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# *In vitro* Regeneration of multiplication shoots in *Catharanthus roseus*- An important medicinal plant

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**ABSTRACT**

*Catharanthus roseus (Linn.) G. Don. syn. Vinca rosea Linn. an important medicinal plant belongs to the family Apocynaceae. The present study describes a simple, efficient and reproducible regeneration system for in vitro propagation of Catharanthus roseus through nodal segment explant. Explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of BAP, NAA and IBA. Multiple shoots were produced on all the concentrations of BAP, NAA and IBA; however BAP (0.5 mg/l) + NAA (1.0 mg/l) concentration proved to be optimal for the production of maximum number of shoots. Best rooting response was observed on half strength MS containing Indole-3-butyric acid (IBA) (0.10 mg/l). Regenerated plantlets were successfully acclimatized and hardened off inside the culture room and then transferred to green house with 100 % survival rate.*

**Keywords:** *Catharanthus roseus*, MS medium, BAP, IBA, Acclimatization.

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## INTRODUCTION

The use of plants as medicines has a long history in the treatment of various diseases. The earliest known records for the use of plants as drugs are from Mesopotamia in 2600 B.C., and these still are a significant part of traditional medicine and herbal remedies [1]. *In vitro* regeneration or micropropagation is the best alternative to overcome these hurdles and it holds tremendous potential for rapid multiplication and production of high quality medicines from them [2]. *Catharanthus roseus* has more than 400 known alkaloids. Some are used by the pharmaceutical industry for the treatment of childhood leukemia, Hodgkin's disease, testicular cancer and cancerous tumors. It is an erect handsome herbaceous perennial plant which is a chief source of patented cancer and hypotensive drugs. It is one of the very few medicinal plants

which have a long history of uses as diuretic, antidysenteric, hemorrhagic and antiseptic. It is known for use in the treatment of diabetes in Jamaica and India. Prevention of cancer, cancer treatment, anti-diabetic, stomachic, reduces high blood pressure, externally against nose bleeding, sore throat and mouth ulcers. In *Catharanthus* too, node culture producing shoots was noticed [3-6]. Optimization for conditions for shoot multiplication has been attempted for continuous production of useful compounds without depleted of natural flora [7-10]. In our studies attempt were made for *in vitro* propagation of *Catharanthus roseus* using nodal explants.

## MATERIALS AND METHODS

***Plant material and explant collection:***

Explants were collected from The Singhania University ground and authenticated by Herbarium Department of Botany, University of Rajasthan Jaipur. Nodal explants were used for establishing maximum number of multiple shoots. The explants (1-2 cm.) were washed thoroughly under running tap water for 10 min. and then treated with few drops of tween-20 (Polyoxyethylene sorbitan monolaurate) for one min. with constant shaking by hand. The shaking followed three successive washings again with distilled water. The surface sterilization was carried out with 0.1% $HgCl_2$ for one min. followed by gentle shaking. After surface sterilization the segmented parts were thoroughly washed for several times with sterile distilled water and explants transferred in 25x 150mm. culture tubes with 15 ml MS media [11] supplemented with hormone BAP and Kn in different concentrations for multiple shoots culture. The culture tubes were incubated room at $25\pm4^0$C under the warm fluorescent light with intensity varying from 2000-3000 lux. The pH was adjusted to 5.8 prior to all autoclaving. The cultures were incubated at $25\pm5$ $^0$Ċ with 8 hours photoperiods.

***Shoot induction and multiplication***

The nodal segment explant were cultured on MS medium supplemented with different concentrations of BAP and Kn. Data for percentage shoot regeneration, shoot number per explant and shoot length was recorded after 6 week of culture. All the cultures were transferred to fresh medium at six week intervals and data for multiplication and growth of shoots was recorded up to three subculture passages.

***In vitro rooting***

For complete plant development regenerated microshoots (4-5 cm) were excised and transferred to rooting medium comprising of MS basal and half strength MS without any growth regulator or with auxins – IBA (0.05, 0.10 mg/l) or IAA (0.50 mg/l). The rooting percentage, number of roots per shoot and root length was recorded after 6 weeks of culturing.

