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Małgorzata MIKICIUK[1], Jacek WRÓBEL[1]
and Paulina JAKOWIENKO[2]

EFFECT OF THE DIVERSE CONCENTRATION OF SODIUM CHLORIDE IN THE MEDIUM ON THE CONTENT OF ASSIMILATION PIGMENTS AND THE BIOMETRIC FEATURES OF BASKET WILLOW (*Salix viminalis* L.) CULTIVATED IN HYDROPONICS

WPŁYW ZRÓŻNICOWANEGO STĘŻENIA CHLORKU SODU W POŻYWCE NA ZAWARTOŚĆ BARWNIKÓW ASYMILACYJNYCH I CECHY BIOMETRYCZNE WIERZBY WICIOWEJ (*Salix viminalis* L.) UPRAWIANEJ W HYDROPONICE

Abstract: In 2006 a hydroponic vegetation experiment was carried out under controlled conditions in the laboratory of the Department of Plant Physiology University of Agriculture in Szczecin. The experiment was conducted using a complete randomization method in a two-factor system in three replications Three series of experiments were performed during the period from April to June. The first experimental factor was a concentration of NaCl in the medium (0.068, 0.136 and 0.170 mol NaCl · dm^{-3}, control test – complete Hoagland's medium), whereas the other factor was a willow clone. The following biometric features were determined: the length of a shoot, the number of leaves on the shoot, fresh and dry matter of shoots. The content of assimilation dyes in the leaves of willow was also determined, 48 and 168 hours after the salinization of the media.

Keywords: *Salix viminalis*, clones 'Bjor', 'Jorr' and 'Tora', salinity stress, assimilation pigments, fresh matter, dry matter

Basket willow (*Salix viminalis*) is used in the protection of environment and in the management of wasteland. Its biomass becomes a very important and economically effective aspect of agricultural production [1]. Its characteristic feature is a low ash content (1–3 %), the ash formed during combustion can be used as a mineral fertilizer [2–4]. The phenomenon of a too strong concentration of salt in a medium resulting in

[1] Chair of Plant Physiology, West Pomeranian University of Technology in Szczecin, ul. J. Słowackiego 17, 71–424 Szczecin, Poland, email: malgorzata.mikiciuk@zut.edu.pl

[2] Department of Horticulture, West Pomeranian University of Technology in Szczecin, ul. J. Słowackiego 17, 71–434 Szczecin, Poland, email: paulina.jakowienko@zut.edu.pl

a salt stress is caused, first of all, by anthropogenic factors, eg by application an excessive amount of salt to fertilizers or by using these substances to clear the roads of ice in winter [5, 6]. Particularly toxic to trees are chlorine and isoosmotic solutions of NaCl [7]. Considering the fact that there is not much information on the effect of salt stress on various genotypes of *Salix viminalis* in available literature, particularly in regards of a juvenile vegetative stage, studies were started in order to assess the physiological reaction of three clones of basket willow ('Bjor', 'Jorr' and 'Tora') to an increased concentration of NaCl in hydroponics.

Material and methods

Biological material for the studies consisted of three clones of basket willow (*Salix viminalis*): 'Bjor', 'Jorr' and 'Tora', the cuttings of which were from the plantation of the Department of Plant Physiology University of Agriculture in Szczecin.

The hydroponic vegetation experiment was carried out under controlled conditions in the laboratory of the Department of Plant Physiology, University of Agriculture in Szczecin in 2006. A method of complete randomization in the two factor system was used in three replications. The first experimental factor was the concentration of NaCl in a medium (0.068, 0.136 and 0.170 mol NaCl · dm^{-3}, the control test – complete Hoagland's medium), the second was a clone of willow. Three independent series of the experiment were carried out in April, May and June and three replications in each series. Willow cuttings were placed in 1 dm^3 glass containers (2 pieces in a container) filled with complete Hoagland's medium. After about 14 days when the cuttings had rooted and the shoots had reached the length of several centimeters, the concentration of NaCl in the media was differentiated according to the experimental combination.

During the juvenile period of willow, ie 168 hours (7 days) from the differentiation of the concentration of NaCl in the media, the following biometric features of the studied clones (the average of the three replications of each experimental combination) were determined: the length of each shoot, the number of leaves on a single shoot, the complete yield of fresh and dry matter of shoots in each plant.

The content of assimilation pigments in the leaves of willow were determined after 48 hours (2 days) and 168 hours (7 days) from the differentiation of the concentration of NaCl in the media, using Lichtenthaler and Wellburn's method [8]. Material for the studies was taken from three representative plants of each experimental combination.

