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AN EFFECTIVE METHOD FOR HIGH FREQUENCY MULTIPLE SHOOTS REGENERATION AND CALLUS INDUCTION OF *CONVOLVULUS PLURICAULIS* CHOISY: AN IMPORTANT MEDICINAL PLANT

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ABSTRACT

Convolvulus pluricaulis Choisy of family convolvulaceae is an indigenous plant mainly advocated as brain tonic. The protocol was developed which produce for shoot regeneration and callus induction. Studies on micro propagation of callus was undertaken in which explants were inoculated on MS medium fortified with various concentration of 2, 4-D(2,4-Dichlorophenoxy acetic acid) and BAP(6-Benzylaminopurine) and Kn(Kinetin).The best callus induction was observed on 2, 4-D (1.0mg/l and 2.0mg/l) also with various concentrations of BAP and Kn. Best regeneration of shoot was achieved when they were cultured on ms medium supplemented with BAP(1- 3.0mg/l) and Kn(1.0 mg/l).The study of micro propagation has given a rapid improvement over conventional technique for multiplication and germplasm preservation of *Convolvulus pluricaulis.*Such medicinal plants can be used for the extraction of medicinally important compounds or for pharmacological studies.

KEYWORDS: ***Convolvulus pluricaulis, callus, shoot regeneration, MS medium, Benzyl Amino Purine, Kinetin.***

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INTRODUCTION

Convolvulus pluricaulis "Shankhpushpi", is an important indigenous drug of Ayurveda[1]. It improves memory power and intelligence. Various chemical constitutes, such as glucose, sucrose, glycosides, alkaloids and various acids etc., are found in the plant[2]. Preclinical *(in vivo* and *in vitro*) investigations have demonstrated nootropic, anxiolytic, tranquillising, antidepressant, antistress, neurodegenerative, antiamnesic, antioxidant, hypolipidemic, immunomodulatory, antiinflammatory, analgesic, antimicrobial, insecticidal, antifungal, antibacterial, antidiabetic, antiulcer, anticatatonic and cardiovascular activity. Clinical studies of its polyherbal formulation justified its potential for the ancient claim of brain tonic[3]. Tissue culture technique has been widely accepted as a tool for biotechnology for vegetative propagation of plants for agricultural, horticultural and forestry. Biotechnological tools are important for multiplication and genetic enhancement. Over 100 years ago, Plant tissue culture has provided the groundwork for the cultivation of plant cells, tissues and organs in culture[4].In conventional cultivation many plants do not germinate, flower and produce seed under certain climatic conditions. Micro propagation ensures a good regular supply of medicinal plants, using minimum space and time[5]. The leaf explants of *Convolvulus pluricaulis* were cultured on MS basal medium supplemented with various combinations of 2, 4-D: kinetin (1:1, 2:2, 3:3, 4:4, 5:5 mg/l) to raise callus and embryoids[6]. In our study various concentrations of auxins (2-4, Dichlorophenoxy acetic acid) and cytokinins(6-Benzylaminopurine&Kinetin) were used to study the induction of callus and shoot regeneration in *Convolvulus pluricaulis*.

MATERIALS AND METHODS

The branches (about 5-6 cm) of shoots of *Convolvulus pluricaulis* plants (Figure1A) were collected from the Herbal Garden, Kota. The branches with nodel explants were washed in running tap water and then washed again thoroughly by adding a few drops of Tween-20 to remove the superficial dust particles as well as fungal and bacterial spores. They were surface sterilized with 0.1% $HgCl_2$ for 5 min followed by rinsing them five times with double distilled water inside the laminar air flow chamber. Nodal segments (with a single axillary bud) about 0.5-0.8 cm were prepared aseptically and were implanted vertically on MS (Murashige and Skoog) medium prepared with specific concentrations of BAP, Kn (1.0-5.0 mg/l) singly or in combination were used for shoot proliferation. Same procedure was repeated for shoot multiplication. The medium containing 3% sucrose was solidified with 0.8% agar (Qualigens). The pH of the media was adjusted to 5.9±0.02 with 1 N NaOH or 1 N HCl solutions prior to autoclaving. Media poured in culture vessels were steam sterilized by autoclaving at 121°C and 15 psi for 15-20 min. The cultures were incubated under controlled conditions of temperature (25±2°C), light (2000- 2500 lux for 16 h/d provided by fluorescent tubes) and 60-70% humidity. For each experiment a minimum of 7 replicates were taken and experiments were repeated thrice. Observations were recorded after an interval of 3 wk. Once culture conditions for shoot induction from explants were established, the shoots produced *in vitro* were sub cultured on fresh medium every 3 wk. The nodal and shoot tip explants were inoculated in various concentrations and combination of 2-4, D, BAP and Kn.

