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Effect of Plant Growth Regulators on Periwinkle (*Catharanthus roseus* L.) G. Don Using Leaves, Hypocotyls and Epicotyl as Explants

KANTA RANI[1]*, RENU SINGH[2], PUSHPA KHARB[2] AND MANJU YADAV[3]

[1]*Department of Biotechnology & Applied Sciences, NCIT College, Israna, Panipat*
[2]*Department of Biotechnology and Molecular Biology, CCS HAU, Hisar*
and [3]*GJU & ST, Hisar 125001, India*
E-mail : kantadahiya@gmail.com
**Correspondence*

Abstract

The effect of plant growth regulators on callus induction using different explants, to regenerate shoot/root from different explants and from calli in cultures of *Catharanthus roseus* (L.) G. Don was studied. Total of 19 shoot regeneration media were used, out of these three were further used to assess the regeneration response and for root regeneration four media were tested supplemented with different concentration of IBA. Based on the results of this study, for shoot proliferation, MS basal medium supplemented with BAP (1.5 mg/liter) + NAA (1.0 mg/liter) was the best followed by MS medium fortified with BAP (3.0 mg/liter) + NAA (4.0 mg/liter) while half strength MS medium supplemented with IBA (2.5 mg/liter) + NAA (0.5 mg/liter) gave best rooting response with quality roots. Besides, shoot regeneration, response from calli both under light and dark conditions was also studied and the media which gave best results was BAP (1.5 mg/liter) + NAA (1.0 mg/liter).

Key words : Auxins, Callus induction, *Catharanthus roseus*, Cytokinins, Shoot induction, Root induction.

Catharanthus roseus (L.) G. Don. (commonly known as sadabahar or periwinkle, family Apocynaceae) gained commercial importance because of its alkaloids in different plant pars. The alkaloids like antineoplastin dimeric vinblastin and vincristine are mainly present in aerial parts, whereas ajmalcine, vinceine, vincamine, raubasin and reserpine are present in roots and basal stem. The dimeric indole alkaloids from *C. roseus* are used for treatment of various human cancers. The extraction of chemicals from intact plants has several problems due to seasonal variation, qualitative and quantitative inconsistency of the product and cost. Micropropagation of different explants using plant tissue culture technique can help, solving these problems. Mujib et al. (1) reported high frequency of plant regeneration in *C. roseus* from shoot tip explant on MS basal medium supplemented with BAP. Sehrawat et al. (2) observed good callus induction from culturing hypocotyls explants under both light and dark condition. Micropropagation studies and effect of various growth regulators were studied here using shoot tip, hypocotyls and leaf explants with and without intervening callus phase in both light and dark conditions.

Methods

The seeds were procured from medicinal aromatic and under-utilized plant section, Department of Plant Breeding, CCS Haryana Agricultural University, Hisar. Seeds were sown in pots and kept in green house. Explants of about 3—4 mm (leaf, shoot tip and hypocotyls) were excised from 10—15 days old seedlings grown in green house. They were washed with tap water containing few drops of teepol, treated with fungicide solution (Bavistin 0.4% wt/vol) for 30 min followed by 4 to 5 times rinsing with distilled water. Different growth regulators namely indole-3-acetic acid (IAA), indolebutyric acid (IBA), naphthalene acetic acid (NAA), 2, 4-dicholophenoxyacetic acid (2,4-D), kinetin, 6-benzylaminopurine (BAP), were tested with full and half strength Murashige and Skoog (MS) basic medium (3). Effectiveness of MS basal medium was also reported earlier in other crops. Standard procedure was followed for the preparation of media (4). The pH of the media was adjusted to 5.7 prior to ster-

Table 1. Days to callus induction from different explants under light and dark conditions in *C. roseus* (L.) G. Don.

