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INFLUENCE OF ROOTSTOCK AND GRAFTING METHOD ON THE GRAFTS SUCCESS AND GROWTH OF *Cedrus deodara* (Roxb. ex Lamb.) 'KARL FUCHS' PLANTS

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ABSTRACT

Experimental studies were conducted in 2015–2017 in the Department of Dendrology, Pomology and Nursery of Poznan University of Life Sciences. The aim of the research was to study the usefulness of three rootstocks: *Larix decidua*, *Pinus armandii*, *Cedrus deodara* and two grafting methods: side grafting and split grafting for propagation purposes of *Cedrus deodara* 'Karl Fuchs'. In addition, it was evaluated how the rootstock and the age of cultivar shoots influenced the level of chloroplast pigments in the needles. A higher percentage of grafts success was obtained on *Pinus armandii* and *Cedrus deodara* using the side grafting method. Grafted scions of 'Karl Fuchs' produced the greatest number of lateral buds and the longest lateral increments of growth on *Cedrus deodara* root stock. Side grafting significantly improved the percentage of plants that restarted their growth in the second year of cultivation. The largest number of buds on two-year-old shoots were counted on plants grafted on *Cedrus deodara* using split grafting method. The rootstocks used in this experiment had a slight effect on the content of chloroplast pigments in the needles. Plants grafted on *Pinus armandii* had the highest level of chlorophyll B, and those grafted on *Larix decidua* – chlorophyll A/B ratio. The content of chloroplast pigments in the needles depended on the age of shoots. The highest level of chlorophyll A, chlorophyll B and carotenoids was observed when needles were collected from the last year's growth.

Key words: deodar cedar, propagation, sprouts growth, number of buds, survival of grafts

INTRODUCTION

So far there have been studies aiming at grafting scions on rootstocks of different coniferous species, especially less frequent ones such as *Cedrus deodara* [Singh and Mahajan 1967, Richards 1972, Layon 1984, Siniscalco 1995, Ishii et al. 2008, Mudge et al. 2009, Joshi and Verman 2010, Melnyk and Meyerowitz 2015, Sevik et al. 2016]. According to Richards [1972], Lyon [1984], Siniscalco [1995] and Pijut [2000] cultivars of *Cedrus* are more routinely propagated by grafting. In their studies Holzer [1970] Schmidtling [1983], Jayawickrama et al. [1991, 1997], Haines and Simpson [1994] confirmed that the used rootstock influences the percentage success of grafts and the subsequent growth of grafted species of coniferous trees. Apart from the rootstock, the success of grafting is also influenced by the quality of scions, and especially the scion position on the branch of the mother plant [Frey et al. 2011], the age of the mother plant [Almqvist 2013, Shu et al. 2013], and the length of time the scions were held in storage [Barnett and

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Weatherhead 1989, Wen-Jun 2007]. The choice of a grafting method is also very important [De-li et al. 2007], as well as the term of the grafting [Frey et al. 2010]. The change in weather conditions after grafting influences the success of grafts and further growth of grafts [Karadeniz 2005].

Phenology, crown and needle characters, mineral contents, organic compounds were also checked in grafted conifers [Jayawickrama et al. 1991].

Studies on chlorophyll and carotenoids content in the needles of different species of coniferous trees were conducted by many authors [Wolf 1956, Lewandowska and Jarvis 1977, Ottander and O'quist 1991, Han and Mukai 1999, Han et al. 2004, Robakowski 2005, Ruiru et al. 2015, Yan-Ping Wu et al. 2015]. Their aim was to evaluate the resistance to a stress factor in the winter season, especially to low temperature. It was found that chlorophyll content is a good bio-indicator of plants physiological condition [Sampson et al. 2003, Zarco-Tejada et al. 2002]. Research shows that chlorophyll content declines more rapidly than carotenoid content when plants are experiencing stress or during leaf senescence [Merzlyak and Gitelson 1995, Sims and Gamon 2002].

The aim of present studies was to check the usefulness of rootstocks and grafting methods for propagation purposes of the selected cultivar of *Cedrus deodara* 'Karl Fuchs'. The content of chloroplast pigments level was also estimated to check the physiological state taking place in grafted plants which result from the compatibility of the considered cultivar and the rootstock used.

