Journal of Research & Development, Vol.3 (2003)
ISSN 0972-5407

In Vitro Plant Regeneration in *Cichorium intybus* L. through Nodal Culture

Azra N. Kamili, Sabeena Bashir, M. Ramzan Paray*, Iqbal H. Qadri and A. M. Shah

Plant Tissue Culture Laboratory, Centre of Research for Development,
The University of Kashmir Srinagar-190006, J&K, India
*Department of Biotechnology, Unique College , Jawahar Chowk, Bhopal

ABSTRACT

A reproducible protocol was developed for micropropagation of *Cichorium intybus* through *in vitro* culture of mature nodal explants. Multiple shoots (axillary and adventitious) were induced on Murashige and Skoog's (half salt strength) medium supplemented with various phytohormonal regimes. Nodular callus proliferation, shoot differentiation and its elongation was observed BAP(15 M) enriched MS(1/2) basal medium. Maximum number of shoots (13.1 ± 0.8) developed on this medium. The shoots (3-6cm long) were separated and transferred to fresh medium for further elongation. A lesser number of multiple adventitious shoots regenerated with a combination of BAP(10 M) +NAA(5 M). The rooting of these shoots was achieved on the basal medium within 2 weeks of culturing. However, with IBA (2.5 m) root initiation occurred after 4 weeks of culturing.

Keywords: *Cichorium intybus*, nodal segments, callus, multiple shoots.

Abbreviations: MS (× ½) - Murashige and Skoog (half salt strength); IBA- Indole-3-butyric acid; NAA-napthaleneacetic acid; 2,4-D- 2,4 dichlorophenoxy acetic acid; IAA-indole-3-acetic acid; BAP-6-benzyl amino purine; Kn-kinetin; CH-casein hydrolysate.

INTRODUCTION

Cichorium intybus Linn. (Asteraceae) is a perennial Mediterranean herb popularly called as Chicory. The plant grows as wild in Kashmir. It has a long history of herbal use and is especially of great value for its tonic effect upon the liver and digestive tract (Chevallier, 1996). The root and leaves are appetizer, cholagogue, depurative, digestive, diuretic, hypoglycaemic, laxative and tonic (Grieve, 1984; Chiej, 1984; Launert, 1981; Lust, 1983; Foster and Duke, 1990). Chicory is rich in the fibrous polysaccharide inulin. Inulin is reported to have a variety of health benefits such as reducing the risk of obesity, heart diseases, non-insulin dependent diabetes, intestinal infection, osteoporosis and colon cancer

(Balasubramanian, 2000). A decoction of the root has proved of benefit in the treatment of Jaundice, liver enlargement, gout and rheumatism (Grieve, 1984). The latex in the stem is applied in warts in order to destroy them (Duke and Ayensu, 1985). The plant merits research for use in heart irregularities (Foster and Duke, 1990). Micropropagation has been successfully used for large scale multiplication of number of medicinal plants (Vincent *et al.*, 1992; Krishan and Seeni, 1994; Komalavalli and Rao, 2000; Mustafa *et al.*, 1997; Manickam *et al.*, 2000; Segio *et al.*, 2000). The present communication describes a protocol for successful micropropagation of *Cichorium intybus* using nodal explants from mature plants.

MATERIAL AND METHODS

Nodal explants were collected from young and healthy plants naturally growing in the Kashmir University Campus. The explants were thoroughly washed with tap water using a detergent cedpol (0.5% v/v) and 2 -4 drops of Tween-20 (surfactant) for 10 minutes and then these were surface sterilized with 0.1% HgCl2 (w/v) solution for 17 minutes, followed by repeated rinsing with autoclaved double distilled water. The explants were then cultured on MS (x½) basal medium (here after referred as MS) enriched with different phytohormones. The rest of the methodology followed was same as in our previous communications.

RESULTS

Effect of various phytohormonal regimes on nodal explants of *C.intybus* are summarized in Table 1.

Caulogenic phase

Nodal explants (1cm long) when cultured on MS medium augmented with 2,4-D (5µM) resulted in light green nodular callus formation all over the surface of the explant (Fig. 1a). Subculturing of this callus on BAP (5µM) enriched medium favoured multiple indirect microshoot regeneration along with nodular callus proliferation (Fig. 1b). Shoot elongation occurred on hormone free medium. In another trial, medium was supplemented with NAA (5µM), which did not favour callus formation, instead a single shoot was formed from the nodal bud. After a period of six weeks root initiation was also recorded from this shoot on the same medium (Fig.1c). These plantlets elongated further upto 6cm showing differentiation of multiple axillary and apical buds (average no. 6) on the same medium.