Medium	Leaf		Hypocotyl		Epicotyl	
Basal salt MS	Light	Dark	Light	Dark	Light	Dark
MS	–	–	–	–	–	–
BAP (2.0)	19	19	18	18	19	19
BAP (0.1)+2,4-D (2.0)	22	19	19	16	12	12
BAP (0.5)+2, 4-D (2.0)	18	18	17	17	15	14
BAP (1.0)+NAA (1.0)	18	17	11	10	16	17
BAP (1.5)+NAA (1.0)	19	18	17	17	17	16
BAP (1.5)+NAA (2.0)	20	19	19	19	18	17
BAP (2.0)+NAA (1.0)	21	20	17	17	17	15
BAP (3.0)+NAA (2.0)	13	12	16	15	14	14
BAP (3.0)+NAA (4.0)	21	19	18	17	17	17
BAP (1.5)+IAA (0.2)	19	19	17	16	16	16
BAP (1.5)+IAA (0.5)	17	17	15	14	15	16
BAP (1.5)+IAA (1.0)	20	18	18	18	17	16
2, 4-D (2.0)	17	15	–	–	–	–
Kinetin (0.5)+ 2, 4-D (2.0)	15	15	14	14	15	14
Kinetin (1.0)+ NAA (1.0)	14	14	13	13	14	14

ilization done at 15 lbs/in^2 for 15 min. All media were solidified with 8g/liter agar. Cultures were maintained at 26 C with 16 h light (light intensity 50μ^2/gms)/8 h darkness. The data of all the experiments conducted under present investigations were presented as mean of the three repeats. Data were analyzed statistically by completely randomized design using one-way analysis of variance (ANOVA).

Results and Discussion

In the *in vitro* studies, the application of growth regulators in the medium disturbs the internal polarity and causes dedifferentiation which results into callus formation. Rohtas (5) found that MS medium supplemented with BAP alone or along with NAA was the best for callus induction.

Callus Induction on Media Supplemented with Different Growth Regulators

Range of days to callus induction for all the explants inoculated on all the media under light and dark conditions was 10—22 days (Table 1; Fig. 1A). With regard to individual explants, days to callus induction from leaf (12—22 days), hypocotyl (10—19 days) and epicotyl (12—19 days) were almost in the same range under both light and dark conditions. Out of various explants used, callus induction was found to the earliest (10—11 days) in hypocotyl explants on media supplemented with MS + BAP (1.5 mg/liter) + NAA (1.0 mg/liter) under light and dark conditions. Similar results were observed by Ramavat et al. (6) in *C. roseus*. Callus induction took maximum (22 days)

Table 2. Callus induction response from leaf explants under light and dark conditions in *C. roseus* (L.) G. Don. **CD = 1.318 (Light); CD=1.434 (Dark); ***CD=1.544 (Light); CD=1.548 (Dark); ****CD=1.351 (Light); CD=1.212 (Dark); CV=1.472, CV=1.561, CV=1.517, CV=1.490, CV=1.515, CV=1.328.

Medium	Leaf**		Hypocotyl***		Epicotyl****
	Mean percent response (Mean ± SE)*	Mean percent response (Mean ± SE)*	Mean percent response (Mean ± SE)*	Mean percent response (Mean ± SE)*	Mean percent response (Mean ± SE)*
Basal salt MS	Light	Dark	Light	Dark	Light
MS	0 (4.05 ± 0.01)	0 (4.05 ± 0.01)	0 (4.05 ± 0.01)	0 (4.05 ± 0.01)	0 (4.05 ± 0.01)
BAP (2.0)	68.4 (56.08 ± 0.01)	69.4 (56.75 ± 0.33)	78.0 (62.40 ± 0.19)	81.8 (65.09 ± 0.01)	64.1 (53.51 ± 0.19)
BAP (0.1) + 2, 4-D (2.0)	70.2 (57.25 ± 0.17)	73.6 (59.36 ± 0.09)	84.4 (67.16 ± 0.21)	85.4 (67.97 ± 0.19)	87.9 (70.10 ± 0.58)
BAP (0.5) + 2, 4-D (2.0)	64.9 (53.95 ± 0.06)	68.4 (56.08 ± 0.01)	88.9 (70.89 ± 0.56)	90.0 (72.02 ± 0.01)	76.4 (61.26 ± 0.55)

Table 2. Continued.

