Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **1 (5):** 42-50 (2013)

Research Article

International Journal of Pure & Applied Bioscience

Efficient shoot regeneration from node explant of *Catharanthus roseus* *(A milestone in cancer chemotherapy)*

Monika Sain, Vandana Sharma*

Department of Botany, Govt. College, Kota, University of Kota, Kota- 324009 (Rajasthan)
E-mail address: monikasain24@gmail.com

ABSTRACT

Catharanthus roseus (L.) G. Don, which also known as "an anticancerous drug yielding plant" is a tropical and subtropical plant belonging to the family Apocynaceae. The alkaloids like Vinblastine and vincristine are mainly present in aerial parts of C. roseus, which are used in treatment of various human cancers, so it is considered as mile stone in cancer chemotherapy.

This review highlights the recent development and achievements made for the micropropagation of Catharanthus roseus in Kota region of South East rajasthan. Based on results of this study, maximum shoot proliferation from nodal explant was observed on MS medium supplemented with BAP (0.5 mg/l) + Kn (2.0 mg/l). For rooting, different concentrations of IBA were used and highest rooting (78%) was observed on ¼ strength of MS medium fortified with IBA (5.0 mg/l). The rooted plantlets were transferred to plastic bags containing a mixture of garden soil and vermiculite (1:1). The hardened plants were successfully established in the soil.

Keywords: *- Catharanthus roseus, nodal, anti-cancer, Vinblastine and vincristine.*

INTRODUCTION

Plants are known for the production of a large array of natural products, also referred economically important to man due to their multiple applications, such as pharmaceuticals, flavour, fragrances, insecticidal, dyes, food additives, toxins etc. The majority of pharmaceutically important secondary metabolites are obtained from wild or cultivated plants, although some attempts have been made, their chemical synthesis in most cases has not been economically feasible. Therefore, production of plant secondary metabolites by cultivation of plants and chemical synthesis, are important agronomic and industrial objectives. As a promising alternative to produce plant secondary metabolites, plant cell culture technology (Micropropagation) has many advantages over traditional field cultivation and chemical synthesis[1].

Catharanthus roseus L. (Apocynaceae) is a medicinal plant which contains a virtual cornucopia of useful alkaloids, used in diabetes, blood pressure, asthma, constipation and menstrual problems[2]. The roots contains, the major alkaloids are ajmalicine and serpentine, which are used in the treatment of circulatory disease[3]. More recently, vinblastine and vincristine alkaloids obtained from *Catharanthus roseus* have been shown to be effective in the treatment of various kinds of cancer such as Leukamia, skin cancer , Lymph cancer, breast cancer and hodgkin's disease[4,5,6,7]. Therefore, *Catharanthus* alkaloids are considered as mile stone in cancer chemotherapy by the nature.

C. roseus has opposite glassy leaves of 2-3 cm; the flowers are white to dark pink with darker red centre, with a basal tube of 2.5-3 cm long and a corolla of about 2-5 cm diameter with five petals like lobes[5]. Fruits of *C. roseus* have many small black and cylindrical seeds[8].

The climatic condition and soil properties of some European countries are unfavourable for the cultivation of *C. roseus*, while there is a great demand for alkaloids and their preces are high, the content of alkaloids

in the raw material derived from naturally occurring whole plant is low. Hence, the importance of studies on the recovery of indole alkaloids from plant tissues and organs. The development of efficient plant regeneration techniques and methods for large scale in-vitro cultivation is of great importance and many become the proper solution for collecting the raw material for recovery of indole alkaloids. The aim of this studyis to develop conditions for efficient shoot and root regeneration from nodal explant of *C. roseus* by the manipulation of different combination of plant growth regulators with the aim of inducing alkaloids production.

