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

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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 mg.L⁻¹ BAP (6-benzylaminopurine) and 0.25 mg.L⁻¹ Kinetic, and optimal rooting (75.6%) was achieved on the MS medium containing 0.25 mg.L⁻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 hydroponi-
cally grown species was recorded also for
Thymus spp. (Sargsyan et al., 2011), while fresh and dry
weight of Achillea millefolium grown in hydropon-
ics was ten times higher than plants grown con-
vventionally (Pedneault et al., 2014).

There is a possibility that an optimal produc-
tion system could be established by the micropro-
pagation of selected elite genotypes, and then by
growing the obtained plants hydroponically. For
this reason, in order to increase production effi-
ciency, 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
disinfectected 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 fol-
dowing parameters were measured: rooting per-
centage, 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 transferred in hydro-
ponic culture for growing in a half-strength mod-
ified Hoagland nutrient solution (Dunisijević
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 trans-
ferred 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 suc-
cessfully, the 77.3% of green cuttings and 67% of
woody cuttings regenerated shoots, but this dif-
ference 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 num-
ber 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 cut-
tings.

The rooting rate on the half-strength MS
media ranged from 17% to 79%, and it was sig-
ificantly influenced by auxine type (Table 1).
Significantly better results were achieved on the
media containing IBA, with a higher rooting per-
centage, 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 ac-
climatization in the modified Hoagland hydropon-
ics nutrient solution. The composition of rooting
medium influenced the acclimatization rate and
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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
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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**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.
REFERENCES

Borade A. S., Kale B. N., Shete R. V. (2011): A phytopharmacological review on *Lawsonia inermis* (Linn), Int. J. Pharm. Life Sci., 2 (1) (536-541)

Chaudhary G., Goyal S., Poonia P. (2010): *Lawsonia inermis* Linnaeus: A phytopharmacological review, Int. J. Pharm. Sci. Res., 2 (91-98)

Đunisijević Bojović D., Đukić M., Maksimović V., Skočajić D., Suručić Lj. (2012): The Effects of iron deficiency on lead accumulation in *Ailanthus altissima* (Mill.) Swingle Seedlings. J. Environ. Qual., 41 (1517-1524) DOI: 10.2134/jeq2011.0450

Fira A., Clapa D. (2009): Ex-Vitro Acclimation of some Horticultural Species in Hydroculture, *Bulletin UASVM Horticulture*, 66 (1) (44-50) DOI: http://dx.doi.org/10.15835/buasvm-cn-hort:3782

Mairapetyan S.K., Tadevosyan A.H. (1999): Optimisation of *Lawsonia inermis* L. and *Indigofera articulata* Gouan., Nutrient solution in opan-air hydroponics. *Acta Hortic.*, 481 (321-326)

Marković M., Skočajić D., Grbić M., Đukić M., Obratov - Petković D., Đunisijević - Bojović D., Borovica M. (2015): Micropropagation of *Achillea millefolium* L. on half - strength MS medium and direct rooting and acclimatization of microshoots in hydroponic culture, Bulletin of Faculty of Forestry, 111 (99-112) https://doi.org/10.2298/GSF1511099M

Murashige T., Skoog F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol. Plant., 15 (473-497)

Pedneault K., Dorais M., Leonhart S., Angers P., Gosselin A. (2014): Time-course accumulation of flavonoids in hydroponically grown *Achillea millefolium* L., *Can J Plant Sci.*, 94 (383-395)

Ram K., Shekhawat N.S. (2011): Micropropagation of commercially cultivated Henna (*Lawsonia inermis*) using nodal explants, Physiol Mol Biol Plant., 17 (281-289)

Rout G.R., Das G., Samantaray, S., Das, P. (2001): In Vitro micropropagation of *Lawsonia inermis* (Lythraceae), *Rev Biol Trop.*, 49 (3-4) (957-963)

Sargsyan E., Vardanyan A., Ghalachyan L., Bulgaladary S. (2011): Cultivation of Thymus by In Vitro And Hydroponics Combined Method. Engineering and Technology., 5 (119-122)

Singh M., Jindal S. K., Singh D. (2005): Natural Variability, Propagation, Phenology and Reproductive Biology of Henna, Henna, Cultivation, Improvement and Trade: Jodhpur, India: Central Arid Zone Research Institute, (13-18)

Zafar S., Ahmad S., Jameel S. (2006): *Lawsonia inermis* Linn. – A Review, „Medicinal Plants: Traditional Knowledge“, Trivedi, P.C. (Ed.), IK International Publishing House Pvt. Ltd., New Delhi, (34-49)

Zapata E. V., Morales G.S., Lauzardo A.N.H., Bonfil B.M., Tapia G.T., Sánchez A., Del Valle M.V., Aparicio A.J. (2003): In vitro regeneration and acclimatization of plants of Turmeric (*Curcuma longa* L.) in a hydroponic system, Biotecnología Aplicada, 20 (1) (25-31)

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