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**EFFECTS OF 2-HYDROXYBENZOIC ACID ON THE EFFICIENCY
OF THE PHOTOSYNTHETIC APPARATUS OF BASKET WILLOW
(*Salix viminalis* L.) PLANT GROWING UNDER CONDITIONS
OF SALT STRESS**

**WPŁYW KWASU 2-HYDROKSYBENZOESOWEGO NA WYDAJNOŚĆ
APARATU FOTOSYNTETYCZNEGO WIERZBY WICIOWEJ
(*Salix viminalis* L.) ROSNĄCEJ W WARUNKACH STRESU SOLNEGO**

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Streszczenie. Celem badań, przeprowadzonych w 2016 r. w Katedrze Fizjologii Roślin i Biochemii Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie, była ocena wpływu egzogennej aplikacji kwasu 2-hydroksybenzoowego (kwasu salicylowego) na wydajność aparatu fotosyntetycznego wierzby wiciowej rosnącej w warunkach podwyższonego stężenia soli. Pierwszym czynnikiem doświadczalnym było stężenie chlorku sodu (kontrola – pożywka pełna Hoaglanda, $3,0 \text{ g NaCl l} \cdot \text{dm}^{-3}$) drugim czynnikiem – stężenie kwasu 3-hydroksybenzoowego (kontrola – pożywka pełna, $138 \text{ mg} \cdot \text{dm}^{-3}$). Klon Tora dobrze tolerował krótki okres ekspozycji na zastosowane stężenie NaCl, o czym świadczą duże wartości takich wskaźników, jak wskaźnik witalności PSII (PI), wskaźnik Area oraz maksymalna potencjalna efektywność reakcji fotochemicznej w PSII, wyznaczona po adaptacji ciemniowej (F_V/F_M). Dłuższy okres ekspozycji roślin na zasolenie spowodował zmniejszenie u klonu Tora wartości parametru T_{FM} (czasu wzrostu fluorescencji chlorofilu od początku pomiaru do osiągnięcia maksimum), u klonu 'Bjor' natomiast – PI oraz F_V/F_M . Aplikacja kwasu 2-hydroksybenzoowego wywarła zróżnicowany wpływ na parametry indukcji fluorescencji chlorofilu „a” wierzby wiciowej rosnącej w warunkach stresu solnego. Zastosowanie kwasu 2-hydroksybenzoowego, przy dłuższej ekspozycji roślin na podwyższone stężenie NaCl, zwiększyło wartości takich parametrów, jak T_{FM} oraz F_V/F_M u klonu Bjor wierzby wiciowej; w przypadku klonu Tora zmniejszyło wartość wskaźnika PI.

Key words: basket willow, fluorescence of chlorophyll a, salt stress.

Słowa kluczowe: wierzba wiciowa, fluorescencja chlorofilu a, stres solny.

INTRODUCTION

Salix viminalis L. crop has become popular not only for its value as a energy crop, but also for its usefulness in the reclamation of industrial devastated areas (Stolarski 2004). Despite its rapid growth and easy cultivation in nutrient-poor substrate, the use of this plant may be difficult, because of the detrimental effects of salts of various elements, often found in degraded

soils in toxic amounts. It is worth noting that amount of sodium chloride, a salt commonly found in mining areas, is rising in the soil globally due to the occurrence of the phenomenon of global warming (Jakubiak and Śliwka 2008). Toxic impact of NaCl give damage to cell structures, leading to impairment of metabolic pathways and physiological processes of plants. According to many authors, excessive salinity is a limiting factor for photosynthesis. It may damage the PSII pigments system (Stępień and Kłobus 2006; Wrochna et al. 2007) and may cause disturbances in the transport of electrons. However, there are also reports of high activity of this photosystem under conditions of salt stress, which may be associated with the latest reports on the lack of interaction of reactive oxygen species produced in the plant under the influence of adverse environmental factors on PSII (Photosystem II) light-damaging (Lu et al. 2002; Nishiyama and Murata 2014). Studies on the potential for salt stress reduction show beneficial effects on plants of certain elements such as potassium, silicon and selenium or organic compounds, including 2-hydroxybenzoic acid (salicylic acid). It is a compound belonging to growth regulators group, phenol derivatives, which in conditions of biotic and abiotic stress, is in charge of synthesis induction and accumulation of immune proteins in plant (Ogórek et al. 2011). Studies show that there are beneficial effects of external application of salicylic acid on plants, manifested in the reduction of stress, in many species growing under conditions of elevated salt concentration in soil (Shakirova et al. 2003; Hussein et al. 2007; Faried et al. 2017).

