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Original article

Effects of auxin doses on rooting of *Juniperus* L.

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Summary. The present study was conducted to investigate the rooting ratios of cuttings taken from naturally grown Greek juniper trees of Gevaş and Çatak (Van) regions at different dates in 2013 and 2014 and under different hormone doses. The cuttings taken in October and December of 2013 and April of 2014 were immersed into water for 24 hours, then they were dipped into 3000 ppm, 5000 ppm and 7000 ppm NAA and IBA hormone solutions for 5 seconds. Cuttings were planted into growth mediums for rooting. For reliable rooting, the most proper hormone concentration was identified as 5000 ppm NAA and the most proper planting time was identified as April.

Key words: *Juniperus*, cutting, hormone

Introduction

About 97% of juniper forests are constituted by Greek juniper and foetid juniper. Annual precipitations in juniper forests are between 400-600 mm. Junipers are quiet resistant to extreme soil conditions, thus they are among the valuable tree species (1). Juniper is also an ornamental plant used in urban landscapes or used as median tree.

In landscape works of today, junipers may be used to create near-normal plant compositions. Such uses both minimize the potential problems to be experienced and provide an economic application.

Juniper species with resistant, fine textured and redolent wood have a great place in forestry of Turkey. They are commonly used for functional purposes in landscaping, wind and snow brake construction, green zone formation, erosion control and forestation works and for esthetical purposes in park arrangements.

Junipers are commonly preferred in forestation works since they can develop at low temperatures, benefit from winter precipitations at uppermost level, minimize life activities in dry periods and be resistant to drought, cover their leaves with a waxy layer to prevent water losses, develop a taproot system in a short time and these roots can penetrate into deep soil profiles rapidly.

With the overcome of germination obstacle, today juniper is able to be propagated with seeds. However, high empty and rotten seed ratios and slow development process entail the use of vegetative production techniques.

It was observed in studies about cutting-propagation of these natural plant species with various uses that rooting ratios varied from one variety to another. In this study, effects of auxins on adventive root formation of Greek juniper were investigated and the best proper time to take cuttings were tried to be determined based on internal hormone levels.

In juniper stands, it was sometimes observed that naturally growing young species have turned into shrub forms because of excessive grazing. When these young junipers were put under preservation, it was observed that they developed smooth top shoots and grew taller (2).

Foliolate species can easily be rooted with root, branch, leaf and etc. cuttings. In some foliolate species, seed propagation has almost totally given up because of easy rooting characteristics of the cuttings and easier transmission of genetic superiorities to new generations with cuttings. In coniferous species on the other hand, the difficulties experienced in cutting rooting significantly restricts the productions.

Except for some stands already put under protection, majority of juniper sites are gradually constricting and some sites are almost faced to extinction. The objective of the present study was to put forth the possible use of cuttings in sapling production, thus to investigate possible contributions of cutting propagation in juniper production.

Material and Method

The cuttings composed of two-years old shoots were used in this study. Cuttings were taken from different locations with altitudes of between 1400-1800 meters at 3 periods (October, December, April). About 2-2,5 cm bottom sections of cuttings were cleaned from needles and cut in aslant fashion. Prepared cuttings were immersed into water for 24 hours and they were dipped into 2 hormone solutions at 3 different doses (3000, 5000, 7000ppm IBA and 3000, 5000, 7000 ppm NAA) for 5 seconds. Then cuttings were planted into tubes filled with sand, fertilizer and forest soil mixture. A control treatment without any hormones was also used.

IAA, ABA, GA3 and zeatin amounts

About 10 g cutting piece was weighed for hormone analysis. Samples were broken into pieces in a mortar with liquid nitrogen, 60 ml 80% ethanol was added over the samples and resultant samples were then homogenized for 10 minutes. The resultant extract was taken into a beaker and kept in a shaker set in a cold storage at +4°C for a night. The extract was then filtered through Whatman number 1 filter paper and evaporated in a rotary evaporator at 35°C.