MATERIAL AND METHODS

The branches (about 5-6 cm) of shoots of *Catharanthus roseus* plant were collected from the Garden of govt. college, Kota, Rajasthan.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. 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. 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.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 explants were inoculated in various concentrations and combination of growth regulators (BAP, Kn, NAA, IAA). Among these, the maximum number of shoots (8.35±0.5) was developed on MS media fortified with 0.5 BAP± 2.0 Kn. Maximum shoot length was observed as 5.97±0.17 on medium supplemented with 0.5mg/l BAP+1.0mg/l NAA. Rooting of elongated shoots was attempted under *in vitro* conditions. Auxins (IBA) alone in different concentrations (1.0-5.0 mg/l) were incorporated in the agar (0.8%) solidified medium containing 1/4 MS salts and 3% sucrose. The *in vitro*-rooted plantlets were transferred to plastic bags 1/4th filled with garden soil land vermiculite (1:1) and irrigated with 1/4 MS salt solution. These bags 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

It is well known that the balance of cytokinin and auxin in plant tissues control the direction of organogenesis. To keep this balance ideal for development of new organs exogenous cytokinin or auxin is usually added to the plant regeneration medium. In this research, nodal tissue of *Catharanthus roseus* were found to be sensitive to the presence and concentration of cytokinin and auxin seemed critical for shoot regeneration. Addition of exogenous plant growth regulator to the medium was necessary for plant regeneration of *C. roseus.*

In present study, The nodal explants, when inoculated on MS medium containing single cytokinin(BAP , Kn)and auxin(NAA,IAA) in the range 1.0-5.0 mg/l showed enhanced shoot proliferation. (Table-1, 2). BAP at its 3.0 mg/l concentration evoked best response.

Shoots after their initial proliferation on medium containing 3.0 mg/l BAP were sub-cultured on same fresh medium after every 21 days. Incorporation of single BAP , Kn, NAA and IAA into MS medium supported multiplication of shoots in culture, BAP proved to be a better choice than other and the maximum number of shoots were obtained on its 3.0 mg/l concentration (Table-1, Figure-1-A, B, Figure-2). When BAP was used in combination with Kn and NAA a variety of responses were observed (Table-3,4 Figure-1 C, D and Figure-3). But best response was observed on medium containing 0.5 mg/l BAP +

2.0 mg/l Kn (Average number of shoots 8.35±0.5) and best shoot length was observed on medium containing 0.5 mg/l BAP + 1.0 mg/l NAA (Average shoot length 5.97±0.17). 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. IBA in different concentrations (1.0-5.0 mg/l) induced rooting when incorporated in the medium containing ¼ of MS salts. The best rooting response, however, was observed on medium containing 5.0 mg/l IBA, where roots measuring 1.06±0.05cm (average) were formed (Table-5, Figure-1-D, 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).

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

Hormone Concentration (mg/ l)	Hormone Concentration (mg/ l)	Response (%)	Number of Shoots/explant (mean±SD)	Shoot length (in cm) (mean±SD)
BAP	**Kn**			
1.0	-	80	7.12±0.45	1.80±0.28
2.0	-	75	5.40±0.81	2.54±0.65
3.0	-	60	3.40±0.24	1.36±0.74
4.0	-	55	2.30 ±0.31	1.19±0.21
5.0	-	40	1.73±0.87	1.00±0.15
	1.0	78	6.67±1.22	2.70±1.50
	2.0	74	5.80±0.24	2.50±0.94
	3.0	82	7.13±0.65	3.36±0.29
	4.0	50	4.50±0.20	2.23±0.11
	5.0	45	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)

Table-2: Effect of Auxin (NAA and IAA) on shoot proliferation from nodal shoot explant of *C. roseus*

Hormone Con. (mg/ l)	Hormone Con. (mg/ l)	Response (%)	No. of Shoot/explant (mean±SD)	Shoot length (in cm) (mean±SD)
NAA	**IAA**			
1.0	-	87	5.23±0.12	1.77±0.39
2.0	-	60	4.40±0.94	2.15±0.28
3.0	-	75	5.75±0.34	3.31±0.30
4.0	-	70	5.80±0.24	2.50±0.94
5.0	-	65	3.95±0.03	1.65±0.65
-	1.0	70	1.57±0.40	1.62±0.28
-	2.0	68	2.42±0.39	2.28±0.71
-	3.0	75	2.28±0.36	0.53±0.33
-	4.0	55	1.08±0.19	0.28±0.13
-	5.0	30	1.28±0.36	0.15±0.18

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)

Table-3: Interactive effect of cytokine (BAP+ NAA) on shoot multiplication by sub culture of shoot Clumps of *C. roseus*

