CONTEMPORARY AGRICULTURE
SAVREMENA POLJOPRIVREDA
The Serbian Journal of Agricultural Sciences
Srpski časopis za poljoprivredne nauke
Vol. 64, No. 3 - 4, Pp. 158 - 163, 2015.
www.contagri.info
ISSN: 0350-1205 UDC: 63(497.1)(051)-"540.2"
University of Novi Sad, Serbia
Published by Faculty of Agriculture, Novi Sad

Original scientific paper **UDC: 631.535**

EFFECTS OF DIFFERENT ROOT GROWTH STIMULATORS ON WEEPING FIG (*FICUS BENJAMINA* L.) 'EXOTICA' PROPAGATION

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Summary:*The most effective method of propagation as well as the optimal content of plant growth regulators and appropriate supplements to apply were determined for Weeping fig (Ficus benjamina L.) 'Exotica', a common woody species grown in indoor conditions. Both, vegetative propagation by cuttings and micropropagation were successfully established in this study. Results of the two-year study on vegetative propagation showed that stem cuttings treated with plant growth regulators containing 0.5% α-naphthalene acetic acid had the greatest percentage of rooting (91.67%). In the study on micropropagation, the effects of cytokinins and activated charcoal on multiplication and rooting were tested. The multiplication faze showed to be the most efficient on MS medium containing 4 mg l^{-1} BA and 1 mg l^{-1} IBA. A higher percentage of rooting (50.3%), root length (27.9 mm) and number of roots (5.4) were obtained in plants grown in medium with activated charcoal. Our study showed that the use of adequate plant growth regulators and appropriate supplements (e.g. active charcoal) positively influences the quality and the rapidity of Weeping Fig (Ficus benjamina L.) "Exotica" propagation.*

Key words: *vegetative propagation; Ficus benjamina L.; micropropagation; plant growth regulators; stem cuttings; active charcoal*

INTRODUCTION

Weeping Fig (*Ficus benjamina* L.) is a woody plant originating from tropical and South East Asia. Extensive worldwide use of this plant is justified by its high aesthetic value and the ability to adapt to interior conditions (Veneklaas et al. 2002). Moreover, it has proved to be very efficient in the elimination of gassy formaldehyde from a confined space (Kwang et al. 2008).

Weeping Fig (*Ficus benjamina* L.) can be vegetative propagated through stem cuttings, as well as via micropropagation.

When it comes to propagation with cuttings, the features of a shoot, as well as the period in which the cuttings are taken, are largely significant (Klein et al. 2000; Swamy et al. 2002; de Andre´s et al. 2004). Also, the plant hormone auxin plays an important role in the rooting of the cuttings. The most important naturally-occurring auxin is indole-3-acetic acid (IAA). In their research, Ford et al. (2001) examined the effectof auxinon the rooting of difficult-to-root (*Syringa vulgaris* L.) and easy-to-root (*Forsythiaintermedia* Zab.) cuttings, and concluded that there

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exists no strong relation between the basal IAA concentration and the rooting ability. However, it has been shown that the difference in polar auxin transport ability has a major influence on rooting in general. According to Balestri and Lardicci (2006), rooting stimulation of difficult-to-root species is achieved via the usage of various hormonal treatments, such as synthetic auxin, indolebutyric acid (IBA) and1-naphthalene acetic acid (NAA). However, cuttings of some species can successfully root without auxin treatment (Grifftih 1998; Hartmann et al. 2002).

Micropropagation is a form of *in vitro* cultivation of allparts of a plant, under sterile conditions. According to Kozai and Kubota (2001), micropropagation represents a modern technology for the production of a large number of genetically superior woody plants, free of viruses, in limited time and space. *In vitro* culture propagation of *Ficus* species has so far been widely studied as an alternative method for mass production of high quality plant material (Rout et al. 2006; Rzepka-Plevnes and Kurek 2001; Dijkshoorn-Dekker 1996; Amo and Picazo 1992).

The aim of this study was to find the optimal method and the fastest way to induce vegetative propagation of Weeping Fig (*Ficus benjamina* L.) "Exotica", grown in indoor conditions.

