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Original Scientific Paper

Improved alkaloid content in callus culture of *Catharanthus roseus*

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ABSTRACT: The low yield and high market price of the pharmaceutically important alkaloids of *Catharanthus roseus* (L.) G. Don viz. vincristine, vinblastine and ajmalicine have created interest in improved alternative routes for their production such as using cell and tissue culture. The callus developed on Murashige and Skoog (MS) media supplemented with different concentrations of auxins and cytokinins was found to have variable alkaloid contents. Combinations of auxins with cytokinins were found to be better for leaf callus growth and enhancement of alkaloid content. The highest enhancement of total alkaloid content was found in 0.50 mg/l of 2, 4-D and 1.0 mg/l of BA, compared with other combinations. Our findings indicated that in addition to plant growth regulators and strength of the MS media, various carbon sources and their concentrations had a significant influence on leaf callus growth and total alkaloid content. It was found that half strength MS basal medium supplemented with 2, 4-D and BA (0.5mg/l and 1.0 mg/l, respectively) and 6% sucrose was best for biomass production of leaf callus and enhancement of alkaloid accumulation in *C. roseus*.

Key words: *Catharanthus roseus*, callus culture, leaf callus, callus biomass, alkaloid.

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INTRODUCTION

Plants are known to produce a large array of natural products, also referred to as secondary metabolites. Plant secondary metabolites are a huge number of natural compounds with a wide diversity in chemical structure. They are economically important to man due to their multiple applications, such as pharmaceuticals, flavors, fragrances, insecticides, dyes, food additives, toxins (Taha *et al.* 2009).

However, it is well known that their production is frequently low and depends on the physiological and developmental stage of the plant. The majority of pharmaceutically important secondary metabolites are obtained from wild or cultivated plants, and 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 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 synthesize with chemical methods (Zhao & Verpoorte 2007).

Catharanthus roseus, produces several commercially valuable secondary metabolites including the anti-cancerous vinblastine, vincristine and anti-hypertensive alkaloids ajmalicine and serpentine. Bioengineering efforts to synthesize these indole alkaloids in plant tissue cultures of *C. roseus* have yielded varying responses (Moreno *et al*. 1995; Stern 2000; Gragg & Newman 2005). The low yield of dimeric indole alkaloids from the plant (approximately 0.0005%) and their consequent high price have stimulated numerous efforts to develop alternative strategies for their production.

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In the present investigation, *in vitro* culture experiments were conducted on *Catharanthus roseus* using MS (Murashige & Skoog, 1962) media as basal media for assessing the effect of plant hormone combinations, MS salt content (full strength and half strength), type of energy sources (carbohydrate) on growth and total alkaloid content in leaf callus as well as for standardization of culture media for biomass production of alkaloids.

MATERIALS AND METHODS

Healthy seeds of *Catharanthus roseus,* variety Nirmal were washed in running tap water for about three h, then treated with bavistine (disinfectant) for 10 min with continuous shaking. A few drops of detergent (Tween 20) were added before final washing, which helps in removing the waxy coating, also reducing the surface tension and acting as surfactant. Thereafter, seeds were washed five times in sterilized distilled water. Further sterilization was carried out under laminar air flow. Aseptic transfer was done under the laminar air flow. The entire internal surface was swabbed with 70% ethanol prior to starting the work. Washed seeds were treated with ethanol for 2 min then washed 2-3 times with sterilized double-distilled water then treated with 0.1% $HgCl_2$ for 6-8 min followed by 5-6 rinses in double-distilled sterilized water to remove traces of $HgCl_2$. After sterilizations, seeds were aseptically transferred onto the medium. Five seeds were inoculated in each culture tube containing 20 ml of 0.7% agarified MS medium (without any growth regulator). The inoculated seeds were incubated at 25 ± 2^0 C in a culture room, in the dark. *In vitro* germinated seedlings were utilized as a source of leaf explants (Fig-A). Segments of leaf explant were cultured in standardized MS medium fortified with BAP (1 mg/l) and NAA (1 mg/L) for callus initiation. The cultures were incubated under a 16 h photoperiod at 25 ± 2^0C. After 7 weeks, developed calli were sub-cultured in the same medium for mass increase. Thereafter, established callus cultures were grown on different modified media for assessing the effect of phytohormones, strength of MS (full strength and half strength) medium and type of energy source (carbohydrate) on callus multiplication and total alkaloid accumulation (after 7 weeks of subculture). Data on fresh weight and total alkaloid content were recorded.

