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SHORT COMMUNICATION

Effects of auxin and cytokinin on callus induction in *Catharanthus roseus* (L.) G. Don

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ABSTRACT

The study was conducted to observe the effect of different concentration and combination of auxin and cytokinin towards the callus induction of *C. roseus*. Explants comprising of basal leaf with petioles of *Catharanthus roseus* were cultured onto MS media supplemented with different types and concentrations of auxins (naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic (2,4-D)) and cytokinins (benzyl amino purine (BAP), and kinetin). Calli produced from explants showed differences in response in each of the treatment combinations. Treatments with kinetin and NAA, BAP with 2,4-D (Experiment B) did not differ significantly. Treatment with 3.0 mg L^{-1} BAP + 3.0 mg L^{-1} NAA (Experiment C) gave the highest dry weight (2.776 g) suggesting an optimum level of combination for callus induction.

Keywords: *Catharanthus roseus* (L.) G. Don, callus induction, auxin, cytokinin

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1 Introduction

The species *Catharanthus roseus* has been extensively studied because of its valuable secondary metabolites having anti-cancer properties (Mukherjee et al., 2001; Aslam et al., 2010). These compounds have been reported to be in high demand in the pharmacological industry, but exist in minute quantities, making them extremely expensive (Suttipanta et al., 2011). Plant tissue culture offers the potential of producing cells, callus, tissue, organs or whole plants in a conducive and controlled environment for their subsequent use in the production of secondary metabolites. Driven by the fact that extensive research can lead to endangering the plant population, scientists have come out with various research on the ideal condition for callus initiation and plant regeneration. One of the methods was manipulation of nutrient and plant growth hormone. The present study aims at developing a protocol for the development of callus in *C. roseus* utilizing auxins and cytokinins.

2 Materials and Methods

Sterilized leaf petioles of *C. roseus* previously raised in a rain-sheltered environment were cultured onto MS media supplemented with different types and concen-

trations of plant growth regulators (PGRs) of auxins and cytokinins, *viz*. (A) kinetin + naphthalene acetic acid (NAA), (B) benzyl amino purine (BAP) + 2,4-dichlorophenoxy acetic (2,4-D), and (C) BAP + NAA. The concentrations of PGRs (auxins and cytokinins) used were each 0, 1, 2 and 3 mg L^{-1}, respectively. Each treatment combination had ten replications with one explant in each replication.

Each set of treatments was repeated three times. The cultures were incubated in a culture room, maintained at 24 ± 2 °C for 16 hour-photoperiod under fluorescent light of 2000 - 3000 lux intensity. Callus formation was evaluated at six weeks after initial culture. The parameters recorded were dry weight, fresh weight and organogenesis of callus. All experiments were conducted in a completely randomized design (CRD) with each treatment combination replicated ten times. Each set of auxin and cytokinin experiment was repeated three times. Data on the differences between treatments were analyzed using analysis of variance (ANOVA) with the level of significance at $P<0.05$.

3 Results and Discussion

Explants of *C. roseus* responded differently in terms of callus biomass when cultured on media supplemented with various PGRs. Explants wrinkled and showed callus formation at the cut edges within two weeks. Explants in treatments with no auxin and/or cytokinin showed no callus formation suggesting that exogenous plant growth regulators were critical in promoting callus formation (Fig. 1a).

Treatment with kinetin and NAA showed no callus formation when the medium was supplemented with only kinetin at 1, 2 and 3 mg L^{-1}. Media supplemented with only NAA and in combination with kinetin yielded compact and green callus formation (Table 1, Fig. 1b). Similar observations were reported by Udhaya et al. (2012) where explants of *Sauropus endogynous* cultured in low concentrations of kinetin alone did not produce any callus when compared with media supplemented with NAA. In the present study, shoot formation was observed in the treatment with 3.0 mg L^{-1} (Fig. 1a). No organogenesis was observed in both experiments B and C.

In Experiment B where media were supplemented with 2,4-D and BAP alone did not produce any response. When BAP was combined with 2,4-D, friable, whitish-green callus formed (Table 2, Fig. 1c and Fig. 1d). Such callus is said to be favourable for suspension culture for industrial-scale alkaloid extraction (Chattopadhyay et al., 2002). Our results are in agreement with Negi (2011) where 2,4-D alone did not trigger any callus formation in *C. roseus*, but when in combination with BAP at 0.1 mg L^{-1} and 2 mg L^{-1}, copious amounts of callus was recorded.

In Experiment C, no callus formation was observed in treatments with BAP or NAA alone. Treatments supplemented with both BAP and NAA produced compact green callus (Table 3, Fig. 1f). Interaction between cytokinin and auxin appears to play a significant role in callus induction in *C. roseus*. Haq et al. (2013) reported similar observations when leaf, node and fruit explants of *C. roseus* produced callus when exposed to media supplemented with 2,4-D and kinetin.

