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Possibility of Using Green Cuttings in Vegetative Propagation of *Sequoiadendron giganteum* (Lindl.) J. Buchh.)

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Abstract

An experiment was undertaken studying the effects of genotype, treatment with growth regulators and duration of rooting period on the percentage of rooting and quality of the root system of green cuttings of giant sequoia (*Sequoiadendron giganteum* (Lindl.) J.Buchh.). Cuttings were taken from 18 ten-year seedlings of 10 species of origin. The duration of rooting period (3 months vs. 5 months) was statistically reliable for root- and callus formation rates. The extension of rooting period for two month affords 57.76% more rooted cuttings. The genotype is the main factor influencing the percentage of rooting, number of roots formed and the length of the main root. Treatment with auxin also increase the percentage of rooting, number of roots formed and the length of the main root. Treatment with 4000 ppm indole butyric acid (IBA) gives good results at the end of the third and fourth month, but a five-month rooting period cuttings treated with 4000 ppm IBA showed the highest percentage of rooting.

Key words: giant sequoia, rooting, green cuttings, IBA dose

Introduction

Giant sequoia (*Sequoiadendron giganteum* (Lindl.) J. Buchh.) has been noted for its enormous size and age, and its rugged, awe – inspiring beauty.

Giant sequoia is highly regarded as an ornamental and is promising as a major timber – producing species (Piirto et al., 1981). Giant sequoia grows best in deep, well-drainer sandy loams. Soil pH ranges mostly from 5,5 to 7,5, with an average of about 6,5 (Bonnicksen et al., 1981). The tree is monoecious; male and female cone buds form during late summer. Cones bearing fertile seeds have been observed on trees as young as 10 years of age, but the large cone crops associated with reproductive maturity usually do not appear before about 150 or 200 years. Giant sequoias have serotinous cones, which may remain attached to the stems without opening to release seeds. For 20 years or more, cones may retain viable seeds and continue to photosynthesize and grow, their peduncles producing annual rings that can be used to determine cone age (Dulitz, 1986; Fins, 1981). A typical mature giant sequoia produces an average of 1500 new cones each year although variability among trees and from one year to another is great. Natural reproduction in giant sequoia is an unusually tenuous process. In one study, viability of seeds removed from fresh cones and placed on the ground dropped from 45 percent to 0 in 20 days. Seeds collected from the forest floor showed an average viability of about 1 percent (Rogers, 1986; Libby, 1986). Seed dormancy is not evident in giant sequoia, so surviving seeds germinate as soon as conditions are favorable (Harrison, 1986; Heald, 1987). Germination is epigeal. The most significant requirement for germination is an adequate supply of moisture and protection of the seed from desiccation. This is best provided by moist, friable mineral soil that covers the seed to a depth of 1 cm and that is partially shaded to reduce surface drying. A wide range of temperatures is acceptable for germination. The generally sandy soil of the groves normally provide an additional requirement of adequate aeration and the optimal pH range of 6 to 7 (Eyre et al., 1980).

Giant sequoias up to about 20 years of age may produce stump sprouts subsequent to injury (Melchior et al., 1987). Cuttings from juvenile donors root quickly and in high percentage - up to 94 percent (Zinke et al., 1992; Rundel, et al., 1991). Limited success has been achieved in rooting cuttings from older – 30-or 40-years-old trees (Dulitz, 1986; Piirto et al. 1981). Differences in vegetative regeneration capacities between juvenile and older donors may be reduced if cuttings are taken at the time of budbreak, instead of during the dormant period (Berthonet al., 1987; Stark, 1998). The use of vegetative propagation is very important to tree improvement and has become one of the most important tools of the tree improvement forester (Worrall et al., 1989). It can provide multiplication of new clones of *Sequoiadendron giganteum* (Lindley) Buchholz with superior growth, adapted to adverse ecological conditions and resistant or tolerant to *Heterobasidion annosum, Armillaria mellea, Poria incrassanta* and *Poria albipellucida*, which are needed for environmental protection, preservation, timber production and crop protection.

