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Micropropagation of *Coleus blumei* from nodal segments and shoot tips

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Abstract

A rapid and highly-effective method for micropropagation from nodal segment and shoot tip explants was established for *Coleus blumei* Benth. Nodal segments and shoot tips were inoculated on MS medium containing 0.7 % agar, 3 % commercial sugar, and different combinations of 6-benzyladenine (BA) with indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) or α-naphthaleneacetic acid (NAA). Hundred percent shoot induction from both explants was achieved on the medium containing BA (2 mg dm^{-3}) and NAA (1 mg dm^{-3}). Shoot tips were proved to be the better explant in comparison to nodal segments in having high rate of shoot induction and more number of shoots. The same media conditions were found suitable for shoot multiplication. Multiplied shoots rooted best on MS medium supplemented with IBA (2 mg dm^{-3}). Micropropagated plants were successfully established in soil after hardening, with 100 % survival rate.

Additional key words: agar concentration, carbon source, growth regulators, shoot differentiation, tissue culture.

Introduction

Ornamental plant, *Coleus blumei* Benth., which belongs to the family Lamiaceae, is a natural hybrid of several *Coleus* species. It has attractive foliage and, therefore, it is planted extensively as a decorative indoor and outdoor plant. It is generally vegetatively propagated through cuttings. It is also used for the synthesis of rosmarinic acid, a pharmaceutically important ester of caffeic acid (Petersen *et al.* 1995). In the preceding years, an ample amount of work was done on *Coleus* suspension cultures for the production of rosmarinic acid. Previous attempts on *C. blumei* cultivation include its propagation by seeds and shoots in MS medium (Murashige and Skoog 1962) with 3 mg dm^{-3} 6-benzyladenine (BA) (Marcotrigiano *et al.* 1990), apical meristems with 0.1 - 3.0 mg dm^{-3} indole-3-acetic acid (IAA) (Smith and Murashige 1982), leaf disc with 2 mg dm^{-3} BA and 1 mg dm^{-3} NAA (Ibrahim *et al.* 1992), from stem explants with 10 μM BA (Meinhard *et al.* 1993), and from nodal, internodal and leaf explants with BA alone or BA in combination with IAA (Zagrajski *et al.* 1997). Though a number of workers have investigated *Coleus* species, a standardized protocol for micropropagation of *C. blumei* is yet to be evolved. This paper describes a micropropagation technique for *C. blumei* using nodal segments and shoot tips that can be used for commercial purpose.

Materials and methods

The plants of *Coleus blumei* grown in the glasshouse (Botanic Garden), Guru Nanak Dev University, Amritsar (India) were used as experimental material. Nodal segments containing axillary bud (1.5 - 2.0 cm) and shoot tip (0.5 - 1.0 cm) explants were excised from young shoots. The explants were washed with 5 % (v/v) *Teepol* solution for 10 min, surface-sterilized with 0.05 % (m/v) mercuric chloride for 3 - 4 min and rinsed 3 - 4 times

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Abbreviations: BA - 6-benzyladenine; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; NAA - α-naphthaleneacetic acid; MS medium - Murashige and Skoog's (1962) medium.

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with sterile double-distilled water. An oblique cut was done at the basal end of nodal segments, followed by a vertical cut in the stem in order to increase the area for fast absorption of nutrients from the medium. The explants were cultured singly in culture tubes (150 × 25 mm) containing MS medium supplemented with different concentrations of BA alone and in combination with IAA, indole-3-butyric acid (IBA) and NAA. The pH of each medium was adjusted to 5.6 before the addition of agar (*Qualigens*, Mumbai, India). The cultures were incubated under 16-h photoperiod (irradiance of 40 μmol m^{-2} s^{-1} provided by cool-white fluorescent tubes) and temperature of 25 ± 2 ºC. After 30 d of culture, the shoots were subcultured to their respective fresh medium for shoot multiplication. Different concentrations (0.6 - 0.8 %) of agar were tested to find out its optimum level for maximum shoot induction. The sucrose (3 %) was also replaced with commercial sugar and *shakkar* (locally available crude form of sugar) in order to check the efficiency of the protocol.

For root induction, multiplied shoots of both explants with an average length of 2 cm were separated into single ones and cultured on MS medium supplemented with various concentrations of IBA and NAA. The cultures were incubated at the same conditions. After 30 d, rooted plantlets were transferred to small plastic pots containing garden soil and sand (1:1) mixture. The pots with plants were initially covered with plastic polythene for 12 - 15 d to avoid desiccation. Thereafter, the plastic polythene was removed, and the plants were kept in the culture room (25 ± 2 ºC and 16-h photoperiod) for another 15 d before transfer to the greenhouse for one month and establishment in the field.