Total alkaloid content in dry calli was estimated by the use of Dragendroff's reagent (Narshiman & Mehrotra 2003). Data were recorded in triplicate and statistically analyzed. All statistical analyses were performed using STATISTICA (version 6.0).

RESULTS

Effects of different concentrations of auxins and cytokinins singly on callus proliferation and total alkaloid content. 2,4-Dichlorophenoxyacetic acid (2, 4-D) showed stimulatory effects on callus proliferation and total alkaloid content (Table 1). Maximum callusing response (76%) was noted at 4.0 mg/l and the minimum at 0.25 mg/l. Of six concentrations of 2, 4-D used in MS media, 2.00 mg/l was better for callus proliferation while 6 mg/l for increase in total leaf alkaloid content. It was noted that the higher concentration of 2, 4-D in media had an inhibitory effect on callus proliferation but stimulated a gradual increase in total alkaloid content. Fresh weight and total alkaloid content in leaf callus at different concentrations of 2, 4-D were significantly higher than for 0.25 mg/l 2, 4-D except for 0.50 mg/l and 1.00 mg/l (Fig.1B).

MS media fortified with different concentrations (0.25–6.0 mg/l) of 1-naphthaleneacetic acid (NAA) showed stimulatory effects on callus growth and total alkaloid content (Table 2). Maximum callusing response (66%) was noted at 4.0 mg/l of NAA and the minimum at 0.25 mg/l. Of six concentrations of NAA used in MS media, a concentration of 2.00 mg/l was found to be better for increase in total alkaloid content while 4mg/l was better for callus proliferation. NAA concentrations up to

Table 1. Effect of various concentrations of auxins on *C. roseus* leaf callus biomass and total alkaloid content (incubation period 42 days)

Phyto-hormones (mg/l)	Responsive callus (%)	2, 4-D		NAA	
		Fresh weight (g)	Total alkaloid content (mg/g DW)	Fresh weight (g)	Total alkaloid content (mg/g DW)
0.25	39	0.16 ± 0.03	3.93 ± 0.40	0.35 ± 0.03	4.17 ± 0.37
0.50	48	0.24 ± 0.02^{ns}	7.40 ± 0.55*	0.51 ± 0.04*	5.63 ± 0.65^{ns}
1.00	57	0.37 ± 0.02*	9.37 ± 0.40**	0.57 ± 0.03*	5.45 ± 0.32*
2.00	73	0.97 ± 0.08**	10.85 ± 0.45**	0.66 ± 0.03**	7.34 ± 0.41**
4.00	76	0.78 ± 0.08**	25.23 ± 1.79**	0.98 ± 0.10**	6.95 ± 0.42**
6.00	51	0.52 ± 0.06**	31.06 ± 1.33**	0.62 ± 0.04**	6.60 ± 0.38**

ns- p>0.05, *- p<0.05, **- p<0.01- in comparison with 0.25 mg/l

Table2. Effect of various combinations of growth hormones on *C. roseus* leaf callus biomass and total alkaloid content (incubation period 42 days).

