JK Knowledge Initiative, 2(1): 9-15, March 2018 ISSN: 2457-0974

Catharanthus roseus (L.) G. Don and In Vitro Techniques: A Review

Mehpara Maqsood *[1] , Mir Khusrau[2] and **Abdul Mujib** [3]

[1]* *Govt. College for Women M.A. Road, Srinagar,* [2] *Govt. Degree College (Boys), Anantnag,*
[3]*Department of Botany, Jamia Hamdard, New Delhi*

Abstract: *Catharanthus rosues* is an immensely important medicinal plant. Tissue culture finds huge scope in most aspects of *C. roseus* viz., micropropagation, somatic embryogenesis, cryopreservation, protoplast isolation, synthetic seed development, elicitation in reference to alteration in alkaloid content, etc. Various concentrations and combinations of plant growth regulators were used by different workers for the callus induction, somatic embryogenesis and regeneration (direct and indirect). Mostly these biotechnological techniques have been employed in an attempt to increase the alkaloid content of this plant. Different alkaloids are derived from its roots, leaves and stem. Important to mention, vinblastine and vincristine are an important set of anti-cancerous alkaloids derived from this plant. The lower yield and high market value of the medicinally important alkaloids of *C. roseus* viz. vincristine, vinblastine and ajmalicine have created interest in improved alternative ways for their better yield such as cell and tissue culture. Elicitation by using biotic elicitors (fungus and yeast extract), abiotic elicitors and employing different digestive enzymes like cellulase, pectinase, driselase, macerozyme (protoplast isolation) constitute some of the important methodologies to increase the yield of these alkaloids. This review highlights the attempts in tissue culture that have been undertaken so far to propagate, conserve and enhance the alkaloid content of this plant.

Key words: *Catharanthus roseus*, alkaloids, micropropagation, alkaloids, elicitation.

Introduction

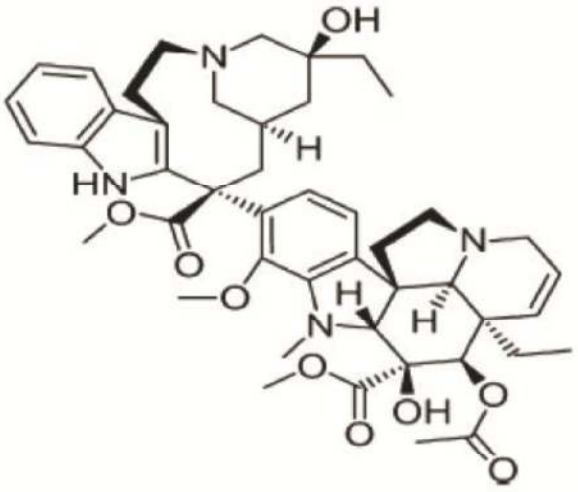
Plant cell cultures are more comparable to mammalian cell cultures from the view of biochemical engineering. These systems promise to be more cost-effective due to the simple media using cheaper medium components (mostly an organic salts and sucrose) and the lack of human pathogenic particles that have to be eliminated in the downstream processing (Boehm 2006).

Catharanthus roseus, popularly known as Madagascar periwinkle, is an important medicinal plant considering its importance in pharmacology. Belonging to the family apocynaceae, Madagaskar Periwinkle contains more than 400 alkaloids of which some have been categorised as antineoplastic agents for inhibiting or preventing the growth and spread of tumours or malignant cells such as rhabdomyosarcoma, leukaemia, neuroblastoma, malignant lymphomas and Hodgkin's disease (Ethalsha and Retna 2014). Ethno-botanical information on medicinal plants and their usage by indigenous cultures is useful in conservation of traditional cultures, biodiversity, community healthcare and drug development (Haq et al. 2011). In human body, the chemical constituents of medicinal plants interact directly or indirectly with the body chemistry (Asma et al. 2016).

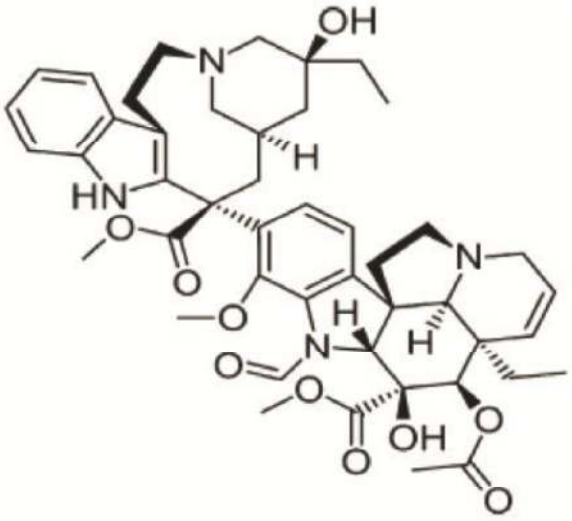
Two of the dimeric alkaloids vinblastine and vincristine mainly present in the aerial parts, have found extensive application in the treatment of human neoplasmas. Among the monomeric alkaloids ajmalicine (raubacine) found in the roots has been confirmed to have a broad application in the treatment of circulatory diseases, especially in the relief of obstruction of normal cerebral blood flow.