Hormone Concentration (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	70
0.5 BAP + 1.0 NAA	7.30±0.64	5.97±0.17	80
0.5 BAP + 2.0 NAA	5.02±0.76	3.06±0.22	86
0.5 BAP + 3.0 NAA	3.78 ±0.57	2.17±0.47	75
0.5BAP + 4.0 NAA	1.52±0.15	1.19±0.21	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-4: Interactive effect of cytokine (BAP+ Kn) on shoot multiplication by sub culture of shoot Clumps of *C. roseus*

Hormone Concentration (mg/ l)	Number of Shoots/explant	Shoot length (in cm)	Shooting Response (%)
0.5 BAP + 0.5 Kn	2.14±0.51	1.41±0.28	60
0.5 BAP + 1.0 Kn	7.10±0.28	2.71±0.36	75
0.5 BAP + 2.0 Kn	8.35±0.51	3.42±0.39	65
0.5 BAP + 3.0 Kn	3.10 ±0.41	2.41±0.38	38
0.5BAP + 4.0 Kn	2.57±0.40	1.52±0.36	45

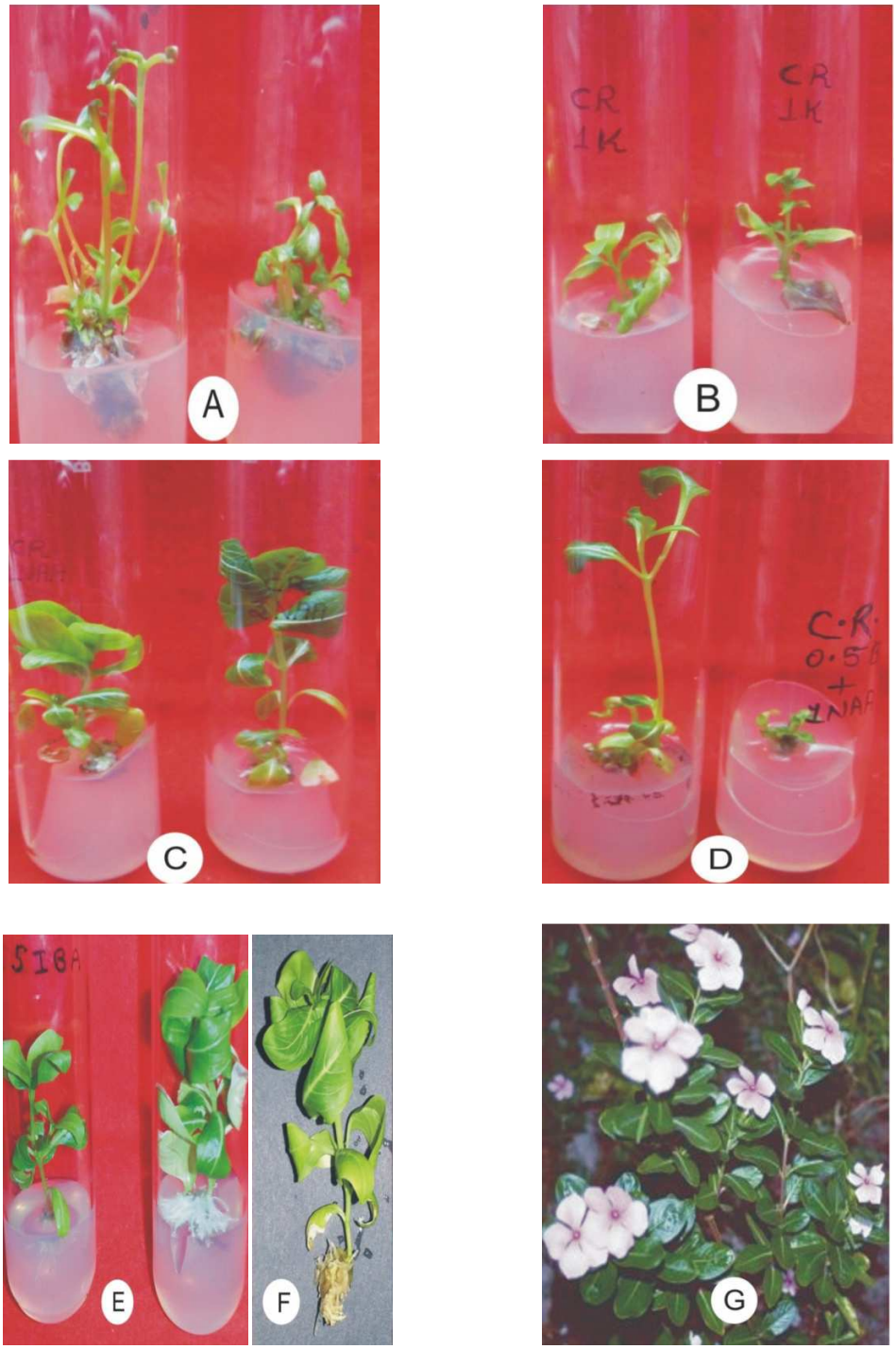
Medium: MS+ additives; mean± SD, n= 7 replicates
Means having the same letter in each Column do not different significantly at P< 0.05 (Tukey's test.)

