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ORIGINAL PAPER

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INFLUENCE OF ROOTSTOCKS AND THE TIME OF GRAFTING PROCEDURE ON THE EFFICIENCY OF PROPAGATION BY GRAFTING TWO CULTIVARS OF MOUNTAIN PINE (*Pinus mugo* Turra) AND ESTIMATION OF CHLOROPLAST PIGMENTS LEVEL IN THE NEEDLES

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ABSTRACT

The experimental studies were conducted from 2015–2017 years in two cycles. The aim of the research was to determine a better grafting time and a choice of an appropriate rootstock for the propagation of two cultivars of mountain pine (*Pinus mugo* Turra). The influence of the grafted cultivar and rootstock used on the level of chloroplast pigments in the needles was also checked. The studies concerned two cultivars of mountain pine (*Pinus mugo*) 'Grześ' and 'Zundert', which were grafted on four different rootstocks: *Pinus contorta* (Dougl. ex Loud.), *Pinus nigra* (Arn.), *Pinus sylvestris* (L.) and *Pinus mugo* subsp. *uncinata* (Ramond Domin) in two dates: 20 January and 15 March. The highest percentage of graft success was obtained for the two cultivars on *Pinus uncinata* and *Pinus contorta* rootstocks. A later term of grafting procedure affected bigger effectiveness of grafting of the two studied cultivars, except for the graft success on *Pinus sylvestris* rootstock. The highest increments of side shoots and lengths of main stems for 'Grześ' cultivar were obtained on *Pinus nigra*, and for 'Zundert' the influence of the rootstock on the above mentioned parameters was not evident. No significant differences in the number of increments in the first and second year of studies were observed. The highest level of chlorophyll A and B was found in the needles of 'Grześ' cultivar, independently from the rootstock used. Among tested rootstocks, only *Pinus nigra* had a significant impact on a higher content of chlorophyll B in the needles of the studied cultivars of mountain pine trees.

Key words: pine, graft success, photosynthesis activity, growth, development of plants

INTRODUCTION

As many studies proved a rootstock which is used influences graft success and the power of growth of the grafted scions of conifer trees [Holzer 1970, Copes 1980, Schmidtling 1983, Jayawickrama et al. 1991, Haines and Simpson 1994, Jayawickrama et al. 1997]. Also the quality of scions, and specially the place they were taken from their scion plant [Frey et al. 2011], the age of the scion plant [Almqvist 2013, Shu et al. 2013] and the storage life of the scions [Barnett and Weatherhead 1989, Wen-Jun 2007] decide on the graft success and a further growth of propagated plants. Also a grafting method [De-li et al. 2007] and the term of this procedure [Frey et al. 2010] has an important influence of the effectiveness of graft success and on the growth of the grafts of conifer plants. Changes in weather conditions after grafting influence

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graft success and their further growth [Karadeniz 2005]. So far there are very few research results concerning the usefulness of rootstocks for grafting various cultivars and species of pine trees [Ahlgren and Wilderness 1972].

A very interesting Polish cultivar of *Pinus mugo* is 'Grześ'. In winter the needles of this cultivar discolour into golden-yellow. 'Zundert' is also a dwarf cultivar of *Pinus mugo* which has a shrubby form and which needles change colour into yellow after first frost [Welch and Haddow 1993].

For many years scientists have been interested in the chloroplast pigments content in the needles of conifer plants [Bolhàr-Nordenkampf and Öquist 1993, Adams and Demmig-Adams 1994, Ottander et al. 1995, Strand 1995, Grassi et al. 2000, Colom et al. 2003, Oquist and Huner 2003]. It was found that stress usually causes a decrease in the content of chlorophyll in leaves [Hendry and Brown 1987] and a rise in the content of carotenoids [Peñuelas and Filella 1998]. It is thought that a low concentration of chlorophyll can directly restrict the potential of the photosynthesis process [Curran et al. 1990, Filella et al. 1995, Rybus-Zając 2005, 2010].

Chlorophyll serves as an indicator of: photosynthesis activity, growth, development, production and biochemical aspects of different plant cultivars, providing a lot of important information on the physiological condition of plants [Sims and Gamon 2002, Steele et al. 2008]. Determination of the chlorophyll content gives also, but indirectly, knowledge about the nutrients content [Filella et al. 1995, Moran et al. 2000].

The relative level of chlorophyll undergoes changes with abiotic factors, such as, for example light [Larcher 1995], so determination of their number can give important information about connections between the plants and their environment. Valladares et al. [2000] say that the chlorophyll content is higher in leaves taken from a shadowed part of the plant than in ones from a sunny surface, the content of carotenoids, however, increases with the exposure to sun. On the other hand, Niinemets [1997] gives an opinion that the decrease in solar radiation intensifies the synthesis of chlorophyll. What is more, the content of chlorophyll and chlorophyll A to chlorophyll B ratio in some plants is dependent on temperature [Ottander et al. 1995].

