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EFFECTS OF DIFFERENT MEDIA AND HORMONES ON PROPAGATION BY CUTTINGS OF EUROPEAN YEW (*Taxus baccata* L.)

Utjecaj različitih supstrata i hormona na zakorjenjivanje reznica obične tise (*Taxus baccata* L.)

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ABSTRACT

European yew (*Taxus baccata* L.), native in North and Central Europe, Mediterranean countries, Azores, Turkey and Caucasus, has a wide range of uses as a non-wood forest product. Because the species is reduced in nature as a result of widespread utilization, it is necessary to protect and reproduce yew. The effects of different greenhouse media, rooting media and hormones were investigated on propagation by cutting of European yew. For the experiment, three greenhouse media (Greenhouse-1 media with air temperature at 20±2°C, rooting table temperature at 20±2°C, Greenhouse-2 media with air temperature at 20±2°C, rooting table temperature at 25±2°C and Greenhouse-3 media without temperature adjustment), two rooting media (perlite and peat) and four hormones (IBA 1000 ppm, IBA 5000 ppm, NAA 1000 ppm and NAA 5000 ppm) were determined in the present study. The rooting percentage, callus percentage, root length and the number of roots were determined. The results showed that the highest rooting percentage was 80% in IBA 5000 ppm treatment in perlite rooting media of Greenhouse-2 media. It can be suggested that the rooting table temperature should be 5°C higher than the air temperature, perlite rooting media and 5000 ppm dosage of IBA hormone should be used for high rooting success.

KEY WORDS: *Taxus baccata*, Cutting propagation, Greenhouse media, Rooting media, Auxin

1. INTRODUCTION
1. UVOD

European yew (*Taxus baccata* L.), belonging to *Taxaceae* family, spreads over North and Central Europe, Mediterranean countries, Azores, Turkey and Caucasus. It is often multi-stemmed and its height is generally 15-25 m, but its trunks can be very large: up to 4 m in diameter. Mature fruits are red fleshy succulents looking. Propagation of the species can be made with seed, cutting or grafting. It is a preferred species in parks and gardens suitable for pruning. But shoots, leaves and seeds are extremely poisonous (Thomas and Polwart, 2003; Anşin and Özkan, 2006; Mamıkoğlu, 2015; Benham et al. 2016). There has been a decrease in many places because it is extremely toxic. At the present time, it is not considered to be a commercial crop because of its extremely slow growth rate. However, it is valued as an amenity tree for hedging. In recent years, the species has become important due to the taxane alkaloids found in its foliage, and also in its bark in minute amounts, that have been developed as an anticancer drug. Today, forests harbouring yew have been designated as special protection areas (Shemluck, 2003; Benham et al. 2016). The

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genus *Taxus* has created a great interest due to its content of diterpene alkaloids, especially taxol (Malik et al. 2011). The production of taxol and baccatin III by callus and cell suspension cultures of different *Taxus* species has been expressed (Cusidó et al. 1999). The species must be reproduced for reasons such as the existence of wide usage areas and need to be protected.

The effectiveness of nursery management is seriously affected by delay in germination. Thus, alternative planting materials are required (Akinyele, 2010). One of the plant propagation techniques is the vegetative propagation by cuttings. Cutting are prepared by the vegetative portions of the plant including stems, modified stems (rhizomes, tubers, corms and bulbs), leaves or roots. Stem cuttings can be divided into four groups to be hardwood, semi-hardwood, softwood and herbaceous (Hartmann et al. 2002). After leaves abscise, hardwood cuttings are made of matured, dormant firm wood. The hardwood cutting is one of the cheapest and easiest methods of vegetative propagation. Hardwood cuttings, prepared during the dormant season (Hambrick et al. 1991), are easy to prepare and require little or no special equipment during rooting (Fourrier, 1984).

Perlite is widely used in rooting of plants for moisture retention and good aeration characteristics, sterility and light weight (Şimşek, 1993; Hartmann et al. 2002). Peat is an organic material commonly used in greenhouse cultivation, floriculture, seedling production and similar horticultural works in many countries of the world (Çolak and Günay, 2011).

