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Effect of plant growth regulators on relative growth rate, multiple shoot formation and alkaloid accumulation in *Catharanthus roseus* (L.) G. Don

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Abstract

The effect of plant growth regulators on callus induction and growth, multiple shoot formation from juveline explants and alkaloid accumulation in cultures of *Catharanthus roseus* was studied. Hypocotyl explant were the best for callus induction both under light and dark. A combination of NAA (1.0/2.0 mg/l) with BAP (1.5 mg/l) in MS basal medium significantly improved the growth of hypocotyl derived calli both under light and dark conditions. Cotyledonary leaf explants exhibited maximum shoot formation. Detection of leurocristine (vincristine) in the remaining calli after shoot formation raises the possibility of producing pharmaceutically important dimeric anticancerous alkaloids in the cultures.

Key words : *Catharanthus roseus*, callus induction, multiple shoots, relative growth rate, alkaloids

Introduction

Catharanthus roseus (L.) G. Don (*Sadabahar*, family apocynaceae) has tremendous medicinal value because of the presence of cytotoxic alkaloids in different plant parts. The extraction of alkaloids from intact plants poses several problems due to seasonal variation, qualitative and quantitative inconsistency of the product and cost. Manipulation of the plant cell culture environment and culture media can affect the rates of both cell growth and accumulation of secondary metabolites. The low yield of highly valued antileukemic alkaloids in the plant [1] were the major motives to study the possibility of their production in cell or tissue cultures. Datta and Srivastava [2] detected vinblastine (bisindole-alkaloid) in leaf organ cultures and callus lines established from different explants of *C. roseus*. However, vinblastine yield in multiple shoots raised from callus was found to be equal to that of *in vitro* seedlings of same age. High biomass yield resulted in an increase of alkaloid accumulation as reported by Courtois and Guern [3] and Scragg *et al.* [4]. Plant growth regulators and light play a significant role in growth and production of alkaloids in plants [1]. In the present investigation we report the effect of plant growth regulators on callus induction, callus growth both under light and dark, multiple shoot formation and alkaloid accumulation in the cultures.

Materials and methods

The seeds were procured from the section of Medicinal and Aromatic Plants, Department of Plant Breeding, CCS Haryana Agricultural University, Hisar. The surface sterilized seeds (70% ethanol for 1 min. and 1% sodium hypochlorite for 20 min.) of *C. roseus* were germinated on medium containing 30 g/l sucrose and 8 g/l agar at 25 ± 1°C under dark. Explants (3-4 mm) from hypocotyl (H), epicotyl (E), cotyledonary leaves (CL) and roots (R) of 7-10 days old seedlings were cultured on Murashige and Skoog [5] basal medium supplemented with plant growth regulators (Fig.1). The calli were sub-cultured on selected media. Fresh and dry weights of calli were recorded after 15, 30 and 45 days of subculture (Tables 1, 2) under light and dark conditions. The relative growth rate (RGR) was calculated as per Jyoti *et al.* [6] as under :

$$\frac{\text{Change in weight}}{\text{Initial weight}} \times \frac{1}{\text{t (timeinterva)}}$$

The effect of NAA with BAP/kinetin to induce direct multiple shoot formation from cotyledonary leaves was also studied (Table 3). The data were analysed statistically by completely randomised design. All the cultures were assessed for the presence of alkaloids [7].

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Results and discussion

Callus could be successfully induced in hypocotyl, epicotyl, cotyledonary leaf and root explants. The