Phytohormones (mg/l)		Responsive callus (%)	Fresh weight (g)	Total alkaloid content (mg/g DW)
2, 4-D+ NAA	(1.0mg/l+0.5mg/l)-A	65	1.46 ± 0.18	28.98 ± 1.04
2,4-D + NAA	(0.5mg/l+1.0mg/l)-B	58	0.66 ± 0.06**	25.56 ± 1.37ns
2,4-D + IAA	(1.0mg/l+0.5mg/l)-C	60	1.14 ± 0.23*	27.96 ± 2.09ns
2,4-D + IAA	(0.5mg/l+1.0mg/l)-D	49	0.63 ± 0.04**	21.62 ± 1.68*
2,4-D + BA	(1.0mg/l+0.5mg/l)-E	78	1.56 ± 0.12ns	39.94 ± 1.59**
2,4-D + BA	(0.5mg/l+1.0mg/l)-F	70	0.98 ± 0.04**	47.92 ± 2.85**
2,4-D + KIN	(1.0mg/l+0.5mg/l)-G	69	0.94 ± 0.06**	24.88 ± 1.45ns
2,4-D + KIN	(0.5mg/l+1.0mg/l)-H	63	0.64 ± 0.03**	23.48 ± 1.90ns
NAA + IAA	(1.0mg/l+0.5mg/l)-I	59	0.47 ± 0.04**	18.00 ± 1.14**
NAA + IAA	(0.5mg/l+1.0mg/l)-J	53	0.42 ± 0.06**	14.62 ± 0.86**
NAA + BA	(1.0mg/l+0.5mg/l)-K	67	1.04 ± 0.03*	20.92 ± 1.04**
NAA + BA	(0.5mg/l+1.0mg/l)-L	59	0.74 ± 0.05**	11.14 ± 1.09**
NAA + KIN	(1.0mg/l+0.5mg/l)-M	71	1.10 ± 0.06*	8.30 ± 0.99**
NAA + KIN	(0.5mg/l+1.0mg/l)-N	64	0.78 ± 0.08**	7.00 ± 1.05**
IAA + BA	(1.0mg/l+0.5mg/l)-O	58	0.37 ± 0.02**	7.30 ± 0.34**
IAA + BA	(0.5mg/l+1.0mg/l)-P	47	0.27 ± 0.04**	7.00 ± 0.71**
IAA + Kn	(1.0mg/l+0.5mg/l)-Q	60	0.60 ± 0.06**	5.30 ± 0.13**
IAA + Kn	(0.5mg/l+1.0mg/l)-R	53	0.33 ± 0.02**	4.98 ± 0.54**

ns- p>0.05, *- p<0.05, **- p<0.01- in comparison with 2, 4-D + NAA (1.0 mg/l+0.5 mg/l).

4mg/l showed stimulatory effects on callus growth and up to 2mg/l on increasing total alkaloid content.

MS media fortified with different concentrations (0.25–6.0 mg/l) of indole-3-acetic acid (IAA) showed slight initiation of callus growth at an early stage followed by necrosis of callus (Fig.1D).

MS media fortified with different concentrations (0.25–6.0 mg/l) of 6-benzylaminopurine (BA) showed negative effects on callus growth along with necrosis.

No callusing was noted at various concentrations of kinetin (KIN) with the transferred callus segment suffering from necrosis.

Effects of different concentration and combination of growth hormones on leaf callus proliferation and total alkaloid content. 2, 4-D in combination with NAA at all concentrations induced callus proliferation in sub-cultured callus (Table 2). The callus that formed was white and did not show any morphogenic response while callus proliferation with rhizogenesis (Fig. 1E) was noted on 2, 4-D supplemented with IAA.

When 2, 4-D was applied together with BA, callus proliferation was induced at all concentrations (Table 3) and the resulting callus was granular and showed morphogenic responses. When 2, 4-D was added in combination with KIN callus formation was initiated (Fig. 1F).

NAA in combination with IAA promoted callus proliferation along with rhizogenesis (Table 2). Callus was generally compact and yellowish brown. NAA with cytokinins viz. BA and KIN induced callus proliferation at all concentrations applied (Table 2).

IAA in combination with the cytokinins BA and KIN in all concentrations induced callus proliferation but this response was in general relatively lower than the other combinations of auxins and cytokinins.

MS media with 2,4-D and BA (1.0mg/l+0.5mg/l) (Fig. 1E), 2,4-D and BA (0.5mg/l+1.0mg/l) (Fig. 1F) were found to be the most promising media in relation to callus biomass increase and total alkaloid content. This increase was also found to be statistically significant (Table 2).

Effects of strength of MS media on leaf callus proliferation and total alkaloid content. For this study, media E, F were used as standard media. Decrease in strength of MS media had no effects on callus biomass though it enhanced total alkaloid content (Table 3). Of ten combinations that were used, half strength MS medium with 2, 4-D and BA (0.5 mg/l +1.0 mg/l) was found to be the best for production of adequate callus biomass along with high alkaloid content.