Differences in the content of chlorophyll among different species of plants found so far, were connected with the stage of leaf's development and its senescen [Gamon and Surfus 1999, Carter and Knapp 2001]. It was also found that trees had a higher content of chlorophyll than shrubs. The content of chlorophyll B and carotenoids did not differ between various forms of the plants' growth [Uvalle Sauceda et al. 2008]. The amount of photosynthesis pigments in trees changes with their growth and development, also including their age, location of stems in the crown, light conditions and access to minerals and water [Merzlyak et al. 1999, Silkina and Vinokurova 2009].

The aim of the consider experiment was the determination of the usefulness of four rootstocks and two dates of grafting procedure for the propagation of two mountain pine cultivars. An attempt was also taken to determine the influence of the rootstock and the cultivar on the content of chloroplast pigments in needles, which had not been studied before.

MATERIAL AND METHODS

The experiment was conducted twice in years 2015–2017 in Kobylin in the south region of Great Poland in a private nursery of trees and ornamental shrubs belonging Mr. and Mrs. Nowaczyk. The plant material were scions of *Pinus mugo* of 'Grześ' and 'Zundert' cultivars and the rootstocks of *Pinus nigra*, *Pinus uncinata*, *Pinus contorta* and *Pinus sylvestris*. The experiment consisted of 16 combinations (4 rootstocks × 2 cultivars × 2 dates). Each combination consisted of 4 repetitions with 20 plants in each one. Altogether the experiment included 1280 grafted rootstocks.

One year before the experiment started the purchased rootstocks had been planted into P9 pots filled with a mixture of peat, bark and perlite in 10 : 1 : 1 ratio and added of slowly realized fertilizer Osmocote Exact in the amount of $4 g \cdot l^{-1}$ of the substrate. Grafting procedure was conducted in two dates: on 20 January and 15 March of 2015 and 2016 year. At the end of November the rootstocks were placed in a foil tunnel, where their selection took place. The ones with the diameter of 6–7 mm were chosen and lateral shoots were removed from the bottom. All scions were taken in the period of the scion plant's full dormancy.

The rootstocks were graft by the use of side grafting method [Hrynkiewicz-Sudnik et al. 1999]. Next the scions were tied with a rubber band. All plants from each combination were labeled and placed in a heated foil tunnel in the temperature between 8–12ºC during winter months. To ensure higher moisture all grafts were covered with a low foil tunnel. After a period of six weeks from the day of grafting procedure, cold hardening – by gradual airing – started. At the end of April the plants were taken from the tunnel and planted into C 21 pots filled with the mixture of peat, bark and perlite in 10 : 1 : 1 ratio with the addition of Osmocote Exact in the amount of 5 $g \cdot l^{-1}$ of the substrate. In the same time the rootstocks were pruned in the middle of their height and the shoots growing in the direct vicinity of the grafted cultivar were removed. After following six weeks all rootstocks individually were cut just above the growing cultivar. The measurements and observations of the plants were conducted twice after the end of the plants' growth in the first and second year of their cultivation. They concerned the graft success, lengths of their main stems as well as the number of lateral shoots.

The needles used for the determination of the level of chloroplast pigments were collected in the first week of September. The determination was carried out according to Hiscox i Israelstam [1979], method, which allows to extract pigments form the plant material by means of dimethyl sulfoxide (DMSO) without tissue maceration. Test portions of 0.05 g were cut into 2–3 cm segments and they were treated with 5 cm^3 DMSO and incubated in water bath at 65°C for 60 min. The content of individual pigments in the extract was determined with a spectrophotometer at an appropriate wavelength. For chlorophyll A the measurement of the extract's absorbance was carried out at the wavelength of 665 nm, for chlorophyll B at 649 nm and for carotenoids at 480 nm. The content of pigments was calculated according to modified Arnon's formulae [1949].

$$\text{Chlorophyll A} = (12.19 \times A_{665} - 3.45 \times A_{649}) \times \times V \times (1000\ W)^{-1}\ [mg \cdot g^{-1}\ \text{fresh mass}]$$

$$\text{Chlorophyll B} = (21.99 \times A_{649} - 5.32 \times A_{665}) \times \times V \times (1000\ W)^{-1}\ [mg \cdot g^{-1}\ \text{fresh mass}]$$

$$\text{Sum A + B} = (18.09 \times A_{649} + 7.05 \times A_{665}) \times \times V \times (1000\ W)^{-1}\ [mg \cdot g^{-1}\ \text{fresh mass}]$$

$$\text{Carotenoids} = 1000 \times A_{480} - 2.14\ \text{chl. A} - - 70.16\ \text{chl. B} \times 214^{-1}\ [mg \cdot g^{-1}\ \text{fresh mass}]$$

where: A – absorbance at a given wavelength, V – total volume of extract (cm^3), W – mass of a sample (g).

To compare the obtained results two-factor variance analysis (rootstocks and grafting dates for growth features and grafts success, and the rootstocks and cultivars for the content of chloroplast pigments) was applied. The significance of differences among combinations was evaluated on the basis of confidence intervals, using Duncan test for significance level α = 0.05. To calculate percentage values Bliss transformation was used.

