CHAPTER - 2

EFFECT OF GROWTH REGULATORS ON SEED GERMINATION; GROWTH, DEVELOPMENT AND REGENERATION OF PLBs in *Arundina, Dendrobium* AND *Geodorum*

2.1 INTRODUCTION

The vast majority of orchids are propagated by seeds rather than by vegetative methods (Mitra 1971). The seeds of orchids which are produced in large numbers in each capsule, are highly fragile structures and posseses virtually no endosperm and no food materials as most other seeds contain. Though the orchids produce many seeds, they require mycorrhizal association for germination (Arditti 1967). However, only less than 5% seeds germinate in nature (Rao 1977, Sharma and Tandon 1986). On the other hand the conventional methods of vegetative propagation is a time consuming and tedious process (Sagawa and Kunisaki 1984). Moreover, seeds sown in the nursery beds not only require long period for germination, but also slight change in soil pH or other physical conditions destroy the whole population (Northen 1962). Due to so many peculiar factors associated with the structure of orchid seeds and the requirement of fungal association for germination under *in vivo* condition, seed germination of orchids had been an engima for a long time.

The subject of mycorrhizal association and effect of several other factors like photoperiod, intensity and quality of light, temperature and pH on seed germination has been reviewed in details by Arditti (1967). Knudson for the first time showed that orchid seed germination is possible in simple nutrient medium and without any fungal association and studied various aspects of *in vitro* germination of orchid seeds (Knudson 1922, 1924, 1925 and 1930). Following Knudson's discovery the propagation of orchids through *in vitro* germination of seeds has been emphasized by several groups (Arditti 1967, Arditti *et al* 1981, Arditti *et al* 1982, Clement 1973, Clement and Ellyard 1979, Clement 1981, Curtis 1943, Ernst 1975, Knudson 1946, 1951; Mitra 1971, Soutamere 1964,1974 and1981). These investigations stressed the importance of nutrient media and several other factors associated with orchid seed germination.

Different media and their modifications in respect of macro- and micro nutrients (Arditti *et al* 1981, Henrich *et al* 1981, Ziegler *et al* 1967,), amino acids (Curtis 1947, Foneshbech 1972, Raghavan and Torrey 1964, Sporel 1948, Sporel and Curtis 1948,), vitamins (Mead and Bullard 1973 and 1979, Noggle and Wynd 1943) and carbohydrates (Ernst 1967, Foneshbech 1972) have been used.

These early works have emphasized the importance of vitamins, amino acids and other growth factors for germination of orchid seeds and the development of PLBs to plantlets. Moreover, these investigations clearly established the fact that the physico - chemical requirement of orchid seed germination and seedling growth vary considerably from species to species.

To promote the seed germination and growth of PLBs / callus various plant growth regulators like IAA, IBA, NAA, BAP, GA3, Kn have been tested (Hadley and Harvais 1968, Hadley 1970, Goh 1970, Pierik and Steegmans 1972, Sharma and Tandon 1986 Straus and Resinger 1976,). Experiments with auxins, cytokinins and gibberellins on orchid seed germination as well as seedling growth gave inconsistent and therefore inconclusive results (Arditti 1979): This may be due to the physiological reasons or the doses of hormones used. A perusal of earlier literature revealed that IAA, IBA, NAA enhance seed germination and seedling growth in a large number of orchid species (Singh, 1991). Upto 80% seed germination was reported in *Cymbidium mastersii* when lower concentrations of IAA were used (Mitra 1986). Lower concentrations of NAA promoted seed germinationm in some orchids (Mathews and Rao 1980, Viz *et al* 1981). Inhibitory effects of these auxins were reported in a few other cases (Arditti 1979, Singh 1986). Cytokinins were known to have different effects on different species. Cytokinin alone or in combination with auxins promotes seed germination in a number of orchids (Mitrta,1986). BAP, 2,4-D, and NAA had rarely been used in seed germination of orchids (Singh, 1991).

During the last few years tissue culture techniques have been extensively exploited for rapid and large scale propagation of orchids (Nayek *et al* 1997, Park *et al* 1996, Prakash *et al* 1995) and huge literature have been accumulated. But only a few of them critrically examined the role of growth regulators on seed germination and seedling development of orchids. Meagre information is available in this regard in *Arundina graminifolia* and *Dendrobium moschatum*. Moreover, so far no information at all is available on seed germination or the role of growth regulators on seed germination and growth of PLB / callus in *Geodorum densiflorum*. Due to lack of information in this regard in orchids in general and the taxa undertaken in the present study in particular, the present investigation was directed at elucidating the effects of different growth regulators on seed germination and growth, development of PLBs / callus and their regeneration.