One of the non-invasive methods to determine the efficiency of a photodynamic apparatus and to evaluate the physiological state of a plant is based on the measurement of fluorescence capacity of chlorophyll a. It uses the natural luminescence capacity of assimilation pigments to evaluate the functioning of the PSII (Murchie and Lawson 2013). Fluorimetric methods allow rapid and highly sensitive assessment of both plant responses to stressors as well as the effectiveness of repair mechanisms and the ability of plants to maintain homeostasis under stressful conditions (Murkowski 2002; Skórska 2002).

The purpose of the study was:

- evaluation of the effect of exogenous application of 2-hydroxybenzoic acid on the state of the photosynthetic apparatus of willow growing in elevated salt concentrations
- evaluation of usefulness of the fluorescent method for characterization the physiological state of the different willow genotype under stressed salt conditions

MATERIAL AND METHODS

The research include two clones of the basket willow (*Salix viminalis* L.) – Tora and Bjor, whose seedlings (20 centimeters sections of annual shoots) came from the plantation of the Department of Plant Physiology and Biochemistry of the West Pomeranian University of Technology in Szczecin.

With each willow clone selected for testing, in 2016, a two-factor hydroponic experience was performed in the laboratory of the Department of Plant Physiology and Biochemistry in a complete randomized triplication. The first experimental factor was the concentration of sodium chloride (control – complete Hoagland medium, 3.0 g NaCl · dm⁻³) and second was the concentration of 3-hydroxybenzoic acid (control – complete Hoagland medium,

138 mg · dm⁻³). A single repetition was 5 pieces of plants placed in one container – in one experiment there were 24 containers. Glass containers of 1 dm³ were used for the experiment. By the time the willow seedlings were rooted and about five centimeters of shoots were formed, they were filled with full medium. After approximately 14 days from the time of placing the plants in the hydroponics, the composition of the media was varied according to the accepted system of experiment.

Chlorophyll fluorescence parameters were recorded using a Handy PEA spectrofluorometer (Hansatech) based on the standard camera procedure. Measurements were made twice during the course of the experiment, after 5 (I term) and 10 days (II term) from the beginning of the experimental exposure period, each in 15 leaves (selected one representative leaf from the plant) from each experimental combination, in the same position on the plant, which was shaded 20 minutes before the measurement with factory clips. By the spectrofluorometer was measured and calculated the following chlorophyll fluorescence induction parameters:

F_0 – initial fluorescence (zero), excitation energy loss indicator in energy antennas;

F_M – maximum fluorescence, after reduction of acceptors in PS II and after darkening adaptation;

$F_V = F_M - F_0$ – variable fluorescence, determined after darkening adaptation, parameter dependent on maximum PSII quantum yield;

F_V/F_M – maximum, potential efficiency of photochemical reaction in PS II determined after darkening adaptation, after reduction of acceptors in PS II (Bolhár – Nordenkamp and Öquist 1993);

T_{FM} – chlorophyll fluorescence growth time from the beginning of the measurement to the maximum (F_M);

PI (Performance Index) – PS II vitality indicator, which relates to the overall vitality of this system;

A_M (Area) – surface above the chlorophyll fluorescence induction curve between the F_0 and F_M points proportional to the pool size of the reduced plastochinon electron acceptors in PS II. Expressed in units bms (bitomilisecond).

