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EFFECTS OF DIFFERENT SUBSTRATES AND IBA CONCENTRATIONS ON ADVENTITIOUS ROOTING OF NATIVE *Vitex agnus-castus* L. CUTTINGS

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Abstract. *Vitex agnus-castus* L. (chaste tree) is a native plant species carrying valuable ornamental, functional and medicinal properties. It was aimed to determine the most practical and optimum rooting conditions for successful vegetative production of *V. agnus-castus*. It was focused on definition of optimal cutting collection time, and specification of the effects of different rooting substrates and IBA concentrations on rooting abilities and growth of *V. agnus-castus* cuttings. Two rooting experiments (Exp.) were performed; at the beginning and end of its deciduous season. Sand and sand:sphagnum peat (1:1 v/v) mixture were tested as substrate in Exp. I, and perlite in addition to former substrates in Exp. II. The hardwood stem cuttings were treated with different concentrations of IBA in both experiments. The results of Exp. II indicated both substrate and concentration, and also their interaction had significant effects on adventitious rooting formation. In addition, it was revealed the maximum rooting ratio (100%) and the most vigorous growth generated in *V. agnus-castus* cuttings collected at the end of its deciduous season and treated IBA concentrations in perlit medium.

Key words: Auxin, cutting, domestication, ornamental plant, substrate, *Vitex agnus-castus*

INTRODUCTION

An ascending tendency for the native plant use in recent landscape plannings has drawn the attention. This steadily growing interest has encouraged the studies on domestication and breeding of native plants in many countries. In particular, the large-scale studies supported by the governments in USA, Australia and Brazil are significantly remarkable. Beside the exploration and domestication researches, the number of the survey studies on native plant use [Waterstrat 1997, Potts et al. 2002, Helfand et al. 2006, Brzuszek et al. 2007, 2010, Brzuszek and Harkess 2009, Ari et al. 2010, Humair et al. 2014] has also

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being increased. They were performed mainly to explore the market value and outlook of landscape sector among the consumer, producer and professionals orienting the market.

In a previous local questionnaire study, Ari et al. [2010] also tried to determine the perspective and inclinities of the representatives of landscape sector in Antalya, Turkey, for the use of native plants. Their findings were just about the same with those of Brzuszek et al. [2007 and 2010] and Brzuszek and Harkess [2009]'s surveys' in terms of the preference reasons for native plant use and the expectations from the sector. In addition, *Vitex agnus-castus* and *Erica manipuliflora* were identified as the most desired native plant species in the study of Ari et al. [2010].

V. agnus-castus L. (chaste tree) (moved from Verbenaceae to Lamiaceae) [Judd et al. 2002, Hershberger 2010] originated from arid and semi arid regions of Mediterranean and Western Asia [Schopmeyer 1974, Dogan et al. 2011] up to 1200 m [Boissier 1963]. It is widely distributed throughout the coastal lane of Anatolia [Davis 1982] in Turkey. Southern Anatolia, particularly the east coast of Antalya Province, has extremely rich native populations of *V. agnus castus* [Karaguzel and Girmen 2009]. It is a deciduous shrub/tree species growing up to 2–4 m. Raceme type inflorescence shows the colors varying from white to lavender blue including also the shades of pink from June to September [Dirr 1990]. It has been used mainly for treatment of premenstrual and menopausal problems since the ancient times because of valuable bioactive compounds of fruits [Milewicz et al. 1993, Hobbs 1996, Lucks et al. 2002, Kuruüzüm-Uz et al. 2003, Cossuta et al. 2008].

V. agnus castus carries multiuse plant properties. Besides being an important medicinal-aromatic plant, it carries also ornamental value for outdoor use. It has advantage to be used in hot summer climate conditions as in Antalya and other regions under the Mediterranean conditions. Some wild accessions have superior functional characteristics important for landscape use such as resistance/tolerance to drought, saline and poor soil conditions. Travlos [2009] declared *V. agnus-castus* is quite favoured for some rehabilitation programs of degraded lands [Hirobe et al. 1997] since it can grow in poor soils and dry regions [Miyamoto 2008]. In addition, it was included in the list of enduring plants for xeriscape-type landscapes by Wade et al. [2010].