Vinblastine sulphate (sold as Velban) is used particularly to treat Hodgkin's disease

*Corresponding author(s):
meh_heem@yahoo.co.in (Mehpara Maqsood)



Vinblastine



Vincristine

besides lymphocarcinoma, choriocarcinoma, neuroblastoma, carcinoma of breasts, lungs and in treating acute and chronic leukemia. Vincristine sulphate (sold as Oncovin) arrests mitosis in metaphase and is very effective for treating acute leukemia in children and lymphocytic leukemia (Junaid et al. 2010).

Habit and habitat of *C. roseus*

C. roseus is grown as medicinal as well as ornamental plant. It is 1m tall perennial herb with oppositely arranged leaves. Leaves are around 3cm long, oval and oblong. The fruit is a pair of follicles with each follicle about 3-4 cm long.

Medicinal importance

C. roseus is used by different countries for treating different diseases (Aruna et al. 2015). In Africa, the leaves are used for treating rheumatism and menorrhagia. In Madagascar, the leaves are used in inducing vomiting, the roots are purgative, anthelmintic, hemostatic and for stopping toothache. In Malaysia, the plant is used for treating hypertension, diabetes, cancer and insomnia. In India, the leave extracts are used against wasp sting. In Mauritius, the leaves are used for treating indigestion and constipation. In Jamaica and Cuba, the flowers are used as eyewash for infants. In America, the plant extracts are used for easing sore throats and laryngitis. In Philippines, the leaves are used for treating diabetes and stomach cramps while the root decoction is used in intestinal parasitism. The roots are also used in treatment of dysentery. In Bahamas, the decoction prepared from flowers is used to treat flatulence, asthma and tuberculosis. In Hawaii,

the boiled plant extract is used for stopping excessive bleeding.

C. roseus and Tissue culture

In order to provide enough plant material for commercial exploitation, the cultivation of these plants using conventional method is not sufficient. Thus mass propagation through *in vitro* culture to get alkaloids and to make them available throughout the year is an urgent necessity (Rashmi and Trivedi, 2015). Tissue culture has been suggested as a feasible technology for the production of many plant secondary metabolites (Verma et al. 2012). The varying responses in *in vitro* culture of *C. roseus* were noted at different phytohormonal concentrations and combinations from leaf callus. The auxins were found to be the best for callus proliferations and growth. Among auxins, 2, 4-D was better for increase in callus biomass and total alkaloid content. 2, 4-D was also reported as the most effective auxin in various medicinal plants (Junaid et al. 2008), while combinations of auxins with cytokinins were found to be better for leaf callus growth and enhancement in alkaloid content. These results are in accordance with the view of Zenk et al. (1977) and Brown (1990) that plant growth regulators have remarkable effects on growth and differentiation and thus metabolism of cultured cells. Somatic embryogenesis has been reported in a wide variety of plant genera (Mujib and Samaj, 2006), in *Catharanthus roseus* it has been reported for the first time by Junaid et al. (2006). In both developmental pathways, the use of exogenous auxins/auxin analogues like 2, 4-D efficiently trigger embryogenesis (Dipti et al. 2016).

As a promising alternative to produce plant secondary metabolites, plant tissue culture technology has many advantages over traditional field cultivation and chemical synthesis, particularly for many natural compounds that are either derived from slow growing plants or difficult to be synthesized with chemical methods (Zhao et al. 2007)].