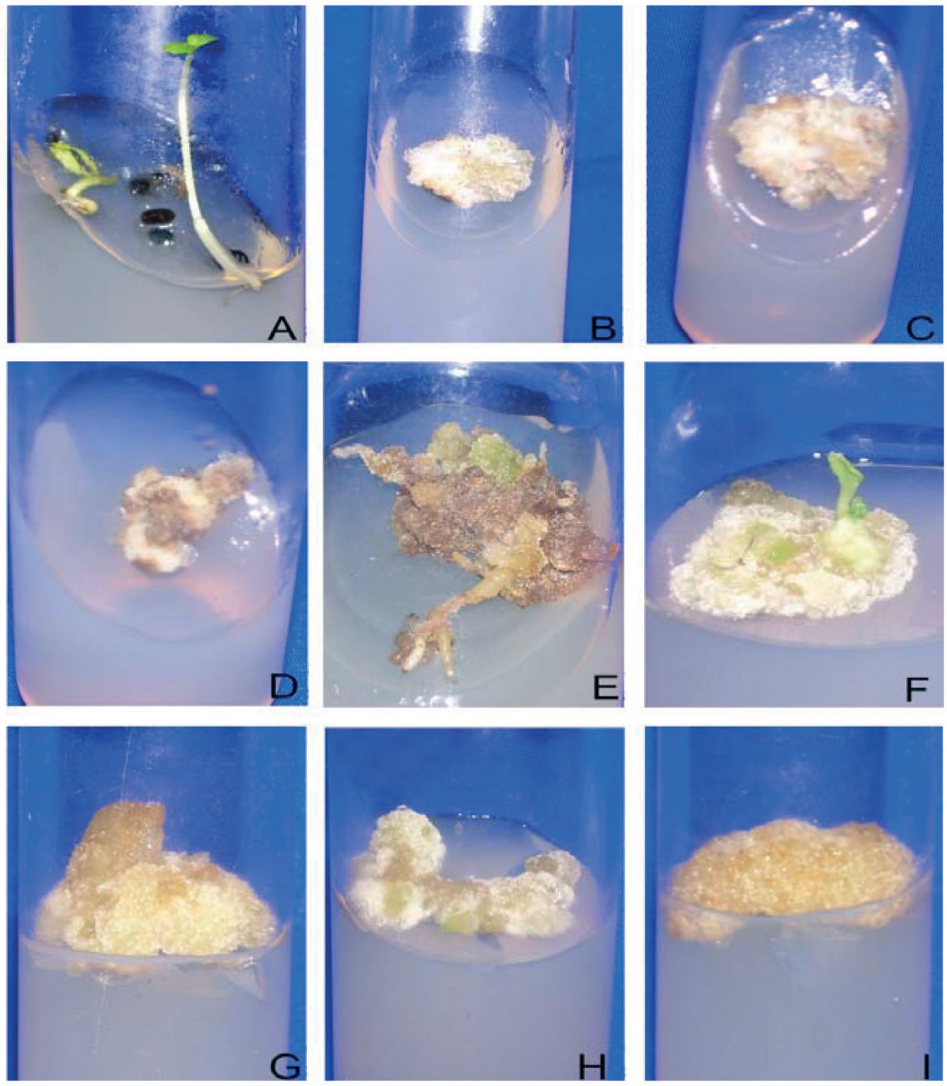

Figure 1. A: *in vitro* germinated seedling of *C. roseus*; B – D: leaf callus at initial stage; E: rhizogenesis; F: organogenesis from leaf callus; G-I: leaf callus.

