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FREE PROLINE SYNTHESIS IN LEAVES OF THREE CLONES OF BASKET WILLOW (*SALIX VIMINALIS*) AS A RESPONSE TO SUBSTRATE SALINITY

A two-factor hydroponic experiment was conducted on nutrient medium whose concentrations were as follows: 0.068, 0.136 and 0.170 mol·dm^{-3}. During the experiment, the proline content was determined 12, 48 and 168 hours after applying the stress agent in the leaves of three basket willow clones using the method of BATES at al. [1]. The study aimed at determining the effect of different NaCl concentrations in substrate as well as the influence of stress time on the proline content in the leaves of basket willow clones ("Bjor", "Jorr" and "Tora"). The "Bjor" clone was found to be characterised by the highest proline content, whose concentration rose together with an increase in the salinity of nutrient medium and the time of osmotic stress applied. The "Jorr" clone was characterised by the lowest proline content in leaf fresh mass, with its maximum value being found after 48 hours of stress duration.

1. INTRODUCTION

The basket willow is one of the most widespread species of the genus *Salix*. It is used as a fuel and in soil protection, in bringing land into cultivation, in the air and water protection [2]. The studies have been carried for several years in order to obtain inter-specific willow genotypes with increased tolerance, also to abiotic stress agents [3]. In spite of a broad application of the basket willow in land reclamation, there are no reports related to its biochemical indicators. Biochemical studies aim at finding sensitive metabolic indicators that will be of use in evaluating the plant tolerance to different abiotic environmental stress agents. During the last 10 years, many papers have been published that refer to the relationship between a plant defensive response and metabolic changes, including in particular the effect of salt stress on proline synthesis. From among 400 papers collected, none has dealt with this problem in the case of the basket willow, despite a broad ecological application of this tree.

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2. RESEARCH METHODS

2.1. MATERIAL AND METHODS

A hydroponic experiment was carried out in three replications. The basket willow cuttings grew only in full-nutrient medium until they formed an appropriate root system and reached a height of 40 cm. Thereafter, the cuttings of each basket willow clone examined were placed in twos into hydroponic culture units. The cultures were supplemented with NaCl solutions in appropriate concentrations and aerated.

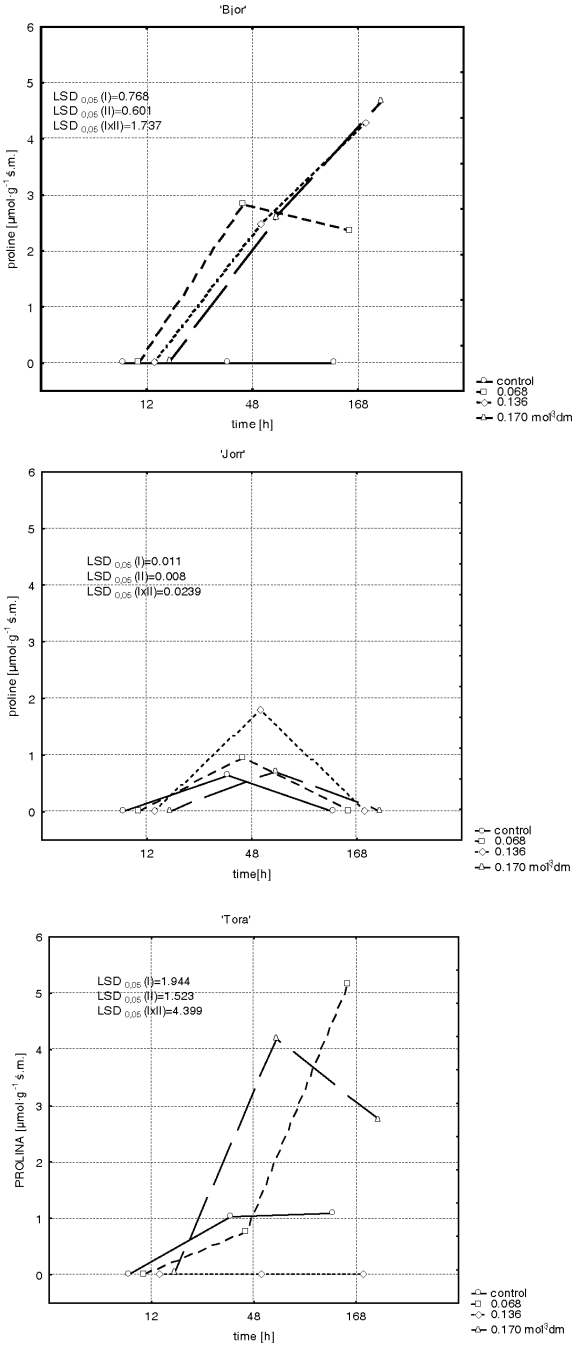
A two-factor experiment was carried out for each clone separately, with salinity levels being the first factor (control (0.000), 0.068, 0.136 and 0.170 mol dm^{-3}), and the time of experiment – the second one (12, 48 and 168 h). In this period of time, free proline content in plant leaves (adjusted to $mg \cdot g^{-1}$ of fresh mass) was determined by the method of BATES et al. [1]. The content of potassium, calcium, magnesium and sodium in leaves ($mg \cdot kg^{-1}$) was determined using the ASA method.

