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OPTIMISATION OF A *LAWSONIA INERMIS* L. MICROPROPAGATION PROTOCOL AND ACCLIMATIZATION IN A HYDROPONIC CULTURE

Marija Marković, PhD, associate profesor, University of Belgrade - Faculty of Forestry, Belgrade, Serbia (marija.markovic@sfb.bg.ac.rs)
Danijela Đunisijević-Bojović, PhD, associate profesor, University of Belgrade - Faculty of Forestry, Belgrade, Serbia
MSc Marijana Milutinović, University of Belgrade - Faculty of Forestry, Belgrade, Serbia
MSc Jelena Petrović, University of Belgrade - Faculty of Forestry, Belgrade, Serbia

Abstract: *Lawsonia inermis* is an important industrial and medicinal plant, cultivated mostly for dye production which is used in cosmetic industry. The objective of this study was establishing an efficient *in vitro* propagation system in order to obtain plants that will be acclimatized and grown hydroponically. The effect of different media on *in vitro* rooting were examined, followed by growing obtained microplants in a hydroponic culture, in a half-strength modified Hoagland nutrient solution. The highest rooting rate (79.2%) was recorded on the half-strength MS media containing 0.50 mg.L^{-1} IBA, with a high average number of roots (7.4). Those microplants acclimatized well in a hydroponic culture, where very rapid growth was recorded, and well developed roots and shoots were formed. After transplanting plants from hydroponic to soil, the survival rate was 100%. This is the first study reporting an acclimatization procedure in a hydroponic for the henna plant.

Key words: acclimatization, Henna, hydroponic, *in vitro* rooting, plant growth regulators

INTRODUCTION

Lawsonia inermis L., commonly known as henna, is an important industrial and medicinal plant. It is cultivated mostly for a dye production which is derived from henna leaves and used in a cosmetic industry (Zafar *et al*., 2006). In addition, many studies reported that henna had shown antidiabetic, hepatoprotective, antibacterial, antifungal, immunomodulatory, antioxidant and analgesic effect (Chaudhary *et al*., 2010; Borade *et al*., 2011). Besides traditional propagation techniques, from seeds or by cuttings (Singh *et al*., 2005), propagation of henna using *in vitro* culture is convenient for a rapid production of high-quality, uniform plants (Ram, Shekhawat, 2011). Rout *et al*. (2001) established sterile culture of *L. inermis* from apical and axillary meristems and obtained a multiplication rate that was the highest on the MS medium (Murashige, Skoog, 1962) supplemented with 0.25 mgL^{-1} BAP (6-benzylaminopurine) and 0.25 mgL^{-1} Kinetin, and optimal rooting (75.6%) was achieved on the MS medium containing 0.25 mgL^{-1} IBA (Indole-3-butyric acid). Ram, Shekhawat (2011) reported an improved protocol for the micropropagation of henna using nodal explants obtained from mature plants. They successfully performed *ex vitro* rooting of obtained

microshoots in the autoclaved soilrite containing 1/4 strength of MS macro salts, and survival rate of obtained rooted plants was 80% after transfer in greenhouse conditions. However, Mairapeytan, Tadevosyan (1999) stated that the content of dye was 3 times greater in leaves collected from plants grown in the open-air soilless conditions than from plants grown in soil. The higher content of important secondary metabolites in hydroponically grown species was recorded also for *Thymus* spp. (Sargsyan *et al*., 2011), while fresh and dry weight of *Achillea millefolium* grown in hydroponics was ten times higher than plants grown conventionally (Pedneault *et al*., 2014).

There is a possibility that an optimal production system could be established by the micropropagation of selected elite genotypes, and then by growing the obtained plants hydroponically. For this reason, in order to increase production efficiency, we decided to research the possibility of rooting and acclimatization of *in vitro* produced henna microplants in hydroponic culture.

