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SELECTED PHYSIOLOGICAL PARAMETERS OF THREE VARIETIES OF BASKET WILLOW (*SALIX VIMINALIS* L.) FROM PERENNIAL PLANTATIONS

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A b s t r a c t. The aim of the research conducted in 2010-2012 on one-year, two-year and three-year shoots of three verities of *Salix viminalis*: 'Bjor', 'Jorr' and 'Tora', grown from 14, 15 and 16 year old stumps, was to compare the shoots of different age in terms of selected physiological characteristics, that is the concentration of chloroplast pigments, water balance of leaves and water content and dry matter of shoots. The content of chloroplast pigments (chlorophyll a, chlorophyll b and carotenoids) and the water balance were determined on the basis of RWC-relative water content and WSD-water saturation deficit indicators. Moreover, the water and dry matter content of one-year, two-year and three-year old willow shoots were determined. It was found that the age of the shoots differentiated the physiological parameters of willow varieties. The highest concentration of chlorophylls was reported in the leaves of three-year old shoots of the Bjor variety, in turn the highest concentration of carotenoids was found in the leaves of one-year old shoots of Jorr. Regardless of the age of the shoots, the highest rate of hydration of leaves and stems was a characteristic trait of the Tora variety, and the lowest was found in Jorr. High levels of the measured physiological parameters, such as chlorophyll a and b, carotenoids and relative water content in leaves obtained in the 5th rotation, that is 14th, 15th and 16th year of running the plantation, indicate a high physiological activity and hence high productivity of the studied willow varieties.

K e y w o r d s: *Salix viminalis* L., relative water content (RWC), photosynthetic pigments

INTRODUCTION

In recent years, there have been ongoing discussions on energy security, reduction of greenhouse gas emissions and the strong increase in the share of renewable energy in the global production of heat and electricity. It is expected that biomass will be a very important component of renewable energy sources, and energy crops

production, especially trees and shrubs, will be one of the most important sources of biomass (Kisiel *et al*. 2001, Berndes *et al.* 2005, Szczukowski *et al*. 2012). A priority in the national energy action plan is to meet the high requirements of the European Union regarding the increase of the consumption of renewable energy to 15-20% by 2020 (GUS 2009). In Poland, biomass comes mainly from forest residues, wood industry, agriculture and municipal waste. However, its share is too small to fulfil the obligations relating to biomass usage, which forces the need to acquire it from the plantation of perennial plants which have higher energy value than annual plants (Szczukowski *et al.* 2012, Kuś and Faber 2009).

Currently in Poland perennial energy crops occupy a small area – over 10 thousand hectares, however, according to specialists, up to 4 million hectares can be used for this purpose. An advantage should be taken of this opportunity and increase gradually the area of perennial energy crops. Yet, the selection of plants is important, depending on the region and its climate and soil conditions. This should warrant many years of high yield. For this purpose, long-lived energy plants plantations with high productivity of biomass are the best. Certainly, basket willow (*Salix viminalis* L.) is a species that meets these criteria. Rarely are they grown as species of pure varieties. Research on new willow growing forms has been conducted for many years, improving their immune, physiological and morphological features, and thus their production capacity. On the basket willow plantation the biomass production may proceed without any interference for 20-25 years. Its harvest usually takes place in cycles of one, two, three or four years, depending on the plant density (Dubas *et al.* 2004, Szczukowski *et al*. 2004, Wróbel 2006).

The physiological characteristics of the plants, among other things, are used to evaluate their production capacity. Therefore, a study was undertaken in order to compare shoots of different age of three varieties of basket willow, derived from perennial plantation grown in the rotation cycles, regarding the selected physiological characteristics, such as the concentration of assimilation pigments and water balance of leaves and water, and dry matter content in shoots.

MATERIAL AND METHODS

The study was conducted in the period of 2010-2012 on a 450 m^2 perennial plantation of basket willow (*Salix viminalis* L.) which was established in 1997, grown in a 3-year rotation cycle, followed by a collection of biomass shoots. This is a part of the research conducted by Wróbel and Wróbel (2015). Plant arrangement was approx. 20 thousand pcs/ha. Cycles were counted since 1998 because after the first year the annual shoots were cut off for better pullulation of plants in the second year. The biological material for the experiment consisted of three varieties of *Salix viminalis:* Bjor, Jorr and Tora, the seedlings (stem cuttings) of which came from

the parent certified plantations Hvidsten Energy Forest in Denmark. The three-year study was carried out in the fifth rotation, on one-year (14th crop year – 2010), two-year (15th crop year – 2011), and three-year (16th crop year – 2012) old shoots. The plantation of *Salix viminalis* was established on an urbanised anthropogenic soil of the light soils category (loamy sand and strongly dusty- pgmp), neutral (pH = 6.7), with an average content of phosphorus and organic carbon and a high total content of nitrogen, potassium, calcium and magnesium. The basket willow vegetation area has a high average annual rainfall of 600 mm (Wróbel and Wróbel 2015).