Callus induction from the leaf explants of Convolvulus pluricaulis

The leaf explants of *C.pluricaulis* were transferred to MS media supplemented with different concentration of 2-4,D [1.0,2.0,3.0 mg/l] (Table-1) and cytokinin (BAP and Kn) [0.5,1.0,1.5 mg/l](Table-2) and different combination of 2-4,D(1-3mg/l)+BAP(0.5-1.5mg/l) and 2-4,D(1-3mg/l) + Kn (0.5-1.5mg/L).

Shoot regeneration from the nodal explants of Convolvulus pluricaulis

The nodal explants of *Convolvulus pluricaulis* was inoculated on MS media supplemented with different concentration of BAP (0.5, 1.0, 1.5mg/l) and Kn (0.5, 1.0, 1.5mg/l) (Table-2).

RESULTS

Callus formation

Callus formation was observed in 15 days after inoculation from the leaf sheath explants on MS medium supplemented with 2,4- D (1.0,2.0,3.0,4.0,5.0 mg/l)(Figure 1B). Maximum callus induction was observed after 3 week on 2, 4-D (1mg/l) (Table-1), minimum callus induction was observed on of 2-4, D (3 mg/l). No callus induction was seen on higher concentrations of 2-4, D. Best callus were seen on 2-4,D(1mg/l)+ Kn (1 mg/l)(Figure 1E) and 2-4,D (1mg/l)+ BAP(0.5mg/l)(Figure 1C) and2,4,D (1mg/l)+ BAP (1mg/l)(Figure 1D) respectively. The callus was green, compact and globular in shape.

Shoot regeneration from nodal explants

Among different concentration of BAP and Kn, tried highest shoot regenerating capacity (94%) (Table-2) was recorded on Kn(2mg/l)+BAP (0.5mg/l) combination. Maximum shoot length was observed on Kn (1mg/l). The minimum (29%) shoot regeneration was observed on BAP (1.0mg/l) +Kn (0.5mg/l). Maximum shoot production (62%) was seen on BAP (2mg/l)(Figure1G) and BAP 3mg/l) (Figure1H).Where as minimum shoot production (29%) was seen on BAP (1.0mg/l).Maximum shoot production (74%) (Figure1F) was seen on Kn (1mg/l). Among the all concentrations tried best results were obtained Kn (2mg/l) +BAP (0.5mg/l) and Kn alone (1mg/l).The average mean number of shoots on Kn (2.0mg/l) +BAP (0.5mg/l) (Table-2) was $5.8\pm0.57$and average shoot length observed 6.6 ± 2.46 cm.InKn(1mg/l) (Table-2) average mean number of shoots was $5.1\pm1.04$and average height of shoots was 6.5 ± 1.32 cm respectively.