In order to supply this desired native plant species in large quantities, the production systems should be well documented. It is possible to produce *V. agnus-castus* by both generative and vegetative methods. For the generative production, Belhadj et al. [1998] and Travlos and Karamanos [2007] reported its seeds had certainly physiological dormancy. However, Ari et al. [2014] obtained very good germination ratios ranging from 58 to 96% without any pretreatment as suggested by Dirr and Heuser [1987]. Even though germination of *V. agnus-castus* seems not very arduous, the germination ratios are not stable and generally non-repeatable. Also, the seed-produced individual plants will show segregation in their phenotypes and ingredients carrying medicinally economic importance. In contrast, the vegetative production methods can provide the mass clonal plant production which is important in particular for the reproduction of individual plants with superior plant characteristics, and also the mutant genotypes.

The adventitious rooting (AR) of the cuttings is the most widely used method in vegetative production. Many factors have effect on the success ratio of AR such as rooting substrates, exogenous plant growth regulator (PGR) type and concentrations, cutting properties and preparation beside the environmental conditions. Substrate is one of the most important factors among them. Besides holding the cutting in place, a good substrate should provide the correct degree of moisture to the cutting base while allowing aeration [Hartmann et al. 2010, Fornes et al. 2013]. On the other hand, the AR formation has been shown to correlate closely with endogenous auxin concentrations in several studies [Haissig 1970, Eliasson and Areblad 1984, Guerrero et al. 1999, Tworkoski and Takeda 2007]. Exogenous auxins also play a very big role in determination of rooting capacity [Kotis et al. 2009, Ling et al. 2013]. Indole-3-butyric acid (IBA) is the most common used synthetic PGR due to higher root inducing capability owing to its greater stability to light than others [Kurepin et al. 2011, Pacurar et al. 2014].

Even though it is possible to encounter with a very general statement saying "the vegetative production of *V. agnus-castus* is easy", the number of the detailed studies in literature seems rather limited. Moura et al. [2009], Coelho et al. [2011] and Alkurdi et al. [2013] set up some experiments for its vegetative propagation. All of these studies were carried out in different dates and rooting media such as soil, cattle manure, compost, sand, river sand, peat most and different mixture of them, but without any PGR use.

The target was to establish the most effective and practical vegetative production method for *V. agnus-castus* in order to provide its successful nursery productivity. For the purpose, it was focused in this study; i) to identify the optimum time of cutting collection, ii) to determine the effect of different rooting substrates and IBA concentrations on AR abilities and growth of *V. agnus-castus* cuttings.

MATERIAL AND METHOD

Plant material. The present study was carried out in Batı Akdeniz Agricultural Research Institute (BATEM) in Aksu, Antalya (lat. 36°56'N, long. 30°53'E), Turkey, during 2007–2008, under the project of 106G020, sub project of KAMAG-105G068, supported by TUBITAK. The donor plants of *V. agnus-castus* cuttings were located in Gazipaşa (lat. 36°08'N, long. 32°26'E). Both Aksu and Gazipaşa are districts of Antalya and under Mediterranean conditions with dry, hot summers and mild, wet winters. The climatic parameters of the months in which cuttings were collected for rooting experiments were presented in Table 1. The soil type of donor plants was clay loam (Paralithic Xerortent) with 2% $CaNO_3$ ingredient, 1.7% organic matter content, electrical conductivity (EC) of 59 µS cm^{-1}, pH 7.6, available phosphorus (P) level of 5 mg kg^{-1}, changable potassium (K) level of 123 mg kg^{-1}, changable calcium (Ca) level of 5628 mg kg^{-1} and changable magnesium (Mg) level of 191 mg kg^{-1}.

Table 1. The meteorological data of the months in which cuttings were sampled for rooting experiments of *V. agnus-castus*

	Gazipaşa/ Antalya		
	October 2007*	November 2007	February 2008
Mean minimum air temperature (ºC)	19.0	14.3	8.0
Mean maximum air temperature (ºC)	28.2	22.1	16.5
Mean air temperature (ºC)	22.9	17.4	11.8
Mean relative humidity (%)	65.6	66.6	65.5
Mean maximum precipitation (mm)	6.8	41.8	54.6
Mean total precipitation (mm)	9.3	189.1	85.5
Mean wind speed ($m.s^{-1}$) precipitation	1.7	1.8	1.9

* – meteorological data of October 2007 were given in order to show at least one-month-data since the Exp. I was set up at the beginning of November 2007

