ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Auxiliary bud proliferation and multiplication of medicinally important plant *Catharanthus roseus* (L) G. Don through internodal tissue culture

Rajender Vadluri, Naga Chandra Bandari, Rani Biyyani, Srinivas Gorripati, Sandeep Sadanapalli, Mohmmad Mazeed, Allenku Jyoshna, Devulapelly Amulya, Murali Krishna Thupurani*, Bhargavi Katta*, Vijay Bomma*

Department of Biotechnology, Chaitanya Degree and Postgraduate College (Autonomous), Hanamkonda, Warangal, 506001-Telangana –India.

Manuscript Info

Manuscript History:

Received: 14 September 2015
Final Accepted: 26 October 2015
Published Online: November 2015

Key words:

Benyl amino purine, Catharanthus roseus, Kinetin, Madagascar, Murashigee and Skoog

Corresponding Author

Murali Krishna Thupurani, Bhargavi Katta, Vijay Bomma

(*all the authors are contributed equally)

Abstract

Catharanthus roseus (L.) G. Don (Apocynaceae) is native to Indian Ocean Island and found in tropical and subtropical regions worldwide. It is commonly called as Madagascar periwinkle and given immense importance for its attributed therapeutic properties. In this study, an efficient protocol was developed for in vitro propagation of Vinca rosea L. via nodal tissue explants. The explants were decontaminated and inoculated on to the Murashige and Skoog's media (pH 5.8) supplemented with various concentrations of benzyl amino purine (1.0-3.5 mg/ml) and Kinetin (1.0-3.5 mg/ml) single and in combinations. The cultures were incubated at 25˚C ± 2˚C, 16-h photoperiod (16: 8) of cool-white fluorescent tubes (PPF 45 mmol·m^{-2}·s^{-1}) and 55- 60% relative humidity. The data analysis was carried out using SAS V.9.0 software following Student't' test. Shoot formation capacity was noticed Murashigee and Skoog media with concentrations of BAP (Benyl amino purine) and KN (Kinetin). The highest number of shoots was observed with nodal segments that were maintained BAP+IAA (2.3 + 0.6 mg/L) and showed 95 % shoot initiation response. The results are expressed in SEM values and P< 0.5 is considered. The study concludes that, the nodal segments of C. *roseus* cultivated on MS mediam supplemented with 2.3 mg/L BAP and 0.7 mg/L IAA was found best choice for shoot multiplication.

INTRODUCTION

Catharanthus *roseus* (L.) G. Don (Apocynaceae) is native to Indian Ocean Island and found in tropical and subtropical regions worldwide. It is commonly called as Madagascar periwinkle and given immense importance for their possessed therapeutic properties. Monoterpenoid indole alkaloids isolated from the extracts of C. *roseus* are highly studied for their active role in growth inhibition of new blood vessels that supports the growth of tumor (**Noble, 1990**). Anti-leukematic alkaloids such as vinblastine and vincristin isolated from this plant are extensively used in treatment of cancer chemotheraphy (Magnotta etal., 2006). Moreover, these alkaloids also possess anti-oxidants, anti-diabetic properties (**Soon Huat Tiong et al., 2013**). It has been reported that these alkaloids regenerate β-cells of pancreas and stimulate the production of insulin. Literature survey revealed that C. *roseus* contains over 130(**Hisiger and jolicoeur., 2007**) compounds and many of them possess cytotoxicity (**Noble, 1990; Zu et al., 2006**). From medicinal and biodiversity point of view, the current investigation was carried out to regenerate this plant through auxiliary bud proliferation and development using BAP, KN and IAA.

2.0 MATERIAL AND METHODS

2.1. EXPLANTS SOURCE AND SURFACE DECONTAMINATION METHODS

Young shoots were collected from the crowns of mature tree (3-4 years old) growing in the green house of Chaitanya Postgraduate College (Autonomous), Hanamkonda, Warangal. Healthy nodal segments measuring 2.0-3.0 cm were cut, defoliated and rinsed thoroughly in running tap water for 30 minutes. The explants were treated with tween 20 and washing was continued under running tap water furthermore 5min. Nodal segments were sterilized with 70 % (v/v) ethyl alcohol (2- 3 min) and continued to repeated washings with sterile distilled water followed by antibiotic treatment (10 µg/mL ciproflaxicin) . These treated explants were used for inoculation.