MATERIAL AND METHODS

The study involved two methods of propagation of Weeping Fig (*Ficus benjamina* L.) 'Exotica': vegetative propagation using stem cuttings and micropropagation.

Stem cuttings of 8-10 cm in length were taken in April 2013 and 2014 from a 30-year-old mother plant. Motherplantgrown intermsof the interiorwithoutlargetemperature variations. The cuttings were submerged in lukewarm water to close conductive bundles and prevent dehydration of cuttings. After preparation, the cuttings were treated with plant growth regulator INCIT 1 (Bioplant) and INCIT 5 (Bioplant) and then put into pots of Ø 7cm. The active ingredient of applied plant growth regulator is α-naphthyl acetic acid (NAA), at a concentration of 0.1% in plant growth regulator INCIT 1, and 0.5% in plant growth regulator INCIT 5. The rooting of cuttings was performed in a greenhouse of the Botanical garden at the Faculty of Agriculture, University of Novi Sad, Serbia (45°14′50″N 19°51′04″E). To achieve a high relative humidity, the smaller plastic tunnel was set just above the cuttings. The air temperature ranged from 22-26° C, while the relative humidity was 80-90%. The sample comprised 20 cuttings with three replications. Control plants grown on the medium without any plant growth regulators amounted to 20 cuttings. The study included two measurements, first of which was performed 30 days after the treatment, and the second one was performed 15 days after the first. The root system was measured using a ruler and graph paper.

The reproduction material from the same mother plant was used for the micropropagation. The shoots tips were cut into short segments, 1-1.5 cm in length, so that each contained a single bud. Afterwards, the sterilization of the plant material, crockery, cutlery and the medium was performed. The basal medium Murashige and Skoog (1962) (MS), supplemented with 3% sucrose was used. For the elongation phase, the MS medium, enriched with 0.2 mg l^{-1} BA and 0,01 mg l^{-1} NAA was used. The medium contained agar at the concentration of 6,5 g l^{-1}, while the pH of the medium was regulated with 1M NaOH or 1M HCL to pH 5,7±0,1. For the multiplication phase, two mediums were tested, both having the same MS mineral content, but different concentration of plant growth regulators. The first contained 8 mg l^{-1} BA and 1 mg l^{-1} IBA, while the second contained 4 mg l^{-1} BA and 1 mg l^{-1} IBA. For the rooting phase, two mediums with halved content of micro and macro salts (1/2 MS) and with the same concentration of plant growth regulators (0,1 mg l^{-1} IBA) were tested. The difference between these two mediums implies the presence of activated charcoal (3 g l^{-1}). The rooted plants were planted in Jiffy pots, followed by gradual acclimatization. The research was conducted in a laboratory for micropropagation at the Department of Pomology, Viticulture, Horticulture and Landscape architecture at the Faculty of Agriculture, University of Novi Sad, Serbia.

The "Steckmedium" (Klasmann) substrate was used for both methods of propagation. Substrate composition contains a mixture of poorly cultivated peat and perlite, pH (H_2O) 5.5-6.5.

The data were analyzed using Statistica 10 software (StatSoft OK, Tulsa), which included statistical analysis calculating the mean values ($\overline{x}$), coefficients of variation (CV) and Duncan multiple intervals test.

RESULTS

The rooting of Weeping Fig (*Ficus benjamina* L.) 'Exotica' cuttings, treated with two phytohormones in controlled microclimate conditions on the appropriate substrate, was followed during a two-year period. During both years of testing, the experiment was performed in the second half of April (Table 1).

Table 1. Mean values ($\bar{x}$) and the coefficient of variation (cv) of the studied parameters of Weeping Fig (*Ficus benjamina* L.) 'Exotica