Rooting experiments. Two experiments (Exp.) were performed in *V. agnus-castus* to detect the optimal cutting collection time and to compare the effect of different substrates and IBA concentrations on rooting and growth of the cuttings. The hardwood cuttings were supplied from two vigorous mature trees well branched in Gazipaşa at the beginning (Exp. I) and end (Exp. II) of the deciduous season. For each experiment, only one donor plant used in order to eliminate the genotype effect. The deciduous long shoot cuttings were cut, agglomerated and preserved in damp clothes in sacks one day before the experiment. During the trial installations, they were shortened and cleaned with the sharp pruning shears. Meanwhile, it was tried to prepare homogeneous cuttings in 1 cm diameter, 20 cm length and with 2–3 nodes as much as possible. However, the cuttings ranging from 0.7 to 1.5 cm in diameter and from 15 to 23 cm in length were also had to be used due to the necessity for high number of cuttings from a single plant. 3–4 cm basal ends of the cuttings were quick dipped into different IBA (Merck, Germany), dissolved in 96% ethanol (Merck), concentrations for 10 s, while the control cuttings were immersed in distilled water. After air dried ≈15 min, cuttings of each treatment were inserted in two rows in approximately 5 cm depth into the rooting benches containing different growing substrates in a glasshouse. Rooting benches were bottom heated at 21°C in Exp. II. The cuttings were irrigated with intermittent misting system controlled with a timer. Mist cycles were regulated based to changing climatic conditions within glasshouse and cuttings' need. No fertilizer was applied to the cuttings.

Both experiments were planned in a completely randomized design in changing numbers of factorial arrangements. The survived and rooted cuttings were counted at the end of the experiments. In addition, other important parameters indicating root and shoot growth of the cuttings were recorded. These parameters included fresh and dry root weights and densities of shoots and roots. Of the two rows with 15 cuttings in each, only rooted cuttings of the second rows were used for root weighting. Dried weights were determined after exposing roots to 70°C for 48 h in a chamber (Memmert, Germany). Under "density" title, the shoot and root developments were rated based on a 1–4 visual rating scale, where 1 = poor, 2 = medium, 3 = good, 4 = very good, for evaluation of cutting growth.

Experiment I. In this initial experiment, the hardwood cuttings were treated with 0, 3000 and 6000 mg l^{-1} IBA concentrations. Then, they were stuck in two growing substrates; sand (1–3 mm coarse river sand) or the mixture of sand and sphagnum peat (Klasmann-Deilmann GmbH, Germany), which was referred as peat henceforth, (1:1 v/v) in unheated condition on 2 November 2007. Peat was not used alone due to its inefficiency observed in our previous work. The experiment was set up in a 2 × 3 (substrate × concentration) factorial design with 3 replications, 30 cuttings in each (a total of 540 cuttings). The cuttings of this experiment were harvested on 02 June 2008 for the observation.

Experiment II. This modified experiment was based on the growth performances of the cuttings in Exp. I. The hardwood cuttings having the shoot buds up to burst were treated with 0, 2500 and 5000 mg l^{-1} IBA concentrations, then inserted in one of three growing media; sand, the mixture of sand and peat (1:1 v/v) or perlite (Etiper, Turkey) in heated condition on 28 February 2008. The bottom heating was ceased in the third week of March 2008 due to the increasing air temperature. This experiment was maintained in a 3 × 3 (substrate × concentration) factorial design with 3 replications, 30 cuttings in each (a total of 810 cuttings). The cuttings of this experiment were evaluated on 12 May 2008.

Statistical analysis. Data of the experiments were first subjected to Levene's test to determine homogeneity of the variances. For the homogeneous data, two-way and also one-way, if required, variance analysis (ANOVA) were performed by GLM (general linear model) procedures to test treatment differences, then means were separated by Tukey's multiple range test using SPSS software (IBM, USA) at an alpha 0.05 level. For the nonhomogeneous survival and rooting percentages of the cuttings in Exp. II, the ratios were transformed prior to analysis using arcsine of the square root to raise homogenity. However, the reported means were based on nontransformed data.

RESULTS

Experiment I. There could not be found any significant difference either within substrates or IBA concentrations, and also interaction between both factors in two-way ANOVA except for fresh (p = 0.008) and dry root (p = 0.015) weights. In terms of mean values, 90.4% viability and 58% rooting were obtained irrespective of rooting substrates. On the other hand 90.3% viability and 58% rooting were determined regardless of IBA concentrations (data not shown). However, the highest rooting ratio (68.9%) and the most fresh (8.3 g) and dry root (1.2 g) weights were achieved from the cuttings treated with 6000 mg l^{-1} IBA in sand medium (tab. 2). The best root (3.2) and shoot (2.3) growth regarding density based on a 1–4 visual rating scale happened in the cuttings treated with 3000 mg l^{-1} IBA in sand:peat (1:1).

