UDC 630.5
Original scientific paper

VARIABILITY OF PHYSIOLOGICAL AND GROWTH CHARACTERISTICS OF WHITE WILLOW (*SALIX ALBA* L.) CLONES

Saša ORLOVIĆ[1], Slobodanka PAJEVIĆ[2], Bojana KLAŠNJA[1], Zoran GALIĆ[1] and Miroslav MARKOVIĆ[1]

[1]Institute of Lowland Forestry and Environment Novi Sad
[2]Faculty of Sciences Novi Sad, Department for Biology and Ecology

Orlović Saša, Slobodanka Pajević, Bojana Klašnja, Zoran Galić and Miroslav Marković (2006): *Variability of physiological and growth characteristics of white willow (Salix alba l.) clones.* - Genetika, Vol. 38, No. 2, 145-152.

The samples from field experiment on five clones of white willow (*Salix alba* L.) in the adult phase, was investigated as follows: net photosynthesis and dark respiration, content of photosynthetic pigments (chlorophylls a,b and carotenoids), number and size of stomata and elements of growth (tree diameters and heights). The aim of the research is to determine if there are any relations between these physiological characters and the elements of growth, i.e. if any of them can be utilized in the early selection for growth vigor. The results show that all the characters are characterized by low coefficients of variation, statistically highly significant differences and high coefficients of heritability in a broad sense. As for the net of photosynthesis, the number of stomata on the adaxial and abaxial sides of the leaf are in high correlation with the elements of growth, which indicates that they can be used in the early selection for growth vigor.

Key words: White willow, physiological characters, growth elements

Corresponding author: Saša Orlović, Institute of Lowland Forestry and Environment Novi Sad, Serbia.

INTRODUCTION

Tree-shaped willows in the conditions of Serbia are very important because they grow on the soils of heavier particle size composition and, in contrast to the other tree species on the alluvium, they can tolerate longer flood periods. Of all the tree-shaped willows, the dominant and the most important species is white willow (*Salix alba* L.) which, together with black poplar (*Populus nigra* L.), grows in the mixed association *Saliceto-Populetum*. Nowadays this association is practically disappearing, because by river regulation, the regime of underground waters has been changed and the flood period has been shortened. In the sites where flood periods are too long for poplars, intensive experimental-production plantations of white willow are established from clonal material obtained by long-term breeding.

Because of their high rate of growth, simple methods of regeneration, multiple use primarily in chemical (KOPITOVIĆ *et al.*, 1987) and mechanical processing of wood (KRSTINIĆ, 1966), willows are very suitable for research. Recently, the importance of this species has increased because of the creation of good conditions for the establishment of energy plantations, especially in the Scandinavian countries (RONNBERG - WASTLJUNG, 1996).

Previous work and research of tree-shaped willow breeding, after KRSTINIĆ, (1986), was based on the selection for the straightness of the stem and fast growth, utilization of the effects of spontaneous mutations and inter-species hybridization. In addition, much attention was drawn to the research of its adaptive value, i.e. the width of reaction norm (KRSTINIĆ, 1984; RONNBERG - WASTLJUNG, 1996), requirements of mineral nutrients (KOMLENOVIĆ and KRSTINIĆ, 1982), as well as its ability to form adventitious roots (KRSTINIĆ and KAJBA, 1988), in the aim of the maximum utilization of this species genetic potential. So far the research mainly included quantitative characters and genetic and phenotypic correlation willows (LIN and ZSSUFFA 1983) so as to increase the efficiency of selection. According to the available references, insufficient attention was drawn to the possibility of utilization of physiological characters in the early selection for growth vigor of white willow clones. Nowadays, in forest tree breeding, the trend is to make the time needed for the creation of varieties maximally short. In this aim, structurally functional relations are researched (anatomical structure of plant organs and physiological processes) which enables the definition of characters based on which the genotypes with desirable properties can be recognized in the earliest possible ontogenetical phase (ORLOVIĆ *et al.*,1996). The successful resolving of this problem shortens the period necessary for the research and lowers the financials (CEULEMANS *et al.*, 1987). One of the most desirable properties of willows, along with the low susceptibility to diseases and insects, is growth vigor. It is the result of complex genetic interactions, i.e. interactions of the genotype and the environment (ORLOVIĆ *et al.*, 1996).

This paper presents the results of the research of variability of several physiological characters of white willow clones, in the aim to research their potential utilization in the selection for growth vigor.

