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Multiple shoots regeneration of (anti-cancer plant) *Catharanthus roseus* - An important medicinal plant

Jitendra Mehta[1]* Deeksha Upadhyay[1], Priyanka Paras[1], Rukhshar Ansari[1], Sunil Rathore[1], Shalini Tiwari[1]

1.Plant Tissue Culture Laboratory and Department of Biotechnology, Vital Biotech Research Institute, University of Kota, Kota- 324009 (Rajasthan), INDIA.

ABSTRACT

An efficient and cost effective micropropagation protocol using MS medium developed for Catharanthus roseus, a commercially important medicinal plant. Shootlets were regenerated from nodal explants of stem through axillary shoot proliferation. The induction of multiple shoots from nodal segments were premier in MS medium supplemented with 0.5 mg/l BAP ± 1mg/l NAA. For rooting, different concentration of IBA were used and maximum rooting was recorded on MS medium with 5 mg/l IBA. The rooted plantlets were hardened initially in culture room conditions and then transferred to misthouse.

Keywords: Micropropagation, shoot proliferation, anti-cancer plant

*Corresponding Author Email: jitendra1242@gmail.com

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INTRODUCTION

Herbal medicine have always occupied the nuclear place in every traditional system of medicine.It may be *Ayurveda*, *Unani* or *Sidtha*. More than 21,000 plants possess medicinal values which have been listed by World Health Organization (WHO). 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.[1]. *In vitro* regeneration or micropropagation has tremendous potential for rapid multiplication and production of high quality medicinal plants [2]. *Catharanthus roseus* (L.) G. Don. (Commonly known as *sadabahar* or Periwinkle, family apocynaceae), gained commercial importance because of its medicinal properties. It is an erect handsome herbaceous perennial plant which is a chief source of patented cancer and hypotensive drugs. *Catharanthus roseus* has more than 400 known alkaloids in its different parts. The alkaloids like antineoplastindimeric, vinblastin and vincristine are mainly present in aerial parts, whereas ajmalcine, vinceine, vincamine, raubasin and reserpine are present in roots and basal stem. The dimericindole alkaloids from *C.roseus* are mainly used for treatment of various human cancers. Pharmaceutical industry used it for the treatment of childhood leukemia, Hodgkin's disease, testicular cancer and cancerous tumors. *C.roseus* 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 *C.roseus*, node culture producing shoots were noticed [3-6]. Optimization of conditions for shoot multiplication has been attempted for continuous production of useful compounds without depletion of natural flora [7-10]. Selection of suitable experiments depends on various factors such as physiological stage, type of organ tissue, seasonal variations of parent plant from which the explants is selected [11]. In present study, attempts were made for *in vitro* shoot regeneration of *Catharanthus roseus* using different explants. These efforts are made to study plant regeneration using shoot tip, hypocotyls and epicotyls explants, this have done in light conditions.

MATERIAL AND METHODS

Collection of explant

The branches (about 5-6 cm) of *Catharanthus roseus* plant were collected from the Herbal Garden, Bundi Road, near Nanta farm house, Kota, Rajasthan.

Surface Sterilization

The branch with node 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.

Explant implantation

Nodal segments (with a single axillary bud) about 0.5-0.8 cm were aseptically prepared and were implanted vertically on MS medium prepared with specific concentrations of BAP, Kn (1.0-5.0 mg/l) singly or in combination were used for shoot proliferation. Same experiments were repeated for shoot multiplication.

Media Preparation

The medium containing 3% sucrose was solidified with 0.8% agar (Qualigens). The *p*H of the media was adjusted to 5.2-6.2 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.

Subculturing

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 BAP and NAA. Among these, the maximum number of shoots (7.30±0.64) and maximum shoot length (5.97±0.17) was observed on medium supplemented with 0.5 BAP+1.0 NAA. Rooting of elongated shoots was attempted under *in vitro* conditions.

Auxins Concentration

Auxins (IBA) alone in different concentrations (0.5-5 mg/l) were incorporated in the agar (0.8%) solidified medium containing 1/4 MS salts and 3% sucrose. But the maximaum number of roots (2.80±0.73) and maximum root length was observed on medium supplemented with 5.0 IBA.

Acclimatization

The *in vitro*-rooted plantlets were transferred to culture bottles 1/4th filled with soilrite composition (soil: sand: peat moss) and irrigated with 1/4 MS salt solution. These bottles were kept in controlled environmental conditions of culture room. After 3 wk of growth, the plantlets were transferred to misthouse for further growth.