	Measuring	Treatment	Rooted cuttings (%)		No. of roots		Average root length (mm)		Discarded leaves (%)		No. of new shoots		Wilted cuttings (%)	
			$\bar{x}$	cv(%)	$\bar{x}$	cv(%)	$\bar{x}$	cv(%)	$\bar{x}$	cv(%)	$\bar{x}$	cv(%)	$\bar{x}$	cv(%)
I	I	Control	60.00[a]	7.22	7.00[a]	42.67	7.00[a]	39.14	40.00[c]	10.82	1.00[a]	/	40.00[c]	10.82
		0,1% NAA	83.33[b]	1.00	8.00[a]	10.82	8.00[a]	30.62	16.67[b]	54.83	1.00[a]	/	16.67[b]	54.83
		0,5% NAA	91.67[c]	3.89	20.00[b]	18.87	18.00[b]	7.86	8.33[a]	32.07	1.00[a]	/	8.33[a]	32.07
	II	Control	60.00[a]	7.22	10.00[a]	35.25	15.50[a]	46.63	40.00[c]	10.82	2.00[a]	/	40.00[c]	10.82
		0,1% NAA	83.33[b]	12.00	20.00[b]	13.01	19.00[b]	6.03	16.67[b]	54.83	2.33[a]	21.43	16.67[b]	54.83
		0,5% NAA	91.67[c]	3.89	36.00[c]	8.67	25.43[c]	14.75	8.33[a]	32.07	3.00[a]	/	8.33[a]	32.07
II	I	Control	65.00[a]	7.69	7.00[a]	14.28	7.30[a]	11.11	35.00[c]	14.28	1.00[a]	/	35.00[c]	14.28
		0,1% NAA	81.67[b]	9.35	7.67[a]	7.53	8.00[a]	25.00	18.33[b]	41.66	1.00[a]	/	18.33[b]	41.66
		0,5% NAA	91.67[c]	3.15	21.00[b]	12.60	17.67[b]	3.27	8.33[a]	34.64	1.00[a]	/	8.33[a]	34.64
	II	Control	65.00[a]	7.69	9.00[a]	11.11	15.10[a]	3.31	35.00[c]	14.28	2.00[a]	/	35.00[c]	14.28
		0,1% NAA	81.67[b]	9.35	15.50[b]	39.11	19.38[b]	36.59	18.33[b]	41.66	2.67[a]	21.65	18.33[b]	41.66
		0,5% NAA	91.67[c]	3.15	25.22[c]	18.51	22.67[c]	20.49	8.33[a]	34.64	2.67[a]	21.65	8.33[a]	34.64

* Values marked with different letters are statistically significant at the 0.05 level of significance

From the results obtained, the best rooting was achieved on cuttings treated with plant growth regulator INCIT 5, as confirmed by the Duncan multiple intervals test (Table 1). In both testing years, the percentage of rooting cuttings did not show a statistically significant difference. The rooting percentage (%) of cuttings treated with plant growth regulator INCIT 1 was 83.33% in the first year, and 81.67% in the second year. The rooting percentage (%) of cuttings treated with plant growth regulator INCIT 5 was the same in the first and the second year, and amounted up to 91,67%. Control treatment had a significantly lower rooting percentage in the first, as well as in the second year of study (60 % and 65%, respectively).

The highest number of roots had cuttings treated with plant growth regulator INCIT 5 in the first (20.00) as well as in the second year of study (21.00). Cuttings treated with plant growth regulator INCIT 1 had an average of 8.00 rootlets in the first year, and 7.67 in the second year, while the control cuttings had an average of 7.00 rootlets in the first and the second year. During the second measurement, an increased number of roots in all the cuttings were observed, but the best developed root system was again the one where the cuttings were treated with plant growth regulator INCIT 5 (36.00) in the first, as well as in the second year (25.22).

Cuttings treated with plant growth regulator INCIT 5 had the longest root system in the first (18.00 mm) and in the second year (17.67 mm), followed by the cuttings treated with plant growth regulator INCIT 1 in the first and in the second year (8.00 mm). Cuttings from the control treatment had the shortest root system in the first (7.00 mm) and in the second year (7.30 mm). In the period between the first and the second measurement, the length of the root system had increased in all the treatments. The largest root system was observed in the cuttings treated with plant growth regulator INCIT 5 in the both years, 25.43 mm and 22.67 mm, respectively.

Control treatment had the highest percentage of discarded leaves in the first (40%) and in the second year (35%). Cuttings treated with plant growth regulator INCIT 1 had 16.67% discarded leaves in the first year, and 18.33% in the second year. The lowest percentage of discarded leaves had cuttings treated with plant growth regulator INCIT 5, in both years (8.33%). The percentage of discarded leaves was the same during the second measurement.

