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Callus-Mediated Shoot Organogenesis from Shoot Tips of *Cichorium intybus*

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ABSTRACT

Cichorium intybus L. is a medicinally important plant with anti-cancerous and anti-hepatotoxic properties. An efficient method for totipotent callus formation has been developed in *C. intybus* from the basal portion of shoot tip explants on MS medium supplemented with different concentrations of plant growth regulators (PGRs) like 6-benzylamino purine (BAP) and kinetin (Kn) with an auxin, indole-3-butyric acid (IBA). Cultures growing under the influence of BAP+IBA produced considerably more callus than cytokinins used alone. Re-differentiation of such callus led to multiple shoot formation on the same medium after 3 weeks. Isolated shoots were individually rooted in the presence of different concentrations of IBA. Plantlets obtained were transplanted into small pots containing peat, vermiculite, sand and soil mixture (1:1:1:1), 60% of which survived.

Keywords: Asteraceae, phytohormones, re-differentiation
Abbreviations: BAP, 6-benzylamino purine; **IBA**, indole-3-butyric acid; **Kn**, kinetin; **MS**, Murashige and Skoog; **PGR**, plant growth regulator

INTRODUCTION

Cichorium intybus L. (chicory) is a member of the Asteraceae. It is distributed in Northern and Southern Europe and Turkey (Bais and Ravishanker 2001). Chicory has been successfully cultivated in India since 1918 in Coimbatore and subsequently Nilriris in Tamil Nadu and at Broach, Amalsad and Jamnanagar in Gujrat (Muthuswami and Pappiah 1980). Chicory forms flowering shoots and seeds after overwintering. Initially, chicory seeds were imported into India but are now successfully produced locally (Anonymous 1992). Phytochemicals or plant constituents are distributed throughout the entire chicory plant but the main constituents are present in the roots. The tuberous roots of this plant contain a number of medicinally important compounds such as inulin, lactones, coumarins, flavonoids and vitamins (Varotto and Lucchin 2000). Bitter substances found in this plant are lactucin, lactucopicrin and esculentin (Chopra *et al*. 1956). The plant root is used as an anti-ulcerogenic, anti-inflammatory and appetizer. It is also used to cure various heart diseases and has anti-hepatotoxic (Zafar and Ali 1998) and antibacterial (Petrovic *et al*. 2004) activity. This plant is used in the treatment of AIDS, colon cancer and insomnia (Duke 1983). It is useful in vitiated conditions of cephalalgia, heapatomegaly, inflammation and asthma (Nadkarni 1976). *In vitro* regeneration of *C. intybus* has been previously reported through shoot organogenesis using different explants (e.g. roots, shoots, leaves, nodal buds, petioles, etc.) and with various plant growth regulator (PGR) combinations (Profumo *et al*. 1985; Pieron *et al*. 1993; Kamili *et al*. 2003; Rehman *et al*. 2003). This paper describes the development of an indirect regeneration protocol from cultured shoot tips of *C. intybus*.

MATERIALS AND METHODS

Seeds of *C. intybus* obtained from Pusa, New Delhi, India were used as experimental material. Seeds soaked overnight were washed with a few drops of laboratory detergent (Labolene) and 2-3 drops of Tween-20 (surfactant) after washing under running tap water. Chemical sterilization of seeds was achieved by treating them with 0.2% of $HgCl_2$ for 10 min. Finally they were washed with autoclaved double distilled water 3-4 times to remove all traces of sterilant and then germinated on Murashige and Skoog (MS) basal medium (1962) containing 3% (w/v) sucrose (Hi-Media, Mumbai, India) and 0.8% (w/v) difcobactoagar (Hi-Media). Shoot tip explants obtained from aseptically grown seedlings were inoculated onto MS medium containing different concentrations of Kinetin (Kn), 6-benzylamino purine (BAP), and BAP+IBA (indole-3-butyric acid)(all PGRs from Hi-Media). After 4 weeks shoots were singled out and transferred to rooting medium. The pH of media used was adjusted to 5.5 using 1N HCl or 1N NaOH before autoclaving the medium at 121°C for 20 min. After inoculation, all the cultures were incubated under cool fluorescent tubes in a 16-h photoperiod with a light intensity of 1500–3000 lux at a constant temperature of 25 ± 3°C; relative humidity of 60–70% was maintained. For the hardening-off procedure, plantlets were washed with sterile distilled water to remove traces of medium and agar and then transferred to plastic pots containing peat, vermiculite, sand and soil mixture (1: 1: 1: 1, v/v). Experiments were set up in a Randomised Block Design and all the experiments were repeated three times and 10 replicates were used for each treatment. Observations were recorded for the number of shoots/explant, length of shoots. Mean and standard deviation were calculated for each treatment.