MATERIAL AND METHOD

The research in field conditions was carried out on the samples from field experiment. The experiment was established from the clones of *Salix alba* in 1983 by deep planting with seedlings 1/0, spacing 4.25 m x 4.25 m on fluvisol at Kać forest (45° 17' N, 19° 53' E, Elevation 76 m). In autumn 1995 the samples of completely formed leaves exposed to light were determined as follows: net of photosynthesis and dark respiration, content of photosynthetic pigments, and the number and size of stomata. Diameters were measured at the height of 1.30 m and tree heights precisely to within 10 cm.

The leaf samples were taken from the top of the crown and all analyses and measurements have been done on the completed formed leaves fully exposed to light.

The net photosynthesis and dark respiration were determined polarographically, by using Clark¢s electrode. The net of respiration was determined by the quantity of absorbed oxygen (in the dark) and photosynthesis by the quantity of released oxygen in mmol m^{-2} s^{-1}. In the analysis, very thin leaf slices (up to 0.5 mm) without nerves, after JONES and OSMOND (1973). The process of photosynthesis was carried out under complete saturation with white light produced by quartz -iodine lamp (STANKOVIĆ and WALKER, 1977). The content of photosynthetic pigments was discovered in acetone solution by Wettstein¢s method. The number and size of stomata was determined by the WOLF, (1950) method. By this method, microscopic preparations were made and the number of stomata per mm^2 was determined as well as their length and width in mm. The data were processed by the standard statistical method, and the Tables present the arithmetical means, coefficients of variation, LSD test, correlations and heritability in the broad sense (by the formula: $h^2_{bs} = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_e^2}$; s^2_c - Genotypic variance; s^2_e = Environmental variance) (WRIGHT, 1962)

RESULTS AND DISCUSSION

Table 1. presents the results of the research of net photosynthesis and dark respiration intensities. The results show that the highest net (rate) was that of the clone 107/65/6 with 17.14 mmol $m^{-1}s^{-1}$, and the lowest was 282 (12.52 mmol $m^{-1}s^{-1}$). The highest intraclonal variability of this parameter was measured for the clone 377 (11.00%), and the lowest for the clone 107/65/8 (7.77%). LSD test determined that all the clones except the clone 282 occur within one interval of homogeneity, i.e. that between them there are no statistically significant differences.

Respiration net (rate) was the highest for the clone 73/64/8 (11.70 mmol $m^{-1}s^{-1}$), and the lowest for the clone 107/65/8 (7.47 mmol $m^{-1}s^{-1}$). The characteristic of this parameter is that three clones (378, 107/65/6, 377) had very low, and the other two clones (73/64/8 and 282) had high coefficients of variation. By the

analysis of variance, it was observed that the differences between the clones were statistically highly significant, which is also proved by LSD test, by which the researched clones were grouped into two intervals of homogeneity.

Table 1. - Net photosynthesis and respiration (mmol $m^{-1}s^{-1}$)

Clone	Net Photosynthesis		Respiration	
	Mean value	Vk	Mean value	Vk
378	15.28 a	9.93	8.19 b	2.11
107/65/6	17.14 a	7.77	7.47 b	2.58
377	15.86 a	11.00	9.55 a	2.00
73/64/8	16.24 a	9.55	11.70 a	15.41
282	12.52 b	11.60	10.12 a	14.42
H^2_{bs}	0.91		0.90	
LSD<0.05				

For the net photosynthesis and dark respiration, very high coefficients of heritability in the broad sense were obtained (photosynthesis 0.91 and respiration 0.90).

The results of the research of the quantitative content of photosynthetic pigments (chlorophyll a, b and carotenoids) have been shown in Table 2. They show that the difference between the clones is not statistically significant, regarding the content of pigments in the fresh substance, which is also proved by the fact that by LSD test a new interval of homogeneity was formed. The highest content of chlorophyll was in the clones 377 and 73/64/8 (1.0948 mg), and the lowest 107/65/6 (0.723 mg). The content of chlorophyll b was the highest in the clone 282 (0.4090 mg), and the lowest in the clone 378 (0.3768 mg). The content of chlorophyll a+b was between 1.1231 mg (clone 107/65/6) and 1.6270 mg (clone 378). The contents of these pigments were characterized by low coefficients of variation, which means practically that intraclonal variability was small. The highest content of carotenoid was in the clone 378 (1.2134 mg), and the lowest 107/65/6 (0.6550 mg). The contents of carotenoid varied in the clones 107/65/6 and 73/64/8, while in the other clones it was much more stable, with a low coefficient of variation.