2.2 MATERIALS AND METHODS

8-12 week old mature, green and undehisced capsules containing undifferentiated embryonic masses were used as explant material for observation of seed germination, while PLBs/calluses were used as explant source for observation of their growth and development. Surface sterilization of capsules were done following the same method as described in Chapter I. PLBs/PLB callus masses were collected aseptically from the culture tubes and used directly without sterilization.

Preparation of culture media

Modified VW and KnC media were used in the present study. The composition of the media and the preparation was same as shown in Table 1.2.2 of Chapter I.

Preparation of organic additives

Four organic additives, namely, Coconut Milk, Tomato Juice, Banana Extract and Peptone were mainly usd for the present study. This preparation has already been described in Chapter I. 5, 10, 15 and 20% of CM,TJ and BE and 1 gm, 2 gm, 3 gm and 4 gms of Peptone were used with the medium to observe their effects on seed germination.

Preparation of growth regulators

Various growth regulators like Indole acetic acid (IAA), α Napthalene acetic acid (NAA) 6 Benzyl amino purine (BAP) and Kinetin (Kn) was added with the medium individually and in combination as well. The method for preparation of stock solution was same as described in Chapter I.

Inoculation, Culture conditions and Growth measurements

The inoculation procedure and culture conditions were same as described in details in Chapter I. The PLBs and calluses were weighed by an electric single pan balance in aseptic condition. Initial weight was recorded at the time of inoculation. Fial weight was taken after 8 weeks. Weighing was done at a regular interval of two weeks. Growth index is expressed as the ratio of increase in fresh weight of the PLB or callus to the initial fresh weight of the callus. Each treatment had 5 replicates and repeated thrice.

2.3 OBSERVATIONS

2.3.1. Effects of auxins and cytokinins on seed germination of *Arundina, Dendrobium* and *Geodorum*

Various concentrations of different growth regulators like IAA, NAA, BAP and Kn were added to the organic additive enriched VW and KnC medium to observe their effects on seed germination of three orchid taxa, namely, *Arundina graminifolia, Dendrobium moschatum* and *Geodorum densiflorum*. Varying effects were noted. The germination percentage was calculated by using the formula shown in Section 1.2 of Chapter I. The results obtained in the present study have been summarised, tabulated and presented in Tables 2.3.1, 2.3.2 and 2.3.3. The salient features are as follows.

In *Arundina graminifolia*, lower concentrations of both the auxins i.e. IAA and NAA were found to promote seed germination when added to CM (15%,v/v) supplemented VW medium. Slight increase in germination percentage was noted when 0.5 mg l^{-1} IAA was combined with CM enriched VW medium. But significant increase in seed germination was omly noted when 1 mg l^{-1} IAA was mixed with the medium. The germination percentage was as high as 86 (Table 2.3.1) when 1 mg l^{-1} was added to CM enriched modified VW medium. However, 2 mg l^{-1} IAA resulted in a significant decrease in time requirement for germination without any further increase in germination percentage. Higher concentrations of IAA (3 mg l^{-1} and 4 mg l^{-1}) resulted in a significant decrease in seed germination percentage thus being inhibitory to the process.

NAA at 1mg l^{-1} concentration resulted in a sharp and significant increase in seed germination percentage in *A.graminifolia* (Plate 7). 92% seed germination was recorded when the said concentration of NAA(1mg l^{-1}) was added to CM (15%) modified VW medium (Table 2.3.1). However, like IAA higher concentrations of NAA to were found to inhibit seed germination as it resulted in a gradual decrease in germination percentage with increase in concentration. Only 51 and 46 percentage seeds were germinated respectively when 3 mgl^{-1} and 4 mg l^{-1} NAA was added with the medium (Table 2.3.1).

Table 2.3.1 Effect of growth regulators on seed germination of *A. graminifolia*. Data were taken after 6 weeks following germination. Each treatment had 5 replicates and repeated thrice.

Treatment	Concentration mg l^{-1}	Days required for germination ± SE	Percentage of Germination ± SE
Control (VW + CM)	00	16 ± 0.90	80 ± 3.00
	0.5	16 ± 0.60	82 ± 2.00
	1.0	17 ± 1.00	86** ± 3.00
IAA	2.0	13* ± 0.30	80 ± 3.00
	3.0	16 ± 0 .70	64***± 4.08
	4.0	18 ± 0 .20	52*** ± 0.70
	0.5	19 ± 0.30	79 ± 1.00
	1.0	17 ± 0.50	92***± 2.00
NAA	2.0	21 ± 1.09	87**± 1.00
	3.0	19 ± 0.70	51*** ± 5.00
	4.0	17 ± 0.20	46*** ± 3.00
	0.5	46***± 1.00	26*** ± 1.50
	1.0	48***± 0.20	30** ± 3.00
BAP	2.0	43** ± 0.30	30*** ± 4.00
	3.0	43***± 0.50	19***± 2.50
	4.0	46***± 0.90	21***± 2.00
	0.5	39** ± 0.40	22***± 1.00
	1.0	39** ± 1.50	28***± 3.60
Kn	2.0	36***± 0.70	34***± 2.00
	3.0	41***± 1.07	31***± 1.09
	4.0	36**± 0.80	19***± 3.00