Table 2. Effect of different IBA concentrations on survival and rooting ratios, and some growth parameters of *Vitex agnus-castus* cuttings in two substrates of Experiment I[1]

Substrate	IBA (mg l^{-1})	Viability (%)	Rooting (%)	Density[2]		Root weight (g)	
				root	shoot	fresh	dry
		M ±SE	M ±SE	M ±SE	M ±SE	M ±SE	M ±SE
Sand	control	84.4 ±2.9	47.8 ± 9.5	2.6 ±0.1	1.9 ±0.2	3.4 ±0.9	0.4 ±0.2
	3000	90.0 ±5.8	62.2 ±20.6	2.4 ±0.4	2.2 ±0.4	7.7 ±5.1	1.1 ±0.7
	6000	94.4 ±2.9	68.9 ±15.5	3.0 ±0.3	2.2 ±0.3	8.3 ±4.5	1.2 ±0.7
Sand:peat (1:1 v/v)	control	96.7 ±1.9	53.3 ± 3.3	2.6 ±0.2	2.0 ±0.2	0.5 ±0.3	0.1 ±0.0
	3000	88.9 ±4.0	61.1 ±12.5	3.2 ±0.4	2.3 ±0.4	5.4 ±4.0	0.9 ±0.7
	6000	87.8 ±2.9	54.4 ± 4.0	2.6 ±0.2	2.2 ±0.1	3.0 ±0.8	0.6 ±0.2

[1] Values are mean (M) and standart error (SE). Means were found non-significant at $p \leq 0.05$ in a previously applied two-way ANOVA for the statistical comparison
[2] These data were obtained based on a 1–4 visual rating scale (1 – poor, 2 – medium, 3 – good, 4 – very good)

Experiment II. The two-way ANOVA indicating variances were significantly different among overall substrates and IBA concentrations for all observation parameters including viability and rooting percentages, shoot and root densities and fresh and dry root weights (tab. 3). Also, there were significant interactions for rooting ratio, shoot density, both root weights between substrates and IBA concentrations.

Table 3. Effects of different substrates and IBA concentrations on survival and rooting ratios and some growth parameters of *Vitex agnus-castus* cuttings in Exp. II

		viability (%)	rooting (%)	Density[1] shoot	Density[1] root	Root weights (g) fresh	Root weights (g) dry
		M ±SE	M ±SE	M ±SE	M ±SE	M ±SE	M ±SE
Substrate	sand	97.8 ±1.0 a[2]	43.7 ±8.8 c	1.9 ±0.1 b	2.2 ±0.1 b	2.0 ±0.5 b	0.4 ±0.1 b
	sand:peat (1:1)	94.1 ±1.8 b	51.5 ±6.8 b	1.8 ±0.1 b	2.4 ±0.2 ab	7.3 ±2.9 a	1.6 ±0.6 a
	perlit	100.0 ±0.0 a	91.9 ±4.7 a	2.6 ±0.2 a	2.8 ±0.2 a	4.4 ±1.4 ab	0.8 ±0.2 ab
IBA (mg l^{-1})	control	94.8 ±2.1 b	40.1 ±10.1 b	1.7 ±0.1 b	2.0 ±0.2 b	1.0 ±0.3 b	0.2 ±0.1 b
	2500	99.3 ±0.5 a	71.8 ± 8.2 a	2.2 ±0.1 a	2.5 ±0.1 a	3.3 ±0.3 b	0.7 ±0.1 b
	5000	97.8 ±0.8 ab	75.2 ± 7.3 a	2.5 ±0.2 a	2.9 ±0.2 a	9.2 ±2.7 a	1.8 ±0.6 a
Significance	substrate (S)	0.002**	0.000***	0.000***	0.007**	0.020*	0.019*
	IBA concentr. (C)	0.014*	0.000***	0.001***	0.000***	0.000***	0.002**
	S × C	0.125NS	0.002**	0.047*	0.456NS	0.026*	0.050*

[1] These data were obtained based on a 1–4 visual rating scale (1 – poor, 2 – medium, 3 – good, 4 – very good)
[2] Values are mean (M) and standart error (SE). Two-way ANOVA was used for the comparison of the means and they were separated by using Tukey's multiple range test. Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.
NS: Nonsignificant; *, **, *** – significant at $p \leq 0.05$, 0.01, 0.001, respectively

As seen in Table 3, mean differences for rooting ratio among both growing substrates and IBA concentrations were very significant ($p < 0.001$). The interaction between these factors was also found highly significant ($p < 0.01$). The cuttings in perlite exhibited the superior rooting ratio (91.9%) than that of in sand:peat (51.5%) and sand (43.7%). IBA treatments made difference (75.2 and 71.8% for 5000 and 2500 mg l^{-1} IBA, respectively) than the control cuttings (40.1%).

