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Strategies for the enhancement of vincristine and vinblastine in *Catharanthus roseus* - A review.

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ABSTRACT
India is blessed with ancient knowledge of Ayurveda that is using many plants as medicines since thousands of years. *Catharanthus roseus* also known as Madagascar periwinkle is one of the pharmacologically important plants which is extremely beneficial to mankind owing to their medicinal properties. *C. roseus* shows many essential therapeutic properties such as anti-diabetic, anti-microbial, antioxidant activities, memory enhancer and anti-cancerous activity. Vincristine and vinblastine in *C. roseus* have shown excellent anticancerous properties. But all these medically important constituents are present in very small quantities. Enhancement of these compounds by establishing sustainable hydroponic culture can fulfill the consumption and market demands in years to come. Treatment of these cultivars by various biotic and abiotic elicitors seems to be a great alternative for this purpose.

Keywords *Catharanthus roseus*; Vincristine; Vinblastine; hydroponics, Biotic and abiotic elicitor.

INTRODUCTION

Catharanthus roseus is a plant from apocynaceae family, It is also known as Madagascar periwinkle. In Marathi it is called as 'Sadafuli'. It is widely spread in India and mostly occurs in two varieties depending on the colour of flower. A pink variety is known as *rosea* and white variety is called as *alba* (1). This plant has come out to be a miracle in the inhibition and treatment of variety of cancer types especially childhood leukemia.

The plant is known to be rich source of secondary metabolites.The chemical extracts of plant leaves, flower and stem showed promising activity in killing cancer cells. Dimeric alkaloids like vincristine and vinblastine from *Catharanthus roseus* are widely known for their cytotoxicity and are also used for cancer chemotherapy treatment (2, 3). Both alkaloids differ in their molecular structure by only one carbonyl group and show structural similarity but they differ significantly in their activities on cancer cells and their toxic properties. Of the two components, vincristine has a role in preventing leukemia and Hodgkin's disease (4). Leukemia is a cancer of blood and bone marrow which involves the formation of excessive amounts of WBCs. Vincristine inhibit cell proliferation by altering the dynamics of tubulin addition and loss at the ends of mitotic spindle microtubules. It is marketed under the name *Oncovin*. Other compound, Vinblastine was isolated at the University of Ontario and was used in the treatment of advanced testicular and breast cancer. It has also shown promising activity against Hodgkin's lymphoma. The drug functions by preventing mitosis in metaphase by binding to tubulin fibres, thus preventing the cells from making the spindles needed for segregation of the chromosome and division resulting in the overall reduction of white blood cells. Vinblastine is marketed under the name *Elban*.

Due to the lesser natural concentration of these extremely beneficial compounds; it needs approximately 500 kg of dried leaves for the production of 1 kg of vinblastine. To increase the yield of these compounds various strategies have been employed. One approach could be growing the plant under *in vitro* conditions but it again faces various challenges such as the high expenditure incurred and can increase the cost of drug. The possibility to increase the yield through synthetic biology can also be one way to overcome the challenges of having a steady supply and possibly decrease the price of the drugs, allowing more people to have access to treatment. A slight change in structural confirmation may lead to hazardous effects of toxicity. Thus, the best way forward is to synthesize the drug *in situ* and increase the growth rate of plant with higher concentration of these anticancer components, for which, hydroponic cultures are very promising.

Hydroponics

Hydroponics is a method of growing plants without soil using mineral nutrient solutions in water. Terrestrial plants can be grown with their roots submerged in the mineral solution and can grow upto 30-50 % faster than a soil plant. It needs lower nutrient due to control over nutrient levels. It has lower water requirement as water stays in the system and can also be reused.

Thus, establishment and elicitation of hydroponic cultures of *C. roseus* can prove beneficial with respect to application of the treated plant extracts on cancer cells. The overall increase of secondary metabolites and their role in expression (upregulation or downregulation) of various apoptosis related genes of cancer cells has also been observed by performing studies on the basis of correlation between dosage and cytotoxicity (Fig. 2). Thus, an economically beneficial technological approach for harvesting of higher biomass (leaf and stem) with concentrated anticancer compounds is desirable.

Elicitor treatments

Elicitation of the plant cell cultures has also been employed for the enhancement of the secondary metabolites (5, 6). Under stress, increase of alkaloid content has been reported in *C. roseus* (1). Application of elicitors like jasmonate and salicylic acid has positive effect on alkaloid biosynthesis. Combination of tetramethyl ammonium bromide and *A. niger* mycelial homogenate also showed highest ajmalicine and catharanthine production. Use of yeast extract too shows positive effects on the production of secondary metabolites. Comparative studies of enzymatic activities of the elicitor treated plants are needful in establishing a link between increase in metabolite concentration and enhanced anticancer properties of *C roseus* plant extracts.