MATERIAL AND METHODS

The experiment was conducted in two series within two years of cultivation. The experiment consisted of 6 combinations (2 grafting methods × 3 rootstocks). Each combination had three replications of 20 plants in each. The plant material consisted of two-year-old *Cedrus deodara*, *Pinus armandii* and *Larix decidua* rootstocks. Two grafting methods: side grafting and split grafting were used. *Cedrus deodara* and *Pinus armandii* rootstocks were planted in C3 pots filled with a mixture of high peat and keramzyt in 10 : 1 ratio and fed with Osmocote Exact in the amount of 3 $g \cdot l^{-1}$of substrate. *Larix decidua* rootstocks were planted into P9 pots filled with a mixture of peat with perlite in 10 : 1 ratio and fed with the same fertilizer but in the amount of 1.5 $g \cdot l^{-1}$ of the substrate.

Grafting was conducted in the third week of March. Scions 10 cm long were taken from the mother *Cedrus deodara* 'Karl Fuchs' growing in the field a few days before grafting and they were stored in a cold store at 2−3°C. Scions were taken from the top of the main shoots, from a well sunlit part of the crown, from the last year's growth. After the scion was grafted to the rootstock with a rubber band, the grafts were relocated to plant beds in a 9 × 30 m^2 tunnel. The plants were watered by means of micro-sprinklers to maintain appropriate humidity. At the beginning of May, airing of the tunnel started. In the second week of June the plants were moved to field beds. In the third week of August the rootstocks were cut diagonally above the graft and the percentage of grafts success was evaluated. The measurements were carried out after 8 months from the beginning of the experiment as well as in May of the successive year after overwintering of plants.

Determination of chloroplast pigment level in the needles was conducted according to the method of Hiscox and Israelstam [1979], which extract dyes from plant material with di-methyl sulfoxide (DMSO) without maceration of the tissue. The needles were collected at the end of the first and second year of cultivation. The needles came from shoots from current year's and last year's growth. Needle samples were collected in the first days of September.

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 minutes. The amount of individual chloroplast 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].

$$\text{Chlorophyll A} = (12.19 \times A_{665} - 3.45 \times A_{649}) \times V \times (1000\ W)^{-1}\ (mg \cdot g^{-1}\ f.m.)$$

$$\text{Chlorophyll B} = (21.99 \times A_{649} - 5.32 \times A_{665}) \times V \times (1000\ W)^{-1}\ (mg \cdot g^{-1}\ f.m.)$$

$$\text{Sum A + B} = (18.09 \times A_{649} + 7.05 \times A_{665}) \times \times V \times (1000\ W)^{-1}\ (mg \cdot g^{-1}\ f.m.)$$

$$\text{Carotenoids} = (1000 \times A_{480} - 2.14\ \text{chl.}\ A - - 70.16\ \text{chl.}\ B) \times 214^{-1}\ (mg \cdot g^{-1}\ f.m.)$$

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 analysis of variance (rootstocks, grafting methods) was applied. The significance of differences among combinations was evaluated on the basis of confidence intervals, using Duncan test for significance level $\alpha = 0.05$. To calculate percentage values Bliss transformation was used.

RESULTS

The type of a rootstock as well as the grafting method significantly influenced the results of graft success (Tab. 1). Independently from a grafting method the use of *Pinus armandii* and *Cedrus deodara* rootstocks gave much better results than grafting on *Larix decidua*. For each rootstock side grafting increased the percentage of graft success.

Independent of grafting method the greatest average number of buds and increase in growth increments of shoots in the first year of cultivation of the studied cultivar was obtained when *Cedrus deodara* was used as a rootstock (Tabs 2 and 3). For all the rootstock used in this study, a grafting method did not significantly influence the number of buds.

Grafting method did not have any significant influence on the thickness of the rootstock. Observed statistical differences depended on the species of the rootstock used. As far as this feature is concerned the greatest thickness was observed for *Larix decidua*, followed by *Cedrus deodara* and *Pinus armandii*. The combinations did not differ significantly with each other (Tab. 4).

In the second year of cultivation the influence of both rootstock and grafting method on the number of buds was observed. The significant increase in the number of buds was reported when *Cedrus deodara* was used as the rootstock and split grafting method was used (Tab. 5).

On the basis of measurements it was found that a side grafting method had a significantly positive effect on the percentage of graft survival in the second year of cultivation. However, the influence of rootstock used turned out to be not significant as far as this parameter is concerned (Tab. 6).