As for the content of photosynthetic pigments, different coefficients of heritability in the broad sense were determined, i.e.: for the content of chlorophyll a 0.93; chlorophyll b 0.45; chlorophyll a+b 0.63 and carotenoids 0.88.

The number and size of leaf stomata in the researched white willow clones have been shown in Table 3. The results of the research show that all the clones had a higher number of stomata on the abaxial compared to the adaxial surface. The highest number of stomata per mm^2 of leaf area was in the clone 377 (81 adaxial and 91 abaxial), and the lowest number was in the clone 282 (51 adaxial and 61 abaxial). LSD test produced several intervals of homogeneity, which means that the clones showed the highly significant statistical difference.

Table 2. - Content of photosynthetic pigments (mg/g of fresh substance)

Clone	Chlorophyll a		Chlorophyll b		Chlorophyll a+b		Carotenoids	
	Mean value	Vk	Mean value	Vk	Mean value	Vk	Mean value	Vk
378	1.0705 a	1.73	0.3768 a	3.99	1.6270 a	3.06	1.2134 a	2.29
107/65/6	0.7230 a	9.49	0.3957 a	1.65	1.1231 a	8.07	0.6550 b	8.34
377	1.0948 a	3.84	0.3824 a	8.87	1.4564 a	1.31	0.9434 a	2.35
73/64/8	1.0948 a	10.64	0.4042 a	9.23	1.4991 a	1.72	1.0565 a	11.40
282	0.9248 a	2.50	0.4090 a	4.22	1.3272 a	2.71	0.7950 a	2.93
H^2_{bs}	0.93		0.45		0.63		0.88	
LSD<0.05								

Table 3. - Number, length and width of stomata

Clone	Stoma number per mm^2				Stoma length (μm)				Stoma width (μm)			
	Adaxial		Abaxial		Adaxial		Abaxial		Adaxial		Abaxial	
	Mean value	Vk	Mean value	Vk	Mean value	Vk	Mean value	Vk	Mean value.	Vk	Mean value	Vk
378	55 c	4.21	76 b	5.18	21.92 c	3.18	22.12 c	4.22	16.44 ab	4.13	19.00 b	5.16
107/65/6	71 b	6.16	78 b	6.33	22.90 b	5.11	23.75 b	6.12	14.40 b	5.10	16.48 c	6.16
377	81 a	4.11	91 a	2.82	24.28 b	5.82	24.08 b	5.93	22.60 a	4.18	19.76 a	4.00
73/64/8	57 c	5.17	64 c	5.43	26.60 a	5.66	24.36 b	4.21	18.28 ab	3.82	15.88 c	4.61
282	51 d	4.82	61 d	5.88	24.76 b	4.88	29.76 a	3.16	18.24 ab	3.89	16.40 c	3.33
h^2_{bs}	0.89		0.90		0.82		0.89		0.73		0.88	

The stomatal length on the upper epidermis (adaxial) ranged between 21.92 mm (clone 378) to 26.60 mm (clone 73/64/8). On the lower epidermis (abaxial), stomatal length ranged between 22.12 mm to 29.76 mm. As for this parameter, statistically highly significant differences were observed on both surfaces of the leaf.

The largest width of stomata on both surfaces of the leaf was found for the clone 377 (22.60 mm adaxial and 19.76 mm abaxial), and the smallest width, for the clone 107/65/6 (14.40 mm) on the adaxial and 73/64/8 (15.88 mm) on the abaxial epidermis.

All the stomatal characters (number, length and width) are characterized by high coefficients of heritability in the broad sense and low coefficients of variation.

The largest tree diameters (Table 4) were in the clone 377 (15.75 cm), and the smallest - the clone 282 (14.55 cm). As for this parameter, the analysis of variance showed statistically highly significant differences between the clones, which is also proved by the differentiation of the clones in several intervals of homogeneity.

Height growth (Table 4.) was best for the clone 377 (18.45 m), and the least for the clone 378 (12.45 m). LSD test showed the existence of two intervals

of homogeneity, which means that, as regards this parameter, the clones showed statistically highly significant differences.

