- Short communication

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The Effect of Hormonal Composition, Type of Explant and Light Condition on Callus Production in Periwinkle (*Catharanthus roseus* L.)

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Periwinkle plant contains different indole alkaloids such as Ajmalicine, Vincristine and Vinblastine that have many medicinal applications. An approach to produce these compounds is made through cell culture. Therefore, it seems that optimization of callus culture is necessary for this goal. So, in this experiment 16 hormonal treatments, 2 types of explants and 2 light conditions were investigated. Maximum callus percentage fresh and dry weight were obtained from root explants with 1.5 mg/l NAA and 0.1 mg/l Kn and growing in dark condition.

Plant secondary metabolites are known to supply essential compounds for human medical needs.. Production of these compounds in plants has therefore assumed great significance. Cell culture technology is assumed to be a promising approach for the production of secondary metabolites with many benefits in contrast to conventional agriculture methods (Junaid et al. 2010). So, plant tissue culture has been accepted as a popular and effective procedure for the study of medicinal plants under *in vitro* conditions for production of natural compounds with biological applications (Doran 2000, Abdin et al. 2003). Periwinkle plant (*Catharanthus roseus* L.) is one of the most important ornamental and medicinal plants in world. This plant has a large number of terpenoids, indole alkaloids (Ataie-Azimi et al. 2008). One of the most expensive medicinal compounds which include vincristine and vinblastine that accumulate in leaves and ajmalicine and serpentine that accumulate in roots and are effective in cancer

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treatment and as anti-stress and melancholia treatment drugs (Taha et al. 2009). Hitherto, many studies have been done by El-Sayed and Vrepoorte (2002), Senoussi et al. (2007), Taha et al. (2009) and Kalidass et al. (2010). They reported the optimization of indole alkaloid production in this plant. The present experiment, likewise, was made to optimize callus culture conditions in periwinkle plant as a prerequisite for further research. For example, the establishment of cell suspension culture and gene transformation. The goal would be to study the qualitative and quantitative production of secondary metabolites in *C. roseus* L.

Seeds after surface sterilization were cultured in free hormonal MS. Thus, 1 cm pieces from root and 1 cm^2 from leaf were used as explants in B5 medium including 2 g/l glutamic acid with different treatments of 1.5 mg/l IAA, IBA, NAA and 2,4-D and BAP and Kn with 0.1 and 0.5 mg/l for callus production. In latter step, light conditions were investigated on callus production. The best explant was selected and cultured in the most appropriate hormonal treatment. Different light conditions (dark and light) were applied on samples. After two cycles of subculture (3 month-calli), traits such as callus percentage, callus index, fresh and dry weight, color and tissue of callus were investigated to calculate each of traits:

Calli were kept in oven at 70^0C for 24 hrs for the measurement of their weight.

Analysis of data was done by using of SAS software (9.1). For normal distribution of data, first angle conversion (Arcsine) for percentage data and then analysis of data were performed.

Calculation of callus percentage in leaf and root explants were indicated that medium containing NAA is the most suitable for callus production. So that, root explants showed cent per cent callus at each four media containing NAA (0.1 and 0.5 mg/l BAP and Kn). While in leave explants, the most callus percentage indicated at medium containing NAA + 0.5 mg/l Kn (Table 1).

Maximum callus index was related to root explant and NAA + 0.1 mg/l Kn that it was investigated 12.70. While callus index in leaf explant was displayed in NAA + 0.5 mg/l Kn that it was 11.87. Callus index represents explant ability for callus production and rate of callus formation in plant tissue. In other words, investigation process of callus production within a specified period can represent the process of callus induction (fast or slow) in plant tissue that determination of tissue ability for callus production, too. Hence, it can be concluded that root explant has the greater ability to callus formation.

Highest weight of calli was related to root explant and NAA hormone that it fresh and dry weight was 2.92 and 2.62 g, respectively. While cultured of leaf

Table1. Effect of different hormonal treatment on callus percentage. Callus index, fresh and dry weight of callus, color and tissue of callus in root and leaf explants.