Results of the study were statistically analyzed using a two-way analysis of variation in complete randomization. The significance of differences between means was determined using Duncan's test, with significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The metabolic process that regulates the accumulation of biomass by plants, which is particularly sensitive to stress factors, is photosynthesis. An effective method to assess changes in the activity of their photosynthetic apparatus is to analyze the fluorescence signal of the chlorophyll "a". Under natural conditions, the photodynamic apparatus loses from 0.5 to 5% of the previously absorbed energy in the form of chlorophyll fluorescence. The effective course of the reaction of energetic changes in chloroplasts is characterized by low level of chlorophyll steady fluorescence and the characteristic course of its induction. The impact of stress factors causes both the intensity change and the nature of chlorophyll fluorescence induction (Kalaji and Łoboda 2007; Kuckenberget al. 2009; Murkowski and Mila 2010).

In the case of the Tora clone, the influence of experimental factors on the value of T_{FM} parameter, ie the time of chlorophyll fluorescence growth from the beginning of the measurement to the maximum was not showed. Longer plant exposure times for applied NaCl (measured at II term) resulted in a decrease in T_{FM} values compared to control plants. At the same time of measurement, the significance of interaction was also determined. The highest values of T_{FM} were found in plants grown in complete medium without the addition of 2-hydroxybenzoic acid (Table 1).

Table 1. Chlorophyll fluorescence growth time from the beginning of the measurement to the maximum T_{FM} in the Tora willow clone

Tabela 1. Czas wzrostu fluorescencji chlorofilu od początku pomiaru do osiągnięcia maksimum T_{FM} u klonu Tora wierzby wiciowej

	2-hydroxybenzoic acid Kwas 2-hydroksybenzoesowy 138 mg · dm ⁻³	Control Hoagland medium Kontrola – żywka pełna Hoaglanda	Average Średnia
I term – I termin			
NaCl 3.0 g · dm ⁻³	307.00 ^a	270.00 ^a	288.50 ^a
Control Hoagland medium Kontrola – żywka pełna Hoaglanda	356.00 ^a	296.00 ^a	326.00 ^a
Average – Średnia	363.00 ^a	283.00 ^a	
II term – II termin			
NaCl	288.00 ^a	257.00 ^a	272.50 ^a
Control – Kontrola	294.00 ^a	407.00 ^b	350.50 ^b
Average – Średnia	291.00 ^a	332.00 ^a	

Averages marked with the same letters do not differ significantly with significance level $\alpha = 0.05$ – Średnie oznaczone tymi samymi literami nie różnią się istotnie przy poziomie istotności $\alpha = 0,05$.

Addition of this compound to the high salt concentration medium did not affect the value of the T_{FM} parameter. For the Bjor clone at the first date of measurement, no significant main effects of the experience were found but the significance of the interaction of factors was shown. The smallest T_{FM} value, significantly different from that found in plants grown under elevated salinity and the interaction of 2-hydroxybenzoic acid, has been demonstrated for plants grown in control medium supplemented with 2-hydroxybenzoic acid. At the second date of measurement in Bjor clone a higher T_{FM} value was found for plants growing at elevated NaCl levels and in salicylic acid medium. It was also found that addition of this compound to media with NaCl and control media increased the T_{FM} value (Table 2).

The pool of reduced plastochinone electron acceptors in PSII (Area) are one of the best indicators of plant assimilation efficiency measured by detection and analysis of chlorophyll „a” fluorescence signal. In the case of blocking electron transport from reaction centers to plastochinones, which may occur eg. in stress conditions, the value of the A_M parameter decreases (Augustynowicz et al. 2010). In the Tora and Bjor clones, no significant influence of the major experience factors on the value of the A_M parameter was found in both measurement dates. The significance of their interaction was shown. In the first measurement, in the case of the Tora clone, the highest value of the A_M parameter was found in plants grown in the medium without addition of NaCl, enriched with salicylic acid. In the Bjor clone, on the second measurement date, after prolonged exposure, showed that the addition of salicylic acid to the medium containing NaCl reduced the value of the A_M parameter (Table 3, 4).