RESULTS

The results of the percentage of graft success for 'Grześ' cultivar were differentiated by the rootstock used and the date of grafting procedure. In the earlier date the best result was obtained on *Pinus sylvestris*, and the worst on *Pinus nigra*. Another dependence of the percentage of graft success on the rootstocks was found in the second date of grafting. Here the best results were observed on the two rootstocks: *Pinus uncinata* and *Pinus contorta* (Tabs. 1, 2 and 3).

Analyzing the rootstocks use and date of grafting the biggest increments of lateral shoots were obtained on *Pinus nigra* in the first date of grafting procedure. The smallest ones were observed on *Pinus contorta* in both terms of grafting procedure, and also on *Pinus uncinata* and *Pinus sylvestris* in the later term (Tabs. 1, 2 and 3).

Significantly the biggest lengths of the main stem for 'Grześ' cultivar were obtained in the first date of grafting procedure for all combinations of rootstocks, and in the second term only for Pinus *nigra* and *Pinus sylvestris* rootstocks (Tabs. 1, 2 and 3).

The rootstocks and grafting procedure date used in the experiment did not have any significant influence on the number of increments of 'Grześ' cultivar in the first and the second year of its growth (Tabs. 1, 2 and 3).

The best percent of graft success for 'Zundert' cultivar in the first date of grafting was observed on *Pinus sylvestris* and *Pinus uncinata.* In the second date, however, significantly better graft success was observed for *Pinus contorta* and *Pinus uncinata* (Tab. 4).

Table 1. The values of individual features for *Pinus mugo* 'Grześ' – interaction of factors

Feature	Rootstock	Date of grafting procedure		Femp.
		20 January	15 March	
Percentage of graft success	*Pinus contorta*	73.0 b	87.0 d	38.21 **
	Pinus nigra	63.3 a	73.0 b	
	Pinus sylvestris	80.0 c	73.1 b	
	Pinus uncinata	73.0 b	87.0 d	
The average of increments' lengths in the first year	*Pinus contorta*	4.35 ab	3.65 a	0.19
	Pinus nigra	5.18 c	4.59 bc	
	Pinus sylvestris	4.59 bc	4.23 ab	
	Pinus uncinata	4.46 b	3.88 ab	
The average of the length of the main stem	*Pinus contorta*	5.40 bc	5.00 ab	1.06
	Pinus nigra	6.30 c	6.00 c	
	Pinus sylvestris	5.70 bc	5.70 bc	
	Pinus uncinata	5.50 bc	4.50 a	
The average of the number of increments in the first year	*Pinus contorta*	3.60 a	3.80 a	0.09
	Pinus nigra	3.70 a	3.80 a	
	Pinus sylvestris	3.70 a	3.70 a	
	Pinus uncinata	3.60 a	3.80 a	
The average of number of increments in the second year	*Pinus contorta*	14.20 a	14.70 a	0.42
	Pinus nigra	14.10 a	13.90 a	
	Pinus sylvestris	14.20 a	13.30 a	
	Pinus uncinata	14.50 a	15.60 a	

Data marked with the same letters do not differ among each other for an individual feature at α = 0.05. ** means a very significant difference

Table 2. The values of individual features depended on used rootstocks for *Pinus mugo* 'Grześ'

Feature	Rootstock	Value of feature	Femp.
Percentage of graft success	*Pinus contorta*	80.0 c	47.74 **
	Pinus nigra	68.2 a	
	Pinus sylvestris	76.5 b	
	Pinus uncinata	80.0 c	
The average of increments' lengths in the first year	*Pinus contorta*	4.00 a	5.46 **
	Pinus nigra	4.89 b	
	Pinus sylvestris	4.41 a	
	Pinus uncinata	4.17 a	
The average of the length of the main stem	*Pinus contorta*	5.20 ab	6.45 **
	Pinus nigra	6.15 c	
	Pinus sylvestris	5.70 bc	
	Pinus uncinata	5.00 a	
The average of the number of increments in the first year	*Pinus contorta*	3.70 a	0.03
	Pinus nigra	3.75 a	
	Pinus sylvestris	3.70 a	
	Pinus uncinata	3.70 a	
The average of number of increments in the second year	*Pinus contorta*	14.45 a	0.73
	Pinus nigra	14.00 a	
	Pinus sylvestris	13.75 a	
	Pinus uncinata	15.05 a	

Data marked with the same letters do not differ among each other for an individual feature at α = 0.05. ** means a very significant difference

Table 3. The values of individual features depended on date of grafting for *Pinus mugo* 'Grześ'

Feature	Date of grafting		Femp.
	20 January	15 March	
Percentage of graft success	72.3 a	80.0 b	90.04 **
The average of increments' lengths in the first year	4.65 b	4.09 a	11.36 **
The average of the length of the main stem	5.73 b*	5.30 a	4.36 *
The average of the number of increments in the first year	3.65 a	3.78 a	0.63
The average of number of increments in the second year	14.25 a	14.38 a	0.03