Table 4. - Tree diameters and heights in the 13-year old plantation

Clone	DIAMETER (cm)		HEIGHT (m)	
	Mean value	Vk	Mean value	Vk
378	15.60 a	4.93	12.45 b	5.75
107/65/6	15.45 ab	10.97	14.05 a	2.30
377	15.75 ab	4.95	18.45 a	7.75
73/64/8	14.85 ab	5.55	13.40 a	7.45
282	14.55 b	9.02	13.95 a	8.24
h^2_{bs}	0.65		0.54	

The correlation analysis was performed in order to research the relationship between the analyzed physiological characters and the elements of growth. Table 6 shows the coefficient of correlation between the researched characters and the elements of growth.

The results show that the following were in high positive correlation with tree diameters: net photosynthesis, stoma number (adaxial and abaxial) and stoma width (adaxial), and with tree height: net photosynthesis, stoma number (adaxial) and stoma width (adaxial) (Table 5.).

Table 5. - Coefficients of correlation between the researched physiological characters and the elements of growth

	1*	2	3	4	5	6	7	8	9	10	11	12
Diameter	0.73	-0.63	0.72	0.94	-0.62	-0.80	0.12	0.79	0.09	-0.11	0.16	0.21
Height	0.77	0.06	0.84	0.68	0.22	0.03	0.80	0.53	0.19	-0.21	-0.10	-0.23

* 1-net photosynthesis; 2-respiration; 3-stoma number - adaxial; 4-stoma number - abaxial; 5-stoma length - adaxial; 6-stoma length abaxial; 7-stoma width - adaxial; 8- stoma width - abaxial; 9-content of chlorophyll a; 10-content of chlorophyll b; 11-content of chlorophyll a+b; 12-content of carotenoids

DISCUSSION

The results of the research of several physiological characters and elements of growth indicate that there is a very marked variability within the species *Salix alba*. The greatest number of these characters is characterized by low coefficients of variation and by statistically significant differences between the clones (except the contents of photosynthetic pigments), which leads to a conclusion that these characters are under genetic control to a high extent. The net photosynthesis and respiration were within the limits of previous researches by several authors (PELKONEN, 1984; CEULEMANS and SAUGIER, 1991). The net photosynthesis was in a high positive correlation with the elements of growth,

which has so far been observed for several woody species: poplars (ISEBRANDS *et al.*, 1988; ORLOVIĆ, 1995), larch (MATYSSEK and SCHULZE, 1987; 1987a). This means practically that this parameter can be used unfailingly in the selection for growth vigor. The content of photosynthetic pigments varied depending on the clone and it was not in high correlation with the elements of growth. This parameter is important for the total productivity and for growth elements if another criterion also used in the early selection is the period of leaf surface duration, which is today very successfully applied in maize breeding by stay green method. The number of stomata on the upper epidermis (adaxial) was also in the positive correlation with the elements of growth, which has already been observed for the clones of black poplar (ORLOVIĆ, 1993). The researched physiological characters are characterized by high coefficients of heritability in the broad sense (h^2_{bs}), which has a high significance in the enhancement of selection methods in the aim of maximum utilization of the genetic potential of tree-shaped willows.

REFERENCES

CEULEMANS, R., IMPENS, I. (1983): Net CO_2 exchange rate shoot growth of young poplar (*Populus*) clones. Journal of Experimental Botany., 34:866-870.

CEULEMANS, R., IMPENS, I., STEENACKERS, V. (1987): Variations in photosynthetic, anatomical and enzymatic leaf traits and correlations with growth in recently selected *Populus* hybrids. Canadian Journal of Forest Research., 17: 273-283,

CEULEMANS, R., SAUGIER, B., (1991): Photosynthesis. In: PHYSIOLOGY OF TREES. A.S. Raghavendra (Editor) N° 2::21-50. John Wiley & Sons, Inc.

ISEBRANDS, J., CEULEMANS, R., WIARD, B. M., (1988); Genetic variation in photosynthetic traits among *Populus* clones in relation to yield. Plant physiology and Biochemistry., 26: 427-437

JONES, H. G. OSMOND, C. B., (1973): Photosynthesis by thin leaf slices in solution. I Properties of leaf slices and comparison with whole leaves. Australian Journal of Biological Sciences, 26:15-24

KOMLENOVIĆ, N., KRSTINIĆ, A., (1982): Genotipske razlike između nekih klonova stablastih vrba s obzirom na stanje ishrane. Topola 133-134:29-40

KOPITOVIĆ, S., KLASNJA, B., KORALIJA Z., (1987): Uticaj svojstava drveta pojedinih klonova topole i vrbe na osobine poluceluloze po neutralno-sulfitnom (NSSC) postupku. Radovi Instituta za topolarsvo 18: 215-234

