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IN VITRO MICROPROPAGATION OF *COLDENIA PROCUMBENS* L. FROM NODAL EXPLANTS

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ABSTRACT

An efficient protocol for *in vitro* micropropagation of *Coldenia procumbens* L. has been developed. The nodal explants were cultured on MS basal medium supplemented with different concentrations of BAP and KIN. The two cytokinins tested, BAP was found to develop in shoot multiplication and higher number of shoots from the nodal explants when compared to KIN. higher number of shoots was produced from all the concentrations of both BAP and KIN. The highest frequency of 100% shoot induction was observed on MS basal medium supplemented with 10 µM BAP and 8 µM KIN. The number of shoots observed on the MS basal medium supplemented with BAP obtained 15 as well as 10.6 in KIN. The data's are recorded after 30days of inoculation.

KEYWORDS: *Coldenia procumbens L.*, cytokinins, nodal explants.

INTRODUCTION

Coldenia procumbens Linn.(Boraginaceae) is an annual herb, common weed in India. It is found widely in south india on waste lands, common in dry rice grounds. The genus having 24 species of prostate.[1] [2] *Coldenia procumbens* is only species of its genus has a place both in the *Hortus Bengalensis* and Moon's Catalogue of ceylon.[3] This plant is widely used in

traditional medicines in india, Africa, malaysia. Acetone, water, methanolic extract of dried aerial parts shows weak angiotensin-converting enzyme inhibition in vitro.[4]

Taxonomic classification of *Coldenia procumbens Linn.*

Kingdom: Plantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Boraginales

Family: Boraginaceae

Genus: Coldenia

Species: *Coldenia procumbens Linn.*

Synonyms : Creeping Coldenia

Ayurvedic : Tripakshee

Vernacular Names:

Sanskrit - Tripakshee

English - Creeping Coldenia

Hindi - tripungkee;

Telugu -hamsapadu

Tamil - seruppadai or cherupadai

It is a prostrate herb usually lying quite flat on the ground,common on dry rice grounds, stems reaching 10-50cm long, shaggy with white hairs, branches often numerous. It is distributed in tropical and subtropical zones and found widely in south India3-6. **Leaves:** Crisped, alternate, short and sessaile 2-6×1.2-2cm, obovate to oblong, rounded at the apex, warty hair with rosette of basal cells, stomata anomocytic, palisade adaxial, veins 4-6 pairs on each side. [5],[6] **Flowers:** Small, 3-4mm long, white, sessile, inconspicuous, solitary, axillary, nearly sessile; calyx- 1-1.5cm, lobes lanceolate to ovate lanceolate, corolla a pale yellow and very small.Sepals-5, broadly lanceolate, 2.5mm long, petals-5, united below into a short tube, stamens-5, inserted styles-2.[6],[7] **Fruit:**3-4mm long, Composed of four cells wrapped in calyx, with single seed in each cell. Fruit initially splitting into 2 halves, later each half into 2 one seeded nutlets with distinct beak.[3]

Fresh leaves of *Coldenia pro*cumbens ground and applied to Rheumatic Swellings, equal parts of dried powder mixed with seeds of fenugreek causes Suppurations of boils. Fresh leaves are used to promote maturation, the decoction of *Coldenia procumbens* leaves when

mixed with *Centella asiatica* and *Madhuca longifolia* and *Ixora coccinia* would give significant result in wound healing, plant extract also used in treatment of piles, Leucorrhoea, Menorrhagia.[1], [2], [3], [4], [8], [9] Coldenia procumbens having the glycosides, phytosterols, proteins, amino acids, fixed oils flavonoids, gums and mucilage as a chief constituents. Alkaloids and tannins are higher in alcoholic extract than in water extract. Reducing sugars and phenols are higher in water extract than in alcoholic extract. Non-reducing sugars and steroids are equally present in both the extracts. Saponins and fixed oils & fats are present only in water and alcoholic extracts respectively.[10], [11]

Micropropagation is one of the important tool for conservation of medicinally important palnt. Its also a viable tool for rapid multiplication with in a short duration. It is a method for production of clones. This method conducted under laboratory condition and it has a chance to produce genetically homogenous plants. micropropagation method is highly impact in Plant breeding, Horticulture and Forestry. It is a suitable method for obtaining a large quantity of genetically homogenous and healthy plant material which can be used for planting. **[12]** This technique is an alternative method of propagation as there is an increase in the propagation rate of plants, availability of plants throughout the year, protection of plants against pests and pathogens under controlled conditions and the availability of uniform clones and uniform production of secondary metabolites.[13]

MATERIALS AND METHODS

Source of Explants

The field grown *Coldenia procumbens* Linn. (Boraginaceae) was selected for the source of explants in the present Study. Nodal regions with axillary bud of two weeks older plants were used as explants for micropropagation.