Data marked with the same letters do not differ among each other for an individual feature at α = 0.05. * means a significant difference, ** means a very significant difference

Table 4. The values of individual features for *Pinus mugo* 'Zundert' – interaction of factors

Feature	Rootstock	Date of grafting		Femp.
		20 January	15 March	
Percentage of graft success	*Pinus contorta*	67.0 b	80.0 d	145.38 **
	Pinus nigra	67.0 b	60.0 a	
	Pinus sylvestris	73.1 c	67.0 b	
	Pinus uncinata	73.1 c	80.0 d	
The average of increments' lengths in the first year	*Pinus contorta*	4.30 abc	4.70 bc	3.82*
	Pinus nigra	4.48 abc	4.24 abc	
	Pinus sylvestris	3.93 ab	4.80 c	
	Pinus uncinata	4.53 abc	3.70 a	
The average of the length of the main stem	*Pinus contorta*	5.20 ab	6.20 b	4.52 **
	Pinus nigra	5.80 ab	5.40 ab	
	Pinus sylvestris	5.10 ab	6.00 ab	
	Pinus uncinata	6.10 b	4.90 a	
The average of the number of increments in the first year	*Pinus contorta*	3.70 a	3.70 a	0.02
	Pinus nigra	3.70 a	3.70 a	
	Pinus sylvestris	3.60 a	3.60 a	
	Pinus uncinata	3.60 a	3.70 a	
The average of number of increments in the second year	*Pinus contorta*	14.50 a	14.10 a	0.39
	Pinus nigra	14.10 a	15.20 a	
	Pinus sylvestris	14.90 a	14.00 a	
	Pinus uncinata	14.20 a	14.80 a	

Data marked with the same letters do not differ among each other for an individual feature at α = 0.05. * means a significant difference, ** means a very significant difference

Table 5. The values of individual features depended on used rootstocks for *Pinus mugo* 'Zundert'

Feature	Rootstock	Value of feature	Femp.
Percentage of graft success	*Pinus contorta*	73.5 c	187.38 **
	Pinus nigra	63.5 a	
	Pinus sylvestris	70.0 b	
	Pinus uncinata	76.5 d	
The average of increments' lengths in the first year	*Pinus contorta*	4.50 a	0.70
	Pinus nigra	4.36 a	
	Pinus sylvestris	4.36 a	
	Pinus uncinata	4.12 a	
The average of the length of the main stem	*Pinus contorta*	5.70 a	0.12
	Pinus nigra	5.60 a	
	Pinus sylvestris	5.55 a	
	Pinus uncinata	5.50 a	
The average of the number of increments in the first year	*Pinus contorta*	3.70 a	0.08
	Pinus nigra	3.70 a	
	Pinus sylvestris	3.60 a	
	Pinus uncinata	3.65 a	
The average of number of increments in the second year	*Pinus contorta*	14.30 a	0.04
	Pinus nigra	14.65 a	
	Pinus sylvestris	14.45 a	
	Pinus uncinata	14.50 a	

Data marked with the same letters do not differ among each other for an individual feature at α = 0.05. ** means a very significant difference

Table 6. The values of individual features depended on date of grafting for *Pinus mugo* 'Zundert'

Feature	Date of grafting		Femp.
	20 January	15 March	
Percentage of graft success	70.0 a	71.8 b	18.38 **
The average of increments' lengths in the first year	4.31 a	4.36 a	0.07
The average of the length of the main stem	5.55 a	5.63 a	0.09
The average of the number of increments in the first year	3.65 a	3.68 a	0.02
The average of number of increments in the second year	14.43 a	14.53 a	0.02

Data marked with the same letters do not differ among each other for an individual feature at α = 0.05. ** means a very significant difference

The length of increments in the first grafting date did not differ significantly. In the later term of the procedure the highest value of this parameter was obtained on *Pinus sylvestris*, which did not differ much from the lowest value obtained on *Pinus uncinata*.

Significantly the biggest value of the main stem's length for 'Zundert' cultivar was obtained in the second date of grafting procedure on *Pinus contorta* and in the first one on *Pinus uncinata*. These results were different only from those obtained for the later date of grafting carried out on *Pinus uncinata* rootstock (Tabs. 4, 5 and 6).

Rootstock combinations and dates of grafting procedure did not have any big impact on the number of increments, independently from the year of measurements (Tabs. 4, 5 and 6). Also the two grafting dates

Table 7. Content of pigments in the needles depending on the combination

Combination Cultivar/rootstock	Chlorophyll A	Chlorophyll B	Carotene
Grześ / *Pinus nigra*	0.5198 c	0.1795 bc	0.5330 c–e
Grześ / *Pinus sylvestris*	0.5717 c	0.2006 c	0.5924 de
Grześ / *Pinus uncinata*	0.5452 c	0.1908 c	0.5933 de
Grześ / *Pinus contorta*	0.5552 c	0.1944 c	0.6197 e
Zundert / *Pinus nigra*	0.2630 b	0.1383 b	0.4804 b–d
Zundert / *Pinus sylvestris*	0.1991 b	0.0691 a	0.4367 abc
Zundert / *Pinus uncinata*	0.1604 ab	0.0547 a	0.3896 ab
Zundert / *Pinus contorta*	0.0712 a	0.0271 a	0.3575 a
Femp.	3.03 °	7.51 **	3.02 °