2.2. CULTURE MEDIUM AND SHOOT MULTIPLICATION

The surface sterilized explants were inoculated on to the Murashige and Skoog's media (pH 5.8) supplemented with various concentrations of benzyl amino purine (1.0-3.5 mg/ml) and Kinetin (1.0-3.5 mg/ml) single and in combinations. The cultures were incubated at 25˚C ± 2˚C, 16-h photoperiod (16: 8) of cool-white fluorescent tubes (PPF 45 mmol·m^{-2}·s^{-1}) and 55- 60% relative humidity. Numbers of days taken for bud break and its percentage, number of shoots per explants and mean shoot length were measured.

2.3 STATISTICAL ANALYSIS

Data for shown for shoot multiplication experiment is expressed as a mean ± SE. The data were analyzed statistically using SAS 9.0 version and followed student't' test. The p value is considered when less than $P< 0.5$.

3.0 RESULTS

3.1 EFFECT OF BENZYL AMINO PURINE ON SHOOT INITIATION

The MS medium supplemented with BAP (1.0-3.5 mg/L) was used for proliferation and development of auxiliary buds. The number of shoot buds and shoot length was noticed after 6 weeks. The healthy growth of shoots was observed with the nodal plants that were maintained on 1.9-2.5 mg/L. Nodal segments that are received BAP 1.0-1.8 mg/L were resulted in no growth and negligible growth was found in nodal segments that were supplemented with BAP 2.6-3.5 mg/L (Data not shown). The shoot number, length ranged from 2-10 and 1-4 cm respectively. (Table 1.0). The highest number of shoots 9.9±2.5 and longest shoot 3.91±1.0 cm ($p<0.05$) were observed with nodal cultures (95 % shoot initiative response) supplemented with 2.3 mg/ L BAP (Table 1.0 and Graph 1.2).

3.2 EFFECT OF BENZYL AMINO PURINE IN COMBINATION WITH 3-INDOLE ACETIC ACID ON SHOOT INITIATION

The effect of BAP (1.0-3.5 mg/L) and IAA (0.2-0.8 mg/L) on nodal shoot bud proliferation and its development was also determined (Table 1.1). The effect of BAA in combination with IAA was found significant compared to the effect of BAP alone. The shoot number, length ranged from 6-14 and 1-4 cm respectively (Table 1.1 and Graph 1.0). The highest number of shoots and the length 14.3±2.0 and 4.20±1.3 ($p<0.05$) was recorded at 2.3 mg/L of BAP in association with 0.6 mg/L IAA (Table 1.1). On the other hand, nodal segments supplemented with BAP 1.0-1.8 mg/L and IAA 0.1-0.9 mg/L were resulted in no growth and negligible growth was found in the nodal segments that were supplemented with BAP 2.6-3.5 mg/L and IAA 1.0-1.9 (Data not shown).

3.3. EFFECT OF KN ON SHOOT INITIATION

The shoot initiation capacity of KN at various concentrations (1.0-3.5 mg/L) was found minimum. The number of shoots and length ranged from 1-4 and 0.7-3 cm (Table 1.0). The highest number of shoots 3.90±2.9 and longest shoot 2.99±1.3cm ($p<0.05$) were observed with nodal cultures (88 % shoot initiative response) supplemented with 2.7 mg/L KN (Table 1.0 and Graph 1.3).On the other hand, nodal segments received below 2.0 mg/L and above 2.8 mg/L KN did not show any shoot initiation and development (Data not shown).

3.4 EFFECT OF KN IN COMBINATION WITH 3-INDOLE ACETIC ACID ON SHOOT INITIATION

KN (1.0-3.5 mg/L) and IAA (0.2-1.0 mg/L) was found slightly better when compared to the effect of single KN. The shoot number, length ranged from 2-9 and 0.5-2.99 cm (Table 1.1). The highest number of shoots and the length 9.63±2.9 and 3.33±2.0 ($p<0.05$) was recorded with nodal segments (95% shoot initiative response) supplemented with 2.7 mg/L of BAP in association with 0.9mg/L IAA (Table 1.1 and Graph 1.1). No results were recorded with nodal segments supplemented with KN 1.0-1.9 mg/L and IAA 0.1-0.9 mg/L and minimum growth was noticed with nodal segments that were supplemented with KN 2.9-3.5 mg/L and IAA 1.1-1.7 (Data not shown).

3.5 EFFECT OF DARK PHASE ON DEVELOPMENT OF NODAL SEGMENTS

Nodal segments of different concentration of both BAP and KN alone or in combination with IAA are subjected different period of dark phases. Among the time periods subjected, 14 h dark treatment was shown with high development of shoots compared to that from 6 h, 8 h, 10, 12, 16 h (Graph 1.4).

