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THE EFFECT OF INCISION WOUNDING OF CUTTINGS AND OF A RHIZOGENESIS PROMOTER ON THE ROOTING OF DAWN REDWOOD (*METASEQUOIA GLYPTOSTROBOIDES HU ET CHENG*)

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ABSTRACT

On the basis of conducted experiments on the rooting of dawn redwood cuttings it was found that the application of Seradix B No 1 mixed with Kaptan Zawiesinowy 50 (1:1, v:v) increased the number of rooted cuttings by 14.5% in comparison to the control. Moreover, an advantageous effect of the growth stimulator was observed on the number of secondary roots on a cutting. However, the application of a growth promoter did not have a marked effect on their length. Incision wounding of cuttings at their base had an adverse effect on the rooting percentage and the length of secondary roots. It results from the conducted observations that at the position of the incision wounding roots are not formed. It can be seen from the performed cross-section that dawn redwood regenerates roots from the cambium (cambial ring) and not from the callus. For this reason the labour-intensive procedure of incision wounding of cuttings is not recommended in the propagation of dawn redwood. The effect of two factors, i.e. incision wounding of cuttings and the application of a growth regulator, on the rhizogenesis process in dawn redwood was investigated in this study.

Key words: dawn redwood, *Metasequoia glyptostroboides* Hu et Cheng, propagation, softwood cuttings, incision wounding of cuttings, stimulation of root formation.

INTRODUCTION

Attempts to improve rooting efficiency in dawn redwood have been undertaken by numerous authors. Cuttings were collected at various dates and at different growth stages, they also differed in size. They were subjected to disinfection, hormone treatments and incision wounding procedures. Organic and mineral substrates were used for rooting. Cuttings were rooted at various ambient temperatures (low and elevated), humidity and available light were also regulated.

Dawn redwood may be propagated by seeding and vegetatively. Imported seeds are used in sowing, since trees growing in Poland do not produce seeds capable of germination. Only the oldest specimen growing in the Botanical Garden of the University of Warsaw in years with especially advantageous weather conditions produces scarce seeds capable of germination (Dolatowski – oral communication). The formation of blind seeds depends on several factors. Staminate flowers frequently do not develop and pollination does not occur [9]. Moreover, specimens in the phase of senile growth are also lacking in Poland. The oldest trees are over 50 years old. Very often plants capable of reproduction grow separately, which makes cross pollination difficult [12]. Stephan [15] reported that obtaining seeds in the temperate zone is impossible due to the vegetation season being too short for dawn redwood. The vegetation season for dawn redwood should last 134 days. It is longer than for other coniferous plants shedding needles in winter.

Dawn redwood may be propagated vegetatively using hardwood or softwood cuttings. Investigations on this method were initiated in 1948 in England [1] and the United States [4]. Maternal plants were 5-month old dawn redwoods obtained from seeds brought from China. Studies on the above mentioned reproduction method in dawn redwood were also carried out by Eleršek, Hočevar and Jurc [7]. They collected shoots from a 29-year old maternal tree. They prepared cuttings with the length ranging from 10 to 21 cm and treated them with preparations Seradix B No 1, 2 or 3. Such prepared cuttings were placed in a substrate consisting of 50% peat, 25% sand and 25% perlite. Cuttings were rooted in a glasshouse. Control cuttings (not treated with the stimulator) rooted only in 25%, while cuttings treated with Seradix B No 1 – in 63%, with Seradix B No 2 – 48% and with Seradix B No 3 in 50%, respectively. ObdrŸálek and Pinc [13] recommended the collection of cuttings at the turn of February and March and incision wounding of their shoots at the base. For hormone treatment they recommended a water solution of IBA (50 mg/1l) or 2% preparation in the form of powder. Macdonald [11] used 0.8% IBA powder. According to that author 15-cm long cuttings have to be prepared from strong, not too thin shoots. The temperature during rhizogenesis should be 21°C. Pokornỳ [14] reported that it is not a very effective method, making it possible to obtain only 30-40% rooted cuttings. Connor [6] was of a different opinion. He obtained approx. 90% rooted cuttings already four months after they were transferred into the substrate. However, he applied a more complex procedure. At present in American nurseries 8 to 20 cm long cuttings are prepared. Both typical softwood cuttings and hardwood cuttings with slightly browned bark are collected. They are rooted in a substrate consisting of 50% perlite and 50% vermiculite. Commercially available preparations, such as e.g. Rootone, are used to stimulate rhizogenesis. Cuttings are put in container trays, which are placed in rooms ensuring high humidity [8]. Monteuuis et al. [12] conducted studies on the propagation of dawn redwood using various types of cuttings. They also investigated the effect of various exogenous substances stimulating rooting, various substrate compositions, as well as the effect of incision wounding of cuttings performed at the base, on the efficiency of their rooting and the quality of their root systems. An advantageous action of Rhizopon AA was shown, giving 76.8% rooted cuttings. The application of other substances, such as Rhizopon A and Rhizopon B, did not have any effect of the percentage of rooted cuttings in comparison with the control, as the figures were 51.5, 59.0 and 59.1%, respectively. In case of the obtained results Monteuuis et al. [12] found a significant effect of the applied substrate on the process of rhizogenesis. The lowest number of cuttings, i.e. 38.6%, rooted in a 1 : 1 (v/v) mixture of ground pine bark and perlite. In turn, in a substrate consisting of 2 parts highmoor peat and 1 part perlite 50% rooted cuttings were obtained. The best results – 77.1%, were achieved using a 1 : 3 mixture of highmoor peat and perlite. Incision wounding of cuttings at the base increased the percentage of rooted cuttings from 36 to 54%. However, the authors reported that at the site of the incision wounding roots were not formed. The root system of the new plants was weaker than that in the plants not subjected to incision wounding.