2.2. STATISTICAL METHODS

The results obtained, i.e. the proline content in leaves, were processed statistically. A two-factor analysis of variance was carried out, and the significance of the factors was tested using the Tukey's test at $\alpha = 0.05$. In order to examine the relationship between the content of chemical elements examined and proline concentration, a simple regression analysis was performed.

3. RESULTS AND DISCUSSION

Many authors [4]–[6] report that proline accumulation in plant leaves takes place mainly due to the soil salinity. The basket willow is particularly exposed to such a factor owing to its planting along roads whose ice cover removed in winter contributes to increased salinity of soil. In the present study, the proline content in the basket willow was determined in leaves, since the biggest changes produced by high soil salinity are observed in these organs [7]. When analysing the results presented in the figure, one can infer that the "Jorr" clone was characterised by the lowest mean content of free proline, whereas the "Bjor" clone – by the highest one. According to many authors [8]–[10], free proline accumulation is the indicator of both stress intensity and the plant ability to deal with high salt concentration. In general, it is higher in the plants characterised by higher resistance to stress [11]. Therefore, one may think that the "Bjor" clone is resistant to osmotic stress at the aforesaid salt concentrations applied in hydroponic culture. Synthesis of such an osmoregulator in the "Bjor" clone



Effect of different substrate salinity levels and osmotic stress duration on proline content in the basket willow (*Salix viminalis*)

took place together with the increase in salt concentration in substrate. Similar results were obtained by STOLARSKA et al. [12]. Also HOLLWARTH [13] observed a similar response in the leaves of horse-chestnut (*Aesculus hippocastanum*). In the case of the "Jorr" clone, the maximum proline content was found to accumulate after 48 hours of salt stress at the substrate salinity of 0.136 mol·dm^{-3}. The fact that proline synthesis in the leaves of this clone after 168 hours decreased to the level observed 12 hours after the occurrence of salinity stress is distinctive. In the case of the "Tora" clone, no straightforward trend was observed in changes caused by the stress agents examined.

The study of BANDURSKA [14] suggests that proline level under the same stress conditions may differ within different species, also within varieties of the same species. The results of this study provide evidence of different susceptibility of the basket willow clones examined to stress stimulus intensity.

Based on the findings presented in the table, it was also found that in the case of the "Bjor" clone, the level of proline content in leaves was fairly positively correlated with the content of K^+ and Ca^{2+} ions. This could affect a proper stabilisation of protein structures and plasma membranes in this clone, which at the same time points to its defence against salinity.

Table

Coefficients of correlation between proline content and Na^+, K^+, Ca^{++} and Mg^{++} ions' content (g·kg^{-1})

y	*x*	Coefficients of correlation *r*		
		"Bjor"	"Jorr"	"Tora"
Proline	Na^+	0.55	–0.28	0.31
	K^+	0.76*	–0.38	–0.21
	Ca^{2+}	0.63*	–0.40	–0.17
	Mg^{2+}	0.23	–0.70	–0.19

4. CONCLUSIONS

1. Among the basket willow clones examined, the "Bjor" clone was characterised by the highest proline content (1.60 μmol·g^{-1} f.m.); the concentration of this amino acid rose together with an increase in nutrient medium salinity and osmotic stress duration. The proline content was proportional to that of K^+ and Ca^{2+} ions in the leaves. This clone was characterised by the highest resistance to osmotic stress induced by the excessive amount of sodium chloride in substrate.

2. In the case of the "Jorr" clone, the maximum content of this osmoregulator was observed after 48 hours for all sodium chloride concentrations analysed. 168 hours after osmotic stress application, the proline level decreased to that observed after 12 hours.

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SYNTEZA WOLNEJ PROLINY W LIŚCIACH TRZECH KLONÓW WIERZBY WICIOWEJ (*SALIX VIMINALIS*) JAKO ODPOWIEDŹ NA ZASOLENIE PODŁOŻA

Przeprowadzono dwuczynnikowe doświadczenie hydroponiczne, w którym stężenia pożywki wynosiły kolejno 0,068; 0,136; 0,170 mol·dm^{-3}. Oznaczono zawartość proliny metodą Batesa i wsp. [1] po 12, 48, 168 godzinach od momentu zastosowania czynnika stresowego w liściach trzech klonów wierzby wiciowej. Celem pracy było określenie wpływu zróżnicowanych stężeń NaCl w podłożu oraz czasu oddziaływania stresu na zawartość proliny w liściach klonów wierzby „Bjor", „Jorr", „Tora". Stwierdzono, że klon „Bjor" charakteryzował się największą zawartością proliny, a jej stężenie rosło w miarę zwiększania zasolenia pożywki oraz wydłużania czasu oddziaływania zastosowanego stresu. Klon „Jorr" charakteryzował się najmniejszą ilością proliny w świeżej masie liści, a jej maksymalną ilość stwierdzono po 48 godzinach oddziaływania stresu.