Table 1.0: Showing the effect of BA and Kn for shoot production

BAP Conc (mg^{-1})	KN Conc (mg^{-1})	Response (%)	Mean Shoot No± SEM	Mean Shoot length (Cm)± SEM
1.9	--	15	3.1±0.8	0.79±0.21
2.0	--	23	4.4±1.1	0.91±0.11
2.1	--	36	6.7±1.2 *	1.12±0.19
2.2	--	48	7.9±0.3 *	1.80±0.2*
2.3	--	82	9.9±2.5 *	3.91±1.0*
2.4	--	70	6.3±0.6*	2.3±0.1*
2.5	--	28	2.9±0.1	1.09±0.3
---	2.0	20	0.98±0.1	0.58±1.1
---	2.1	25	1.0±0.3	0.99±0.2
---	2.2	34	1.74±0.5	1.12±0.1
---	2.3	48	1.96±0.9	1.36±0.2
---	2.4	56	1.30±1.2	1.77±0.3
---	2.5	63	1.51±1.5	2.10±0.5*
---	2.6	79	2.32±2.0*	2.54±0.1*
---	2.7	88	3.90±2.9*	2.99±1.3*
----	2.8	72	2.61±2.4*	1.90±0.2*

n=3 for Mean± SEM of shoot number and shoot length *P< 0.05

Table 1.1: Effect of BA and Kn in combination with IAA

BAP Conc (mg^{-1})	KN Conc (mg^{-1})	IAA Conc	Response (%)	Mean Shoot No± SEM	Mean Shoot length (Cm)± SEM
1.9	--	0.2	35	6.84±1.00	0.85±0.17
2.0	--	0.3	55	7.0±1.1	0.99±0.18
2.1	--	0.4	65	9.98±0.1*	1.36±0.26*
2.2	--	0.5	85	12.1±0.9*	1.88±0.3*
2.3	--	0.6	95	14.3±2.0*	4.20±1.3*
2.4	--	0.7	80	10.9±0.6	2.7±0.1
2.5	--	0.8	35	6.95±0.5	1.12±0.1
---	2.0	0.2	20	1.9±0.1	0.72±0.19
---	2.1	0.3	30	2.6±0.2	0.86±0.2
---	2.2	0.4	55	3.1±0.1	1.16±0.13
---	2.3	0.5	64	3.4±0.1	1.39±0.25
---	2.4	0.6	72	2.8±1.1	2.01±0.5
---	2.5	0.7	79	4.7±1.5 *	2.65±0.11*
---	2.6	0.8	87	6.6±2.0 *	2.86±1.7*
---	2.7	0.9	95	9.63±2.9*	3.33±2.0*
----	2.8	1.0	80	7.1±2.1*	2.10±0.96*