The obtained results of the studies were verified by means of a two-factor analysis of variance in the system of complete randomization. In order to determine the differences between the averages and for the interaction, Tukey's half-intervals at confidence of $\alpha = 0.05$ were calculated. Due to the uniformity of variance of error, a synthesis of the results of three experimental series was performed.

The results and their analysis

The length of individual shoots of *Salix viminalis* growing in the media of differentiated concentration of NaCl did not differ significantly. The largest difference of the

length of a single shoot was observed between the combination of 0.170 mol NaCl · dm^{-3} (19.0 cm) and the control combination (26.4 cm). No significant differences were observed in the length of shoots of the compared clones of willow, either. A slightly larger length of the clone shoots than that of the remaining clones was observed in clone 'Jorr' (25.2 cm) – Table 1.

Table 1

The mean length of a single shoot of *Salix viminalis* [pieces]

Concentration of NaCl [mol · dm^{-3}]	Clone			Mean I
	'Bjor'	'Jorr'	'Tora'	
Control	25.2	30.0	24.2	26.4
0.068	17.0	28.0	26.7	23.9
0.136	22.5	23.8	20.0	22.1
0.170	23.2	18.8	15.0	19.0
Mean II	21.9	25.2	21.4	
$LSD_{0.05}$ for:	concentration of NaCl (I) – ns clone (II) – ns interaction I × II and II × I – ns			

ns – non-significant.

A significant effect of NaCl concentration in the medium on the average number of leaves on individual shoots of basket willow was recorded, for the largest number of leaves on a shoot was characteristic of the plants growing under the controlled conditions (24 pieces) and in the medium of the smallest concentration of NaCl (21 pieces). Whereas the smallest number of leaves (18 and 16 pieces) was characteristic of the plants grown hydroponically in the medium of the following concentration: 0.136 and 0.170 mol NaCl · dm^{-3} (Table 2). Similar results of the studies were obtained by Stark [6]. She observed that the strongest inhibition of the growth of leaves occurred in the plants that were in saline stress.

Table 2

The mean number of leaves on a shoot *Salix viminalis* [pieces]

Concentration of NaCl [mol · dm^{-3}]	Clone			Mean I
	'Bjor'	'Jorr'	'Tora'	
Control	21	25	25	24
0.068	15	25	24	21
0.136	18	21	15	18
0.170	19	15	14	16
Mean II	18	21	19	
$LSD_{0.05}$ for:	concentration of NaCl (I) – 5.2 clone (II) – ns interaction I × II and II × I – ns			

ns – non-significant.

Significantly the largest yield of fresh and dry matter of shoots was obtained by willows growing under controlled conditions, whereas the smallest by those growing in the medium of 0.170 mol NaCl · dm^{-3} concentration (Figs. 1, 2). Significantly a smaller amount of fresh and dry matter of shoots than in the remaining clones, was observed in clone 'Bjor'. Comparing all the experimental variants, it can be concluded that the largest yield of fresh matter of shoots was obtained in all the clones of willow under the controlled conditions and in clones 'Jorr' and 'Tora' growing in the medium of 0.068 mol NaCl · dm^{-3} concentration, whereas the smallest yield was observed in all the clones at the highest concentration of NaCl in hydroponic media (Fig. 1). Significantly the largest content of dry matter of willow shoots was recorded under

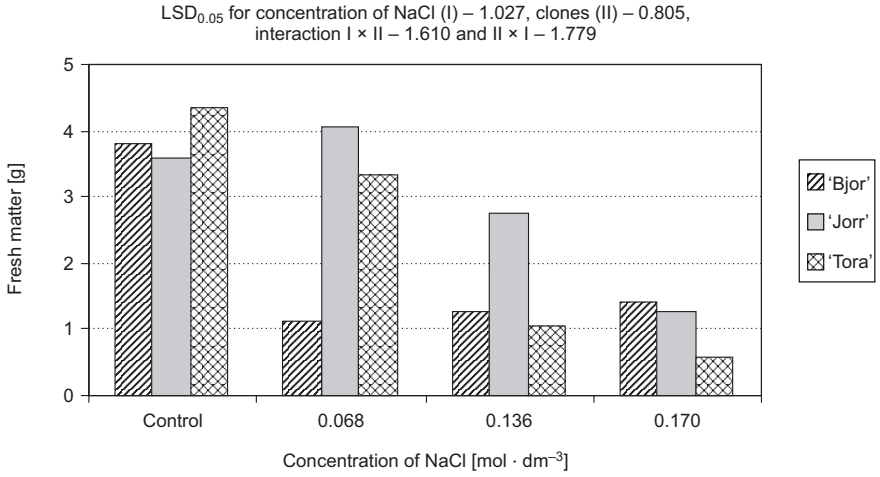

Fig. 1. The total yield of fresh matter of shoots *Salix viminalis* [g · $plant^{-1}$]

