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Micropropagation of *Prunella vulgaris* L. - A Valuable Medicinal Plant

Sabeena Bashir, Azra N. Kamili and A. M. Shah
Plant Tissue Culture Laboratory, Centre of Research for Development, University of Kashmir, Srinagar-190006

ABSTRACT

An efficient procedure for *in vitro* multiplication of *Prunella vulgaris* L.(Lamiaceae) through shoot tip culture is described for the first time. The maximum number of direct shoots was induced from seedling shoot tips on MS medium containing various concentrations of BAP. A combination of BAP and NAA also favoured direct multiple shoot formation in the explant. However, presence of NAA alone in the medium promoted direct root initiation and elongation in the explants after 10-12 weeks of culturing period. Isolated microshoots when subcultured on MS(x1/2) basal medium resulted in maximum shoot elongation (8-10cm) and simultaneous rooting in the explants. Plantlets regenerated were healthy.

Key words: *Prunella vulgaris*, shoot regeneration, microshoots, plantlets.

Abbreviations : M.S (x 1/2) - Murashige and Skoog (half salt strength), BAP-6-benzyl aminopurine, NAA-naphthalene acetic acid, IAA-indole-3-acetic acid.

INTRODUCTION

Prunella vulgaris L., commonly known as Self Heal, belongs to family Lamiaceae. It is a perennial herb distributed all over Europe, temperate Himalaya and western Tibet, from Kashmir to Bhutan. It grows wild in Kashmir valley. The herb has great medicinal importance and is used as antiseptic, antirheumatic, antipyretic and tonic (Kaul, 1997). It is widely used to stop bleeding and to treat diarrhoea and boils. Chinese research shows the herb to have a moderately strong antibiotic action against a broad range of pathogens including *Shigella* spp. and *E. coli*, strains of which can cause enteritis and urinary infection. The infusion of herb is also used as an injection for internal bleeding and for piles.

Prunellin (polysaccharide), an anti-HIV. active compound, has been isolated from aqueous extracts of this medicinal herb(Abba *etal*.,1989). In screening tests of different commonly used herbs *Prunella vulgaris* was found to exert the best anti-HIV activity (John *etal*.,1994). The herb also shows antiviral action against the Herpes Simplex virus(Zheng,1990).

A high content of rosmarinic acid, immuno-modulation effects of the polysaccharide prunellin and antiviral activity of some constituents make the plant interesting from the view point of therapeutical applications (Markova *et al.*,1997).Thus the plant is in great demand for production of traditional and modern medicines in India. Since the rate of exploitation of medicinal herbs by man is very fast, there is every possibility that this treasure may someday be lost from our valley.The promising technique of plant tissue culture has been widely used for mass multiplication and commercial utilisation of number of medicinal plants(Sanchez-Gras and Calvo,1996;Andrade *et al.*,1999;Komalavalli and Rao,2000;Manickam *et al.*,2000;Segio *et al.*,2000;Kamili *et al.*,2001;Dias *et al.*, 2002;Liu *et al.*,2003;Kamili *et al.*,2004).The present study is an attempt to develop an *in vitro* method for mass propagation of *P. vulgaris* by using shoot tip explants obtained from *in vitro* raised seedlings.

MATERIAL AND METHODS

Fresh viable seeds of *P. vulgaris* were collected from healthy plants growing naturally in the Rafiabad area of district Baramulla,Kashmir. Seeds were washed with detergent cedepol (0.5%v/v) and 2-4 drops of Tween-20(surfactant) under running tap water followed by final rinsing with double distilled water. These seeds were soaked for 2-3 days at 4°C in a refrigerator. Surface sterilization of soaked seeds was achieved by using 0.1% $HgCl_2$ for 15-17 minutes followed by three times rinsing with autoclaved double distilled water. The sterilized seeds were then inoculated on MS(x1/2) basal medium(1962)fortified with 3%sucrose.The shoot tip explants were obtained from 4-6 weeks old *in vitro* raised seedlings and cultured on MS(x1/2)medium enriched with various phytohormonal regimes. The pH of the medium was adjusted between 5.5-5.6 by using 0.1N NaOH or 0.1N HCl before jelling the medium with 0.8%Difco- bactoagar. The medium was finally dispensed into culture vials, which were plugged and autoclaved for 20 minutes at 15lb pressure and 121°C temperature. The cultures were maintained at 25±3°C with 55-65%RH and exposed to 16 hour photoperiod provided by cool fluorescent tubes (3000 lux).

RESULTS

The influence of NAA(auxin),BAP(cytokinin) and their combinations on axillary shoot multiplication through shoot tip culture was investigated (Table 1).Shoot tip explants cultured without growth regulator/s did not induce growth and regeneration. However, NAA (2.5mM,5mM, 7.5mM,10mM,) alone favoured elongation and rooting of explants. Various BAP concentrations (2.5 5, 10, 15 or 20mM) stimulated direct multiple axillary shoot initiation, proliferation and elongation within 6-8 weeks of culture period. The maximum number (18±0.4) of shoots per explant was observed on BAP 15mM (Fig.1). However, higher concentration of BAP (20mM) reduced the no. of axillary shoots per explant.