Residual filtrate (about 4 ml) was supplemented with 8 ml of 0,1 M KH2PO4 buffer solution and resultant extract was then centrifuged at 5000 rpm at +4°C for 1 hour. Supernatant section of the centrifuged samples was taken and supplemented with insoluble PVPP and thoroughly mixed. The mixture was left for 5 min as it is. Samples were filtrated through regular filter, filtrates passed through C-18 seppak cartridges and the resultant filtrate was discarded. The hormones remained over C-18 seppak cartridges were dissolved in 80% methanol and collected in colored vials (3, 4). Samples were ultimately injected into High Performance Liquid Chromatography (HPLC) device. The HPLC parameters were as follows: DAD detector, 254 nm wave length, the column is C18 at room temperature. Standard curves were created to determine the arrival time of hormones on chromatogram in HPLC and to calculate the amounts. The r^2 value for the standard curve in ABA analyses was r^2=0.999.

Results and Discussion

The chromatograms for internal hormone standard extracts prepared at different concentrations and injected into HPLC are presented in Figures 1, 2 and 3.

It was observed in Table 1 that there was a remarkable variation in zeatin and abscisic acid amounts with the utilized hormone dose.

Growth and development may reach to certain levels only with the existence of growth regulators and ABA at proper ratios. While auxins, cytokines and gibberellins are increasing with rapid growth in spring, ABA levels decrease in the same period. The case is totally reverse toward the end of growth season. In other words, while ABA levels are increasing, the other hormone levels decrease. The present findings in this sense comply with the findings of Battal *et al.* (5), but inconsistent with the results reported by Yalçın (6). Keskin, (1991) used four different doses of IBA and IAA to investigate cutting rooting of foetid juniper (*Juniperus foetidissima* Willd.) and Greek juniper (*Juniperus excelsa* Bieb.) species at two different periods (March and April) and reported quite lower rooting ratios for Greek juniper than for foetid juniper. In foetid juniper, 0.6% dose of IBA yielded a rooting ratio of 52% and the greatest rooting ratio in Greek juniper was identified as 3.3% with 0.2% dose of IAA hormone. The differences between application times were not found to be significant (Table 2). The present findings were similar to findings of Keskin (7), but significant dif-

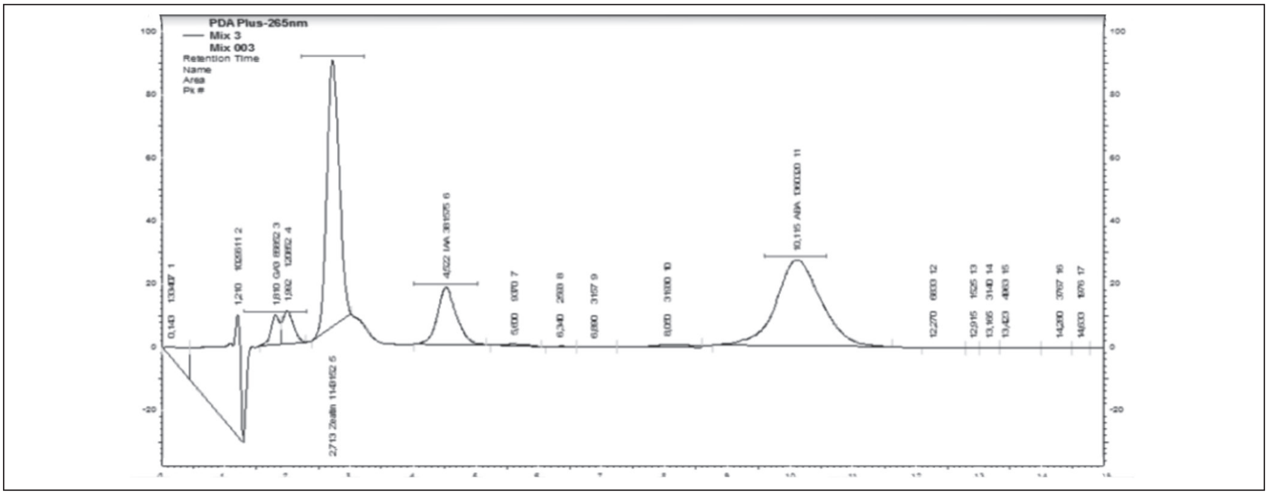

Figure 1. The chromatogram for internal hormone standard extract prepared at 75 ppm concentration and injected into HPLC (1) Gibberellic acid (GA3), (2) Zeatin, (3) Indole acetic acid (IAA), (4) Abscisic acid (ABA).