KRSTINIĆ, A., (1986): Stablaste vrbe. In: POPLARS AND WILLOWS IN YUGOSLAVIA.V. Guzina (Editor) pp. 84-102

LIN, J., ZSUFFA, L. (1993): Quantitative genetic parameters for seven characters in clonal test of *Salix eriocephala*. II Genetic and environmental correlations and efficiency of indirect selection. Silvae Genetica 42: 126-131

MATYSSEK, R., SCHULZE, E. D., (1987): Heterosis in hybrid larch (*Larix decidua leptolepis*). I. The role of leaf characteristics. Trees 1: 218-224

MATYSSEK, R., SCHULZE, E. D. (1987): Heterosis in hybrid larch (*Larix decidua x leptolepis*). II. Growth characteristics. Trees 1: 225-231

ORLOVIĆ, S. (1993): Proučavanje morfologije i varijabilnosti stoma topola. M.Sc. Thessis, Šumarski fakultet Beograd. p.118

ORLOVIĆ, S., PAJEVIĆ, S., KRSTIĆ, B. (1995): Possibility of utilizing some morphological and physiological characters in poplar breeding. IUFRO XX World Congress, Poster abstracts. pp. 70

ORLOVIĆ, S., GUZINA, V., KRSTIĆ, B., MERKULOV, LJ. (1998): Genetic variability in anatomical, physiological and growth characteristics of hybrid poplar (Populus x euramericana Dode (Guinier)) and eastern cottonwood (Populus deltoides Bartr.) clones. Silvae Genetica 47 (4): 183-190

PELKONEN, P., (1984): Carbon dioxide in Willow clones. In Ecology And Management Of Forest Biomass Production Systems. K. Perttu (Editor) Rep. 15: 187-196 Department of Ecology and Environmental Research. Swedish University of Agricultural Sciences

RONNBERG - WASTLJUNG A. S., (1988): Inter- and intra specific variation and genotype x site interaction in *Salix alba* L. *S. dasyclados* Wimm. and *S. viminalis* L. Scandinavian Journal of Forest Research 3: 449-463

RONNBERG - WASTLJUNG A. S., (1996): Genetics of quantitative characters. Dissertation, Swedish University of Agricultural Sciences. Uppsala, p.35

STANKOVIĆ, Z. S., WALKER, D. A., (1977): Photosynthesis by isolated pea chloroplasts. Some effects of adenylates and inorganic pyrophosphate. Plant Physiology 59: 428-432

WETTSTEIN, D., (1957): Chlorophyll-letale und der submikroskopisehe Formweehsel der Plastiden. Exp. Cell. Res. 12 (3), 427-506

WOLF, L., (1950): Mikroskopicka tehnika, Praha

WRIGHT, I. W., (1962): Genetics of forest tree improvement. FAO, Rome, 399 pp.

Received May 17, 2006
Accepted July 3, 2006

VARIJABILNOST FIZIOLOŠKIH I PARAMETARA RASTA KLONOVA VRBA (*SALIX ALBA* L.)

Sasa ORLOVIĆ[1], Slobodanka PAJEVIĆ[2], Bojana KLAŠNJA[1], Zoran GALIĆ[1] i Miroslav MARKOVIĆ [1]

[1]Poljoprivredni fakultet Novi Sad, Institut za nizijsko šumarstvo i životnu sredinu, Novi Sad
[2]Prirodno matematički fakultet Novi Sad, Departman za biologiju i ekologiju, Novi Sad, Srbija

Izvod

Na uzorcima pet klonova bele vrbe (*Salix alba* L.) iz poljskog višegodišnjeg ogleda istraživana je varijabilnost neto fotosinteze i disanja, sadržaja fotosintetičkih pigmenata (a, b i karotenoidi), broja i veličine stoma, i elemenata rastenja. Cilj istraživanja je bio da utvrdi da li postoji veza između istraživanih fizioloških parametara i elemenata rastenja i da li se oni mogu koristiti u ranoj selekciji na bujnost rasta. Rezultati istraživanja su pokazali da svi ispitivani parametri imaju male koeficijente varijacije i visoku naslednost i širem smislu kao i da su razlike uzmeđu klonova bile statistički značajne. Neto fotosinteza i broj stoma su bili u visokoj korelaciji sa elementima rasta što upućuje na to da se mogu koristiti u selekciji na bujnost rasta.

Primljeno 18. maja 2006.
Odobreno 3. jula 2006.