RESULTS AND DISCUSSION

The nodal explants, when inoculated on MS medium containing BAP and Kn in the range 1.0-5.0 mg/l showed enhanced shoot proliferation. BAP at its 1.0 mg/l concentration evoked best response (Table-1, Figure-1 A, B, Figure-2).

Figure-1: (A-G) Micropropagation of *Catharanthus roseus* from nodal shoot explants

A. Shoot multiplication on MS medium supplemented with 1.0 mg/l BAP, **B.** Shoot multiplication on MS medium supplemented with 1.0 mg/l Kn, **C.** Shoot multiplication on MS medium supplemented with 2.0 mg/l NAA, **D.** Shoot multiplication on MS medium supplemented with 0.5 mg/l BAP+1.0 mg/l NAA, **E.** In vitro root induction on ¼ of MS medium supplemented with 5.0 mg/l IBA, **F.** 4 weeks old rooted plant for hardening, **G.** well growing plant in green house.

Table-1:Effect of cytokinin (BAP and Kn) on shoot proliferation from nodal shoot explant of *Catharanthus roseus*

Hormone Concentration (mg/ l)	Hormone Concentration (mg/ l)	Response (%)	Number of Shoot/explant (mean±SD)	Shoot length (in cm) (mean±SD)
BAP	**Kn**			
1.0	-	98	7.12±0.45	1.80±0.28
2.0	-	92	5.40±0.81	2.54±0.65
3.0	-	86	3.40±0.24	1.36±0.74
4.0	-	72	2.30 ±0.31	1.19±0.21
5.0	-	70	1.73±0.87	1.00±0.15
-	1.0	97	6.67±1.22	2.70±1.50
-	2.0	80	5.80±0.24	2.50±0.94
-	3.0	96	3.87±0.39	3.36±0.29
-	4.0	76	4.50±0.20	2.23±0.11
-	5.0	72	3.31±0.30	1.27±0.85

Medium: MS+ additives; mean± SD, n= 7 replicates, Means having the same letter in each Colum do not different significantly at P< 0.05 (Tukey's test)

Shoots after their initial proliferation on medium containing 1.0 mg/l BAP were sub-cultured on same fresh medium after every 21 days. Incorporation of BAP or Kn into MS medium supported multiplication of shoots in culture, BAP proved to be a better choice than Kn and the maximum number of shoot was obtained on its 1.0 mg/l concentration (Table-1, Figure-1 A, B, Figure-2).

When BAP was used in combination with NAA a variety of responses were observed (Table-2, Figure-1 C, D and Figure-3). But best response was observed on medium containing 0.5 mg/l BAP + 1.0 mg/l NAA (Average number of shoots (7.30±0.64) and Average shoot length (5.97±0.17) was observed on medium containing 0.5 mg/l BAP+ 1.0 mg/l NAA.

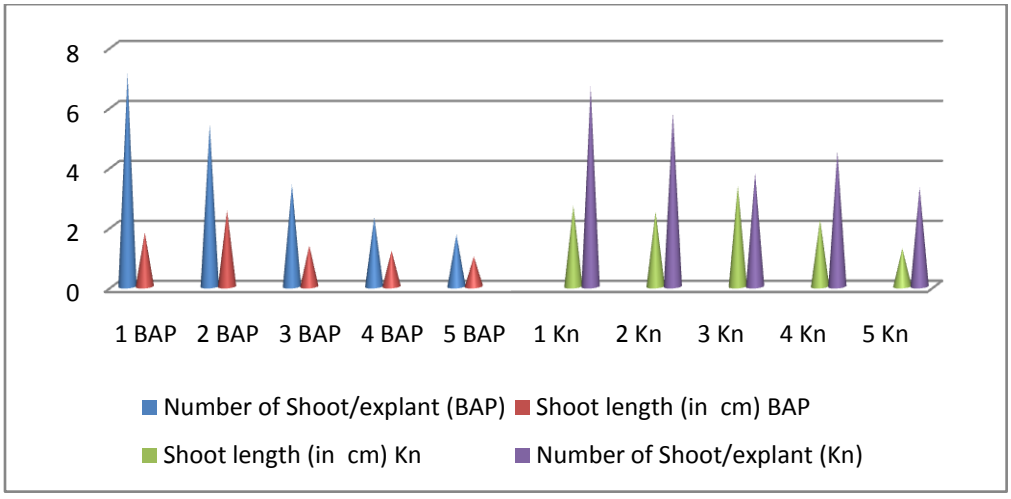

Figure-2: Effect of cytokine (BAP and Kn) on shoot proliferation from nodal shoot explants of *Catharanthus roseus*