Data marked with the same letters do not differ among each other for an individual feature at $\alpha = 0.05$. ** means a very significant difference, ° means a significant difference at $\alpha = 0.01$

Table 8. Content of pigments in the needles depending on used rootstock

Rootstock	Chlorophyll A	Chlorophyll B	Carotene
Pinus nigra	0.3914 a	0.1589 b	0.5067 a
Pinus sylvestris	0.3854 a	0.1349 ab	0.5146 a
Pinus uncinata	0.3528 a	0.1227 a	0.4915 a
Pinus contorta	0.3132 a	0.1107 a	0.4886 a
Femp.	1.81	4.32 *	0.24

Data marked with the same letters do not differ among each other for an individual feature at $\alpha = 0.05$. * means a significant difference

did not differentiate the results of this parameter obtained in the two years of the observation of the growth of 'Zundert' cultivar (Tabs. 4, 5 and 6).

Taking into consideration the influence of the rootstock on the level of chloroplast pigments, a significantly higher content of chlorophyll A and B and carotene was obtained for 'Grześ' cultivar in all rootstock combinations (Tabs. 7 and 8). From all the rootstocks used in the experiment only *Pinus nigra* had a significant influence on a higher level of Chlorophyll B (Tab. 8).

DISCUSSION

The side grafting method, which was used in the experiment is a method propagation suggested for conifer plants [Bärtels 1982, Macdonald 1986, Hartmann et al. 2011]. Taking into account a high percentage of graft success in the conducted experiment one can recommend this method for the propagation of the cultivars of mountain pine trees.

A lot of authors [Kleinschmidt 1975, Bärtels 1982, Hrynkiewicz-Sudnik et al. 1999, Hartmann et al. 2011] pay attention to the choice of appropriate rootstocks for grafting procedure. For two-needled pines the recommended rootstocks are *Pinus sylvestris* and *Pinus contorta*, for three-needled ones *Pinus sylvestris* and also *Pinus ponderosa* and *Pinus jeffreyi*. For five-needled pines a recommended rootstock are *Pinus contorta* and *Pinus strobus* – although the last one is being withdrawn because of its vulnerability to white pine blister rust. Also Bärtels [1982] thinks that *Pinus contorta* is one of the most universal rootstocks for grafting pine trees. Among the studied rootstocks, analyzing the number of taken scions only *Pinus nigra* rootstock influenced the lowering of the effectiveness of grafting procedure, especially in its earlier term.

On the other hand, *Pinus nigra* affected the more intensive growth of grafted pine tree cultivars. However, it took place only in the first year of cultivation. As far as the effectiveness of grafting procedure is concerned, two rootstocks: *Pinus uncinata* and *Pinus contorta* were outstanding, especially in the last term of grafting procedure. Looking into the mutual influence of the rootstock and the cultivar grafted on it, it is hard to explain existing differences in graft success. It could be connected with the depth of dormancy of individual rootstocks or with the intensity of their rasin tapping after their acceleration directly before the grafting procedure. Differentiation in the growth potential at winter rest, depending on the cultivar was confirmed by other researchers [Oribe and Kubo 1997, Oribe et al. 2001, 2003]. On the basis of the conducted experiment and the previous one [Nowaczyk 2014] it can be concluded that *Pinus uncinata* is also a good rootstock, which can be successfully used for the propagation of slowly growing dwarf pine trees. *Pinus nigra* and *Pinus sylvestris*, however, are recommended for fast growing pines, although the graft success in smaller on them in comparison with other rootstocks.

Because of the observed influence of the time of grafting procedure on the graft success and growth of coniferous plants two grafting terms were chosen for the experiment [Mergen 1955, Bärtels 1982, Blazich and Hinesely 1994, Hinesley and Frampton 2002]. The later grafting term in the middle of March turned out to be better as far as the percentage of graft success is concerned, and worse referring to the growth of the plants in the first year of cultivation. In the previous experiment [Nowaczyk 2014] it was also observed that mountain pine cultivars with needles which discolour into yellow in winter, have a bigger percentage of graft success in a later grafting term (the middle of March) compared with the January term. It is not confirmed, however, by Barnett and Miller [1994] who recommend an earlier grafting term for coniferous species.

Some authors [Bärtels 1982, Hrynkiewicz-Sudnik et al. 1999] recommend beginning the grafting procedure of pine trees in December. In the conducted experiment it was found that the earlier term did not have any significant advantage over later ones, and in early spring months even better results are obtained. The advantage of the spring grafting is that such grafts start their vegetation earlier and growing shoots are characterized by bigger increments. On the basis of the conducted studies it can be stated that there are a lot of factors, which affect the percentage of graft success and the pace of growth of the plants propagated by grafting. Therefore it is worth to carry out further studies in this field, as the available literature is very limited.