n=3 for Mean± SEM of shoot number and shoot length *P< 0.05

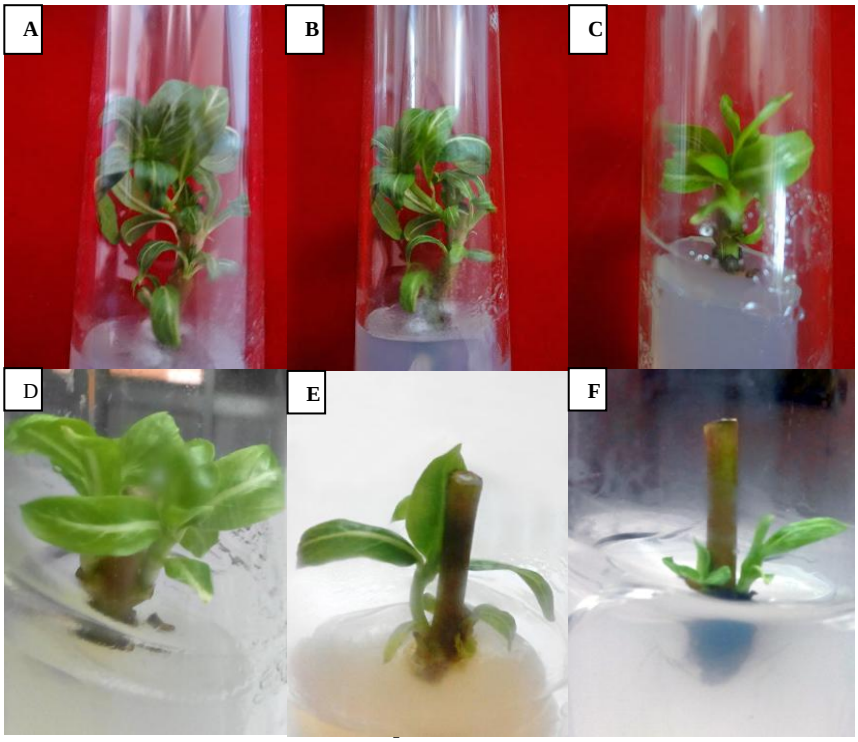


Figure 1A-F. Effect of Benzyl amino purine alone or in combination with IAA on in vitro shoot regeneration from intermodal tissue of Catharanthus roseus. Figure 1A- Nodal segments supplemented with 2.3 mg/l^{-1} of BAP and 0.6 mg/l^{-1}IAA showing high number and larger size of shoots. Figure 1B-D Nodal segments received 2.4 mg/$l^{-1}$of BAP and 0.7 mg/l^{-1}IAA showing healthy number of shoots and their larger size. Figure 1E-F-Showing the minimal growth of nodal segments supplemented with single BAP at, 2.0, 2.1, 2.2 and 2.5 mg/l^{-1}.

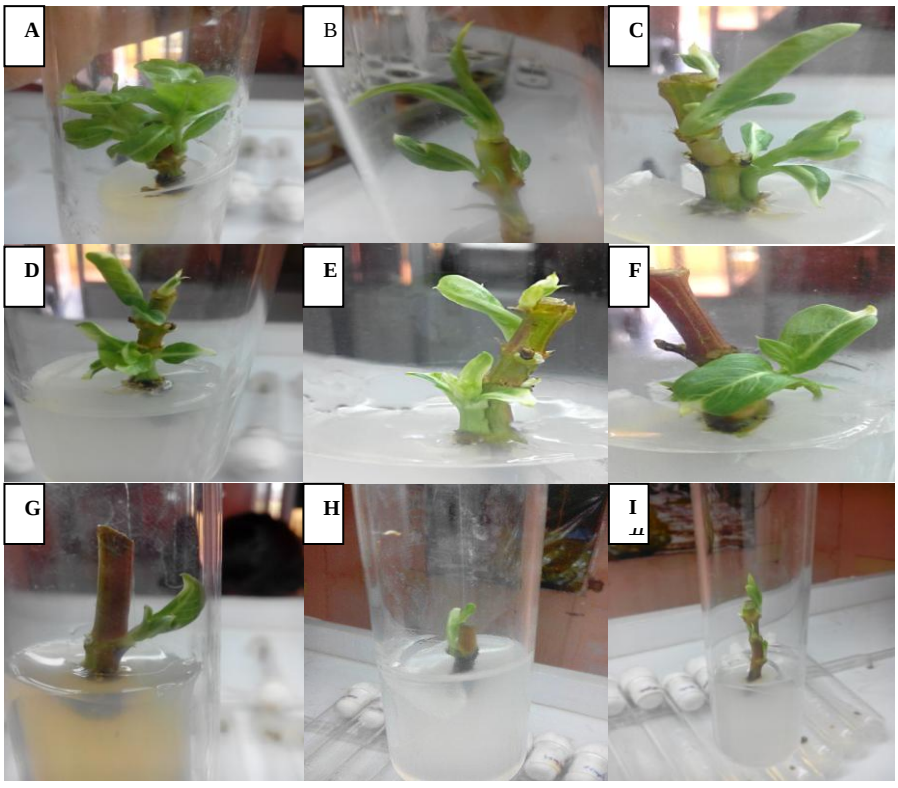
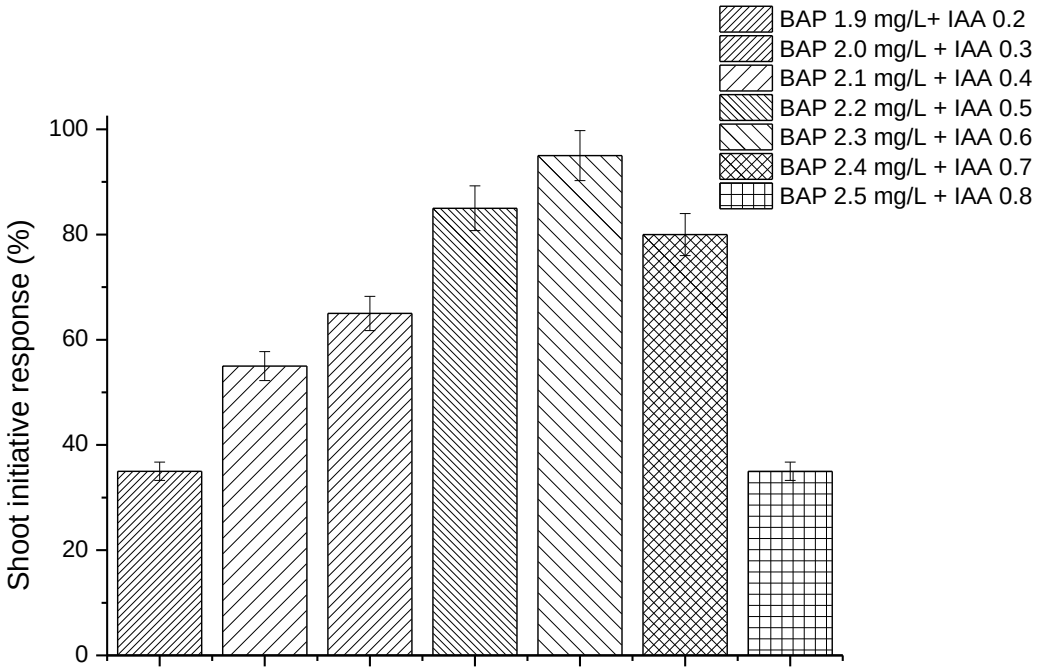


