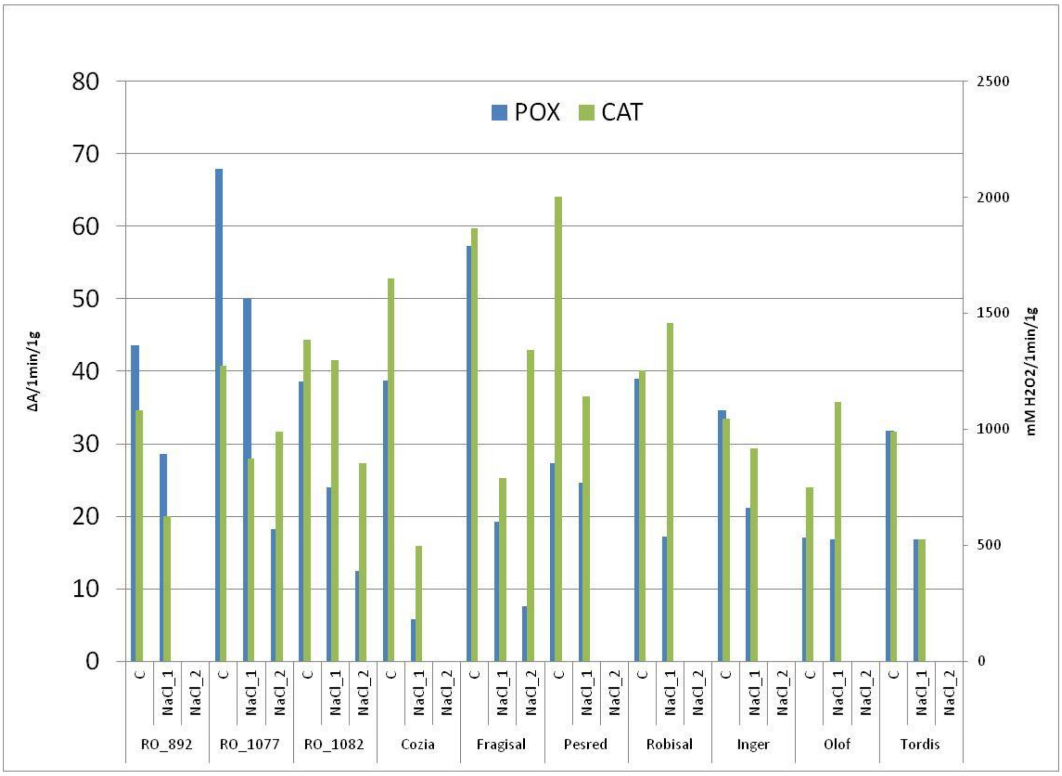

Figure 1. The variation of enzymatic activity in NaCl experiment: catalase (CAT); peroxidase (POX)

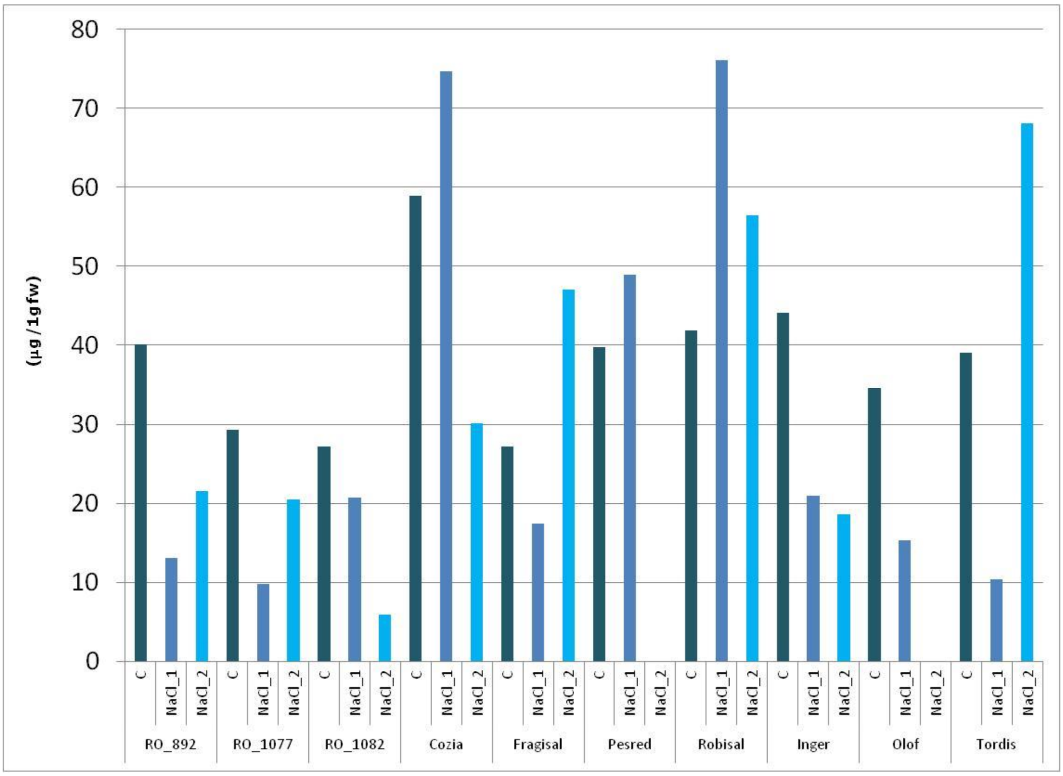

Figure 2. The variation of enzymatic activity in NaCl experiment: proline (PRO)