Studies on the influence of the rootstock and the time of grafting procedure for selected cultivars of mountain pine which discolour in winter, did not give explicit results. However, some dependencies can be observed. The biggest graft success for both cultivars of mountain pine was observed on *Pinus uncinata* and *Pinus contorta* rootstocks. The biggest increments for *Pinus mugo* 'Grześ', however, were obtained on *Pinus nigra* for the earlier grafting term. The smallest increments on *Pinus mugo* 'Grześ' were obtained in both dates on *Pinus contorta* and on *Pinus uncinata* in the second one.

The highest level of chlorophyll A and chlorophyll B in the needles of the studied cultivars was found in 'Grześ' cultivar independently from the rootstock used (Tab. 9). One can draw a conclusion that it is the cultivar with the most intensive growth. It does not confirm

Table 9. Content of pigments in the needles depending on cultivar

Cultivar	Chlorophyll A	Chlorophyll B	Carotene
Grześ	0.5480 b	0.1913 b	0.5846 b
Zundert	0.1734 a	0.0723 a	0.4161 a
Femp.	196.54 **	144.49 **	43.62 **

Data marked with the same letters do not differ among each other for an individual feature at $\alpha = 0.05$. ** means a very significant difference the opinion of Uvalle Sauceda et al. [2008] that the content of chlorophyll B and carotenoids did not differ among growth forms. Among the tested rootstocks only *Pinus nigra* had a significant influence on a higher level of chlorophyll B which confirms a stronger growth of this rootstock in comparison with the other ones used in the experiment. The influence of a rootstock and cultivar on the content of chloroplast pigments in leaves were not studied earlier. The opinion of other authors that studies on the level of chloroplast pigments can provide valuable information about the physiological status of plants potential found its confirmation [Curran et al. 1990, Filella et al. 1995, Sims and Gamon 2002, Steele et al. 2008].

CONCLUSIONS

1. The highest percentage of graft success in the first date of propagation was obtained for 'Grześ' cultivar on *Pinus sylvestris* rootstock, and for 'Zundert' in the second date on *Pinus contorta*, and *Pinus uncinata* rootstocks. The highest percentage of graft success for the studied cultivars was obtained in the second term of grafting procedure.

2. The biggest increments for 'Grześ' were obtained on *Pinus nigra*. For 'Zundert' cultivar no significant influence of the rootstock used was observed.

3. Plants of 'Grześ' cultivar grew more intensively, independently from the rootstock used. This cultivar was characterized also by a higher level of chloroplast pigments.

4. Of all studied rootstocks only *Pinus nigra* had a significant influence on the increase of the chlorophyll B content in needles.

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REFERENCES

Adams, W.W., Demmig-Adams, B. (1994). Carotenoid composition and down regulation of photosystem II in three conifer species during the winter. Physiol. Plant., 92, 451–458. DOI: 10.1111/j.1399-3054.1994.tb08835.x

Ahlgren, C.E., Wilderness, Q.S. (1972). Some effects of inter and intraspecific grafting on growth and flowering of some five-needle pine. Silvae Genet., 21(3/4), 122–126.

Almqvist, C. (2013). Interstock effects on topgraft vitality and strobili production after topgrafting in *Pinus sylvestris*. Can. J. For. Res., 43(6), 584–588. DOI: 10.1139/cjfr-2012-0507

Arnon, D.J. (1949). Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. Plant Physiol., 24, 1–15. DOI: 10.1104/pp.24.1.1

Barnett, J.R., Miller, H. (1994). The effect of applied heat on graft formation in dormant *Picea sitchensis* (Bong.) Carr. J. Exper. Bot., 45(1), 135–143.

Barnett, J.R., Weatherhead, I. (1989). The effect of scion water potential on graft success in Sitka spruce (*Picea sitchensis*). Ann. Bot., 64(1), 9–2. DOI: 10.1093/oxfordjournals.aob.a087813

Bärtels, A. (1982). Rozmnażanie drzew i krzewów ozdobnych [Propagation of ornamental trees and shrubs]. Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa [in Polish].

Blazich, F.A., Hinesely, L.E. (1994). Propagation of fraser fir. J. Environ. Hort., 12, 112–117.

Bolhàr-Nordenkampf, H.R., Öquist, G. (1993). Chlorophyll fluorescence as a tool in photosynthesis research. In: Photosynthesis and production in a changing environment: a field and laboratory manual, Hall, D.O., Scurlock, J.M.O., Bolhàr-Nordenkampf, H.R., Leegood, R.C., Long, S.P. (eds.). Chapman and Hall, London, 193–206.

Carter, G.A., Knapp, A.K. (2001). Leaf optical properties in higher plants: Linking spectral characteristics to stress and chlorophyll concentration. Am. J. Bot., 88, 677–684.

