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PLANT REGENERATION FROM HYPOCOTYL CULTURE OF *PEGANUM HARMALA*

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Abstract

Conditions for plant regeneration from excised hypocotyl of *Peganum harmala* were studied. Complete plants were regenerated directly from hypocotyl segments on MS medium supplemented with BAP, Kinetin and NAA. Multiple shoots were regenerated from 87% of explants. Addition of thiamine hydrochloride to the culture medium, increased the number of explants which regenerated and the number of shoots per explant. Regenerated shoots were rooted on MS medium supplemented with or without NAA. The morphology of whole plants were similar to the original plants.

Introduction

Peganum harmala is a bushy, herbaceous perennial with short creeping roots native to arid parts of North Africa, Mediterranean, the Middle East, Pakistan, India and Iran. It contain numerous alkaloids which have become economically valuable secondary metabolites. Seeds and other explants of *P. harmala* have long been used for medicinal purposes as fungicide and also herbicide due to presence of harmine (Berlin *et al.*, 1993). There have been several attempts to regenerate plants *via* tissue culture technology e.g., mung bean meristem culture (Goel *et al.*, 1983), cotyledon culture (Gulati & Jaiwa, 1990) and protoplast culture of potato cv. Delaware (Ehsanpour & Jones, 2001). It is interesting to note that regeneration of mature plants from *P. harmala in vitro* culture has not been reported. Studies on tissue culture of *P. harmala* have been concentrated largely on improving the production of alkaloids such as serotonin and carboline in cell suspension and hairy root cultures (Courtois *et al.*,1988).

Identification of plant regeneration system from *in vitro* cultures may be important as a source of material for genetic manipulation, physiological studies and alkaloid production (Anju & Pawan, 1992). The establishment of a reliable procedure for plant regeneration from *P. harmala* explants is essential for the recovery of whole plants and the application of techniques of genetic engineering to enhance alkaloid production. The purpose of the present study was to develop efficient procedures for the regeneration of plant from different explants. In this paper we report the successful regeneration of complete plants from hypcotyl culture of *P. harmala*.

Material and Methods

Seeds of *P. harmala* were obtained from seed and seedling production centre in Iran. The seeds were rinsed in 70% alcohol for 1 min., disinfested in 10% (w/v) aqueous sodium hypochlorite solution for 10 min., thoroughly rinsed in sterile distilled water and planted on MS medium (Murashige & Skoog, 1962) containing 3% (w/v) sucrose and

0.7% (w/v) agar (Merck) in 250 ml glass jars. The medium was adjusted to pH 5.8 with 0.1N NaOH or 0.1N HCl before addition of agar and autoclaved at 121°C for 15 min. The seeds were kept in culture room and germinated in 16-h photoperiod (2000 lux) at 25°C. Segments (5-10 mm) of hypocotyl were excised from such aseptically raised hypocotyl and served as explants for shoot regeneration. Explants were cultured on MS medium containing different 10 combinations of cytokinin and auxin as shown in Table 1.

Table 1. Combination of hormones in MS medium for plant regeneration.

	Hormones (mg/l)				
Medium number	**NAA**	**Kin**	**BAP**	**BA**	**TH**
1	0	0	0	0	0
2	0.5	0.5	0.5	0	0
3	0.5	0.5	1.0	0	0
4	0.5	1.0	2.0	0	0
5	0.5	2.0	2.0	0	0
6	0	3.0	2.0	0	0
7	0.5	0.5	0	0.5	5
8	0.5	0.5	0	2.0	5
9	0.5	0.5	0.5	0	5
10	0.5	0.5	2.0	0	10

NAA (a- Naphthalene acetic acid), Kin (Kinetin), BA (6-Benzyladenine)
TH (Thiamine hydrochloride), BAP (6- Benzylaminopurine)

Regenerated shoots were cut from base and were transferred on MS medium without auxin or supplemented with NAA (0.5/L) for rooting, then plantlet with well developed roots were washed in running tap water and transferred to pots containing sterile vermiculite and watered with tap water containing half strength of MS macro elements. All cultures were maintained under the same experimental condition as for seed germination. The effects of treatments was quantified on the bases of percentage of cultures showing response and the number regenerated per culture.

Results and Discussion

Ten different combinations of NAA, Kin, BAP, BA and TH were examined for effectiveness in shoot regeneration from hypocotyl explants. Various number of shoots regenerated in different combinations of media. Addition of different concentration of BA, Kin and NAA to MS medium induced variable amount of callus at the base of the explants followed by multiple adventitious shoot differentiation from explants within 5-6 weeks of culture (Table 2). Adventitious shoot and roots regenerated from hypocotyl cultured on medium 6 (Fig. 1).

In another set of experiment, segments of leaf, stem, epicotyl and roots (10mm) from seedlings were used for regeneration, but they were unresponsive in all media. Where Citric acid @ 2 mg/l was added to the medium, only the calli became clear. However, no shoot or root was observed on these explants. The total number of plant with shoot formation was 87 and 85 out of 87 shoots produced roots in medium No.10.

Table 2. Effect of different combination of cytokinine and auxin on hypocotyl culture of *Peganum harmala*.