Table-5: 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	0.51±0.05	0.32±0.33	30
2.0 IBA	0.92±0.12	0.42±0.31	35
3.0 IBA	3.60±0.51	0.61±0.08	40
4.0 IBA	4.10±0.36	0.92±0.10	80
5.0 IBA	5.80±0.27	1.06±0.05	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-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.

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

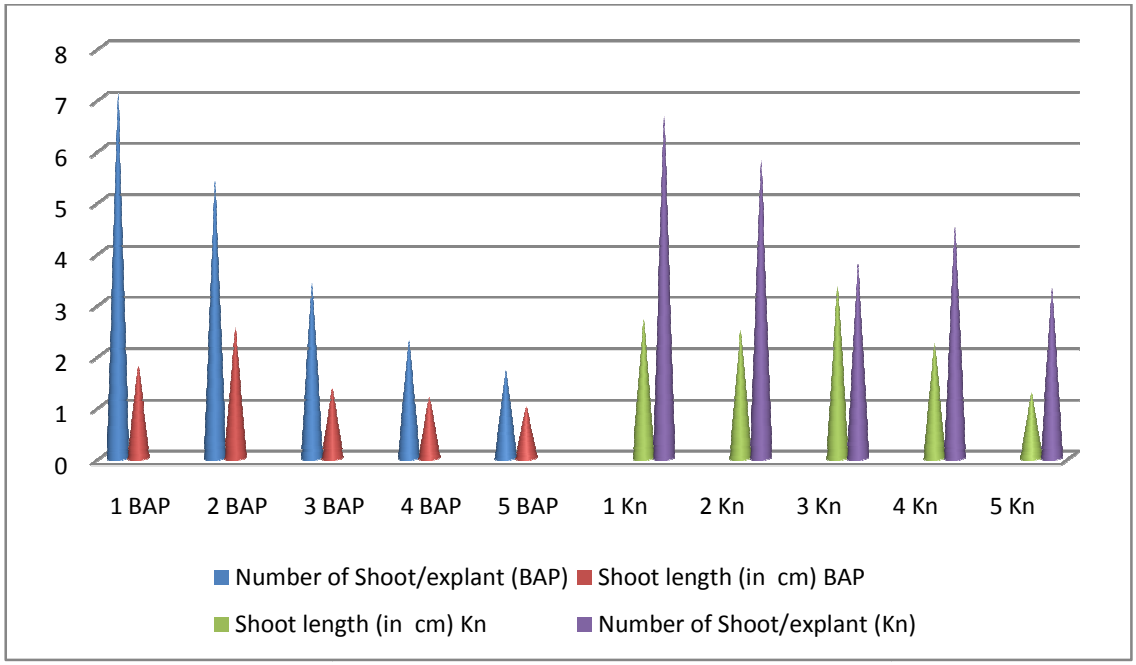


Figure-3: Interactive effect of cytokine (BAP + NAA) on shoot multiplication by subculture of shoot clumps of *Catharanthus roseus*

