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In vitro Clonal Propagation of *Paederia foetida* L. - A Medicinal Plant of Bangladesh

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Abstract

Paederia foetida L. is an important perennial climbing medicinal herb. It becomes woody with age. Mass collection of the plant from natural habitats has led to its depletion. We have established a protocol for its mass propaga-tion that would help cultivation of this valuable plant in the homestead. Shoot tips and nodal segments from field-grown plants were used as explants and cultured on MS medium with 1.0 mg/l BA. Proliferation of shoots took place from axillary buds. Subculture of the nodal segments from initial culture on fresh medium gave a higher number of shoots. For rooting, shoots were excised from the proliferating cultures and implanted individually on root induction medium, consisting of half strength MS salts supplemented with 0.1 mg/l of IBA. Within five weeks of transfer, there was good rooting in 95% shoot cuttings. Plantlets were transferred to plastic pots maintained within a polythene tent. After two weeks of acclimatization they were transferred to the open environment where 80% of the plantlets survived.

Introduction

Mass propagation of plant species through *in vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture technology. Recently, there has been much progress in this technology for some medicinal plants. Tissue culture propagation and its importance in conservation of genetic resources and clonal improvement have been described by many workers: Barz et al. (1977), Datta and Datta (1985), Kukreja et al. (1989) and Jusekutty et al. (1993).

P. foetida is a climbing twining shrub emitting a bad smell. The plant is indigenous to the Indian subcontinent and it grows wild in different forests and village groves of Bangladesh. It is an important gregarious medicinal plant.

The leaves are rich in carotene and vitamin C, also contain a high amount of keto alcohol, keto compound and alkaloid.

Leaf juice is astringent and given to children for treatment of diarrhoea. Poultice of leaves are used to relieve distention due to flatulence in herpes and retention of urine. Decoction of leaves dissolves vesical calculi and acts as diuretic. Leaves and roots are also regarded as tonic and stomachache and given to sick and convalescing patients; also used as remedies for diarrhoea, dysentery and rheumatic affections. Roots and barks are used as emetic and in the treatment of piles, inflammation of spleen and pain in chest and liver. Fruit is a specific against toothache (Ghani 1998)

The present study was undertaken to establish a protocol for *in vitro* clonal propagation of *P. foetida*. *In vitro* propagation has a number of advantages over the sexual one in a large scale propagation programme (Abbott 1978). In sexual methods superior genotypes are sometimes lost through gene recombination. By means of micropropagation, superior gene combinations can be preserved practically unaltered.

Materials and Methods

Tender twigs were collected from field grown mature plants of *P. foetida* L. defoliated and sectioned into 2 - 3 nodal segments. They were washed under continuous flashing of running tap water for 30 min and then treated with a solution of the Savlon (5% v/v) for 10 min and finally surface sterilized with $HgCl_2$ (0.1% w/v) for 10 min. Lastly, the material was washed three times with autoclaved distilled water to remove any trace of $HgCl_2$.

The shoot tip and nodal segments were excised from the disinfected material and divided into 1.0 - 1.5 cm pieces with at least one node in each explant. The basal medium used for all the experiments was MS formulation containing 30 g/l sucrose, 7 - 8 g/l agar and supplemented with BA and Kn, either individually or in different combinations with auxins, NAA, IBA or IAA. The media were adjusted to pH 5.7 ± 0.1 and autoclaved at 1.1 kg/cm^2 for 20 min at 121_C. Cultures were incubated at 25 ± 1_C with a photoperiod of 16 h at 2000 - 3000 lux of cool white fluorescent light.

Cultures were initiated in 150 ∇ 25 mm glass tube and subcultured regularly on fresh medium at four-week intervals in 100 ml flasks. The shoots that proliferated from primary explants were isolated and subcultured on fresh medium several times for bulking up shoot culture material. Shoots (3 - 4 cm) were excised from proliferating cultures and implanted on half strength MS

supplemented with either of NAA, IBA or IAA (0.1 - 1.0 mg/l) for rooting. Rooted shoots were transferred to pots under *ex vitro* condition after proper hardening.

Results and Discussion

Nodal and shoot tip explants from field-grown mature plants of *P. foetida* were cultured on MS supplemented with BA and Kn at different concentrations (viz. 0.2, 0.5, 1.0, 2.0 and 5.0 mg/l) for proliferation of axillary shoots. Initial shoot segments containing nodal zone produced multiple shoot buds on all cytokinin supplemented media. Explants from the *in vitro* grown shoots were also incorporated in this experiment along with those derived from mature field-grown plants for proliferation of axillary shoots.

The proliferation efficiency of nodal explants from mature plants was significantly higher than that of shoot tip explants when evaluated six - seven weeks after proliferation. As a supplement, 1.0 mg/l BA showed the best performance of proliferation that produced shoots in 95% of cultured explants (Fig. A). Explants produced the highest number of 7.80 ± 0.56 shoots per culture on the medium with 4.10 ± 0.18 cm average length of shoots per culture (Figs. B - D). When the explants were cultured on Kn based medium, only 45 - 80% of them proliferated. In this treatment, the highest number of shoots per explant and average shoot lengths were 5.60 ± 0.35 and 3.28 ± 0.15 cm for nodal explants, respectively.