The more vigorous shoot and root development are the important indicators of the cutting growth. These indicators were represented by the phrases "shoot and root density" in the work and the highest values of them formed in the cuttings in perlite (2.6 and 2.8, respectively, over 4). The IBA treated cuttings also presented higher shoot and root densities than the control treatment. The differences of the shoot density within the growing substrates and IBA concentrations were found very significant ($p < 0.001$). Also, the interaction between them was detected significant ($p < 0.05$). On the other hand, the root density differences among the substrates and IBA concentrations were quite significant ($p < 0.01$ and $p < 0.001$, respectively) while there was no interaction between two factors.

In terms of root weights, substrates and interaction between substrate and PGR concentration exhibited significant difference ($p < 0.05$) for both fresh and dry weights. In addition, there was significance among IBA concentrations for both root weights. The cuttings in sand:peat showed higher fresh and dry root weights (7.3 and 1.6 g, respectively) than cuttings in sand or perlit. The 5000 mg l^{-1} IBA treated cuttings exhibited superior root weights (9.2 for fresh and 1.8 g for dry weight) than others.

Since the interaction between substrates and IBA concentrations was found significant ($p < 0.01$), separate one-way ANOVA were made to demonstrate the difference between IBA concentrations for each substrate in terms of rooting performance of the cuttings. The results of them were presented in Fig. 1.

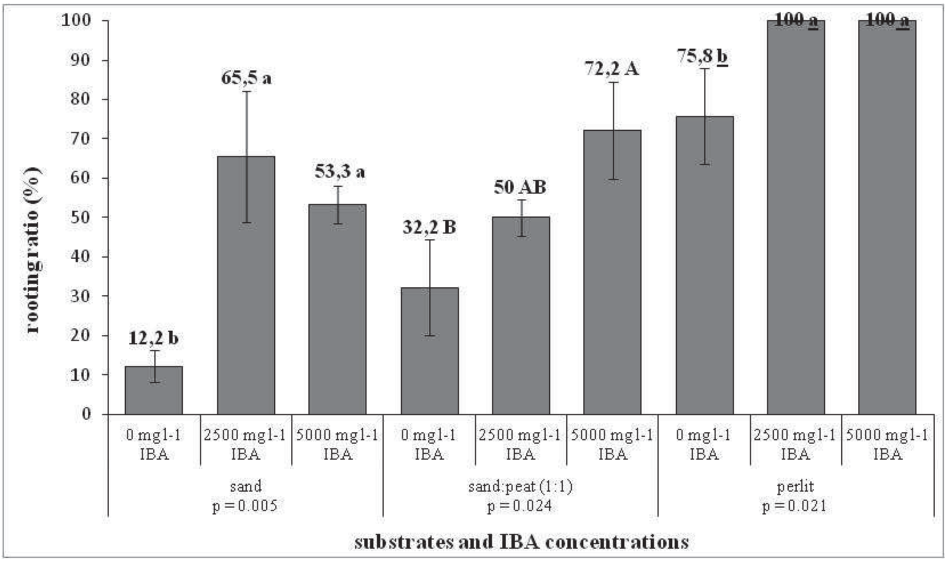

Fig. 1. Effects of different IBA concentrations in each substrate on adventitious rooting of *Vitex agnus-castus* cuttings in Exp. II (Values are mean (M) and ±standard error (SE). Comparison of the means in each substrate was estimated by separate One-Way ANOVA, then TUKEY post hoc test. Means within each substrate followed by the same letter are not significantly different at $p \leq 0.05$)

The highest rooting ratio (100%) formed in the cuttings inserted in perlite media and treated both 2500 and 5000 mg l^{-1} IBA. The IBA treated cuttings in the substrates of sand and sand:peat also presented higher rooting performances than the control treatments. Therefore, it is obvious that IBA treatments created significant positive effect on the rooting ratios of the cuttings in all substrates while there could not be found significant difference between the IBA concentrations. On the other hand, changing responses of the cuttings inserted in different substrates to increasing IBA concentrations regarding the shoot and root densities and both fresh and dry root weights (g) were presented in Fig. 2.