The interaction between auxin and cytokinin has been reported to play significant roles in inducing cellular differentiation and organogenesis in tissue and organ cultures. The complexity of this interaction has not been fully documented. According to Sunderland (1960), fresh weight of the callus was influenced by the absorption of water and other components in the basal medium which resulted in cell enlargement, while cell division and new material synthesis played a role in increasing dry weight of callus.

Statistically, both Treatments A and B showed no significant differences. Treatment with 3.0 mg L^{-1} BAP + 3.0 mg L^{-1} NAA in C showed the highest dry weight of 2.776 g, suggesting that this treatment combination was optimum.

4 Conclusions

Plant tissue culture provides an alternative means of exploiting valuable compounds in medicinal plants as a natural source of treatment. Establishing an effective callus induction method enables researchers to fully utilize its potential in large scale plant cell culturing where secondary metabolites can be extracted. From this study, the combination of auxins and cytokinins proved to play a significant role in inducing callus in *C. roseus*. The combination ratio of 3:3 BAP: NAA showed significance in fresh and dry weight compared to the other treatments used.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

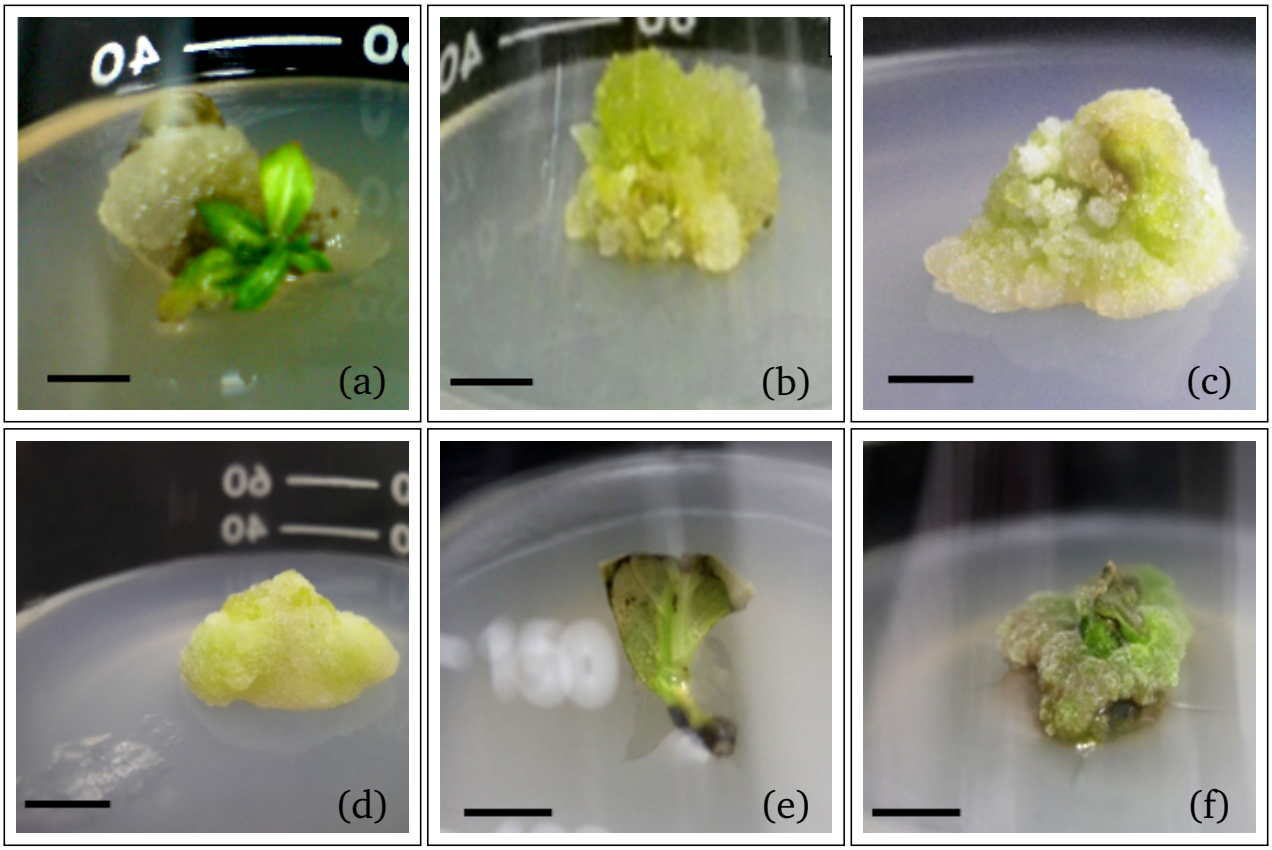

Figure 1. (a) Shoot formation in treatment with 3.0mg L^{-1} NAA, (b) compact green callus in 2.0:1.0 mg L^{-1} Kin:NAA, (c) whitish-green callus formed in 1.0:1.0 mg L^{-1} BAP:2,4-D, (d) friable like structure observed in 1.0:3.0 mg L^{-1} BAP:2,4-D, (e) no callus formation on explants cultured in MS0, (f) callus in 3.0:3.0 mg L^{-1} BAP: NAA. Horizontal bar = 1 cm

