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Efficient Shoot Regeneration in Syrian Rue (*Peganum harmala* L.) Under *in vitro* Conditions

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ABSTRACT

Peganum harmala L., a perennial shrub of medicinal and ornamental importance, was propagated from shoot apices and first axillary buds on agar solidified MS medium supplemented with 0-2.22 µM benzylaminopurine (BAP) in combination with 0.11– 1.07 µM α-naphthalene acetic acid (NAA). No shoot regeneration was observed on MS medium without plant growth regulators. MS medium containing 1.11 µM BAP and 0.11 µM NAA was optimal for shoot regeneration from both explant types at 16 h light. Shoot regeneration from shoot apices was superior to that from first axillary buds. Hyperhydricity of the regenerated shoots was overcome by transferring them to plant growth regulators free MS medium. Rooting was achieved on MS medium containing 4.90 µM IBA. Rooted plants were successfully acclimatized in pots placed in a growth chamber for subsequent transfer to green house.

Key Words: Syrian rue; Axillary buds; Shoot apices; Callus formation; Rooting; Propagation

INTRODUCTION

Peganum harmala L. (Syrian rue), belonging to the family Zygophyllaceae, is a 0.5 to 0.6 m long perennial plant with biochemical, pharmaceutical, medicinal (Baytop, 1999) and ornamental interest. It grows widely in the region extending from the Mediterranean basin to the South Asia. As an ornamental plant, this white flowering plant (Fig 1a) is ideal, because of its low maintenance and drought tolerance. Carboline alkaloids like harmine (7-methoxy-1-methyl-9H-pyrido [3, 4-b] indole) and harmaline (4, 9-dihydro-7-methoxy-1-methyl-3H-pyrido [3,4- b] indole) obtained from various parts of the plant are used against a number of diseases (Aarons, 1977; Sobhani *et al.,* 2002). Medicinally, the fruit and seed are digestive, diuretic, hallucinogenic, narcotic and uterine stimulants (Kartal *et al.,* 2003). A red dye is obtained from the seed and is widely used in Turkey and Iran for coloring carpets (Baytop, 1999). Conventional propagation of *P. harmala* is from seed and it has several limitations including germination. No reliable data are available about seed germination, growth and fruiting of the plant in domesticated or natural settings.

Saini and Jaiwal (2000) and Ehsanpour and Sa-Adat (2002) has reported in vitro propagation of *P. harmala* from cotyledonary node and hypocotyl explants respectively. All other in vitro protocols concentrate on the production of secondary metabolites (Reinhard *et al.,* 1968; Nettleship & Slaytor, 1974 a,b; Sasse *et al.,* 1982 a,b, 1987; Berlin & Sasse, 1988; Courtois *et al.,* 1988; Berlin, 1989, 99) through callus formation. This study was aimed at to identify a suitable explant and a protocol for *in vitro* multiplication of *P. harmala* a) for ornamental purposes, b) improved harvesting of carboline alkaloids like harmine and harmaline of economic and pharmaceutical importance and c) creation of somatic variation and selection thereafter. With these aims in mind, we investigated the possibility of shoot regeneration of *P. harmala* from shoot apices and uppermost first axillary bud (from the top) for *in vitro* organogenesis, which may allow propagation of superior genotypes and the ability to perform genetic transformation experiments.

MATERIALS AND METHODS

Mature fruits of *P. harmala* were harvested during September, 2003; from Elaziğ province in the South Eastern Anatolia, a native stand known for the best *P. harmala* in Turkey. Fruit capsules were pierced opened with a sharp needle to extract mature seeds. Surface sterilization (of seeds) was performed by immersion in 100% (v/v) commercial bleach (Axion, Turkey; 5-6% NaOCl) for 5 minutes, followed by 3 rinses of 3 min each with sterile distilled water. The apical meristem and the first axillary bud from the top were used as explants and were obtained from 10 days old *in vitro* germinated seedlings grown on MS medium (Murashige & Skoog, 1962) without growth regulators supplemented with 30 g L^{-1} sucrose. For organogenesis experiments, the medium was supplemented

with 1.11–2.22 μM BAP and 0.11–1.07 μM NAA (Table I) and 30 g L^{-1} sucrose. The pH of the medium was adjusted to 5.6–5.8 with 1 N HCl or 1 N NaOH. Thereafter, 0.8% agar was added to the respective medium before autoclaving. Medium (35 ml) was poured into 100 x 10 mm sterile Petri dishes under sterile conditions. All cultures were grown at 24±2°C in 16 h light (42 μmol m^{-2} s^{-1}) or complete darkness. All explants were subcultured every 4 weeks, in both propagation and rooting experiments. Hyperhydricity, if any, was overcome by subculture on plant growth regulator free MS medium containing full or half strength of salts and vitamins. Rooting was obtained on ½ and full strength MS (major, minor, salts, & vitamins) medium containing 1.23, 2.45, 4.90 or 7.35 μM 3-indole butyric acid (IBA) in 16 h light (42 μmol m^{-2} s^{-1}) at 24±2°C.