Table 3. Effect of strength of MS media on *C. roseus* leaf callus biomass and total alkaloid content (incubation period 42 days)

S.N.	Concentration of growth regulators (mg/l)	Medium	Responsive callus (%)	Fresh weight (g)	Total alkaloid content (mg/g DW)
1.	2,4D + BA (1.0+0.5)	MS	73	1.49 ± 0.06	44.90 ± 1.74
		½ MS	72	1.45 ± 0.02^{ns}	63.96 ± 3.06**
2.	2,4D + BA (1.0+0.25)	MS	68	1.58 ± 0.05^{ns}	69.08 ± 1.31**
		½ MS	70	1.50 ± 0.04^{ns}	73.74 ± 2.18**
3.	2,4D + BA (0.5+1.0)	MS	67	1.04 ± 0.03**	78.54 ± 2.17**
		½ MS	58	0.95 ± 0.02**	85.66 ± 1.66**

ns- $p>0.05$, **- $p<0.01$- in comparison with MS + 2, 4-D + BA (1.0mg/l+0.5 mg/l)

Table 4: Effect of various types of energy resources on *C. roseus* leaf callus biomass and total alkaloid content (incubation period 42 days)

Dose Groups	Responsive callus (%)	Fresh weight (g)	Total alkaloid content (mg/g DW)
3% sucrose	62	1.03 ± 0.06	82.96 ± 1.93
6% sucrose	68	1.30 ± 0.04**	96.32 ± 1.71**
3% glucose	73	1.60 ± 0.05**	62.54 ± 2.07**
6% glucose	78	1.66 ± 0.04**	67.82 ± 2.39**
3% fructose	64	0.88 ± 0.05*	70.50 ± 1.61**
6% fructose	59	1.13 ± 0.06ns	72.38 ± 0.99**

ns- p>0.05, *- p<0.05, **- p<0.01- in comparison with 3% sucrose

Effects of carbohydrate type on leaf callus proliferation and total alkaloid content. For this study half strength MS medium with 2, 4-D and BA (0.5mg/l + 1.0 mg/l) was selected as the standard medium. Sucrose, glucose and fructose were used as energy sources that were added to the standard medium (Table 4). In relation to callus proliferation and alkaloid content, sucrose was found to be the most promising energy source and sucrose at 6% concentration in the standard medium was found to be the best for callus proliferation and alkaloid yield in comparison to other carbohydrate sources.

Overall, our results showed that half strength MS medium enriched with 2, 4-D and BA (0.5mg/l+1.0 mg/l), with 6% sucrose (Fig1G, 1I), was the best for biomass production of leaf callus and enhancement of alkaloid accumulation in *C. roseus*.

DISCUSSION

Tissue culture has been suggested as a feasible technology for the production of many plant secondary metabolites. For example, ginsenoside from *Panax gingseng*; rosmarinic acid from *Coleus blumei*, shikonin from *Lithosermum erythrohizon*, diosgenin from *Dioscorea*, ubiquinone-10 from *Nicotiana tabacum*, berberin from *Coptis japonica* and podophyllotoxin from *Juniperus chinensis* accumulated at much higher levels in cultured cells than in intact plants (Misawa *et al.* 1985; Smith *et al.* 2002; Premjet *et al.* 2002). According to Kalidass et al. (2010) the production of secondary metabolites in callus cultures is controlled by environmental factors, viz medium component, pH and temperature. To obtain high leaf callus biomass and alkaloids in higher concentration, *in vitro* experiments were carried out varying the composition of the media.

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; Misawa 1994; Asaka *et al.* 1993), 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.

In general, we found low auxin and higher cytokinin concentrations to be better for callus proliferation and growth and enhancement of alkaloid content in leaf callus of *C. roseus,* which is in agreement with findings of Kodja *et al.* (1989). According to Decendit & Liu (1992) cytokinins are able to amplify alkaloid production in *C. roseus* by removal of auxins from the culture medium of non-tumerous cell lines.

The highest enhancement in total alkaloid production resulted from 0.50 mg/l of 2, 4-D and 1.0 mg/l of BA, compared with other combinations. Combinations of 2, 4-D and BA were used by Olivira *et al.* (2001) for enhancement of ramiflorin in callus of *Aspidosperma ramiflorum,* Taha *et al.* (2008) for corydalin in callus of *Corydylis terminalis,* Khan *et al.* (2008) for alkaloid in callus of *Corydylis ophiocarpa,* Yamada & Hashimoto (1982) for tropane alkaloids in callus of *Hyocyamus niger,* Morimoto *et al.* (1994) for rosmarinic acid in callus of *Salvia miltorizzhiza*, and Mirjalili *et al.* (2009) for steroidal lactone in callus of *Withania somnifera*.