Colom, M.R., Pini Prato, E., Giannini, R. (2003). Chlorophyll fluorescence and photosynthetic response to light in 1-year-old needles during spring and early summer in *Pinus leucodermis*. Trees, 17, 207–210. DOI: 10.1007/s00468-002-0222-2

Curran, P.J., Dungan, J.L., Gholz, H.L. (1990). Exploring the relationship between reflectance red edge and chlorophyll content in slash pine. Tree Physiol., 7, 33–48. DOI: 10.1093/treephys/7.1-2-3-4.33

De-li, S.O.N.G., Shao-ling, J.J., Li-min, Z. (2007). Primary study on survival and growth potential of different grafting methods for Korean pine (J.). J. Jilin For. Sci. Tech., 3:002 [in Chinese].

Filella, I., Serrano, L., Serra, J., Peñuelas, J. (1995). Evaluating wheat nitrogen status with canopy reflectance indices and discriminant analysis. Crop Sci., 35, 1400–1405. DOI: 10.2135/cropsci1995.0011183X003500050023x

Frey, H.H., Frampton, J., Blazich, F.A., Hinesley, L.E. (2010). Grafting Fraser Fir (*Abies fraseri*): effect of grafting date, shade and irrigation. HortScience, 45(4), 617–620. DOI: 10.21273/HORTSCI.45.4.617

Frey, H.H., Frampton, J., Blazich, F.A., Hundley, D., Hinesley, L.E. (2011). Grafting fraser fir (*Abies fraseri*): effect of scion origin (crown position and branch order). HortScience, 46(1), 91–94. DOI: 10.21273/HORTSCI.46.1.91

Gamon, J.A., Surfus, J.S. (1999). Assessing leaf pigment content and activity with a reflectometer. New Phytol., 143, 105–117. DOI: 10.1046/j.1469-8137.1999.00424.x

Grassi, G., Colom, M.R., Minotta, G. (2008). Effects of nutrient supply on photosynthetic acclimation and photoinhibition of one-year-old foliage of *Picea abies*. Physiol. Plant., 111, 245–254. DOI: 10.1034/j.1399-3054.2001.1110217.x

Haines, R.J., Simpson, J.A. (1994). Scion-rootstock relationships with respect to height growth and foliar concentrations of nitrogen and phosphorus in reciprocal grafts of *Pinus caribaea* var. *Hondurensis.* New Forests, 8, 71–79. DOI: 10.1007/BF00034132

Hartmann, H.T., Ester, D.E.K, Avies, F.T. Denever, G. (2011). Hartmann and Kester's plant propagation: Principles and practices, 8th ed. Pearson, New York, pp. 838.

Hendry, G.A.F., Houghton, J.D., Brown, S.B. (1987). Tansley review no. 11: the degradation of chlorophyll – a biological enigma. New Phytol., 107, 255–302.

Hiscox, J.D., Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot., 57, 1332–1334. DOI: 10.1139/b79-163

Holzer, K. (1970). Experments in heteroplastic grafting of *Pinus cembra*. Silvae Genet., 19, 164–170 [in German].

Hrynkiewicz-Sudnik, J., Sękowski, B., Wilczkiewicz, M. (1999). Rozmnażanie drzew i krzewów nagozalążkowych [Propagation of Gymnosperm Trees and Shrubs]. Wydawnictwo Naukowe PWN, Warszawa [in Polish].

Jayawickrama, K.J.S., Jett, J.B., McKeand, S.E. (1991). Rootstock effects in grafted conifers: a review. New Forest, 5, 157–173. DOI: 10.1007/BF00029306

Jayawickrama, K.J.S., McKeand, S.E., Jett, J.B. (1997). Rootstock effects on scion growth and reproduction i 8-year-old grafted loblolly pine. Can. J. For. Res., 27(11), 1781–1787.

Hinesley, L.E., Frampton, J. (2002). Grafting fraser fir onto rootstock of selected Abies species. HortScience, 37, 815–818. DOI: 10.21273/HORTSCI.37.5.815

Karadeniz, T. (2005). Relationship between graft success and climatic values in walnut (*Juglans regia* L.) J. Cent. Eur. Agric., 6, 631–634.

Kleinschmidt, J. (1975). Vegetative Vermehrung der Fichte. Mitt. Des Vereins für Forstliche Standortskunde unf Forstpflanzenzüchtung [Vegetative propagation of the spruce]. Mitteilungen 24 [in German].

Larcher, W. (1995). Physiological Plant Ecology, 3rd ed. Springer, Berlin.

Macdonald, B. (1986). Practical woody plant propagation for nursery growers. Timber Press, Portland, Oregon.

Merzlyak, M.N., Gitelson, A.A., Chivkunova, O.B. Rakitin, V.Y.U. (1999). Non-destructive optical detection of pigment changes during leaf senescence and fruit ripening. Physiol. Plant., 106, 135–141. DOI: 10.1034/j.1399-3054.1999.106119.x

Moran, J.A., Mitchell, A.K., Goodmanson, G., Stockburger, K.A. (2000). Differentiation among effects of nitrogen fertilization treatments on conifer seedlings by foliar reflectance: a comparison of methods. Tree Physiol., 20, 1113–1120. DOI: 10.1093/treephys/20.16.1113

Mergen, F. (1955). Grafting slash pine in the field and in the greenhouse. J. For., 53, 836–842.