Table 2: Interactive effect of cytokine (BAP+ NAA) on shoot multiplication by sub culture of shoot Clumps of *Catharanthus roseus*

Hormone Conc.(mg/ l)	Number of Shoots/explant	Shoot length (in cm)	Shooting Response (%)
0.5 BAP + 0.5 NAA	6.50±0.27	3.87±0.39	98
0.5 BAP + 1.0 NAA	7.30±0.64	5.97±0.17	99
0.5 BAP + 2.0 NAA	5.02±0.76	3.06±0.22	97
0.5 BAP + 2.5 NAA	4.98±0.74	2.31±0.48	82
0.5BAP + 3.0 NAA	3.78 ±0.57	2.17±0.47	78

Medium: MS+ additives; mean± SD, n= 7 replicates

Means having the same letter in each Column, do not different significantly at P< 0.05 (Tukey's test)

Table-3: Effect of auxin (NAA) on shoot induction from nodal explant of *Catharanthus roseus*

Hormone Conc. (mg/ l)	Number of shoots/explant	Shoot length (in cm)	Shooting Response (%)
1.0 NAA	5.23 ±0.12	1.77±0.39	92
2.0 NAA	8.75±0.34	3.31±0.30	97
3.0 NAA	5.80±0.24	2.50±0.94	95
4.0 NAA	4.40±0.94	2.15±0.28	86
5.0 NAA	3.95±0.03	1.65±0.65	78

Medium: MS+ additives; mean± SD, n= 7 replicates

Means having the same letter in each Colum do not different significantly at P< 0.05 (Tukey's test)

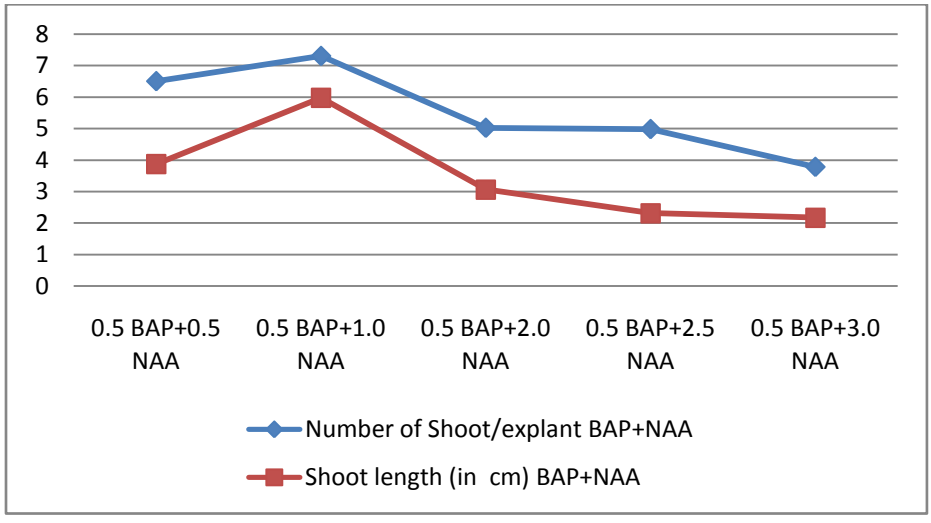

Figure-3: Interactive effect of cytokine (BAP + NAA) on shoot multiplication by subculture of shoot clumps of *Catharanthus roseus*

The full or half strength of MS medium without any PGR was failed to induce rooting of regenerated shoots. However, shoots were capable to induce root when cultured on medium containing auxins.

Table-4: Effect of auxin (IBA) on root induction from isolated shoot of *Catharanthus roseus*

Hormone Concentration(mg/ l)	Number of roots/explants	Root length (in cm)	Rooting Response (%)
1.0 IBA	1.08±0.19	0.51±0.05	73
2.0 IBA	1.38±0.37	0.92±0.10	78
3.0 IBA	1.40±0.52	1.06±0.08	80
4.0 IBA	2.80±0.73	0.43±0.33	85
5.0 IBA	3.60±0.51	1.68±0.32	90

Medium: MS+ additives; mean± SD, n= 7 replicates. Means having the same letter in each Colum do not different significantly at P< 0.05 (Tukey's test)