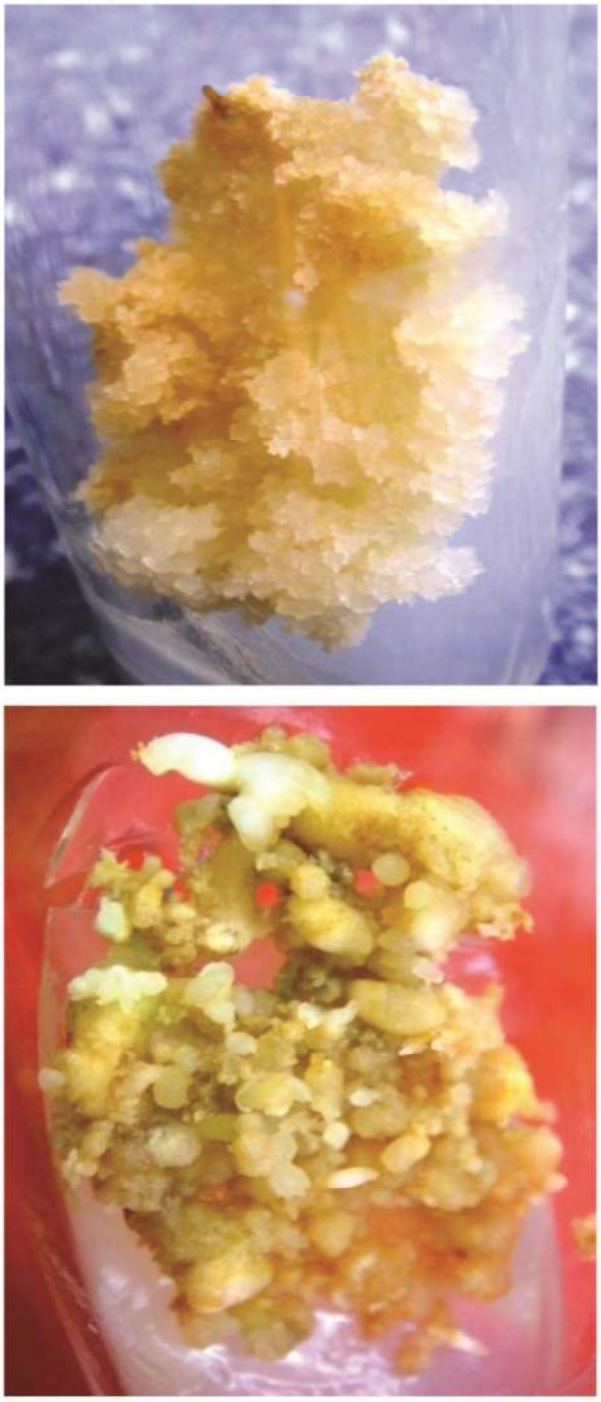


Figure 1: (a) callus culture of *C. roseus* and (b) callus cultures treated with Yeast extract in *C. roseus*. (Bar 1-a and 1-b=3mm)

Catharanthus has been cultured *in vitro* to make it available in large amount by various workers (Mujib et al. 2007). In *C. roseus*, however, the incidence of induction of embryogenic callus has been relatively new (Junaid et al. 2006). It was quite different from non embryogenic callus and was induced from hypocotyl of *in vitro* germinated seeds. Saifullah and Khan (2011) stated that for the cell suspension culture preparation, MS medium along with 1.5 mg/l 2, 4-D and 0.5 mg/l Kn was best for friable callus production in *Catharanthus roseus*. MS + Kn 0.1mg/l and 2, 4-D 1mg/l triggering quick formation of white callus with resin secretion on its surface.

Protoplast isolation method and subsequent plant regeneration were established from embryogenic suspension, derived from hypocotyl callus (Mehpara et al. 2012), Fig. 1a.

The effect of yeast extract on vinblastine and vincristine in protoplast derived tissues/plantlets in *C. roseus* and noted enhanced yield of vinblastine and vincristine in response to the same (Mehpara et al. 2017).

For conservation of elite germlines in *C. rosues*, synthetic seeds were developed from somatic embryos (Mehpara et al. 2012), Fig. 1b.

An efficient cryopreservation protocol was established for embryogenic cell suspension cultures of *Catharanthus roseus* (Samar et al. 2009).

Research on *Catharanthus roseus* in relation to alkaloid content

Quantitative estimation of vincristine was carried out using HPLC from *in vitro* grown tissues and a comparison was made from *ex vitro* raised plantlets. Vincristine content was noted to be tissue specific as leaf callus and germinated embryos produced maximum vincristine (Junaid et al. 2009).

Elicitors

Factors better known as elicitors have been reported to stimulate the production of secondary metabolites in *C. roseus* (Eilert et al 1986). The substance used as an elicitor may be biotic or abiotic in nature. Biotic elicitors include microbial filtrates (Yeast, *Pythium* and other fungal filtrates), while abiotic elicitors comprise of simple inorganic and organic molecules (vanadyl sulphate, oxalate, UV irradiation etc.).