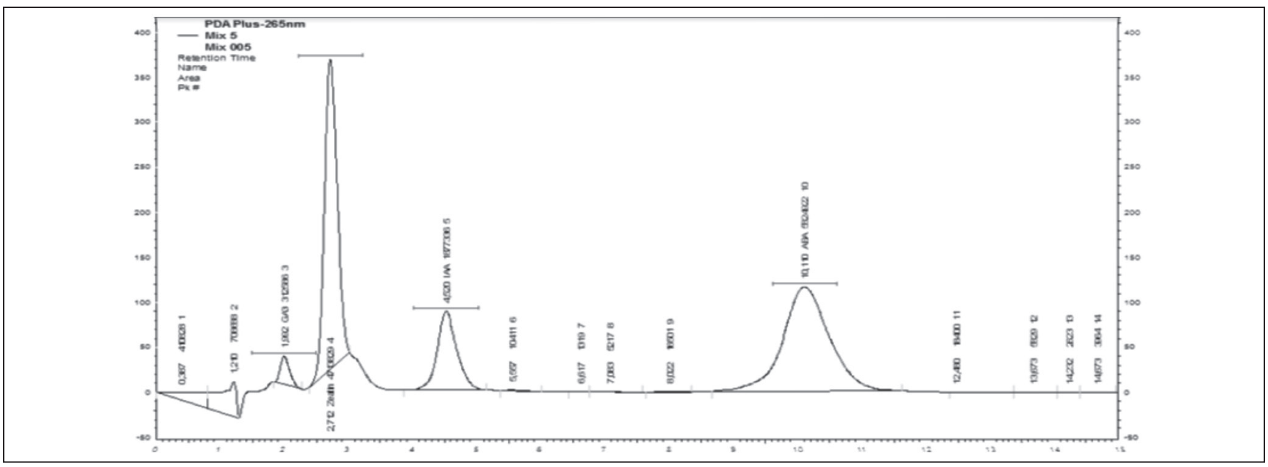

Figure 2. The chromatogram for internal hormone standard extract prepared at 25 ppm concentration and injected into HPLC (1) Gibberellic acid (GA3), (2) Zeatin, (3) Indole acetic acid (IAA), (4) Abscisic acid (ABA).

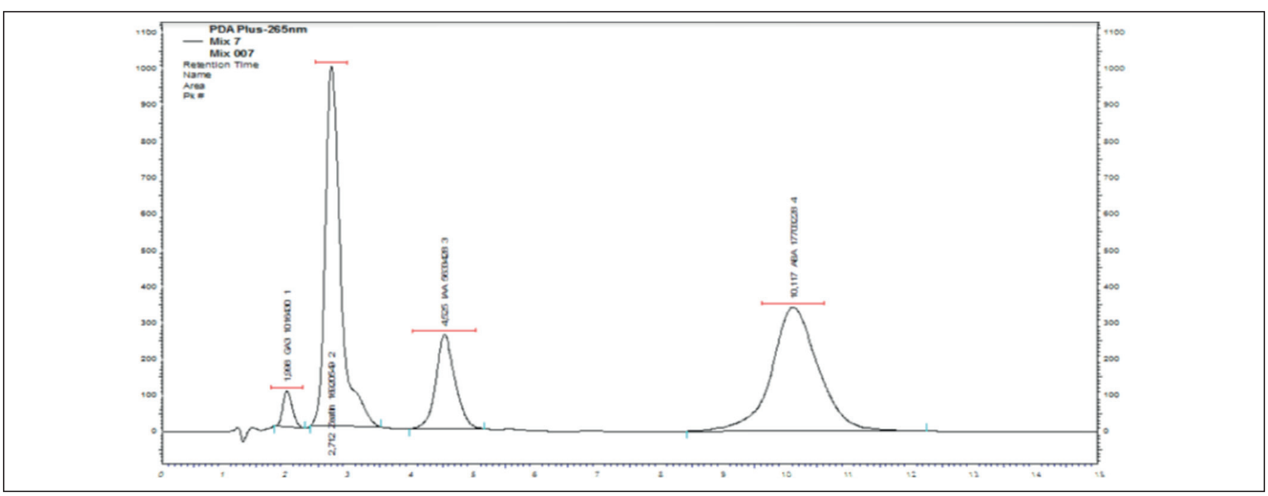

Figure 3. The chromatogram for internal hormone standard extract prepared at 6,25 ppm concentration and injected into HPLC (1) Gibberellic acid (GA3), (2) Zeatin, (3) Indole acetic acid (IAA), (4) Abscisic acid (ABA).