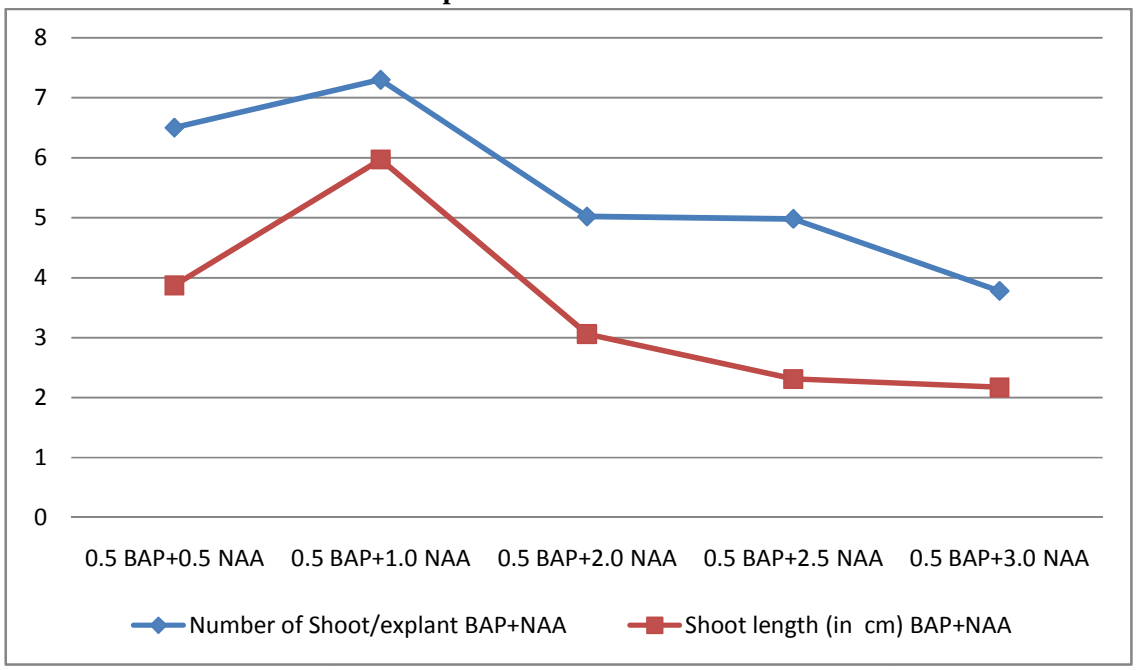


Figure-4: Effect of Auxin (NAA & IAA) on shoot multiplication by subculture of shoot clumps of *Catharanthus roseus*

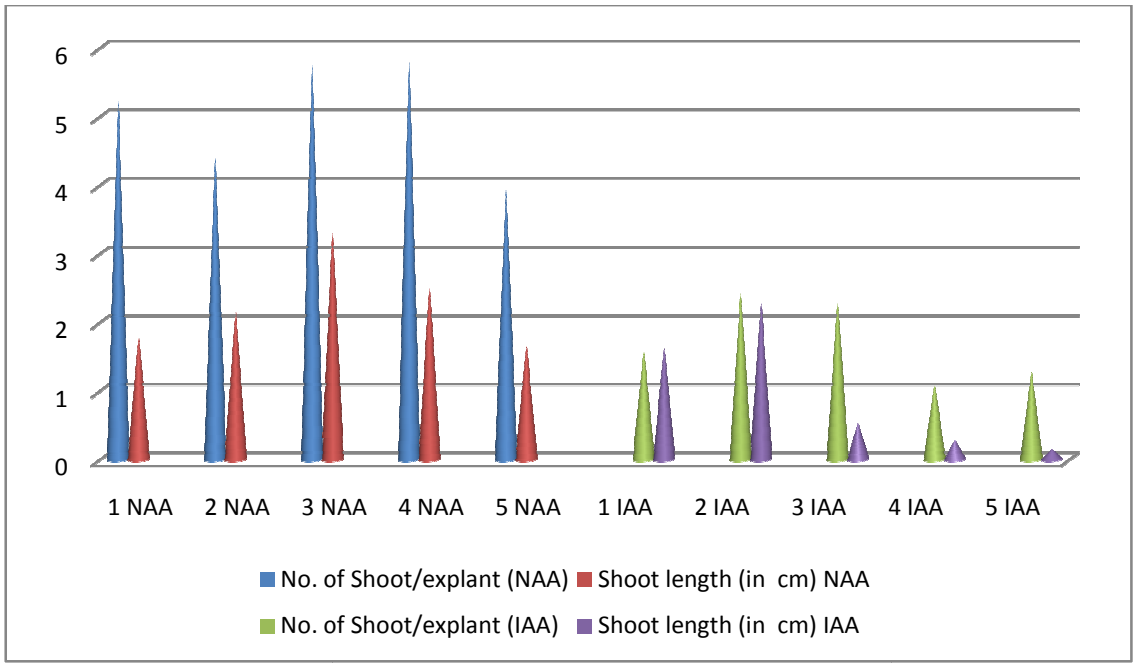


Figure-5: Effect of Auxin (IBA) on root induction from isolated shoots of *Catharanthus roseus*