During the vegetation of the three varieties of basket willow: Bjor, Jorr and Tora in the first decades of: June, July and August in the period 2010-2012, determination of assimilation pigments in the leaves (chlorophyll a, chlorophyll b and carotenoids) was done with the Lichtenthaler and Welburn method (1983), and the balance of water in the leaves was determined on the basis of RWC-relative water content and WSD-water saturation deficit indicators (Yamasaki and Dillenburg 1999). Measurements were taken on the 8th leaf from the tip of the shoot. Results from ten randomly selected plants with specific terms were averaged for each year of the study.

What was also determined was the water and dry matter content in the shoots of annual, biennial and triennial studied varieties of basket willow. They were carried out in the third decade of January of 2011, 2012 and 2013 on a representative sample of 10 plants. To this purpose, the oven-dry method was implemented. The results were converted into percentage values.

The test results were analysed statistically. Two factorial analysis of variance was performed. The first experimental factor was the three varieties of basket willow, and the second – the age of the shoot. In order to determine the significance of the main effects and interactions, the Duncan test statistical analysis was performed at the significance level $\alpha = 0.05$. The homogeneous groups were determined. The results were processed using Statistica 10.0 software.

The Pearson correlation coefficients (r) between the water content in the shoots (WS) and the relative water content in leaves (RWC) were also calculated. When the correlation coefficients (r) between these parameters were significant ($\alpha = 0.05$) or highly significant ($\alpha = 0.01$), they were labelled by one or two asterisks (*, **).

RESULTS AND DISCUSSION

Plant productivity depends on a number of factors such as the quality of plant genetic material or habitat, agronomic and climatic conditions. According to many researchers (Starck 1999, Kozłowski *et al.* 2001, Wróbel *et al.* 2006), assimilation pigments and hydration of leaves tissue are among the most important factors directly affecting the intensity of photosynthesis and biomass production.

The content of chlorophyll a and b and carotenoids in the leaves from the annual, biennial and triennial shoots of basket willow varieties Bjor, Jorr and Tora grown on 14-, 15- and 16-year old stumps are shown in Figs 1-9. There were significant differences determined in the amount of pigments depending on the variety and age of the shoots. The highest amounts of *chlorophyll a and b* were noted in the leaves of the Bjor variety grown on three-year old shoots (respectively approx. 2.1 and 0.96 mg g^{-1} f.w.), and the lowest in those of Tora regardless of shoots age (respectively approx. 1 and 0.4 mg g^{-1} f.w.) and in the case of *chlorophyll a* in the leaves of the Jorr variety grown on three-year old shoots (1 mg g^{-1} f.w.). In the Bjor plants the content of both forms of chlorophyll in the leaves increased significantly with the age of shoots, while in Jorr the content of these pigments significantly decreased. In Tora there was no significant difference in the amount of *chlorophyll a and b* between the shoots of different age (Figs 1 and 4).

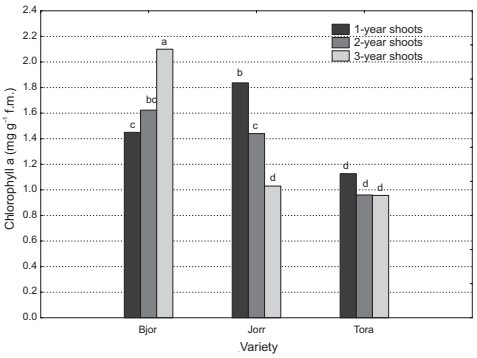

Fig. 1. The content of *chlorophyll a* in the leaves of 1-, 2- and 3-year old shoots depending on the variety of basket willow (*Salix viminalis* L.); values denoted with the same letters do not differ statistically

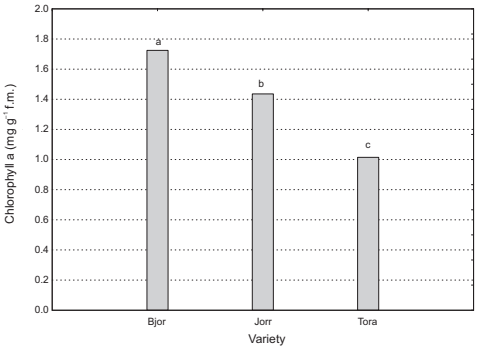

Fig. 2. The content of *chlorophyll a* in the leaves of three varieties of basket willow (*Salix viminalis* L.)