The highest level of chlorophyll A, chlorophyll B and chlorophyll A + B and carotenoids was found in the needles collected from last year's growth of plants grafted on *Pinus armandii*. However, the lowest level of chloroplast pigments was present in current year's growth for the same combination. Other combinations did not differ significantly with each other. In case of chlorophyll A/B its significantly lowest level was found in the needles of plants grafted on *Pinus armandii* on last year's growth (Tab. 7).

Rootstock used in this experiment influenced the level of pigments in the needles only in case of chlorophyll B and its higher content in the needles of plants grafted on *Pinus armandii,* and chlorophyll A/B grafted on *Larix decidua* (Tab. 8).

The age of shoots also significantly impacted the content of chloroplast pigments in the needles. The highest level of chlorophyll A, chlorophyll B, A+B and carotenoids was found in last year's growth (Tab. 9).

DISCUSSION

When grafting coniferous species, several factors influenced the choice of rootstock, including similarity of growth and appropriate age [Jayawickrama et al. 1991]. In the consider experiment recommended by Hrynkiewicz-Sudnik et al. [1995] and Ślaski and Sękowski [1998] rootstocks (*Larix decidua, Cedrus deodara*) for grafting cedar trees were used. *Pinus armandii* rootstock was used because in the previous experiment it gave good results for grafting deodar cedar.

In the opinion of Holzer [1970] Schmidtling [1983], Jayawickram et al. [1991, 1997], Haines and Simpson [1994] the rootstock used influences the grafts success and the growth of grafted coniferous plants. Ahlgren and Wilderness [1972], Rojas and Garcia [1980], and Jayawickrama et al. [1991] claim that the grafting success is also affected by the degree

Table 1. Influence of rootstocks and methods of grafting on the percentage of grafts success

Rootstocks	Methods of grafting		Average for rootstock	F emp. for rootstock
	split grafting	side grafting		
Larix decidua	14.9aˆ	24.9b	19.7a	92.98 **
Cedrus deodara	32.2b	80.4d	57.3b	
Pinus armandii	48.3c	72.5d	60.8 b	
Average for method of grafting	30.9a	59.9b		
F emp. for method of grafting	104.41 **			F emp. for the interaction of factors 15.23**

ˆValues marked with the same letter do not differ at α = 0.05
**The value of F emp. marked with two stars indicates the occurrence of very significant differences at α = 0.05

Table 2. Influence of rootstocks and methods of grafting on the number of buds in the first year of cultivation

Rootstocks	Methods of grafting		Average for rootstock	F emp. for rootstock
	split grafting	side grafting		
Larix decidua	2.1abˆ	1.1a	1.6a	22.23**
Cedrus deodara	3.5c	3.0bc	3.2b	
Pinus armandii	1.0a	1.3a	1.1a	
Average for method of grafting	2.2a	1.8a		
F emp. for method of grafting	2.35			F emp. for the interaction of factors 1.69

Explanations as in Table 1

Table 3. Influence of rootstocks and methods of grafting on the length of sprouts in the first year of cultivation (cm)

Rootstocks	Methods of grafting		Average for rootstock	F emp. for rootstock
	split grafting	side grafting		
Larix decidua	0.8aˆ	0.1a	0.5a	24.19**
Cedrus deodara	2.1b	1.6b	1.9b	
Pinus armandii	0.1a	0.3a	0.2a	
Average for method of grafting	1.0a	0.7a		
F emp. for method of grafting	2.59			F emp. for the interaction of factors 1.84

Explanations as in Table 1

Table 4. Influence of rootstocks and methods of grafting on the diameter of rootstocks in the first year of cultivation (cm)

Rootstocks	Methods of grafting		Average for rootstock	F emp. for rootstock
	split grafting	side grafting		
Larix decidua	1.0bˆ	1.0b	1.0c	72.87**
Cedrus deodara	0.8a	0.9a	0.9b	
Pinus armandii	0.8a	0.8a	0.8a	
Average for method of grafting	0.9a	0.9a		
F emp. for method of grafting	0.02			F emp. for the interaction of factors 0.45

Explanations as in Table 1

Table 5. Influence of rootstocks and methods of grafting on the number of buds in the second year of cultivation

Rootstocks	Methods of grafting		Average for rootstock	F emp. for rootstock
	split grafting	side grafting		
Larix decidua	8.2aˆ	4.1a	6.1a	18.9**
Cedrus deodara	53.5b	15.4a	34.5b	
Pinus armandii	0.9a	1.3a	1.1a	
Average for method of grafting	20.9b	6,9a		
F emp. for method of grafting	8.51*			F emp. for the interaction of factors 6.47*