The nature and concentration of cytokinin used in this study influenced proliferation of axillary shoots derived from nodal and shoot tip segments of mature plants and *in vitro* raised shoots. At most of concentrations BA was more effective in inducing proliferation of axillary shoots while Kn was considerably less effective. The percentage of explants showing proliferation and number of shoots per culture increased gradually with an increase of cytokinin concentrations from 0.2 - 1.0 mg/l. Further increase in cytokinin concentration from 2.0 - 5.0 mg/l did not improve any of the parameters but reduced shoot proliferation. At the highest level (5.0 mg/l) of cytokinin, explants failed to proliferate any shoots. The results of this experiment also indicated that 1.0 mg/l BA was more suitable than 1.0 mg/l Kn for shoot proliferation (Table 1).

In this experiment, different combinations of cytokinin and auxin were tested. Of these, the most effective combination for axillary shoots proliferation was 2.0 mg/l BA + 0.1 mg/l NAA. In this combination, the

highest percentage (85) of shoots per culture, the total number of 6.70 ± 0.42 shoots per culture, the total number of 4.27 ± 0.26 usable shoots per culture, and an average length of 3.62 ± 0.15 cm shoots per culture were observed.

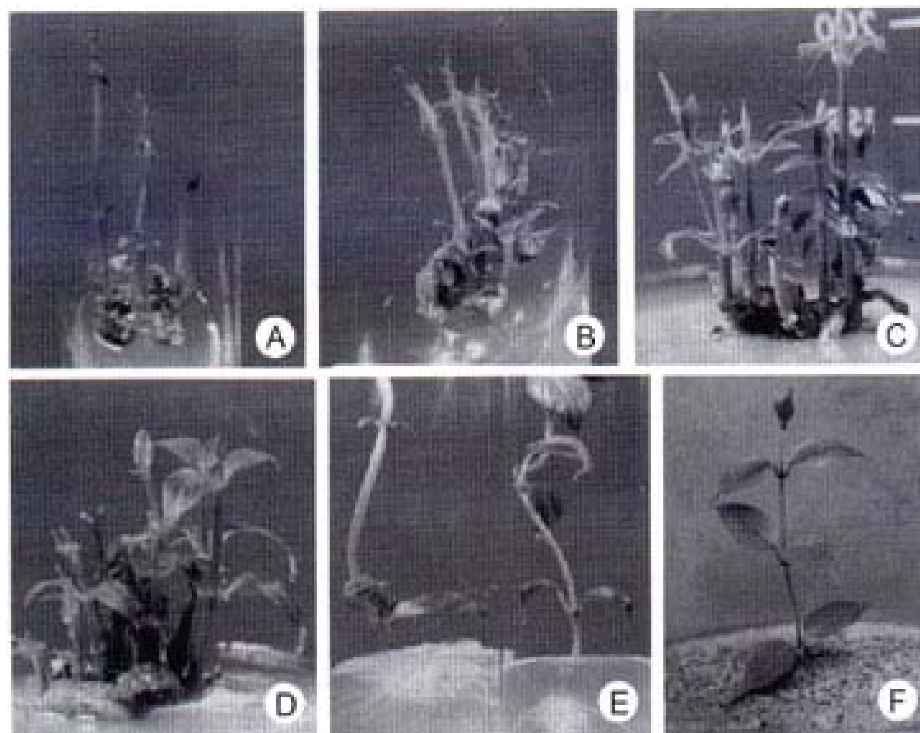
Table 1. Effect of growth regulators on shoot proliferation and number of shoots per culture from nodal explants. Data were taken 8 - 10 weeks after culture.

Growth regulators (mg/l)	% of shoot formation	No. of total shoots/culture	No. of usable shoots/culture	Av. length of shoots/culture
BA				
0.2	60	5.30 ± 0.16	3.10 ± 0.11	3.50 ± 0.24
0.5	80	6.30 ± 0.35	3.50 ± 0.17	3.60 ± 0.16
1.0	95	7.80 ± 0.56	4.50 ± 0.38	4.10 ± 0.18
2.0	55	2.65 ± 0.23	3.00 ± 0.13	3.20 ± 0.25
5.0	-	-	-	-
Kn				
0.2	60	5.10 ± 0.18	3.26 ± 0.16	2.85 ± 0.21
0.5	70	5.45 ± 0.25	3.85 ± 0.29	3.15 ± 0.28
1.0	80	5.60 ± 0.35	4.10 ± 0.30	3.28 ± 0.15
2.0	45	5.15 ± 0.24	3.00 ± 0.13	3.20 ± 0.25
5.0	-	-	-	-
BA + NAA				
0.5 + 0.1	60	5.70 ± 0.12	3.58 ± 0.15	3.00 ± 0.13
0.5 + 0.2	45	4.26 ± 0.42	2.65 ± 0.21	2.63 ± 0.18
1.0 + 0.1	75	6.10 ± 0.35	4.12 ± 0.26	3.25 ± 0.15
1.0 + 0.2	50	4.60 ± 0.16	2.85 ± 0.13	2.80 ± 0.14
2.0 + 0.1	85	6.70 ± 0.42	4.27 ± 0.26	3.62 ± 0.15
2.0 + 0.2	55	5.23 ± 0.13	3.21 ± 0.23	2.95 ± 0.31
BA + IAA				
0.5 + 0.1	40	3.56 ± 0.25	2.56 ± 0.42	2.61 ± 0.24
0.5 + 0.2	50	4.82 ± 0.15	3.25 ± 0.12	2.95 ± 0.13
1.0 + 0.1	40	4.00 ± 0.27	3.00 ± 0.28	2.80 ± 0.25
1.0 + 0.2	60	5.00 ± 0.15	3.58 ± 0.12	3.10 ± 0.13
2.0 + 0.1	35	3.50 ± 0.27	2.50 ± 0.28	2.62 ± 0.25
2.0 + 0.2	45	4.25 ± 0.29	3.10 ± 0.19	2.85 ± 0.17