As indicated in Fig. 2, it seems like the cuttings in almost each substrate showed gradually increased positive reaction to the gradually increased IBA concentration for all cutting growth parameters, except shoot density of the cuttings in sand medium. The highest shoot and root densities (3.22 and 3.24, respectively) were achieved from the cuttings treated with 5000 mg l^{-1} IBA in perlite. However, the most fresh (17.02 g) and dried root (3.46 g) weights were also obtained from the same concentration IBA treated cuttings, but in sand:peat medium. Consequently, it might be possible to say 5000 mg l^{-1} IBA concentration stimulated the most vigorous growth in the *V. agnus-castus* cuttings especially inserted in perlite medium and the highest root weights in sand:peat mixture.

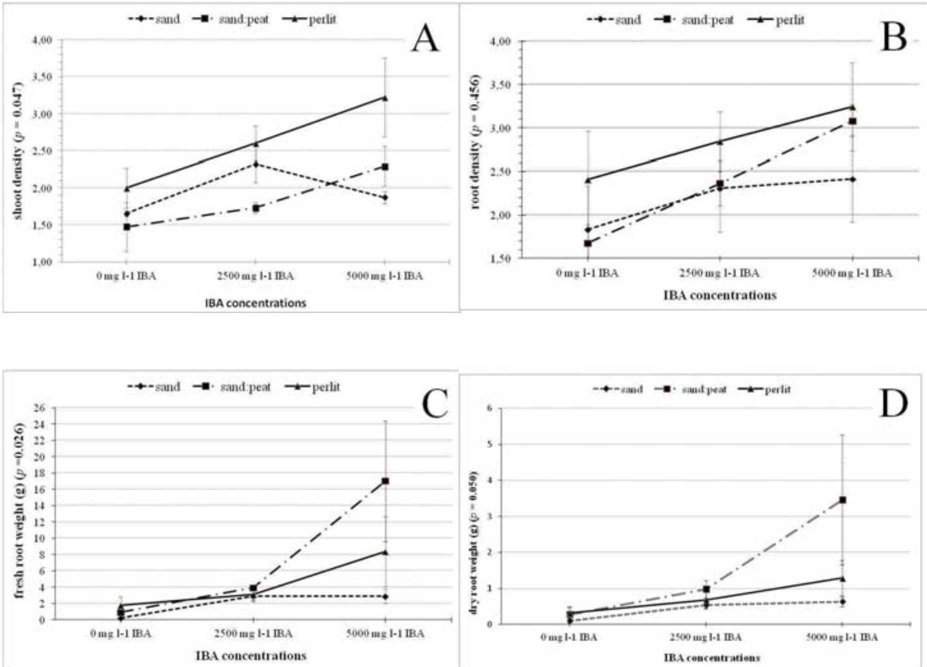

Fig. 2. Differences in the response of *Vitex agnus-castus* cuttings inserted in three rooting substrates (sand, sand:peat (1:1 v/v) and perlit) in Exp. II to increasing IBA concentrations (0.2500 and 5000 mg l^{-1}) for (A) shoot density, (B) root density, (C) fresh root weight (g), (D) dry root weight (g). Shoot and root densities were obtained based on a 1–4 visual rating scale (1 – poor, 2 – medium, 3 – good, 4 – very good). Vertical bars indicate ± SE of the means

It was tried to use the homogeneous cutting thickness mostly in ≈1 cm diameter as well as a range between 0.7–1.5 cm in this work due to the requirement for high number of cuttings from one plant. As an important observation on this feature, it was seen that the best rooting and cutting growth generated in the cuttings in 1 cm thickness (Fig. 3).

DISCUSSION

The results of the present work reveal that it is possible to obtain maximum rooting ratio (100%) in *V. agnus-castus* by collecting the cuttings in appropriate time and culturing them in the suitable substrate and IBA concentrations. The cuttings cultured in February 28 (Exp. II) showed better performance in terms of all observation parameters than those of in November 2 (Exp. I). 100% maximum rooting ratio versus 68.9% formed in the hardwood cuttings cultured at the end of the deciduous season of *V. agnus-castus* in Exp. II against that of at the beginning of the season in Exp. I. The effect of seasonal differences derived from climatic conditions and/or the cutting properties have been shown in different rooting studies by Testolin et al. [1988], Ercişli and

Güleryüz [1999], Guo et al. [2009], Zhang et al. [2009] and Ciubotaru and Roshca [2011] for *Prunus persica*, *Rosa* spp. (rose hips), *Paeonia* 'Yang Fei Chu Yu', *Feijoa sellowiana* and *Thuja occidentalis* 'Danica', respectively. Among the limited literature on *in vivo* adventitious rooting of *V. agnus-castus,* Alkurdi et al. [2013] set up two experiments on January 12 and 26 May to compare the effect of different culturing time, substrate and cutting type. The culture conditions and observation parameters of their study are quite different than those of present study. They used soft and semi-hardwood cuttings without any PGR treatment. Also, their observation criteria did not include rooting ratio (%). However, they determined the increase in number of roots and root length on January 12 and in number of branches and leaves on 26 May.

