Indian J.L.Sci. 4 (1) : 125-128, 2014

ISSN : 2277-1743 (Print)
ISSN : 2278-7879 (Online)

IN-VITRO PROPAGATION OF A MEDICINAL PLANT *Catharanthus roseus* L. (G.) DON

S. PANDEY[a1], A. N. BAHADUR[b], V. K. KANUNGO[c] AND U. TIWARI[d]

[a]Department of Biotechnology, D.P Vipra College Bilaspur, C.G., India
[bd]Department of Botany and Biotechnology, Govt. Agrasen College, Bilha, Bilaspur, C.G, India
[c]Government Nagarjuna P.G. college of Science, Raipur., C.G., India

ABSTRACT

***Catharanthus roseus* L.(G) Don is a very common garden plant widely known as sada-suhagan, rich in medicinal property and known to be used for the treatment of Diabetes, High blood pressure, Malaria, Hodgkin's disease etc. Its anti-bacterial and anti-Cancer property makes it very useful. It prevents the growth of new blood vessels that supports the tumor growth. *Catharanthus roseus* is a potential source for anti-leukemic alkaloids like Vinblastine, Vincaleukoblastine, Vinblastine Sulphate, which is used for the treatment of human tumors. It is also recommended for the treatment of neoplasms and generalized Hodgkin's disease. Vincristine and Vincristine Sulphate are used for the treatment of leukemia in children. Potential value of *Catharanthus roseus* as a medicine requires its commercial production and for that an efficient protocol is needed for large scale harvesting. In this respect, present study was designed to set a protocol for rapid proliferation of plant through shoot bud culture. Shoot bud culture using nodal explants were established on MS Medium supplemented with various concentrations of growth hormone BAP, NAA and Kinetin. After 30 days of incubation, maximum shoots were raised on MS medium supplemented with growth hormone BAP with the concentration of 1.0 mg/l.**

KEYWORDS : In-vitro Propagation, Shoot Bud Culture, Growth Hormone

Human beings have exploited the plants for curing ailments since antiquity. Traditional systems of medicine like Ayurveda, Unani, Homeopathy and Siddha, solely rely on phyto-pharmaceuticals that are obtained from selected medicinal plants. Ethno-botanical and ubiquitous plants serve as a rich resource of natural drugs for research and development (Kong,et.al., 2003). Medicinal plant-based drugs owe the advantage of being simple, effective and exhibit broad spectrum activity (Chin, et. al., 2006). Medicinal plant products, when compared to their synthetic counterparts minimize the adverse side effects.

Despite of increasing advancement in the field of medicine and molecular diagnosis, it is estimated that 80% of the world's population is still dependent on the plant derived pharmaceuticals. WHO report depicts that plant based products or its derivatives accounts for nearly 28% of drugs available in the market (Newman, et.al, 2003). Natural products as such and their derivatives have historically been exploited as a valuable source of novel therapeutic agents (Koehn and Carter, 2005). A large proportion of plant based compounds are used as lead molecules in drug discovery to produce synthetic molecular analogs that have similar skeletons yet intricate structures. This implicates that phytochemicals play a critical role in diversity oriented synthesis (DOS) of natural products like pharma compounds (Marcaurelle and Johannes, 2008). In-vitro culture technique offers a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plant (Ajithkumar and Seeni 1998).

In present study *Catharanthus roseus* popularly known as Madagascar periwinkle, Vinca rosea, Sada Suhagan, was taken which is native to Indian Ocean Island of Madagascar and also common to Tropical and Sub-tropical regions worldwide including Southern United States. *Catharanthus roseus* is commonly known to be an ornamental plant. It is used as folk medicine to treat Diabetes and High blood pressure. It is also known for the treatment of Malaria, Hodgkin's disease; prevent growth of new blood vessels that support Tumor Growth (anticancer property). It exhibits antibacterial property against certain species of bacteria viz. *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus pyrogens*, *Bacillus cereus*, *B.subtilis* (Ramya, et. al., 2008). *Catharanthus roseus* is a potential source of anti-leukemic alkaloids Vinblastine, Vincaleukoblastine and Vinblastine Sulphate which is having growth inhibition effects in certain human tumors. It is used experimentally for the treatment of neoplasms and recommended for generalized Hodgkin's disease and resistant to Choricarcinoma .Vincristine and Vincristine Sulphate are used for the treatment of leukemia in children. Beside above described alkaloids there are 70