RESULTS AND DISCUSSION

After 2 weeks of incubation on MS medium fortified with either Kn (2.5-10 µM) or BAP (1-7 µM) alone, shoot tips started callusing at their cut ends. After 4 weeks both the cytokinins (CKs) used induced shoots via callus. BAP at 7 µM produced a maximum of 15.2 shoots/explant (**Table 1**) with an average length of 3.5 cm (**Fig. 1A**). As the BAP

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Research Note

Table 1 Response of *in vitro* raised shoot tips of *Cichorium intybus* to different concentrations of BAP.

PGR	% Response	Callusing	Shoot no. (Mean ± SD)	Average shoot length (cm) ± SD
BAP (1 µM)	88	++	4.8 ± 1.55	6.5 ± 0.35
BAP (2 µM)	85	++	6.4 ± 1.9	6.25 ± 0.25
BAP (3 µM)	90	+++	8.8 ± 2.8	6 ± 0.70
BAP (4 µM)	90	+++	13.1 ± 2.99	5.75 ± 0.50
BAP (7 µM)	85	+++	15.2 ± 3.8	3.5 ± 0.57

+ (minimum callusing), ++ = (moderate callusing), +++ (maximum callusing); Growth period = 6 weeks; Data represents mean ± SD. The experiment was repeated three times.

Table 2 Response of *in vitro* raised shoot tips of *Cichorium intybus* to different concentrations of kinetin.

PGR	% Response	Callusing	Shoot no. (Mean ± SD)	Average shoot length (cm) ± SD
Kn (2.5 µM)	80	+	4.2 ± 0.77	3.98 ± 0.31
Kn (5.0 µM)	85	+	6.4 ± 1.0	4.1 ± 0.66
Kn (7.5 µM)	85	+	7.6 ± 1.0	4.4 ± 1.31
Kn (10 µM)	85	++	6 ± 1.0	4.7 ± 1.16

+ (minimum callusing), +++ (maximum callusing); Growth period = 6 weeks. Data represents mean ± SD. The experiment was repeated three times.

Table 3 Response of *in vitro* raised shoot tips of *Cichorium intybus* to different concentrations of BAP + IBA.

PGR	% Response	Callusing	Shoot no. (Mean ± SD)	Average shoot length (cm) ± SD
BAP (4 µM) + IBA (1 µM)	88	+++	19.5 ± 4.135	3 ± 0.70
BAP (4 µM) + IBA (2 µM)	85	++++	22.36 ± 5.24	3.2 ± 0.81
BAP (4 µM) + IBA (3 µM)	90	++++	24 ± 4.7	3.5 ± 0.54
BAP (4 µM) + IBA (4 µM)	90	++++	18.4 ± 2.99	3.8 ± 0.81

+ (minimum callusing), +++ (moderate callusing), ++++ (maximum callusing) Growth period = 6 weeks. Data represents mean ± SD. The experiment was repeated three times.