In plant propagation, the induction of roots is a process regulated by environmental and endogenous factors such as temperature, light, plant growth regulators (especially auxin), carbohydrates, mineral salts and other molecules (Gehlot et al. 2014). Auxins involved in most aspects of plant development are a group of tryptophan-derived signals (Woodward and Bartel, 2005). These control growth and development, early stages of embryogenesis, organization of apical meristem (phyllotaxy) and branching of the plant aerial parts (apical dominance), formation of main root, lateral and adventitious root initiation (Went and Thimann, 1937). Auxins have been shown to be efficient inducers of adventitious roots in many woody species (De Klerk et al. 1999). The widely used sources of plant growth regulators for rooting of cuttings are the IBA (indole-3-butyric acid), IAA (indole-3-acetic acid) and NAA (α-naphthalene acetic acid) (Cooper, 1935; Fogaça and Fett-Neto 2005).

The objective of this study is to determine the effects of different greenhouse media (GM), rooting media (RM) and hormones (H) on propagation by cutting of European yew (*Taxus baccata* L.).

2. MATERIALS AND METHODS
2. MATERIJALI I METODE

The study was conducted in The Research and Application Greenhouse at Faculty of Forestry, Karadeniz Technical University (KTU) in 2016. Hardwood cuttings taken in March from the last annual shoots of a 28-year-old male individual located in KTU Kanuni Campus were used as study material. This study was carried out in Greenhouse-1 media (air temperature at 20±2°C, rooting table temperature at 20±2°C), Greenhouse-2 media (air temperature at 20±2°C, rooting table temperature at 25±2°C) with technological systems and Greenhouse-3 media (nylon tunnel greenhouse media without temperature adjustment). Temperatures in the greenhouse media were adjusted with the automation system in technological greenhouse. Perlite

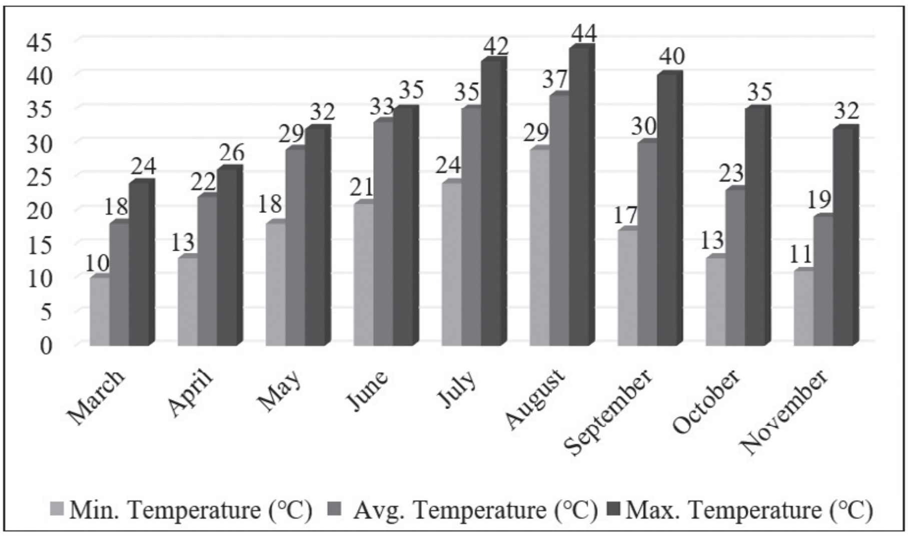

Figure 1. Greenhouse-3 media monthly temperature values
Slika 1. Mjesečne temperaturne vrijednosti supstrata u Stakleniku-3

Figure 2. Planted cuttings in perlite and peat rooting media, rooting status on some cuttings

Slika 2. Pikirane reznice u perlitu i tresetu kao supstratu za ukorjenjivanje, faze rizogeneze na pojedinim reznicama

and peat were used as rooting media in all greenhouse media. IBA (Indole-3-Butyric Acid) and NAA (α-Naphthalene Acetic Acid) of 1000 and 5000 ppm dosages were selected from among the auxin group hormones to induce rooting from plant growth regulators for rooting. All hormone implementations were made in each greenhouse and rooting media.