Fig. 3. Different stages in the rooting experiments of *V. agnus-castus* cuttings. a – the beginning of Exp. II, b – towards the end of Exp. II, c – a 17-days-control cutting in perlit, d – the rooted cuttings inserted in perlit and treated with 5000 mg l^{-1} IBA in Ex. II, e – rooted cuttings in 1 cm diameter, f – vegetatively propagated a *V. agnus-castus* plantlet through the work

In terms of cutting properties, it was reported the higher amount of endogenous auxin in cuttings enabled higher rooting ratio [Haissig 1970, Tworkoski et al. 2006, Geiss et al. 2009]. Tworkoski et al. [2006] found that endogenous IAA levels declined in peach stems after 104 days following bud break and suggested that the cuttings should be collected in peach, and possibly in other plant species, before significant decline occur, shortly earlier in the growing season [Tworkoski and Takeda 2007]. As well as the higher endogenous hormone, the amount of carbohydrate also plays an important role for rooting. Carbohydrates modulate gene expression [Gibson 2005] and interact strongly with plant hormone signaling [Leon and Sheen 2003, Gibson 2004]. Geiss et al. [2009] reported that AR depends on the sufficient amount of carbohydrates in the root regeneration zone, where they can stimulate the root initiation and development [Li and Leung 2000, Calmar and de Klerk 2002]. Beside the higher endogenous auxin content, the more carbohydrate ingredient of the cuttings at the end of the deciduous season is a predictable situation for the cuttings bracing theirselves to a new growing season. The leafless hardwood cuttings were used in both *V. agnus-castus* rooting experiments in the present study. But, the cuttings collected in February 28 had the shoot buds, which would open after 2–3 weeks later. Thus, the reason of the more successful rooting and the more vigorous cutting growth in Exp. II might be mainly attributed to the appropriate cutting collection time, which is the end of its deciduous season.

One another important cutting property for a good AR is the cutting size. In *V. agnus-castus*, Moura et al. [2009] and Coelho et al. [2011] used the cuttings with 2–4 mm and 3–5 mm in diameter, respectively, for their rooting studies. Even though the cutting length was 20 cm in both studies as in this work, their cuttings seem much thinner than that of the present work in which the general cutting thickness was ≈ 1 cm. The wider cutting diameter up to a certain degree might provide better water and nutrient uptake from the substrate, thus more cutting survival and consequently more vigorous rooting and cutting growth. The highest cutting survival ratios were 60 and 31.2% for the shoot cuttings in 20 cm length and the shoot minicuttings in 3–5 cm in the study of Coelho et al. [2011], which also affected the rooting success and cutting growth. However, the lowest cutting survival ratios of the present work were 84.4% in sand medium and 88.9% in sand:peat in the Exp. I and Exp. II, respectively. The highest survival ratio of the cuttings was 100% in all treatments of the perlit medium including control in Exp. II. Hence, the use of cutting in ≈1 cm thickness is recommended for more survival and successful rooting of *V. agnus-castus* cuttings.

In terms of the effect of different rooting substrates used in the present study, there could not be found any significant difference in Exp. I within two substrates (sand and sand:peat), and also interaction between substrates and IBA concentrations used. In contrast, variances and mean differences were significantly different among three substrates for all observed parameters in Exp. II, and also interactions with IBA concentrations for some parameters including rooting ratio ($p < 0.001$). The cuttings in perlite showed the superior rooting performance than those of in sand:peat and sand alone. The superiority of pure perlite use for cutting rooting has been revealed also in different plant species such as *Arbutus andrachne* [Al-Salem and Karam 2001], *Clematis* section Atragene [Kreen et al. 2002], and *Euphorbia* × lomi Thai hybrids [Fascella and Zizzo 2009]. The reason for positive effect of perlite on rooting is likely about physical characteristics of the perlite. Tilt and Bilderback [1987] reported particle size of the media was found less decisive than air and water content for rooting performance in various studies. Meldrum [1979] had also stated there was a direct relationship between rooting and the aeration of media. In addition, Gislerod [1983] explained the probable reason for positive effect of perlite on *Poinsettia* cutting rootings as the increased air content and oxygen diffusion rate. On the other hand, perlit might supply more repeatable rooting results due to its inorganic structure compared to other organic substrates such as peat, compost, etc.