Table 1
Effect of auxin and cytokinin on callus induction from the nodal explants of Convolvulus pluricaulis

Hormone concentration(mg/l)	Response	Colour of Callus	Morphology of Callus
2,4D (1mg/l)	+++++	Light greenish	Compact
2,4D (2mg/l)	+++	Light greenish	Compact
2,4D (3mg/l)	+	- -	- -
2,4D(1mg/l)+BAP (0.5mg/l)	++++	Light greenish	Compact
2,4D(1mg/l)+BAP(1mg/l)	+++	Little greenish	Compact
2,4D(1mg/l)+BAP(1.5mg/l)	+	- -	- -
2,4D(1mg/l)+Kn(0.5mg/l)	++++	Whitish	Friable
2,D(1mg/l)+Kn(1mg/)	++	Whitish	Friable
2,4D(1mg/l)+Kn(1.5mg/l)	+	Brown	- -

Table 2
Effect of Cytokinin (BAP) on shoot induction from the nodal explants of Convolvulus pluricaulis.

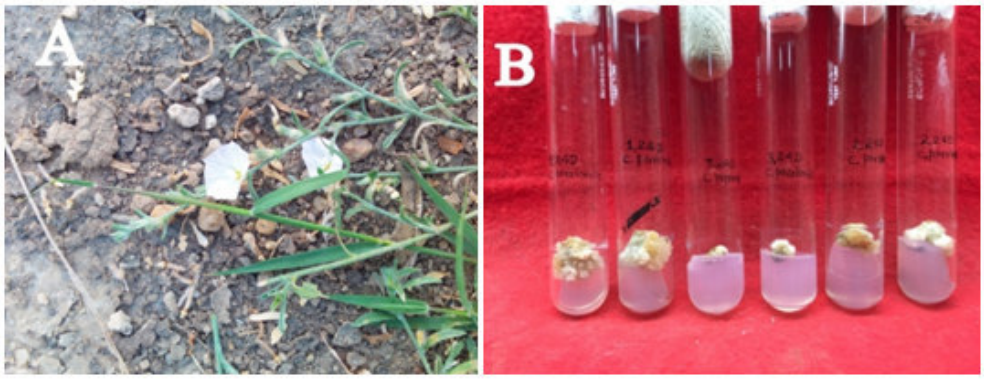
Hormone concentration(mg/l)	% of explants produced shoot	Number of shoots per explants Mean* ± SD	Height of shoots (in cm) Mean* ± SD
BAP (1mg/l)	48	2.06±0.76	3.9±0.36
BAP (2mg/l)	51	4.23±0.25	4.5±1.32
BAP(3mg/l)	62	4.4±0.36	4.53±0.61
KN(1mg/l)	74	5.1±1.04	6.5±1.32
KN(2mg/l)	40	4.1±0.28	4.1±0.15
KN(3mg/l)	32	2.36±0.51	2.06±0.60
BAP(1mg/l)+Kn(0.5mg/l)	29	2.2±0.36	2.26±0.25
BAP(1mg/l)+Kn(1.0mg/l)	26	2.4±0.40	2.0±0.30
BAP(1mg/l)+Kn(1.5mg/l)	20	2.1±0.35	1.9±0.25
Kn(2mg/l)+BAP(0.5mg/l)	94	5.8±0.57	6.6±2.46
Kn(2mg/l)+BAP(1.0mg/l)	54	4.1±0.36	4.4±0.61
Kn(2mg/l)+BAP(1.5mg/l)	47	3.6±0.76	3.5±0.50

** Mean and standard error of 3 replicates each*

Table 3
Anova table (number of shoots and shoot length/explants)

Source of variance	SS	DF	MS	Fcal value	P value	Ftab value **
Between groups	0.601667	1	0.601667	0.281295	0.601166	4.30095
Within groups	47.05612	22	2.138914			
Total	47.65778	23				