Research on the intensity of assimilation conducted on the same varieties of willow (Wróbel and Wróbel 2015) showed that the highest average net assimilation activity was a characteristic trait of the Jorr variety. The intensity of assimilation for this variety decreased significantly with the age of shoots, while for Bjor it increased significantly. A similar relationship was found in the case of the concentration of *chlorophyll a* and *b*. According to Stolarski *et al*. 2002, Stolarski *et al*. 2008, Stolarski *et al*. 2011, Szczukowski and Budny 2003, Szczukowski *et al*. 2005, the most favourable, due to the increase in shoots biomass, was harvesting every three years, for in the third year the biggest increase in wood pulp can be observed, which constituted over 40% of the entire three-year growth. The results of research conducted by Wróbel and Wróbel (2015) confirmed this, as in the case of the Bjor variety such growth amounted to approx. 58%, for Jorr 50%, and for Tora approx.

47%. Probably the much greater increase in the yield of pulp of three-year old shoots of the Bjor variety compared with Jorr and Tora was influenced by its higher photosynthetic activity and a greater concentration of assimilation pigments found in our study. According to Hall and Rao (1999), the concentration of chlorophyll in leaves has a major influence on the activity of the assimilation process in plants. A low chlorophyll concentration may lead to a decrease of photosynthesis activity of plants and therefore reduce their productivity. Alternatively, when the level of chlorophyll is high, an increase in both photosynthesis activity and productivity is observed.

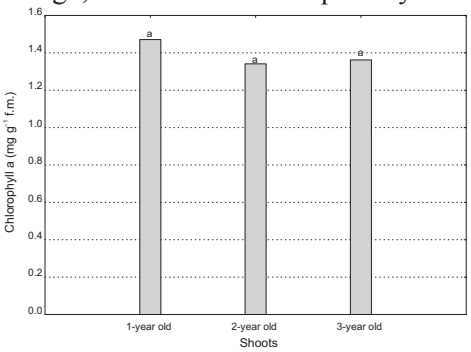

Fig. 3. The content of *chlorophyll a* in the leaves of 1-, 2- and 3-year old shoots of basket willow (*Salix viminalis* L.)

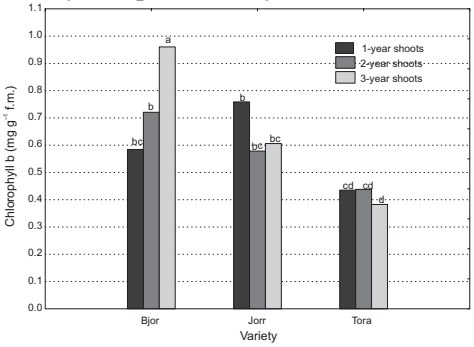

Fig. 4. The content of *chlorophyll b* in the leaves of 1-, 2- and 3-year old shoots depending on the variety of basket willow (*Salix viminalis* L.)

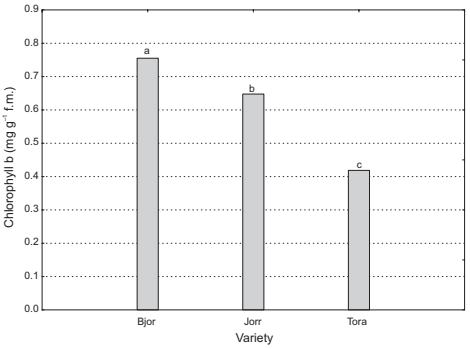

Fig. 5. The content of *chlorophyll b* in the leaves of three varieties of basket willow (*Salix viminalis* L.)