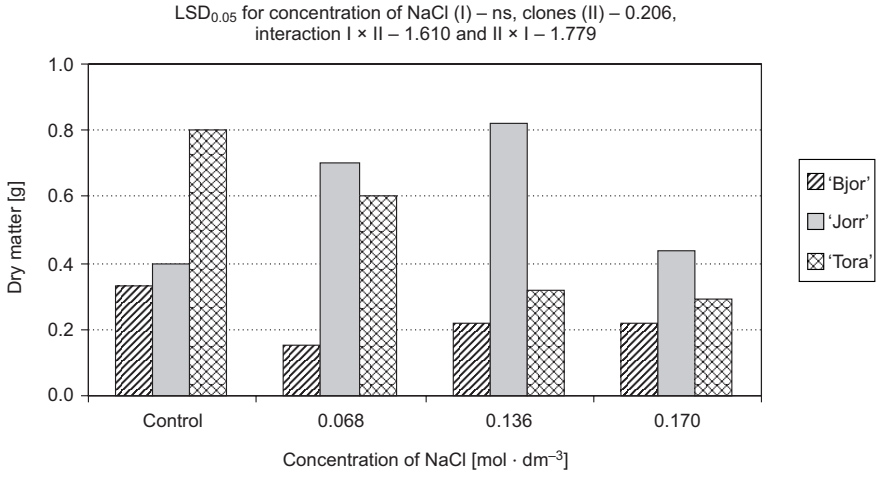

Fig. 2. The total yield of dry matter of shoots *Salix viminalis* [g · $plant^{-1}$]

controlled conditions (0.51 g), whereas the smallest, at the highest dose of NaCl (0.32 g). Significance of the influence of experimental factors on the yield of dry matter of willow shoots was also determined, for the largest dry matter was characteristic of clones 'Tora' and 'Jorr' under conrolled conditions and at the lowest concentration of NaCl and clone 'Jorr' at 0.136 mol NaCl · dm^{-3} concentration (Fig. 2).

It was observed that an increase in the concentration of NaCl in the medium resulted in a decrease in the yield of both fresh and dry matter of *Salix viminalis*. Similar results were obtained by Gregorczyk et al [1].

Under the conditions of excessive salinity of the medium, the photosynthetic apparatus of a plant is damaged. Then the efficiency of both phases of photosynthesis decreases [9]. According to many authors, the reaction to salt stress is also a decrease in the content of assimilation pigments – chlorophyll and carotenoids – in plant leaves [5, 10, 11]. At the first time of the determination (48 hours from the salinization of hydroponic media) no significant influence of experimental factors on the content of all the studied assimilation dyes in the leaves of *Salix viminalis* was observed. Whereas at the second time of the determination, the highest concentration of chlorophyll (*a*, *b* and complete), was characteristic of the leaves of willow growing in the highest concentration of NaCl. A stimulating effect of this concentration of salt on the production of chlorophyll in willow leaves was observed in clone 'Jorr' in particular. Also at this time, clone 'Jorr' was characterized by the largest amount of both chlorophyll and carotenoids, as compared with all the clones. 168 hours after the salinization of hydroponic media, a significant effect of NaCl concentration on the concentration of carotenoids in willow leaves, was also observed, for the largest amount of these dyes was recorded in plants growing in the medium of 0.170 mol NaCl · dm^{-3} concentration (Table 3).

Table 3

Content of chlorophyll *a*, *b*, *a* + *b* and carotenoids in leaves of *Salix viminalis* [mg · g^{-1} f.m.]

Clone	I time					II time				
	Concentration of NaCl [mol · dm^{-3}]				Mean II	Concentration of NaCl [mol · dm^{-3}]				Mean II
	Control	0.068	0.136	0.17		Control	0.068	0.136	0.17	
Chlorophyll *a*										
'Bjor'	1.156	0.757	1.323	0.975	1.053	1.655	1.406	1.563	0.966	1.398
'Jorr'	1.184	1.108	0.899	1.246	1.109	1.523	1.362	1.657	3.436	1.995
'Tora'	1.498	1.332	1.484	1.223	1.384	1.705	1.613	1.581	1.599	1.625
Mean I	1.280	1.066	1.235	1.148		1.628	1.460	1.600	2.000	
$LSD_{0.05}$ for:	concentration of NaCl (I) – ns clone (II) – ns interaction I × II and II × I – ns					concentration of NaCl (I) – 0.408 clone (II) – 0.323 interaction I × II i II × I – 0.646 and 0.707				
Chlorophyll *b*										
'Bjor'	0.510	0.302	0.569	0.439	0.455	0.631	0.551	0.669	0.401	0.563
'Jorr'	0.524	0.498	0.382	0.616	0.505	0.607	0.546	0.727	1.745	0.906

Table 3 contd.