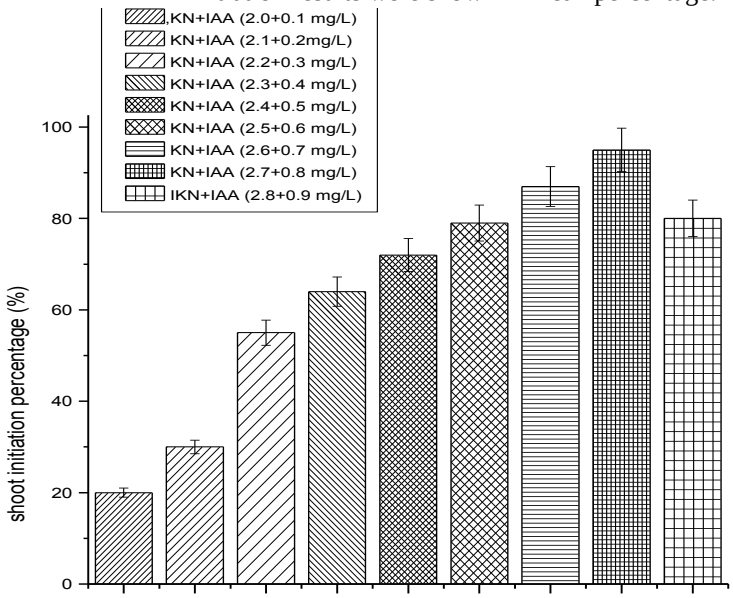
Figure 2A-I. Effect of Kinetin alone or in combination with IAA on in vitro shoot regeneration from intermodal tissue of Catharanthus roseus. Figure 2A- Nodal segments supplemented with 2.7 mg/l^{-1} of KN and 0.8 mg/l^{-1}IAA showing high number and larger size of shoots. Figure 2B- Nodal segments received 2.8 mg/$l^{-1}$of KN and 0.9 mg/l^{-1}IAA showing healthy number of shoots and their larger size. Figure 1C-I-Showing the minimal growth of nodal segments supplemented with single KN at, 2.0, 2.1, 2.2,2.3, 2.4, 2.5 and 2.6 mg/l^{-1}.



Effect of Benzyl amio purine in combination with 3- indole-acetic acid on shoot formation