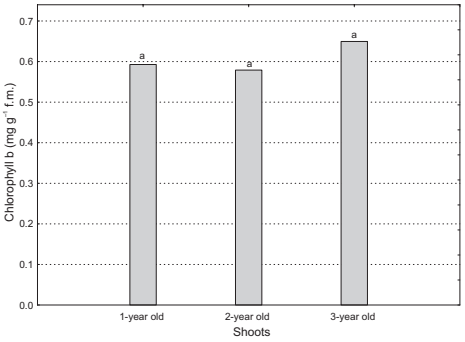

Fig. 6. The content of *chlorophyll b* in the leaves of 1-, 2- and 3-year old shoots of basket willow (*Salix viminalis* L.)

Statistical analysis performed for the main effects (varieties and the age of shoots) showed significant differences in the content of pigments between the varieties – Fig. 2 and 5. Bjor was characterised by the highest content of both forms of *chlorophyll* (respectively approx. 1.7 mg *chl. a* g^{-1} f.w. and approx. 0.86 mg *chl. b* g^{-1} f.w.), and Tora by the lowest (respectively approx. 1 and 0.42 mg g^{-1} f.w.). For the

second main effect, that is the age of shoots, no significant differences were shown (Fig. 3 and 6). The content of chloroplast pigments was characteristic for species and varieties, modified by the influence of environmental and anthropogenic factors (Starck 1999, Kozłowski 2001, Krzesłowska 2004, Malinowska and Wróbel 2015).

The higest concentration of carotenoids was a characteristic trait of leaves of the Jorr variety grown on one-year old shoots (approx. 1.3 mg g^{-1} f.w.). They contained significantly more of these pigments than other combinations. Definitely the least amount of carotenoids was observed in leaves of Tora variety, regardless of shoots age, and in Bjor grown on one-year old shoots (approx. 0.6 mg g^{-1} f.w.) – Fig. 7. In Bjor variety the content of these pigments increased with age of shoots, and in the Jorr variety it decreased. On the other hand, in the Tora variety it did not differ, as in the case of *chlorophyll a and b.*

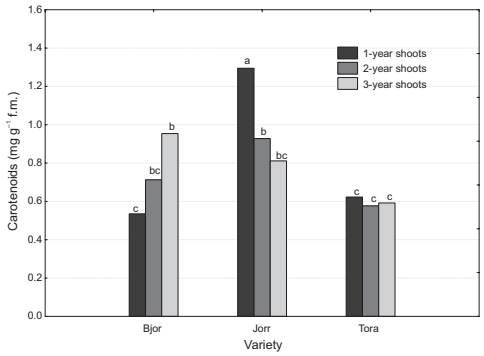

Fig. 7. The content of carotenoids in the leaves of 1-, 2- and 3-year old shoots depending on the variety

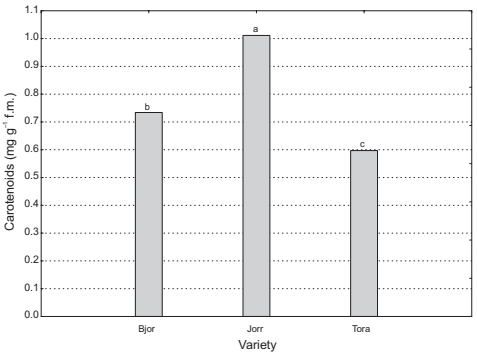

Fig. 8. The content of carotenoids in the leaves of three varieties of basket willow (*Salix viminalis* L.)

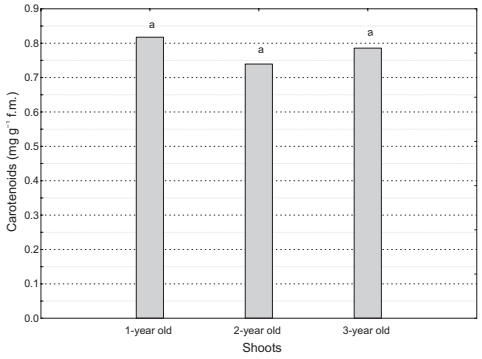

Fig. 9. The content of *carotenoids* in the leaves of 1-, 2- and 3-year old shoots of basket willow (*Salix viminalis* L.)

Jorr variety contained the largest average amount of carotenoids, Bjor approx. 30% less, and Tora approx. 40% – Fig. 8. However, there were no significant differences in the content of carotenoids between shoots of different age. The results

of Stolarska *et al.* (2008) regarding the content of assimilation pigments of the Bjor, Jorr and Tora varieties growing in salinity conditions differed from these results; Tora variety contained more *chlorophyll a and b* and carotenoids than Bjor and Jorr.