Clone	I time					II time				
	Concentration of NaCl [mol · dm^{-3}]				Mean II	Concentration of NaCl [mol · dm^{-3}]				Mean II
	Control	0.068	0.136	0.17		Control	0.068	0.136	0.17	
'Tora'	0.707	0.599	0.708	0.591	0.651	0.704	0.652	0.663	0.738	0.689
Mean I	0.508	0.466	0.553	0.549		0.648	0.583	0.686	0.961	
$LSD_{0.05}$ for:	concentration of NaCl (I) – ns clone (II) – ns interaction I × II and II × I – ns					concentration of NaCl (I) – 0.198 clone (II) – 0.156 interaction I × II and II × I – 0.313 and 0.343				
Chlorophyll *a* + *b*										
'Bjor'	1.666	1.059	1.892	1.414	1.508	2.286	1.957	2.232	1.368	1.961
'Jorr'	1.707	1.610	1.280	1.863	1.615	2.130	1.908	2.385	5.181	2.901
'Tora'	2.206	1.930	2.192	2.008	2.084	2.410	2.265	2.243	2.336	2.314
Mean I	1.860	1.533	1.788	1.762		2.275	2.043	2.287	2.961	
$LSD_{0.05}$ for:	concentration of NaCl (I) – ns clone (II) – ns interaction I × II and II × I – ns					concentration of NaCl (I) – 0.605 clone (II) – 0.478 interaction I × II and II × I – 0.956 and 1.047				
Carotenoids										
'Bjor'	0.478	0.497	0.509	0.440	0.481	0.642	0.570	0.674	0.461	0.587
'Jorr'	0.471	0.455	0.376	0.551	0.463	0.611	0.541	0.731	1.557	0.860
'Tora'	0.619	0.528	0.620	0.614	0.595	0.674	0.632	0.670	0.750	0.682
Mean I	0.523	0.493	0.502	0.535		0.642	0.581	0.691	0.923	
$LSD_{0.05}$ for:	concentration of NaCl (I) – ns clone (II) – ns interaction I × II and II × I – ns					concentration of NaCl (I) – 0.170 clone (II) – 0.134 interaction I × II and II × I – 0.269 and 0.295				

ns – non-significant.

Similar results of the studies concerning the impact of salt concentration on the content of assimilation pigments in leaves of various genotypes of basket willow were obtained by Wrobel and Gregorczyk [5]. According to these authors, clone 'Jorr' in differentiated concentrations of NaCl of the medium was distinguished by the largest amount of photosynthetic dyes.

Conclusions

1. Increasing concentration of NaCl in hydroponic media resulted in the reduction of the mean number of leaves on a shoot of basket willow.

2. Willows growing under the conditions of the highest salinity gave the lowest yield of fresh matter of shoots.

3. The applied concentration of NaCl did not cause any increases in either chlorophyll or carotenoids in willow leaves. Particularly high concentration of assimilation pigments was characteristic of clone 'Jorr'.

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WPŁYW ZRÓŻNICOWANEGO STĘŻENIA CHLORKU SODU W POŻYWCE NA ZAWARTOŚĆ BARWNIKÓW ASYMILACYJNYCH I CECHY BIOMETRYCZNE WIERZBY WICIOWEJ (*Salix viminalis* L.) UPRAWIANEJ W HYDROPONICE

[1] Zakład Fizjologii Roślin
[2] Katedra Ogrodnictwa
Zachodniopomorski Uniwersytet Technologiczny w Szczecinie

Abstrakt: Hydroponiczne doświadczenie wegetacyjne przeprowadzone zostało w 2006 roku, w kontrolowanych warunkach, w laboratorium Katedry Fizjologii Roślin Akademii Rolniczej w Szczecinie. Doświadczenie założono metodą kompletnej randomizacji w układzie dwuczynnikowym, w trzech powtórzeniach. Wykonano trzy serie doświadczeń w miesiącach kwiecień – czerwiec. Pierwszy czynnik doświadczalny stanowiło stężenie NaCl w pożywce (0,068; 0,136 i 0,170 mol NaCl · dm^{-3}, kontrola – pożywka pełna Hoaglanda), drugi natomiast klon wierzby. Określono następujące cechy biometryczne roślin: długość pędu, liczba liści na pędzie, świeża i sucha masa pędów. Oznaczono również zawartość barwników asymilacyjnych w liściach wierzby, 48 oraz 168 godzin po zasoleniu pożywek.

Słowa kluczowe: *Salix viminalis*, klony ‘Bjor’, ‘Jorr’, ‘Tora’, stres solny, barwniki asymilacyjne, świeża masa, sucha masa