Niinemets, U. (1997). Role of foliar nitrogen in light harvesting and shade tolerance of four temperate deciduous woody species. Funct. Ecol., 11, 518–531. DOI: 10.1046/j.1365-2435.1997.00109.x

Nowaczyk, N. (2014). Wpływ podkładki i terminu szczepienia na wzrost dwóch odmian sosen [Impact of rootstock and grafting date on the growth of two pine varieties]. Praca inżynierska, UP Poznań.

Oribe, Y., Kubo, T. (1997). Effect of heat on cambial reactivation during winter dormancy in evergreen and deciduous conifers. Tree Physiol., 17, 81–87. DOI: 10.1093/treephys/17.2.81

Oribe, Y., Funada, R., Shibagaki, M., Kubo, T. (2001). Cambial reactivation in locally heated stems of the evergreen conifer *Abies sachalinensis* (Schmidt) Masters. Planta, 212, 684–691. DOI: 10.1007/s004250000430

Oribe, Y., Funada, R., Kubo, T. (2003). Relationships between cambial activity, cell differentiation and the localization of starch in storage tissues around the cambium in locally heated stems of *Abies sachalinensis* (Schmidt) Masters. Trees, 17, 185–192. DOI: 10.1007/s00468-002-0231-1

Ottander, C., Campbell, D., Öquist, G. (1995). Seasonal changes in photosystem II organization and pigment composition in *Pinus sylvestris*. Planta, 197, 176–183. DOI: 10.1007/BF00239954

Oquist, G., Huner, N.P.A. (2003). Photosynthesis of overwintering evergreen plants. Annu. Rev. Plant Biol., 54, 329–355. DOI: 10.1146/annurev.arplant.54.072402.115741

Peñuelas, J., Filella, I. (1998). Visible and near-infrared reflectance techniques for diagnosing plant physiological status. Trends Plant Sci., 3, 151–156.

Rybus-Zając, M. (2005). Oxidative stress generation in *Taxus baccata* leaves affected by *Pestalotiopsis funerea* Desm. Under different light conditions. Dendrobiology, 54, 51–56.

Rybus-Zając, M. (2010). Wpływ zwiększonego promieniowania UV-B na poziom barwników chloroplastowych w roślinach cisa pospolitego (*Taxus baccata* L.) [The influence of enhanced UV-B radiation on the chloroplast pigments level in common yew (Taxus baccata L.) plants]. Nauka Przyr. Technol., 4(3), 38 [in Polish].

Silkina, O.V., Vinokurova, R.I. (2009). Seasonal dynamics of chlorophyll and microelement content in developing conifer needles of *Abies sibirica* and *Picea abies*. Russ. J. Plant Physiol., 56, 780–786. DOI: 10.1134/S1021443709060077

Sims, D.A., Gamon, J.A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens. Environ., 81, 337–354. DOI: 10.1016/S0034-4257(02)00010-X

Shu, W.B., Liang, Y.Y., Yang, Z.Q., Su, W.B., Liang, X.Z. (2013). The graft technology of high-yield rosin *Pinus massoniana* seed orchard. J. Fujian For. Sci. Tech, 1, 85–88 [in Chinese].

Schmidtling, R.C. (1983). Rootstock influences flowering, growth and survival of loblolly pine grafts. Forest Sci., 29(1), 117–124.

Steele, M., Gitelson, A.A., Rundquist, D. (2008). Non--destructive estimation of leaf chlorophyll content in grapes. Am. J. Enol. Vitic., 59, 299–305.

Strand, M. (1995). Inhibition of photosynthesis in current year needles unfertilized and fertilized Norway spruce [*Picea abies* (L.) Karst.] during autumn and early winter. Trees, 9, 332–340. DOI: 10.1007/BF00202497

Valladares, F., Martinez-Ferri, E., Balaguer, L., Perez-Corona, E., Manrique, E. (2000). Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: A conservative resource-use strategy. New Phytol., 148, 79–91.

Uvalle Sauceda, J.I., Gonzalez Rodriguez, H., Ramirez Lozano, R.G., Cantu Silva, I., Gomez Meza, M.V. (2008). Seasonal trends chlorophylls a and b and carotenoids in native trees and shrubs of Northeastern Mexico. J. Biol. Scien., 8(2), 258–267. DOI: 10.3923/jbs.2008.258.267

Welch, H., Haddow, G. (1993). The World Checklist of Conifers. The World Conifer Data Pool.

Wen-Jun, F. (2007). Effect analysis of twig grafting of *Pinus taiwanensis*. J. Fujian For. Sci. Tech., 4, 95–97 [in Chinese].

Wu, Y.-P., Liang, X., Liu, X.-Y., Zhong, K., Gao, B., Huang, Y.-N., Gao, H. (2015). *Cedrus deodara* pine needle as a potential source of natural antioxidants: Bioactive constituents and antioxidant activities. J. Func. Foods, 14, 605–612. DOI: 10.1016/j.jff.2015.02.023