*Note: * : Mean of 3 replication, ** : Significant F Value @ 5% level*



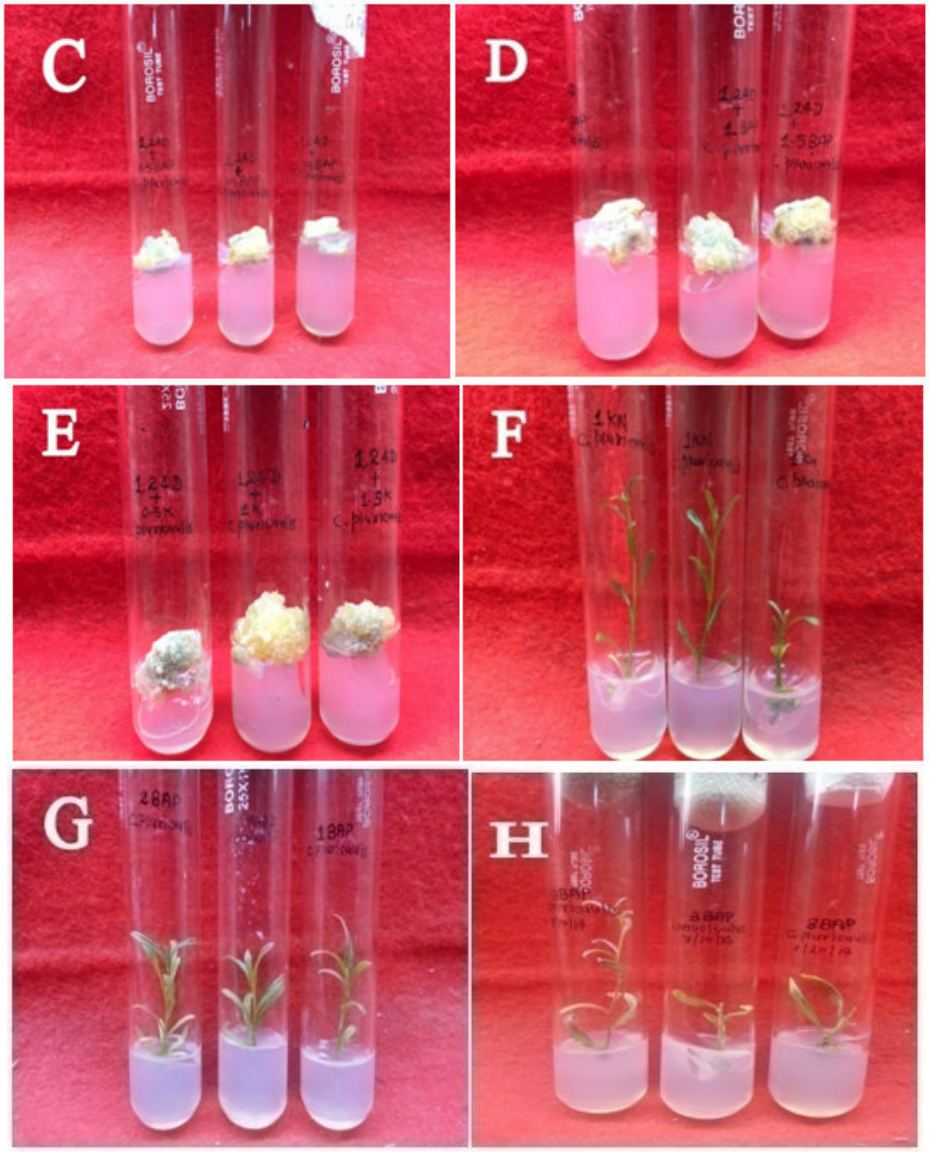


Figure 1
Callusing response and shoot induction of Convolvulus pluricaulis

DISCUSSION

Convolvulus pluricaulis Choisy, commonly known as ‘‘Shankhpushpi’’is a perennial herb, is an ayurvedic medicinal plant recommended as a brain tonic to promote intellect and memory, eliminates nervous disorders and to treat hypertension. Because of increasing demand of the drug, this plant species has been over-exploited[7]. As enabling and emerging technology, plant tissue culture techniques have been developed andused as a novel tool to assist plant breeders in crop improvement perspectives. These novel tools can be used to either increase the speed and/or the efficiency of breeding process in order to improve the accessibility of existing germplasm and to create new genetic variation for crop improvement as well as to be able to achieve the objective which is not possible through conventional breeding methods. Thus, plant tissue culture technology has a vast potential to produce plants of superior quality and selection of useful variants in well adapted high yielding genotypes with better disease resistance and stress tolerance capacities[8]. In the present study of *Bacopa monnieri* nodal explants were inoculated onto MSmedia supplemented with 1mg/l BAP. Further multiplication was obtained on MSmedium+BAP(1.0mg/l).The shoots were rooted and best rooting was observed by IBA when incorporated in MS at different concentrations (0.1-0.3mg/l)and (0.2mg/l) gave good results. After 30 days about 2.2cm length was observed. Almost 70% of the rooted shoots survived during hardening[9]. For the formation of somatic embryoids in *Convolvulus pluricaulis* leaf was used as suitable explants material which when cultured on MS basal medium supplemented with 2-4,D and kinetin(5:5mg/L) in combination gave better response in embryogenic callus initiation while the lower hormonal concentrations produced only non-embryonic callus. The embryonic callus cells were green in colour, compactly packed, globular in shape with uniform margins and pink pigmentation. Maximum numbers of embryoids were seen on media containing NAA (0.25mg/l) and Kinetin (3mg/l)[6]In*Convolvulus alsinoides* Linn. higher concentrations of 2, 4-D (3 mg/l) was more effective forcallus induction on terminal buds and flower explants. For callus induction, the explants were culturedon MS

