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Short Communication

Effect of Plant Growth Regulators on Production of Vindoline in the Callus of *Catharanthus roseus*

Aneela Fatima, Muhammad Zia and M. Fayyaz Chaudhary*
Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

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Abstract. Callus of *Catharanthus roseus* cultured from leaf explant was proliferated on MS medium supplemented with different plant growth regulators either individually or in combination. One month old callus was used for extraction and quantification of vindoline in the callus. The highest amount of vindoline (34.49 µg/g) was found in the callus sub-cultured on MS medium supplemented with 6-benzyl-amino purine (BA, 5 mg/l) while 26.82 µg/g vindoline was observed in callus produced on BA and Kinetin (Kin) at 1.0 mg/l of each in combination. The callus produced at different concentration of auxins failed to produce singly detectable concentration of vindoline. It is concluded that cytokinins supplemented in MS medium enhance the production of vindoline in the callus of *Catharanthus roseus*.

Keywords: *Catharanthus roseus*, callus, vindoline, kinetin, auxins

Besides vincristine and vinblastine, around a hundred other indole alkaloids of medicinal properties have been isolated from *Catharanthus roseus* (van Der Heijden *et al.*, 1992). The alkaloid serpentine is a tranquilizer, while catharanthine and vindoline lower blood sugar level, thus reducing the symptoms of diabetes (Goodman and Gilman, 1990). Ajmalicine is used in the treatment of hypertension and obstructive circulatory diseases (Beck, 1984). Lately, the semi-synthetic drug, vindensine (a vinblastine analogue) has been introduced for the treatment of melanoma and lung cancer (Budavari, 1989).

Attempts to improve the yield of such bioactive compounds through cell and tissue culture techniques and metabolic engineering have led to intensive studies on the indole alkaloid biosynthesis and its regulation (Verpoorte *et al.*, 1999 and 1997; Meijer *et al.*, 1993). However, production of secondary metabolites in these undifferentiated plant material is mostly lower than that in the fully developed plant. The production level can be increased by optimization of the media composition or by elicitation (Verpoorte *et al.*, 1997).

Several studies have been carried out on the influence of the concentration of various growth regulators, especially auxins, on the alkaloid production by *C. roseus* cultures (Ganapathi and Kargi, 1990; van Der Heijden *et al.*, 1989). The present study was conducted to examine the effect of plant growth regulators on the production of vindoline in the callus of *C. roseus*.

For callus production, the leaf explant from ornamentally grown plants was cultured on Murashige and Skoog (MS) (1962) medium supplemented with 1.0 mg/l, 2, 4-D in combination with 0.5 mg/l naphthaline acetic acid (NAA) for callus induction.

*Author for correspondence; E-mail: fayyazchaudhary@yahoo.com

The methanolic extract of plant leaves contains 85.35 µg/g vindoline while stem contains only 10.29 µg/g (Table 1). The results were consistent with the previous experiment of Furmanowa *et al.* (1994) who found that vindoline and catharanthine were main alkaloids in the leaves of *C. roseus* but vindoline was always dominant.

Table 1. Presence of vindoline in *Catharanthus roseus* plant

Plant part	Description	Fresh weight	Vindoline µg/g
Flower petals	Whitish	1 g	-
Stem	Green	1 g	10.29
Leaves	Lush green	1 g	85.35

Callus of *C. roseus* can be a source of indole alkaloids. Plant growth regulators significantly ($p<0.05$) influenced the biosynthesis of vindoline depending on type and concentration in the medium. The callus produced through different hormones, had two margins, green and white. Level of greenness part also affected vindoline production because vindoline is present in photosynthetic parts of plants and so on in callus. Loyola-Vargas *et al.* (1986), observed vinblastine and vincristine in white and green lines of callus of *C. roseus* during testing of the effect of various growth regulators on induction of green callus in 3-year-old cell lines. The green line had approx. twice as much of the above mentioned alkaloids as the white one. Vindoline and catharanthine, (monomeric alkaloids) are precursor for vincristin and vinblastin (dimeric alkaloids) as reported by Verpoorte *et al.* (1997).

Cytokinin at different concentrations had different effect on vindoline production in the callus individually or in combination (Table 2). Decendit *et al.* (1992) reported that cytokinins are able to amplify the increase in alkaloid production due to removal of auxins from the culture medium of non-tumourous *C. roseus* cell lines. Largest amount of vindolin i.e. 34.49 µg/g was produced by the use of BA 5.0 mg/l whereas, Zeatin (Z) 2 mg/l produced 29.58 µg/g vindoline. Kin in combination with NAA had more effect on vindoline production than individually. Miura *et al.* (1987) found that callus of *C. roseus* grown on MS medium, supplemented with NAA and Kin, in dark, contained vinblastine. Effect of NAA on vindoline production may be due to better callus growth on this growth regulator. Production of vindoline on cytokinin is due to callus texture; the produced callus was light green or more part of callus was greenish while white margins were also present.