MATERIAL AND METHODS

The experiment was conducted in a plastic tunnel of 3.0 × 10.0 × 1.5 m placed in an unheated glasshouse. At the height of 50 cm above cuttings an electronically controlled automatic mist producing system was installed, which turned on depending on the ambient temperature and humidity. The time of mist production was 8 seconds. This made it possible to maintain relative ambient humidity at approx. 90-100%.

Plastic container trays, filled with substrate in which cuttings were rooted, were placed on a prepared bed. Each container tray contained 24 pots of 125 cm^3. New container trays were used each year to root cuttings in order to eliminate the possibility of transferring soil-borne pathogens on the walls of the containers.

The experiment was conducted in three cycles:
from 1997.07.14 to 1998.04.13,
from 1998.07.13 to 1999.04.12,
from 1999.07.12 to 2000.04.10.

Cuttings were collected each time in the second decade of July from 2-year old maternal plants grown in containers in an unheated glasshouse. Shoots for the preparation of cuttings were always collected in the morning, when plants were in full turgor. Whole one-year old growths were cut. They were protected against withering by abundant sprinkling with water and covering with thin plastic foil. Apex cuttings with the length of 10-12 cm were prepared. Branches were reduced at the distance of 5-6 cm from the main shoot to limit excessive transpiration.

In the conducted preliminary investigations on the application of various substrates to root cuttings it turned out that the best substrate was a mixture of highmoor peat and perlite (1:1, v:v). For this reason in further studies on the rooting of dawn redwood this substrate was subsequently used. Substrate salinity was 0.27g KCl · dm^{-3} and its reaction – pH (H_2O) = 5.0.

In each experiment cycle 4 combinations were applied. In one combination a total of 96 cuttings were rooted (in four replications of 24 cuttings each). Altogether 384 cuttings were used in one cycle, while in the whole experiment it was 1152 cuttings.

The first factor differentiating the combinations was the performance of incision wounding of cuttings. Cuttings were divided into two groups. In one of them the shoot was incision wounded along its axis at the length of approx. 1.5-2.0 cm, exposing the cambium to increase the adhesion of the rooting stimulator and to increase water uptake. The other cuttings were not subjected to this procedure.

The other factor was the application of a rooting stimulator. A preparation made in Great Britain, Seradix B No 1 (0.2% indolile-3-butyric acid) was applied, mixed with Kaptan Zawiesinowy 50 (a preparation produced in Poland) in a 1:1 voluminal ratio. For reach combination a control was applied, in which cuttings were not treated with a growth promoter.
Cuttings were planted in the substrate at the depth of 2 cm. On the same day they were preventively sprayed with a fungicide, Topsin M 70 WP at the concentration of 0.1%. The procedure was repeated after 10 days using Euparen 50 WP at the concentration of 0.2%.