Yeast Extract

It is a potent biological enhancer. It is observed that it enhances the concentration of vincristine and vinblastine. Maximum yield was obtained in germinating embryos and in *in vitro* grown leaf (6). Unknown compounds from Yeast extract are known to bind cell membrane bound proteins and activate the secondary metabolism related genes via. hydrogen

peroxide (5). Antioxidant enzymes such as superoxide dismutase, peroxidase, glutathione reductase, catalase, NADPH oxidase are also known to enhance owing to cellular stress (7).

Methyl Jasmonate

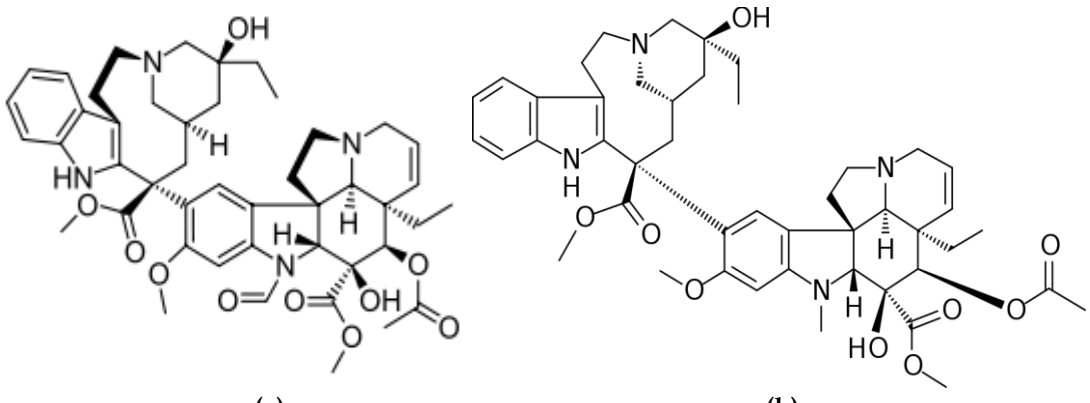
Treatment of different concentration of methyl jasmonate (MeJa) on *Catharanthus roseus* hairy roots has shown to elicit the accumulation of alkaloids such as catharanthine, ajmalicine along with ajmaline and serpentine (8, 9). Also the accumulation of alkaloids in increased concentration was observed in the tissues of *C. roseus*.

Salicylic acid

Salicylic acid (SA) treatment reportedly reverses the adverse effects of salinity in *C. roseus* plants. Salinity causes the overall decrease in the production of alkaloids, ascorbic acid and antioxidant enzymes such as catalase, peroxidase and superoxide dismutase. Foliar spray of SA significantly increases the total alkaloid content and also rapidly enhances the concentration of vincristine and vinblastine in salt and nickel stressed *C. roseus* plants (10).

Molecular mechanism of elicitation

Plant secondary metabolism and the defense response of plants are related to each other. The attack of any herbivore, biotic or abiotic stress induces the elicitation response. Such as biosynthesis of phenolics, xanthones and lignin deposition in the plant cell wall. In elicitation, pathogen origin small molecule is capable of triggering the same response in plants as it causes in the pathogen itself. Signal recognition is the first step in the signal transduction pathway. Recognition of different signals is the unique characteristics of plant cells to initiate the activations of kinases, reactive oxygen species, ion fluxes and cytoplasm acidification. Elicitors binding sites or receptors are located on the plasma membrane of the plant cell. Most likely, transmembrane receptor-like protein kinases (RLKs) and R-proteins (race specific elicitors) are involved to combat the broad range of pathogen stimuli (11, 12). Intracellular signaling systems consist of G-proteins which are mainly involved in the processes linked to hormone signaling, growth and defense responses and in responses related to elicitors (13). Phospholipase C pathway is activated by variety of elicitors causing the production of the second messenger inositol triphosphate (IP_3) and diacylglycerol. In *Arabidopsis* the gene (AtPLC1) was cloned which over-expresses PI-PLC and helped plants against dehydration, salinity and low temperature. Three distinct PI-PLC isoforms (StPLC1, StPLC2 and StPLC3) when cloned in *Solanum tuberosum* leaves provided resistant against drought stress (14). Chitosan treatment of *Rubia tinctorum* cultures showed decrease in the synthesis of anthraquinone and diacylglycerol after treatment with PLC-antagonist neomycin and U-73122 (15). Role of IP_3 in releasing the intracellular calcium is well known but the role of diacylglycerol is less clear. Involvement of calcium as the secondary messenger is well known. It mediates the regulation of various cellular processes such as elicitation by affecting the intracellular calcium levels. The involvement of calcium in plant processes have been confirmed by the membrane patch-clamp technique and measurement of calcium by calcium sensitive dye (16).