Since no temperature regulation was made in Greenhouse-3 media, the temperature measurements were taken three times a day (morning, noon and evening) with a thermometer until completion of the study. Monthly temperature values in Greenhouse-3 media are given in Figure 1.

The study was set up to be three replications, according to the "randomised complete block design". A total of 900 cuttings were planted to rooting including 1 species x 2 hormones x 2 doses x 3 greenhouse media x 2 rooting media x 10 cuttings x 3 replications (720 cuttings) and control cuttings in each media (180 cuttings). Cuttings were taken from determined stock plant early in the morning. The cuttings were generally prepared to be 10-12 cm long. The bottoms of the prepared cuttings were immersed in powdered hormone and transferred to the rooting media. The irrigation was made after the cuttings were transferred to rooting media. Thus, it was ensured that the cuttings were fully seated in the rooting media. In the measurements made in the cuttings, the first callus and first root formation dates, rooting percentage (RP), callus percentage (CP), root length (RL) and the number of root (RN) values were determined (Figure 2). Rooting percentage was expressed as a percentage of the total cutting by determining the number of cuttings forming root. Callus percentage was determined to be the percentage of total cutting by determining the number of non-rooted cuttings with callus formation. Root length was to the length of roots formed in cuttings. And root number was the number of roots occurred in cuttings.

Statistical analysis: The data were analyzed using SPSS 23 statistical program. Analysis of variance (univariate) was conducted to determine the effects of different greenhouse media, rooting media and hormones on rooting percentage, callus percentage, root length and the number of root. In addition, Duncan test was performed to determine the groups that were found in terms of hormones and greenhouse media for RP, CP, RL and RN.

3. RESULTS
3. REZULTATI

The values of RP, CP, RL and RN in terms of measured values of greenhouse media, rooting media and hormones used in the study are given in Table 1.

As a result of the study, it was determined that perlite rooting media was more effective in rooting compared to peat rooting media, and peat rooting media was more effective in callus formation by comparison with perlite rooting media in all greenhouse media. Compared to peat rooting media, of the average difference was 82.43% in terms of rooting success in perlite rooting media in Greenhouse-1 media. This difference was 94,12% in Greenhouse-2 media and 85,90% in Greenhouse-3 media. The highest rooting percentages occurred in all greenhouse media were in the perlite rooting media. Accordingly, the highest rooting percentages occurred in NAA 1000 ppm treatment with 76.67% in