Medium number	Shoot regenerated %	Mean shoots per explant %	Culture forming roots %	Shoot length (cm)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	25	2.2 ± 0.3^a	22^a	6.5^a
5	0	0	0	0
6	0	0	0	0
7	20	2.7 ± 0.7^a	17^a	5.6^a
8	50	3.5 ± 1.3^b	35^b	7.4^a
9	24	2.1 ± 0.2^a	18^a	5.4^a
10	87	6.7 ± 0.8^c	50^c	6.2^a

Data with common letter have no significant difference using LSD test ($p < 0.5$)

Fig. 1. Regenerated shoots from hypocotyl culture on medium No. 10

All rooted *In vitro* grown plants were transferred on to 500 ml pots containing sterile vermiculite and watered with distilled water containing half strength of MS salts. In this step 79 young plants survived. For evaluation of somaclonal variation occurring in regenerates, plants were analysed according to the morphology. All examined plants were similar to the original plants.

In many species plant regeneration from different explants have been reported eg., shoot tip (Gulti & Pawan, 1992), leaf segments, cotyledonary nodes, apical meristem and hypocotyl (Isabel *et al.*, 1996; Hosokawa *et al.*, 1996; Hee-Ju *et al.*, 1997; Lin *et al.*, 1997; Agrawal *et al.*, 1997). The plant regeneration under *In vitro* condition are affected by several different parameters such as genotype, the concentration of phytohormones supplemented with the regenerated medium, the physiological condition of the explants and developmental stage of the original plants as source of plant materials (Attifield & Evans, 1991; Motoshige *et al.*, 1992). Combination of BAP and Kinetin as a strong source of cytokinin in association with thiamine chloride as a suitable source of nitrogen possibly promoted shoot formation on the calli. However, this system for *P. harmala* can be used for normal regeneration of plant with no somaclonal variation. In future it can also be applied for genetic manipulation (Lin *et al.*, 1997) and alkaloid production under *In vitro* condition.

References

Agraval, D.C., A.K. Banerjee, R.R. Kolala, A.B. Dhage, A.V. Kulkarni, S.M. Nalawade, S. Haxra and K.V. Krishnamurthy. 1997. *In vitro* induction of multiple shoots and plant regeneration in cotton (*Gossypium hirsutum* L.). *Plant Cell Reports*, 16: 647-652.

Anju, G. and K.J. Pawan. 1992. *In vitro* induction of multiple shoot and plant regeneration from shoot tips of mung bean (*Vigna radiata* (L.) Wilczek). *Plant Cell Tissue and Organ Culture*, 29: 199-205.

Attfield, S.M. and P.K. Evans. 1991. Stages in the initiation of root and shoot organogenesis in cultured leaf explants of *Nicotiana tabacum* cv. Xanthi nc. *Journal of Experimental Botany*, 42: 59-63.

Berlin, J., R. Christian, G. Norbert, N.K. Inna, W. Luder and W. Victor. 1993. Biosynthesis of serotonin and B-carboline alkaloids in hairy root cultures of *Peganum harmala*. *Phytochemistry*, 33: 593-597.

Courtois, D., D. Yvernel, B. Florin and V. Petiaro. 1988. Conversion of tryptamin to serotonin by cell suspension cultures of *Peganum harmala*. *Phytochemistry*, 27: 3137-3142.

Ehsanpour, A.A. and M.G.K. Jones. 2001. Plant regeneration from mesophyll protoplasts of potato (*Solanum tuberosum*) cultivar Delaware using silver thiosulfate (STS). *Journal of Science*, 12: 103-110.

Goel, S., A.K. Mudgal and S.V. Gupta. 1983. Development of *in vitro* cultured shoot tips of *Vigna mungo* and *V. radiata*. *Trop. Plant Sci. Res.*, 1: 31-33.

Gulati, A. and P.K. Jaiwal. 1990. Culture conditions effecting plant regeneration from cotyledon of *Vigna radiata*. *Plant Cell Tissue and Organ Culture*, 23: 1-7.

Hee-Ju, Y., Oh. Kyung, O. Manho, C. Dong-Woog, M.K. Young and K. Sang-Gu. 1997. Plant regeneration from callus cultures of *Lithospemum erythrohizon*. *Plant Cell Reports*, 16: 261-266.

Hosokawa, K., M. Nakano, Y. Oikawa and S. Yamamura. 1996. Adventitious shoot regeneration from leaf, stem and root explants of commercial cultivars of *Gentiana*. *Plant Cell Reports* 15: 578-581.

Isabelle, R., D. Fredric, S.S. Rajbir and S.S. Brigitte. 1996. In plants 2, 3, 5 triiodobenzoic acid treatment promotes high frequency and routine *in vitro* regeneration of sugarbeet plants. *Plant Cell Reports*, 16: 142-146.

Lin, H.S., M.N. DeJeu and E. Jacobsen. 1997. Direct shoot regeneration from excised leaf explants of *in vitro* grown seedling of *Alstroemeria* L. *Plant Cell Reports*, 16: 770-774.

Motoshige, K., H. Satoshi, A. Baltazar and O. Kiyohoru. 1992. Protoclonal variation of plant regeneration in rice. *Plant Cell Tissue and Organ Culture*, 28: 1-10.

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

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