Values followed by asterisks in each organic additive treatment within the same column are significantly different from the control (no growth regulators) using Students t-test at *5% level; ** 1% level and *** 0.1% level.

BAP and Kn at any concentration on the other hand was not only found to significantly decrease the percentage of seed germination, but also delayed it by 3-4 weeks in *A. graminifolia* when added to CM supplemented modified VW medium. Seeds were germinated after 36- 4 8 days in BAP or Kn supplemented medium . Moreover, germination percentage was very low when these two growth regulators were added to the medium. Only 19-34 percent seeds were germinated in BAP / Kn supplemented medium which is significantly lower in comparison to control.

Effects of growth regulators on seed germination of *D. moschatum* was recorded and shown in Table 2.3.2 . Optimum germination was recorded when lower concentrations of IAA / NAA were added to the medium (Plate 8) . IAA at 2 mg l^{-1} concentration resulted in a sharp and significant increase in germination over control. 81% seed germination was noted when 2 mg l^{-1} IAA was added to CM (15%) supplemented modified KnC medium. Further increase in IAA concentration decreased the germination percentage (Table 2.3.2). However, an increase in germination was recorded when 1mg l^{-1} NAA was supplemented with CM enriched modified KnC medium. The germinmation percentage at this concentration of NAA was as high as 84. Higher concentrations of NAA than 2 mg l^{-1} was found to have inhibitory effect on germination.

BAP had a very little effect on seed germination of *D. moschatum*. Slight increase in seed germination was noted at lower concentrations (0.5 mg l^{-1}). Kinetin at any concentration was found to decrease the seed germination percentage which was significantly lower than control (Table 2.3.2).

Table 2.3.3 reveals that in *G. densiflorum*, IAA had almost no effect on seed germination. Slight increase in germination percentage was noted at lower concentrations of IAA. NAA on the other hand was found very effectivein increasing the percentage of seed germination of *G. densiflorum*. High germination percentage was recorded when 1mg l^{-1} NAA was added with Peptone (2gm l^{-1}) supplemented KnC medium.

Table 2.3.2 Effect of growth regulators on seed germination of *D.moschatum* All data were taken after 6 weeks following germination. Each treatment had 5 replicates and repeated thrice

Treatment	Concentration mg l^{-1}	Days required for germination ± SE	Percentage of Germination ± SE
Control (KnC + CM 15%)	00	22 ± 0.50	72 ± 3.00
	0.5	21 ± 0.60	72 ± 2.60
	1.0	21 ± 0.30	75* ± 3.00
IAA	2.0	19 ± 0.50	81*** ± 3.51
	3.0	19 ± 0.90	69 ± 1.09
	4.0	20 ± 1.20	49*** ± 2.70
	0.5	22 ± 1.00	75** ± 2.50
	1.0	18 ± 0.40	84*** ± 2.00
NAA	2.0	18 ± 0.20	79** ± 1.00
	3.0	23 ± 1.70	63* ± 1.70
	4.0	21 ± 1.00	52*** ± 3.00
	0.5	21 ± 0.50	73 ± 2.00
	1.0	21 ± 1.00	71 ± 2.80
BAP	2.0	23 ± 0.80	64* ± 3.00
	3.0	21± 0.50	68* ± 2.00
	4.0	24 ± 0.20	57*** ± 5.80
	0.5	24 ± 0.30	58*** ± 3.73
	1.0	19 ± 0.90	58*** ± 1.00
Kn	2.0	21 ± 0.70	56** ± 0.90
	3.0	24 ± 0.20	59* ± 1.00
	4.0	23 ± 0.40	50*** ± 2.64

Values followed by asterisks in each organic additive treatment within the same column are significantly different from the control (no growth regulators) using Students t-test at *5%level; ** 1% level and *** 0.1% level.

Table 2.3.3 Effect of growth regulators on seed germination of *G. densiflorum* All data were taken after 6 weeks following germination. Each treatment had 5 replicates and repeated at least thrice.

