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SALIX ACCESSIONS WITH POTENTIAL FOR NEW HYBRIDS. A CASE STUDY FROM BANAT AREA

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Abstract

Willows are fast-growing species with a large capacity for sprouting and easy breeding. In order to identify Salix accessions suitable for different conditions, a willow plantation was established in the Didactic Station of BUASVM from Timisoara, Timis County, Romania. 19 Salix genotypes (nine species: Salix alba L., S. cinerea L., S. caprea L., S. daphnoides L., S. fragilis L., S. incana L., S. purpurea L., S. pentandra L., S. triandra L.) were collected from a polluted area (copper and uranium mining area) but also from old willow farms, all from Caras-Severin County. The sprouting capacity and biometric observations were made in an experimental trial with uncut and cutback shoots and biomass was estimated after one growing season. Large variability was observed in terms of survival rate but also the number of shoots per stool, maximum height, and diameter. To evaluate the tolerance of heavy metal stress, four accessions were selected and a laboratory experiment was developed. The enzymatic activity varied according to with stress abiotic factor and also with genotype.

Key words: *willow, biometric observation, heavy metal stress, abiotic stress.*

INTRODUCTION

In the last decade, the interest for willow short rotation coppice in Romania increased. Willows are fast-growing species with a large capacity for sprouting and easy breeding, high transpiration rate, and a high potential for land reclamation (Landberg and Greger, 1994; Pulford and Watson, 2003). The clones from Europe, dedicated to biomass production were created in a wetter and cooler climate. They are suitable in a breeding program to provide valuable traits, but native germplasm is of special interest, due to the adaptation to regional conditions with hot and often dry summers. Native species of *Salix* were considered valuable sources of resistance genes in many breeding programs (Kopp et al., 2001; Iori et al., 2015; Stolarski et al., 2020). Climate change, due to the greenhouse gas effect will affect in the future the agricultural crops, by water stress (Wolfe et al., 2018). The area, affected by drought will increase in the future decades and breeding efforts has to be focused on crops tolerant to abiotic stress (Fabio et al., 2019). Heavy metals are one of the abiotic stresses that became a major threat to crop production (Hassanuzzman et al., 2020). Many researchers investigated the growth performance of willow and their capability for phytoremediation (Greger and Landberg, 1999; Jensen et al., 2009; Dos Santos Utmazian et al., 2017). The aim of this study was to evaluate the potential of willow accessions to be used in land reclamation or as genitors in a breeding program.

MATERIALS AND METHODS

Field trial. Willows genotypes collected from the Banat area represent the biological material for this research. These genotypes were planted in 2015 in a collection in Experimental Didactic Station from Banat University, in the Western Plain of Romania, with an elevation of 88m (N 45047'06" E 21012'50"). The soil is cambic chernozem with a slightly acidic pH value (6.1). The amount of precipitation is 592 mm (Hernea et al., 2016). 19 willows genotypes have been planted in twin rows 70 cm apart and with 140 cm between each set of twin rows. 46 cuttings from each genotype were established in prepared soil at a distance of 80 cm between them. Because weed control is critical in willow plantation, herbicide and also mechanical weed control were applied. After the first growing season, one row from each genotype was cut back and the survival rates were calculated. Biometrical observations were made: (i) no of shoots per stool; (ii) the maximum height of the shoot using a measuring pole (precision cm); (iii) the diameter at the base of the shoots using an electronic caliper (0.01mm precision). To select genotypes for hybridization, in spring 2016, the biomass was estimated.

Hydroponic experiment. In 2017 one laboratory experiments was initiated. 9 cuttings per experimental variants with 10 cm length, no more than 1cm diameter, and 2-4 buds were used. Cuttings were placed in 150 CMC plastic glasses, in Hoagland solution and were daily aerated. The experiment tested the resistance to heavy metals (cadmium, copper, nickel, and lead) for four genotypes: P3 - *S. fragilis,* A2 - *S. daphnoides,* L1 - *S. purpurea,* L2 - *S.* alba. It was tested two concentration from each metal: Cd (Cd_1=5.0; Cd_2=10.0 mg/l), Cu (Cu_1=250 mg/l; Cu_2=500 mg/l) Ni (Ni_1=200 mg/l; Ni_2=500 mg/l), Pb (Pb_1=250 mg/l; Pb_2=1000 mg/l) and the control (Hoagland solution). Observations were made at the end of heavy metal treatment: (i) initial (green) mass; (ii) initial (green) shoot mass; (iii) initial (green) root mass; (iv) dry shoot mass; (v) dry root mass. The dry mass was determined by putting shoots and roots in metallic boxes and dries until constant weight. The tolerance of studied genotypes to heavy metals have been evaluated at the end of the experiments according to with 5 predefine vitality classes: (5: high, leaves and shoots green; 4: medium, leaves up to 50% necrotic and shoots green ; 3: low, leaves more than 50% necrotic and shoots green; 2: leaves necrotic and shoots partial necrotic; 1: all necrotic, shoot dry mass index (100 x shoots dry mass: cutting initial mass (SDMI)), roots dry mass index (100 x roots dry mass: cutting initial mass (RDMI)), shoots dry mass: roots dry mass (S/R) and vitality in a hydroponic heavy metal experiment (Heike et al., 2014 modified).

