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SHORT COMMUNICATION

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Rooting of Mugo pine (*Pinus mugo*) cuttings as affected by IBA, NAA and planting substrate

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Abstract

Aim of study: The effect of planting substrate and concentrations of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) hormones was studied on the rooting of mugo pine cuttings.

Area of study: The research was carried out in Rasht city, Guilan province, Iran.

Material and methods: Both hormones (IBA and NAA) were applied at four concentrations of 0, 1000, 2000 and 4000 mg/l. Planting substrates included sand, perlite, cocopeat, sand + perlite, and sand + cocopeat (1:1).

Main results: The highest rooting percentage (55%) was obtained under the trilateral treatment $a_2b_4c_1$ (sand × 4000 mg/l NAA × 1000 mg/l IBA). Sand + cocopeat was found to be the best rooting substrate.

Research highlights: It is recommended to apply sand with 4000 mg/l and 1000 mg/l concentration of experimental hormones (NAA and IBA, respectively).

Keywords: auxin; rooting; *Pinus mugo*; vegetative propagation.

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Introduction

Mugo pine (*Pinus mugo*) is used as bonsai and Christmas tree. Also, it is recommended for median strip plantings in the highways (Edward & Dennis, 1994). Today, mugo pine is cultivated in commercial forests to produce wood and timber and it is used as a tree in reforestation projects (Jorgensen, 2010). It is propagated by seed, rooting of cuttings and grafting (Edward & Dennis, 1994). Cutting is an important method for propagating of ornamental trees and shrubs (Reezi *et al*., 2006). Pine cuttings are difficult to root (Hartmann *et al*., 2011).

Auxins have the greatest effect on root formation in cuttings. Plants produce natural auxin (IAA) in their young branches and leaves, but exogenous auxin needs to be applied for improved rooting (Štefančič *et al*., 2007). Haugh *et al.* (1974) found that auxin improved the rooting of some clones of mugo pine in June but was detrimental to others, particularly at higher concentrations. Henrique *et al*. (2006) found that the most effective treatment for the rooting of *Pinus caribaea* was 4000 mg L^{-1} IBA plus 100 mg L^{-1} paclobutrazol. The rooting media are another effective factor in improving the rooting of cuttings (Ofori *et al*., 1996; Hemati & Khanjani Shiraz, 2009). In most cases, the rooting percentage and the quality of the roots are directly associated with planting substrate (MacDonald, 2000; Sadhu, 1998). More rooted cuttings and more roots per cutting occurred on junipers when 100% peat (0% polystyrene) or 25% polystyrene were used than when higher rates of polystyrene were applied. More arborvitae cuttings rooted under 50% or 75% polystyrene. More roots and longer roots formed on juniper cuttings with perlite than with the same concentration of polystyrene in the substrate (Cole & Dunn, 2002). In general, conifers have been found to root best in December and January after a period of chilling (Haugh *et al*., 1974).

Mugo pine is a hard- to- root species that producers have not been able to propagate by rooting the cuttings in the nursery. The present study was aimed at evaluating the effect of rooting substrate and type of auxin on semi-hardwood cuttings of mugo pine.

Materials and Methods

Experimental site and plant material

The cuttings of mugo pine were collected from International Flowers and Plants Center in Ramsar, Iran, where the study was conducted. The experiment was carried out in a cold plastic-covered greenhouse with bottom heat and mist irrigation system. The leaf stem cuttings were taken from several maternal plants in November. The maternal orchard consisted of 30 mugo pine trees. The stock trees were 15 years old and about three meters tall. The length and diameter of the cuttings were about 10-15 and 5-6 mm, respectively. The shoot tip cuttings were gathered from a same place and were randomly treated. They were cut from the beneath of the node. The substrates of sand, perlite, cocopeat, sand + perlite and sand + cocopeat were applied with the same volume ratios. The environment and media temperature during the rooting period were 10-15°C and 18-21°C, respectively.

Experimental design, treatments and traits

The study was carried out as factorial experiment based on a Randomized Complete Block Design with 80 treatments at three replications. There were three cuttings in each experimental plot. It included 720 cuttings of mugo pine. The treatments included IBA and NAA hormones, both at four levels of 0, 1000, 2000 and 4000 mg/l and rooting substrate at five levels (sand, perlite, cocopeat, sand + perlite and sand + cocopeat). Before sticking the cuttings in the rooting substrate, 2-3 cm of their basal zones was immersed in the synthetic IBA and NAA for 10 seconds. The cuttings were monitored for 7 months. After about 7 months, rooting percentage, root length, root fresh weight, root dry weight, number of lateral roots and root area were measured.