Treatment	Concentration (mg l^{-1})	Days required for germination ± SE	Percentage of Germination ± SE
Control (KnC + PEP 2 gm l^{-1})	00	37 ± 0.50	63 ± 1.90
	0.5	36 ± 0.70	65 ± 2.00
	1.0	38 ± 1.00	67* ± 3.00
IAA	2.0	38 ± 0.40	67* ± 2.00
	3.0	37 ± 0.90	50** ± 3.00
	4.0	40 ± 1.00	42*** ± 2.20
	0.5	32 ± 1.50	70** ± 2.50
	1.0	32 ± 1.00	75***± 4.00
NAA	2.0	35 ± 0.90	72**± 2.00
	3.0	38 ± 1.00	56*± 2.00
	4.0	34 ± 0.70	41***± 0.90
	0.5	42 ± 0.30	62 ± 2.00
	1.0	36 ± 0.70	64 ± 2.60
BAP	2.0	38 ± 0.70	69**± 3.00
	3.0	34 ± 1.00	72***± 2.50
	4.0	39 ± 0.20	58* ± 2.00
	0.5	39 ± 0.60	64 ± 3.00
	1.0	42 ± 0.90	60 ± 3.50
Kn	2.0	42 ± 0.40	65 ± 4.00
	3.0	46 ± 0.90	59 ± 3.00
	4.0	46 ± 0.20	56*± 4.80

Values followed by asterisks in each organic additive treatment within the same column are significantly different from the control (growth regulators) using Students t-test at *5% level; ** 1% level and *** 0.1% level.

However, higher concentrations of NAA yielded sharp decrease in germination percentage (Table 2.3.3) and was thus found inhibitory.

BAP at 3 mg l^{-1} concentration resulted in a sharp and significant increase in germination percentage. 72% seed germination was recorded when 3 mg l^{-1} BAP was added with Peptone supplemented KnC medium. However, Kn had no effect on seed germination of *G. densiflorum*.

2.3.2 Effects of auxins and cytokinins on growth, development and regeneration of PLBs/calluses in *A. graminifolia, D. moschatum* and *G. densiflorum*

To asses their effect on growth, development and regeneration of PLBs, different concentrations of various growth regulators like IAA, NAA, BAP and Kn were added to organic additive enriched VW and KnC media. Data obtained in the present study clearly showed the differential response of different growth regulators on the growth and development of PLBs in the three investigated taxa. Growth index was measured as the ratio of increase in fresh weight of the PLBs / calluses to the initial weight of the PLBs / calluses. In each calculation an average of 5 samples were taken.

Effects of various growth regulators on growth, development and regeneration of PLBs in *A. graminifolia* were recorded and presented in Table 2.3.4. The results presented in the table clearly showed that lower concentrations of IAA and NAA was very effective in promoting the growth of PLBs in *A. graminifolia* (Plate 7). Sharp increase in fresh weight of PLBs were obtained when 1 mg l^{-1} IAA was added to CM enriched VW medium. The growth index too was very high. Number of leaves and roots were optimum at the said concentration of IAA(Plate 7). However, higher concentrations of IAA than 2 mgl^{-1} was found inhibitory as it resulted in a significant decrease in fresh weight and growth index than control. The PLBs were deep green in colour. Enlargement of PLBs were initiated at the end of first week following inoculation which is followed by direct regeneration of almost all the PLBs into plantlets. Further increase in fresh weight of PLBs in *A. graminifolia* was noted when 1 mg l^{-1} NAA was added to the media. Nearly tenfold increase in fresh weight of PLBs was achieved when 1 mg l^{-1} NAA (Table 2.3.4) was added to CM enriched VW medium. The number and development of leaves and roots were optimum at this concentration of NAA. But the growth and development of PLBs were retarded when higher concentrations (3mg l^{-1} or 4 mgl^{-1}) of NAA was added. BAP and Kn at any concentration, on the other hand inhibit the growth and development of PLBs of *A. graminifolia*. The increase in fresh weight of PLBs and the growth index was significantly low in comparison to control. Only three to fourfold increase in fresh weight of PLBs was achieved in 8 weeks which is significantly less than control.

Plate 7

Table 2.3.4 Effect of growth regulators on growth, development and regeneration of PLBs in *A.graminifolia* All data were collected after 8 weeks following germination.