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Figure 3. The reaction of the analyzed genotypes at the end of the treatment with different NaCl concentration (NaCl_1=50 mM/l; NaCl_2=100.0 mM/l; NaCl_3=150 mM/l; M = Control)

Conclusions

The response of willow genotypes in terms of biomass index showed the tolerance for the first and second levels of NaCl in the case of genotypes RO1077, RO1082, Pesred and Inger. There was no resistance to the third level of NaCl. The enzymatic activity varied with genotype and NaCl level. A decrease in activity was observed for peroxidase and proline activity. No pattern was defined for catalase activity.

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References

[1] Ahirwal, J., Maiti, S.K.; Singh, A.K. (2017), Ecological Restoration of Coal Mine-Degraded Lands in Dry Tropical Climate: What Has Been Done and What Needs to Be Done? Environ. Qual. Manag. 26, 25–36.

[2] Babeanu, C., Paunescu G., Popa, D., Badita, A.A. (2008), *Changes of some antioxidant enzyme activities in leaves of drought tolerant varieties of wheat from Oltenia during vegetation stages*. Bulletin UASVM, Agriculture 65(1), 30-33.

[3] Bates, L.S., Waldren, R.P., Teare, I.D. (1973), *Rapid determination of free proline for water-stress studies*. Plant Soil 39, 205–207. https://doi.org/10.1007/BF00018060

[4] Buzatu-Goanta, C., Cornenau, M., Babeanu, C., Dodocioiu, A.M. (2021), *Screening of willow for saline stress in a hydroponic experiment.* Journal of Horticulture, Forestry and Biotechnology. 25(1), 47 - 513.

[5] Ghezehei, S.B.; Nichols, E.G.; Hazel, D.W. (2019), Productivity and cost-effectiveness of short-rotation hardwoods on various land types in the southeastern USA. Int. J. Phytoremed. 22, 98–110.

[6] Hernea, C., Corneanu, M., Băbeanu, C., Sarac, I. (2017), Effect of naCl on willow hydroponic experiment, Journal of Horticulture, Forestry and Biotechnology. 21(4), 30-35.

[7] Flynn, H. & Holder C. Eds. (2001), *A guide of useful wood of the world*. Forest Products Society. p. 618.

[8] Keita, N., Bourgeois, B., Evette, A., Tisserant, M., González, E., Breton, V., Goulet, C., & Poulin, M. (2021), *Growth response of cuttings to drought and intermittent flooding for three Salix species and implications for riverbank soil bioengineering*. Environmental Management, 67(6), 1137–1144.

[9] Machado, R.M. A., & Serralheiro, R. P. (2017), *Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization*. Horticulturae, 3(2), 30.

[10] Mittova, V, Volokita, M, Guy M. (2015), *Antioxidative systems and stress tolerance: insight from wild and cultivated tomato species*, in Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants, eds K. J. Gupta and Igamberdiev (Switzerland: Springer International Publishing), 89–131.

[11] Pimentel, D., Berger, B., Filiberto, D., Newton, M., Wolfe, B, Karabinakis, E., Clark, S., Poon, E., Abbett, E., Nandaopal, S. (2004), *Water Resources: Agricultural and Environmental Issues*. BioScience 54, 909–918.

[12] Ran, X., Wang, X., Gao, X., Liang, H., Liu, B., Huang, X. (2021), *Effects of salt stress on thephotosynthetic physiology and mineral ionabsorption and distribution in white willow (Salixalba L.)*. PLoS ONE 16(11): e0260086. https://doi.org/10.1371/journal.pone.0260086

[13] Sinha, A.K. (1972), *Colorimetric Assay of Catalase*. Analytical Biochemistry, 47, 389-394.2.

[14] Zalesny, R.S., Headlee, W.L., Gopalakrishnan, G., Bauer, E.O., Hall, R.B., Hazel, D.W., Isebrands, J.G., Licht, L.A., Negri, M.C., Nichols, E.G., et al. (2019), *Ecosystem services are popular at long-term phytoremediation sites in the Midwest and Southeast, United States*. Energy Environ. 8, e349.

[15] Wang, Q.Z., Liu, Q., Gao, Y.N. (2017), *Review on the mechanisms of the response to salinity-alkalinity stress in plants*. Acta Ecologica Sinica 37(16): 5565–5577. [in Chinese]