Table 1. Effect of kinetin and NAA on mean dry weight and mean fresh weight of tissue biomass (Experiment A)

Treatment	Kinetin (mg L^{-1})	NAA (mg L^{-1})	Dry weight (g)	Fresh Weight (g)
A1	0	0	0.011 ± 0.006d	0.333 ± 0.074ef
A2	0	1	0.028 ± 0.011cd	0.036 ± 0.042ef
A3	0	2	0.018 ± 0.004cd	0.440 ± 0.044def
A4	0	3	0.028 ± 0.009cd	0.617 ± 0.148def
A5	1	0	0.008 ± 0.003d	0.263 ± 0.003f
A6	1	1	0.035 ± 0.015bcd	0.690 ± 0.275cdef
A7	1	2	0.060 ± 0.010abc	1.277 ± 0.171abc
A8	1	3	0.017 ± 0.002cd	0.453 ± 0.096def
A9	2	0	0.019 ± 0.011cd	0.577 ± 0.184def
A10	2	1	0.078 ± 0.013a	1.477 ± 0.152a
A11	2	2	0.045 ± 0.019abcd	0.823 ± 0.168abcde
A12	2	3	0.043 ± 0.007abcd	0.953 ± 0.165abcde
A13	3	0	0.018 ± 0.011cd	0.460 ± 0.006def
A14	3	1	0.055 ± 0.021abc	1.060 ± 0.368abcd
A15	3	2	0.075 ± 0.028ab	1.340 ± 0.400ab
A16	3	3	0.044 ± 0.003abcd	1.067 ± 0.052abcd

Means followed by the same letter are not significantly different at $P < 0.05$.

Table 2. Effect of BAP and 2,4-D on mean dry weight and mean fresh weight of tissue biomass (Experiment B)

Treatment	BAP (mg L^{-1})	2,4-D (mg L^{-1})	Dry weight (g)	Fresh weight (g)
B1	0	0	0.007 ± 0.002b	0.049 ± 0.033c
B2	0	1	0.039 ± 0.030ab	0.410 ± 0.313bc
B3	0	2	0.026 ± 0.006ab	0.319 ± 0.071c
B4	0	3	0.059 ± 0.018a	0.952 ± 0.326ab
B5	1	0	0.024 ± 0.010ab	0.239 ± 0.095c
B6	1	1	0.032 ± 0.015ab	0.434 ± 0.231abc
B7	1	2	0.033 ± 0.014ab	0.522 ± 0.220abc
B8	1	3	0.037 ± 0.006ab	0.518 ± 0.072abc
B9	2	0	0.028 ± 0.003ab	0.255 ± 0.014c
B10	2	1	0.034 ± 0.007ab	0.419 ± 0.120bc
B11	2	2	0.024 ± 0.013ab	0.423 ± 0.216abc
B12	2	3	0.038 ± 0.006ab	0.529 ± 0.125abc
B13	3	0	0.009 ± 0.006b	0.019 ± 0.017c
B14	3	1	0.065 ± 0.016a	1.032 ± 0.287a
B15	3	2	0.041 ± 0.010ab	0.571 ± 0.154abc
B16	3	3	0.036 ± 0.009ab	0.600 ± 0.146abc

Means followed by the same letter are not significantly different at $P < 0.05$.

Table 3. Effect of BAP and NAA on mean dry weight and mean fresh weight of tissue biomass (Experiment C)

Treatment	BAP (mg L^{-1})	NAA (mg L^{-1})	Dry weight (g)	Fresh weight (g)
C1	0	0	0.433 ± 0.217ef	0.186 ± 0.062ef
C2	0	1	0.152 ± 0.057f	0.429 ± 0.242cdef
C3	0	2	0.261 ± 0.111f	0.275 ± 0.115def
C4	0	3	0.125 ± 0.014f	0.150 ± 0.019f
C5	1	0	0.179 ± 0.064f	0.188 ± 0.071ef
C6	1	1	0.779 ± 0.196def	0.839 ± 0.209bcdef
C7	1	2	0.345 ± 0.135ef	0.373 ± 0.147def
C8	1	3	1.017 ± 0.158cde	1.007 ± 0.255bcdef
C9	2	0	0.304 ± 0.086ef	0.330 ± 0.095def
C10	2	1	1.414 ± 0.283bcd	1.539 ± 0.303bcdef
C11	2	2	1.556 ± 1.057bc	1.679 ± 1.146abcd
C12	2	3	1.544 ± 0.382bc	1.659 ± 0.411abcde
C13	3	0	0.175 ± 0.048f	0.186 ± 0.073ef
C14	3	1	1.727 ± 0.606bc	1.831 ± 0.620abc
C15	3	2	1.881 ± 0.162b	2.023 ± 0.169ab
C16	3	3	2.776 ± 0.842a	2.981 ± 0.908a

Means followed by the same letter are not significantly different at $P < 0.05$.

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