[1]**Corresponding author**

other alkaloids have been isolated which are effective in treating various types of cancer like breast cancer, lung cancer, uterine cancer, melanomas etc., (Verpoorte, 1998).Number of alkaloids was recently been updated as 130 (Hisiger and Jolicoeur., 2006), amongst them 11 alkaloids have been studied and analyzed frequently and 08 are commercially available.

Catharanthus roseus has rich medicinal value because of variety of alkaloids are produced by the plant and it cannot be consumed raw as decoction or in any raw form hence, refinement and purification is required prior to consumption. For commercial refining and purification, large scale production is required and which can be achieved with the help of in-vitro culture technique. In-vitro culture technique offers a viable tool for mass multiplication; therefore, shoot bud culture study was planned to obtain maximum and rapid proliferation of phytomass.

MATERIALS AND METHODS

The present study aims to establish and standardize a protocol for the rapid proliferation of *Catharanthus roseus* by in-vitro culture. Shoot bud culture was done by taking healthy shoots with 2-3 nodes excised from a healthy plant of *Catharanthus roseus* collected from the Botanical garden of the college, where study was conducted. The leaves were removed from the shoot and thoroughly washed under running tap water for 20 minutes. Later, shoots were washed with detergent Labolene for 5 minutes, followed by rinsing with distilled water 6-7 times. After thorough rinsing explants were cut into 0.5-1.0 cm pieces and then treated with 0.1 (w/v) Mercuric Chloride for 10 minutes, later it was rinsed with distilled water for several times. Further explants were dipped into 12% (v/v) Hydrogen Peroxide for 5 minutes and washed thoroughly with distilled water, thereafter; these explants were surface dried on pre-sterilized filter paper.

To initiate micro-propagation, auxiliary nodal explants of 0.5-1.0 cm were vertically inoculated on MS medium. To promote the shoot induction, MS medium was fortified with BAP, Kinetin, and NAA. After establishment of micro-shoots, they were sub-cultured after every 4 weeks. The cultures were maintained at 25±2°C under photoperiod of 15-16 hours. The micro-shoots were then transferred to rooting medium (full strength and half strength rooting medium). Hardening was done in two consecutive stages, liquid and then solid. Liquid hardened plantlets were rinsed with distilled water and dipped in 0.2% (w/v) solution of Bavistin (fungicide) for 30 minutes and then subsequently on IBA solution for 10 minutes. After this, it was transferred to sterile sand, Soilrite and garden soil (2:1:1) in maintained humidity.

RESULTS AND DISCUSSION

In in-vitro propagation, shoots per explants, average shoot length, nodes per shoot, leaves per shoot and bud break percentage was studied for *Catharanthus roseus* on MS medium supplemented with phyto-hormones like BAP, NAA and Kinetin in concentration of 0.0, 0.10, 0.25, 0.50, 0.75, 1.0 and 1.50 mg/l. Similar study was conducted in *Lippia alba* by Gupta et. al.(2001) and in *Phyla nodiflora* by Ali et. al. (2005).Shoots per explants, average shoots length, nodes per shoot and leaves per shoot were observed maximum (2.4±0.3, 5.2±0.4, 6.1±0.1 and 12.5±1.5) on MS medium supplemented with 1.0 mg/l BAP. The bud break percentage was observed 100% in MS medium

Table 1: Effect of Different Levels of Bap on Auxiliary Shoot Growth, Raised from Nodal Explants of *Catharanthus Roseus* After 30 Days of Incubation in MS Medium.