Each year rooting cuttings were protected against frost by covering the container trays with a 2-cm layer of dry sawdust. In wintertime ambient temperature in the plastic tunnel was checked several times.

In the second decade of March the quality of wintered cuttings was examined organoleptically. Observations concerned non-measurable characters, such as frost damage and the degree of pathogen infestation. Moreover, the following were also determined:

– the percentage of rooted cuttings,
– the number of secondary roots on the cutting,
– root length on the cutting (cm).

The results concerning the number and length of secondary roots on the cutting were elaborated statistically using the analysis of variance. Data defining the percentage of rooted cuttings were subjected to the Bliss transformation. Means were compared using the Newman-Keuls test. Moreover, the results of three-year experiments were synthesized, with years treated as a random factor. This made it possible to eliminate the effect of time on the investigated characters and to determine the actual effect of individual experimental factors.

RESULTS

The first secondary roots on cuttings of dawn redwood were observed 14 days after they were planted in the substrate. Depending on the applied factors, in the years of the experiment the average number of rooted cuttings ranged from 74.4 to 99.5% (tab. 1). Incision wounding of cuttings in each experimental cycle decreased the rhizogenesis ability of cuttings. Control samples rooted even by 6.1% better than those subjected to incision wounding. In the conducted synthesis, in spite of obtaining varying values (91.1 and 94.9%), no positive effect of this procedure was shown on the rooting percentage, which was confirmed statistically. In all the years of the experiment an advantageous effect of the growth promoter was found. The rooting efficiency of cuttings, depending on the year, increased from 1.6 to 6.4%. Treating cutting with a growth promoter increased the percentage of rooted cuttings from 91.0 to 95.0%. The synthesis of all the experimental years made it possible to prove this dependency statistically (tab. 1).

Table 1. Mean percentage of rooted cuttings in individual years of the experiment in terms of applied factors

Year	Factors					
	incision wounding		F emp.	growth promoter		F emp.
	incision wounding applied	no incision wounding		no growth promoter	Seradix + Kaptan (1:1)	
1997	93.8 a[1]	98.6 b	2.84 °	94.4 a	97.9 b	6.21*
1998	98.4 a	99.0 a	0.05	97.9 a	99.5 b	2.98 °
1999	74.4 a	80.3 b	3.66*	74.1 a	80.5 b	8.76**
Synthesis of the years	91.1 a	94.9 a	0.21	91.0 a	95.0 b	0.24

[1] values for individual factors denoted with identical letters do not differ statistically at the level of significance α = 0.05 in individual years

In each year of the study the application of the substance stimulating rooting increased the number of roots per cutting. While applying the growth promoter on average from 3.0 to 6.5 roots were obtained per cutting, whereas in the control it was from 2.7 to 3.7, respectively. After the synthesis for the three experimental years was conducted it was found that cuttings treated with a growth stimulator generated on average 4.0 and those untreated 3.3 roots. This dependency was confirmed statistically (tab. 2). Incision wounding of cuttings at the base increased the number of secondary roots at the base from 0.2 to 0.7 in relation to the control. After the results were synthesized an average of 3.7 roots were obtained for incision wounded cuttings and controls, respectively. Incision wounding of cuttings did not have a significant effect on this trait (tab. 2).

Table 2. Mean number of roots per cutting in individual years of the experiment depending on the applied factors

Year	Factors					
	incision wounding		F emp.	growth promoter		F emp.
	incision wounding applied	no incision wounding		no growth promoter	Seradix + Kaptan (1:1)	
1997	3.7 a[1]	4.4 b	16.45**	3.7 a	4.3 b	12.15**
1998	5.0 a	5.2 a	0.74	3.7 a	6.5 b	371.70**
1999	2.8 a	3.0 a	2.28	2.7 a	3.0 b	6.93*
Synthesis of the years	3.7 a	3.7 a	0.02	3.3 a	4.0 b	61.77**

[1] values for individual factors denoted with identical letters do not differ statistically at the level of significance α = 0.05 in individual years

Two factors affected the mean length of secondary roots: the applied growth promoter and the applied procedure of their treatment. The procedure of their treatment needs to be considered as the more important factor in this case.