Table 2. Chlorophyll fluorescence growth time from the beginning of the measurement to the maximum T_{FM} in the Bjor willow cloneTabela 2. Czas wzrostu fluorescencji chlorofilu od początku pomiaru do osiągnięcia maksimum T_{FM} u klonu Bjor wierzby wiciowej

	2-hydroxybenzoic acid Kwas 2-hydroksybenzoesowy	Control Kontrola	Average Średnia
I term – I termin			
NaCl**	446.00 ^b	343.00 ^{ab}	394.50 ^a
Control – Kontrola	304.00 ^a	347.00 ^{ab}	325.50 ^a
Average – Średnia	375.00 ^a	345.00 ^a	
II term – II termin			
NaCl	394.00 ^a	300.00 ^b	347.00 ^b
Control – Kontrola	292.00 ^b	243.00 ^a	267.50 ^a
Average – Średnia	343.00 ^b	271.50 ^a	

Explanation see Table 1 – Objaśnienia jak w tab. 1.

Table 3. Surface above the chlorophyll „a” fluorescence induction curve proportional to the pool size of the reduced plastochinon electron acceptors in PSII – A_M (Area) [bms], in the Tora willow cloneTabela 3. Powierzchnia nad krzywą indukcji fluorescencji chlorofilu „a”, proporcjonalna do wielkości puli zredukowanych plastochinonowych akceptorów elektronów w PSII – A_M (Area) [bms] u klonu Tora wierzby wiciowej

	2-hydroxybenzoic acid Kwas 2-hydroksybenzoesowy	Control Kontrola	Average Średnia
I term – I termin			
NaCl	50 471.50 ^a	53 017.80 ^a	51 744.65 ^a
Control – Kontrola	63 081.90 ^b	47 215.80 ^a	55 148.85 ^a
Average – Średnia	56 776.70 ^a	50 116.80 ^a	
II term – II termin			
NaCl	47 913.80 ^a	42 627.60 ^a	45 270.70 ^a
Control – Kontrola	48 015.50 ^a	43 469.70 ^a	45 742.60 ^a
Average – Średnia	47 964.65 ^a	43 048.65 ^a	

Explanation see Table 1 – Objaśnienia jak w tab. 1.

Table 4. Surface above the chlorophyll „a” fluorescence induction curve proportional to the pool size of the reduced plastochinon electron acceptors in PSII – A_M (Area) [bms], in the Bjor willow clone

Tabela 4. Powierzchnia nad krzywą indukcji fluorescencji chlorofilu „a”, proporcjonalna do wielkości puli zredukowanych plastochinonowych akceptorów elektronów w PSII – Area [bms], u klonu Bjor wierzby wiciowej

	2-hydroxybenzoic acid Kwas 2-hydroksybenzoesowy	Control Kontrola	Average Średnia
I term – I termin			
NaCl	46 106.70 ^a	53 238.00 ^a	49 672.35 ^a
Control – Kontrola	55 948.50 ^a	52 886.50 ^a	54 417.50 ^a
Average – Średnia	51 027.60 ^a	53 062.25 ^a	
II term – II termin			
NaCl	34 266.70 ^a	46 023.30 ^c	40 145.00 ^a
Control – Kontrola	45 256.20 ^{bc}	38 681.60 ^{ab}	41 968.90 ^a
Average – Średnia	39 761.45 ^a	42 352.45 ^a	

Explanation see Table 1 – Objaśnienia jak w tab. 1.