For each treatment, 24 tubes were inoculated with the desired explant and the experiments were repeated twice. The data pertaining to number of shoots or roots per culture, shoot and root length were subjected to a one-way analysis of variance (*ANOVA*) and the differences among means were compared by high-range statistical domain (HSD) using Tukey's test (Meyers and Grossen 1974).

Results and discussion

Shoot initiation occurred from axillary buds of nodal segments after 6 - 7 d. In all cultures maximum number and height of shoots were achieved at 2 mg dm^{-3} BA and 1 mg dm^{-3} NAA (Fig. 1, Table 1). BA at 2 mg dm^{-3} in combination with IBA or NAA resulted in increase in number and height of shoots with increase in concentration of IBA or NAA, however, in combination with IAA resulted in decrease in number and height of shoots with increase in concentration of IAA. Shoot tip explant also showed a similar pattern of growth with various hormonal treatments (Table 2). Maximum number and height of shoots were also achieved at 2 mg dm^{-3} BA in combination with 1 mg dm^{-3} NAA (Fig. 2). Our results are in line with some of the previous studies, where BA and NAA were found to be useful in shoot induction from nodal segments and shoot tip explants of various other plants, *e.g. Jasminum officinale* (Bhattacharya and Bhattacharya 1997), *Vanilla planifolia* (George and Ravishankar 1997), *Aristolochia indica* (Manjula *et al.* 1997), *Vitex negundo* (Kannan and Jasrai 1998), *Syzygium travancoricum* (Anand *et al.* 1999) and *Ancistrocladus abbreviatus* (Bringmann *et al.* 1999). Similar combinations of plant hormones were also found to be responsible for callus and plantlet initiation in leaf discs of *C. blumei* (Ibrahim *et al.* 1992). However, in certain other *Coleus* species, *C. forskohlii* (Sen and Sharma 1991) and *C. parviflorus* (Ponsamuel *et al.* 1994), BA alone was sufficient for formation of multiple shoots from nodal segments and shoot tips. Similarly Hiregoudar *et al.* (2005) also reported that addition of BA (2 μM) alone to MS medium is responsible for shoot induction and multiplication in *in vitro* culture of hypocotyls and internodal explants of *Feronia limonia*. In some other studies, kinetin and IAA were also

Table 1. Effect of BA in combination with auxins (IAA, IBA and NAA) on shoot induction from nodal segments of *C. blumei* after 30 days of culture on MS medium containing 0.7 % agar and 3 % commercial sugar. Means ± SE. Mean followed by the same letter are not significantly different using HSD multiple comparison test within the different treatment regimes with particular growth regulator and/or particular combination of plant growth regulators.

Growth regulators [mg dm^{-3}]				Shoot formation	Number of shoots	Height of shoots
BA	IAA	IBA	NAA	[%]	[explant^{-1}]	[cm]
–	–	–	–	41.67±1.00	1.40±0.11	0.95±0.03
1	–	–	–	58.33±1.00	1.68±0.10^{ab}	0.93±0.03^{ab}
2	–	–	–	64.58±0.50	2.16±0.12^{a}	1.08±0.02^{a}
3	–	–	–	56.25±0.50	1.78±0.11^{a}	1.02±0.02^{a}
4	–	–	–	52.08±0.50	1.52±0.10^{ab}	0.90±0.02^{ab}
5	–	–	–	45.83±1.00	1.36±0.10^{ab}	0.77±0.02^{c}
2	0.2	–	–	100.00±0.00	3.19±0.10^{a}	1.04±0.10^{a}
2	0.4	–	–	75.00±1.00	2.50±0.09^{b}	0.94±0.07^{b}
2	0.6	–	–	75.00±0.00	2.33±0.08^{b}	0.87±0.07^{c}
2	0.8	–	–	70.83±1.00	2.20±0.09^{b}	0.70±0.11^{d}
2	1.0	–	–	66.67±1.00	2.09±0.09^{bc}	0.63±0.12^{e}
2	–	0.2	–	75.00±1.00	2.19±0.08^{c}	0.95±0.06^{d}
2	–	0.4	–	81.25±0.50	2.31±0.07^{c}	0.93±0.07^{d}
2	–	0.6	–	83.33±0.00	3.12±0.09^{ab}	1.04±0.07^{c}
2	–	0.8	–	83.33±0.00	3.30±0.09^{a}	1.29±0.08^{b}
2	–	1.0	–	87.50±1.00	3.45±0.08^{a}	1.32±0.08^{a}
2	–	–	0.2	66.67±1.00	2.12±0.09^{c}	0.71±0.11^{e}
2	–	–	0.4	75.00±0.00	2.39±0.08^{c}	1.00±0.08^{bcd}
2	–	–	0.6	91.67±2.00	2.98±0.09^{b}	1.05±0.09^{bc}
2	–	–	0.8	97.92±0.50	3.34±0.09^{a}	1.09±0.08^{b}
2	–	–	1.0	100.00±0.00	3.54±0.07^{a}	1.37±0.08^{a}

