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RESEARCH ARTICLE

IN VITRO SHOOT REGENERATION IN *Cichorium Intybus* L. –AN IMPORTANT MEDICINAL PLANT

Iqbal H Qadri

Centre of Research for Development,(CORD), The University of Kashmir Srinager--190006, J & K, India

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ABSTRACT

An efficient method for totipotent callus formation and whole plant regeneration has been developed for chicory (*Cichorium intybus* L.). Totipotent calli of chicory were induced from leaf, intermodal and root explants on MS medium supplemented with different concentrations of NAA and 2,4-D at 0.5-10 µM in combination with BAP (2 µM).The best callus was developed on medium supplemented with 2,4-D (10 µM) and BAP(2 µM) combination .These calli were transferred to shoot regeneration medium containing MS basal medium with different concentrations and combinations of BAP, KIN and IAA. Maximum number of shoots was obtained on MS medium with BAP (4 µM) + IAA (1 µM).

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INTRODUCTION

Chicory (*Cichorium intybus* L.), a member of *Asteraceae*, is a biennial plant, and is one of the important medicinal plants cultivated throughout India. The whole plant (root, shoot and leaf, is used medicinally, because of thepresence of inulin, a major reserve carbohydrate, bitter sesquiterpene lactones, coumarins, flavonoids and vitamins. It is used as anti-hepatotoxic, antiulcerogenic, anti-inflammatory, appetiser, digestive, stomachic, liver tonic, cholagogue, cardiotonic, depurative, diuretic, emmenagogue, febrifuge,alexeteric and tonic. The chicory plant is used for treating AIDS, cancer, diabetes, dismenorrhoea, impotence, insomnia, splenitis, and tachycardia. The plant grows wild in Kashmir and has along history of herbal use as liver tonic and correcting digestive disorders (Chevallier, 1996, Grieve, 1984, Launert. 1981, lust, 1983, Foster and Duke,1990).Chicory is rich in inulin, which has variety of health benefits, such as reducing risk of obesity, osteoporosis and risk of colon cancers Balasubramanniam, 2000). Although different workers have reported invitro studies on regeneration of different species of chicory (Vincet et al,1992; Krishan and Seeni,1994,Komalavalli and Rao,2000) but the present study has worked out a complete protocol for invitri regeneration of *Cichorium intybus* plantlets from the leaf and root calli .

Corresponding author: **Iqbal H Qadri,**
Centre of Research for Development,(CORD), The University of Kashmir Srinager--190006, J & K, India.

MATERIALS AND METHODS

Leaf and root explants from the young and healthy field grown plants were collected from the Kashmir university campus. The explants were thoroughly washed with tap water using a lab detergent Cedpol(0.5%) along with 2-3 drops of Tween-20 (Surfactant) for 10 minutes, followed by surface sterilization with 0.1% Mercuric chloride solution for 10 minutes. Sterilization treatment was followed by three times rinsing with double distilled water. The explants were then cultured on MS (half basal) medium, enriched with different concentrations of Auxins for callus induction.

RESULTS

Callus Induction: Callus induction was observed in Leaf and root segments after four weeks of culture period on Ms (half salt) medium supplemented with NAA or 2,4-D(5-10µM) in combination with BAP(2 µM). The best type of callus was developed on medium supplemented with 2,4-D (10 µM) and BAP(2 µM) combination .Leaf explants developed aggressive friable callus as compared to root explants after subculturing on same medium after eight weeks of culture period (Fig.1&2).Among both types of auxins (NAA and 2,4-D) used for callus induction,2,4-D proved to be more efficient in callus induction in leaf segments as compared to root segements. The nodular callus developed so was subjected to shoot regeneration medium.

Table 1. Morphogenetic response of leaf and root explants of Cichorium intybus on various phytohormonal combinations

BAP	NAA	2,4-D	IAA	Kn	Response%	Degree of callogenesis	Percentage shoot development
2 µM	5 µM	---	---	---	30	Mild growth of callus	---
2 µM	7 µM	---	---	---	60	Moderate growth	---
2 µM	10 µM	---	---	---	70	Moderate growth	---
2 µM	---	5 µM	---	---	50	Nodular with slow growth	---
2 µM	---	7 µM	---	---	80	Nodular with moderate growth	---
2 µM	---	10 µM	---	---	100	Nodular green callus with aggressive growth	--
2 µM	---		1 µM	---	10	-	-
4µM	---	---	1 µM	---	80	Highest shoot initiation	80
2 µM	---	---	---	1 µM	30	Mild response	10
4µM	---	---	---	1 µM	50	Moderate Response	40

Data scored after 4 weeks of culture period.