The combined interaction of BAP (5, 10, 15mM) and NAA (2.5,5mM)also favoured direct

multiple shoot regeneration. However one combination of BAP (10mM) and NAA (5mM) was found optimum for both multiple shoot and root regeneration (Fig.2). Subculturing of primary cultures with multiple axillary shoots regenerated on various phytohormonal regimes onto hormone free MS(x1/2) medium stimulated maximum microshoot elongation (4-10cm). The elongation phase was followed by isolation of elongated shoots for rooting trials. Direct root initiation (100%) and elongation was again observed after subculturing on half strength basal medium (Fig.3). Complete plantlets (6-10cm) with long(5-12cm) and thread like roots were recovered after 6-8 weeks on rooting medium(Fig.4). The healthy plantlets were deflasked and transferred in small pots containing sand, soil and vermiculite mixture(1:1:1)(Fig5).

Table1. Effect of NAA, BAP and NAA+BAP on axillary shoot multiplication from shoot tip explants of *P. vulgaris* after 6-weeks of culture period on MS(x1/2) basal medium.

NAA(mM)	BAP(mM)	Response	Percentage response	Average No. of axillary shoots/explant*
MS(x1/2)Basal	(Control)	-	-	-
2.5	0	Elongation of explant	60	-
5	0	Elongation of explant accompanied with rooting	75	-
7.5	0	do	80	-
10	0	do	80	-
0.0	5	Multiple axillary shoot regeneration	80	8+0.7
0.0	10	do	90	15 ± 0.5
0.0	15	do	95	18 ± 0.4
0.0	20	do	70	7 ± 0.7
2.5	5	do	65	3 ±0.3
5	10	Multiple axillary shoot regeneration accompanied with rooting	85	7 ± 0.6
2.5	15	Multiple axillary shoot regeneration	90	8 ±0.4
5	15	do	80	6 ± 0.6

*M±S.E., 10 replicates/ treatment.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 1 - 5: *In vitro* response of shoot tips of *Prunella vulgaris L.*

Fig. 1. Multiple shoot regeneration and elongation on MS(x1/2) + BAP(15μM). (after 6 weeks)

Fig. 2. Multiple shoot regeneration and root formation on MS(x1/2) + BAP(10μM) + NAA(5μM). (after 10 weeks)

Fig. 3. Isolated plantlets on MS(x1/2) basal medium. (after 4 weeks)

Fig. 4. Fully elongated plantlet on MS(x1/2) basal medium. (after 6 weeks)

Fig. 5. Plantlet in Sand : Soil: Vermiculite mixture.

DISCUSSION

The present investigation carried on seedling shoot tip explants of *P. vulgaris* offers a potential and efficient protocol for mass propagation and conservation of this medicinal herb.Scanning of literature revealed that there is no published report on *in vitro* studies of *P. vulgaris* and the present brief study is,thus the first report of its kind on this plant spp. Since there are no published reports on *P. vulgaris* the results obtained in the present study are discussed in light of a well exploited member of the family Lamiaceae i.e, *Lavendula* spp.

In the present study good shoot multiplication response was observed on various BAP concentrations. Such findings are in accordance with those of Sanchez-Gras and Calvo (1996) who also reported best shoot multiplication rate in nodal bud cultures of *Lavandula latifolia* on BAP(5mM) fortified MS medium. Multiple shoot regeneration has also been reported by Andrade *et al* (1999)in nodal segments of *L. vera* on BAP(4.4mM,8.8mM) augmented MS medium. Similar reports of multiple shoot regeneration in shoot tip cultures of *L. officinalis* on various BAP were recorded by Tyub *et al*.(2004). However, Panizza and Tognoni(1991) and Nobre(1996)while working on nodal cuttings of Lavadin and *L. stoeches*, respectively , observed no effect of BAP on the axillary bud multiplication. In the present investigation addition of NAA to BAP containing induction medium generally reduced number of shoots per explant. This observation is in conformity with those of Sanchez-Gras and Calvo (1996)in nodal bud cultures of *L. latifolia*. However, these results are in contrast with those reported for *L. vera*(Quazi,1980) and *L. latifolia*(Quazi,1980;Calvo and Segura,1989),in which a significant increase in the number of shoots was promoted on media supplemented with combination of BAP and NAA or IAA. Present data also reveals both axillary shoot regeneration and subsequent rooting in the microshoots on BAP(10mM) +NAA(5mM) fortified basal medium. In *L. officinalis* quite similar response was observed (Chishti *et al*.,2003) on BAP(2mg/l)+IAA(1mg/l) supplemented MS(x1/2) medium. Rooting of isolated shoots was achieved on half strength basal medium. Similar results were recorded by Sanchez-Gras and Calvo (1996) in *L. latifolia* and Jordan *et al* (1998) in *L. dentata* on MS(x1/2) basal medium. However, the studies of Andrede *et al*. (1999) revealed enhanced rooting by reducing MS salt concentration to ¼ in *L. latifolia*.

The propagation procedure described in present communication allows the multiple production of plants from a single shoot tip explant. Therefore, the technique offers a high potential for rapid and massive propagation of *P. vulgaris*-a valuable medicinal plant.

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