Fig. 1. Shoot formation from nodal segment of *Coleus blumei* on MS medium containing BA (2 mg dm^{-3}) and NAA (1 mg dm^{-3}).
Fig. 2. Shoot formation from shoot tip on MS medium containing BA (2 mg dm^{-3}) and NAA (1 mg dm^{-3}).
Fig. 3. Shoot multiplication on the same medium.
Fig. 4. Rooting of shoots on MS medium containing IBA (2 mg dm^{-3}).
Fig. 5. Transplanted plant in pot.

found to be suitable for shoot induction and multiplication from nodal segments and shoot tips of *C. forskohlii* (Sharma *et al.* 1991, Bhattacharya and Bhattacharya 2001). Zagrajski *et al.* (1997) studied the shoot induction from nodal, internodal and leaf explants of *C. blumei* Benth with the use of BA alone or in combination with IAA. They reported that nodal explants were best for shoot induction and multiplication while internodal and leaf explants did not give consistent results.

Among various concentrations of agar (0.6 - 0.8 %), added to MS medium, 0.7 % agar was found to be optimal for shoot induction in maximum number of cultures and production of the highest number and height of shoots (Table 3). Similarly, when different sugars (sucrose, commercial sugar and *shakkar*) were added to MS medium at 3 % concentration, commercial sugar was found to be equally effective as sucrose in producing shoots from nodal segments and shoot tips (Table 4). However, there was a decline in shoot induction percentage, and number and heights of shoots with *shakkar*. Commercial sugar can be used instead of sucrose, as the former is 10 times cheaper than the latter. Similar results of using table sugar as effective for micropropagation of *Solanum tuberosum* and *Rotula aquatica* have been reported by Bains (1991) and Martin (2003), respectively.

Routine sub-culturing of shoots from both the explants in the similar medium (2 mg dm^{-3} BA and 1 mg dm^{-3} NAA) after every 4 weeks resulted in high frequency of multiple shoots (15 - 20 shoots per flask) (Fig. 3). Echeverrigaray *et al.* (2005) reported the highest

Table 2. Effect of BA in combination with auxins (IAA, IBA and NAA) on shoot formation from shoot tips of *C. blumei* after 30 d of culture on MS medium containing 0.7 % agar and 3 % commercial sugar. Means ± SE. Means followed by the same letter are not significantly different within the different treatment regimes with particular growth regulator and/or particular combination of plant growth regulators.

Growth regulators [mg dm^{-3}]				Shoot formation	Number of shoots	Height of shoots
BA	IAA	IBA	NAA	[%]	[explant^{-1}]	[cm]
–	–	–	–	45.83±1.00	1.54±0.11	1.01±0.02
1	–	–	–	62.50±1.00	1.77±0.10^{a}	0.98±0.02^{ab}
2	–	–	–	70.83±1.00	2.20±0.12^{a}	1.12±0.02^{a}
3	–	–	–	64.58±1.50	1.83±0.11^{a}	1.04±0.02^{a}
4	–	–	–	56.25±0.50	1.63±0.12^{ab}	0.93±0.02^{c}
5	–	–	–	47.92±0.50	1.43±0.11^{ab}	0.82±0.03^{d}
2	0.2	–	–	100.00±0.00	3.52±0.07^{a}	2.06±0.09^{a}
2	0.4	–	–	100.00±0.00	2.92±0.08^{b}	1.78±0.07^{b}
2	0.6	–	–	100.00±0.00	2.54±0.07^{c}	1.50±0.06^{c}
2	0.8	–	–	91.67±1.00	2.39±0.07^{cd}	1.27±0.07^{d}
2	1.0	–	–	85.42±0.50	2.17±0.08^{cd}	1.06±0.08^{e}
2	–	0.2	–	81.25±0.50	2.15±0.11^{d}	1.03±0.07^{e}
2	–	0.4	–	83.33±0.00	2.30±0.09^{d}	1.28±0.08^{d}
2	–	0.6	–	85.42±0.50	3.12±0.09^{c}	1.45±0.08^{c}
2	–	0.8	–	100.00±0.00	3.54±0.07^{b}	1.79±0.07^{b}
2	–	1.0	–	100.00±0.00	3.56±0.07^{a}	2.08 0.09^{a}
2	–	–	0.2	75.00±0.00	2.39±0.08^{cd}	1.02±0.09^{e}
2	–	–	0.4	75.00±1.00	2.58±0.10^{cd}	1.24±0.09^{d}
2	–	–	0.6	97.92±0.50	2.89±0.09^{c}	1.49±0.07^{c}
2	–	–	0.8	100.00±0.00	3.31±0.10^{b}	1.85±0.06^{b}
2	–	–	1.0	100.00±0.00	3.75±0.09^{a}	2.25±0.06^{a}

Table 3. Effect of agar concentration on shoot formation from nodal segments and shoot tips of *C. blumei* on MS medium containing BA (2 mg dm^{-3}) and NAA (1 mg dm^{-3}) along with 3 % commercial sugar. Means ± SE. Means followed by the same letter are not significantly different within the different concentrations of agar for each explant.