(a) **(b)**

Fig. 1 Chemical structure of vincristine (a) and vinblastine (b)

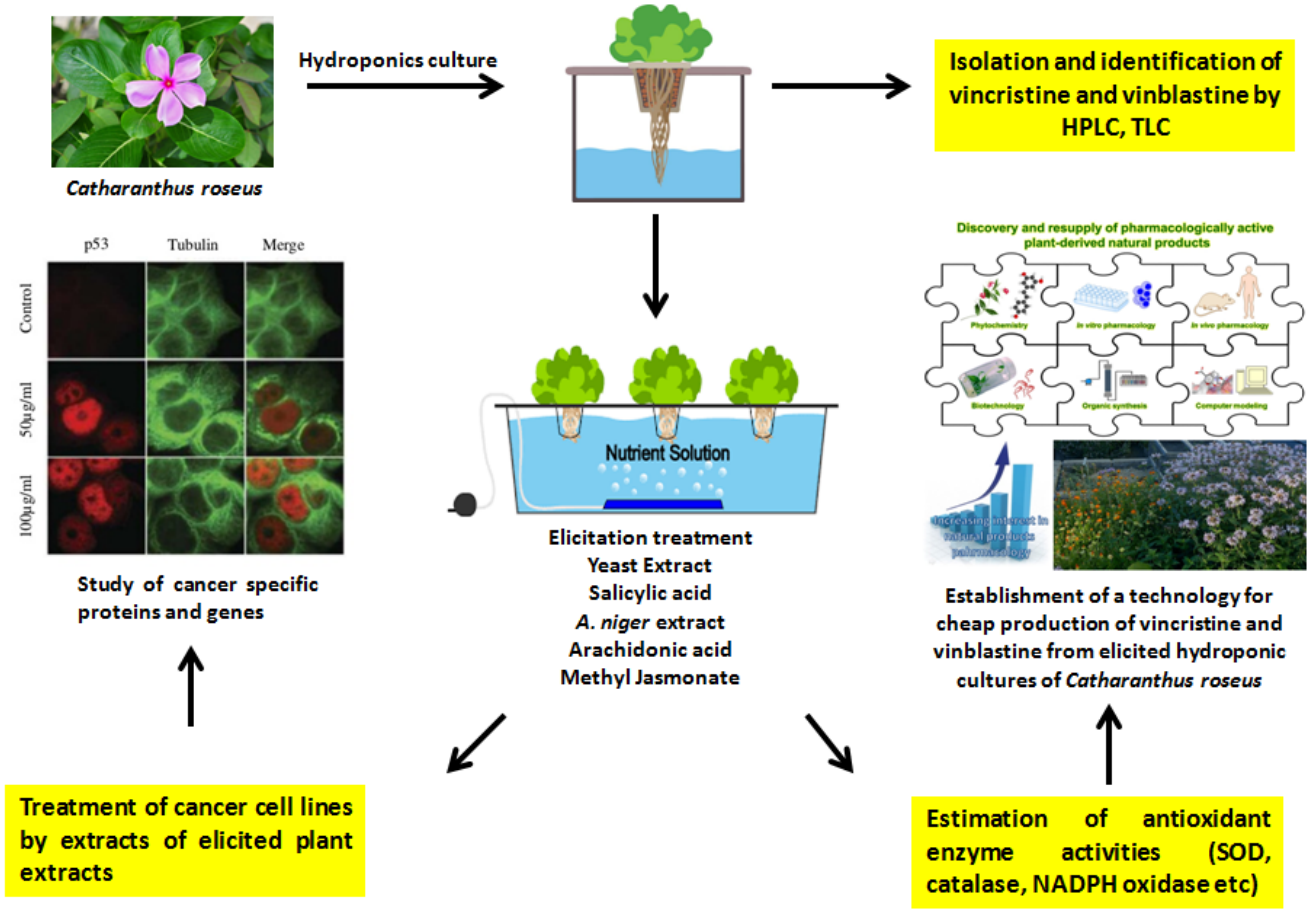


Fig. 2 Establishment of hydroponic culture and elicitation in enhancement of anticancer and antioxidant capacities