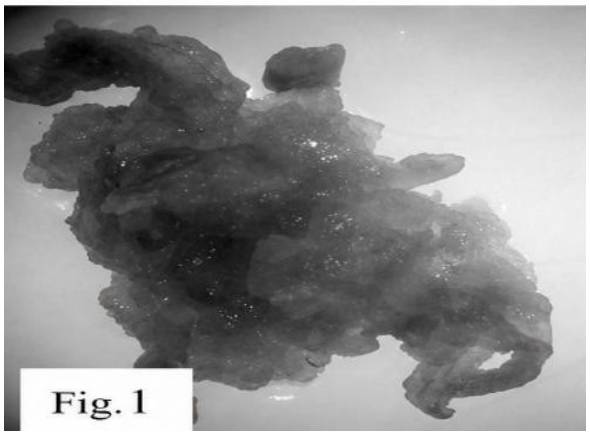

Fig.1-Leaf callus on MS medium + 2,4-D(10 µM) + BAP(2 µM)

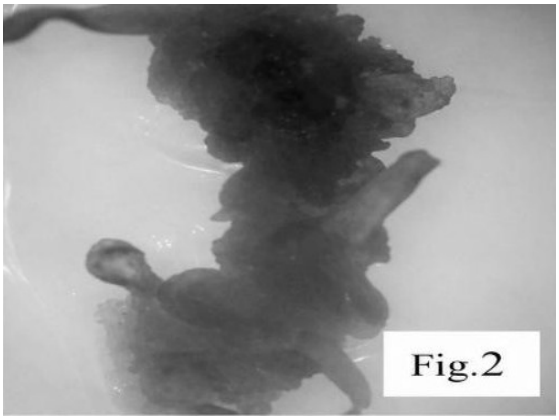

Fig.2- Root callus on MS medium + 2,4-D(10 µM) + BAP(2 µM)

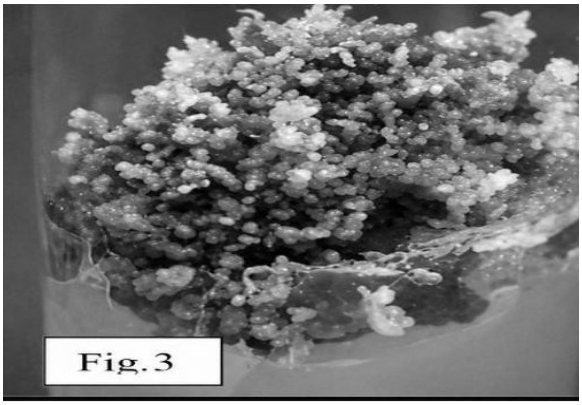

Fig.3- Shoot Regeneration on MS +BAP(4 µM) + IAA(1 µM). after 4 weeks

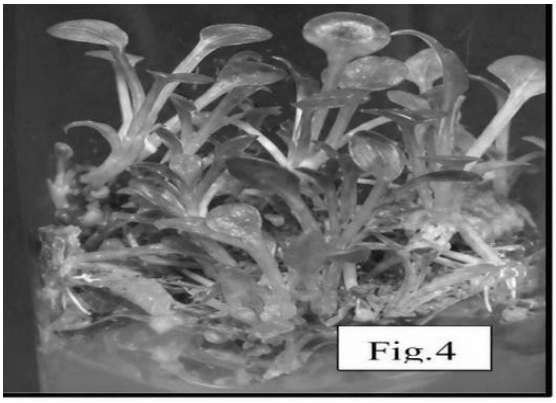

Fig.4- Shoot Regeneration on MS +BAP(4 µM) + IAA(1 µM). after 8 weeks

Shoot proliferation: Nodular callus developed from leaf and root segments were then cultured on shoot proliferation medium, consisting of MS(x1/2) medium supplemented with BAP (1-5 µM) and IAA (0.5-1 µM). Some explants were also cultured on BAP (1-5 µM) and KIN (0.5-1 µM).after four weeks of culture period shoot induction was visible in almost all calli. The best results were obtained on medium supplemented with BAP (4 µM) and IAA (1 µM) as compared to medium supplemented with BAP (4µM) and KIN(1 µM). *Fig-3 & 4*. The results of all treatments are presented in *table - 1*

DISCUSSION

The present study was carried out on internodal,leaf and root segments of C*ichorium intybus*. These explants showed great potential of plant regeneration under different phytohormonal combinations.

In present study maximum callogenesis was observed on MS medium with 2,4-D(10 µM) and BAP(2 µM) combination , which are in contradiction to studies of other workers in different medicinal plants (Whipkey et al,1992,Sebastian et al,2002). The present studies are also in partial contradiction to the studies of Velayutham (2006) in Chicory. In present study shoot induction was observed from the callus under the influence of BAP(4 µM) and IAA(1 µM) which are in contrast to studies of Rehman et al (2002) on *Cichorium intybus*, in which maximum shoot regeneration was observed on IAA (2 µM) and Kn (5 µM) .Indirect shoot regeneration was also observed in other medicinal plants on different phytohormonal combinations (Arora and Bhojwani,1984 in *Saussurea*; Nin et al,1996 in *Artemesia* and Kamili et al, 2001 in *Artemesia*) .All these studies are in partial line with our studies on *Cichorium intybus.*

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Abbreviations: BAP – 6-benzylaminopurine; 2,4-D – 2,4-dichlorophenoxyacetic acid; IAA – indoleacetic acid; IBA – indolebutric acid; KIN – kinetin (6-furfuryl aminopurine); NAA –naphthaleneacetic acid; PGRs – plant growth regulators.

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