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MULTIPLE SHOOT REGENERATION IN *CLERODENDRUM INCISUM* L., – AN ORNAMENTAL WOODY SHRUB

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Abstract

A rapid and efficient protocol for induction of multiple shoots from nodal explants of an ornamental woody shrub, *Clerodendrum incisum* L., var. macrosiphon was developed. Nodal explants were inoculated on MS medium containing different concentrations of 6-Benzyladenine (BA) or Kinetin (Kn) alone. Murashige & Skoog (MS) medium supplemented with BA (5 µM) induced maximum number of shoots. The shoots were rooted on half strength of MS medium supplemented with α-naphthalene acetic acid (NAA) and rooted plantlets were established in soil as phenotypically normal mature plants.

Introduction

India has a long tradition of growing flowers. It is however only recently that economic aspects of flower have been exploited both in domestic and in international market. Two important components of the floriculture industry are trade of cut flower and foliage and supply of propagation material including seeds, bulbs, tubers, cutting-raised plants and tissue culture raised plants. However there are a large number of ornamental plants which are in high demand with limited availability, because of slow propagation through conventional techniques, which are in needs of urgent attention for *In vitro* multiplication. One of such species is *Clerodendrum incisum,* which is recently recognized as a beautiful ornamental species because of its shapes of musical notes.

Clerodendrum incisum L., commonly known as **"**musical notes**"** and **"**morning kiss**"** belongs to the family Verbenaceae, is cultivated as an ornamental plant. It is an erect-shrub, intermittently; native to Africa, in Java, it is cultivated as a garden- ornamental (Shrivastava & Patel, 2007). Buds are more interesting than the open flower and the buds look like pure white musical notes standing at the top of the stems. It blooms periodically throughout the warmer months (Ress, 1964).

The leaf extract of the plant has been shown to contain insecticidal properties against mosquitoes (Kalyanasundaram *et al*., 1985). *Clerodendrum incisum* is propagated through cuttings as seed setting is very low. Plant tissue culture is useful tool for the conservation and rapid propagation of plants (Prakash *et al*., 1999) and is also the prerequisite for its genetic transformation (Hu *et al*., 2005). This paper reports a high frequency multiple shoot regeneration in *Clerodendrum incisum* through nodal explants obtained from field grown plants.

Materials and Methods

Healthy plants of *Clerodendrum incisum* L., var. macrosiphon growing in the natural habits in Botanical Gardens, Aligarh Muslim University, Aligarh, India were used to obtain the nodal explants. Nodes (1 cm long), excised from healthy plants were pretreated with Teepol (5% w/v) for 5 min., surface sterilized with 0.1% mercuric chloride for 2-3 min., and finally rinsed with 3-4 times in a sterile double distilled water to remove traces

of mercuric chloride. The nodal explants were inoculated by inserting their cut-ends in the MS medium supplemented with 1, 2, 5 and 10 µM of BA and Kn to induce multiple shoots. The medium contained 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The pH of the medium was adjusted to 5.8 prior to the addition of agar and autoclaved at 121^{o}C at 15 lb pressure for 15 min. The culture was maintained at 25±2^{o}C under a light intensity of 3000 lux provided by cool-white fluorescent lamps and 50-55% relative humidity. For each treatment, 10 replicates were maintained.

For instance of roots, *In- vitro* raised shoots were excised from the culture and inoculated on MS full and half basal medium supplemented with Indole butyric acid (IBA), Indole acetic acid (IAA) and Naphthalen acetic acid (NAA). The shootlets were also cultured on auxin free full and half strength MS medium.

Rooted plantlets were transferred to paper cups containing sterile soilrite (a white granular chemically inert supporting powder) for hardening at diffused light (16/8 hr) photoperiod conditions and covered with plastic bag to maintain 80-90% humidity. Subsequently, the plantlets were transferred to greenhouse and after one month they were planted in the soil.