Medium	Leaf**		Hypocotyl***		Epicotyl****
Basal salt MS	Mean percent response (Mean ± SE)* Light	Mean percent response (Mean ± SE)* Dark	Mean percent response (Mean ± SE)* Light	Mean percent response (Mean ± SE)* Dark	Mean percent response (Mean ± SE)* Light
BAP (1.0) + NAA (1.0)	80.0 (63.78 ± 0.66)	80.9 (64.44 ± 0.66)	92.2 (74.41 ± 0.88)	94.9 (77.64 ± 0.40)	80.6 (64.24 ± 0.58)
BAP (1.5) + NAA (1.0)	74.6 (60.05 ± 0.60)	78.9 (62.98 ± 0.01)	89.2 (71.32 ± 0.35)	89.6 (71.67 ± 0.35)	82.0 (65.28 ± 0.51)
BAP (1.5) + NAA (2.0)	70.6 (57.46 ± 0.001)	72.3 (58.59 ± 0.60)	88.7 (70.77 ± 0.62)	89.5 (71.62 ±1.09)	84.1 (66.88 ± 0.64)
BAP (2.0) + NAA (1.0)	72.5 (58.70 ± 0.63)	77.8 (62.22 ± 0.48)	89.9 (71.97 ± 1.04)	91.2 (73.36 ± 1.34)	73.9 (59.56 ± 0.37)
BAP (3.0) + NAA (2.0)	90.2 (72.25 ± 0.81)	92.2 (73.90 ± 1.17)	88.0 (70.15 ± 0.01)	89.3 (71.40 ± 0.62)	78.1 (62.45 ± 0.43)
BAP (3.0) + NAA (4.0)	67.7 (55.67 ± 0.32)	68.9 (56.44 ± 0.48)	71.6 (58.12 ± 0.66)	73.7 (59.45 ± 0.01)	70.0 (57.08 ± 0.38)
BAP (1.5) + IAA (0.2)	71.4 (57.97 ± 0.29)	74.0 (59.69 ±0.31)	75.5 (60.65 ± 0.60)	76.7 (61.43 ± 0.18)	72.5 (58.70 ± 0.63)
BAP (1.5) + IAA (0.5)	76.2 (61.12 ± 0.44)	78.3 (62.59 ± 0.40)	77.5 (62.01 ± 0.51)	78.3 (62.59 ± 0.40)	75.0 (60.36 ± 0.69)
BAP (1.5) + IAA (1.0)	51.3 (46.05 ± 0.78)	53.0 (47.51 ± 0.06)	55.6 (48.48 ± 0.01)	60.6 (51.40 ± 0.53)	65.9 (54.54 ± 0.50)
2, 4-D (2.0)	68.9 (56.42 ± 0.33)	71.2 (57.87 ± 0.79)	59.4 (50.69 ± 0.50)	61.1 (51.69 ± 0.01)	0 (4.05 ± 0.01)
Kinetin (0.5) + 2,4-D (2.0)	57.0 (49.10 ± 0.39)	57.1 (49.37 ± 0.44)	83.2 (66.22 ± 0.57)	85.2 (67.76 ± 0.41)	69.0 (56.50 ± 0.30)
Kinetin (1.0) + NAA (1.0)	54.0 (47.60 ± 0.44)	55.0 (48.14 ± 0.35)	82.3 (65.48 ± 0.38)	83.1 (66.12 ± 0.51)	67.8 (55.73 ± 0.35)

in leaf explants on media MS + BAP (0.5 mg/liter) + 2, 4-D (2.0 mg/liter). Highest percentage of callus induction was obtained from hypocotyl (94.9% and 92.2%) followed by leaf (92.2 and 90.2%) under light

Table 3. Effect of different growth regulators on plant regeneration using leaf, hypocotyl and epicotyl as explants in *C. roseus* (L.) G. Don. CD=1.269, (at 5% level of significance), *Transformed value, CV=1.366.