Plants metabolic changes to abiotic stress (hydric or heavy metals stress) were evaluated by guaiacol-peroxidase and catalase activities

The activity of catalase (CAT) was determined colorimetrically at λ= 570 nm (Sinha, 1972). The activity of guaiacol peroxidase (POX) was determined colorimetrically at λ = 470 nm (Babeanu et al., 2008).

All statistical analyses were conducted with STATISTICA 10.0 software. The variables: survival rate, no of shoots per stool, maximum height of the shoots, the diameter at the base of the shoots, dry matter yield was analyzed statistically by a repeated- measures ANOVA, with genotype and management practice as grouping factors. The statistics F for these analyses are shown in the table of results. Duncan's multiple range test with $p < 0.05$ was used to evaluate the significance of differences between the cut back and uncut groups.

RESULTS AND DISCUSSIONS

Nineteen genotypes have been evaluated in a willow plantation: 1 Pojejena - *S. fragilis* (P1), 2 Pojejena - *S. fragilis* (P2), 3 Pojejena - *S. purpurea* (P3), 4 Pojejena - *S. pentandra* (P4), 5 Pojejena - *Salix purpurea* (P5), 6 Tausani - *S. alba* (T1), 7 Sasca - *S. incana* (S1), 8 Sasca - *S. caprea* (S2), 9 Sasca - *S. purpurea* (S3), 10 Sasca - *S. purpurea* (S4), 11 Agadici - *S. fragilis* (A1), 12 Agadici - *S. daphnoides* (A2), 13 Agadici - *S. daphnoides* (A3), 14 Agadici - *S. caprea* (A4), 15 Agadici - *S. cinerea* (A5), 16 Lisava - *S. purpurea* (L1), 17 Lisava - *S.daphnoides* (L2), 18 Lisava - *S. caprea* (L3), 19 Lisava - *S. fragilis* (L4). One of the most important aspects after the establishment of a culture is the survival rate of the plants. In short rotation coppice, 90% of the survival rates are expected but not less than 75% (Bennick et al, 2008).

A variability in survival rate, during the harvest rotations, as well as the decrease in time was observed also, by other reserchers, in a field trial in Canada (Amichev et al., 2018) and in Poland (Stolarski et al., 2020). Four genotypes had good results (Figure 1), for others, the results were satisfactory according to the fact that all the cutting used were collected from one plant, sometimes with low biometric characteristics (abandoned willow farms) or grown in difficult condition (sterile dumps).

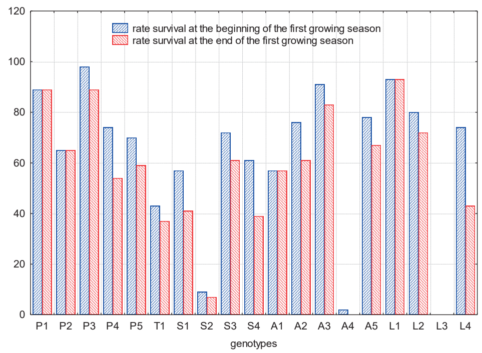

Figure 1. *Salix* genotypes survival rates at the beginning and the end of the first growing season