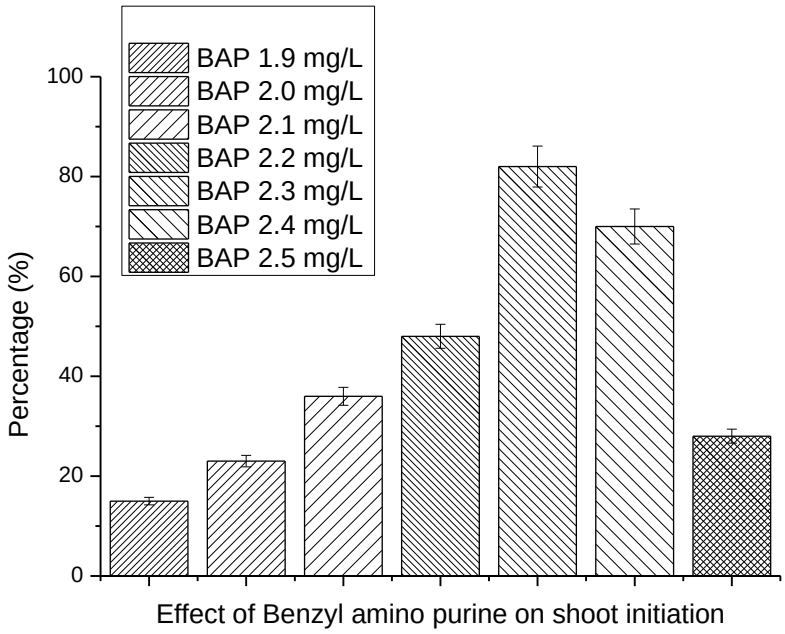
Gr

aph 1.0 This graph shows the high shoot formation potency of Catharanthus *roseus* nodal tissue responded against various concentrations of Benzyl amino Purine in combination with 3-indole-acetic acid. The shoot initiation results were shown in mean percentage.



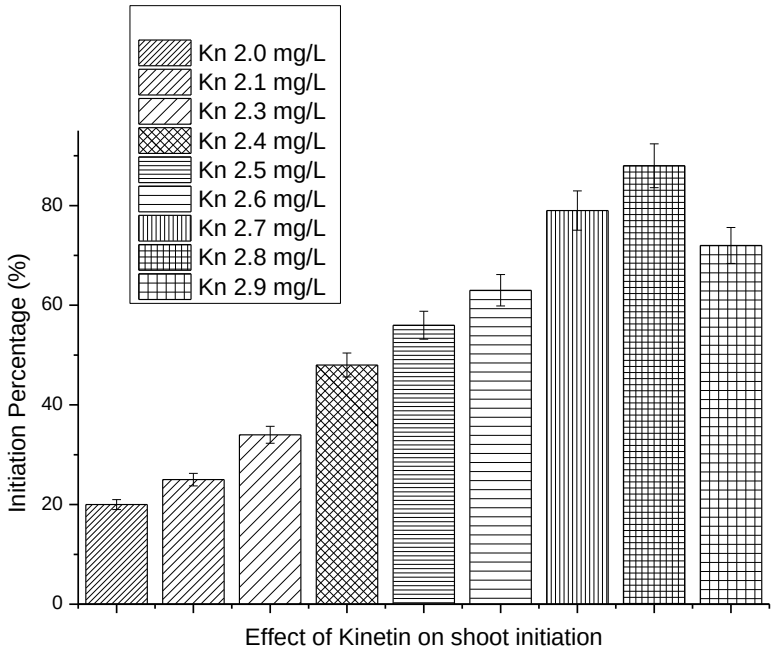
Effect of Kinetin in combination with 3-indole-acetic acid on shoot formation

Graph 1.1 This graph shows the moderate shoot formation potency of Catharanthus *roseus* nodal tissue responded against various concentrations of Kinetin in combination with 3-indole-acetic acid. The shoot initiation results were shown in mean percentage.

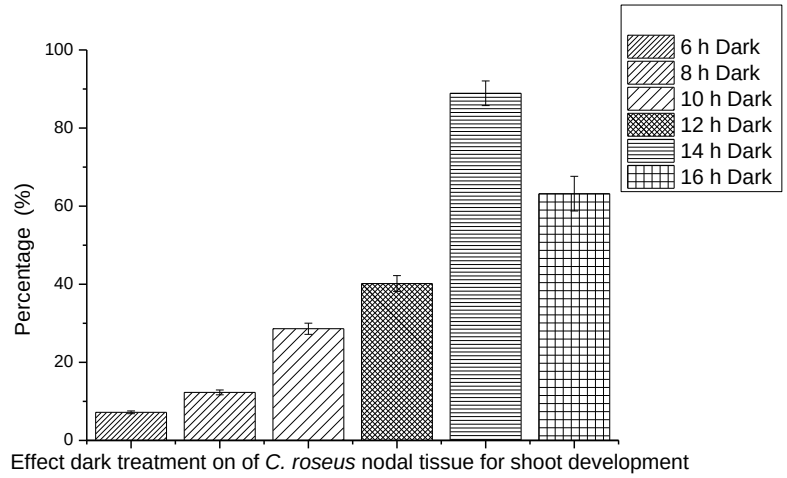


Graph 1.2

This graph shows the average shoot formation potency of Catharanthus *roseus* nodal tissue responded against various concentrations of Benzyl amino Purine alone. The shoot initiation results were shown in mean percentage.