Explant	Agar conc. [%]	Shoot formation [%]	Number of shoots [explant^{-1}]	Height of shoots [cm]
Nodal segments	0.6	83.33±0.00	2.57±0.09^{c}	1.04±0.07^{c}
	0.7	100.00±0.00	3.54±0.08^{a}	1.38±0.08^{a}
	0.8	91.67±1.00	3.22±0.07^{b}	1.26±0.07^{b}
Shoot tips	0.6	85.42±0.50	2.90±0.10^{c}	1.28±0.08^{c}
	0.7	100.00±0.00	3.75±0.09^{a}	2.25±0.06^{a}
	0.8	100.00±0.00	3.42±0.07^{b}	1.77±0.07^{b}

multiplication rate on MS medium with a combination of BA and IBA in *Lavandula dentata*. Multiplied shoots from both the explants were separated into single ones and transferred to MS medium without plant growth regulators or containing IBA or NAA. Root initiation was observed after 8 - 10 d in MS medium containing IBA (2 mg dm^{-3}) in 100 % cultures and the number of roots was recorded after 30 d of culture (Table 5, Fig. 4). Root induction decreased with increase in concentration of IBA. NAA resulted in comparatively lesser number of roots. IBA at 2 mg dm^{-3} was found to be the best treatment for induction of roots. Our results are in accordance with similar findings on some other plants, *e.g. Elaeagnus angustifolia* (Iriondo *et al.* 1995), *Asparagus robustus* (Nayak and Sen 1998), *Eucalyptus tereticornis* (Sharma and Ramamurthy 2000) and *Hemidesmus indicus* (Sreekumar *et al.* 2000).

Table 4. Effect of different carbon sources (3 % sucrose, commercial sugar or *shakkar*) on shoot formation from nodal segments and shoot tips of *C. blumei* on MS medium containing BA (2 mg dm^{-3}) and NAA (1 mg dm^{-3}) along with 0.7 % agar. Means ± SE. Means followed by the same letter are not significantly different within the different carbon sources for each explant.

Explant	Carbon source	Shoot formation [%]	Number of shoots [explant^{-1}]	Height of shoots [cm]
Nodal segments	sucrose	100.00±0.00	3.54±0.07^{a}	1.38±0.08^{a}
	sugar	100.00±0.00	3.50±0.07^{a}	1.37±0.08^{a}
	shakkar	75.00±0.00	2.30±0.08^{b}	1.02±0.07^{b}
Shoot tips	sucrose	100.00±0.00	3.75±0.09^{a}	2.25±0.06^{a}
	sugar	100.00±0.00	3.56±0.09^{a}	2.23±0.05^{a}
	shakkar	75.00±1.00	2.86±0.08^{b}	1.26±0.08^{b}

Table 5. Effect of IBA and NAA [mg dm^{-3}] on root induction from shoots obtained from nodal segments and shoot tips of *C. blumei* on MS medium. Means ± SE. Means followed by the same letter are not significantly different within the different concentrations with particular growth regulator.

IBA	NAA	Root induction [%]	Number of roots [shoot^{-1}]	Root induction [%]	Number of roots [shoot^{-1}]
–	–	72.92±0.50	11.20±0.08	75.00±1.00	11.60±0.17
2	–	100.00±0.00	31.52±0.33^{a}	100.00±0.00	31.73±0.35^{a}
4	–	91.67±1.00	28.66±0.33^{b}	93.75±0.50	29.87±0.34^{b}
6	–	81.25±0.50	25.28±0.39^{c}	83.33±1.00	24.72±0.37^{c}
–	2	85.42±0.50	24.32±0.32^{a}	87.50±1.00	25.07±0.31^{a}
–	4	75.00±1.00	22.36±0.38^{b}	75.00±1.00	22.05±0.35^{b}
–	6	68.75±0.50	20.03±0.39^{c}	70.83±0.00	18.32±0.35^{c}

Rooted plantlets were established in soil with 100 % survival (Fig. 5). The present study showed shoot tip to be a better explant producing higher number and height of shoots as compared to nodal segments on MS medium containing 0.7 % agar and 3 % commercial sugar along with BA (2 mg dm^{-3}) and NAA (1 mg dm^{-3}). The advantages of the present protocol include simple

medium, cost-effectiveness and hundred percent survival rate of *in vitro*-raised plants in *ex vitro* conditions. Hence, the protocol can be used for commercial production of rosmarinic acid from *C. blumei.*

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