***Hardening and acclimatization***

Plantlets with well developed root system were removed from the rooting medium, washed properly under running tap water to remove any adherent gel and transferred to thermocol cups containing sterilized soilrite. Thermocol cups were covered with transparent polythene bags to ensure high humidity and irrigated with ¼ strength MS salt solution (without vitamins) for initial 2 weeks followed by tap water. Hardening and acclimatization was done under diffuse light conditions (16:8 h photoperiod). Polythene bags were removed gradually in order to acclimatize

plantlets, after 4 weeks they were successfully transferred to earthen pots containing garden soil and maintained in green house under normal day length conditions.

***Data collection and statistical analysis***

All the experiments were conducted with 10 replicates per treatment and repeated three times. Data for shoot induction shoot multiplication and rooting experiments were recorded after 6 weeks of culture. The data were analyzed statistically and expressed as a mean ± SE of three repeated experiments.

## RESULTS AND DISCUSSION

***Induction of multiple shoots***

The response of various cytokinins (BAP and Kn) for shoot regeneration from nodal segment explants is. The explants cultured on MS basal medium without growth regulators (control) did not show any regeneration response. However, the addition of plant growth regulators enhanced the multiplication rate and the number of shoots per explant.

**Table 1. Effect of cytokinins with MS-medium on multiplication of nodal segment of *Catharanthus roseus***

| Growth regulators concentration (mg/l) | No. of shoot/explant *Mean ± $t_{0.05}$ S.E. ($\overline{X}$) | Shoot lengths in cm |
|---|---|---|
| **BAP** | | |
| 0.5 | 9.2 ±0.37 | 3.4 ±0.12 |
| 1.0 | 5.4 ±0.81 | 3.2 ±0.17 |
| 1.5 | 7.12 ±0.45 | 2.93±0.55 |
| 2.0 | 4.0 ±0.36 | 2.3 ±0.31 |
| 2.5 | 3.4±0.24 | 1.8 ±0.28 |

The percentage response varied with the type of growth regulator used and its concentration. During the experiment when 0.5 – 2.5 mg/l concentrations of BAP were tried on the results proved that BAP (0.5 mg/l) elicited (9.0-11.0) shoots with 3.4 cm shoot length per explant within 7 weeks of inoculation **(Fig-a) (Table-1)**. On increasing the concentration of BAP the response was delayed further and a reduced number of shoots were obtained. Manicakam *et al.* (2000) [12] reported that the maximum number of shoots (8.0) was obtained in *Withania somnifera (L.)* Dunal from the nodal explants MS medium was supplemented with 1.0 mg /l BAP. The superiority of BAP over other cytokinins on shoot bud production and proliferation of shoots has been reported for several medicinal and aromatic plant species such as *Prosalea* corydifolia [13], *Eclipta alba* [14] and *Mentha viridis* [15]. By increasing or decreasing the concentration of BAP beyond the optimal level, a gradual reduction in the number of shoots was also reported in *Tylophora indica* [16].

As a result of synergism between cytokinin- auxin combinations, the frequency of shoot proliferation was better than cytokinin alone. Different auxins viz. NAA/IBA and 2, 4-D in combination with BAP (0.5 mg/l) were tried to multiple shoot proliferation. In the present study maximum multiple shoot (35.0 – 36.0) and shoot length (6.56 cm) was observed on MS medium supplemented BAP (0.5 mg/l) + NAA (1.0 mg/l) + 3 % activated charcoal **(Fig-B)**. Higher concentration of NAA (1.5 mg/l) reduced the regeneration percentage and resulted dark greenish

thick form at the base of shoots. Lower concentration of NAA (0.5 mg/l) reduced the regeneration percentage and resulted light greenish color formed at the base of shoots. In increase level of NAA (1.5 mg/l) response was found 70% with 11.0 – 12.0 numbers of shoots. In decrease level of NAA (0.5 mg/l) response was found 40% with 7.0 – 8.0 numbers of shoots (Fig-5, a, b). However, different concentration of IBA used with BAP (0.5 mg/l) was tried to multiplication. MS + 0.5 mg/l BAP +1.5 mg/l IBA proved that elicited (1.0 – 2.0) shoots with 7.5 cm (higher shoot length) shoot length per explant within 7 weeks of inoculation **(Fig-C)**.