For the micropropagation of Weeping Fig (*Ficus benjamina* L.) 'Exotica', the MS mineral media with the addition of different concentrations of plant growth regulators was used.

The successful establishment of aseptic culture where an infection had not occurred was followed by the multiplication phase.

Better effect of multiplication was achieved in the medium which had a lower content of cytokinins. Plants grown on a medium with 4 mg l^{-1} BA and 1 mgl^{-1} IBA had an average of 4 new shoots per explants, while the plants grown on a medium with 8 mg l^{-1} and 1 mg l^{-1} IBA had an average of 2 new shoots per explants. Plants multiplicated on a medium with 4 mg l^{-1} BA and 1 mg l^{-1} IBA were better developed in comparison with plants multiplicated on a medium with the 8 mg l^{-1} BA and 1 mg l^{-1} IBA (Fig. 1 and Fig. 2).

Figure1. Multiplication obtained by medium with 4 mg l^{-1} BA and 1 mg l^{-1} IBA

Figure 2. Multiplication obtained by medium with 8 mg l^{-1} BA and 1 mg l^{-1} IBA

The results indicate that the higher percent of rooted plants was achieved on the medium with activated charcoal (Tab. 2).

Table 2. Influence of activated charcoal on the rooting of Weeping Fig (Ficus benjamina L.) 'Exotica'

IBA mg l^{-1}	AC g l^{-1}	Rooting (%)	Root length (mm)	No. of roots
0.1	/	20.7	7.0^{b}	2.2^{b}
0.1	3	50.3	27.9^{a}	5.4^{a}

* Values marked with different letters are statistically significant at the 0.05 level of significance

In fact, the percentage of rooting in plants grown on the medium without activated charcoal amounts to approximately 20.7%, while the percentage of rooting in plants grown on the medium with activated charcoal amounted approximately 50.3%.

Plants grown in a medium without activated charcoal had an average root length of 7.0 mm, and the plants grown in a medium with activated charcoal had an average root length of 27.9 mm. Also, the number of roots was significantly higher in plants grown in a medium with activated charcoal and amounted approximately 5.4, while the number of roots in plants grown in a medium without activated charcoal amounts to approximately 2.2.

DISCUSSION

Growth regulators are mostly being applied to the basal part of the cuttings, using liquid or powder formulations of auxin.

Researches, Blythe et al. (2004) examined the effect of basal and foliar application of auxin on the rooting of terminal cuttings of *Aglaonema modestum* Schott., *Ficus benjamina* L., *Gardenia augusta* Merr. and *Hedera helix* L. The results of their study showed that foliar application of auxin does not have major influence on the rooting of

Weeping Fig (*Ficus benjamina* L.), while the treatment of the basal part of cuttings showed to be significantly more efficient.

The results of our study showed that plant growth regulators INCIT 1 and INCIT 5 have a positive impact on the root system development. The best rooting was obtained in cuttings treated with plant growth regulator INCIT 5, which implies the longest roots with the highest number of roots and the lowest percentage of wilting.

The same results were obtained in the second year of study. Synthetic auxin α-naphthyl acetic acid (NAA) had a positive effect on the root system formation of Weeping Fig (*Ficus benjamina* L.) 'Exotica'. This is also in direct correlation with the period of time needed for the rooting of Weeping Fig (*Ficus benjamina* L.) 'Exotica' cuttings. Cuttings treated with plant growth regulator INCIT 5 were the first to form callus and the roots as well.

Results in this study have also shown that micropropagation of Weeping Fig (*Ficus benjamina* L.) 'Exotica', with the use of an adequate medium and the application of appropriate concentrations of plant growth regulators, can give a large number of plants in a short period of time. The first phase of micropropagation is very important due to its function to establish a sterile *in vitro* culture. In our study, infection had not occurred during the process of plant implementation into the culture, primarily due to a high-quality herbal material that was previously kept indoors, in addition to a well-executed sterilization.