Growth regulator **BAP** conc. mg/l	Shoot per Explants	Average Shoot Length (cm)	Nodes per Shoot	Leaves per Shoot	Bud Break %
0.00	[illegible]	0.6±0.2	Nil	Nil	40
0.10	1.6±0.1	0.6±0.1	1.2±0.1	5.2±0.9	80
0.25	1.5±0.2	2.1±0.3	3.3±0.2	10.5±0.4	100
0.50	2.2±0.3	2.5±0.4	2.7±0.4	11.8±1.9	100
0.75	2.0±1.0	3.4±0.3	2.7±0.3	11.2±1.5	100
1.00	2.4±0.3	5.2±0.4	6.1±0.1	12.5±1.5	100
1.50	1.4±0.2	0.4±0.1	Nil	Nil	30

Table 2 : Effect of Different Levels of Naa on Auxiliary Shoot Growth, Raised From Nodal Explants of *Catharanthus Roseus* After 30 Days of Incubation in MS Medium.

Growth regulator **NAA** conc. mg/l	Shoot per Explants	Average Shoot Length (cm)	Nodes per Shoot	Leaves per Shoot	Bud Break %
0.00	Nil	Nil	Nil	Nil	Nil
0.10	1.0±0.00	0.8±0.03	1.2±0.1	6.0±0.8	100
0.25	1.0±0.00	1.3±0.1	2.8±0.1	7.60±0.4	100
0.50	1.1±0.00	1.5±0.8	3.4±0.2	8.0±1.2	80
0.75	1.2±0.1	2.3±0.3	4.0±0.4	9.5±1.3	80
1.00	1.4±0.1	4.5±0.2	5.1±0.5	12.2±1.6	100
1.50	1.2±0.1	2.1±0.2	2.4±0.4	10.2±2.0	80

Table 3 : Effect of Different Levels of Kinetin on Auxiliary Shoot Growth, Raised From Nodal Explants of *Catharanthus Roseus* After 30 Days of Incubation in MS Medium.

Growth regulator **Kn** conc. mg/l	Shoot per Explants	Average Shoot Length (cm)	Nodes per Shoot	Leaves per Shoot	Bud Break %
0.00	Nil	Nil	Nil	Nil	Nil
0.10	1±0.0	2.4±0.1	5.2±0.1	09.2±0.6	100
0.25	1±0.0	2.4±0.1	5.0±0.2	10.6±1.2	100
0.50	1±0.0	2.7±0.1	5.2±0.1	10.0±1.2	100
0.75	1±0.0	3.4±0.1	5.6±0.2	14.2±1.7	100
1.00	1±0.0	1.7±0.09	4.1±0.3	11.3±1.4	100
1.50	1±0.0	1.5±0.06	2.2±0.1	10.2±0.6	100

supplemented with 0.25, 0.50, 0.75 and 1.0 mg/l BAP (Table-1). When nodal explants were inoculated on MS medium, Murashige and Skoog (1962), fortified with NAA, 80-100% bud break response was observed. The number of shoots per explants, average shoots length, nodes per shoot and leaves per shoot were observed maximum 1.4±0.1, 4.5±0.2 and 5.1±0.5 and 12.2±1.6 at the concentration of 1.0 mg/l NAA. The bud break percentage was observed 100% in MS medium supplemented with 0.10, 0.25, and 1.0 mg/l NAA (Table-2). When MS medium supplemented with Kinetin, 100% bud break and shoots per explants (1±0.0) was observed for all the concentrations. Maximum values of average shoot length (3.4±0.1), nodes per shoot (5.6±0.2) and leaves per shoot (14.2±1.7) were observed at the concentration of 0.75 mg/l of Kinetin (Table-3).