Graph 1.3 This graph shows the minimum shoot formation potency of Catharanthus *roseus* nodal tissue responded against various concentrations of Kinetin alone. The shoot initiation results were shown in mean percentage.



Graph 1.4 A-I. Effect of Dark phase on in vitro shoot regeneration from intermodal tissue of Catharanthus roseus. Fifth column showing highest shoot development percentage achived with the 14 h dark treatment.

4.0 DISCUSSION

In the current research, shoot initiation response was varied in Murashigee and Skoog medium supplemented with different PGRs. The initiation of auxiliary bud growth was noticed after 1st week of incubation and the number of shoots and shoot length was determined after 6th week. According to the data obtained in the present study, the analysis of variance of shoot initiative response was highly noticed. The optimum concentration of Benzyl amino purine in combination with 3-Indole-acetic acid was found significant in the adventitious bud development. Nodal segments supplemented with auxin and cytokinin ratio1:2 (0.6 mg/L IAA and 2.3 mg/L of BAP) produced high number of shoots with larger size (**Makunga and Staden, 2008; Sen et al., 2009**) (See Fig. 1A). On the other hand nodal segments received auxin and cytokinin in 1:3(0.7 mg/L IAA and 2.4 mg/Lof BAP) also resulted in good number of shoot with large size (See Fig. 1B). Even though, the development was observed with the nodal segments supplemented with BAP 1.9, 2.0, 2.1, 2.2 and 2.5 mg/l^{-1} in combination with IAA 0.2, 0.3, 0.4, 0.5, 0.8 mg/l^{-1}, the further growth was not seen (See Figures 1C, 1D, 1E, 1F). The capacity of auxiliary bud proliferation and development of shoots were found low with Benzyl amino purine alone. The decrease or increase in the concentration of Benzyl amino purine from the optimal level, a gradual reduction in the number of shoots was noticed. On the other hand, the growth rate of the nodal segments supplemented various concentrations of Kinetin alone or in combination with 3-Indole-acetic acid showed poor ability on the shoot development. The number of shoots and their length was found slightly more in the nodal segments that were maintained on Kinetin in combination with IAA (See Figures 8, 9, 10, 11). It was published that, the nodal segments of C. *roseus* showed high shoot formation capacity using Benzyl amino purine alone at 6.0 mg/L (**Rupesh Kumar et al., 2013**). In the same year it was also published that, the nodal segments of C. *roseus* was effectively showed 98% of shoot development at 1.0 mg/l BAP alone (shoot number 7.12±0.45 and shoot length 1.80±0.28) and further on sub culturing, these regenerated shoots on to 0.5 mg/l BAP in combination with 1.0 mg/l NAA resulted even more high in shoot number and shoot length (shoot number 7.30±0.64 and shoot length 5.97±0.17) (**Jitendra Mehta et al., 2013**). The above studies were in confusion that, whether the BAP or NAA induce the shoot formation.

It is well fact that known that, Benzyl amino purine induces larger number of adventitious shoots comparing to other PGRs in microprogation of ornamental plants like C. *roseus* (**Tripepi, 1997**). The present studies correlated with previously reported work at the same makes differs in several aspects such as methodology of nodal tissue sterilization, the use of BAP and IAA at different concentrations, the number of shoots produced and their length(**Bakrudeen et al., 2013; Pandey et al., 2014; Rukhama et al., 2013; Mohammed Faheem et al., 2011**). The effect of dark phase was also determined in this study.(Graph 1.5) Nodal segments maintained at 14 h dark showed increased rate of regeneration capacity (**Choi et al., 2001; Dai and Castillo, 2007; Dai et al., 2004**). This is

due etiolating and subsequent in decrease of deposition in the cell wall makes the cells to easily absorb the exogenous plant growth regulators (PGRs) and speed up the process of organogenesis of C. *roseus*. The data of the nodal segments which shown negligible and without response were not shown in the results.

5.0 CONCLUSION

The study concludes that, the nodal segments of C. *roseus* cultivated on MS mediam supplemented with 2.3 mg/L BAP and 0.7 mg/L IAA was found best choice for shoot multiplication. It also concluded that BAP is more suitable PGR compared with from Kinetin for shoot proliferation and development from C. *roseus* nodal tissue.