CONCLUSION

Catharanthus roseus plant species is like a gift to mankind due to its numerous applications. Each part of this plant is advantageous in many ways. Some rural communities use the extracts of flowers and leaves in dried or wet forms to make a paste and apply it on wounds for fast healing. Fresh juice from flowers made into a tea is a routine procedure in Indian Ayurveda to treat certain common skin problems like acne, dermatitis and eczema. Plant extracts have been found to contain alkaloids and anti-cancerous compounds like vincristine, vinblastine. *C. roseus* plant extract is a potential source of many antioxidants. It also shows antimicrobial activities against *Bacillus megaterium* and Shigella. Antioxidant enzymes for e.g. catalase, peroxidase, glutathione reductase are present in *C. roseus* plant extracts. The yield of these useful medicinal compounds and enzymes in the plant can be increased by many folds by applying certain new techniques like hydroponics and by giving different enhancer treatments. The enhancement treatments are also helpful for the study of biochemical pathways leading to the production of alkaloids and vincristine & vinblastine; well known anti-cancerous compounds.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

1. Rai V, Tandon PK, Khatoon S. Effect of chromium on antioxidant potential of *Catharanthus roseus* varieties and production of their anticancer alkaloids: vincristine and vinblastine. BioMed research International, 2014; 934182: 1-10.

2. Erdogrul. Antibacterial activities of some plant extract used in folk medicines. Pharmaceutical Biology, 2002; 40(1): 269-273.
3. Jaleel CA, Gopi R, Lakshmanan, MA, Pannerselvam, R. Tradimefon induced hanges in the antioxidant metabolism and ajmalicine in *Catharanthus roseus* (L.) G. Don. Plant Science, 2006; 171(2); 271-276.
4. Heijden RV, Jacobs DI, Snoijer W, Hallard D, Verpoorte R. The *Catharanthus* alkaloids: Pharmacognosy and biotechnology. Current Medicinal Chemistry, 2004; 11(1): 607-628.
5. Moon UR, Mitra A. A mechanistic insight into hydrogen peroxide mediated elicitation of bioactive xanthones in Hoppea fastigiata shoot cultures. Planta, 2016; 244(1): 259-274.
6. Moon UR, Durge AA, Kumar M. Value addition of khus (*Vetiveria zizanioides* L. Nash) root extracts by elicitor and heat treatment. Industrial Crops and Products, 2020; 144(1): 112037.
7. Maqsood M, Abdul M. Yeast extract elicitation increases vinblastine and vincristine yield in protoplast derived tissues and plantlets in *Catharanthus roseus*. Revista Brasileira de Farmacognosia, 2017; 27(5): 549-556.
8. Ruiz-May E, Galaz-Avalos RM, Loyola-Vargas VM. Differential secretion and accumulation of terpene indole alkaloids in hairy roots of *Catharanthus roseus* treated with methyl jasmonate. Molecular Biotechnology, 2008; 41: 278.
9. Lee-Parsons, C.W.T., Ertik S., Tengtrakool, J. Enhancement of ajmalicine production in *Catharanthus roseus* cell cultures with methyl jasmonate is dependent on timing and dosage of elicitation. Biotechnology Letters, 2004; 26(1): 1595-1599.
10. Idrees M, Naeem M, Aftab T, Khan MMA, Moinuddin. Salicylic acid mitigates salinity stress by improving antioxidant defense system and enhances vincristine and vinblastine alkaloids production in periwinkle (*Catharanthus roseus* (L.) G. Don. Acta Physiologiae Plantarum, 2011; 33(1): 987-999.
11. Montesano M., Brader G, Palva, ET. Pathogen derived elicitors: searching for receptors in plants. Molecular Plant Pathology, 2003; 4: 73-79.
12. Gabriel DW, Rolfe BG. Working models of specific recognition in plant-microbe interactions. Annual Reviews on Phytopathology, 1990; 28: 365-391.
13. Jones AM. G-protein-coupled signaling in Arabidopsis, Current Opinion in Plant Biology, 2002; 5: 402-407.
14. Kopka J, Pical C, Gray JE, Müller-Röber B. Molecular and enzymatic characterization of three phosphoinoside-specific Phospholipase C isoforms from potato. Plant Physiology 1998; 116: 239-250.
15. Vasconsuelo A, Maria Giuletti A, Picotto G, Rodriguez-Talou J, Boland R. Involvement of the PLC/PKC pathway in chitosan-induced anthraquinone production by *Rubia tinctorum* L. cell cultures. Plant Science, 2003; 165: 429-436.
16. Kuchitsu K, Ward JM, Allen GJ, Schelle I, Schroeder JI. Loading acetoxymethyl ester fluorescent dyes into the cytoplasm of Arabidopsis and Commelina guard cells. New Phytologist, 2002; 153: 527-533.