In the Tora clone, in both measurement periods the higher PI value was found in plants grown in elevated salinity and in willow grown in salicylic acid-free medium. The addition of this compound to the NaCl-containing medium reduced the value of the PI index, as determined on the two measurement dates. At the first term, the same dependency was also noted for plants growing in the control medium. In the Bjor clone, after a longer exposure to experimental agents, a higher PI value showed plants grown in NaCl-free medium. The supply of salicylic acid to plants did not affect this feature in both control growing and high salt concentrations (Table 5, 6).

Table 5. PS II vitality indicator – PI (Performance Index) in the Tora willow clone
Tabela 5. Wskaźnik witalności systemu PS II – PI u klonu Tora wierzby wiciowej

	2-hydroxybenzoic acid Kwas 2-hydroksybenzoesowy	Control Kontrola	Average Średnia
I term – I termin			
NaCl	1.505 ^b	1.776 ^c	1.640 ^b
Control – Kontrola	1.224 ^a	1.468 ^b	1.346 ^a
Average – Średnia	1.346 ^a	1.622 ^b	
II term – II termin			
NaCl	1.231 ^a	1.777 ^b	1.504 ^b
Control – Kontrola	1.074 ^a	1.169 ^a	1.121 ^a
Average – Średnia	1.152 ^a	1.473 ^b	

Explanation see Table 1 – Objaśnienia jak w tab. 1.

Table 6. PS II vitality indicator – PI (Performance Index) in the Bjor willow clone
Tabela 6. Wskaźnik witalności systemu PS II – PI u klonu wierzby wiciowej Bjor

	2-hydroxybenzoic acid Kwas 2-hydroksybenzoesowy	Control Kontrola	Average Średnia
I term – I termin			
NaCl	1.119 ^a	1.376 ^{ab}	1.247 ^a
Control – Kontrola	1.459 ^b	1.440 ^b	1.450 ^a
Average – Średnia	1.289 ^a	1.408 ^a	
II term – II termin			
NaCl	0.827 ^a	0.890 ^a	0.858 ^a
Control – Kontrola	1.277 ^b	1.221 ^b	1.249 ^b
Average – Średnia	1.052 ^a	1.055 ^a	

Explanation see Table 1 – Objaśnienia jak w tab. 1.

Table 7. Maximum, potential efficiency of photochemical reaction in PS II determined after darkening adaptation F_v/F_M in the Tora willow clone
Tabela 7. Maksymalna potencjalna efektywność reakcji fotochemicznej w PS II wyznaczona po adaptacji ciemniowej F_v/F_M u klonu Tora wierzby wiciowej

	2-hydroxybenzoic acid Kwas 2-hydroksybenzoesowy	Control Kontrola	Average Średnia
I term – I termin			
NaCl	0.819 ^b	0.829 ^b	0.824 ^b
Control – Kontrola	0.797 ^a	0.823 ^b	0.810 ^a
Average – Średnia	0.808 ^a	0.826 ^b	
II term – II termin			
NaCl	0.831 ^a	0.830 ^a	0.806 ^a
Control – Kontrola	0.804 ^a	0.808 ^a	0.831 ^a
Average – Średnia	0.818 ^a	0.819 ^a	

Explanation see Table 1 – Objaśnienia jak w tab. 1.

Table 8. Maximum, potential efficiency of photochemical reaction in PS II determined after darkening adaptation F_v/F_M in the Bjor willow clone
Tabela 8. Maksymalna potencjalna efektywność reakcji fotochemicznej w PS II wyznaczona po adaptacji ciemniowej F_v/F_M u klonu wierzby wiciowej Bjor

	2-hydroxybenzoic acid Kwas 2-hydroksybenzoesowy	Control Kontrola	Average Średnia
I term – I termin			
NaCl	0.740 ^a	0.808 ^b	0.774 ^a
Control – Kontrola	0.809 ^b	0.815 ^b	0.812 ^a
Average – Średnia	0.774 ^a	0.811 ^a	
II term – II termin			
NaCl	0.699 ^b	0.592 ^a	0.645 ^a
Control – Kontrola	0.814 ^c	0.791 ^c	0.802 ^b
Average – Średnia	0.756 ^a	0.691 ^a	

Explanation see Table 1 – Objaśnienia jak w tab. 1.