Three genotypes did not performed at all (S2, A4, and L3), but it is known from the literature how difficult is to reproduce goat willow by cuttings.
The number of shoots per stool was counted, the highest and the thickest shoots were measured and biomass was estimated (Table 1). Diameter at the base of the shoots, height, and also the number of shoots per stool is genetically determined, but also influenced by pedoclimatic conditions (Stolarski et al., 2020). The sprouting capacity increased in all cut back variants, but only a few differences were significant compared to the uncut ones (P4, S1, A2, A3, A5, L1) (Table 1).
The highest mean values were observed for genotypes A2 (7.6 and 17.4) and A3 (7.6 and 15.1) for both uncut and cut back practice. Differences were observed for height and the diameter at the base of the shoots also. The lowest height was registered for genotypes L4 (96 cm for uncut management practice and 111 for cut back management practice) followed by genotypes A5 (111 cm and 103 cm). The highest values were registered for genotype P3 (220 cm and 225). The maximum diameter was registered for genotype A1 (35.6 mm and 18.8 mm) and T1 (30.97mm and 21.74 mm). In terms of biomass, the most productive genotype (initial estimation) was P3 with very good height characteristics and also large diameter values. It should be noted that in most genotypes with significant differences in sprouting, between the cut back - uncut variants, there are no significant differences in the height or diameter of the shoots. Analysis of variance showed that the effect of each factor and also all factors analyzed (genotypes and management practice) is significant (Table 2). This analysis was the base for genotype selection to establish the heavy metal experiment. Some researchers consider that to evaluate the heavy metal tolerance of plants, the hydroponic experiment can be a first step (Torabi et al., 2012) before field experiment, but it is not a certainity that plants will react the same (Zabtudowska et al., 2009).

Hidroponic experiment. In the experiment, the effect of genotypes and heavy metal treatment was analyzed according to shoots and roots dry biomass (Table 3) and the analysis of variance was performed (Table 4).
The experiment reveals the influence of heavy metal on different genotypes. All genotypes react very well on cadmium treatment. It can be seen an increase of SDMI and RDMI value with increasing concentration for all genotypes. For the other variants, it can be seen a decrease of SDMI with increasing the heavy metal concentration. But it not the same in terms of RDMI where the parameters decrease with the increase of copper concentration, increase with the increasing of nickel concentration and it is increasing or decreasing with lead increasing. The best results in terms of vitality were obtained for cadmium and lead treatment and the worst with nickel treatment.
Genotypes P3 and L1 react very well to cadmium, lead, and the lower analyzed concentration of copper but very bad to nickel and the higher analyzed concentration of copper. The genotype A2 shows a very high sensitivity to copper but reacts better than all others to nickel in the lower analyzed concentration. Willows are species with phytoremediation potential having tolerance/resistance to heavy metals from air, water and soil (Watson, 2002)). The importance of genotype in the stress response of the plant is highlighted by the analyses of variance for the shoots and roots dry mass index. The Fisher test applied for each heavy metal reveal the hierarchy of heavy metal toxicity. The most toxic metal is copper followed by nickel and lead on one side and cadmium, with a stimulating effect on the other side. General reduction of willow plant growth under copper concentrations was highlighted by Mleczek et al. (2013).

Table 1. Characteristics of willow plants after two growing seasons according with genotypes and management practice (uncut and cut back) and biomass production after one growing season

Genotypes	Uncut/Cut back	No shoots/stool Mean ± SD	Maxim height (cm) Mean ± SD	Maxim diameter at the base of the shoots (mm) Mean ± SD	Biomass (t/ha)
P1	Uncut	3.8 ± 2.6	205 ± 58	23.98 ± 7.71	2.786
	Cut back	6.6 ± 3.0	203 ± 72	17.28^{0} ± 7.08	
P2	Uncut	2.8 ± 1.3	191 ± 78	26.24 ± 7.60	1.141
	Cut back	5.1 ± 2.7	123** ± 84	13.55^{000} ± 6.65	
P3	Uncut	3.4 ± 1.9	220 ± 52	24.18 ± 7.22	4.211
	Cut back	7.9 ± 3.7	225 ± 55	20.65 ± 5.51	
P4	Uncut	4.0 ± 1.5	199 ± 36	17.72 ± 3.97	1.328
	Cut back	11.8*** ± 5.8	185 ± 22	12.91 ± 3.69	
P5	Uncut	3.0 ± 1.4	235 ± 42	20.78 ± 7.17	1.069
	Cut back	6.2 ± 4.5	211 ± 37	17.22 ± 5.48	
T1	Uncut	3.4 ± 1.2	183 ± 37	30.97 ± 5.94	0.307
	Cut back	5.3 ± 4.6	159 ± 45	21.74^{000} ± 3.95	
S1	Uncut	2.1 ± 0.9	139 ± 39	20.69 ± 5.87	0.589
	Cut back	9.0*** ± 4.8	151 ± 42	19.07 ± 4.04	
S3	Uncut	7.6 ± 4.0	154 ± 28	18.43 ± 3.54	2.903
	Cut back	9.1 ± 4.2	146 ± 50	15.87 ± 1.58	
S4	Uncut	5.9 ± 2.9	197 ± 31	23.03 ± 5.22	1.971
	Cut back	7,9 ± 2.7	186 ± 39	11.78^{000} ± 2.25	
A1	Uncut	4.7 ± 2.9	249 ± 46	35.60 ± 6.73	1.868
	Cut back	9.4 ± 4.0	175^{00} ± 33	18.80^{000} ± 6.40	
A2	Uncut	7.6 ± 3.6	175 ± 40	22.80 ± 8.40	2.958
	Cut back	17.4*** ± 6.7	170 ± 33	14.48^{00} ± 2.72	
A3	Uncut	7.6 ± 4.2	163 ± 41	14.68 ± 5.40	1.978
	Cut back	15.1*** ± 4.0	134 ± 20	12.65 ± 3.18	
A5	Uncut	5.4 ± 2.9	111 ± 21	16.75 ± 4.88	1.039
	Cut back	11.9*** ± 5.4	103 ± 15	11.25 ± 3.05	
L1	Uncut	4.1 ± 2.0	166 ± 54	18.80 ± 7.03	3.698
	Cut back	9.9** ± 4.3	170 ± 43	15.10 ± 3.58	
L2	Uncut	5.4 ± 2.5	189 ± 76	22.07 ± 6.53	2.836
	Cut back	8.7 ± 3.6	180 ± 57	16.93 ± 6.17	
L4	Uncut	2.9 ± 1.0	96 ± 29	16.96 ± 8.09	0.918
	Cut back	6.1 ± 2.6	111 ± 34	15.17 ± 3.61	