Culture Medium

The nutrient medium consists of inorganic salts, carbon source and organic supplements. In addition, vitamins and growth regulators are also added to the medium. In the present study, the basal medium consists of the mineral salts and organic nutrients of Murashige and Skoog (MS) salts [14] with B5 vitamins[15] are used. For convenience, throughout this chapter, MS medium with MS salts plus B_5 vitamins is being referred as MS medium. The basal medium is supplemented with various concentrations and combinations of different growth regulators.

Growth regulators

The prwsent study the hormone concentration was used in µM (micromole). These growth regulators were used as supplement to the basal medium individually as well as in different combinations.

Cytokinins: BAP (6-benzylaminopurine)

KIN (6-furfurylaminopurine)

Preparation of culture medium

The present study was performed in the basal medium with MS salts, B5 vitamins, 3% sucrose and 0.8% agar.

The basal medium was variously supplemented with factorial combinations of different growth regulators ranging from 2 – 10 µM BAP or KIN alone for shoot multiplication or in combination of both the cytokinins. After adding all the supplements (various concentrations of different hormones) to the basal medium, the pH of the medium was adjusted to 5.8. The molten medium was dispensed in culture tubes or culture bottles or conical flasks and was capped with cotton plugs.

Sterilization of Culture Medium and Glassware's

The culture medium containing high concentration of sucrose supports the growth of several microorganisms. These microbes generally grow much faster than the explants and finally spoil the culture. So it is very essential to maintain a complete aseptic environment inside the culture tube. Therefore, the culture medium, glassware's, forceps and scalpels was sterilized by autoclaving at 1.06 kg cm^{-2} and 121°C for 15 min. As well as the same procedure to follow the sterilization of. During this period much care was taken to avoid denaturation of growth regulators and vitamins that were incorporated into the medium. The culture tubes left free until agar in the medium become solidified. Then the tubes were transferred to inoculation chamber for inoculation.

Sterilization of explant

The explants were taken from the field grown mature plants. The explants consisting of the nodal regions with axillary bud were surface sterilized by rinsing in running tap water for 30 minutes. Then they were washed in an agitated solution of liquid detergent (Teepol) for 5

minutes and followed by distilled water for 2-3 times for removing the traces of liquid detergent. After thorough washing, the materials were taken in to the Laminar Flow Chamber where they were disinfected with 70% alcohol for 30-60 seconds followed by 0.1% mercuric chloride for 3-5 minutes. Finally, the materials were thoroughly rinsed with sterile distilled water for 4-5 times to remove the traces of mercuric chloride.

Inoculation Procedure

Before starting inoculation all the requirements such as culture tubes, containing media, spirit lamp, sterile water, glassware and explants, were placed in the laminar air flow chamber. The platform surface of the chamber was swapped with 70% alcohol. After swapping the chamber with 70% alcohol, the UV light was switched on for 30 minutes. After 30 minutes, the UV light was switched off and the white fluorescent light was switched on. Before inoculation, hands were rinsed with absolute alcohol. The instruments were sterilized by dipping in absolute alcohol followed by flaming and cooling. The inoculation was carried out in the vicinity of flame. The surface sterilized explants were aseptically transferred to the respective culture media in the Laminar Flow Chamber. The explants were taken out from beaker and at the same time the cotton plug of the culture tube was slightly opened in front of the spirit lamp flame, the explant was put it the medium and immediately covered with cotton plug. The explants with nodal regions were inserted in the medium vertically. Cultures were transferred to fresh media with the same hormone concentration at 4 week intervals.

Culture Conditions

The cultures were maintained in a culture room at 25±2°C under 16 hr photoperiod with a light intensity of 30-40 µM m^{-2} s^{-1} supplied by cool white fluorescent tubes. These growth conditions were referred to as standard culture conditions for *in vitro* studies.

Culture Maintenance

The nodal explants regions, were initially cultured on MS solid medium in test tubes. After 4 weeks, the initiated shoot multiples were subcultured on MS basal medium fortified with the same growth regulator concentrations and combinations or whichever is the best for further multiplication. To facilitate higher number of shoot formation, the explants were also subcultured on conical flasks and/or culture bottles which can provide more space and more medium for growth and multiplication.