The highest rate of RWC was recorded in leaves of one-year old shoots of Tora and Bjor varieties (94 and 93%) and significantly lower in Jorr (86%). The leaves from the 3-year old shoots of Jorr were characterised by the lowest relative water content (75%), 9% less than the Bjor and 14% less than Tora (Fig. 10).

Wróbel and Gregorczyk (2004) and Wróbel and Mikiciuk (2010), when examining the effect of salinity on the water balance of one-year old leaves of shoots of the same basket willow variety, showed differences in water balance. In controlled conditions, the highest relative water content (RWC) was reported in the Tora variety (approx. 93-94%), which is similar to the result obtained in the present study. Lower RWC was reported for the Jorr and Bjor varieties (89 and 82%). In turn, the Bjor variety, compared with Jorr and Tora, was characterised by far more favourable water balance in conditions of high salinity, which may indicate greater resistance of this variety to salt stress. Research conducted in central Sweden by Weih and Nordh (2002) on 14 clones of basket willow, including Bjor, Torr and Tora, showed that of these three clones the Jorr verity had the highest rate of relative water content (RWC) – approx. 80%. A clone of Tora had lower RWC by about 4%, and Bjor by 8%. These authors suggest that the water balance is not constant for each plant genotype, it is variable and determined by numerous environmental factors.

The assessment of the basket willow shoots biomass yield and its energy value takes into account the water and dry matter content (Szczukowski and Tworkowski 2001; 2004, Szczukowski *et al*. 2004, Stolarski *et al*. 2011). Differences in the water and dry mass content of shoots of different age in the three varieties of *Salix viminalis* are shown in Fig. 11. It may be clearly noted that one-year old shoots contained the highest levels water (Tora – 56, Bjor – 54.8, Jorr – 50.7%) and the least of dry matter. The concentration of water in shoots decreased with their age. In the Jorr and Bjor varieties the drop of water content in the three-year old shoots was 3.1 and in Tora – 2%. Similar levels of water content in the wood of basket willow collected in the 3-year cycle were shown by Stolarski *et al.* 2002 and Szczukowski and Budny (2003). Moisture of one-year old shoots stood at 53%, in two-year old shoots it decreased to 50% and in three-year old ones – to 46%. Szczukowski and Tworkowski (2001) reported that the water content in *Salix sp.* wood varied, and at the time of harvesting one-year old shoots it was 52%, two-year old shoots – 50%, three-year old shoots – 46%. In turn, Ager *et al*. (1986), when studying numerous clones of basket willow, showed that the moisture content of one-year old shoots ranged from 50 to 61.5%. Moisture of the biomass of different clones of *Salix viminalis* collected in the 4-year rotation was at the level of 46.1-47.1% (Szczukowski *et al*. 2014).