Significance of the differences are indicated as * P < 0.05, ** P < 0.01, *** P < 0.001, positive differences and ^{0} P < 0.05, ^{00} P < 0.01, ^{000} P < 0.001, for the negative ones.

Table 2. The effect of genotype and management practice on main characteristic and biomass of *Salix* sp. plantation (Fisher Test)

Characteristc	Analysis of Variance: Marked effects are significant at p < .05000 Factors: 1- Genotyp; 2- management practice					
	1		2		1x2	
	F	P	F	p	F	p
No shoot	9.73290***	0.000000	141.2449***	0.000000	16.46291***	0.000000
Shoot height	14.41945***	0.000000	8.0453**	0.004761	8.79235***	0.000000
Diameter at the base of the shoot	8.35212***	0.000000	89.8912***	0.000000	11.38638***	0.000000
Dry biomass	2.754475***	0.000642				

Table 3. SDMI, RDMI and vitality in hydroponic heavy metal experiment

Genotype	Characteristic	Control	Cd		Cu		Ni		Pb	
			1	2	1	2	1	2	1	2
P3	SDMI	3.00	2.42	2.86	1.71	0.77	2.05	1.28	3.08	1.90
	RDMI	0.59	0.33	0.45	0.61	0.59	0.28	0.44	0.48	0.61
	Ratio S/R	5.19	9.43	6.41	3.17	1.77	8.22	6.09	6.44	3.29
	Vitality	5	5	5	5	2	2	1	5	5
A2	SDMI	1.04	0.78	0.85	0.41	0.27	0.54	0.45	1.12	0.27
	RDMI	0.32	0.19	0.34	0.53	0.40	0.12	0.14	0.38	0.21
	Ratio S/R	3.39	4.67	2.67	0.87	0.71	5.33	4.78	2.97	1.67
	Vitality	5	5	4	1	1	4	2	5	5
L1	SDMI	2.56	1.96	2.54	1.48	0.61	1.52	1.19	2.62	1.48
	RDMI	0.53	0.29	0.58	0.80	0.58	0.30	0.43	0.55	0.56
	Ratio S/R	4.87	6.76	4.39	1.95	1.07	5.00	4.36	5.00	3.02
	Vitality	5	5	5	5	2	3	2	5	5
L2	SDMI	2.19	2.03	2.42	1.07	0.55	0.90	0.30	2.17	0.45
	RDMI	0.60	0.54	0.61	1.09	0.88	0.24	0.35	0.47	0.34
	Ratio S/R	4.02	5.33	4.72	0.98	0.58	4.00	1.44	4.5	2.22
	Vitality	5	5	5	4	2	1	1	5	3

Table 4. Analysis of variance for hydroponic heavy metal experiment. The effect of genotype and heavy metal treatment on shoots and roots dry biomass

Character	Marked effects are significant at p < .05000 Factors: 1 - Genotype; 2 - Cd treatment; 3 - Cu treatment; 4 - Ni treatment; 5 - Pb treatment; 6 - All treatment													
	1		2		3		4		5		6		1 x 6	
	F	p	F	p	F	p	F	p	F	p	F	p	F	P
IBL	19.618 ***	0.00	0.93	0.405	21.656 ***	0.0	9.907 ***	0.0	8.655 ***	0.00	9.333 ***	0.00	14.74 ***	0.00
IBR	7.082 ***	0.00	2.34	0.112	2.696 *	0.08	5.556 **	0.01	0.613	0.55	5.101 ***	0.00	2.95 ***	0.00

According to the calculated indices (SDBI and RDBI) and plant vitality, an initial selection for tolerant willow clones can be made. All genotypes showed tolerance to cadmium and lead treatment. In other researches it was observed a large variation in willow clones Cd tolerance (Vyslouzilova et al., 2003; Zacchini et al., 2009).