Table 1. Results from measured values
Tabela 1. Rezultati izmjerenih vrijednosti

GM (SS)	RM (SU)	H (H)	RP (%) (PZ)	CP (%) (PK)	RL (cm) (DK)	RN (roots) (BK)
Greenhouse-1 Media Supstrat Staklenika-1	Perlite Perlit	Control / Kontrola	36.67	43.33	1.82±3.60	1.83±3.27
		IBA 1000	40.00	53.33	1.42±2.94	1.07±2.24
		IBA 5000	50.00	26.67	2.25±3.60	2.07±3.26
		NAA 1000	76.67	20.00	3.15±3.14	3.30±3.09
		NAA 5000	43.33	56.67	1.41±2.12	1.60±2.85
		Average / Prosjek	49.33	40.00	2.01±3.15	1.97±3.02
	Peat Treset	Control / Kontrola	6.67	90.00	0.68±3.43	0.10±0.40
		IBA 1000	6.67	86.67	0.49±2.35	0.10±0.40
		IBA 5000	6.67	93.33	0.38±1.76	0.10±0.40
		NAA 1000	16.67	83.33	1.64±5.47	0.47±1.53
		NAA 5000	6.67	86.67	0.37±1.64	0.10±0.40
		Average / Prosjek	8.67	88.00	0.71±3.25	0.17±0.78
Greenhouse-2 Media Supstrat Staklenika-2	Perlite Perlit	Control / Kontrola	63.33	10.00	2.77±3.08	3.40±3.28
		IBA 1000	60.00	20.00	3.15±3.85	3.77±4.70
		IBA 5000	80.00	13.33	3.26±2.97	3.80±3.20
		NAA 1000	76.67	6.67	4.51±3.25	4.00±3.52
		NAA 5000	60.00	16.67	3.44±3.69	2.70±2.81
		Average / Prosjek	68.00	13.33	3.43±3.39	3.53±3.54
	Peat Treset	Control / Kontrola	6.67	83.33	0.62±2.94	0.23±0.90
		IBA 1000	6.67	90.00	1.16±4.48	0.10±0.40
		IBA 5000	0.00	93.33	0.00±0.00	0.00±0.00
		NAA 1000	6.67	63.33	0.55±2.64	0.27±1.29
		NAA 5000	0.00	90.00	0.00±0.00	0.00±0.00
		Average / Prosjek	4.00	84.00	0.47±2.67	0.12±0.72
Greenhouse-3 Media Supstrat Staklenika-3	Perlite Perlit	Control / Kontrola	23.33	73.33	0.36±0.77	0.40±0.77
		IBA 1000	70.00	26.67	4.60±4.42	2.20±2.25
		IBA 5000	66.67	30.00	2.85±3.71	1.43±1.38
		NAA 1000	56.67	43.33	2.96±4.13	1.63±1.97
		NAA 5000	43.33	53.33	2.40±4.37	1.27±2.30
		Average / Prosjek	52.00	45.33	2.64±3.94	1.39±1.90
	Peat Treset	Control / Kontrola	13.33	76.67	0.97±3.41	0.30±0.99
		IBA 1000	10.00	90.00	0.16±0.58	0.27±0.98
		IBA 5000	6.67	90.00	0.55±2.66	0.07±0.25
		NAA 1000	6.67	93.33	0.83±3.17	0.07±0.25
		NAA 5000	0.00	96.67	0.00±0.00	0.00±0.00
		Average / Prosjek	7.33	89.33	0.50±2.41	0.14±0.65

Table 2. Results of analysis of variance (univariate)
Tabela 2. Rezultati analize varijance (univarijatna)

	RP (PZ)		CP (PK)		RL (DK)		RN (BK)	
	F	P	F	P	F	P	F	P
GM (SS)	124,37	0,000*	743,37	0,000*	2,68	0,069	20,43	0,000*
RM (SU)	15485,31	0,000*	16537,64	0,000*	104,23	0,000*	237,52	0,000*
H (H)	203,34	0,000*	143,81	0,000*	3,56	0,007*	2,77	0,026*
GM x RM	324,38	0,000*	387,66	0,000*	5,30	0,005*	21,64	0,000*
GM x H	77,32	0,000*	77,69	0,000*	1,14	0,334	1,54	0,139
RM x H	172,40	0,000*	118,99	0,000*	2,22	0,065	1,82	0,124
GM x RM x H	56,64	0,000*	115,87	0,000*	2,03	0,040*	1,09	0,370

* P<0,05 (There is a statistically significant difference.)