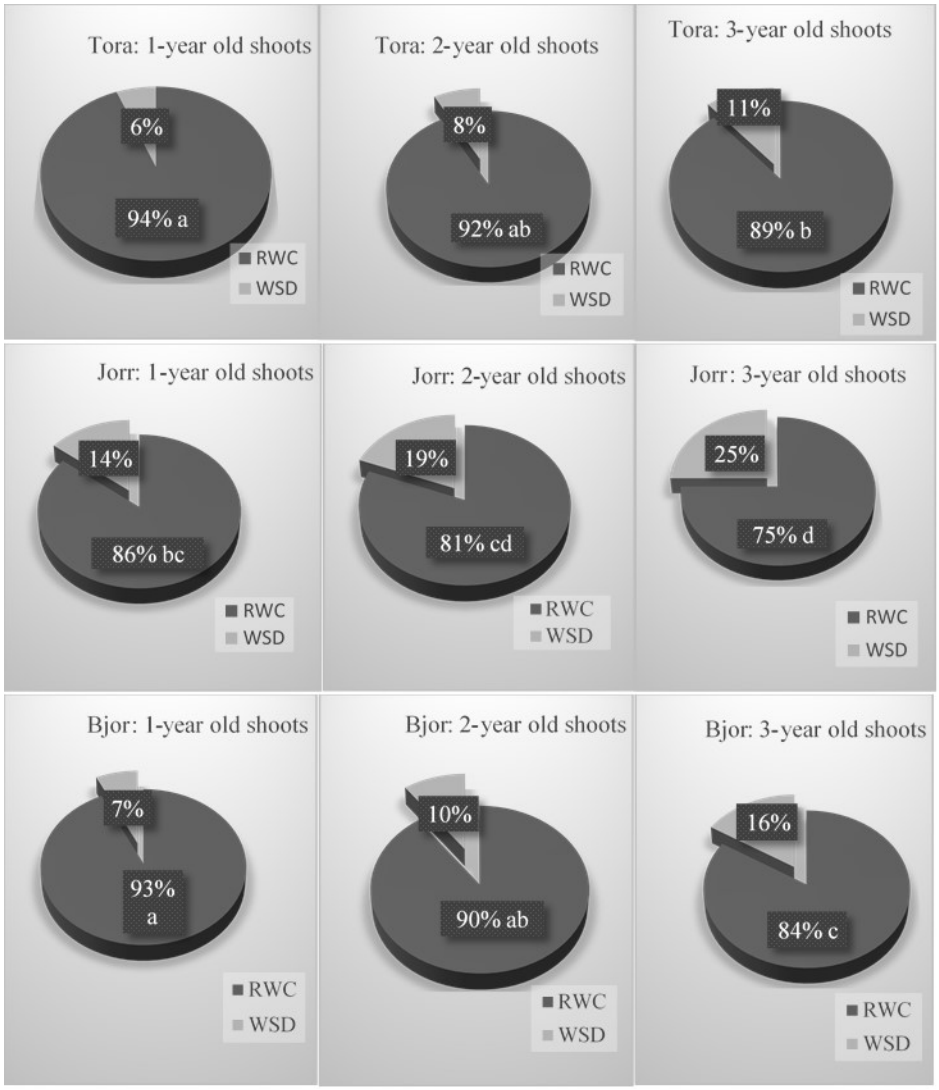

Fig. 10. Leave hydration indicators (RWC – relative water content and WSD –water saturation deficit) of one-, two- and three-year old shoots depending on the varieties of basket willow (*Salix viminalis* L.)

Of the three studied varieties of basket willow, regardless of the age of shoots, the Tora variety was characterised by the largest water content by far (54.9%) and the lowest dry matter content (45.1%). On the other hand, Jorr was characterised by the lowest hydration of the shoots (49.2%) and substantially the highest content of dry matter (50.8%). Three-year old shoots of this variety contained 52.4% of dry matter which was 4.1% more than Bjor and 6.4% more than Tora – Fig. 11.

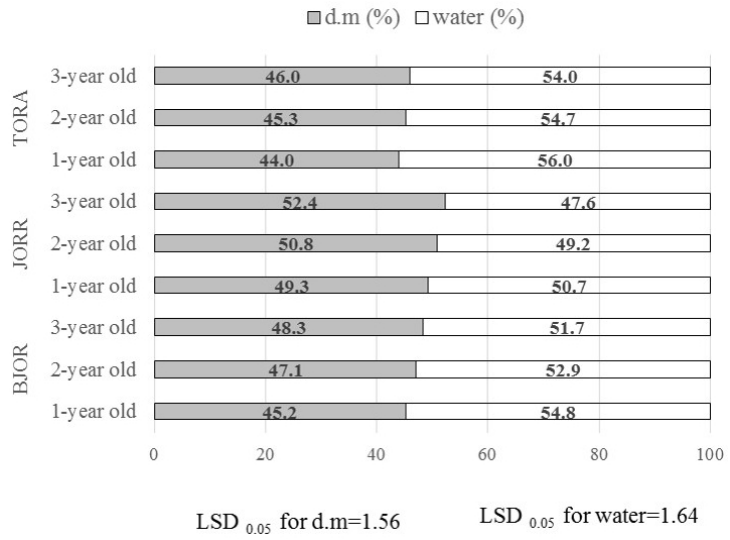

Fig. 11. Content of dry matter and water (%) in the willow shoots depending on their age and variety

According to Szczukowski *et al*. (2004) and Szczukowski and Budny (2003), when determining the yield of dry matter of timber, the water content of basket willow shoots, which at the time of collection is an average of about 50%, must be taken into account.

On the basis of values of linear correlation coefficients in all tested varieties of basket willow, regardless of the age of shoots, we found a highly significant and substantial positive correlations between the relative water content in leaves (RWC) measured during the growing season and the water content in shoots (WS) at the time of harvesting (tab. 1).