The successful establishment of aseptic culture, as well as their elongation, was followed by the multiplication phase. At this stage, better reproduction was achieved on the medium with the lower content of cytokinins, which does not coincide with the results of Al Malki and Elmeer (2010). This indicates that each genotype corresponds to a different composition of the medium and plant growth regultators.

Since the primary functions of the root system are the absorption of water solution and anchorage of plants to the substrate, it is essential that plants have a well-established root system. In the rooting stage, the presence or absence of activated charcoal in the medium significantly affects the formation of the root system. Effects of activated charcoal can usually be attributed to the establishment of a darkened environment, the adsorption of undesirable substances, adsorption of growth regulators and other organic elements (Pan and van Staden, 1998). In the plants grown on a medium without activated charcoal, the root system formed directly from callus. These plants are characterized by a limited flow of nutrients from roots to the aboveground part, due to nutrient retention in the callus. In plants grown on a medium with activated charcoal, the root system formed directly from the aboveground part, thus, the flow of nutrients from the roots to the aboveground part of the plant is undisturbed.

In our study, activated charcoal showed to have a positive impact on the formation of the rooting system of Weeping Fig (*Ficus benjamina* L.) 'Exotica'. These results are confirmed by experiments of Al Malki and Elmeer K. M. (2009), which tested the impact of the medium and active charcoal on the rooting of Weeping Fig (*Ficus benjamina* L.) 'Anastasia'. In their study, the percentage of rooting in plants grown on the medium with active charcoal was approximately 78%, in contrast to those grown on the medium without active charcoal where the percentage of rooting was approximately 58%.

Overall, this study showed that the use of adequate plant growth regulators and appropriate supplements (e.g. active charcoal) positively influences the quality and the rapidity of Weeping Fig (*Ficus benjamina* L.) "Exotica" propagation.

CONCLUSION

Plant growth regulators INCIT 1 and INCIT 5 have a positive impact on the root system development. The longest roots with the highest number of roots and the lowest percentage of wilting was obtained in cuttings treated with plant growth regulator INCIT 5. Micropropagation of Weeping Fig (*Ficus benjamina* L.) 'Exotica', with the use of an adequate medium and the application of appropriate concentrations of plant growth regulators, showedpositive results. Propagation with appropriate supplements (e.g. active charcoal) indicatespositiveresults on the formation of the rooting system of Weeping Fig (*Ficus benjamina* L.) 'Exotica'.

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UTICAJ RAZLIČITIH REGULATORA RASTA NA RAZMNOŽAVANJE SITNOLISNOG FIKUSA (*FICUS BENJAMINA* L.) 'EXOTICA'

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Izvod: Cilj rada bio je da se ispita najbolji metod razmnožavanja sitnolisnog fikusa (*Ficus benjamina L.*)'Exotica', kao i optimalna koncentracija regulatora rasta i dodatih suplemenata. U radu su primenjene metode vegetativnog razmnožavanja, reznicama stabla, kao i mikropropagacija. Rezultati dvogodišnjeg istraživanja pokazali su da su reznice stabla tretirane regulatorom rasta koncentraciije 0,5% α-naftilsirćetne kiseline imale najveći procenat ožiljavanja (91,67%). Efekat citokinina i aktivnog uglja testiran je kod razmnožavanja mikropropagacijom. Faza umnožavanja pokazala se najefikasnijom na MS hranljivoj podlozi koja sadrži 4 mg l^{-1} BAi 1 mg l^{-1} IBA. Veći procenat ožiljavanja (50,3%), dužina korena (*27.9 mm*) i broj korenova (5,4) uočeni su kod biljaka gejenih na hranljivoj podlozi sa aktivnim ugljem. Rezultati su pokazali da je upotreba adekvatrnih regulatora rasta i suplemenata (na pr. aktivni ugalj) pozitivno uticala na kvalitet i brzinu razmnožavanja sitnolisnog fikusa (*Ficus benjamina L.*)'Exotica'.

Ključne reči: vegetativno razmnožavanje, *Ficus benjamina* L., mikropropagacija, regulatori rasta, reznice, aktivni ugalj

Received / Primljen: 29.06.2015.
Accepted / Prihvaćen: 11.9.2015.