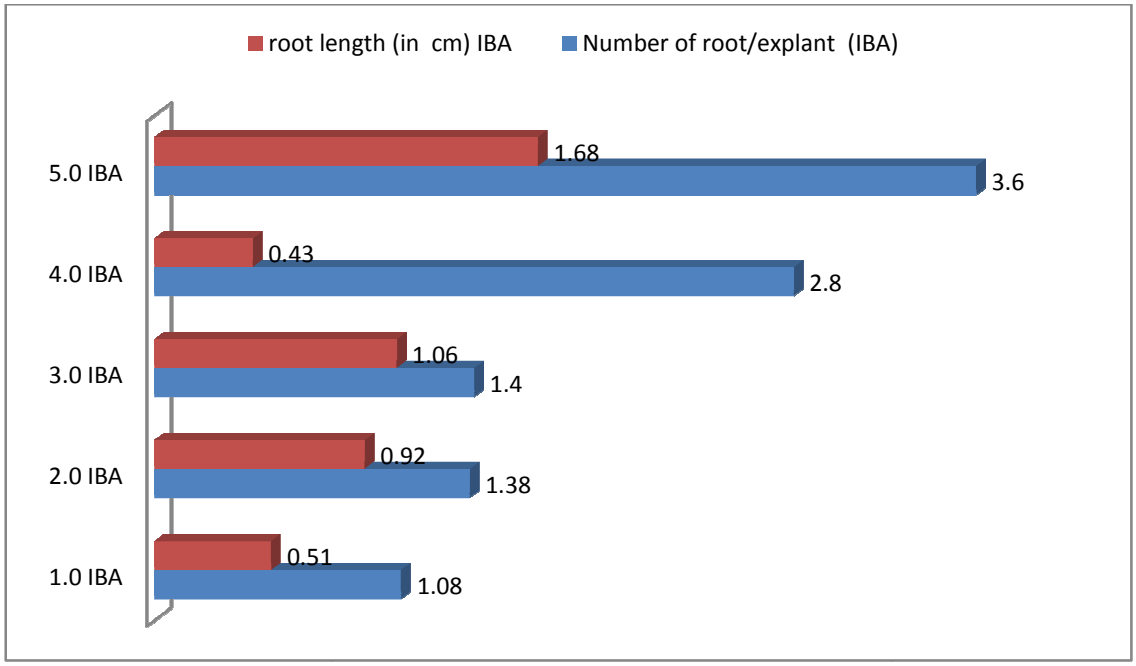
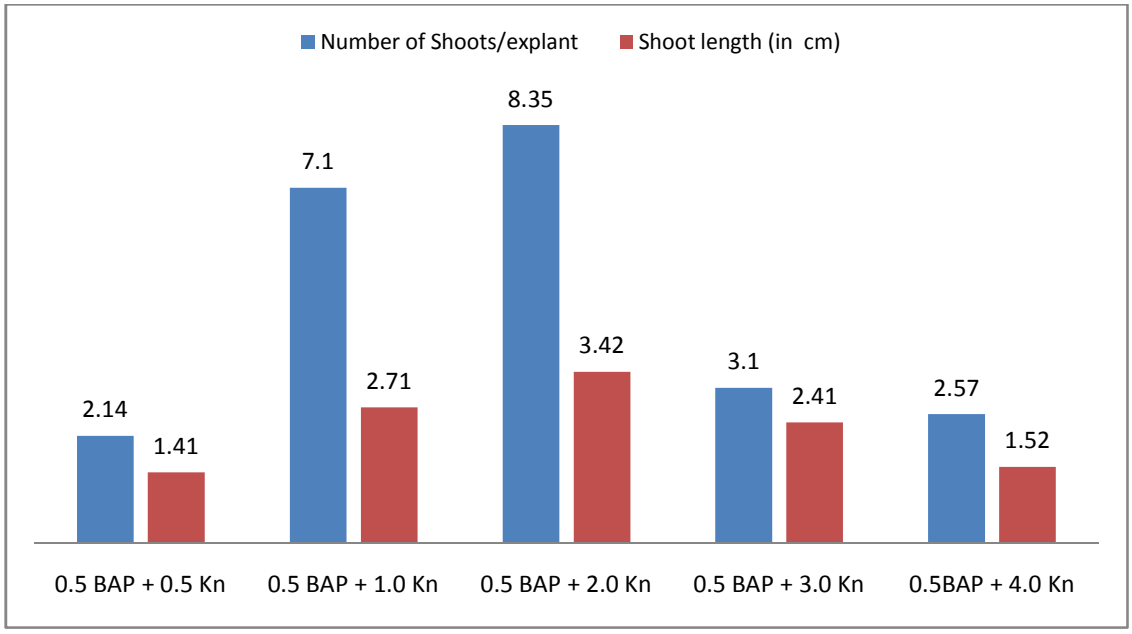


