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PHYTOHORMONE INDUCED VARIATION IN *IN VITRO* SEED CULTURE OF *DENDROBIUM APHYLLUM* (Roxb.)

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Knudson-C medium is quite effective in inducing seed germination in *Dendrobium aphyllum* (Roxb.). Both IAA and α NAA significantly enhanced seedling differentiation and growth when added to basal medium enriched with coconut water 15% and banana extract 6%. Kinetin although induces root shoot formation, is partially inhibitory to growth. 2, 4-D has negative effect as it induces callus formation instead of seedling differentiation. Kinetin induces multiple shoot formation which is not seen in the case of the other hormones.

Keywords: *Dendrobium aphyllum;* Hormone, *In vitro* seed culture; Orchid.

Introduction

Massive deforestation particularly in underdeveloped and developing countries are understandably causing global concern. But another overlooked and equally serious concern is the threat to the epiphytic plants for which tree trunks and branches are the natural habitats. Many epiphytic plants which were abundant till a few years back have suddenly become endangered due to sudden elimination of their natural habitat. *Dendrobium aphyllum* (Roxb.) is one such epiphytic orchid that grows on both deciduous as well as non-deciduous trees and abundant in the forests of plain and low altitude areas of North East India. However, due to massive deforestation since 1990 it has become rare and its status has become a matter of serious concern. As in many other orchids natural regeneration in *D.aphyllum* is slow and inefficient. During rainy season, new shoots develop vegetatively which grow into pendulus cylindrical stem. The species is deciduous because young tender stems which bear lanceolate sessile leaves during summer fall off in winter. The plant produces many capsules and each contain numerous tiny seeds. However, in nature the seeds rarely germinate and grow into seedlings. In view of this, for their rapid propagation for commercial as well as for conservation purpose it is necessary to develop a protocol for rapid propagation by *in vitro* seed culture method. In case of orchids, *in vitro* seed culture has been standardised for many species and seed culture has been found to be more convenient and effective than using vegetative parts as explants. Hence seed culture was chosen for the present study.

Materials and Methods

Knudson C media was used for germination of seeds. The orchid capsules were surface sterilised with 0.2% mercuric chloride solution for 8 minutes followed by treatment with 70% alcohol for 30 seconds. Finally it was washed with sterile distilled water. Seed suspension made in sterile distilled water was inoculated in the media. Knudson C media enriched with coconut water 15% and banana extract 6% was used as the basal media for sub-culture of protocorms. The basal media were separately supplemented with Indole-acetic acid, α - Napthelene acetic acid, Kinetin and 2, 4-D; at the concentrations of 2 mgL^{-1}, 5 mgL^{-1} and 10 mgL^{-1} to study the effect of hormones on plantlet differentiation from protocorm and seedling growth. Protocorms which had developed 40 days after inoculation of seed were transfered to hormone containing media. After six weeks of first subculture observations were recorded with respect to fresh weight of seedling, length of root and shoot. At the same time another set of seedlings were transfered to fresh media contain-

ing identical concentration of hormones for second sub-culture. Following second sub-culture observations were recorded after a total of 13 weeks of sub-culture. Basal media without hormone served as control. Each treatment was replicated thrice and the results were statistically analysed using Fisher's method of analysis of variance ratio.

Results and Discussion

Germination of seed was observed 20-22 days after inoculation of seed suspension in Knudson C media, which was indicated by the appearance of greenish yellow colouration of seeds. The germinated seeds were allowed to grow for further 40 days after which they developed into protocorms indicated by development of rudimentary leaf. Following sub-culture for 6 weeks it was found that out of four hormones IAA and α - NAA showed stimulatory effect on seedling differentiation and growth in terms of fresh weight per seedling. Kinetin also induced plantlet differen-

Table 1. Growth of plantlet in terms of fresh weight after different period of culture following basal media supplemented with various concentrations of hormones.