Our findings indicate that callus growth was found to be more efficient on a MS medium with full strength, rather than half strength which is consistent with the findings of Kadkade (1981, 1982). The results agreed with those of Drewes & Staden (1995) for solasodine production in *Solanum mauritianum* and of Rosli *et al.* (2009) for 9-methoxycanthin-6-one production in *Eurycoma longifolia* callus cultures. The nutrient concentration of a particular basal medium was also previously reported to be the greatest contribution towards the variation of solasodine production in callus cultures of *Solanum aviculare* (Kittipongpatana *et al.* 1998). According

to Rhodes *et al.* (1990) and Drewes & Staden (1995), the capability of different basal media formulation in supporting plant cell growth and the synthesis of plant secondary metabolites were linked to the ionic balance in the medium. Drewes & Staden (1995) and Lipavaska & Vreugdenhil (1996) stated that it is important to find a suitable nutrient concentration of basal medium because a lower concentration of nutrient components is not enough to support cell growth and higher concentrations of nutrients may become toxic and cause an osmotic stress for plant cell cultures.

Besides, plant growth regulators and the strength of MS media, various carbon sources and their concentrations were found to have a significant influence on leaf callus growth and total alkaloid content. In general higher concentrations of carbon resources caused an enhancement in callus biomass as well as total alkaloid content. Our study showed that the maximum callus biomass production occurred with 6% glucose while maximum alkaloid production occurred at a concentration of 6% sucrose. This is in accordance with the finding of Woo *et al.* (1998) that higher concentrations of monosaccharide caused the maximum production of biomass. Studies on callus of *Hyoscyamus niger* showed that increasing sucrose concentration caused an increase in biomass as well as stimulation of production of scopolamine alkaloid (Hilton & Rhodes 1994). In contrast, Schripsema & Verpoorte (1992) showed that biomass production of *Datura stramonium* root culture in sucrose-enriched growth medium was higher than in a monosaccharide medium, while alkaloid production was highest in a monosaccharide medium. Although 6% sucrose decreased overall callus biomass slightly, the markedly increased production of alkaloid in comparison with other carbon resources make it the most effective concentration for large scale production of *Catharanthus* alkaloids. This is in line with findings of Scragg & Ashton (1990) in *Catharanthus roseus* callus culture. Shaoxiong *et al.* (1996) reported that higher concentrations of sucrose cause enhancement in steroidal alkaloid production in hairy roots culture of *Solanum aviculare*.

The present study showed that half strength MS basal medium supplemented with 2, 4-D and BA (0.5mg/l+1.0 mg/l) with 6% sucrose was the best for biomass production of leaf callus and enhancement of alkaloid accumulation in *C. roseus*. The results indicate that the combination of different basal media, carbon sources and phytohormones could be a useful tool in developing rational strategies to enhance the production of various bioactive molecules *in vitro*.

REFERENCES

Asaka ILI, Hirotani M, Asada Y & Furuya T. 1993. Production of ginsenoside saponins by culturing ginseng (*Panax ginseng*) embryonic tissue in biorectors. *Biotechnol. Lett*. **15**:1259-1264.

Brown TJ. 1990. The initiation and maintenance of callus cultures. In: Pollard JW & Walker JM (eds.) Methods in Molecular Biology, vol.6, Plant cell and Tissue culture. The Human Press, New Jersey, pp:57-63.

Decendit A & Liu D. 1992. Cytokinin-enhanced accumulation of indole alkaloids in *Catharanthus roseus* cell cultures: The factors affecting the cytokinin response. *Plant Cell Reports* **11**: 400-403.

Drewes FE & Staden JV. 1995. Initiation of and solasodine production in hairy root cultures of *Solanum mauritianum* Scop. *Plant Growth Regulation* **17**: 27–31.

Gragg GM & Newman DJ. 2005. Plants as a source of anti-cancer agents. *J. Ethnopharmacol.* **100**: 72-79.