Figure-6: Interactive effect of cytokine (BAP+ Kn) on shoot multiplication by sub culture of shoot Clumps of *Catharanthus roseus*



CONCLUSION

In conclusion, an efficient regeneration system through organogenesis using nodal explant was developed. Effect of single and various combinations of plant growth regulators were elucidated, which will largely facilitate developing an efficient and universal genetic transformation system of *Catharanthus roseus.*

ACKNOWLEDGEMENT

We are thankful to department of botany, Govt. College Kota for providing faculties and also thankful to Dr. Vandana Sharma and staffs members of botany department, Govt. College Kota for encouragement.

REFERENCES

1. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov. **4:** 206–220 (2005)
2. Murch SJ, Krishna Raj S, Saxena PK. Tryptophan is a precursor for melatonin and serotonin biosynthesis in in vitro regenerated St. John's wort (Hypericum perforatum L. cv. Anthos) plants. Plant Cell Rep. **19:** 698-704 (2000)
3. Constabel F, Gaudet-La Prairie P, Kurz WGW, Kutney JP. Alkaloid production in *Catharanthus roseus* cell cultures. XII. Biosynthetic capacity of callus from original explants and regenerated shoots. Plant Cell Rep. **1:** 139-142 (1982)
4. Endo T, Goodbody A, Misawa M. Alkaloid production in root and shoot culture of *catharanthus roseus.* Planta Med,. **53(5):** 479-482 (1987)
5. Hirata K, Horiuchi M, Ando T, Miyamoto K, Miura Y. Vindoline and catharanthine production in multiple shoot cultures of *Catharanthus roseus*. Journal of Fermentation and Bioengineering. **70:**193–195 (1990)
6. Miura Y, Hirata K, Kurano N, Miyamoto K, Uchida K, Formation of vinblastine in multiple shoot culture of *Catharanthus roseus,* Planta Med., **54:** 18-20 (**1988**)
7. Hirata K, Horiuchi M, And T, Miyamoto K & Miura Y, Effect of near-ultraviolet light on alkaloid production in multiple shoot culture of *Catharanthus roseus.* Planta Med., **57:** 499-500 (1991)
8. Yuan Y J, Hu T T & Yang Y M, Effect of auxins and cytokinins on formation of *Catharanthus roseus* G. Don multiple shoots, *Plant. Cell Tiss. Org. Cult.*, **37:** 193-196 (1994)

9. Mitra A, Khan B , Rawal S, Rapid in vitro multiplication of plants from mature nodal explants of *Catharanthus roseus* (L.) G. Don, *Planta Med.,* **64 (4):** 390 (1998)
10. Monard P, Henry H, Optimization of mineral composition of *in vitro* culture media. *Journal Plant Nutrition,* **21 (8):** 1565-1576 (1998)
11. Murashige, T., Clonal crops through tissue culture. In: Barz, W., E. Reinhard and M.H. Zenk, (Eds.). Proc. Life Sciences: Plant Tissue Culture and its Biotechnological Applications.Springer-Verlag, Berlin, pp: 392-403 **(**1977)