MATERIALS AND METHODS

In vitro culture was established using green and woody single-node cuttings, collected from 2-year-old plants grown in a greenhouse. The leaves were removed and nodal segments were disinfected using 3.5% NaOCl with the addition of 2-3 drops of Tween 20, for 20 minutes, and rinsed five times using sterile distilled water. The explants were cultured on the MS medium supplemented with 30 gL^{-1} sucrose, 8 gL^{-1} agar, 0.5 mgL^{-1} BAP and 0.1 mgL^{-1}. After 25 days, the shoots developed from axillary buds were excised and cultured on the same medium for another two subcultures. *In vitro* rooting was performed on half-strength MS media with different concentrations (0.25 mL, 0.5 mL, 1.0 mL) of IBA or NAA (1-Naphthaleneacetic acid). For all media tested, the pH was adjusted to 5.8 before autoclaving at 121 °C for 15 min. All cultures were grown under a 16/8h light/dark photoperiod at 25±2 °C. After 25 days, the following parameters were measured: rooting percentage, number of roots per explant, length of the longest root and length of the longest shoot of each explant. Rooted *in vitro* microplants and *in vitro* obtained shoots were transfered in hydroponic culture for growing in a half-strength modified Hoagland nutrient solution (Đunisijević Bojović *et al*., 2012) during the next 4 weeks. The solution was changed in 7 days intervals. After 4 weeks in hydroponic, the following parameters were measured: the number of leaves, the length of the longest root and the longest shoot of each plant. Plants grown hydroponically were transferred to soil mixture of sand, peat and vermiculite in a ratio of 1: 1: 1.

The obtained data were statistically analyzed using the program Statgraphics Plus ver. 2.1. The significance of differences between the means was determined using the analysis of variance (ANOVA) and the LSD method ($p < 0.05$).

RESULTS

Sterile *in vitro* culture was established successfully, the 77.3% of green cuttings and 67% of woody cuttings regenerated shoots, but this difference wasn't statistically significant (p= 0.34). In the multiplication phase all explants in both subcultures formed normally developed shoots, without vitrification or necrosis. The mean number of shoots per explant ranged from 2.7 to 3.8, the mean length of the longest shoots per explant ranged from 5.7 to 8.6 mm, and there were no significant differences among the results obtained from shoots developed on green and woody cuttings.

The rooting rate on the half-strength MS media ranged from 17% to 79%, and it was significantly influenced by auxine type (Table 1). Significantly better results were achieved on the media containing IBA, with a higher rooting percentage, longer roots and even with longer shoots than on the media with NAA (Table 1). The highest rooting rate was recorded on the half-strength MS containing 0.5 mgL^{-1} IBA (Fig. 1).

For this reason, only the microplants rooted on media containing IBA were transferred on acclimatization in the modified Hoagland hydroponics nutrient solution. The composition of rooting medium influenced the acclimatization rate and

Table 1. The effect of auxine treatment on rooting percentage, the mean number and length of roots and the length of shoots

NAA mgL^{-1}	IBA mgL^{-1}	Rooting rate (%)	Mean number of roots/explant	Mean length of the longest root/explant (mm)	Mean length of the longest shoot/explant (mm)
1.00	0.00	42.7 c	2.4 d	2.1 d	8.7 bc
0.50	0.00	17.7 d	2.0 d	3.0 d	9.5 bc
0.25	0.00	25.0 cd	2.0 d	3.0 d	9.5 bc
0.00	1.00	63.5 b	8.9 a	9.7 ab	13.6 a
0.00	0.50	79.2 a	7.4 ab	13.4 a	10.2 b
0.00	0.25	66.7 b	5.4 bc	8.0 b	7.4 c
0.00	0.00	37.8 c	1.4 d	13.5 a	10.0 b

Note: The values within a column followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

the growth of acclimatized plants (Table 2) and the longest roots and shoots developed from microplants rooted on the medium with 0.5 mgL^{-1} IBA (Fig. 2). After 4 weeks in a hydroponic culture, the growth of plants was considerable, the mean length of roots increased 25 times, from 13.4 mm to 346 mm; and the mean length of shoots increased 11 times, from 10.2 mm to 115 mm (Tables 1, 2). After transplanting to soil, survival rate was 100 % for all plants.