Table 1. Morphogenetic response of Nodal explants (primary cultures)of *Cichorium intybus* to various Phytohormonal regimes.

Growth medium	Response [a]	Degree of callus formation	Percentage response		No. of shoots/ Nodal explant [b]
			Callus	Caulogenesis	
MS Basal	--	---	---	--	---
MS +2,4-D (5 µM)	Light green nodular callus formation.	+	100	--	--
MS +NAA (5 µM)	Single shoot formed from the nodal bud, short lateral adven-- titious roots at the basal end of shoot.	--	--	100	1
MS + BAP (15 µM) 0	Light green nodular callus formation at basal end of explant, axillary shoot formation, mult-iple shoot regeneration via callus redifferen- tiation.	++	100	100	13.1 ± 0.8
MS + BAP (10µM)+ NAA(5µM)	Green nodular callus at basal end, multiple adventitious shoot rege-neration via callus.	++	100	80	6.5 ± 0.4

a. Data scored after 6- weeks of culture period.
b. Mean ± SE, 10 replicates/treatment
no growth; + low growth; ++ moderate growth.

When nodal segments with axillary buds were cultured on BAP (15μM) fortified medium, besides axillary shoot initiation, nodular callus proliferation was also observed at the basal end of the explant. Multiple shoot formation via callus regeneration was stimulated on the same medium. Maximum shoot elongation (3-6 cm) and its proliferation was recorded after sub-culturing on the same medium (Fig. 1d).

Under the combined influence of BAP (10μM) and NAA (5μM) nodular callus proliferation was observed at the basal end of the explant. This was again followed by multiple shoot regeneration and elongation.

Rhizogenic phase

The *in vitro* raised multiple shoots produced in different trials were separated and sub-cultured either on MS basal medium or IBA (2.5μM) enriched medium for root initiation. On basal medium shoot elongation and multiple root formation was observed in 100% shoots after 2 weeks of culturing (Fig. 1e). Shoot elongation and dense root formation (100%) was also recorded on IBA (2.5μM) supplemented medium after 4 weeks. The roots were thin and associated with numerous white tufts of root hairs.

DISCUSSION

The present investigation carried on nodal explants of *Cichorium intybus* has shown that the explant possesses great potential for plant regeneration at a much faster rate. The regenerants were recovered both from direct and indirect pathway. Present study revealed that 2,4-D (5μM) fortified medium favoured nodular callus formation which after subculturing on BAP (5μM) enriched medium produced multiple adventitious shoots. Maximum number of shoot regeneration was achieved on higher BAP (15μM) concentration through callus as well as directly from the nodal region. Multiple shoot regeneration has also been reported in number of medicinally important plants on BAP supplemented medium viz *Artemesia* annua (Whipkey *et al.*, 1992), *Plectranthus vetiveroides* (Sivasubramanian *et al.*, 2002) and *Rotula aquatica* (Sebastian *et al.*, 2002). Very recently Rehman et al., (2002) also reported indirect multiple plant regeneration in leaf explants of *Cichorium intybus* in presence of IAA (2μM) + Kn (5μM) + CH (1000mg/litre). Present results also reveal multiple shoot regeneration under the combined influence of auxin (NAA) and cytokinin (BAP). Although not much published data on micropropagation of *C.intybus* is available but in other members of family Asteraceae plant regeneration has also been reported under the influence of auxin cytokinin interaction (Arora and Bhojwani, 1984 in *Saussurea*