The trees of *Sequqiadendron, giganteum* (Lindley) Buchholz in Bulgaria have not suffered from infection by the above-mentioned diseases. The present paper reports an experiment on propagation of 18 genotypes (denoted as G1– G18) of *Sequoiadendron* from 10 Bulgarian provenances of the species by softwood stem cuttings. The study investigated the influence of the rooting period, genotype and auxin treatment on the rooting percentage and root quality.

Materials and Methods

The experiment was carried out in a rooting bed equipped with bottom heating and mist system in a greenhouse. Paper pots filled with peat and perlite in 1:1 v/v were used to place the rooting material. During the experiment ground temperature of 22^0C and ambient temperature of 21^0C were maintained. The mist system was adjusted to provide 95% air humidity. Photoperiod of 18 hours was ensured, with a high-pressure lamp installed at 1.5 m above the rooting bed. A total of 18 cutting donors were chosen among 5-year-old seedlings, originating from ten Bulgarian provenances of *Sequoiadendron giganteum* (Lindl.) J.Buchh.) and grown in the nursery of Introduction Ltd.company. The plants were exposed to preliminary rejuvenation by hedging in December 2014. The rooting material was collected in mid-December 2014. Homogeneous softwood cuttings 12-15 cm in length and 2-4 mm in diameter were prepared. The cuttings from each genotype were grouped in three bundles one of which was left for control while the other two were treated by basal dip in concentrated solution (2000 ppm and 4000 ppm) of indole-3-butyric acid (IBA) for 5 seconds. The bundles were left to dry for two minutes and all three groups were swirled in captan powder (25% captan) before sticking into the rooting medium.

During the time of the experiment, the cuttings were sprayed with water solution of captan (1.5 g/l) at two-week periods. The application of combined NPK (20:5:30) fertilizer (2g/l) at 10-day periods started one and a half month after the initiation of the experiment and continued until the end. By the end of the third month, all cuttings were examined for rooting and the percentage of rooting, percentage of callus, number of roots and length of the main root were recorded. The non-rooted cuttings were left in the rooting bed and checked for rooting and callus formation one month later.

The experiment was performed in a split-plot design with three replications. Eighteen main plots – genotypes, arranged randomly in each replication were divided in subplots, randomly assigned to three different treatments.

Results and Discussion

The results of rooting cuttings is shown in Table 1. As it can be seen, the percentage of rooted cuttings depends on the genotype and on the concentration of IBA and the length of the rooting period. Out of the total of 18 tested genotypes, genotype G5 had the best rooting ability, where at the end of the fifth month all cuttings were rooted. In terms of this indicator, good results were also evident in genotype G1, genotype G8 and genotype G15, with 98.43 % and 89.27 % rooted cuttings respectively. The lowest percentage of rooted cuttings were those of genotype G13 – 19.11 %, followed by genotype G7 – 21.07 % and genotype G6 – 29.41%. Spethman et al. (1988) stated that for a successful root induction, plants generally need to contain a certain quantity of auxins. It is common to use IBA for rooting of tree-type plants.

Regarding the concentration of IBA within the frame of each period there is a tendency to increase the percentage of rooted cuttings with increasing the concentration of a growth regulator. Untreated variants had the lowest values in all three periods of rooting. In the control variants of genotype G7, G13 and G17 there were no rooted cuttings. Weaver (1973) reported that the increasing doses of IBA could increase the number of roots but could also change the rooting condition. Regarding the length of rooted period, it is evident that the longest period of rooting - five months - is most suitable for rooting of green cuttings from *Sequoiadendron giganteum* (Lindl.) J.Buchh.). Genotype with 100% rooted cuttings - G5 - at the end of the third month had 42.24 % rooted cuttings; after the fourth month – 78.81 % at the end of the fifth month- 100%.

A similar trend was observed in the majority of other genotypes except in genotype G7, genotype G13 and genotype G17 where rooting begins as late as in the fifth month. In genotypes G6, G13 and G17 rooting process starts during the fourth month, i.e. in 77.78 % of the genotypes, the rooting process starts in the third month, and in 16.67% - respectively in the fourth and the fifth month. The rate of rooting is the fastest in genotype G1 and genotype G8 – even at the end of the third month respectively 73.21 % and 79.07 % of the cuttings are rooted in treatment with 4000 ppm IBA. The same trend continued in the fourth and fifth month of rooting.