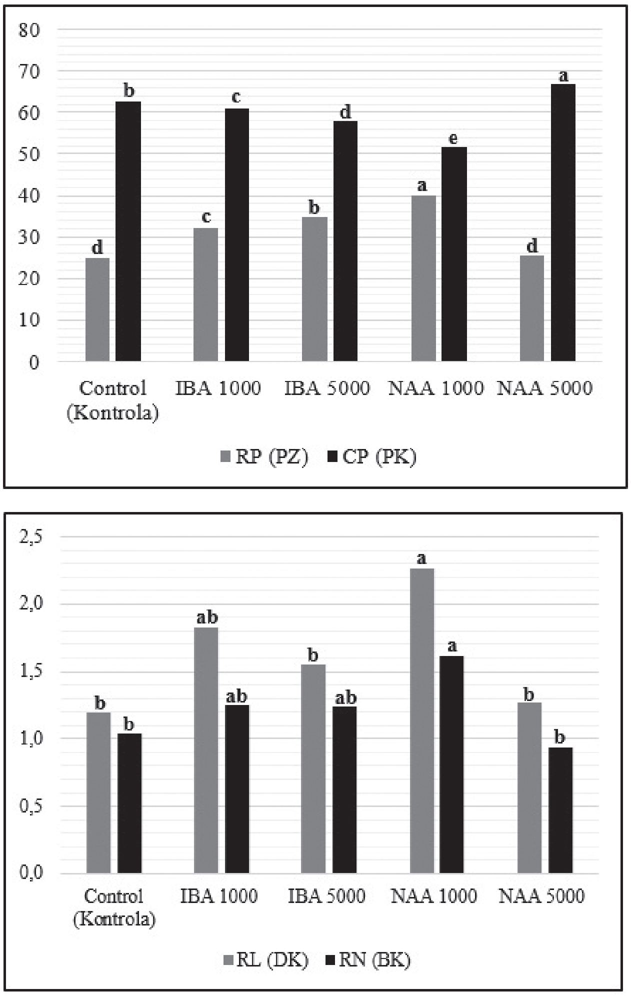

Figure 3. Duncan test results for hormones
Slika 3. Rezultati Duncanovog testa za hormone

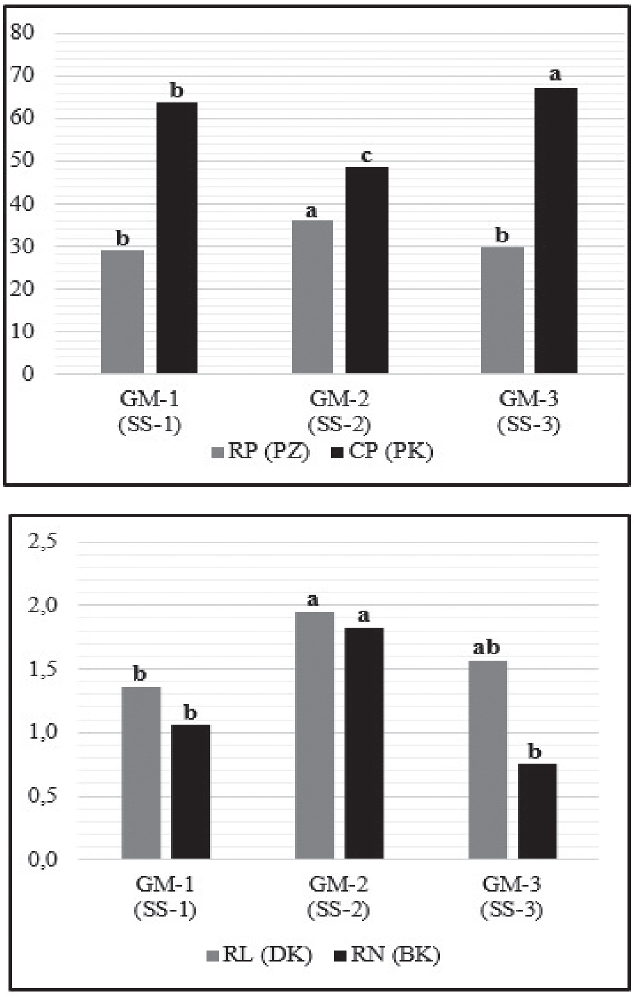

Figure 4. Duncan test results for greenhouse media
Slika 4. Rezultati Duncanovog testa za stakleničke supstrate

Greenhouse-1 media, in IBA 5000 ppm treatment with 80% in Greenhouse-2 media and in IBA 1000 ppm treatment with 70% Greenhouse-3 media. In contrast to rooting percentage, callus percentage values were higher in peat rooting media. The best results in root length and the number of root values were found in the perlite rooting media. For root length and the number of roots values, NAA 1000 ppm treatment in Greenhouse-1 media revealed 3,15 cm and 3,30 roots, respectively. This values determined as 4,51 cm and 4,00 roots in NAA 1000 ppm treatment in Greenhouse-2 media. And finally, they were 4,60 cm and 2,20 roots in IBA 1000 ppm treatment in Greenhouse-3 media. The results of analysis of variance (univariate) are given in Table 2.