Experimental Design, Data Collection and Statistical Analysis

The design of all the experiments was a complete randomized block and each experiment consisted of five explants per flask and five replicate culture flasks per plant growth regulator treatment. The parameters recorded were frequency (number of cultures responding in terms of multiple shoot proliferation and root development), number of shoots per explant, shoot length, number of roots per shoot, root length and survival rate (%). All of the experiments were repeated five times. The analysis of variance (ANOVA) appropriate for the design was carried out to detect the significance of differences among the treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% level of significance.[16]

RESULTS AND DISCUSSION

Shoot Multiplication

Nodal explants of *Coldenia procumbens* Linn.. were cultured on MS basal medium supplemented with different concentrations of BAP and KIN of both these cytokinins ranging from 2 - 10 µM for shoot multiplication. Multiple shoots were initiated within 12 days of inoculation. Maximum number of shoots was observed in 20-30 days. The data in respect of shoot induction frequency, number of shoots and length of shoots on different concentrations of each hormone on nodal explants were presented in **Table 1 and Fig.1**.

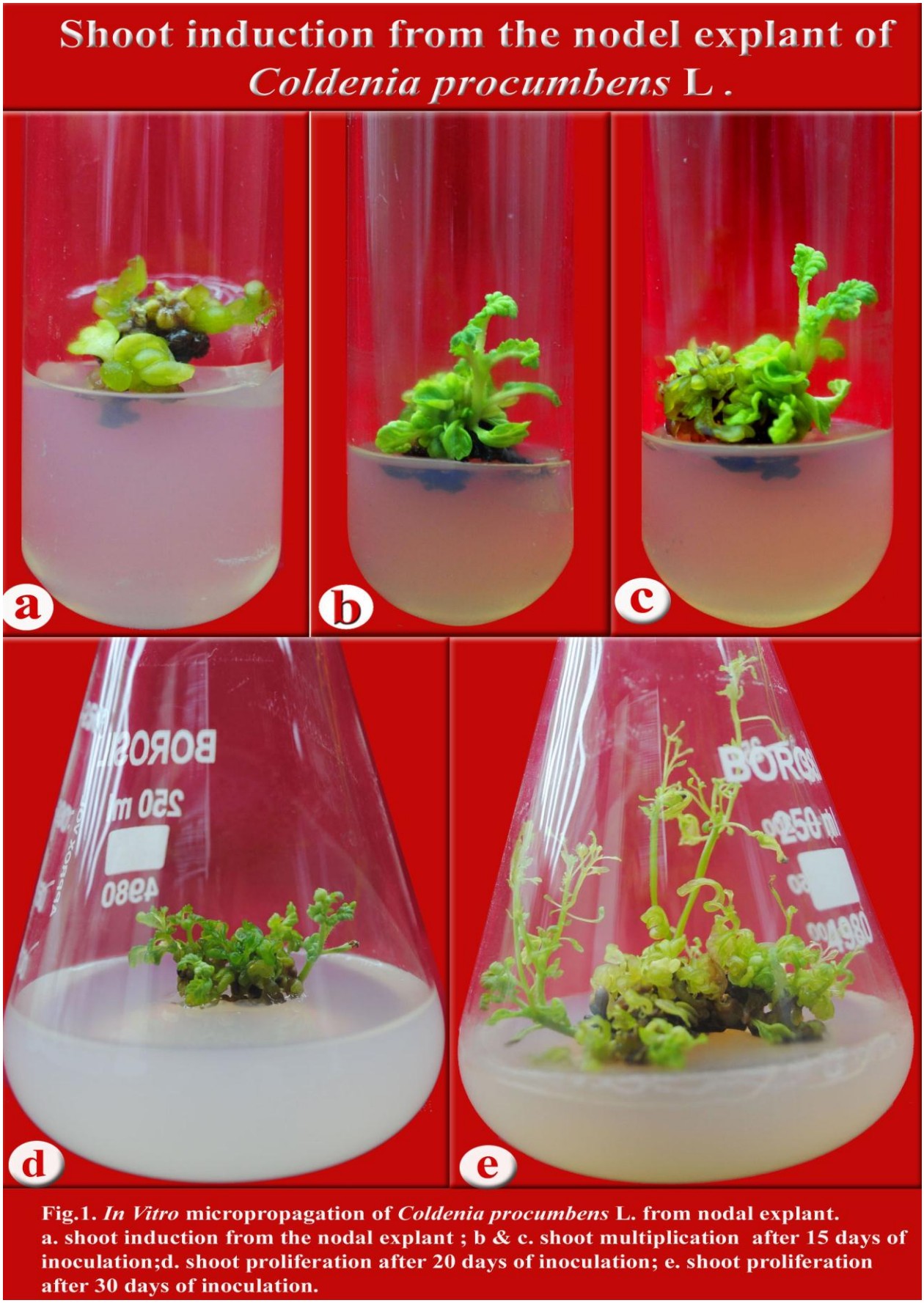

Fig. 1. Shoot induction from the nodal explant of *Coldenia procumbens* L.