The results regarding the number of roots are presented in Table 2. It is visible that the greatest number of roots are in the plants from genotype G5 - 5.6, followed by genotype G1 - 4.7. In other genotypes, the number of roots ranged from 0.7 - 3.1 to genotype G13 - genotype G18, respectively.

Tab. 1. Rooting of cuttings in *Sequoiadendron giganteum* (Lindl.) J.Buchh.) (%)
Укоријењене резнице Sequoiadendron giganteum (Lindl.) J.Buchh.) (%)

Variants *Варијанте*	3 months *3 мјесеца*				4 months *4 мјесеца*				5 months *5 мјесеци*			
	K	2000	3000	4000	K	2000	3000	4000	K	2000	3000	4000
G_1	10.11	28.31	57.32	73.21	18.12	45.71	71.89	84.11	21.54	56.76	90.66	98.43
G_2	9.21	17.44	20.45	20.77	10.43	37.28	44.76	64.23	14.45	74.56	87.78	87.66
G_3	7.44	14.87	31.76	35.65	22.09	25.37	56.56	60.21	27.61	37.34	67.99	67.75
G_4	38.07	45.65	58.43	67.44	44.80	57.62	67.41	70.65	47.23	67.21	69.45	70.43
G_5	31.53	48.73	48.76	42.24	48.78	58.05	69.29	78.81	57.41	61.19	83.22	100.23
G_6	0.0	7.09	13.91	24.06	0	18.67	20.17	20.98	12.24	24.82	27.30	29.41
G_7	0.0	0	0	3.98	0	070	0	18.72	0	4.54	7.91	21.07
G_8	43.18	57.78	68.57	79.07	56.12	68.50	73.56	80.03	56.87	72.63	73.76	89.27
G_9	11.26	24.65	31.18	44.98	18.32	38.69	39.43	59.90	23.69	57.27	59.55	67.05
G_{10}	34.70	34.88	44.87	48.91	39.31	48.93	67.27	75.78	47.09	58.87	73.65	87.66
G_{11}	5.51	11.70	11.51	47.87	21.56	27.87	27.37	58.65	31.03	43.65	54.55	67.78
G_{12}	7.67	7.77	10.12	12.89	11.61	18.72	21.43	29.46	18.45	27.49	39.71	44.65
G_{13}	0.0	2.34	7.65	28.78	0	5.68	11.87	11.54	0	8.09	18.62	19.11
G_{14}	11.76	13.54	24.43	53.56	18.38	23.65	38.69	63.76	27.45	45.78	49.89	78.60
G_{15}	21.64	27.12	34.98	41.52	31.86	45.45	51.57	70.89	44.34	63.09	63.94	89.13
G_{16}	20.33	33.07	38.67	45.56	21.54	51.71	63.45	67.09	27.23	74.56	78.33	78.44
G_{17}	0.0	7.87	18.59	31.09	0	18.20	27.32	27.89	0	31.70	31.48	35.30
G_{18}	4.12	12.55	15.36	17.89	14.73	23.54	25.45	43.09	25.86	38.33	38.66	64.23
5%	0.34	1.23	0.45	2.54	2.97	4.34	3.45	2.54	1.45	3.45	2.43	4.35
GD 1%	1.54	1.98	2.65	2.79	3.45	4.65	4.03	2.68	1.98	3.69	2.90	4.65
0.1%	2.76	2.45	2.97	3.09	3.97	4.99	4.67	3.12	2.33	4.12	3.55	4.89

The data in the table shows that the roots of a cutting grow the fastest in the first three months of rooting, as the increase in this indicator over the coming months is quite minor, moving from 0.0 % to 17.6 % in the fourth month and by 0.0 % to 60.0% in the fifth month.

Regarding IBA dose, a tendency is to increase the number of roots with increasing concentration of growth regulator, as in the first three months of investigation, cuttings treated with 4000 ppm IBA had the highest values. The results are demonstrated in higher values of GD.

Regarding the length of roots it was established highly warranted between genotype, length of the rooting period and dose of IBA Table 3. Genotype G5 - 15.8 cm had the longest roots again, and the shortest were - cuttings from the G7 - 4.0 cm.