According with Liu et al. (2011) the Cd accumulation increase during a short time course and have a slow absorption rate during a long time course. Zhivotovsky et al. (2011) showed that willow clones exposed to different external lead concentrations can tolerate and accumulate Pb to varied degrees. Except for genotype A2 which showed tolerance for a low concentration of nickel all the others showed no tolerance for this metal. In opposite with treatment with nickel, are the results for treatment with copper. In this case, the genotype A2 showed no tolerance, all the others showed tolerance for the lower copper concentration analyzed.

Heavy metal stress cause various biochemical responses of plants.

For two genotype, P3 and L1, the catalase increase with cadmium concentration increment or it quite similar like in genotype L2. Only genotype A2 does not follow this pattern. The peroxidase decrease with cadmium concentration increment for genotypes A2 and L2. The other genotypes react differently to cadmium increment, increase for P3 and slightly decrease with cadmium increment and decrease with cadmium increment for L2 (Figure 2).

In the copper experiment, for genotype P3 and L2 the catalase increase with metal increment, and the peroxidase decrease. The peroxidase increase also for the lower copper concentration and then decrease with copper increment in the case of A2 and L1 (Figure 3).

The same pattern can be shown for plant reaction to lead increment, a decrease of peroxidase. The catalase increase in case of a small lead concentration and decreases in case of high concentration for P3 and A2 and decreases for others (Figure 4).

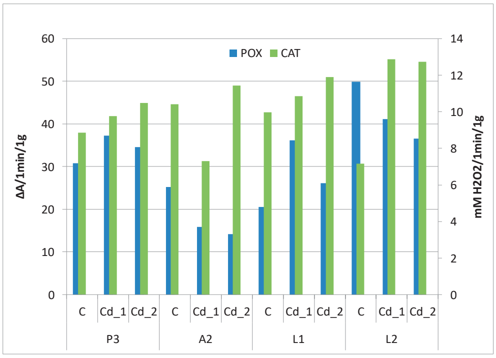

Figure 2. The variation of enzymatic activity, catalase (CAT) and peroxidase (POX) in cadmium experiment

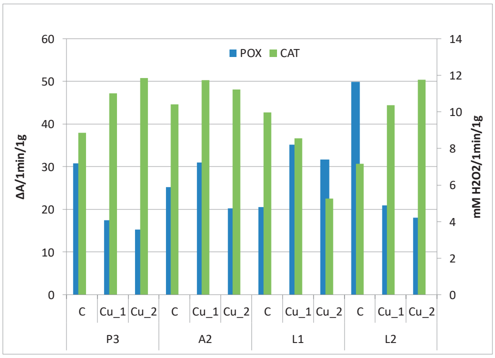

Figure 3. The variation of enzymatic activity, catalase (CAT) and peroxidase (POX) in copper experiment

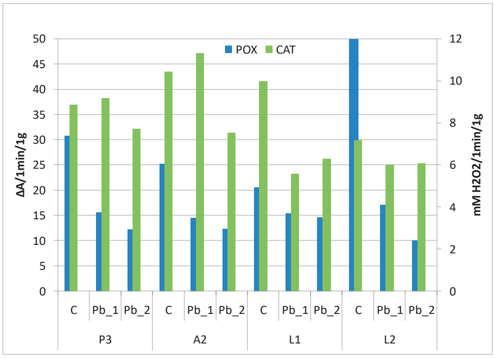

Figure 4. The variation of enzymatic activity, catalase (CAT) and peroxidase (POX) in lead experiment

The enzymatic activity, catalase, and peroxidase decrease with a lead increment for all genotypes and also for P3 and L2 in the copper experiment and L2 in the cadmium experiment. For the other experimental variants, there are no patterns.

CONCLUSIONS

The biometric characters varied significantly with genotype and management practice.
The behavior of willow cuttings varied according to heavy metal (cadmium, copper, nickel ad lead) level.
Cadmium acting like a stimulant for all genotypes. SDMI increase with cadmium increment level and decrease for all other heavy metal.
The enzymatic activity varied according to with stress abiotic factor and also with genotype.

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