**Table 2. Effect of BAP with Auxin + MS- medium on multiplication /elongation of nodal segment of *Catharanthus roseus.***

| Growth regulators concentration (mg/l) | No. of shoot/explant *Mean ± $t_{0.05}$ S.E. ($\overline{X}$) | Shoot lengths in cm |
|---|---|---|
| **BAP + NAA** | | |
| 0.5 + 0.5 | 7.22 ± 1.40 | 4.12 ± 0.15 |
| 0.5 + 1.0 | 35.10 ± 0.74 | 6.56 ± 0.24 |
| 0.5 + 1.5 | 19.82 ± 0.26 | 3.36 ± 0.12 |
| 0.5 + 2.0 | 9.20 ± 0.11 | 5.36 ± 0.51 |
| 0.5 + 2.5 | 8.80 ± 0.17 | 4.35 ± 0.42 |
| 0.5 + 3.0 | 6.36 ± 0.12 | 2.12 ± 0.18 |
| 0.5 + 3.5 | 1.52 ± 0.15 | 1.19 ± 0.21 |
| 0.5 + 4.0 – 8.0 | Nill | Nill |
| **BAP + IBA** | | |
| 0.5 + 0.5 | 1.0 ± 0.35 | 2.15 ± 0.33 |
| 0.5 + 1.0 | 1.0 ± 0.26 | 4.35 ± 0.12 |
| 0.5 + 1.5 | 2.0 ± 0.37 | 7.50 ± 0.21 |
| 0.5 + 2.0 | 1.0 ± 0.22 | 5.25 ± 0.54 |
| 0.5 + 2.5 | 1.0 ± 0.32 | 4.22 ± 0.74 |
| 0.5 + 3.0 | 1.0 ±0.15 | 3.32 ± 0.15 |

On increasing or decreasing concentration of IBA the response was reduced shoot length were obtained **(Table-2)**. Similarly effect of a NAA in combination with BAP on the enhancement of shoot multiplication was observed in *Buchanania lanzan* [17]. While in *Peganum harmala* multiple shoots were induced with the combination of BAP (5.0 mg/l) + NAA (1.0 mg/l) [18]. When the increase or decrease $GA_3$ concentration then found slow response (3.43 ± 0.48 to 4.10 ± 0.42) shoots with 1.5 cm shoot length. Similarly effect of $GA_3$ (0.4 mg/l) with BAP (1.5 mg/l) + Kn (1.5 mg/l) on the enhancement of shoot multiplication was observed in *Sida cordiforlia* [19]. The effect of AC on growth regulator uptake is still unclear but some workers believe that AC may gradually release certain adsorbed products, such as nutrients and growth regulators which become available to plants [20].