Tab. 2. Number of root *Sequoiadendron giganteum* (Lindl.) J.Buchh.) cuttings
Број корјенова на резницама Sequoiadendron giganteum (Lindl.) J.Buchh.)

Variants *Варијанте*	3 months *3 мјесеца*				4 months *4 мјесеца*				5 months *5 мјесеци*			
	K	2000	3000	4000	K	2000	3000	4000	K	2000	3000	4000
G_1	2.1	3.4	4.3	4.5	2.1	3.4	4.3	4.7	2.1	3.4	4.3	4.7
G_2	2.0	2.2	3.0	3.3	2.0	2.5	3.4	3.6	3.0	3.2	3.5	3.6
G_3	0.9	1.3	1.5	1.7	1.0	1.4	1.5	2.0	1.0	1.6	1.9	2.1
G_4	1.9	2.0	2.3	2.6	2.0	2.0	2.1	2.7	2.1	2.0	2.1	2.7
G_5	1.3	2.4	3.7	5.5	1.3	2.6	3.9	5.5	1.5	2.8	3.9	5.6
G_6	0	1.1	1.5	1.7	0	1.2	1.7	1.7	1.3	1.5	1.7	1.7
G_7	0	0	0	1.0	0	0	0	1.0	0	0.7	1.0	1.6
G_8	1.7	2.2	2.5	3.3	2.0	2.2	2.5	3.3	2.0	2.7	3.0	3.4
G_9	2.0	2.2	2.6	2.8	2.0	2.3	2.7	2.8	2.2	2.5	2.7	2.8
G_{10}	2.2	2.7	2.9	3.0	2.2	2.8	2.8	3.2	2.4	3.1	3.3	3.4
G_{11}	1.1	1.6	1.8	2.0	1.1	1.6	1.9	2.2	1.2	1.6	2.1	2.3
G_{12}	0.9	1.3	1.4	1.6	0.9	1.5	1.6	1.6	1.1	1.4	1.6	1.7
G_{13}	0	0.3	0.5	0.7	0	0.3	0.5	0.7	0	0.3	0.6	0.7
G_{14}	1.5	1.7	2.5	2.7	1.6	1.9	2.5	2.9	1.6	2.0	2.5	3.0
G_{15}	2.5	2.9	3.2	3.5	2.6	3.0	3.3	3.5	2.6	3.0	3.3	3.5
G_{16}	2.0	2.5	2.5	2.7	2.2	2.6	2.6	2.7	2.5	2.5	2.7	3.0
G_{17}	0	0.4	0.5	1.0	0	0.5	0.7	1.0	0	0.5	0.7	1.1
G_{18}	2.0	2.5	2.9	3.0	2.2	2.7	3.0	3.0	2.4	2.7	3.0	3.1
5%	1.3	2.1	2.6	1.7	0.8	1.5	2.4	2.3	1.9	2.3	1.4	1.4
GD 1%	1.6	2.3	2.9	1.9	1.6	1.8	2.6	2.7	2.4	2.5	1.6	1.7
0.1%	1.8	2.6	3.5	2.1	1.8	1.9	2.9	2.9	2.8	2.8	1.7	1.8

Cuttings of control variants during the three periods of rooting are with the shortest roots - ranging from 0.0 cm to 6.8 cm in three months; and from 4.0 cm to 15.8 cm in five months.

There is a tendency for increasing the length of roots with increasing the dose of IBA.

Tab. 3. Root length of *Sequoiadendron giganteum* (Lindl.) J.Buchh.) cuttings (cm)
Дужина корјена на резницама Sequoiadendron giganteum(Lindl.) J. Buchh.) (cm)