In perlite medium of Exp. II, IBA treatments made bigger difference than the control treatments. The highest rooting ratio (100%) formed in the cuttings treated both 2500 and 5000 mg l^{-1} IBA. In addition, the highest shoot and root densities and both root weights were achieved from the cuttings treated with 5000 mg l^{-1} IBA. As in perlite, the IBA treated cuttings in the substrates of sand alone and sand:peat also performed higher rooting performances than the control treatments. Therefore, it is obvious that IBA treatments created significant positive effect on AR of *V. agnus-castus* cuttings in Exp. II. The stimulator effect of IBA use on AR has also been reported in different ornamental and woody plant species such as *Prunus cerasus* [Eşitken et al. 2003], Paeonia 'Yang Fei Chu Yu' [Guo et al. 2009], *Prunus persica* [Tworkoski et al. 2006], *Cotinus coggygria* [Pacholczak et al. 2013], and *Teucrium fruticans* [Sabatino et al. 2014]. It is known that exogenously applied auxins, mostly IBA among the available ones, have presumable and consistent influence in initiating AR [Kurepin et al. 2011, Pacurar et al. 2014].

When the rooting performances of the overall control treatments in both experiments were evaluated, it was seen that the highest control rooting (75.8%) generated in perlit medium in Exp.II. This ratio is higher than the best rooting ratio (68.9%) of IBA applied treatments in sand media of Exp. I. Also, it was observed that AR initiated in the second week in control cuttings inserted in perlit in Exp. II. These results show that the use of IBA is not compulsory for rooting of *V. agnus-castus* cuttings, but it has an enhancer effect on maximum rooting up to 100%, especially in perlit medium. Thus, it might be expressed that it is likely to start rooting of the *V. agnus-castus* cuttings up to 76% without any PGR application as long as they were collected in appropriate time and cultured in perlit medium.

CONCLUSION

The hardwood stem cuttings collected at the end of the deciduous season are the best production material for vegetative propagation of *V. agnus-castus* through adventitious rooting. The perlite medium to be the best substrate and the IBA concentrations between 2500–5000 mg l^{-1} as the suitable IBA concentrations generated 100% rooting ratio and most vigorous cutting growth. It is thought that these conditions might supply the repeatable and successful nursery productivity for *V. agnus-castus*.

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WPŁYW RÓŻNYCH PODŁOŻY I STĘŻEŃ IBA NA PRZYPADKOWE UKORZENIENIE RODZIMYCH SADZONEK *Vitex agnus-castus* L.

Streszczenie. *Vitex agnus-castus* L. (niepokalanek pospolity) jest rodzimym gatunkiem roślin o wartościowych dekoracyjnych, funkcjonalnych i medycznych właściwościach. Celem badań było określenie najbardziej praktycznych i optymalnych warunków ukorzeniania dla udanej produkcji *Vitex agnus-castus* L. Skupiono się na określeniu optymalnego czasu zbioru sadzonek oraz wpływu różnych podłoży i stężeń IBA na zdolności ukorzeniania i rozwoju sadzonek *Vitex agnus-castus* L. Przeprowadzono dwa doświadczenia z ukorzenianiem: na początku i na końcu sezonu liściastego. W Dośw. I jako podłoże przetestowano mieszankę piasek i piasek: torfowiec (1:1 v/v), a w Dośw. II perlit dodany do poprzednich podłoży. Sadzonki pędów zdrewniałych traktowano różnymi stężeniami IBA w obu doświadczeniach. Na podstawie wyników Dośw. II wnioskuje się, że zarówno podłoże jak i stężenie, a także ich interakcja, miały znaczny wpływ na przypadkowe ukorzenienie. Poza tym wykazano, że maksymalny wskaźnik ukorzenienia (100%) oraz najbardziej intensywny wzrost sadzonek *V. agnus-castus* miał miejsce przy końcu pory liści w podłożu z perlitu potraktowanego stężeniami IBA.

Słowa kluczowe: auksyny, sadzonka, udomowienie, roślina ozdobna, podłoże, *Vitex agnus-castus*

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