The F_V/F_M parameter is considered to be a measure of the photochemical activity of a plant photosynthetic apparatus. Under optimal growth conditions according to Angelini et al. (2001) its value should be about 0.85 relative units. The reduction in this parameter is a symptom of the stress of photoinhibition. The effect of elevated salt concentrations on the photochemical activity of the plant's photosynthetic apparatus depends on the salinity, the exposure time to this stressor, and the plant species. Jimenez et al. (1997) in rose, Stępień and Kolbus (2006) in cucumber and Wrochna et al. (2007) in *Amaranthus paniculatus* found that F_V/F_M parameter was increased after a short time after treatment plant with NaCl and was decreased when the exposure time to salinity was increased. In the case of rose and cucumber, higher decreases of F_V/F_M parameter were observed after treatment of the plants with higher doses of salt. In Lu et al. (2002) research, there was no negative effect of salinity on *Suaeda salsa* photosynthetic apparatus. In this study, in the Tora clone, after 5 days of exposure to experimental factors, a higher F_V/F_M value was found in plants grown in elevated salinity and in plants from medium with salicylic acid. The addition of this compound to the medium containing NaCl did not affect the F_V/F_M value. For the Bjor clone, in the second term of the study a higher F_V/F_M value was found in plants grown in medium without NaCl. At the same time, in the case of plants growing in elevated salts, F_V/F_M was also increased due to salicylic acid action. The inverse relationship was noted – the enrichment of NaCl medium and salicylic acid resulted in a decrease in F_V/F_M in the first term of the study.

CONCLUSIONS

1. The Tora, the clone of basket willow, well tolerated a short exposure period for the NaCl concentration, as indicated by high values of PI, A_M , and F_V/F_M .
2. Longer plant exposure to salinity resulted in a decrease T_{FM} parameters in Tora clone, and PI and F_V/F_M in Bjor clone.
3. The application of 2-hydroxybenzoic acid had a different effect on chlorophyll "a" fluorescence induction parameters of basket willow growing under stressed salt conditions.
4. The use of 2-hydroxybenzoic acid in prolonged exposure of plants to elevated NaCl increased parameters such as T_{FM} and F_V/F_M in the Bjor clone.
5. The use of 2-hydroxybenzoic acid under saline stress conditions reduced the value of the PI index in the basket willow.

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Abstract. The study conducted in 2016 at the Department of Plant Physiology and Biochemistry at the West Pomeranian University of Technology in Szczecin was to evaluate the effect of exogenous application of 2-hydroxybenzoic acid (salicylic acid) on the efficiency of the photosynthetic apparatus of basket willow growing under conditions of elevated salt concentration. The first experimental factor was the concentration of sodium chloride (control – complete Hoagland medium, $3.0 \text{ g NaCl} \cdot \text{dm}^{-3}$) and second was the concentration of 3-hydroxybenzoic acid (control – complete Hoagland medium, $138 \text{ mg} \cdot \text{dm}^{-3}$). The Tora clone tolerated a short period of exposure to the NaCl concentration, as evidenced by high values of Performance Index (PI), Area, and maximum, potential efficiency of photochemical reaction in PS II determined after darkening adaptation, after reduction of acceptors in PS II (F_V/F_M). Longer plant exposure to salinity resulted in a decrease value of T_{FM} parameter in the Tora clone, and PI and F_V/F_M in Bjor clone. The application of 2-hydroxybenzoic acid had a varied effect on chlorophyll "a" fluorescence induction parameters of basket willow growing under salt stress conditions. The use of 2-hydroxybenzoic acid increased the parameters such as chlorophyll fluorescence growth time from the beginning of the measurement to the maximum (T_{FM}) and F_V/F_M in the Bjor clone with prolonged exposure to higher NaCl concentrations. For the Tora clone, the PI value decreased.