The length of secondary roots in cuttings incision wounded along their shoot was always bigger (tab. 3). In individual years of the experiment the generated roots were longer by 0.5 to 0.7 cm than roots in the control. This dependency was also confirmed in the synthesis of the experiment. Cuttings which were incision wounded had roots of 9.0 cm, while the length of roots in control cuttings was 8.4 cm.

Table 3. Mean length of roots (cm) per cutting in individual years of the experiment depending on the applied factors

Year	Factors					
	incision wounding		F emp.	growth promoter		F emp.
	incision wounding applied	no incision wounding		no growth promoter	Seradix + Kaptan (1:1)	
1997	8.4 b[1]	7.7 a	5.97*	8.0 a	8.2 a	0.24
1998	9.4 b	8.9 a	6.83*	10.7 b	7.5 a	279.41**
1999	9.1 b	8.5 a	4.41*	8.1 a	9.4 b	21.36**
Synthesis of the years	9.0 b	8.4 a	0.09	9.1 a	8.8 a	0.02

[1] values for individual factors denoted with identical letters do not differ statistically at the level of significance α = 0.05 in individual years

The application of a growth promoter had a varying effect on the length of secondary roots in successive years of the experiment (tab. 3). Their length ranged from 7.5 to 10.7 cm. Mean values from the three years were 9.1 cm for control cuttings, while for those treated with a growth regulator it was 8.8 cm; the means did not differ statistically.
Treating cuttings with a growth promoter had an advantageous effect on the total length of roots in each experimental year. The total length of roots per cutting ranged from 20.5 to 47.3 cm, depending on the cycle of the experiment and the application of the growth regulator. Irrespective of the year the total length of roots was on average 28.6 cm for control cuttings and 33.2 cm for cuttings treated with a growth promoter. The significance of differences was confirmed in the synthesis (tab. 4).

Table 4. Mean total length of roots (cm) per cutting in individual years of the experiment depending on the applied factors

Year	Factors					
	incision wounding		F emp.	growth promoter		F emp.
	incision wounding applied	no incision wounding		no growth promoter	Seradix + Kaptan (1:1)	
1997	29.3 a[1]	33.6 b	6.23*	28.6 a	34.3 b	10.99**
1998	42.0 a	41.9 a	0.01	36.6 a	47.3 b	54.60**
1999	23.8 a	23.9 a	0.00	20.5 a	27.2 b	43.08**
Synthesis of the years	30.5 a	31.3 a	1.34	28.6 a	33.2 b	41.49**

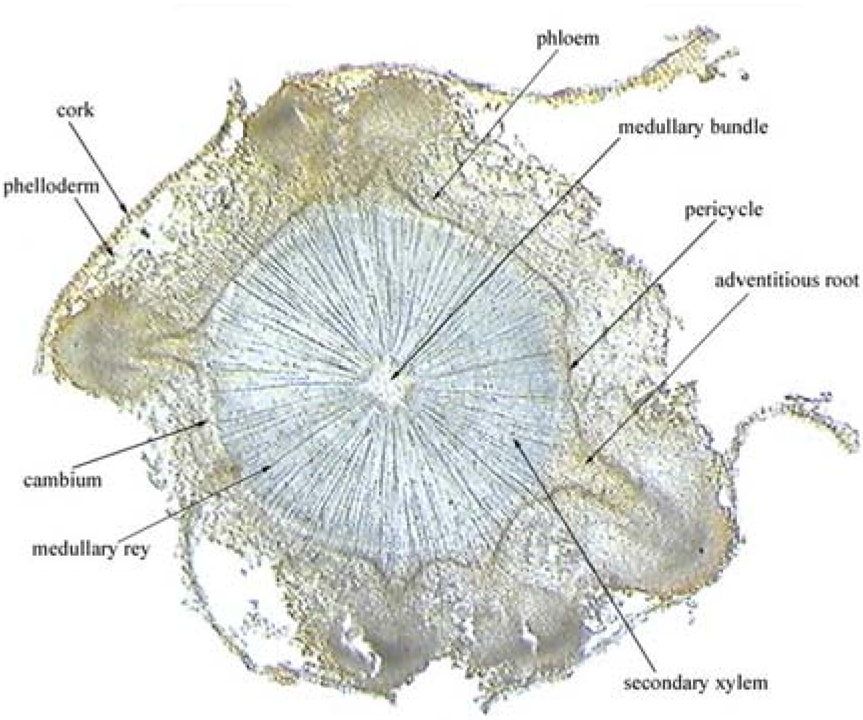
[1] values for individual factors denoted with identical letters do not differ statistically at the level of significance α = 0.05 in individual years

Incision wounding of cuttings had a negative effect on the investigated characters in the first cycle of the experiment. In the other two years no differences were observed in the total length of roots per cutting (tab. 4). Also in the performed synthesis no statistically significant differences were found in the values in case of the performed incision wounding of cuttings – 30.5 cm and no such measure – 31.3 cm.