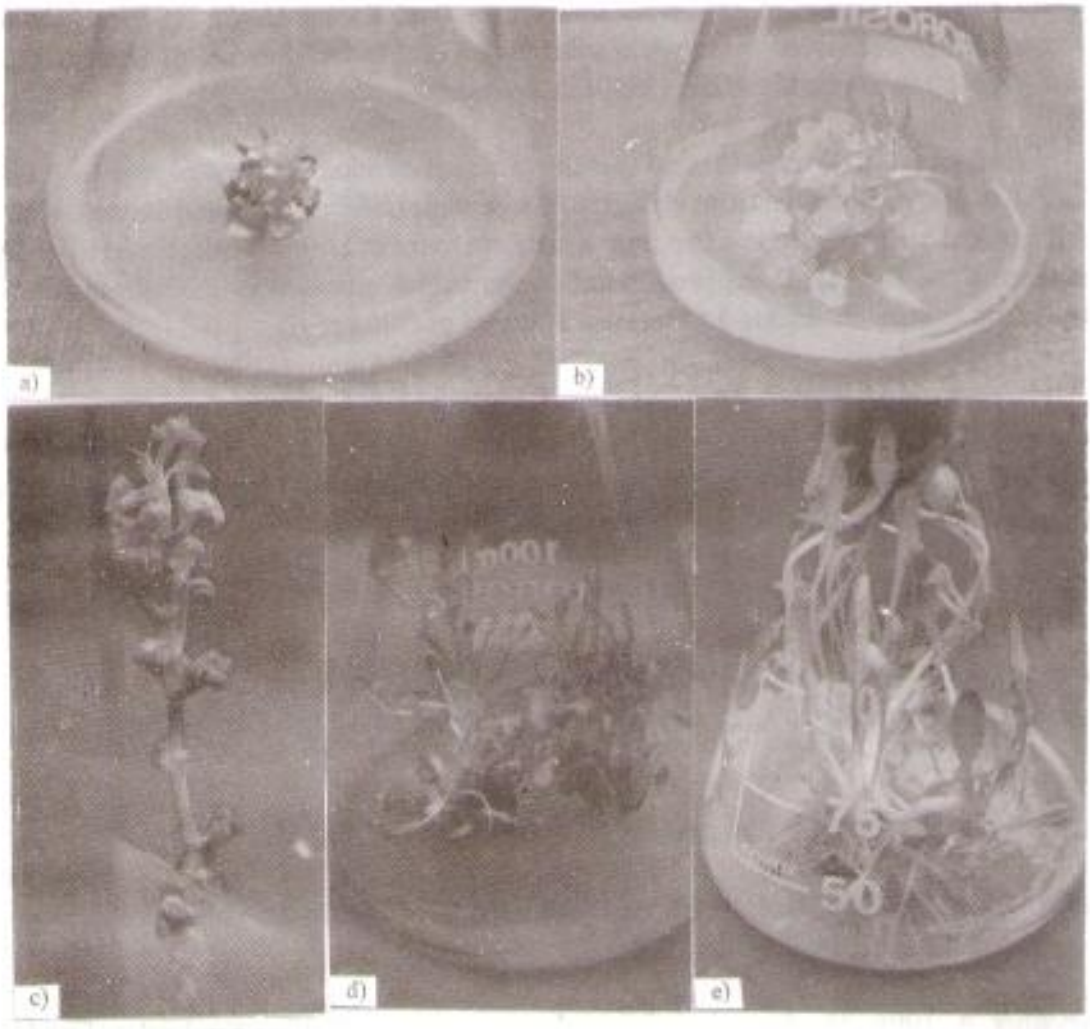

Fig. 1: (a-e) Morphogenetic response of nodal explants of Cichorium intybus L. to various phytohormonal regimes.

a) Formation of nodular callus on MS + 2,4-D (5 M). (After 6 weeks).
b) Nodular callus proliferation and multiple shoot regeneration on MS + BAP (5 M). (After 6 weeks).
c) Formation of plantlet with many adventitious roots on MS + NAA (5 M). (After 8 weeks).
d) Multiple shoot proliferation and elongation on MS + BAP (15 M). (After 6 weeks).
e) Profuse rooting and full elongation of shoots on MS basal medium. (After 2 weeks).

lappa., Nin *et al*., 1996 in *Artemesia absinthium* and Kamili *et al*., 2001 in *A.annua*) Isolated shoots developed roots when subcultured on MS basal medium. Similar results were achieved by Benjamin *et al*., (1990) in *Artemesia pallens*.

The results of the paper reveal that the protocol developed for plant regeneration in *C. intybus* has the potential to be utilized for large scale multiplication and conservation of the medicinal herb. The studies also form a platform to initiate other work related to genetic transformation and increased production of secondary metabolites through callus and suspension culture at various developmental stages of cultures.

ACKNOWLEDGEMENT

The authors are highly thankful to Director, CORD for providing laboratory facilities to carry these investigations.

REFERENCES

Arora, R. and Bhojwani, S.S. 1989. *In vitro* propagation and low temperature storage of *Saussurea lappa* C.B. Clarke-an endangered medicinal plant. *Plant Cell Rep.* **8**: 44-47.