Vindoline production was very low at different concentrations of auxins. At 1.0 mg/l IBA (indole butyric acid) and 2.0 mg/l IAA (indole acetic acid), 19.15 µg/g and 18.46 µg/g vindoline was produced, respectively. In both the cases, the produced callus was light green and soft. Zenk *et al.* (1977) reported that addition of IAA resulted in the highest biomass as well as the highest yield of alkaloid in *C. roseus*. Addition of ABA to suspension culture of *C. roseus* stimulated intracellular accumulation of catharanthine and ajmalicine (Smith *et al.*, 1987). Vindoline was not produced by the two concentrations of 2,4-D; same result was obtained by high concentration of GA_3 (gibbrellic acid). Zenk *et al.*, (1977) reported that addition of NAA and GA_3 suppressed alkaloid formation in *C. roseus* while Knobloch and Berlin (1980) reported that 2, 4-D played inhibitory role on alkaloid accumulation in *C. roseus*. Naaranlahti *et al.* (1989) also reported detection of vindoline in newly induced calli and with high production of vindoline in large amount by the cell suspension cultures of *C. roseus*.

Present study reveals that vindoline can be produced in the callus of *C. roseus* under the influence of plant growth

Table 2. Effect of different growth regulators on production of vindoline in *Catharanthus roseus* callus

Hormone conc. (mg/l)	Callus description	Callogenic responses after 30 days	Vindoline* (µg/g)
1.0 IBA	yellowish green, soft	+ + +	19.15 ^{DE}
2.0 IBA	greenish brown with white spots	+ + +	4.6 ^{FG}
1.0 NAA	yellowish green, compact in centre and soft at margins and also whitish	+ + +	7.21 ^{EF}
2.0 NAA	soft and friable callus, pale green	+ + +	7.9 ^{E}
1.0 IAA	whitish green, looks hard and compact	+ + +	6.31 ^{F}
2.0 IAA	light green, soft friable	+ + + +	18.46 ^{DEF}
1.0 2-4, D	yellowish green and soft friable	+ + + +	-
2.0 2-4,D	yellowish green, soft and friable	+ + + +	-
2.0 BA	green friable with white margins	+ + +	7.26 ^{EF}
5.0 BA	green and hard clumps	+ + +	34.49 ^{A}
2.0 Kin	compact, hard, green	+ + + +	8.31 ^{EF}
5.0 Kin	compact green with white spots	+ + + +	18.10 ^{DEF}
1.0 Z	yellowish green and hard	+ + +	18.08 ^{DEF}
2.0 Z	light green in centre and whitish green margins, hard	+ + + +	29.58 ^{AB}
2.0 GA_3	pale yellow, soft, friable	+ + +	24.47 ^{C}
5.0 GA_3	yellowish brown and soft	+ + +	-
5.0 BA + 2.0 NAA	light green with yellow margins and friable	+ + + +	Traces
6.0 BA + 2.0 NAA	yellowish green, friable and soft	+ + + +	25.09 ^{BC}
5.0 Kin + 2.0 NAA	yellowish green, soft and friable	+ + + +	24.20 ^{CD}
6.0 Kin + 2.0 NAA	yellowish green, soft and friable	+ + + +	25.11 ^{BC}
1.0 BA + 1.0 Kin	light green with white margins, little hard	+ + + +	0.72 ^{G}
2.0 BA + 2.0 Kin	dark brown in centre with light green margins and hard	+ + +	26.82 ^{B}
MS without any regulator	brown	+	Traces

* = results were significant at $P<0.05$; ^{ABCDEFG} = LSD values; IBA = indole butyric acid; NAA = naphthalene acetic acid; IAA = indole acetic acid; BA = 6-butyl amino purine; Kin = kinetin; Z = zeatin; GA_3 = gibberellic acid; MS = Murashige and Skoog medium

regulators. But it is necessary to get primary metabolites from initial stages of culture because after some time, these primary metabolites are converted into secondary metabolites or are rapidly metabolised. Thus the time for culture as well as the texture of callus with effecters in the medium are important factors for production of metabolites in the callus either on solid medium or in suspension culture.

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