Table 1. Descriptive statistics and comparison results for internal hormone types

		N	Avr.	St. Err.	Min.	Max	p
GA3	NAA	14	55,14	5,61	1,53	68,28	0,93
	IBA	12	55,80	5,15	0,00	64,63	
	General	26	55,45	3,77	0,00	68,2 8	
Zeatin	NAA	14	0,90	0,54	0,00	7,43	0,79
	IBA	12	0,68	0,61	0,00	7,34	
	General	26	0,80	0,40	0,00	7,43	
IAA	NAA	14	3,12	0,58	0,10	7,03	0,97
	IBA	12	3,08	0,53	0,00	7,54	
	General	26	3,10	0, 39	0,00	7,54	
ABA	NAA	14	1,24	0,25	0,00	3,55	0,47
	IBA	12	1,00	0,18	0,00	1,68	
	Genera!	26	1,13	0,16	0,00	3,55	

Table 2. Descriptive statistics and comparison results for October and April

		N	Avr.	St. Err.	Min.	Max	p
GA3	October	12	50,75	6,80	0,00	64,63	0,26
	April	14	59,47	3,80	10,51	68,28	
Zeatin	October	12	1,26	0,83	0,00	7,43	0,30
	April	14	0,41	0,20	0,00	2,37	
IAA	October	12	2,52	0,57	0,00	7,54	0,17
	April	14	3,59	0,52	0,10	7,03	
ABA	October	12	0,94	0,17	0,00	1,68	0,28
	April	14	1,29	0,26	0,00	3,55	

ferences were observed between cutting taking times in this study.

Variation of investigated traits in plant parts and comparison results are provided in Table 3. Zeatin and IAA amounts were higher in root sections than in upper sections, but gibberellic acid amount was higher in upper sections than in root sections of the cuttings.

Variation of investigated traits and comparison results for different doses of growth regulators are provided in Table 3. The differences between the doses were not found to be significant. Despite these insignificant differences, GA_3 reached to highest level at 5000 ppm and Zeatin with a value of 2.75 had a quite higher trend at 3000 ppm than the other doses. On the

Table 3.Descriptive statistics and comparison results for plant sections

		N	Avr.	St. Err.	Min.	Max	p
GA3	Upper	13	56,56	4,64	1,53	63,22	0,77
	Root	13	54,33	6,11	0,00	68,28	
Zeatin	Upper	13	0,60	0,5	0,00	7,43	0,63
	Root	13	1,00	0,5	0,00	7,34	
IAA	Upper	13	2,74	0,35	0,12	5,11	0,36
	Root	13	3,47	0,70	0,00	7,54	
ABA	Upper	13	1,01	0,17	0,00	1,68	0,48
	Root	13	1,25	0,28	0,00	3,55	

other hand, IAA reached to maximum value with 3.47 at 5000 ppm dose.

Similarly, GA_3 was measured as 45.90 mg at 3000 ppm and about 63.80 mg at 5000 ppm treatment (Table 4). The least zeatin amount (0.18 mg) was observed in 7000 ppm IBA treatment and the greatest value was seen in 3000 ppm IBA treatment.

Increasing abscisic acid contents are observed with increasing auxin contents. According to Okduman (8) IAA degraded faster in cuttings prepared from hardly-rooting plants, such a case then result in less auxin concentrations at cutting bottoms, IAA transport was slower in hardly-rooting cuttings and these cuttings might contain higher concentrations of rooting inhibitors.

Number of roots, cutting length, root length and root thickness values were also investigated at different doses in Gevaş and Çatak cultivars and the re-

Table 4. Descriptive statistics and comparison results for different doses ons

		N	Avr.	St. Err.	Min.	Max	p
GA3	3000	8	45,91	9,91	0,00	64,11	0,14
	5000	10	63,83	0,62	61,79	68,28	
	7000	8	54,50	6,34	10,51	63,92	
Zeatin	3000	8	2,16	1,18	0,00	7,43	0,06
	5000	10	0,28	0,18	0,00	1,50	
	7000	8	0,08	0,05	0,00	0,35	
IAA	3000	8	2, 5	0,81	0,00	7,03	0,74
	5000	10	3,47	0,52	1,92	6,15	
	7000	8	2,97	0,79	0,10	7,54	
ABA	3000	8	0,84	0,25	0,00	1,68	0,50
	5000	10	1,28	0,19	0,00	2,51	
	7000	8	1,22	0,39	0,00	3,55	