Root area (cm^2) was calculated with the following formula (Atkinson, 1991):

$$\text{Root area} = 2(\text{root volume} \times \pi \times \text{root length})^{0.5}$$

The data were analyzed with MSTATC software package and the means were compared by LSD test.

Results and Discussion

Analysis of variance showed that the interaction between substrate and different concentrations of IBA and NAA was significant for rooting percentage, number of lateral roots, root fresh and dry weight (Table 1). According to means comparison for trilateral interaction of "substrate × NAA × IBA" (Table 2), it was found that the highest rooting percentage of 55% was obtained from the treatment $a_2b_4c_1$ (sand × 4000 mg/l NAA × 1000 mg/l IBA). No rooting was observed in 41 treatments, out of which 21 treatments had the substrate of perlite or sand + perlite mixture and in the other 14 treatments, the concentration of either IBA or NAA was 0 or 1000 mg/l. The choice of appropriate rooting substrate is regarded as an important stage in the propagation of horticultural crops that can increase the rooting rate and improve the number of rooted cuttings (Hartmann *et al*. 2011; Hemati & Khanjani Shiraz, 2009). Griffin *et al*. (1999) reported that IBA and NAA concentrations of 0.25 and 0.5% increased rooting rate, rooting percentage, number of roots and rooting potential of *Magnolia* cv. Santa Rosa, which confirms the results of the present study.

Means comparison of the trilateral interaction on the number of lateral roots of cuttings (Table 2) revealed that the highest number of lateral roots (19.33) was obtained from the treatment $a_4b_2c_4$ (sand + cocopeat × 1000 mg/l NAA × 4000 mg/l IBA). The application of auxin significantly increased the number of roots per cutting (Sadhu, 1998). Saffari & Saffari (2012) stated

Table 1. Analysis of variances of simple and interaction effects of substrates and hormones on traits

SOV	df	Square mean					
		Rooting %	Root fresh weight	Root dry weight	Lateral root number	Root length	Root area
Replication	2	94.88^{ns}	0.05^{ns}	0.001^{ns}	13.62^{ns}	33.93^{ns}	6.35^{ns}
IBA (a)	3	18.21^{ns}	0.10^{ns}	0.002^{ns}	25.70^{ns}	23.16^{ns}	9.66^{ns}
NAA (b)	3	308^{ns}	0.03^{ns}	0.001^{ns}	25.85^{ns}	23.68^{ns}	7.61^{ns}
AB	9	211.89^{ns}	0.12^{ns}	0.002^{ns}	42.23^{ns}	65.19^{ns}	16.47^{ns}
Substrates (C)	4	1423.27**	0.37**	0.007**	151.05**	156.27**	48.76**
AC	12	399.58*	0.14*	0.003**	39.42^{ns}	56.88^{ns}	16.20^{ns}
BC	12	478.69*	0.16*	0.003**	60.91*	57.91^{ns}	16.45^{ns}
ABC	36	412.27*	0.14*	0.003**	47.01*	49.28^{ns}	13.75^{ns}
Error	158	251.47	0.08	0.001	31.7	43.29	10.47

**,* and ^{ns}: significant at 1%, 5% and non-significant, respectively.

that substrate type, hormone concentration and their interaction significantly influenced the number of roots of hopbush (*Dodoneae viscosa* L.) cuttings which is consistent with our results.

Based on Table 2, the highest fresh weight of roots was produced under the treatment $a_4b_3c_4$ (sand + perlite × 2000 mg/l NAA × 4000 mg/l IBA). Hormones stimulate the initial meristem activities and also motivate the growth and development of the formed roots (Hartmann *et al*., 2011; Wiesman *et al*., 1989). According to Table 2, the highest root dry weight of 0.14 g was found under the trilateral treatment $a_2b_3c_1$ (sand × 4000 mg/l NAA × 1000 mg/l IBA). High oxygen content of the root zone accelerates the growth of healthy and strong roots (Wiesman & Lavee, 1995). It was reported that auxins improved root dry weight and higher IBA concentration resulted in higher root dry weight (Hammo *et al*., 2013).

Analysis of variance (Table 1) revealed that among the experimental factors and their interactions, only the simple effect of substrate type (factor C) was significant on root length of mugo cutting ($p<0.01$). Means comparison showed that the highest root length (about 5 cm) was produced in sand + cocopeat substrate with no significant difference with sand (control), sand + perlite and cocopeat substrates.

One important factor in successful rooting of the cuttings is the selection of a proper substrate (Sadhu, 1998). Wright *et al*. (1992) revealed that an appropriate balance between water and air must be maintained in planting substrate in order to absorb enough moisture and oxygen to cutting. Cocopeat holds more water than perlite and it can supply enough oxygen and moisture for cuttings (Hartmann *et al*., 2011).