Root formation was induced in *in vitro* regenerated shoots by culturing them on half strength of MS with 0.1 - 1.0 mg/l either of NAA, IBA and IAA. Among the three types of auxin, IBA was found to be most effective at different concentrations tested for root production on cut margins of the shoot (Table 2).

Among different concentrations, 0.1 mg/l IBA was found to be the best for proper rooting in which 95% shoots rooted within four weeks of culture (Fig. E).

The *in vitro* derived plants acclimated better under *ex vitro* condition when they were transferred on specially made plastic trays containing coco-peat as potting mix and moistened uniformly at periodic intervals taking special care not to damage the roots. The rest of the procedure, followed from this stage up to their establishment in soil was as usual (Fig. F).



Figs. **A - F** : Regeneration of plantlets *in vitro* from the nodal explants obtained from field grown *P. foetida* plants. **A**. Development of axillary shoots from the nodal explant after four weeks of culture. **B**. Development and multiplication of the axillary shoots on MS containing 1.0 mg/l BA after six weeks of culture. **C**. Development and multiplica-tion of axillary shoots on same medium after eight weeks of culture. **D**. Elongation of multiple axillary shoots from the same explant and on the same medium after ten weeks of culture. **E**. Adventitious root formation on regenerated shoots. **F.** *In vitro* raised plantlets grown under *ex vitro* environment in earthen pots.

The shoot tip and nodal explants were cultured on half strength of MS supplemented with BA and Kn at a concentration range of 0.1 - 2.0 mg/l for

assessing the optimum concentration of cytokinins for early sprouting and maximum proliferation of axillary shoots. The proliferation efficiency of nodal explants was significantly higher than that of shoot tip explants, when evaluated 5 - 6 weeks after proliferation. Xim and Zhang (1987) obtained better *in vitro* plantlet regeneration from nodal explants. Begum et al. (2002) also reported that BA was more effective than Kn for proliferation and the development shoots.

Table 2. Effect of different concentration and combination of auxins on adventi-tious root formation from the *in vitro* grown micro-cutting cultured on half strength MS medium. There were 15 - 20 micro-cuttings in each treatment. Data ($\bar{X}$ ± S.E.) were recorded after 6 - 8 weeks of culture.

Types of auxin	Different conc. of auxin (mg/l)	% of micro-cutting rooted	No. of root/micro-cutting	Av. length of the root (cm)
NAA	0.1	85	2.50 ± 0.31	2.10 ± 0.23
	0.2	70	1.90 ± 0.21	1.85 ± 0.41
	0.5	40	1.53 ± 0.18	1.80 ± 0.19
	1.0	-	-	-
IBA	0.1	95	3.50 ± 0.32	3.00 ± 0.25
	0.2	80	2.30 ± 0.23	2.50 ± 0.28
	0.5	55	1.65 ± 0.16	2.45 ± 0.23
	1.0	-	-	-
IAA	0.1	65	2.10 ± 0.21	2.00 ± 0.18
	0.2	40	1.50 ± 0.20	1.85 ± 0.12
	0.5	-	-	-
	1.0	-	-	-

Among the three types of auxin used, IBA was found to be the best for root induction. The findings are in agreement with those observed in other plant species such as *Vitis vinifera* (Islam et al. 2001), *Adhatoda vasica* (Azad and Amin 1998).

About 80 - 95% of the regenerated plantlets could tolerate and survive under *ex vitro* environment or field conditions. A number of plantlets were lost due to damping off and necrosis during acclimatization in *ex vitro* condition. Loss of regenerants due to such symptoms was also observed in *Eucalyptus tereticornis* (Gill et al. 1993), *Solanum nigrum* (Ara et al. 1993), *Rauvolfia serpentina* (Ilahi 1993) and *Rosa damascena* (Kumar et al. 1995). Through this study a protocol for regeneration of complete plantlets has been established. This is perhaps the first report on *in vitro* plant regeneration of *Paederia*

foetida Linn. The results may be of some importance as a pioneering study on tissue culture of this medicinal plant.

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