Conflict of interest

The authors of this paper declare no conflict of interest

ACKNOWLEDGEMENTS

We sincerely, to Dr. C.H.V.Purshothamm Reddy, Chairmen of Chaitanya Colleges for self funding this research work.

REFERENCES

Bakrudeen, A.A.A., Subha Shanthi, G., Gouthaman, T., Kavitha, M.S., Rao, M.V., Rosna, M.T.(2013): In Vitro Propagation, Rooting and Acclimatization of Catharanthus *roseus* (White Variety) - A Green Economy Conservation. Trans. Malaysian Soc. Plant Physiol., 198-202.

Choi, J., Kim, H.J., Lee, Y., Bae, Y., Chung, J., Shin Hyung, N. (2001): Efficient and simple plant regeneration via organogenesis from leaf segments cultures of Persimmon (Diospyros kaki Thunb.). In vitro Cell. Dev. Biol. Plant., 37: 274-279.

Dai, W., Jacques, V., Walla, J., Cheng, Z. (2004): Plant regeneration of chokecherry (Prunnus virgininana L.) from in vitro leaf tissues. J. Environ. Hort., 22: 225-228.

Dai, W and Castillo, C. (2007): Factors affecting plant regeneration from leaf tissues of Buddleia species. Hort Science., 42: 1670-1673.

Hisiger, S and Jolicoeur, M. (2007) Analysis of Catharanthus *roseus* a lka loids by HPCL. J. Phytochemistry Review., 6: 207-234.

Jitendra, M., Deeksha, U., Priyanka, P., Rukhshar, A., Sunil, R., Shalini, T. (2013): Multiple shoot regeneration of (anti-Cancer plant) Catharanthus *roseus* - An important medicinal plant. Am. J. PharmTech Res., 3: 786-793.

Magnotta, M., Murata, J., Chen, J., De Luca, V. (2006): Identification of a low vindoline accumulating cultivar of Catharanthus *roseus* (L.) G Don. by alkaloid and enzymatic profiling. Phytochemistry., 67: 1758–1764.

Makunga N, Staden J (2008). An efficient system for ther production of clonal plant lets of the medicinally important aromatic plant: Salvia Africana-lutea L. Plant Cell, Tiss. Org, Cult,. 92:63-72.

Mohammed, F., Satyapal, S., Babeet, S.T., Moinuddin, K., Anwar, S. (2011): In vitro Regeneration of multiplication shoots in Catharanthus roseus- An important medicinal plant, Adv. in App. Sci. Res., 2: 208-213.

Pandey, S., Bahadur, A.N., Kanungo, V.K., Tiwari, U. (2014): In-vitro propagation of a medicinal plant Catharanthus *roseus* l. (g.) Don. Indian, J.L.Sci., 4: 125-128.

Rukhama, H., Shagufta, N., Farah, A., Farkhanda, M. (2013): Comparison of in vitro response of micropropagation and callogenesis of medicinal plant, Vinca rosea L. J. Agric. Res., 51: 9-17.

Rupesh, K.R., Naresh, K.R., Sharma, V. (2013): Effect of plant growth regulators on micropropagation of Catharanthus roseus, Int. J. Adv. Biotech. and Res., 4: 123-130.

Sen A, Sharma MM, Grover D, Batra A (2009). In vitro regeneration of Phyllanthus amarus Schun. And Thonn: An important medicinal plant. Our Nat. 7:110-115.

Soon Huat, T., Chung Yeng, L., Hazrina, H., Aditya, A., Mohammad javad, P., Won Fen, W., Shiau-Chuen, C., Mohd Raisd, M., Khalijah, A. (2013): Antidiabetic and Antioxidant Properties of Alkaloids from Catharanthus *roseus* (L.) G. Don. Molecules., 18: 9770-9784.

Tripepi, R. (1997): Adventitious shoot regeneration. In Geneve R, Preece J, Merkle S (EDS.), Biotechnology of Ornamental plants. Biotechnology in Agriculture Series. No 16. CAB International. Wallingford. UK, pp. 1997. 45-71.

Zu, Y.G., Luo, M., Mu, F.S., Fu, Y.J., Wang, L. (2006): Advances on alkaloids in Catharanthus *roseus* and their pharmacological activities. Nat. Prod. Res. Dev., 2006. 18: 325–329.