Among the simple effect of planting substrate, sand + cocopeat produced the highest root area. The lowest root area (0.1 cm^2) was formed in perlite. Root area was 27 times greater in superior treatment than in perlite. Root is the water and the nutrient absorbing organ of the plants. Higher root area in the cutting media results in higher survival chance of the cuttings. Rooting substrate not only impacts the number of rooted cuttings, but also affects the quality of the roots (Sadhu, 1998). However, other factors such as bottom heat, mist system and enough water can improve the development and quality of the roots (Hartmann *et al*., 2011).

Finally, without considering the effect of trial auxins, "sand + cocopeat" was found to be a more appropriate substrate. In addition, the significant interaction of substrates and rooting hormones on the most studied traits shows the helpful impact of auxins on the rooting of mugo pine cuttings. The increase in rooting percentage from 14% under no hormone treatment (control) to 55% under IBA and NAA application confirms auxin positive effect on mugo rooting.

Table 2. Interaction effect of "substrate × NAA × IBA" on the some traits of mugo pine

Media	IBA 0 ppm (a_1)				IBA 1000 ppm (a_2)				IBA 2000 ppm (a_3)				IBA 4000 ppm (a_4)				Traits
	NAA 0 ppm (b_1)	NAA 1000 ppm (b_2)	NAA 2000 ppm (b_3)	NAA 4000 ppm (b_4)	NAA 0 ppm (b_1)	NAA 1000 ppm (b_2)	NAA 2000 ppm (b_3)	NAA 4000 ppm (b_4)	NAA 0 ppm (b_1)	NAA 1000 ppm (b_2)	NAA 2000 ppm (b_3)	NAA 4000 ppm (b_4)	NAA 0 ppm (b_1)	NAA 1000 ppm (b_2)	NAA 2000 ppm (b_3)	NAA 4000 ppm (b_4)	
Sand(C_1)	0	22	22	0	0	11	0	55	22	22	11	33	11	0	11	0	Rooting percentage
Perlite(C_2)	0	0	0	0	0	0	11	0	0	0	0	0	0	11	0	0	
Cocopeat(C_3)	11	0	0	0	22	11	11	0	0	11	0	11	0	22	0	0	
Sand+Perlite(C_4)	11	0	22	0	0	0	11	11	22	11	0	22	0	22	22	0	
Sand+Cocopeat(C_5)	33	0	0	44	11	22	0	11	11	0	0	11	22	11	11	44	
Sand(C_1)	0	5.66	4.66	0	0	5.66	0	8.33	1.66	2.33	1.66	9.66	2.33	0	1.66	0	Lateral root number
Perlite(C_2)	0	0	0	0	0	0	1	0	0	0	0	0	0	2.66	0	0	
Cocopeat(C_3)	5.66	0	0	0	11.67	3	2.66	0	0	7.66	0	1.33	0	7	0	0	
Sand+Perlite(C_4)	5.66	0	3	0	0	0	6.33	3.66	2.66	4	0	12.33	0	19.33	13.33	0	
Sand+Cocopeat(C_5)	5	0	0	14.33	6.66	5.66	0	7	4.33	0	0	3.33	7.66	2	7	11	
Sand (C_1)	0	0.53	0.4	0	0	0.21	0	0.82	0.13	0.38	0.06	0.59	0.12	0	0.11	0	Root fresh weight
Perlite(C_2)	0	0	0	0	0	0	0.09	0	0	0	0	0	0	0.18	0	0	
Cocopeat(C_3)	0.33	0	0	0	0.33	0.15	0.17	0	0	0.27	0	0.18	0	0.49	0	0	
Sand+Perlite(C_4)	0.18	0	0.16	0	0	0	0.96	0.08	0.32	0.16	0	0.72	0	0.59	0.87	0	
Sand+Cocopeat(C_5)	0.24	0	0	0.26	0.14	0.31	0	0.14	0.14	0	0	0.17	0.79	0.22	0.21	0.6	
Sand (C_1)	0	0.043	0.04	0	0	0.026	0	0.146	0.013	0.046	0.003	0.07	0.006	0	0.01	0	Root dry weight
Perlite(C_2)	0	0	0	0	0	0	0.01	0	0	0	0	0	0	0.026	0	0	
Cocopeat(C_3)	0.06	0	0	0	0.046	0.016	0.016	0	0	0.033	0	0.013	0	0.053	0	0	
Sand+Perlite(C_4)	0.016	0	0.016	0	0	0	0.06	0.01	0.033	0.033	0	0.093	0	0.076	0.136	0	
Sand+Cocopeat(C_5)	0.026	0	0	0.023	0.016	0.063	0	0.02	0.02	0	0	0.026	0.09	0.023	0.026	0.126	

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