No.	Treatments	Type of explant	Callus (%)	Callus index	Fresh wt. (g)	Dry wt. (g)	Callus color	Callus tissue
1	1.5 IAA + 0.1 BA	Leaf	70 d	5.87 j	1.48 j-l	1.17 j-l	Brownish	Compact
2	1.5 IAA + 0.5 BA	Leaf	80 b-d	6.00 ij	1.21 l	0.90 l	Brownish	Compact
3	1.5 IAA + 0.1 Kn	Leaf	90 a-c	8.33 c-j	1.63 h-l	1.32 h-l	Brownish	Compact
4	1.5 IAA + 0.5 Kn	Leaf	80 b-d	6.25 g-i	1.74 g-k	1.43 g-k	Brownish	Compact
5	1.5 IBA + 0.1 BA	Leaf	85 a-d	7.00 e-j	1.82 g-k	1.51 g-k	Brownish	Compact
6	1.5 IBA + 0.5 BA	Leaf	85 a-d	6.95 e-j	1.35 kl	1.04 kl	Brownish	Compact
7	1.5 IBA + 0.1 Kn	Leaf	85 a-d	7.20 d-j	1.61 h-l	1.30 j-l	Brownish	Compact
8	1.5 IBA + 0.5 Kn	Leaf	80 b-d	5.95 ij	2.18 c-g	1.87 b-g	Brownish	Compact
9	1.5 NAA + 0.1 BA	Leaf	95 ab	9.25 b-i	2.20 c-g	1.89 b-g	Green	Compact
10	1.5 NAA + 0.5 BA	Leaf	95 ab	9.79 a-g	2.49 a-e	2.18 a-d	Green	Compact
11	1.5 NAA + 0.1 Kn	Leaf	95 ab	9.79 a-g	2.61 a-c	2.30 ab	Cream	Soft
12	1.5 NAA + 0.5 Kn	Leaf	100 a	11.87 ab	2.56 a-d	2.25 a-c	Cream	Soft
13	1.5 2,4D + 0.1 BA	Leaf	90 a-c	8.83 b-j	1.46 j-l	1.15 j-l	Green	Compact
14	1.5 2,4D + 0.5 BA	Leaf	85 a-c	7.70 d-j	2.02 e-h	1.71 d-i	Green	Compact
15	1.5 2,4D + 0.1 Kn	Leaf	100 a	10.20 a-e	1.72 g-k	1.41 g-k	Cream	Soft
16	1.5 2,4D + 0.5 Kn	Leaf	85 a-d	8.75 b-j	2.05 e-h	1.79 c-h	Cream	Soft
17	1.5 IAA + 0.1 BA	Root	75 dc	6.70 f-j	1.86 f-j	1.58 f-j	Brownish	Compact
18	1.5 IAA + 0.5 BA	Root	85 a-d	6.62 g-j	1.52 i-l	1.23 j-l	Brownish	Compact
19	1.5 IAA + 0.1 Kn	Root	90 a-c	7.45 d-j	1.88 f-j	1.59 f-j	Brownish	Soft
20	1.5 IAA + 0.5 Kn	Root	90 a-c	8.79 b-j	1.98 f-i	1.70 e-i	Brownish	Soft
21	1.5 IBA + 0.1 BA	Root	90 a-c	6.50 g-j	2.07 d-h	1.78 c-h	Brownish	Compact
22	1.5 IBA + 0.5 BA	Root	85 a-d	7.12 d-j	1.59 h-l	1.30 h-l	Brownish	Compact
23	1.5 IBA + 0.1 Kn	Root	85 a-d	8.29 c-j	1.85 f-j	1.56 f-j	Brownish	Compact
24	1.5 IBA + 0.5 Kn	Root	85 a-d	6.79 f-j	2.34 b-f	2.06 b-f	Brownish	Compact
25	1.5 NAA + 0.1 BA	Root	100 a	10.00 a-f	2.50 a-e	2.21 a-d	Cream	Soft
26	1.5 NAA + 0.5 BA	Root	100 a	10.41 a-d	2.78 ab	2.57 a	Green	Compact
27	1.5 NAA + 0.1 Kn	Root	100 a	12.70 a	2.92 a	2.62 a	Cream	Soft
28	1.5 NAA + 0.5 Kn	Root	100 a	11.45 a-c	2.91 a	2.62 a	Cream	Soft
29	1.5 2,4D + 0.1 BA	Root	80 b-d	7.54 d-j	1.78 g-k	1.49 g-k	Cream	Soft
30	1.5 2,4D + 0.5 BA	Root	90 a-c	8.16 c-j	2.34 b-f	2.05 b-f	Green	Compact
31	1.5 2,4D + 0.1 Kn	Root	95 ab	9.37 a-h	2.04 e-h	1.75 d-h	Cream	Soft
32	1.5 2,4D + 0.5 Kn	Root	95 a-c	9.70 a-g	2.32 b-f	2.04 b-f	Cream	Soft