Statistical analysis. Both regeneration and rooting experiments were replicated 4 times with 4 explants in each replication and were repeated twice. After eight weeks of culture on respective *in vitro* regeneration or rooting medium, data were recorded and subjected to the statistical analysis using SPSS for Windows version 12.00. Separation of treatments was made by Fischer's LSD test. Data given in percentages were subjected to arcsine (√X) transformation before statistical analysis (Snedecor & Cochran, 1967).

RESULTS AND DISCUSSION

The need to continuously improve specific characters like number and color of flowers and carboline alkaloids (like harmine and harmaline) used for various medicinal purposes, make plant tissue culture studies essential for Syrian rue plant. Results indicated that shoot regeneration response was dependent both on BAP and NAA concentration in the medium and on explant type (Table I). Thus, MS medium containing any concentration of plant growth regulators (BAP–NAA) affected the callus formation, shoot regeneration, shoot length and the level of hyperhydricity ($p<0.01$). Callus formation along with regeneration of adventitious shoots is desirable in order to induce somatic variation; which, in turn, aids in selection of plants with desirable traits. All combinations of growth regulators induced callus formation of 66.67 to 100% on the shoot apices and 33.33 to 100% on the uppermost first axillary buds. No callus formation or shoot regeneration was recorded on either shoot apices or first axillary bud explants placed on MS medium without growth regulators (Table I).

The frequency of adventitious shoot formation on all media was better on shoot apices than on axillary buds (Table I). Data further revealed that 1.07 μM NAA in combination with 1.11 or 2.22 μM BAP reduced the number of adventitious shoot regeneration and shoot length compared to when the MS medium containing 0.11 μM NAA with 1.11 or 2.22 μM BAP (Table I). However, reduced concentration of NAA (0.11) in combination with reduced concentration of BAP (1.11 μM) stimulated callus formation on both explants. Shoot primordia were recorded within 12-15 days preceded by an early growth of callus on both explant types (Fig 1b). Variations among treatments and between explants were very distinctive. The highest number shoots (3.30) per explant with mean length of 5.50 cm on apical meristem and 1.67 shoots per explant with a mean length of 2.50 cm on first axillary node were induced on MS medium supplemented with 2.22 μM BAP relative to 0.11–1.07 μM NAA but with considerable hyperdricity especially on younger shoots (Fig 1c). No improvement in regeneration was observed from explants grown under complete darkness (data not shown).

It is speculated that shoot apices and axillary buds are most likely to contain different levels of internal auxin, cytokinin and abscisic acid (ABA) concentrations which influenced the level of adventitious shoot regeneration from first axillary buds and shoot apices. However, in general high hyperhydricity appears to be responsible for reduced number of adventitious shoots (from shoot apices & first axillary buds) under all treatments (Table I). Similarly, a comparison of the shoot length and development on medium containing NAA in combination with 2.22 or 1.11 μM BAP showed that the higher level of NAA (1.07 μM) was responsible for reduced shoot length from shoot apices.

Fig. 1. Shoot regeneration from shoot apices of *Peganum harmala* L. (a) Blooming under natural conditions (b) development of shoots in callus regenerated from shoot apex (c) hyperhydricity of younger shoots (d, e) reversion of hyperhydric shoots to normal when transferred to MS medium. Bar = 1.25 cm.

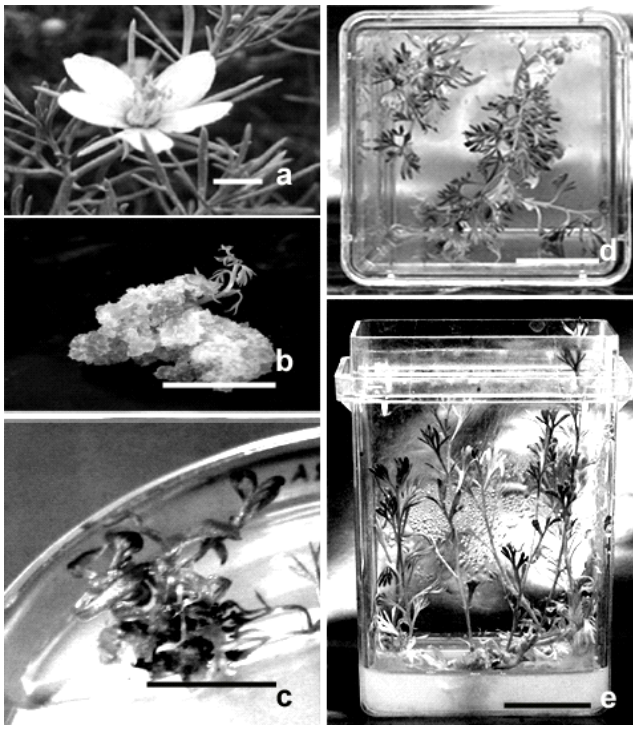

Table I. Shoot organogenesis from shoot apices and first axillary buds of *Peganum. harmala* under *in vitro* conditions after eight weeks of culture.