Figure 1 presents the location of incision wounding of a dawn redwood cutting. The performed procedure turned out to be irrelevant, as roots grew out of the pericycle and not the callus. The callus only healed the created wound.

Fig. 1. A cutting incision wounded along the shoot axis at the distance of 1.5-2.0 cm from the base

Fig. 2. A cross-section of a shoot in the radical zone



The formation of secondary roots on a cutting is presented on a cross-section (fig. 2). Dawn redwood cuttings started to form secondary roots two weeks after they were transferred into the substrate. Initially groups of cells initiating the process of rhizogenesis, the so-called primordia, differentiated from the cambium. From these cells radicles were generated with a formed apical meristem covered with a calyptra. In the course of root growth the phloem was ruptured followed by the rupture of the phelloderm and phellem, covered by the epidermis. Dawn redwood produced secondary roots from tissues located in the vicinity of the pericycle.

DISCUSSION

Dawn redwood may be propagated vegetatively by two methods: using hardwood cuttings (collected in winter or early spring) and in summer from softwood cuttings [9]. Monteuuis et al. [12] obtained better results using softwood cuttings. According to Zan [16], softwood cuttings have to be collected in mid-June. Hrynkiewicz-Sudnik et al. [9] reported that under Polish conditions the most appropriate month for cutting collection from maternal plants is the first half of July. On the basis of the above mentioned literature data and the results of pilot studies in this experiment the date of dawn redwood propagation using softwood cuttings was decided to be the second decade of July.

An important aspect of vegetative propagation is the formation of secondary roots. Primordia on cuttings are most frequently formed at their base. They may also be located in nodes, internodes and lenticells as well as the callus tissue, forming in the basal part of the cutting. The differentiation of roots from the callus is found in woody plants, the cuttings of which root with much difficulty [5]. Buraczyk and Zakrzewski [3] reported that roots on cuttings are rarely initiated from the callus. The callus is characterized by intensive development and uses most nutrient reserves accumulated in the cutting, which may have an adverse effect on root growth.

The most important substances regulating the process of rhizogenesis are plant hormones belonging to the group of auxins [2]. Their positive effect on the initiation of secondary roots was known as early as the 1930's [10]. They belong to the so-called growth regulators. Both endogenous compounds – produced by the plant, and synthetic agents belong to this group [17].

In this experiment a substance stimulating processes of rhizogenesis was applied as one of the factors. The preparation Seradix B No 1 mixed with Kaptan Zawiesinowy 50 in a 1:1 voluminal ratio was used. The addition of the fungicide protected cuttings against fungal diseases and also served as a co-factor of the active substance. Monteuuis et al. [12] showed a positive action of 0.5% IBA (Rhizopon AA) on the percentage of rooted cuttings. The application of other active substances (0.5% IAA – Rhizopon A and 0.1% NAA – Rhizopon B) did not result in increased rooting efficiency in comparison to the control. In the investigations conducted by the author of this study much better results were obtained using a growth promoting substance. The application of 0.2% IBA increased the number of rooted cuttings even by as much as 14.5%.

Various authors frequently cited in literature recommend incision wounding of bases of cuttings by cutting off a narrow strip of bark exposing the cambium. This procedure is to improve the rooting of cuttings, with no adverse effect on their health. An advantageous effect of incision wounding on the rooting of cuttings consists among other things in the fact that cuttings absorb water and growth stimulators more easily. Better rooting in cuttings wounded at the base may also be affected by the generation of the so-called wound hormones [2]. In the experiment of Monteuuis et al. [12] cuttings were wounded by the longitudinal incision at the length of 1 cm from the base of the last internode. An advantageous effect of this measure was observed on the rooting percentage of the cuttings. In hardwood cuttings the incision wounding procedure increased the percentage of rooted cutting from 24.2 to 40.3%, while in softwood cuttings it was from 36 to 54%, respectively. However, it was found that secondary roots did not form roots at the incision position.