Nicotinamide in *C. roseus* cell lines was used to enhance the anthocyanin accumulation (Berglund et al. 1993a). The extract of *Pythium aphanidermatum* in a hormone free cell line responded well and induced enzymes (TDC and anthranilate synthase (AS) which catalyse the biosynthesis of several intermediates in alkaloid production and subsequently accumulated tryptamine (Moreno et al 1993). Several inorganic compounds (sodium chloride, potassium chloride and sorbitol) had also a positive effect on catharanthine accumulation (Smith et al. 1987a).

Fungal elicitation

Dipti et al. (2016) investigated the influence of fungal elicitor *Aspergillus flavus* on alkaloid production in *Catharanthus roseus*. Their study revealed increased yield of vinblastine and vincristine in cultivated tissues. They applied different concentrations of extract to solid MS medium. The quantitative analysis of vinblastine and vincristine yield was conducted in different elicitor treated tissues by the use of HPTLC. Studies have reported maximum vinblastine content in germinating

embryos (0.837µg gm^{-1} dry weight of culture). However, *A. flavus* elicitation further improved vinblastine yield (0.903µg gm^{-1} dry weight). Compared to vinblastine, the yield of vincristine is normally low but upon *A. flavus* addition, maximum vincristine yield was noted (0.216 µg gm^{-1} dry weight). The highest 7.88 and 15.50 % increase in yield of vinblastine and vincristine respectively was noted on *A. flavus* elicitated tissues.

Elicitation due to yeast extract

The yield of two most investigated alkaloids vinblastine and vincristine is unfortunately very low under natural conditions. A vast array of techniques involving elicitation of alkaloid content has recently been employed to augment alkaloid content in *catharanthus* cultures. One such elicitor is Yeast extract. It is a biotic elicitor; the polysaccharide and the peptide moiety have been recognized as a signaling element in enriching secondary metabolites. According to Mehpara et al. (2017), the yeast extract elicitation on vinblastine and vincristine was studied in various protoplast derived tissues and plantlets. Four different yeast extract concentrations (T_1 = 0.5 g/l, T_2 = 1.0 g/l, T_3 = 1.5 g/land T_4 = 2.0 g/l) were used. The alkaloid was quantified using High performance thin layer chromatography. The addition of yeast extract in the medium improved vinblastine and vincristine yield in cultures, maximum being in germinating embryos and in vitro raised leaf. The highest yield was in T_3 (1.5 mg/l) in which 22.74% vinblastine and 48.49% vincristine enrichment was noted in germinating embryos.

Conclusion

C. roseus is plant that has been micropropagated because of the interest of researchers as it contains numerous alkaloids. Numerous biotechnological techniques had been applied to the plant in order to analyse the effect on its growth, conservation and enhancement in alkaloid contents. Vinblastine and vincristine are important anti-cancerous alkaloids derived from this plant. *C. roseus* has been analyzed by various researchers by using different parameters to enhance the alkaloid content. Investigations are on to work out the rate determining steps in the biosynthetic pathway of these alkaloids.

Acknowledgement

The authors acknowledge the financial support by UGC and Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. The encouragement by the Department of Higher Education, J & K Govt., is also highly acknowledged.

References

Aruna MS, Prabha MS, Priya NS and Nadendla R (2015). *Catharanthus roseus*: Ornamental plant is now medicinal boutique. Journal of Drug Delivery & Therapeutics, 5: 1-4.

Asma N, Awing SM and Md IH, Muhammad SA and Muhammad SA (2016). An updated review of *Catharanthus roseus*: pharmaceutical and pharmacological analysis. Indian Research Journal of Pharmacy and Science, 3: 631-653.

Berglund T, Ohlsson AB and Rydstrom J (1993a). Nicotinamide increases glutathione and anthocyanins in tissue culture of *Catharanthus roseus*. Journal of Plant Physiology, 141: 596-600.

Boehm R (2006). The Use of Plants and Plant Cell Cultures for the Bioproduction of Proteins. J. Verbr. Lebensm. 1: 120–125.

Dipti T, Mujib A, Mehpara M, Muzamil A and Nadia Z (2016). *Aspergillus flavus* fungus elicitation improves vincristine and vinblastine yield by augmenting callus biomass growth in *Catharanthus roseus*. Plant Cell Tissue Organ Culture. DOI 10.1007/s11240-016-0998-1.

Dipti T, Mujib A, Mehpara M, Muzamil A and Nadia Z (2016). *Aspergillus flavus* fungus elicitation improves vincristine and vinblastine yield by augmenting callus biomass growth in *Catharanthus roseus.* Plant Cell Tiss. Organ Cult. 126: 291-303.

Eilert U, Constable F and Kurz WGW (1986). Elicitor stimulation of monoterpene indole alkaloids formation in suspension cultures of *C. roseus*. Journal of Plant Physiology, 126: 11-22.