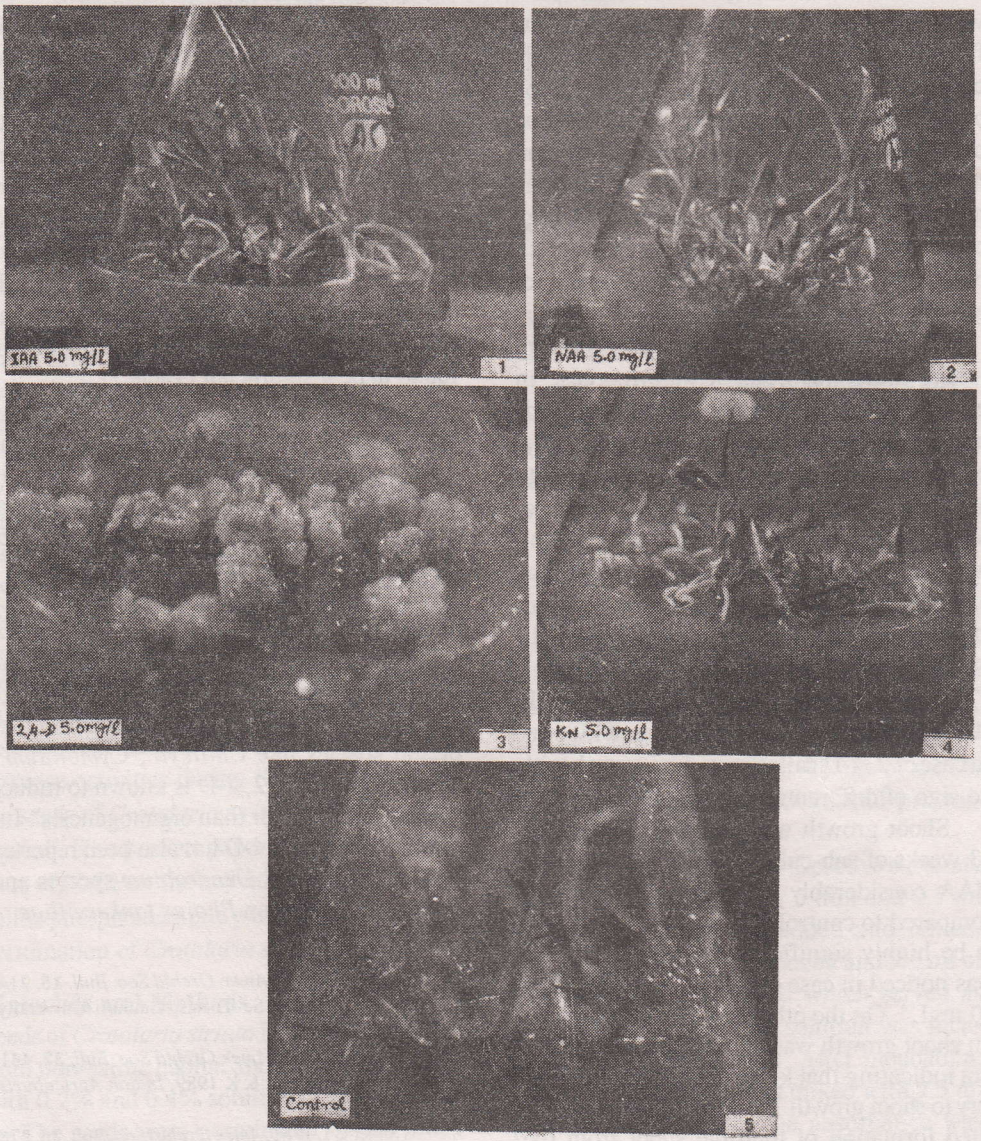
Treatment	Fresh weight per plantlet (mg)	
	After 6 weeks	After 13 weeks
Control	4.72	38.0
IAA - 2 mgL^{-1}	6.36	35.00
IAA - 5 mgL^{-1}	7.91	51.20
IAA - 10 mgL^{-1}	8.59	62.90
α -NAA - 2mgL^{-1}	13.54	157.50
α -NAA - 5mgL^{-1}	8.78	125.50
α -NAA - 10mgL^{-1}	6.93	108.06
Kn - 2mgL^{-1}	1.16	22.72
Kn - 5mgL^{-1}	1.01	26.21
Kn - 10mgL^{-1}	2.31	46.07

C.D. for treatment: At 5% probability level - 26.4332; At 1% probability level - 35.2531
C.D. for time interval: At 5% probability level - 11.8213; At 1% probability level - 15.7656

Table 2: Growth of shoot and root in terms of length after different periods of culture following basal media supplemented with various hormone concentrations.