Variants *Варијанте*	3 months *3 мјесеца*				4 months *4 мјесеца*				5 months *5 мјесеци*			
	K	2000	3000	4000	K	2000	3000	4000	K	2000	3000	4000
G_1	1.7	2.8	3.3	4.8	2.4	3.2	3.5	5.1	4.1	4.6	5.1	6.8
G_2	3.8	4.9	5.9	6.8	4.6	5.1	6.3	7.4	5.2	6.7	8.3	11.5
G_3	2.9	4.5	5.8	7.8	4.7	6.3	7.9	9.2	4.9	6.9	8.4	9.7
G_4	5.7	6.3	7.2	8.9	6.7	7.8	8.4	9.4	7.1	9.1	9.7	10.2
G_5	5.9	7.3	8.2	9.8	7.1	8.1	9.4	10.5	9.9	10.3	13.5	15.8
G_6	0	0.5	1.5	2.4	0	0.7	1.9	3.0	0.8	1.9	2.4	3.4
G_7	0	0	0	2.5	0	0	0	3.6	0	0.7	1.8	4.0
G_8	5.1	6.2	7.8	8.7	5.9	6.7	7.9	8.8	6.7	7.5	8.4	10.5
G_9	4.7	5.1	6.8	7.4	5.0	5.7	6.9	8.2	5.3	6.3	8.2	9.7
G_{10}	6.2	7.2	8.1	9.8	6.8	7.7	9.3	10.1	7.3	8.4	10.5	11.8
G_{11}	4.7	5.4	6.9	7.5	5.1	6.8	7.3	8.4	6.3	7.3	8.1	9.2
G_{12}	4.9	5.1	6.0	6.9	5.3	6.4	7.3	7.5	6.7	7.8	8.0	8.3
G_{13}	0	2.1	3.7	4.0	0	2.9	4.1	4.9	0	3.3	4.8	5.3
G_{14}	5.4	6.1	7.3	8.7	6.9	7.3	8.3	9.8	7.8	8.1	9.4	10.5
G_{15}	6.8	7.8	8.1	9.3	7.3	8.4	9.2	11.5	8.4	9.3	10.5	12.3
G_{16}	5.3	6.5	7.0	7.5	6.1	7.0	7.7	8.7	7.3	8.7	9.3	10.3
G_{17}	0	1.7	2.5	3.7	0	2.3	3.7	4.0	0	3.5	4.7	5.4
G_{18}	1.7	3.5	4.3	5.6	3.8	4.5	5.4	6.3	4.1	5.2	6.8	7.8
5%	4.5	3.1	5.2	4.3	6.3	4.5	3.5	2.7	3.6	4.5	5.4	6.5
GD 1%	4.7	3.5	5.6	4.6	6.7	4.7	3.9	2.9	3.8	4.6	5.7	6.7
0.1%	4.9	3.7	6.1	4.8	6.9	4.8	4.3	3.6	4..2	4.8	5.9	6.9

Conclusion

Based on the results of the present investigation the folowing was concluded: according to the results it appeared that 4000ppm of IBA is good for rooting of *Sequoiadendron giganteum* (Lindley) Buchholz.

Our suggestion is to put the cuttings for rooting over a five-month rooting period.

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Могућност коришћења зелених резница у вегетативној пропагацији *Sequoiadendron giganteum* (Lindl.) J. Buchh.)

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[2] *Introduction Ltd.,* Стара Загора, Бугарска

Сажетак

Обављен је експеримент, који обухвата проучавање утицаја генотипа, стимулатора раста и трајања периода укорјењивања на проценат укорјењивања и квалитет корјеновог система зелених резница џиновске секвоје (*Sequoiadendron giganteum* (Lindl.) J. Buchholz). Резнице су добијене од 18 десетогодишњих садница од 10 различитих генотипова. Трајање периода укорјењивања (3 мјесеца и 4 мјесеца) је био статистички значајан у погледу брзине формирања корјена и калуса. Продужење периода укорјењивања за два мјесеца омогућује 57,76% више укорјењених резница. Највећи утицај на постотак укорјењених резница, број формираних корјенова и дужину главног корјена имао је генотип. Третман са ауксином такође повећава проценат укорењивања, број формираних корјенова и дужину главног корена. Третман са 2000 ppm индолил бутерне киселине (IBA) даје најбоље резултате на крају трећег и четвртог мјесеца, док су саднице са периодом укорјењивања од пет мјесеци и третиране са 4000 ppm IBA имале највећи проценат укорјењивања.

Кључне ријечи: џиновска секвоја, укорјењивање, резнице, доза IBA

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