Fig. 1 Rooted shoots on the half-strength MS supplemented with 0.5 mgL^{-1} IBA

Fig. 2 *L. inermis* plants acclimatized in hydroponics

Table 2. The effect of origin of microplants on their acclimatization in the hydroponic culture

IBA mg.L^{-1}	Acclimatization rate (%)	Mean number of leaves/plant	Mean length of the longest root/explant (cm)	Mean length of the longest shoot (cm)
1.00	70.0 a	11.9 a	13.0 b	4.5 b
0.50	63.2 a	14.1 a	34.6 a	11.5 a
0.25	40.0 b	12.4 a	15.1 b	7.0 ab

Note: The values within a column followed by different letters are significantly different at the P < 0.05 level according to the LSD test

The percentage of rooted shoots placed in the hydroponic solution for *ex vitro* rooting was low, only 13%.

DISCUSSION

The mean number of shoots obtained in our research was relatively low (3.8), but similar results (3.2 - 4.5) were also obtained by Rout *et al*. (2001) on the MS medium containing 0.25 mg.L^{-1} BAP and 0.25 mg.L^{-1} Kinetin. Ram, Shekhawat (2011) obtained a higher number of shoots (4.9) on a modified MS medium with 2 mg.L^{-1} BAP and with additives (50 mg.L^{-1} ascorbic acid, 25 mg.L^{-1} adenine sulphate, 25 mg.L^{-1} arginine and 25 mg.L^{-1} citric acid). They also conducted repeated transfers of shoot clumps on different media investigating the growth after 4 successive subcultures, observing a significant improvement in shoot multiplication and average shoot length when ammonium sulphate was added in the medium.

Rooting percentage obtained in our research on a half-strength MS medium with 1.0 mg.L^{-1} IBA was 70%, while Rout *et al*. (2001) recorded the highest number of 75% rooted shoots on an MS medium with 0.25 mg.L^{-1} IBA, with a decreasing rooting rate on media with higher concentrations of IBA. In addition, in our study, there were 37.8% rooted shoots on a half-strength hormone-free MS medium, but in the research conducted by Rout *et al*. (2001), there were no rooted shoots on the MS medium without hormones. This indicates that the concentration of MS salts influenced the rooting of henna shoots. In addition to that, Ram, Shekhawat (2011) recorded the highest rooting percentage (90%) and the highest average number of roots (5.8) on the 1/4th strength of MS medium supplemented with 5.0 mg.L^{-1} IBA. These results suggest that lower concentrations of MS salts combined with higher concentrations of IBA have a favorable effect on rooting percentage, but the number of shoots is lower compared to the number obtained in our study (8.9) on a half-strength MS medium.

The acclimatization rate in hydroponics (63-70%) was lower than expected compared to the results obtained with some other species (Fira, Clapa, 2009; Marković *et al*., 2015; Zapata *et al*., 2003), but it was similar to the results obtained by Ram, Shekhawat (2011) for henna microplants rooted *in vitro* and acclimatized in soilrite (70%). However, despite the lower rooting rate, Rout *et al*. (2001) recorded high percentage of acclimatized microplants (96%) in a mixture of garden soil, sand and cowdung at the ratio of 2: 1: 1 (v/v).

The presented results showed that *L. inermis* microplants can be acclimatized successfully in a hydroponic culture, when grown in a half-strength modified Hoagland nutrient solution. Thus, the growth of plants is considerable, and after only 4 weeks in a hydroponic culture the mean length of the roots increased 25 times and the mean length of shoots increased 11 times. After being acclimatized in a hydroponic culture, these plants can be transfered to soil with a survival rate of 100%.

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