*Numbers with the same letters have no significant difference in a Duncan's multiple range tests ($p \leq 0.05$).

explant in similar medium, fresh and dry weight was 2.61 g and 2.30 g, respectively. Kalidass et al. (2010) by application of 32 different hormonal treatments were viewed highest dry weight in treatment containing of 2 μM NAA + 2 μM BAP. Therefore, NAA has effective role at enhancement of callus production in periwinkle plant.

Induced calli from various medium and different explants were investigated for color (green, brownish and cream) and tissue (soft or compact). Maximum number of brownish calli were indicated at IAA and IBA treatments. So that 95 percentage of induced calli had brown color in these media. Production of brown color in callus and explant cause their gradual decline. Therefore, they have no suitable growth duration of time and will be lost by multiple cycles of subculture. Also, developed calli in these media had compact tissues. Type of used cytokinin can be effect on growth characteristics of calli. Investigation of quality characteristics at induced calli in different hormonal treatments showed that the most appropriate medium for callus induction (quality characteristics) in medium containing 2,4-D and NAA in combination with Kn (Fig. 1). The type of callus tissue can be effective in later steps of researches for example cell suspension culture. Saifullah and Saifullah (2011) showed that growth response of white calli with soft tissue would be more appropriate in liquid medium and they are more suitable for suspension.

Fig. 1. The effect of NAA in combination with 0.1 mg/l Kn for callus induction in root (right) and leaf (left) explants.

By identification of the most suitable explant for callus production and by aims of determination of the appropriate growth conditions for its induction, root explant was cultured in different hormonal treatments (1.5 mg/l NAA and 2,4-D) in combination of various concentrations of BAP and Kn (0.1 and 0.5 mg/l). Thus, explants were maintained in different light conditions (dark and light) at growth room.

Ninety five per cent callus productions were observed in 1.5 mg/l NAA and 0.5 mg/l BAP treatment. Also, 100% callus was obtained in medium containing 2,4-D + 0.1 mg/l Kn. But, because of low dry weight in this samples, this treatment was not considered. In comparison between NAA different treatments

in light conditions, maximum fresh and dry weight was related to 0.1 mg/l Kn while maximum callus index was assessed in 0.5 mg/l Kn that was equal to 12.49. It seems, increasing Kn concentration had effective on callus induction and its growth. While at developed explants in dark conditions, medium containing NAA was identified as an effective one for increasing callus production and there was no difference on callus percentage between used types and cytokinin concentrations, but explants weight were different. So that, maximum fresh and dry weight were indicated in 0.1 mg/l Kn, 0.5 mg/l BAP, 0.5 mg/l Kn and 0.1 mg/l BAP treatments (Table 2).

Table 2. Effect of different hormonal treatments on callus production at root explant in two different light conditions.