medium supplemented with IAA, 2, 4-D,BA, Kn either alone or in combinations. Profused and higher amount of calli was produced from explants with 2, 4-D (1mg/l) and BA (1mg/l).There are reports on the regeneration from inflorescence pieces of*Bowiea volubilis*on2, 4-D (1mg/l)+BA(1mg/l). (Afolayan and Adebola[10].Induction of callus from root portion of seedlings of *Convolvulus pluricaulis* has been reported on a MS (basal) medium supplemented with sucrose(1.5%), casein hydrolysate(1250 mg/l).IAA (15mg/l), Kinetin (1.5 mg/l) and GA (0.5 mg/l).Maximum hypotensive activity was noticed in the extract of callus tissue cultured on MS (basal) medium supplemented with a high concentration of casein hydrolysate[11]. *Evolvulus alsinoides* L. is used for preparation of 'Shankhapushpi', an important popular ayurvedic drug that contributes considerably to the improvement of memory power. This report describes importance of cotyledonary node and leaf explants for callus formation. The best response was obtained on MS medium fortified with 5.0 µM 6-benzyladenine (BA) in which 96 % of cultures produced 7.6 ± 0.6 shoots per explant. Regenerated shoots were rooted on MS medium with 5.0 µM indole-3-acetic acid (IAA). Plantlets were successfully acclimatized and established in soil. MS medium fortified with 10 µM BA + 5.0 µM IAA showed maximum growth and accumulation of scopoletin in cell cultures[12]. The study of micropropogation has given a rapid improvement over conventional technique for multiplication and germplasm preservation of *Convolvulus pluricaulis*. Therefore callus induction and regeneration has been used for further improvement using biotechnological techniques as well.

CONCLUSION

Tissue culture technology makes it possible to produced large number of disease free, uniform plants of medicinal importance in short span of time. Shoot regeneration and callus induction of *C. pluricaulis* were best on MS media supplemented with kinetin and 2, 4-D.Higher amount of kinetin and 2, 4-D could enhance the production of callus and gave maximum number of shoots. It was observed that the callus induction gave was best (95%) on MS medium supplemented with 2, 4-D (1mg/l) and Kinetin (1mg/l). Maximum shoot length was observed on medium supplemented with Kn (1mg/l).Since the calculated value is smaller than table value so, there is no significant difference in sample mean .Such medicinal plants can be used for the extraction of medicinally important compounds or for pharmacological studies.

CONFLICT OF INTEREST

Conflict of interest declared none.

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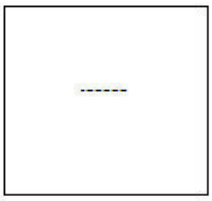

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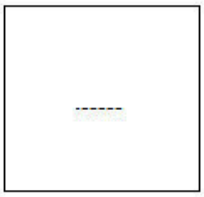

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