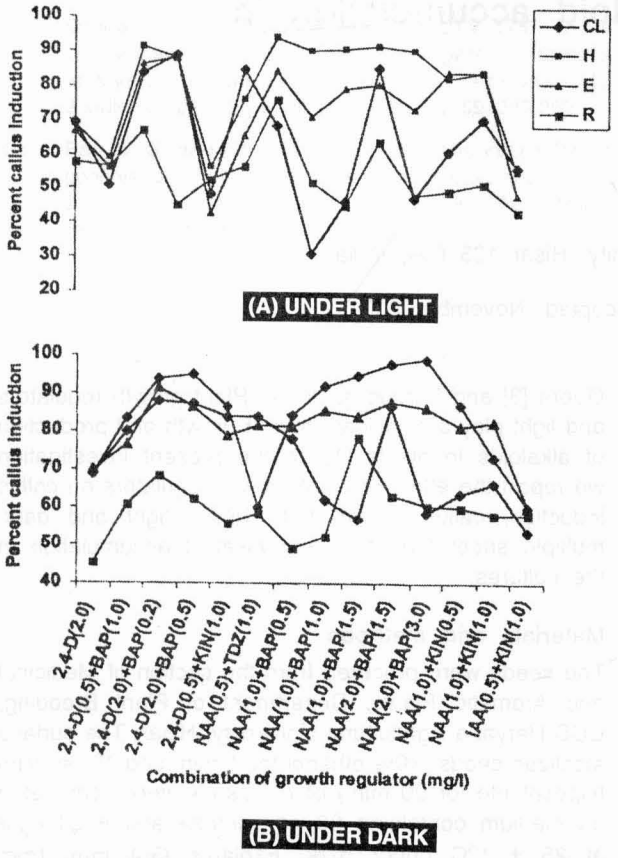

Fig. 1. Effect of plant growth regulators on percentage of callus induction from different explants of *Catharanthus roseus* under (A) light (B) dark

hypocotyl was found to be the best for callus induction under both light (16h photoperiod) and dark conditions (Fig. 1). In order to assess growth characteristics the hypocotyl derived calli (Fig. 2) were further cultured on some selected combinations of growth regulators (Tables 1, 2). In general, increased fresh weight and RGR of hypocotyl derived calli were recorded on MS medium with NAA (1.0 and 2.0 mg/l) + BAP (1.5 mg/l), whereas these were the lowest on MS medium supplemented with 2, 4-D (2.0 mg/l) + BAP (0.2 mg/l). There was a

Table 1. Effect of plant growth regulators in MS basal medium on fresh weight (g) and relative growth rate of hypocotyl derived calli of *Catharanthus roseus* under light and dark

Combination of growth regulators (mg/l)	Fresh weight of calli in g (Relative growth rate of calli per day) Days after inoculation					
	15		30		45	
	Light	Dark	Light	Dark	Light	Dark
2,4-D (2.0) + BAP (0.2)	4.75 (0.08)	3.57 (0.08)	13.25 (0.18)	13.47 (0.26)	16.67 (0.16)	16.22 (0.21)
NAA (1.0) + BAP (0.5)	12.17 (0.59)	07.37 (0.21)	24.93 (0.65)	16.53 (0.28)	29.34 (0.51)	20.42 (0.18)
NAA (1.0) + BAP (1.0)	12.61 (0.58)	06.33 (0.32)	26.47 (0.60)	12.60 (0.41)	31.22 (0.52)	15.68 (0.35)
NAA (1.0) + BAP (1.5)	19.13 (0.64)	12.97 (0.42)	46.49 (0.83)	41.86 (0.75)	47.47 (0.57)	42.78 (0.51)
NAA (2.0) + BAP (1.5)	18.64 (0.83)	14.18 (0.45)	42.08 (0.97)	47.86 (0.92)	44.78 (0.69)	50.23 (0.61)
NAA (1.0) + Kn (0.5)	05.80 (0.30)	08.56 (0.26)	17.23 (0.53)	23.29 (0.45)	22.97 (0.47)	27.76 (0.36)
NAA (1.0) + Kn (1.0)	06.17 (0.23)	07.70 (0.42)	14.44 (0.31)	16.08 (0.47)	18.09 (0.27)	20.08 (0.40)
Mean	11.32 (0.46)	8.67 (0.32)	26.41 (0.58)	24.53 (0.53)	30.08 (0.45)	27.59 (0.37)
CD at 5%	02.49 (0.16)	04.14 (0.19)	09.65 (0.27)	02.35 (0.24)	06.84 (0.16)	05.36 (0.19)