All the factors were statistically significant on the rooting and callus percentage. According to analysis of variance results related to root length, rooting media and hormone factors, greenhouse media x rooting media, greenhouse media x rooting media x hormone interactions produced a statistically significant difference ($P<0,05$). The greenhouse media, rooting media, hormone factors and greenhouse media x rooting media interaction provided a statistically significant difference according to the results of the number of roots. The results of Duncan test were shown in Figure 3 (for hormones) and Figure 4 (for greenhouse media).

When Duncan test results for hormones were evaluated, 4 different groups in terms of RP appeared. While NAA 1000 ppm treatment with 40% formed the first group, Control with 25% created the last group. It was determined that there were 5 different groups in terms of CP. Accordingly, the highest result with 66.67% was revealed by NAA 5000 ppm treatment. Additionally, three different groups arose related to RL and RN. NAA 1000 ppm treatment produced the highest results for both root length (2.27 cm) and root number (1.62 roots). The most effective hormone implementation was NAA 1000 ppm treatment, which gave the highest results in terms of RP, RL and RN values. As expected, this hormone, which has the best rooting status, is the last place in terms of CP.

According to Duncan test results for greenhouse media, 2 different groups emerged in terms of RP and the first group formed in Greenhouse-2 media with 36%. There were 3 different groups for CP. According to this, the highest callus percentage was found in Greenhouse-3 media with 67.33%. When RL values taken into consideration, 3 different groups were come to the fore. The longest root length values were determined in Greenhouse-2 media (1.95 cm). In terms of RN values, 2 different groups took place and Greenhouse-2 media (1.83 roots) was ranked first. In line with all this information, Greenhouse-2 media was identified as the most effective greenhouse media.

In the scope of the study, cuttings were traced from the time they were transferred in the rooting media. The cuttings prepared on 11 March 2016 were planted. The cuttings were inspected every three days. 245 days after the planting (10 November 2016), they were removed from rooting media. The first callus formation of the species occurred in Greenhouse-1 media (in NAA 1000 ppm and NAA 5000 ppm treatments in perlite and peat rooting media), and also in Greenhouse-2 media (in NAA 1000 ppm treatment in peat rooting media), after 33 days from planting. The first root formation took place in NAA 5000 ppm treatment in perlite rooting media of Greenhouse-2 media, 56 days later from planting.