Table 5. Descriptive statistics and comparison results for root quality parameter

		N	Avr.	St. Err.	Min.	Max.	p
Number of roots	Gevaş 1 5000	30	0,80 a	0,22	0,00	4,00	0,01
	Gevaş 2 5000	29	0,66 ab	0,17	0,00	3,00	
	Çatak 5000	30	0,27 bc	0,10	0,00	2,00	
	Gevaş 3000	30	0,20 c	0,07	0,00	1,00	
	General	119	0,47	0,07	0,00	4,00	
Cutting length	Gevaş 1 5000	30	1,64	0,41	0,00	7,44	0,63
	Gevaş 2 5000	29	1,67	0,42	0,00	7,64	
	Çatak 5000	29	1,17	0,41	0,00	6,83	
	Gevaş 3000	29	1,06	0,41	0,00	7,45	
	General	117	1,39	0,21	0,00	7,64	
Root length	Gevaş 1 5000	30	1,92	0,51	0,00	11,23	0,29
	Gevaş 2 5000	29	1,70	0,43	0,00	7,14	
	Çatak 5000	29	1,15	0,43	0,00	9,72	
	Gevaş 3000	28	0,87	0,33	0,00	4,85	
	General	116	1,42	0,22	0,00	11,23	
Root thickness	Gevaş 1 5000	30	0,14	0,05	0,00	0,97	0,10
	Gevaş 2 5000	29	0,15	0,05	0,00	1,13	
	Çatak 5000	30	0,06	0,02	0,00	0,44	
	Gevaş 3000	29	0,05	0,02	0,00	0,34	
	General	118	0,10	0,02	0,00	1,13	

sults are provided in Table 5. Except for the number of roots, the differences in other traits of the groups were not found to be significant. With regard to number of roots, the greatest value (0,80) was observed in 5000 ppm treatment of Gevaş 1 and it was followed by 5000 ppm treatment of Gevaş 2 (0,66). The lowest value was observed in Gevaş 3000 ppm treatment with 0,20 and Çatak 5000 ppm treatment with 0,27.

Maghenini and Nin (9) used 600-1000 ppm IBA concentrations in ligneous side cuttings of basswood and indicated that IBA increased rooting of poorly rooted ligneous side cuttings. The low rooting rates of atlas cedar were also improved with 3000 ppm IBA treatments. The rooting ratio of magnolia increased to 80% with 6000 ppm hormone treatment (10). The greatest rooting ratio in ligneous side cuttings of *Magnolia soulangeana* taken in July was observed as 98% with 1000 ppm IBA treatment. Number of roots, cutting length, root length and root thickness values were also investigated at different doses in Gevaş and Çatak cultivars and the results are provided in Table 4 and 5. Except for the number of roots, the differences in other traits of the groups were not found to be significant. With regard to number of roots, the greatest value (0,80) was observed in 5000 ppm treatment of Gevaş 1 and it was followed by 5000 ppm treatment of Gevaş 2 (0,66). The lowest value was observed in Gevaş 3000 ppm treatment with 0,20 and Çatak 5000 ppm treatment with 0,27. Insignificant differences in other traits of the groups indicated that treatments did not have any significant effects on these traits.

Conclusion

Compared to other juniper species, Greek juniper is a relatively hard-rooted cultivar. Thin and poor structure of juniper shoots from which the cuttings are taken may result in different outcomes for rooting ratios. Therefore, possible differences in rooting ratios should be investigated when the cuttings older than 2 years were used for rooting. The greatest rooting ratio was observed at 5000 ppm in spring months. Superficial scalds were observed at the bottom sections of cuttings at 7000 ppm hormone treatments. Root formation was quite low probably because of callus deformation with environmental impacts, fungus infections, insufficient leaf development parallel to rooting, insufficient nutrient supply through photosynthesis.

Considering the time factor as a significant factor in rooting, March and April can be recommended as proper times to take cuttings for better rooting ratios. The success ratios could greatly be improved under controlled conditions (moisture, soilless culture). Present findings may provide significant outcomes for further studies to be carried out about the rooting ratios in Greek juniper.

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