Table 1. Values of coefficients of linear correlation between relative water content in the leaves (RWC) and water content in the shoots (WS)

Varieties	Shoots	Parameters		Correlation coefficient (r)
	1-year old	RWC	WS	0.887**
Tora	2-year old	RWC	WS	0.692**
	3-year old	RWC	WS	0.511*
	1-year old	RWC	WS	0.758**
Jorr	2-year old	RWC	WS	0.587*
	3-year old	RWC	WS	0.435*
	1-year old	RWC	WS	0.638*
Bjor	2-year old	RWC	WS	0.593*
	3-year old	RWC	WS	0.431*

Letter designation of the parameters: RWC – relative water content; WS – water content in the shoots; (r) * – significant at $\alpha = 0.05$,** – highly significant at $\alpha = 0.01$

The highest values of correlation coefficients were recorded for the Tora variety (0.511-0.887), and the lowest for Bjor (0.431-0.638). The water content in the shoots was determined by the water content in the leaves. Based on the obtained results it may be concluded that the high rate of hydration of leaves in the Tora and Bjor varieties, regardless of the age of shoots, translated into equally high water content in the harvested shoots of these varieties. In turn, the lower water content of the leaves of the Jorr variety translated into a lower water content in the shoots (Figs 10-11).

CONCLUSIONS

1. The age of shoots differentiated the physiological parameters of willow varieties.

2. The content of *chlorophyll a and b* and carotenoids in the Bjor variety increased with the age of shoots, unlike in Jorr, where it decreased. Tora leaves showed no difference in the amount of pigments depending on the age of shoots. The highest concentration of chlorophylls was reported in the leaves of three-year old shoots of the Bjor variety, but the highest concentration of carotenoids was found in the leaves of one-year old shoots of the Jorr variety.

3. The highest leaf and stem hydration rate was observed in Tora, and the lowest in Jorr. The concentration of water in shoots decreased with age, regardless of the variety.

4. High level of the measured physiological indicators such as chlorophyll a and b, carotenoids, relative water content in leaves which were obtained in the 5th rotation (in 14th, 15th and 16th year of the plantation) indicate a high physiological activity of the studied varieties of willow. At the same time, the Bjor and Jorr varieties were characterised by more favourable values of individual physiological indicators affecting productivity than the Tora variety.

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WYBRANE WSKAŹNIKI FIZJOLOGICZNE TRZECH ODMIAN WIERZBY WICIOWEJ (*SALIX VIMINALIS* L.) POCHODZĄCYCH Z WIELOLETNIEJ PLANTACJI

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Streszczenie. Celem badań przeprowadzonych w latach 2010-2012 na jednorocznych, dwu- i trzyletnich pędach trzech odmian *Salix viminalis*: Bjor, Jorr i Tora, wyrosłych z 14- 15- i 16-letnich karp, było porównanie różnych wiekowo pędów pod względem wybranych cech fizjologicznych, tj. koncentracji barwników asymilacyjnych i bilansu wodnego liści oraz zawartości wody i suchej masy w pędach. Wykonano oznaczenia zawartości barwników asymilacyjnych w liściach (chlorofil a, chlorofil b i karotenoidy) oraz określono bilans wody w liściach na podstawie wskaźników RWC-względna zawartość wody i WSD-deficyt wysycenia wodą. Wykonano także oznaczenie zawartości wody i suchej masy w pędach jednorocznych, dwuletnich i trzyletnich badanych odmian wierzby. Na podstawie uzyskanych wyników stwierdzono, że wiek pędów różnicował badane wskaźniki fizjologiczne u odmian wierzby wiciowej. Największą koncentrację barwników chlorofilowych odnotowano w liściach pędów trzyletnich u odmiany Bjor, z kolei karotenoidów w liściach pędów jednorocznych u odmiany Jorr. Spośród badanych odmian, niezależnie od wieku pędów, najwyższym wskaźnikiem uwodnienia liści i pędów charakteryzowała się odmiana Tora, a najmniejszym odmiana Jorr. Wysokie wartości mierzonych wskaźników fizjologicznych tj. chlorofilu a i b, karotenoidów, względnej zawartości wody w liściach uzyskane w 5. rotacji, czyli w 14., 15. i 16. roku prowadzenia plantacji wskazują na wysoką aktywność fizjologiczną, a tym samym na znaczącą produktywność badanych odmian wierzby.

Słowa kluczowe: *Salix viminalis* L., względna zawartość wody (RWC), barwniki asymilacyjne