4. DISCUSSION
4. RASPRAVA

The highest rooting percentage in the cuttings taken from European yew (*Taxus baccata* L.) was found as 80% in IBA 5000 ppm treatment in perlite rooting media of Greenhouse-2 media. The highest callus percentage was detected as 96.67% in NAA 5000 ppm treatment in peat rooting media of Greenhouse-3 media. When the root length values were examined, the longest root length occurred with 4,60 cm in IBA 1000 ppm treatment in perlite rooting media of Greenhouse-3 media. The highest number of roots was found in NAA 1000 ppm treatment (4,00 roots) in perlite rooting media of Greenhouse-2 media. In a study investigating the effects of chemicals on root formation for European yew, rooting success was obtained as 55% in cuttings from male trees in the combined treatment of IBA+NAA (0.25 mM each), compared to cuttings taken from female trees (15%). Additionally, Bavistin was highly effective both callus (≥90%) and root formation (80%) (Nandi et al. 1996). In our study, IBA and NAA hormones were more effective when used alone. The important point here is to achieve higher success with lower cost and labor. It should also be noted that high rooting percentage was achieved even in Control treatment (63.33%) without any hormone use when the appropriate temperature (air temperature at 20±2°C, rooting table temperature at 25±2°C) and rooting media (perlite) conditions were set. Aslam et al. (2007) reported that IBA 500 ppm treatment showed the best results in terms of root length, root number, rooting and callus percentage. In another study on the rooting of the cuttings of *Taxus baccata* L., the highest average rooting percentage and the longest root length in cuttings taken on February 5 was found to be 43.30% and cuttings treated with 0.8% IBA, respectively (Çetin, 1991). Anjum et al. (2011) pointed out that IBA treatment was the most appropriate hormone in terms of rooting and callus percentage, the number of roots and root length. This was followed by NAA and IAA treatment. As a result, it was found that the cuttings treated with IBA 500 ppm gave the best results on rooting. There are many more studies provided rooting success using IBA and NAA auxins on cutting propagation (Stumpf et al. 2001; Kalyoncu et al. 2008; Khoshnevis et al. 2008; Vakouftsis et al. 2009; Abu-Zahra et al. 2011; Kara et al. 2011). In one of these, the effects of IBA hormone and wound were investigated. At the end of the study, it was determined that the IBA treatment had a positive effect on rooting (Collado et al. 2010). Şeker et al. (2010) aimed to determine the effects on rooting of IBA, NAA and IBA+NAA hormones in softwood and semi-hardwood cuttings taken at different periods. The highest root number (3.1 roots), rooting (63.3%) and viability (68%) rates were obtained from IBA 6000 ppm treatment. In another study, the effects on rooting of time of taking cuttings, IBA and NAA hormones (0, 2000, 4000, 5000 and 8000 ppm) were researched. According to the results, while the highest rooting percentage (24.4%) was obtained at IBA 8000 ppm treatment, the longest root length and the highest number of adventitious roots was obtained at IBA 5000 ppm treatment (Rizi et al. 2006). New practices must be made by testing different methods in order to determine the most suitable propagation conditions in our country.

5. CONCLUSION
5. ZAKLJUČAK

Since the highest rooting percentage was found as 80% in IBA 5000 ppm treatment in perlite rooting media of Greenhouse-2 media, it can be suggested that the rooting table temperature should be 5ă higher than the air temperature, perlite rooting media and 5000 ppm dosage of IBA hormones should be used for high rooting success in propagation by hardwood cuttings of European yew.

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SAŽETAK

Obična tisa (*Taxus baccata* L.), prirodno rasprostranjena u sjevernoj i srednjoj Europi, mediteranskim zemljama, Azorima, Turskoj i Kavkazu, ima vrlo široku primjenu kao nedrvni šumski proizvod. S obzirom da je tisa u prirodi reducirana zbog učestalog korištenja, potrebno ju je zaštititi i razmnažati. Istraživali smo utjecaj različitih stakleničkih supstrata (SS), supstrata za ukorjenjivanje (SU) i hormona (H) na razmnožavanje obične tise reznicama. Za svrhu eksperimenta u ovoj su studiji odabrana tri staklenička supstrata (supstrati Staklenika-1 s temperaturom zraka od 20±2°C, temperaturom stola za ukorjenjivanje od 20±2°C, supstrati Staklenika-2 s temperaturom zraka od 20±2°C, temperaturom stola za ukorjenjivanje od 25±2°C te supstrati Staklenika-3 bez temperaturnih prilagodbi). Dva supstrata za ukorjenjivanje (perlit i treset) i četiri hormona (IBA 1000 ppm, IBA 5000 ppm, NAA 1000 ppm and NAA 5000 ppm). Određeni su postotak zakorjenjivanja (PZ), postotak kalusa (PK), duljina korijena (DK) i broj korijena (BK). Rezultati pokazuju da je najviši postotak ukorjenjivanja bio 80% u tretmanu IBA 5000 ppm te perlitu kao supstratu za ukorjenjivanje u supstratu Staklenika-2. Poželjno je da temperatura stola za ukorjenjivanje bude 5°C viša od temperature zraka, a za visok uspjeh zakorijenjivanja treba koristiti perlit i primjenjivati doze IBA hormona od 5000 ppm.

KLJUČNE RIJEČI: *Taxus baccata*, razmnožavanje reznicama, supstrati za staklenike, supstrat za ukorjenjivanje, auksin