Treatment	Shoot, root length per plant (cm)			
	After 6 weeks		After 13 weeks	
	Shoot	Root	Shoot	Root
Control	0.58	0.22	1.96	1.20
IAA -2 mgL^{-1}	0.77	0.33	2.30	1.92
IAA -5 mgL^{-1}	0.70	0.38	3.24	2.01
IAA -10 mgL^{-1}	0.79	0.45	4.26	2.60
α -NAA -2 mgL^{-1}	0.86	0.43	4.19	2.81
α -NAA -5 mgL^{-1}	0.57	0.17	5.08	0.89
α -NAA -10 mgL^{-1}	0.82	0.07	5.24	0.56
Kn -2 mgL^{-1}	0.00	0.00	1.43	0.93
Kn - 5 mgL^{-1}	0.00	0.00	1.33	0.57
Kn - 10 mgL^{-1}	0.00	0.00	1.80	0.94

C.D for treatment: At 5% probability level for shoot = 0.7778; For root = 0.4056
At 1% probability level for shoot = 1.0374; For root = 0.5409
C.D. for time interval: At 5% probability level for shoot = 0.3478; For root = 0.1813
At 1% probability level for shoot = 0.4639; For root = 0.2419


IAA 5.0 mg/l
1
NAA 5.0 mg/l
2
2,4-D 5.0 mg/l
3
KN 5.0 mg/l
4
Control
5

tiation but growth was slow compared to that of control. However, 2, 4-D failed to induce seedling differentiation; it only induced callus formation. In case of IAA with rise in concentration from 2 mgL^{-1} to 10 mgL^{-1} there was gradual rise in fresh weight per seedling from 6.36 to 8.59 mg. On the other hand in case of α - NAA although low concentration of 2 mgL^{-1} exhibited best growth of 13.54 mg per seedling; subsequent increase in concentration resulted in gradual fall of seedling growth. This shows that in case of IAA high concentration (10 mgL^{-1}) and in case of α - NAA low concentration (2 mgL^{-1}) is best for growth enhancement (Table 1).

Following second sub-culture, after 13 weeks there was considerable increase in growth with respect to fresh weight per seedling and differentiation of root and shoot. The pattern of growth enhancement was found to be same as that of after first sub-culture. Maximum growth of 157.5 mg per seedling was found in case of α - NAA 2 mgL^{-1} as against 38 mg in control. However, kinetin 10 mgL^{-1} showed increase in seedling weight (46.07 mg per plant) which is above control. This increase in weight is due to multiple shoot formation seen only in case of kinetin. In case of 2, 4-D callus remained as such with no sign of differentiation.

Shoot growth was prominent only after 13 weeks of sub-culture. Both IAA and α - NAA considerably enhanced shoot growth compared to control which have been found to be highly significant. Best shoot growth was noticed in case of α - NAA 5 mgL^{-1} and 10 mgL^{-1}. On the other hand in case of kinetin shoot growth was lesser than that of control indicating that kinetin is partially inhibitory to shoot growth. However, Kinetin stimulated formation of multiple shoot from the base of the main shoot. The frequency of multiple shoot was found to increase with the increase in concentration. In 10 mgL^{-1} kinetin, the number of shoot per plantlet was 2-7; the highest number observed in the highest concentration of the present study.

In case of root growth IAA considerably enhanced root growth over control and there was gradual increase in growth with increase in concentration. Interestingly, while low concentration of α - NAA exhibited best root growth with increase in concentration there was sharp decline in root growth and at 10 mgL^{-1} root growth was reduced to half of control. This clearly shows that while low concentration of α - NAA is stimulatory to root growth at high concentration it is partially inhibitory. In case of kinetin like shoot growth, root growth was also found to be lesser than that of control; however statistically it was found to be insignificant (Table 2).

As a whole both IAA and α - NAA have been found to be favourable for growth and development of *D. aphyllum*, which is in consistance with similar findings by several other workers on *Dendrobium*. Nath[2] observed that compared to IAA and IBA, α - NAA showed growth and development on *D. nobile*. Favourable effect of α - NAA *Dendrobium* species were also reported by Israel[3] and Das and Ghosal[4]. α - NAA is also known to exert favourable effect on a number of other orchid species like *Cattleya*[5], *Cymbidium*[6], *Epidendrum*[7], etc. 2, 4-D is known to induce callus growth rather than organogenesis[8]. Inhibitory effect of 2, 4-D has also been reported by Goh[9] on several *Dendrobium* species and by Vij and Kaur[10] on *Phaius tankervilliae*.

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