Results and Discussion

Nodal explants of *Clerodendrum incisum* when cultured on MS basal medium supplemented with BA (1, 2, 5 and 10 µM) and Kn (1, 2, 5 and 10 µM) showed direct shoot bud differentiation after 2-3 weeks of inoculation. Low concentrations of BA (1 and 2 µM) were effective but BA (5 µM) was found to be optimal for highest number of shoot multiplication (Fig. 1; a,b,c). A reduction in number of shoots was noticed at 10 µM. BA was found more effective than Kn for shoot regeneration (Tables 1 & 2).

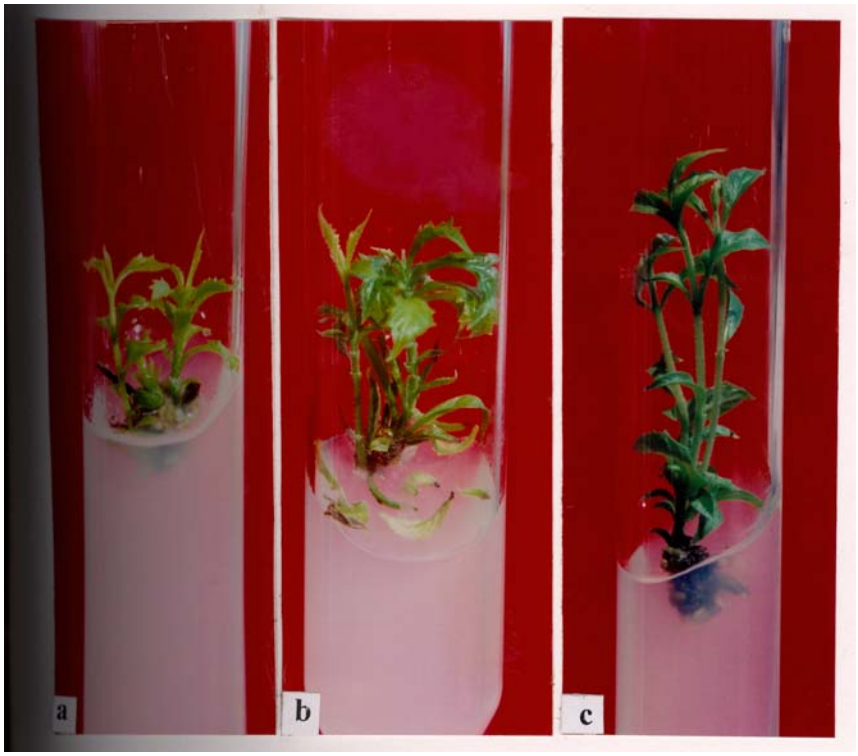

Fig. 1. (a) Induction of multiple shoots from nodal explant on MS+BA (2 µM) – 2 weeks old culture. (b) Proliferation of multiple shoots on MS+BA (2µM) – 3 weeks old culture. (c) Multiple shoot regeneration from nodal explant on MS+BA (5 µM) – 3 weeks old culture.

Table 1. Effect of cytokinin (BA) in different concentrations on shoot regeneration.

S. No.	Medium	% Response	No. of shoot $\overline{X}$ ± S.E.	Shoot length (cm) $\overline{X}$ ±S.E.
1.	MS +BA (1μM)	90	0.10 ± 0.32	0.07 ± 0.22
2.	MS + BA (2 μM)	95	0.09 ± 0.28	0.07 ± 0.23
3.	MS + BA (5 μM)	**98**	0.07 ± 0.24	0.06 ± 0.21
4.	MS + BA (10 μM)	**80**	0.09 ± 0.284	0.04 ± 0.153

Table 2. Effect of cytokinin (Kn) in different concentrations on shoot regeneration.

S. No.	Medium	% Response	No. of shoot $\overline{X}$ ± S.E.	Shoot length (cm) $\overline{X}$ ± S.E.
1.	MS + Kn (1μM)	50	1.5±0.21	1.82±0.148
2.	MS + Kn (2 μM)	60	1.7±0.20	3.3±0.159
3.	MS + Kn (5 μM)	60	1.4±0.15	3.2±0.156
4.	MS + Kn (10 μM)	40	1.2±0.12	2.3±0.147

Table 3. Effect of MS strength and different auxins on root induction from *In vitro* raised shoots.