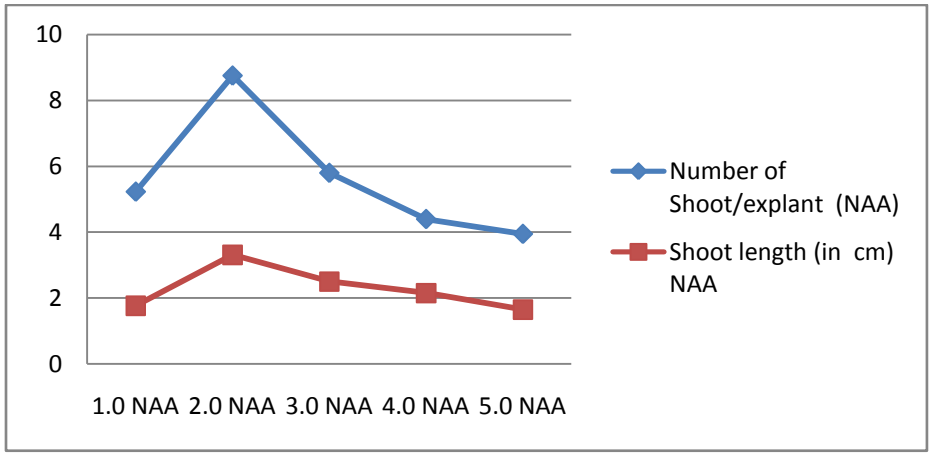

Figure-4: Effect of Auxin (NAA) on shoot multiplication by subculture of shoot clumps of *Catharanthus roseus*

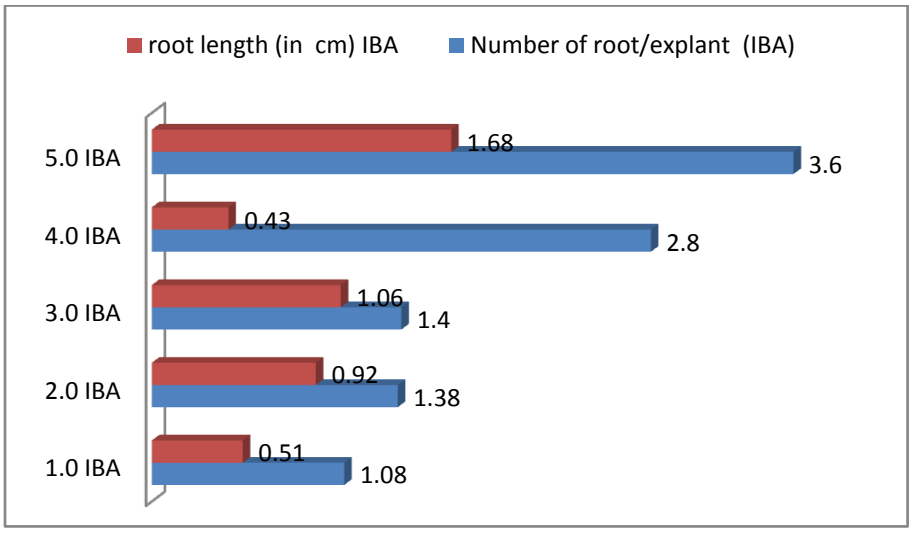

Figure-5: Effect of Auxin (IBA) on root induction from isolated shoots of *Catharanthus roseus*

Auxins in different concentration induced rooting when incorporated in the medium containing ¼ of MS salts. The best rooting response, however, was observed on medium containing 5mg/l IBA, where roots measuring 2.80±0.73 cm (average) were formed (Table-4, Figure-1 E, Figure-4).

In vitro rooted plantlets were initially hardened in culture room conditions where leaves expanded. After 3 weeks, the plantlets were shifted to mist house. There was an increase in length of shoots and new leaves emerged which expanded quickly (Figure-E).

CONCLUSION

The seedling derived explants, being juvenile, are frequently used for micropropagation, as they are easy to establish in culture. In *Catharanthus roseus*, MS medium containing 1.0 mg/l BAP was best for culture initiation. We have found that *Catharanthus roseus* culture grew better on MS medium in comparison to other media. In *Catharanthus roseus* 1.0 mg/l BAP was most suitable for shoot multiplication. We also observed improvement in shoot multiplication by different concentrations of NAA (0.5-3.0 mg/l) in medium along with BAP (0.5 mg/l). Best shooting response was observed on media containing 0.5 mg/l BAP+ 1.0 mg/l NAA (Average number of shoots 7.30±0.64) and 0.5 mg/l BAP+ 1.0 mg/l NAA (Average shoot length 5.97±0.17cm). IBA (Auxin) has been widely used as root induction hormone under *in vitro* and *in vivo* condition. We also found positive role of IBA during *in vitro* rooting. In *Catharanthus roseus*, 5.0 mg/l IBA proved to be the best for in vitro rooting. The *in vitro* rooted plants were hardened first under controlled conditions of culture room and then shifted to mist house where they exhibited and hence, growth and 90% survival.

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