Treatments		Frequency of callus formation (%)		Frequency of adventitious shoot regeneration (%)		Number of shoots per explant		Mean shoot length		Frequency of hyperhydricity (%)	
BAP (µM)	NAA (µM)	Shoot apex	First axillary bud	Shoot apex	First axillary bud	Shoot apex	First axillary bud	Shoot apex	First axillary bud	Shoot apex	First axillary bud
2.22	1.07	91.66[1 a]	33.33[b]	75.00[a]	0.00[c]	2.50[a]	0.00[c]	3.33[ab]	0.00[c]	25.00[c]	0.00[b]
2.22	0.11	83.33[a]	33.33[b]	66.66[b]	8.33[b]	3.00[a]	0.33[ab]	4.33[a]	2.16[ab]	83.33[a]	83.33[a]
1.11	1.07	66.67[b]	100.00[a]	16.66[b]	2.50[c]	0.66[b]	1.33[ab]	1.33[c]	3.00[a]	58.33[b]	83.33[a]
1.11	0.11	100.00[a]	33.33[b]	83.33[a]	33.33[a]	3.30[a]	1.67[a]	5.50[a]	2.50[ab]	33.33[c]	83.33[a]
0.00	0.00	0.00[c]	0.00[c]	0.00[c]	0.00[c]	0.00[c]	0.00[c]	0.00[c]	0.00[c]	0.00[d]	0.00[b]

Each value is the mean of 4 replicates each with 4 explants.
[1]Values with in a column followed by different letters are significantly different at 0.01 level using LSD Test.

Table II. Frequency of rooting of *P. harmala* under *in vitro* conditions on ½ MS (major & minor salts and vitamins) medium, or containing different concentreation of IBA after eight weeks of culture.

IBA µM	Frequency of rooting[1] (%)	Number of roots/shoot	Root length (cm)
1.23	28.33[2] b	1.53 ab	0.92 b
2.45	36.67 a	2.34 b	0.93 a
4.90	83.33 a	4.73 a	2.75 a
7.35	83.33 a	2.73 a	1.96 b

Each value is the mean of 4 replicates each with 4 explants.
[1]Values with in a column followed by different letters are significantly different at 0.01 level using LSD Test.
[2]From shoots that regenerated roots

No effect of NAA levels (on shoot length) was observed on first axillary buds.

All of the hyperhydric shoots reverted to normal when transferred to medium containing full or half strength of MS salts and vitamins without growth regulators (Fig. 1d,e). These results agree with those observed by Bouza, (1997) for *Prunus tenella*, where spontaneous reversion of shoot hyperhydricity on hormone-free medium was induced.

The induction and development of shoots occurred on the same medium, which was in agreement with Phillips *et al.* (1995). A clear effect of explant type and treatment was discerned. Saini and Jaiwal, (2000) observed that out of various seedling explants, the cotyledonary node exhibited maximum shoot regeneration frequency from the axillary region on MS medium supplemented with 5 µM BAP and 0.01 µM NAA and induced 4-5 shoots per explant within 5 weeks on 75% of explants. Similarly, Ehsanpour and Sa-Adat (2002) also regenerated multiple shoots from excised hypocotyl segments of *P. harmala* on MS medium supplemented with BAP, Kinetin and NAA.

Rooting was better when ½ strength MS (major & minor salts and vitamins) medium was used in combination with different concentrations of IBA compared to full strength of MS (major and minor salts & vitamins) medium; which seemed to induce inhibition. Rooting frequency of 28.33–83.33% was obtained on all concentration of IBA in ½ strength of MS major and minor salts and vitamins in the respective medium ($p<0.01$; Table II). 4.90 µM IBA was found optimal with maximum number of 4.73 roots and the longest mean root length of 2.75 cm. Rooted plantlets were transferred to pots for acclimatization and subsequent transfer to the green house.

It was concluded that the apical meristem may have more regenerative tissue responsible for setting up a local hormone concentration gradient that might stimulate the high frequency of shoot regeneration. The results further indicate that shoot apices are highly regenerative and have the potential to develop higher number of shoots.