Balasubramanian, D.2000. Nutraceuticals- functional food. *The Hindu* April 27. pp.22.

Benjamin, B.D., Sipahimalani, A.T. and Heble, M.R. 1990. Tissue culture of *Artemisia pallens*. *Plant Cell Tiss. Org. Cult.* **21**: 159-164.

Chevallier. A. 1996. *The Encyclopedia of Medicinal plants*. Dorling Kindersley, London.

Chiej. R. 1984. *Encyclopedia of Medicinal plants*. Macdonald.

Duke, J.A. and Ayensu, E.S.1985. *Medicinal plants of China*. Reference Publications, Inc.

Foster, S. and Duke, J.A. 1990. *A Field Guide to Medicinal plants of Eastern and Central N. America*. Houghton Mifflin Co.

Grieve, 1984. *A Modern Herbal*. Penguin.

Juliani, H.R (Jr.)., Koroch, A.R., Juliani, H.R. and Trippi, V.S. 1999. Micro propogation of *Lippia junelliana* (Mold.) *Tronc. Plant Cell Tiss. Org. Cult.* **59**: 175-179.

Kamili, A.N., Kaloo, Z.A. and Shah, A.M. 2001. Plant Regeneration from Callus Cultures of *Artemisia annua* Linn. *J.Res. Dev.* **1**: 100-106.

Komalavalli, N. and Rao, M.V. 2000. *In vitro* Propagation of *Gymnema sylvestre*- a multipurpose medicinal plant. *Plant Cell Tiss. Org. Cult.* **61**: 97-105.

Krishan, P.N. and Seeni, S. 1994. Rapid micropropagation of *Woodfordia fruticosa* (L.) Kurz (Lythraceae) a rare medicinal Plant. *Plant Cell Tiss. Org. Cult.* **14**: 55-58.

Launert, E. 1981. *Edible and Medicinal Plants*. Hamlyn.

Lust, J. 1983. *The Herb Book*. Bentam Books.

Manickam, V.S., Elango Mathavan, R. and Antonisamy, R. 2000. Regeneration of Indian ginseng plantlets from stem callus. *Plant Cell Tiss. & Org. Cult.* **62**: 181-185.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and biossays with tobacco tissue cultures. *Physiol. Plant.* **15**: 473-493.

Mustafa, A., Harikrishnan, P.H., Martin, K.P. and Harikrishnan, M. 1997. *In vitro* propagation of *Kaempferia rotunda* L. Indian crocus- a medicinal plant. *Phytomorphology*. **47**: 281-286.

Nin, S., Morosai, E., Schiff, S. and Bennici, A. 1996. Callus cultures of *Artemisia absinthium* L. : initiation, growth optimization and organogenesis. *Plant Cell Tiss. Org. Cult.* **45** (1) : 67-72.

Rehman, R.U., Israr, M., Ahmad, S., Fazili, I.S., Jamal, A., Srivastava, P.S. and Abdin, M. Z. 2002. Regeneration and esculin accumulation in Chicory (Cichorium intybus L.). pp. 24. *Proc. Natl. Semin. Recent Res. Trends in Life Sci*. CORD., University of Kashmir, Srinagar (Abstract).

Sebastian Delse, P., Benjamin, S. and Hariharan, M. 2002. Micropropagation of *Rotula aquatica* Lour.-an important woody medicinal plant. *Phytomorphology* **52** (2 & 3): 137-144.

Segio, E., Simone, B., Fernando, F. and Lucian, A. 2000. Clonal micropropagation of Roman chamomile (*Anthemis nobilis* L). *J. Herbs, Spices and Medicinal Plants*. 7: 35-41.

Sivasubramanian, S., Vallinayagam, S., Patric Raja, D. and Manickam, V.S. 2002. Micropropagation of *Plectranthus vetiveroides* (Jacob) Singh and Sharma- a medicinal Plant. *Phytomorphology*. **52** (1): 55-59.

Vincent, A., Mathew, K.M. and Hariharan, M. 1992. Micropropagation of *Kaempferia galanga* L.- a medicinal plant. *Plant Cell Tiss. Org. Cult.* **28**: 229-230.

Whipkey, A., Simon, J.E., Charles, D.J. and Janick, D.J.1992. *In vitro* production of *Artemisia annua* L. *J. Herbs, Spices and Medicinal Plants*. **1** (182): 15-22.