Hilton MG & Rhodes MJC. 1994. The effect of varying levels of gamborgs B5 salt and temperature on the accumulation of starch and Hyoscyamine in batch culture of transformed roots of *Datura stramonium*. *Plant Cell Tissue Org. Cult.* **38**: 45-51.

Junaid A, Mujid A, Fatima S & Sharma MP. 2008. Cultural conditions affect somatic embryogenesis in *Catharanthus roseus* L. (G.) Don. *Plant Biotechnology Rep*. **8**: 60-69.

Kadkade PG. 1981. Formation of podophyllotoxin in *Podophyllum peltatum* tissue cultures. *Naturwissenschaften* **68**: 481-482.

Kadkade PG 1982. Growth and podophyllotoxin production in callus tissue of *Podophyllum peltatum*. *Plant Sci. Lett.* **25**: 107-115.

Kalidass C, Mohan VR & Daniel A. 2010. Effect of auxin and cytokinin on vincristine production by callus culture of *Catharanthus roseus* L. (Apocynaceae). *Tropical and Subtropical Agroecosystems* **12**: 283-288.

Khan T, Krupadanam D & Anwar Y. 2008. The role of phytohormone on the production of berberine in the calli culture of an endangered medicinal plant, turmeric (*Coscinium fenustratum* L.). *Afr. J. Biotechnol.* **7**: 3244-3246.

Kittipongpatana N, Hock RS & Porter JR. 1998. Production of solasodine by hairy root, callus, and cell suspension cultures of *Solanum aviculare* Forst. *Plant Cell Tiss. Organ Cult.* **52**: 133–143.

Kodja HP, Liu D, Merillon JM, Andreau F, Raideau M & Chenieux JC. 1989. Stimulation par les cytokinins de I, accumulation d' alcaloides indoliques dans des suspensions cellularies de *Catharanthus roseus* (L.) G. Don. *Z. Naturforsch.* **35c**: 551-556.

Lipavaska H & Vreugdenhil D. 1996. Uptake of mannitol from media by *in vitro* grown plants. *Plant Cell Tissue Org. Cult.* **45**: 103-107.

Mirjalili MH, Moyano E, Bonfill M, Cusido RM & Palazon J. 2009. Steroidal lactones from *Withania somnifera*, an antioxidant plant for novel medicine. *Molecules* **14**: 2373-2393.

Misawa M. 1994. Plant Tissue Culture: An Alternative for Production of useful metabolites (FAO Agricultural services Bulletin), Bio International Inc, Toronto, Canada, pp. 18-19.

Misawa M, Hayashi M & Takayama S. 1985. Accumulation of antineoplastic agents by plant tissue cultures. In: Neumann KH (ed.) Primary and Secondary Metabolism of Plant Cell Cultures, Springer-Verlag, Berlin, Heidelberg, pp. 235.

Moreno PRH, van der Heijden R & Verpoorte R. 1995. Cell and tissue cultures of *Catharanthus roseus*: A literature survey. *Plant Cell Tissue Org. Cult*. **42**: 1-25.

Morimoto S, Goto YD & Shoyama Y. 1994. Production of lithospermic acid B and rosmarinic acid in callus tissue and regenerated plantlets of *Salvia miltiorrhiza*. *J. Nat. Prod*. **57**: 817-823.

Murashige T & Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. *Physiol. Plant* **15**: 473-497.

Narasimhan S & Mehrotra S. 2003. Spectrophotometric method for estimation of alkaloid-precipitable with Dragendroff's reagent in plant materials. *J. AOAC Intern.* 86: 1124-1127.

Olivira AJB, Koika L, Reis FAM & Shepherd SL. 2001. Callus culture of *Aspidosperma ramiflorum* Muell.-Arg. Growth and alkaloid production. *Acta Scientia* **23**: 609-612.

Premjet D, Itoh K & Tachibana S. 2002. Stimulation of the production of podophyllotoxin by biogenetic precursors and elicitor in *Juniperus chinensis* stem-derived callus cultures. *Pakistan J. Biol. Sci*. **5**: 313-316.