No.	Treatments	Light conditions	Callus (%)	Callus index	Fresh wt. (g)	Dry wt. (g)	Callus color	Callus tissue
1	1.5 NAA + 0.1BA	Light	100 a	11.04 a-d	2.19 c-f	1.80 c-f	Cream	Soft
2	1.5 NAA + 0.5BA	Light	95 ab	10.04 a-e	2.18 a-d	1.79 c-f	Green	Compact
3	1.5 NAA + 0.1 Kn	Light	100 a	11.25 a-d	2.45 c-f	1.97 c-e	Cream	Soft
4	1.5 NAA + 0.5 Kn	Light	100 a	12.49 ab	2.37 c-f	1.95 c-e	Cream	Soft
5	1.5 2,4D + 0.1BA	Light	85 bc	8.12 de	1.30 g	0.92 h	Cream	Soft
6	1.52,4D+0.5BA	Light	95 ab	9.16 c-e	1.87 d-f	1.48 d-g	Green	Compact
7	1.5 2,4D + 0.1 Kn	Light	100 a	9.79 a-e	1.57 fg	1.18 gh	Cream	Soft
8	1.5 2,4D + 0.5 Kn	Light	95 ab	10.21 a-e	2.05 c-f	1.65 c-f	Cream	Soft
9	1.5 NAA + 0.1 BA	Dark	100 a	10.00a-e	2.50 a-c	2.21 a-c	Green	Compact
10	1.5 NAA + 0.5 BA	Dark	100 a	10.41 a-e	2.86 ab	2.57 a	Green	Compact
11	1.5 NAA + 0.1 Kn	Dark	100 a	12.71 a	2.91 a	2.62 a	Cream	Soft
12	1.5 NAA + 0.5 Kn	Dark	100 a	11.45 a-c	2.78 ab	2.49 ab	Cream	Soft
13	1.5 2,4D + 0.1 BA	Dark	80 c	7.54 e	1.78 e-g	1.49 d-g	Green	Compact
14	1.5 2,4D + 0.5 BA	Dark	90 a-c	8.16 de	2.34 a-d	2.05 b-d	Green	Compact
15	1.5 2,4D + 0.1 Kn	Dark	95 ab	9.37 b-e	2.04 c-f	1.75 c-f	Cream	Soft
16	1.5 2,4D + 0.5 Kn	Dark	90 a-c	9.71 a-e	2.32 c-e	2.04 cd	Cream	Soft

*Numbers with the same letters have no significant difference in DMRT. ($p \leq 0.05$).

Results showed that Kn in high concentrations reduced callus production and development if high concentrations of BAP had effective on callus production and its development. Perhaps this concentration can be interpreted between light and dark conditions. The callus induction showed that the standing of explants in growth room at dark conditions will increase callus percentage and its index. So that, the amounts of fresh and dry weight increased 0.65 and 0.46 g than light state (Table 2). Light conditions and temperature are the effective factors on indole alkaloids production (Zhao et al. 2005). So, many researchers introduced dark conditions in growth room as suitable conditions for callus

induction and its development in periwinkle plant (Zhao et al. 2001, Taha et al. 2009). Type of cytokinin will have high efficacy on color and type of tissue in produced callus. So the samples in medium containing BAP had calli with green color and compact tissue while Kn produced calli with cream color and soft tissue. All samples were treated with BAP and kept in dark conditions, produced calli with green color and compact tissue. So BAP in dark conditions has greater effect on color and tissue of callus. While in light conditions higher concentrations of BAP produced green calli (Table 2).

Results of this experiment showed that the use of root tissues as explant, in B5 containing 1.5 mg/l NAA + 0.1 mg/l Kn + 2 g/l glutamic acid and then transferred to growth room in dark conditions at 25ºC can be considered as the most appropriate for induction of callus and their maximum development in periwinkle plant. This experiment was made initially for optimization of callus culture in periwinkle plant so that in the next stages of study for the purpose of increasing production of valuable indole alkaloids such as ajmalicine, vinblastine and vincristine.

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