Basal salt MS	No. of explants cultured	No. of explants responding	Mean percent response (mean ± SE)*
MS	86	22	25.6 (30.69 ± 0.37)
BAP (0.1)	94	44	46.8 (43.46 ± 0.06)
BAP (1.0)	90	58	64.3 (53.63 ± 0.40)
BAP (2.0)	80	57	71.2 (57.86 ± 0.32)
BAP (1.0)+ NAA (1.0)	94	64	68.0 (55.90 ± 0.61)
BAP (1.5) + NAA (1.0)	107	95	88.9 (71.00 ± 0.85)

Table 3. Continued.

Basal salt MS	No. of explants cultured	No. of explants responding	Mean percent response (mean ± SE)*
BAP (1.5) + NAA (2.0)	111	97	87.4 (69.62 ± 0.43)
BAP (2.0) + NAA (1.0)	114	78	68.4 (56.10 ± 0.34)
BAP (3.0) + NAA (2.0)	102	71	67.6 (55.59 ± 0.36)
BAP (1.5) + IAA (0.2)	120	85	70.8 (57.61 ± 0.64)
BAP (1.5) + IAA (0.5)	108	79	73.1 (59.08 ± 0.31)
BAP (1.5) + IAA (1.0)	110	86	78.2 (62.52 ± 0.42)
Kinetin (1.0) + NAA (1.0)	115	66	57.4 (49.53 ± 0.19)
Kinetin (2.5)+NAA (0.05)	97	59	59.8 (50.90 ± 0.14)

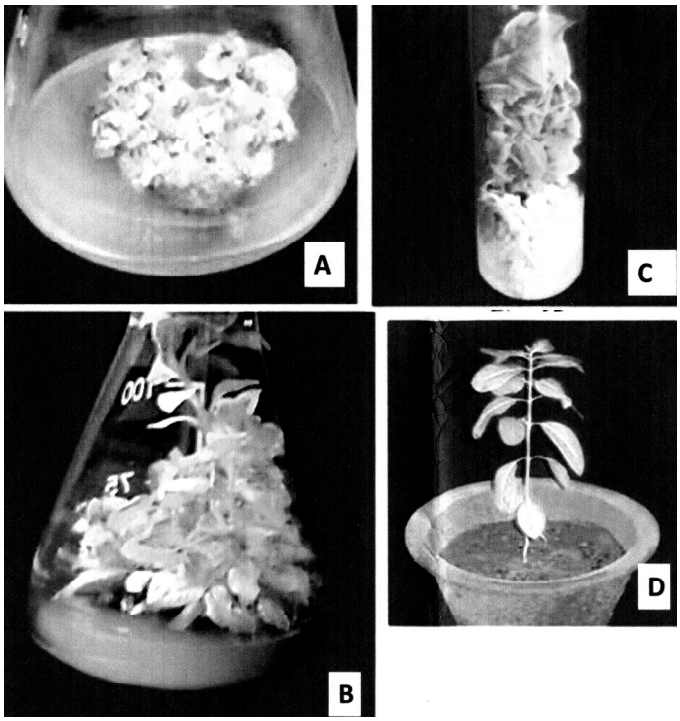

Figure 1. Effect of plant growth regulators on periw-inkle (*Catharanthus roseus* L.). (A) Initiation of callus formation; (B) Multiple shoot formation on shooting medium in *C. roseus*; (C) Regenerated roots on rooting medium; (D) Transfer of regenerated plantlets into pot.

and dark conditions, respectively.

Leaf Explants. Maximum callus induction response (90.2 and 92.2%) was obtained on medium supplemented with BAP (3.0 mg/liter) + and NAA (4.0 mg/liter) under light and dark conditions, respectively (Table 2). Minimum callus induction response (51.3 and 53.0%) was observed on medium supplemented with MS + 2, 4-D (2.0 mg/liter) under light and dark conditions, respectively. No callus formation was observed on plane medium i.e. MS with no growth regulators.