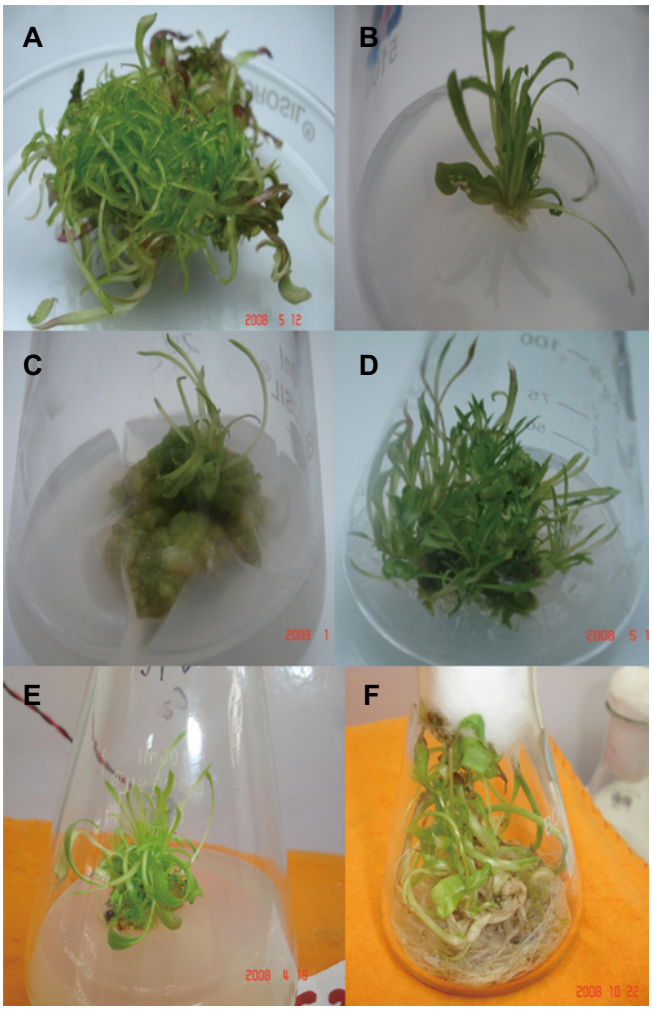

Fig. 1 ***In vitro*** **propagation and acclimatization of** ***Cichorium intybus*** **through indirect callus culture.** (**A**) MS+ BAP (7µM), (**B**) MS+ Kn (7.5 µM), (**C**) MS+ BAP (4 µM) + IBA (3 µM), (**D**) MS+ BAP (4 µM) + IBA (3 µM), (**E**) MS+ Kin (7.5 µM) + IBA (3 µM), (**F**) MS+ IBA (10 µM).

concentration increased, shoot length decreased. Multiple shoot formation from nodal explants via callus with 15 µM BAP was reported by Kamili *et al*. (2003); Veklaylayutham *et al.* (2003) also reported multiple shoots of *C. intybus* from callus using 4.4 µM BAP. Induction of shoots using BAP has been documented in other related important medicinal herbs like *Niger* (Nikame and Shitole 1993), *Anageisus* (Kaul *et al.* 1992), *Thevetia* (Kumar and Kumar 1995), and *Piper* spp. (Bhat *et al.* 1995). Among various concentration of Kn used (2.5-10 µM), the maximum average number of shoots/explant formed in the presence of 7.5 µM Kn was 7.6 and average shoot length was 4.4 cm (**Table 2; Fig. 1B**). Yucesan *et al.* (2007) also reported that Kn alone at 35.36 µM induced most shoots from lamina explants of *C. intybus* and also reported BAP to be more effective than Kn in terms of callus formation and mean number of shoots/explant.

In another trial auxin (IBA) + CK (BAP) and auxin (IBA) + another CK (kinetin) were used together interactively, enhancing callus formation and shoot multiplication. Callus was more compact, nodular and green than all other concentrations and combinations of different PGRs used and maximum callus was obtained on MS medium supplemented with 4 µM BAP + 2 µM IBA, 4 µM BAP + 3 µM IBA and 4 µM BAP + 4 µM IBA and a maximum number of 24 shoots/explants were regenerated from 4 µM BAP + 3 µM IBA acquiring a length of 3.5 cm (**Table 3; Fig. 1C, 1D**). However, at 7.5 µM Kn + 3 µM IBA a maximum of 13.0 shoots were recorded with an average length of 3.8 cm (**Table 4; Fig. 1E**) Yucesan *et al.* (2007), Rehman *et al.* (2000) and Velayutham *et al.* (2006) all reported an increase in shoot multiplication in *C. intybus* when a combination of auxins and CKs were used.

For rooting, multiple shoots were singled out and cultured on MS medium containing different concentrations of PGRs; however, 10 µM IBA produced dense and healthy roots (**Fig. 1F**). Acclimatized plantlets showed a 60% survival rate. This study resulted in the establishment of a protocol for callus-mediated organogenesis of *C. intybus* through *in vitro* raised shoot tips. Through callus a number of shoots can be achieved which could improve the growth and yield of *C. intybus* to meet the demand of the pharmaceutical industry.

Table 4 Response of *in vitro* raised shoot tips of *Cichorium intybus* to different concentrations of Kn + IBA.

PGR	% Response	Callusing	Shoot no. (Mean ± SD)	Average shoot length (cm) ± SD
Kn (7.5 µM) + IBA (1 µM)	75	++	6.2 ± 0.81	3 ± 0.90
Kn (7.5 µM) + IBA (2 µM)	85	++	9.0 ± 0.77	3.8 ± 0.81
Kn (7.5 µM) + IBA (3 µM)	85	+++	13 ± 0.77	3.5 ± 0.74
Kn (7.5 µM) + IBA (4 µM)	80	++	8.2 ± 1.99	3.3 ± 0.71

+ (minimum callusing), ++ (moderate callusing), +++ (maximum callusing) Growth period = 6 weeks. Data represents mean ± SD. The experiment was repeated three times.

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