S. No.	Medium	% Rooting	No. of roots $\overline{X}$ ± S.E.	Root length (cm) $\overline{X}$ ± S.E.
1.	MS	0	-	-
2.	MS ½	20	1.3 ± 0.145	1.6 ± 0.103
3.	MS + IBA (5 μM)	90	2.4 ± 0.290	5.3 ± 0.135
4.	MS½+ IBA (5 μM)	100	3.4 ± 0.352	7.42 ± 0.134
5.	MS + NAA (5 μM)	100	3.5 ± 0.255	4.47 ± 0.109
6.	MS½ + NAA (5 μM)	100	27.8 ± 0.486	2.45 ± 0.114
7.	MS + IAA (5 μM)	70	1.3 ± 0.144	3.59 ± 0.114
8.	MS½ + IAA (5 μM)	90	3.3 ± 0.202	4.32 ± 0.160

The shoots were transferred on full or half strength of MS medium supplemented with IBA, NAA and IAA for the induction of rooting. Half strength of MS medium with NAA was found to be more suitable as compared to full strength of MS medium for rooting of shoots (Table 3). On half strength with NAA (5 μM), a maximum of 27-30 roots with 2-3 cm in length were achieved after 4 weeks of inoculation (Fig. 2; a,b,c).

The plantlets were removed from the culture tubes carefully without destruction of the root system and after washing the roots in water, plantlets were transferred to the plastic cups with soilrite and nurtured with 1/4 MS liquid medium without sucrose (Fig. 3; a,b,c). The cups were covered with polythene bags to maintain the high humidity around the plants. They were kept in growth chamber. The well established plants from cups were transferred to earthen pots with soilrite, manure and sand in 1:1:1 ratio by volume and after one month, acclimatized in natural habits where all plants grew normally. The rooted plantlets obtained from half strength of MS medium with NAA showed 95% survival rate in field condition.

Fig. 2. (a) *In vitro* root induction in microshoot on MS½ + IBA (5 µM) – 5 weeks old culture. (b) *In vitro* root induction from the basal cut end of microshoot on MS½ + NAA (5 µM) – 4 weeks old culture. (c) Induction of roots from basal cut end of microshoot on MS½ + IAA (5 µM) – 4 weeks old culture.

Fig. 3. (a) Acclimatized plantlets – 4 weeks old. (b) An acclimatized plant in soilrite – 5 weeks old. (c) An acclimatized plant in soilrite + soil filled pot, showing branching – 10 weeks old.

Clonal multiplication through various explants is advantageous over conventional propagation method because a large number of plants can be produced within a short duration. The most effective medium for shoot induction and multiplication (4 shoots/node) in *Clerodendrum incisum* L. was achieved on MS medium containing BA, as compared to Kn, at an optimal concentration of 5 µM. This indicates that these explants contain sufficient endogenous level of auxins or capable of its *de novo* synthesis which can induce shoot formation even in a medium containing cytokinin alone (Julliard *et al*., 1992). BA proved superior to other cytokinins for multiple shoot induction in *Clerodendrum colebrookianum* (Mao *et al*., 1995). At higher concentration of BA or Kn the rate of shoot proliferation declined. Similar findings have also been reported in *Psoralea corylifolia* (Saxena *et al*., 1998), *Cleistanthus callinus* (Quraishi *et al*., 1996), *Decalepis arayalpathra* (Gangaprashad *et al*., 2005).

Number of shoots were more in BA containing medium as compared to Kn. Superiority of BA over Kn was also demonstrated by several workers (Rech & Pirer, 1986; Kukreja *et al*., 1991; Van & Kitto, 1990; Sen & Sharma, 1991; Mishra & Bhatnagar, 1995).

Thus, the present findings could be used for conservation and large-scale propagation of this important and economic plant species.

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