ˆValues marked with the same letter do not differ at α = 0.05
*The value of F emp. marked with star indicates the occurrence of significant differences at α = 0.05
**The value of F emp. marked with two stars indicates the occurrence of very significant differences at α = 0.05

Table 6. Influence of rootstocks and methods of grafting on the percent of grafts survival at the beginning of second year of cultivation

Rootstocks	Methods of grafting		Average for rootstock	F emp. for rootstock
	split grafting	side grafting		
Larix decidua	100.0cˆ	97.0bc	98.5a	3.0°
Cedrus deodara	70.2a	100.0c	85.1a	
Pinus armandii	83.6ab	98.7c	90.2a	
Average for method of grafting	84,6a	98.5b		
F emp. for method of grafting	10.29**			F emp. for the interaction of factors 8.97**

ˆ Values marked with the same letter do not differ at α = 0.05
°The value of F emp. marked with ° indicates the occurrence of significant differences at α = 0.01
**The value of F emp. marked with two stars indicates the occurrence of very significant differences at α = 0.05

Table 7. The influence of rootstocks and age of shoots on the level of chloroplast pigments in the needles of 'Karl Fuchs' (mg g^{-1} f.m.)

Combinations	Chlorophyll A	Chlorophyll B	Chlorophyll A + B	Chlorophyll A/B	Carotenoids
Larix decidua / current year's growth	1.1104 bˆ	0.3886 b	1.5009 b	2.8688 b	3.9550 b
Larix decidua / last year's growth	1.2263 b	0.4235 b	1.6519 b	2.8906 b	4.0251 b
Cedrus deodara / current year's growth	0.9295 ab	0.3270 ab	1.2581 ab	2.8491 b	3.3883 b
Cedrus deodara /last year's growth	1.0294 b	0.3614 b	1.3926 b	2.8719 b	3.3900 b
Pinus armandii /current year's growth	0.6985 a	0.2413 a	0.9410 a	2.8844 b	2.4428 a
Pinus armandii /last year's growth	1.5702 c	0.6294 c	2.2023 c	2.5111 a	4.9922 c
F emp.	11.00**	14.29**	12.12**	4.01*	10.52**

Explanations as in Table 5

Table 8. Average level of chloroplast pigments in the needles of 'Karl Fuchs' depending on rootstocks (mg g^{-1} f.m.)

Rootstocks	Chlorophyll A	Chlorophyll B	Chlorophyll A + B	Chlorophyll A/B	Carotenoids
Larix decidua	1.1684 aˆ	0.4060 ab	1.5764 a	2.8797 b	3.9900 a
Cedrus deodara	0.9794 a	0.3442 a	1.3253 a	2.8605 ab	3.3892 a
Pinus armandii	1.1344 a	0.4353 b	1.5717 a	2.6978 a	3.7175 a
F emp.	2.29	2.97°	2.40	3.07°	1.81

ˆValues marked with the same letter do not differ at α = 0.05
°The value of F emp. marked with ° indicates the occurrence of significant differences at α = 0.01

Table 9. Average level of chloroplast pigments in the needles of 'Karl Fuchs' depending on the age of sprouts (mg g^{-1} f.m.)

Shoots	Chlorofil A	Chlorofil B	Chlorofil A + B	Chlorofil A/B	Carotenoids
Curent year's growth	0.9128 aˆ	0.3189 a	1.2333 a	2.8674 a	3.2620 a
Last year's growth	1.2753 b	0.4714 b	1.7489 b	2.7579 a	4.1358 b
F emp.	22.28**	23.95**	23.17**	2.77	11.43**

Explanations as in Table 1

of relationship between the rootstock and the scion. The results of the current study confirm these previous studies in that the rootstock most closely related to the scion of the cultivar was *Cedrus deodara*. It was the *Cedrus* rootstock as well as *Pinus armandii* on which better graft success was observed in comparison with *Larix decidua*. Also grafts of the 'Karl Fuchs' cultivar had the longest increments and number of buds when grafted on *Cedrus deodara* rootstock. Plants grafted on *Larix decidua* and *Pinus armandii* were characterized by small length of increments and fewer buds. This may suggest a smaller affinity of these two rootstock to the 'Karl Fuchs' cultivar.