***In vitro rooting and plant acclimatization***

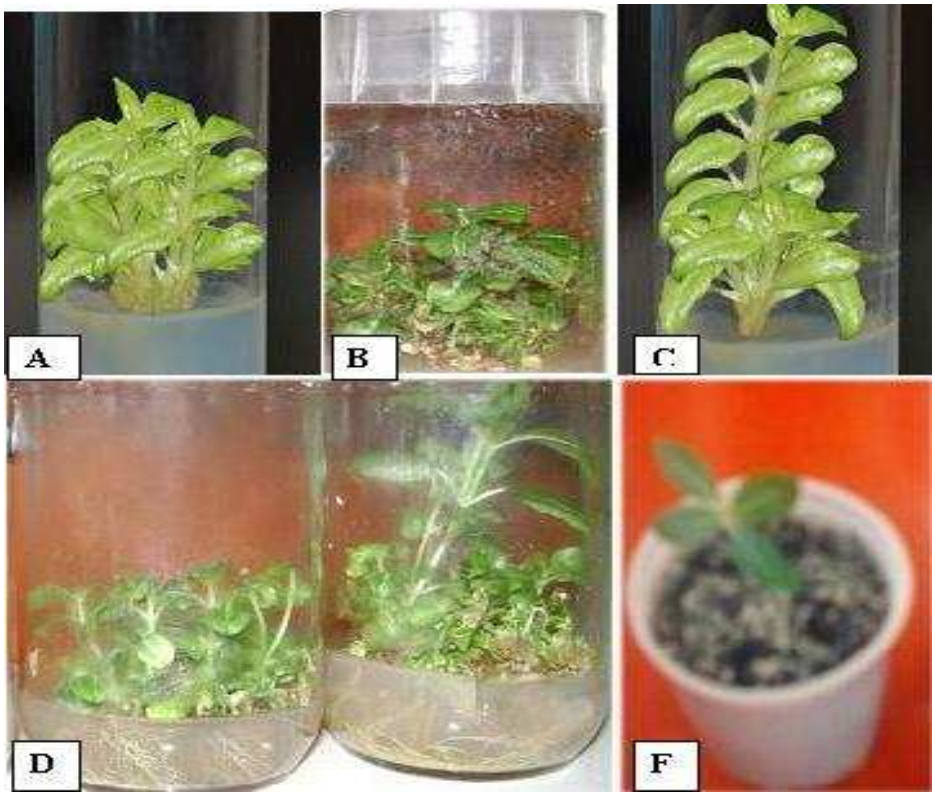
For the development of complete plantlets, *in vitro* raised microshoots of appropriate length (4-5 cm) were excised from the parent tissue and transferred to rooting medium comprised of half or full strength MS medium with or without auxins (IBA) at different concentrations. All the treatments applied in the present study induced rooting, with root initiation occurring approx. 2 week after the transfer of shoots to the rooting medium. Medium free of growth regulator induced relatively few and smaller roots in 65.0 % and 42.3 % of the shoots in half and full strength media respectively.

**Table 3. Effect of IBA on root induction of shoots of *Catharanthus roseus* on MS- medium**

| Auxin concentrations in mg/l | Cultures with roots % | Roots Number | Root lengths cm | Days of rooting In week |
|---|---|---|---|---|
| **IBA** | | | | |
| 0.05 | 82 | 10.57±0.37 | 1.09±0.17 | 3 week |
| **0.10** | **93** | **30.05±0.47** | **6.87±0.57** | **4 week** |
| 0.50 | 70 | 5.12±0.53 | 3.67±0.31 | 3 week |
| 1.00 | 62 | 5.54±0.67 | 4.87±0.57 | 3 week |

The induction of roots in auxin free medium is due the endogenous level of hormones in the regenerated microshoots. Best rooting response (93.0 %) were obtained on half strength MS medium containing IBA (0.10 mg/l) where 30.0 - 31.0 healthy roots/shoots were produced with 6.87 cm of root length after 4 weeks of culture **(Fig.-D) (Table 3).**

**Fig-1. *In vitro* shoot regeneration and plant development in *Catharanthus roseus* through nodal explants**




***A.*** *Induction of shoots on MS basal Media supplemented with BAP (0.5 mg/l).*
***B.*** *Shoots proliferation on MS- media +BAP 90.5 mg/l) + NAA (1.0 mg/l) + AC 3%.*
***C.*** *Elongation of induced shoots on MS-medium + BAP (0.5 mg/l) + IBA (1.5 mg/l).*
***D.*** *In vitro rooting on ½ MS + IBA (0.10 mg/l) after 4 week of culture.*
***E.*** *A well developed acclimatized plant in soil.*

Roots were healthy, thick and having well developed secondary branches. Superiority of IBA over other auxins in root formation has also been reported in other plant species such as *Cunila galoides* [21] and *Clitoria ternatea* [22]. Plantlets with well developed root system were isolated from the rooting medium and hardened off inside the growth room as described in materials and

methods. Regenerated plantlets were successfully transferred to greenhouse conditions with 90 % survival rate and no morphological variation was recorded in leaf morphology, inflorescence pattern and fruit development when compared to *in vivo* plants **(Fig.-E).**

## CONCLUSION

In conclusion, the protocol described in this study can be used for the efficient production of *Catharanthus roseus*. Such plants could be used as a source of tissues for the biochemical characterization of medicinally active compounds and will increase the opportunities for the use of this medicinal plant in both the traditional and modern medical health care.

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