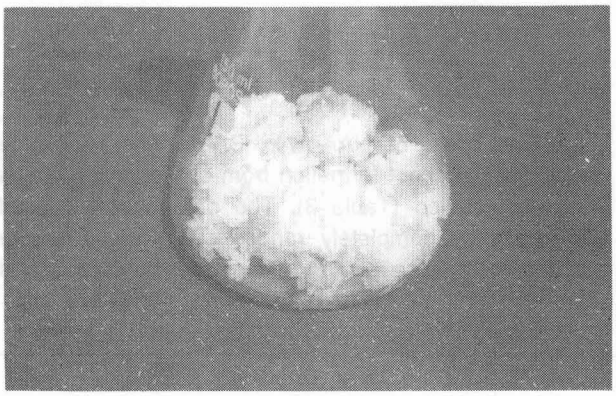

Fig. 2. Hypocotyl derived calli of *Catharanthus roseus*

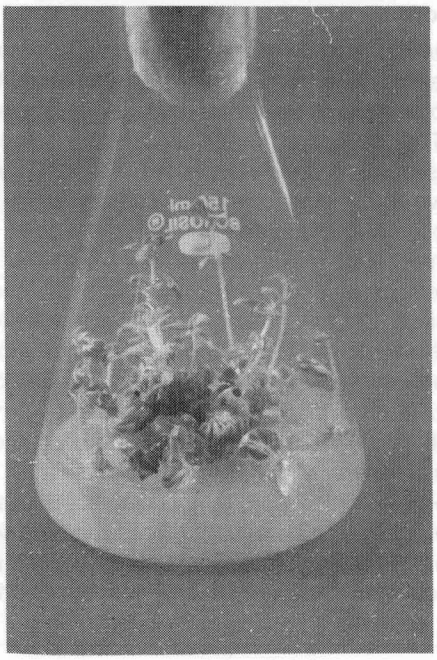

Fig. 3. Multiple shoots from cotyledonary leaves of *Catharanthus roseus*

Table 2. Effect of plant growth regulators in MS basal medium on dry weight (g) and relative growth rate of hypocotyl derived calli of *Catharanthus roseus* under light and dark

Combination of plant growth regulators (mgl)	Dry weight of calli in g (Relative growth rate of calli per day) Days after inoculation					
	15		30		45	
	Light	Dark	Light	Dark	Light	Dark
2,4-D(2.0) + BAP (0.2)	0.24 (0.09)	0.18 (0.09)	0.62 (0.18)	0.58 (0.22)	0.78 (0.16)	0.76 (0.20)
NAA (1.0) + BAP (0.5)	0.36 (0.61)	0.21 (0.19)	0.72 (0.65)	0.51 (0.27)	0.91 (0.55)	0.74 (0.28)
NAA (1.0) + BAP (1.0)	0.48 (0.59)	0.17 (0.24)	1.00 (0.64)	0.60 (0.51)	1.18 (0.52)	0.69 (0.41)
NAA (1.0) + BAP (1.5)	0.50 (0.71)	0.35 (0.43)	1.02 (0.77)	1.20 (0.80)	1.08 (0.54)	1.22 (0.55)
NAA (2.0) + BAP (1.5)	0.45 (0.79)	0.45 (0.52)	1.13 (1.05)	1.17 (0.80)	1.15 (0.72)	1.26 (0.57)
NAA (1.0) + Kn (0.5)	0.30 (0.30)	0.48 (0.29)	0.90 (0.52)	1.32 (0.45)	1.20 (0.47)	1.57 (0.37)
NAA (1.0) + Kn (1.0)	0.24 (0.24)	0.43 (0.42)	0.56 (0.32)	0.90 (0.48)	0.71 (0.28)	1.12 (0.40)
Mean	0.37 (0.47)	0.32 (0.31)	0.85 (0.59)	0.89 (0.50)	1.00 (0.46)	1.05 (0.39)
CD at 5%	0.09 (0.16)	0.13 (0.16)	0.31 (0.25)	0.24 (0.27)	0.18 (0.17)	0.25 (0.21)

Figures written in parenthesis are RGR values

Table 3. Effect of plant growth regulators in MS basal medium on multiple shoot formation from cotyledonary leaves of *C. roseus*

Combination of plant growth regulators (mg/l)	% morphogenetic response	No. of shoots per explant
BAP (0.01)	62.50	2-3
BAP(0.1)	73.33	2-7
BAP(1.0)	90.00	7-11
BAP (0.5) + NAA (1.0)	19.29	2-5
BAP (0.1) + NAA (0.01)	80.00	2-8
BAP (1.5) + NAA (1.0)	31.25	2-7
BAP (3.0) + NAA (2.0)	37.50	2-9
BAP (7.0) + NAA (1.0)	80.00	2-9
BAP (2.0) + NAA (0.01) + CH (1.0)	80.00	2-9
Kn(1.0) + NAA (1.0)	04.76	2-5
Kn (2.5) + NAA (0.05)	73.33	9-13

CH = Casein hydrolysate
NAA = α-Naphthalene acetic acid
Kn = Kinetin
BAP = 6-Benzyl amino purine

significant increase in fresh weight over days to callus induction. RGR was in increasing order from 15 to 30 days but further it decreased both under light and dark conditions (Table 1). Mean dry weight increased gradually from 15 to 45 days of inoculation (0.37, 0.32 to 1.00, 1.05), whereas RGR increased (0.47, 0.31 to 0.59, 0.50) only upto 30 days of inoculation under both the conditions (Table 2). It appeared that use of NAA with BAP/kinetin instead of 2, 4-D with BAP/kinetin was favourable for initial RGR. Though the growth of calli was observed upto 45 days of culturing but RGR after 30 days slowed down under both light and dark conditions. Biomass production rate can be influenced by conditioning factors in the medium [8]. Plant growth regulators affect growth and the effects vary with the type and the quantity of phytohormone applied [1].