In present study BAP was found to be the most effective hormone in 1.0 mg/l concentration for raising maximum shoots per explants, average shoot length and nodes per shoot in MS medium, while kinetin in 0.75mg/l concentration was found suitable for the formation of maximum number of leaves/shoot. BAP alone is reported as most effective by Patil (1998) as was found in present study. Swamy et. al. (1992) also reported BAP as the most efficient cytokinin for the axillary bud initiation and subsequent proliferation of axillary buds. A similar result was obtained by Baskaran and Jayabalan, (2005) for *Ceropegia jainii* and *C. bulbosa*. MS medium supplemented with Kn showed the higher number of shoots reported by Beena et.al. (2003) in *Ceropegia delabrum* but in present study Kn was found suitable for the formation of maximum number of leaves/shoot. However, a synergistic effect of BAP and Kn in promoting the shoot initiation has been reported by Emmannel et al. (2000) was also observed in present study.

ACKNOWLEDGEMENT

The authors are thankful to the Principal, Govt. E.Raghvendra Rao Post Graduate Science College, Bilaspur (C.G), for providing necessary lab facilities.

REFERENCES

Ahmed , Abdul Bakrudeen Ali, Gouthaman, T., Rao, S.A. and Rao, M.A., 2005. Micropropagation of Phyla nodiflora (L.) Greene: An important medicinal plant. Iranian Journal of Biotechnology, 3(3): 2005.

Ajithkumar D. and Seeni S., 1998. Rapid clonal multiplication through in vitro axillry shoot proliferation of Aegle marmelos (L) Corr. A Medical Tree. Plant Cell Reproduction.17:422-426.

Baskaran P. and Jayabalan N. 2005. An efficient micropropagation system for Eclipta alba A valuable medicinal herb. In vitro Cell Dev Biol. 41:532-539.

Beena M. R., Martin K. P., Kirti P. B. and Hariharan M., 2003. Rapid in vitro propagation of medicinally important Ceropegia candelabrum. Plant Cell Tiss Org Cult. 72;285-289.

Chin Y., Balunas M. J., Chai H. B. and Kinghorn A. D., 2006. Drug discovery from natural resources. AAPS J, 8:E239-53.

Emmanuel S., Ignacimuthu S., Kathiravan K., 2000. Micropropagation of Wedelia calendulacea Less. A Medicinal plant. Phytomorphology. 50: 195-200.

Gupta S. K., Khanuja S. P. S., Kumar S, 2001. In vitro propagation of Lippia alba. Curr Sci. 81: 206-210.

Hisiger S. and Jolicoeur M., 2007. Analysis of Catharanthus roseus alkaloids by HPCL, journal-phytochemistry Review. Vol. 6, No. 2-3, july 2007 : 207-234.

Koehn F.E. and Carter G. T., 2005. The evolving role of natural products in drug discovery. Nat Rev Drug Discov. 4:206-20.

Kong J. M., Goh N. K., Chia L. S. and Chia T. F., 2003. Recent Advances in Tradational plant drugs and orchids. Acta Pharmacol Sin 24:7-21.

Marcaurelle L. A. and Johannes C. W., 2008. Application of natural product inspireddiversity oriented synthesis to drug discovery. Prog Drug Res. 66(187):89-216.

Murashige T. Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-479

Newman D. J., Cragg G. M. and Snader K. M., 2003. Natural products as sources of new drugs over the period 1981-2002. J Nat Prod 6:1022-37.

Patil V. M., 1998. Micropropagation studies in Ceropegia spp. In vitro Cell Dev Biol. 34:240-243.

Raghava Swamy B. V., Himabindu K. and Lakshmisita G., 1992. In vitro propagation of the elite rose wood Dalbergia latifolia Roxb.Plant Cell Rep. 11:126-131.

Ramya S., Govindaraji V., Navaneetha Kannan K. and Jayakumararaj R., 2008. In vitro evaluation of antibacterial activity using crude extracts of Catharanthus roseus L. (G.) Don. Ethnobotanical Leaflets 12: 1067-72.

Verpoorte R., 1998. Exploration of nature's chemo-diversity: the role of secondary metabolites as lead for drug development. Drug Dev Today 3:232-238.