Media supplemented with NAA (1.0 mg/liter) + BAP (1.5) was observed to be more effective for callus induction as per cent callus induction response was decreased when it was replaced by IAA (1.0 mg/liter) + BAP (1.5).

Hypocotyl Explants. Highest percentage of callus induction (92.2 and 94.9%) was obtained from hypocotyl explants on medium having MS + BAP (1.5 mg/liter) + NAA (1.0 mg/liter) medium under light and dark conditions, respectively (Table 2). Minimum callus induction response (55.6 and 60.6%) was obtained on media with 2, 4-D (2.0 mg/liter) under both light and dark conditions.

Epicotyl Explant. Superiority of media with MS + BAP (0.5 mg/liter) + 2, 4-D (2.0 mg/liter) medium for epicotyl-derived callus (87.9 and 90.8%) under both light and dark conditions was observed (Table 2).

Table 4. Shoot regeneration response from calli obtained from different explants in *C. roseus* (L.) G. Don. on media supplemented with BAP and NAA. CD=1.452 (at 5% level of significance), *Transformed value, CV=1.466.

NAA (mg/l)	BAP (mg/l)	Explants	No. of shoots regenerated per calli	Mean percent response (Mean±SE)*
1.0	1.5	Hypocotyl	10—15	72.4 (38.98 ± 0.58)
2.0	3.0	Hypocotyl	10—12	55.2 (58.65 ± 0.36)
4.0	3.0	Leaf	5—7	39.1 (48.27 ± 0.21)

Table 5. Root induction response on different media in *C. roseus* (L.) G. Don. CD = 1.745 (at 5% level of significance), + = Low moderate; ++ = Moderate; *Transformed value, CV = 1.576.

MS strength	IBA (mg/l)	NAA (mg/l)	Quality of roots	Mean (%) response (Mean ± SE)*
$^1/_2$ MS	1.0	–	+	50.0 (45.27 ± 0.01)
MS	1.0	–	+	63.9 (53.36 ± 0.84)
$^1/_2$ MS	25	–	++	75.9 (60.94 ± 0.63)
$^1/_2$ MS + MS	25	0.5	+++	90.0 (72.02 ± 0.01)

Callus induction response from epicotyl explants ranged between 64.1 to 90.8% under light and dark conditions, respectively. No callus induction response was observed on MS medium without any growth regulators.

Shoot Regeneration Response from Calli Obtained from Different Explants in C. roseus

Shoot regeneration from calli was observed on different media i.e. MS + BAP (1.5 mg/liter) + NAA (1.0 mg/liter), MS + BAP (3.0 mg/liter) + NAA (2.0 mg/liter) and MS + BAP (3.0 mg/liter), 4.0 mg/liter NAA (Tables 3 and 4). Maximum (72.4%) shoot regeneration from hypocotyl derived calli was observed on BAP (1.5) + NAA (1.0) medium (Fig. 1B) and minimum (39.1%) shoot regeneration from leaf derived callus was observed on BAP (3.0) + NAA (4.0) medium.

Root Induction and Transfer of Regenerated Plantlets to Soil

The shoots obtained from callus were surgically excised under aseptic conditions and inoculated on rooting media (Table 5). Rooting was best observed (90%) when excised shoots were pre treated for two hours with liquid 1/2 MS medium containing IBA (25.0 mg/liter) and then transferred on solid MS media containing NAA (0.5 mg/liter) (Fig. 1 C). This result confirmed by the previous study by Mujib et al. (1). They also reported root induction on MS basal medium containing 0.5 mg/liter NAA. Root induction response was observed to be 63.9 and 50.0 per cent on full strength MS + IBA (1.0 mg/liter) and $^1/_2$ MS + IBA (1.0 mg/liter) media, respectively. Regenerated roots were varies in quality from hard, thick and hairy to thin, feeble with less hair.

Survival rate of regenerated plantlets on MS media supplemented with various synthetic growth regulators was observed to be 50%. The regenerated plantlets were later on transferred to pots containing F : Y : M in 1:1:1 ration. Plantlets grew vigorously in the net house (Fig. 1D).

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