Rhodes MJC, Robins RJ, Hamill JD, Parr AJ, Hilton MH & Walton NJ. 1990. Properties of transformed root culture. In: Charlwood BV & Rhodes MJC (eds.) Proceedings of the Phytochemical Society of Europe Secondary Product from Plant Tissue Culture. Clarendon Press, Oxford, pp. 201-225.

Rosli N, Maziah M, Chan KL & Sreeramanan S. 2009. Factors affecting the accumulation of 9-methoxycanthin-6-one in callus cultures of *Eurycoma longifolia*. *J. Forest. Res.* **20**: 54-58.

Schripsema J & Verpoorte R. 1992. Search of factors related to the indole alkaloid production in cell suspension culture of *Talernaemontana divaicata*. *Plant Med.* **58**: 245-249.

Scragg AH & Ashton S. 1990. Growth of *Catharanthus roseus* suspensions for maximum biomass and alkaloid accumulation. *Enzyme and Microbial Technology* **12**: 292-298.

Shaoxiong Y, Kian HK & Pauline MD. 1996. Effect of sucrose, exogenous product concentration, and other culture conditions on growth and steroidal alkaloid production by *Solanum aviculare* hairy roots. *Enzyme and Microbial Technology* **18**: 238-243.

Smith M, Kobayashi H, Gawienowski M & Briskin DP. 2002. An *in vitro* approach to investigate chemical synthesis by three herbal plants. *Plant Cell Tissue Org. Cult.* **70**: 105-111.

Stern S. 2000. Introductory Plant Biology, 8th ed., McGraw-Hill Companies Inc, pp. 238-247.

Taha HS, El-Bahr MK & Seif-El-Nasr MM. 2009. *In vitro* studies on Egyptian *Catharanthus roseus* (L.). II. Effect of biotic and abiotic stress on indole alkaloids production. *J. Appl. Sci. Res.* **5**: 1826-1831.

Woo HS, Park JM & Yang JW. 1998. Production of scopolamine by normal root culture of *H. niger*. *Biotechnol. Lett*. **17**: 921-926.

Yamada Y & Hashimoto T. 1982. Production of tropane alkaloids in cultured cells of *Hyoscyamus niger. Plant Cell Reports* **1**: 101-103.

Zenk MH, El-Shagi H, Arens H, Stockigt J, Weiler EW & Dues B. 1977. Formation of indole alkaloids serpentine and ajmalicine in cell suspension cultures of *Catharanthus roseus.* In: Barz W. Reinhard E & Zenk MH (eds.). Plant Tissue Culture and its Biotechnological Applications, pp. 27-44, Springer, Berlin.

Zhao R & Verpoorte R. 2007. Manipulating indole alkaloid production by *Catharanthus roseus* cell cultures in bioreactors: from biochemical processing to metabolic engineering. *Phytochem. Rev.* **6**: 435-457.

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Povećanje sadržaja alkaloida u kulturama kalusa *Catharanthus roseus*

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Slab prinos i visoke cene na tržistu farmaceutski važnog alkaloida iz biljke *Catharanthus roseus* (L.) G. Don pre svega vinkristina, vinblastina i ajmalicina postavljaju zahtev za postizanjem boljih rezultata u alternativnoj proizvodnji ovih alkaloida putem kulture tkiva. Kalus je razvijen na MS podlozi Murashige-Skoog u koju su dodati auksini i citokinini. NA različitim koncentracijama ovih regulatora rastenja sadržaj alkaloida varira. Kombinovanje obe grupe regulatora rastenja poboljšava produkciju kalusa poreklom iz lista i sadržaj alkaloida u kalusu. Najbolja produkcija alkaloida zabeležena je na 0.50 mg/l 2,4-D i 1.0 mg/l BA, u odnosu na druge testirane kombinacije. Osim regulatora rastenja, jačina medijuma, različiti izvori ugljenika i njihove koncentracije znatno utiču na produkciju kalusa i sadržaj alkaloida. Polovina bazalnog MS medijuma u koji su dodati 2,4-D i BA (0.5mg/l i 1.0 mg/l) i 6% saharoze se pokazao kao najbolji za visoku biomasu i povećan sadržaj alkaloida u kalusu *C. roseus*.

Ključne reči: *Catharanthus roseus*, kultura kalusa, biomasa, alkaloid.