In the discussed study the greatest percentage of graft success was obtained by means of side grafting. Also Hartman et al. [2002] suggest this method for propagating coniferous plants. Similarly Asaah et al. [2011] obtained much better results of graft success using side grafting in comparison to the split grafting method in which the percentage of grafts success was 50% smaller and the result was highly unsatisfactory (30.9%). Adversely, the success of a split grafting method has been reported for several woody species, [Pio et al. 2008, Asaah et al. 2011, Wendling et al. 2016]. However, contrasting results on the best grafting method of choice were reported by other researchers for different species. For instance, Mugerwa and Okullo [2010] demonstrated that vegetative propagation of *Pinus caribaea* was effective when top cleft grafting rather than a split grafting method was used. Another report by Rezaee et al. [2008] concerning walnut established that highest graft success rate was obtained using bark grafting out of seven tested grafting methods. Similarly, variable grafting success rates for different grafting methods were reported for numerous ornamental and horticultural plants [Islam et al. 2004, Sanou et al. 2004, Blada and Panea 2011, Izadi et al. 2013, Munjuga et al. 2014]. Therefore, it is clearly evident that the choice of grafting method is largely dependent on the species to be grafted as reported by Anjarwalla et al. [2017] and also depend on the type of rootstock used.

Improper grafting technique is one of the limiting factors of its success, especially by poor contact in the cambial region. Observations carried out in spring in the second year of cultivation proved that plants grafted by side grafting restarted their growth in higher percentages. This is compatible with the results obtained by Asaah et al. [2011]. However, the advantage of split grafting lies in the fact that there is a higher surface contact with the vascular cambium between the scion and the rootstock [Ofori et al. 2008]. Higher survival of *Cedrus deodara* plants than the one reported by Edwards [2009] (55%) was obtained in the present experiment. However, the result obtained by the above mentioned author came from a hardly representative sample of plants (there were only a few) so high randomness of the result can be suspected.

Some studies linked low levels of graft survival to some sort of incompatibility between the rootstock and the scion [Han et al. 2013, Munjunga et al. 2014]. Most often, this incompatibility results from the lack of belonging of the rootstock and the propagated cultivar to the same species or kind. In the experiment being considered, it was shown in the percentage of grafts success. The rootstock used did not affect the percentage of plants which restarted their growth in the second year of cultivation. However, starting in the first year small percentage of grafting success was observed when scions were grafted on *Larix decidua*. The scions that were accepted in nearly 100% restarted their growth in the second year. The greatest number of buds of 'Karl Fuchs' cultivar were counted in the second year on split grafted *Cedrus deodara* rootstock. This dependence was observed in the first year of this study.

Rootstock used in this study influenced the level of chloroplast pigments in the needles to a small degree. The highest level of chlorophyll B was obtained from scions grafted on *Pinus armandii* and of chlorophyll A/B those grafted on *Larix decidua.* On the basis of the content of chloroplast pigments in the needles, no clear differences in physiological condition of the plants were observed which could result from inconsistency of the studied rootstock with *Cedrus deodara* 'Karl Fuchs'. Less related species of *Pinus armandii* and *Larix decidua* increased the level of some chloroplast pigments. This is a surprising dependence that requires further observation. On the other hand, the age of shoots significantly influenced the content of pigments in the needles. The highest level of chlorophyll A, chlorophyll B, chlorophyll A + B and carotenoids was found in last year's growth. No similar conclusions were drawn by Rybus-Zając [2010], who

tested the level of chloroplast pigments in *Taxus baccata* (L.) needles. In this author's research, only the level of carotenoids in needles from older shoots was higher. However, the level of chlorophyll in needles from younger shoots was higher than in older ones. Differences in results may have resulted from late sampling of needles in the discussed experiment in September, compared to the experiment caried out by Rybus-Zając [2010] in June. Also changes in the content of chloroplast pigments depend on environmental factors as well as on the plant itself (its origin, genetic variability, age and adaptability) [Wyka et al. 2008].

CONCLUSIONS

1. Obtained results confirm the significance of grafting method and rootstock for achieving the best grafts success and grafts vigor of *Cedrus deodara* 'Karl Fuchs'.

2. The experiment findings highlight that side grafting is a suitable method for obtaining high grafts success and good scion vigor of *Cedrus deodara* 'Karl Fuchs'.

3. A higher percentage of graft success and greater vigorous growth was observed in scions grafted on *Cedrus deodara* rootstock.

4. A higher level of chloroplast pigments was found in the needles of *Cedrus deodara* 'Karl Fuchs' taken from two-year-old shoots.

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