Table 1: Effect of different concentrations of cytokinins on shoot induction from the nodal explant of *Coldenia procumbens* L.

BAP	KIN	Percentage of response (%)	Number of shoots	Shoots length
2 µM	--	80	7.8±1.78	3.94±0.84
4 µM	--	90	5.4±1.14	4.92±0.73
6 µM	--	90	6.2±1.92	5.3±0.29
8 µM	--	95	5±1.58	4.88±0.21
10 µM	--	100	15±2.23	5.48±0.35
--	2 µM	85	5.6±1.51	4.08±0.48
--	4 µM	90	5.8±1.92	4.6±0.27
--	6 µM	95	6.8±1.30	4.78±0.29
--	8 µM	100	10.6±1.14	5.78±0.23
--	10 µM	90	6.6±1.14	4.6±0.36

Shoot multiplication from the Nodal explants (Table 1, Fig.1)

The nodal explants with axillary buds were initially grown on MS basal medium supplemented with BAP or KIN alone in different concentrations ranging from 2 - 10 µM. These two cytokinins BAP was found to induce more number of shoots when compared to KIN. Among different concentrations of BAP, the MS basal medium supplemented with 10 µM BAP showed the highest number of 15 shoots per explant and mean shoot length of 5.48cm.

The basal medium fortified with different concentrations of KIN induced less number of shoots when compared to BAP. Maximum number of 10.6 shoots per explant was induced on MS basal medium containing 8 µM KIN and mean shoot length 5.78 cm. These results showed that both the cytokinins tested were found to initiate and proliferate shoots from the nodal explants. However, BAP was found to be more suitable than KIN for shoot multiplication.

In micropropagation technique, shoots are directly induced from the nodal explant with axillary buds where meristematic tissue is present. This technique is primarily used to produce pathogen free plantlets. Nowadays, it is widely used to get a mass propagation within a short period. Since the meristematic region is the very active site, the axillary buds are readily proliferated. The efficiency of shoot multiplication depends on plant growth regulators and types of explants.[17], [18]

In many plants, multiple shoots were obtained from the shoot tips or axillary buds by administering BAP or KIN. [19], [20], [21], [22], [23], [24] In the present study nodal explants with

axillary bud were taken as explants source. The nodal explants showed active site of positive morphogenetic response and readily developed multiple shoots. The propagation rate and morphogenetic response significantly varied to a greater extent according to the explant type. Shoot tips have always been preferred for *in vitro* studies because they can be handled easily and restore their regeneration potential over other explants. Some earlier findings showed that more number of shoots were produced from the nodal explants. [23], [25], [26]

The cytokinins, BAP and KIN, were independently used to induce shoot multiplication. The induction of multiple shoots varied with the cytokinin types and concentrations. Though several growth regulators are available for shoot multiplication, BAP and KIN are widely used. In the present study both these cytokinins are used independently to find their efficiency in shoot multiplication. Of the two cytokinins used, BAP was found to be more effective in shoot induction and proliferation than KIN. In several studies BAP was most effective in inducing bud break resulting in the sprouting of a large number of shoots.[23],[27],[28],[29]

The cytokinin, BAP has been commonly used for the induction of organogenesis in many plants . A comparison of the relative effect of different cytokinins for multiple shoot formation revealed in order of effectiveness was BAP >KIN > Zeatin > Adenine. [30] It was stated that BAP is effective for meristem, shoot tip and bud cultures.[31] reported that higher concentrations of cytokinins reduced the number of micropropagated shoots. A similar phenomenon was observed earlier [32] and also in the present study. The potential for shoot multiplication appeared to be strong in the presence of BAP alone in the culture medium . The stimulatory effect of multiple shoot formation was similar to that reported in other plant species.[17],[23] The number of shoots produced per explant in the medium supplemented with BAP was significantly higher than other cytokinins. In general, KIN was less effective than BAP and 2iP (2-isopentenyl adenine) in shoot induction. The MS medium containing BAP was more effective than KIN for induction and proliferation of axillary buds as in previous reports.[33],[34],[35],[36], [37],[38] The superior effect of BAP for shoot bud induction on clonal multiplication had been reported in pink ginger [39] and *Solanum nigrum L.*. [40]

CONCLUSION

Micropropagation was carried out from the nodal explants with axillary buds of *Coldenia procumbens* Linn. Nodal explants with axillary buds were grown on MS basal medium supplemented with different concentrations BAP or KIN. Of the two cytokinins BAP was

found to induce more number of shoots from nodal explants when compared to KIN. The MS basal medium supplemented with 10 µM BAP showed the maximum number of 15 shoots per nodal explant and 8 µM KIN produced the maximum number of 10.6 shoots per node.

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