Ethalsha P and Retna AM (2014). Evaluation of antioxidant potential and antibacterial activity of crude extracts *Catharanthus roseus*. International Journal of Pharmaceutical Sciences and Research, 8: 3490-3495.

Haq F, Ahmed H and Alam M (2011). Traditional uses of medicinal plants of Nandiar Khuwar catchment (District Battagam), Pakistan. Journal of Medicinal Plants Research, 5:39-48.

Junaid A, Mujib A, Bhat MA, Sharma MP, and Samaj J (2007). Somatic embryogenesis and plant regeneration in *Catharanthus roseus*. Biologia Plantarum. 51: 641-646.

Junaid A, Mujib A and Sharma MP (2009). Screening of vincristine yield in *ex vitro* and *in vitro* somatic embryos derived plantlets of *Catharanthus roseus* L. (G) Don. Scientia Horticulture 119:325–329.

Junaid A, Mujib A, Bhatt MA and Sharma MP (2006). Somatic embryo proliferation maturation and germination in *Catharanthus roseus*. Plant Cell Tiss. Org Cult. 84: 325-332.

Junaid A, Sheba HK, Zahid HS, Zohra F, Mehpara M, Mukthar AB, Sekh AN, Abdul I, Iffat ZA**,** Saeed AK, Mujib A and Maheshwar PS (2010). *Catharanthus roseus* (L.) G. Don. An important drug: Its application and production. International Journal of Comprehensive Pharmacy, 4 (12).

Mehpara M and Mujib A (2017). Yeast extract elicitation increases vinblastine and vincristine yield in protoplast derived tissues and plantlets in *Catharanthus roseus*. Revista Brasileira de Farmacognosia , http://dx.doi.org/10.1016/j.bjp.2017.05.008.

Mehpara M, Mujib A, Dipti T and Abdin MZ (2012). Protoplast Isolation, Culture and Plant Regeneration in *Catharanthus roseus* (L.) G. Don *via* Somatic Embryogenesis. Curr. Biotechnol. 1: 217-226

Mehpara M, Mujib A, Siddiqui ZH (2012). Synthetic seed development and conversion to plantlet in *Catharanthus roseus* (L.) G. Don. Biotechnol. 11:37-43.

Moreno PRH, Van der Heijden R and Verpoorte R (1995). Cell and tissue cultures of *Catharanthus roseus*; a literature survey II. Updating from 1988-1993. Plant Cell Tiss. Org. Cult. 42:1-25.

Mujib A and Samaj J (2006). Somatic embryogenesis. -Springer-Verlag - Berlin- Heidelberg, New –York.

Mujib A, Ilah A, Junaid A, Samar F, Zahid HS and Mehpara M (2012). *Catharanthus roseus* alkaloids: application of biotechnology for improving yield. Plant Growth Reg. DOI 10.1007/s10725-012-9704-4.

Negi RS (2011). Fast In-Vitro Callus Induction in *Catharanthus roseus* - A Medicinally Important Plant Used in Cancer Therapy. Research J. Pharmaceutical, Biol. Chem. Sci. 2: 597-603.

Rashmi R and Trivedi MP (2015). Rapid In-Vitro Regeneration of an Important Medicinal and an Ornamental Plant (*Catharanthus roseus* L) Biochemistry & Analytical Biochemistry. DOI: 10.4172/2161-1009.1000227.

Saifullah and Khan S (2011). Callus induction and cell suspension culture production of catharanthus roseus for biotransformation studies of (–) caryophyllene oxide. Pak. J. Bot. 43: 467-473.

Samar F, Mujib A, Nasim SA and Siddiqui ZH (2009). Cryopreservation of embryogenic cell suspensions of *Catharanthus roseus* L. (G) Don. Plant Cell Tiss. Org. Cult. 98: 1-9.

Smith JI, Smart NJ, Kurz WGW and Misawa M (1987a). Stimulation of indole alkaloid production in cell suspension cultures of *Catharanthus roseus* by abscisic acid. Planta. Med. 53: 470-474.

Verma AK, Singh RR and Seema S (2012). Improved alkaloid content in callus culture of *Catharanthus roseus*. Botanica Serbica. 36: 123-130.

Zhao M, Bai LM, Wang LY, Toki A, Haseqawa T, Kikuchi M, Abe M, Sakai J, Haseqawa R, Bai Y, Mitsui T, Oqura H, Kataoka T, Oka S, Tsushima H, Kiuchi M, Hirose K, Tomida A, Tsuruo TN and Aado M (2007). Bioactive cardenolides from the stems and twigs of *Nerium oleander*. J. Nat. Prod. 701: 1098-1103.