It results from the observations conducted by the author of this study that incision wounding of cuttings had an adverse effect on rooting percentage. However, this dependency was not confirmed in the synthesis of the three years of the experiment. In turn, incision wounding of cuttings affected the length of roots on cuttings. Incision wounded cuttings produced longer roots. Incision wounding of the base of cuttings had an effect on the number of roots formed in individual years. After the performed statistical analysis no positive effect on the investigated character was found. A slight effect of this procedure on the rooting process, at the simultaneous increase in the labour intensity, resulted in a situation when it is not recommended in the propagation of dawn redwood.

CONCLUSIONS

1. The incision wounding procedure did not have a significant effect on rooting of softwood cuttings of dawn redwood.
2. Incision wounding of cuttings is a time-consuming procedure, which significantly increases production costs, at the same time not yielding tangible profits.
3. The application of a growth regulator had an advantageous effect on rooting percentage, the number of roots on a cutting and their total length.

REFERENCES

1. Białobok S., 1949. *Metasequoia* – jeszcze jedna żyjšca kopalina [*Metasequoia* – another living fossil]. Wszechswiat 1, 23-25 [in Polish].
2. Bojarczuk K., 1997. Regulatory ro□linne w szkółkarstwie [Plant regulators in nursery science]. [in:] Jankiewicz L. S. Regulatory wzrostu i rozwoju ro□lin [Growth and development plant regulators]. PWN Warszawa. 137-161 [in Polish].
3. Buraczyk W., Zakrzewski J., 1990. Fizjologiczne aspekty procesu rizogenezy w pędach drzew i krzewów [Physiological aspects of rhizogenesis in shoots of trees and shrubs]. Wiad. Botan. 34 (1), 3-12 [in Polish].
4. Creech J. L., 1948. Propagation of *Metasequoia* by juvenile cuttings. Science 108, 664-665.
5. Czekalski M., 1983. Korzenie przybyszowe i ich powstawanie [Secondary roots and their formation]. Kosmos S. A. Biologia 1, 91-99 [in Polish].
6. Connor D. M., 1982. Cutting propagation of *Metasequoia glyptostroboides*. The International Plant Propagator's Society 32, 327-329.
7. Eler□ek L., Hočevar M., Jurc D., 1984. Propagation of spruce and *Metasequoia* by cuttings. Gozdarski vestnik. 42, 3, 100-108.
8. Hodges G., 2000. Wiadomosc e-mail. redwood@hiwaay.net
9. Hrynkiewicz-Sudnik J., Sękowski B., Wilczkiewicz M., 1999. Rozmnażanie drzew i krzewów nagozalšžkowych [Propagation of gymnospermous tress and shrubs]. Wydanie III. PWN SA Warszawa, 355-357 [in Polish].
10. Loach K., 1988. Hormone application and adventitious root formation in cuttings – a critical review. Acta Horticulturae 227, 126-133.
11. Macdonald B., 1996. Practical woody plant propagation for nursery growers. Timber Press Inc. Oregon USA. 5 edition. 320.
12. Monteuuis O., Goubier P., Pages C., Pezet C., Sarran P., 1987. Metasequoia glyptostroboïdes renseignements spécifiques et bouturage. Extrait des Annales Afocel. 211-253.
13. Obdrzálek I., Pinc M., 1997. Vegetativní mnozeni listnatỳch dřevin [The vegetative propagation of woody plants]. Průhonice. 101 [in Czech].
14. Pokornỳ J., 1972. Some notes to the biology of dawn redwood (*Metasequoia glyptostroboides* Hu et Cheng.). Sivaecultura Tropica et Subtropica 2. 149-154.
15. Stephan B. R., 1971. Über die Fruchtbildung bei *Metasequoia glyptostroboides*. Deutsche Bäumschule 7, 222-223.
16. Zan S., 1960. Obserwacje nad rozmnażaniem metasekwoi [Observations on propagation of metasequioa]. Wiad. Botan. IV (3-4), 319-322 [in Polish].
17. Zurzycki J., Michniewicz M., 1985. Fizjologia roslin [Plant physiology]. Wydanie II. PWRiL Warszawa: 538-552 [in Polish].

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