The regeneration capacity of cotyledonary leaves was determined by culturing them on MS medium containing different concentrations of BAP or NAA with BAP/kinetin (Table 3). Differentiation of nodular structures at the cut ends of explants subsequently formed multiple shoots (Fig. 3) within 20-25 days of inoculation. MS medium supplemented with 1.0 mg/l BAP was found to be the best for shoot formation (90%) while a combination of 2.5 mg/l kinetin and 0.05 mg/l NAA gave maximum range of shoots (9-13) from cotyledonary leaves. Earlier Yuan *et al.* [9] reported stimulation of multiple shoots from shoot explants on MS medium supplemented with BA (7.0 mg/l) and NAA (1.0 mg/l).

An attempt was also made to detect and identify alkaloids by their chromogenic reactions and Rf values [7]. Serpentine, ajmalicine, catharanthine were some of the main alkaloids detected in hypocotyl derived calli. In regenerated shoots and remaining calli after shoot regeneration serpentine, vidolinine, leurosine, ajmalicine, leurocristine, catharanthine, catharicine were identified. Preconditions like differentiation and maturation of the tissues were required for the more complex dimeric alkaloids as reported by Datta and Srivastava [2].

In the present studies leurocristine (vincristine) a dimeric alkaloid in remaining calli indicates the presence of patches of differentiated tissues.

It can be concluded from the present studies that MS medium containing NAA (2.0/1.0 mg/l) with BAP (3.0/0.5 mg/l) was found to be the best for callus induction. A combination of NAA (1.0/2.0 mg/l) with BAP (1.5 mg/l) improved the callus growth under both light and dark conditions. Multiple shoots were directly induced with high frequency from cotyledonary leaves in the presence of BAP alone or NAA with BAP/kinetin. Leurocristine an important anticancerous dimeric alkaloid could also be detected in the remaining calli after shoot regeneration. As a result of such an analysis productivity of alkaloids could further be improved by optimization of medium composition or over expression of key genes in the terpenoid indole alkaloid biosynthetic pathway as suggested by Moreno *et al.* [1]

References

1. **Moreno R. H. P., Heijden R. V. and Verpoorte R.** 1995. Cell and tissue cultures of *Catharanthus roseus* : A literature survey-II updating from 1988 to 1993. Plant Cell, Tissue and Organ Culture, **42**: 1-25.
2. **Datta A. and Srivastava P. S.** 1997. Variation in vinblastine production of *Catharanthus roseus* during *in vivo* and *in vitro* differentiation. Phytochemistry, **46**: 135-137.
3. **Courtois D. and Guern J.** 1980. Temperature response of *Catharanthus roseus* cultivated in liquid medium. Plant Sci. Lett., **17**: 473-482.
4. **Scragg A. H., Ashfon S., York A., Stepan-Sarkissian G. and Grey D.** 1990. Growth of *C. roseus* suspension for maximum biomass and alkaloid accumulation. Enzyme Microb. Technol., **12**: 292-298.
5. **Murashige T. and Skoog F.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., **15**: 473-479.
6. **Jyoti M., Babbar S. and Varghese T. M.** 1995. Effect of cadmium on the viability and growth of callus of *Solanum melongena* L. var. H-7. Indian J. Plant Physiol., **38**: 244-246.
7. **Svendsen A. B. and Verpoorte R.** 1983. Chromatography of alkaloids, Part A: Thin layer chromatography. Chromatograph library 23 a. Amsterdam, Oxford, Tokyo : Elsevier.
8. **Ten Hoopen H. J. G., Van Gulik W. M., Schlatmann J. E., Moreno P. R. H., Vinke J. L. and Verpoorte R.** 1994. Ajmalicine production by cell cultures of *Cataranthus roseus* : From shake flask to bioreactor. Plant Cell. Tiss. and Org. Cult., **38**: 85-91.
9. **Yuan Y. J., Hu T. T. and Yang Y. M.** 1994. Effect of auxins and cytokinins on formation of *Catharathus roseus* G. Don. multiple shoots. Plant Cell, Tiss. & Org. Cult. (Netherlands), **37**: 193-196.