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REFERENCES

Aarons, D.H., G.V. Rossi and R.F. Orzechowski, 1977. Cardiovascular actions of three harmala alkaloids: harmine, harmaline, and harmalol. *J. Pharmaceut. Sci*., 6: 1244–8

Baytop, T., 1999. Herbal treatments in Turkey, (Past & Present) 2. Baski, [Türkiye'de Bitkilerle Tedavi (Geçmiste ve Bugün)] Nobel Tip Kitapevleri Ltd. *Sti., Istanbul Turkey*., (In Turkish)

Berlin, J., 1989. On the formation of secondary metabolites in plant cell cultures, some general observations and some regulatory studies. In Production of metabolites by plant cell cultures. *Proc. Eur. Symp. APRIA, Paris–France*

Berlin, J., 1999. Genetic transformation of *Peganum harmala*. *In:* Bajaj, Y.P.S. (ed.). *Biotechnology in Agriculture and Forestry.* Transgenic medicinal plants. Springer Germany

Berlin, J. and F. Sasse, 1988. a carbolines and indole alkylamines. *In:* Constabel, F. and I.K. Vasil (eds.). *Cell Culture and Somatic Cell Genetics of Plants.* Vol 5. Academic Press. Orlando, *Florida–USA*

Bouza, L., 1997. Micropropagation of *Prunus tenella* (Dwarf Russian Almond), *In:* Bajaj, Y.P.S. (ed.), *Biotechnology in Agriculture and Forestry, High–Tech and Micropropagation VI*, vol. 40, Springer–Verlag, Berlin, Heidelberg Germany

Courtois, D., D. Yvernal, B. Florin and V. Petiard, 1988. Conversion of tryptamine to serotonin by cell suspension cultures of *Peganum harmala*. *Phytochem*., 27: 3137–41

Ehsanpour, A.A., E. Sa–Adat, 2002. Plant regeneration from hypocotyl culture of *Peganum harmala*. *Pakistan J. Bot*., 34: 253–6

Kartal, M., M.L. Altun and S. Kurucu, 2003. HPLC method for the analysis of harmol, harmalol, harmine and harmaline in the seeds of *Peganum harmala* L. *J. Pharmaceut. Biomed. Anal.,* 31: 263–9

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Pl*., 15: 473–97

Nettleship, L. and M. Slaytor, 1974 a. Adaptation of *Peganum harmala* callus to alkaloid production. *J. Exp. Bot.,* 25: 114–23

Nettleship, L. and M. Slaytor, 1974 b. Limitations of feeding experiments in studying alkaloids biosynthesis in *Peganum harmala* callus cultures. *Phytochem*., 13: 735–42

Phillips, G.C., J.F. Hubstenberger and E.E. Hansen, 1995. Plant regeneration by organogenesis from callus and cell suspension cultures. *In:* O.L. Gamborg and G.C. Phillips (eds.). *Plant Cell Tissue and Organ Culture,* Fundamental Methods. Springer Verlag, *Berlin, New York*

Reinhard, E., G. Corduan and H. Volk, 1968. Al ach Weis von Harmin in Gewebekulturan von *Peganum harmala*. *Phytochem*., 7: 503–4

Saini, R. and P.K. Jaiwal, 2000. *In vitro* multiplication of *Peganum harmala* an important medicinal plant. *Indian J. Exp. Biol*., 38: 499–503

Sasse, F., U. Heckenberg and J. Berlin, 1982. a. Accumulation of â carboline and serotonin in cell cultures of *Peganum harmala* L. I. Correlation between plants and cell cultures and influence of medium constituents. *J. Pl. Physiol*., 69: 400–4

Sasse, F., U. Heckenberg and J. Berlin, 1982 b. Accumulation of â carboline alkaloids and serotonin by cell cultures of *Peganum harmala* L. II. Interrelationship between accumulation of serotonin and activities of related enzymes. *Z. Pflanzenphysiol*., 105: 315–22

Sasse, F., L. Witte and J. Berlin, 1987. Biotransformation of tryptamine to serotonin by cell cultures of *Peganum harmala*. *Planta Medica*, 53: 354–9

Snedecor, G.W. and W.G. Cochran, 1967. *Statistical Methods.* pp. 327–9. The Iowa State *Univ Press, Iowa*.

Sobhani, A.M., S.A. Ebrahimi and M. Mahmoudian, 2002. An In Vitro Evaluation of Human DNA Topoisomerase I Inhibition by *Peganum harmala* L. Seeds Extract and Its Carboline Alkaloids. *J. Phar. Pharmaceut. Sci*., 5: 19–2

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