Treatment	Concentration mg l^{-1}	Weight# (Wt) of PLBS		Growth index	Seedling characteristics			
		Initial Wt (mg)	Wt. after 8 week (mg)		Leaf		Root	
					no	Size (mm)	no	size (mm)
	0.00	5.20	32 ± 1.09	5.15	3- 4	6	2	2-3
	0.5	5.20	39* ± 0.90	6.50	4	8	2	2
	1.0	5.20	53*** ± 0.56	9.19	6	16	4	6
IAA	2.0	5.20	37** ± 0.90	6.11	4	12	3	2
	3.0	5.20	26** ± 0.26	4.00	2	5	1	2
	4.0	5.20	20*** ± 0.37	2.84	2	4	6	--
	0.5	5.20	43** ± 0.40	7.26	4	12	3	5
	1.0	5.20	59*** ± 0.73	10.34	6	16	5	4
NAA	2.0	5.20	46** ± 0.30	7.84	6	11	3	6
	3.0	5.20	32 ± 0.21	5.15	4	11	--	--
	4.0	5.2	17*** ±0.90	2.26	2	5	1	1.5
	0.5	5.20	25** ± 0.87	3.80	2	9	--	--
	1.0	5.20	21** ± 0.90	3.03	4	11	--	--
BAP	2.0	5.20	19*** ±1.00	2.65	2	9	--	--
	3.0	5.20	20*** ± 0 .76	2.84	4	10	1	--
	4.0	5.20	17*** ± 0.20	2.26	2	6	--	--
	0.5	5.20	27 ± 0.46	4.19	2	6	1	1.5
	1.0	5.20	20** ± 0.67	2.84	2	7	--	--
Kn	2.0	5.20	18*** ± 0.50	2.46	2	9	1	3
	3.0	5.20	19*** ±1.00	2.65	4	7	3	2
	4.0	5.20	19*** ± 0.69	2.65	2	10	--	--

Values followed by asterisks in each organic additive treatment within the same column are significantly different from the control (nogrowth regulators) using Students t-test at *5% level; ** 1% level and *** 0.1% level.

Mean of 5 samples

Table 2.3.5 Effect of growth regulators on growth, development and regeneration of PLBS on *D. moschatum*. All data were collected after 8 weeks following germination.

Treatment	Concentration mg l^{-1}	Weight#(Wt) of PLBS			Seedling characteristics			
		Initial Wt of PLBs (mg)	Wt after 8 week (mg)	Growth index	Leaf no	Leaf Size (mm)	Root no	Root size (mm)
	0.00	4.60	24 ±0.34	4.21	2	7	1	3
	0.5	4.60	28 ± 0.56	5.08	2	6	2	2
	1.0	4.60	36 **± 0.90	6.82	2	8	2	1.5
IAA	2.0	4.60	41 ***± 0.32	7.91	4	12	3	2
	3.0	4.60	26 *± 1.40	4.65	2	5	1	2
	4.0	4.60	20 ***±1.00	3.34	2	6	--	--
	0.5	4.60	31 **± 1.20	5.73	3	8	1	2
	1.0	4.60	48 ***± 0.89	9.43	6	14	4	2.5
NAA	2.0	4.60	34 *± 0.90	6.39	4	8	3	2
	3.0	4.60	28 **±0.40	5.08	4	8	2	2
	4.0	4.60	17 ***± 0.43	2.69	2	5	1	1.5
	0.5	4.60	25± 0.20	4.21	4	7	2	3
	1.0	4.60	29 *± 0.74	5.30	4	11	3	3
BAP	2.0	4.60	34 ***± 0.90	6.39	5	17	4	2.5
	3.0	4.60	32 ***± 0.52	5.95	6	15	4	2
	4.0	4.60	28 ± 1.06	5.08	4	11	2	2
	0.5	4.60	24 ± 0.40	4.21	2	9	1	1.5
	1.0	4.60	28 *±1.20	5.08	3	7	1	2
Kn	2.0	4.60	31 **± 1.00	5.73	4	10	2	2
	3.0	4.60	26 ± 0.36	4.65	2	7	1	1
	4.0	4.60	14 ***±0.70	1.80	2	8	--	--

Values followed by asterisks in each organic additive treatment within the same column are significantly different from the control (no growth regulators) using Students t-test at *5% level; ** 1% level and *** 0.1% level.
Mean of 5 samples

The PLBs were pale green in colour and number of roots and leaves were less than control. (Plate 7) However, the PLBs directly regenerated into plantlets.

Lower concentrations of both IAA and NAA promoted the growth and development of PLBs in *D. moschatum*. Nearly tenfold increase in fresh weight in PLBs were achieved when 2 mg l^{-1} IAA was added to CM (15%) enriched KnC medium. 1 mgl^{-1} IAA too was very effective for the growth and development of PLBs in *D. moschatum* (Plate 8). Optimum number of leaves were formed when IAA was added. But the number of root was less. The PLBs started to increase in size at the middle or end of second week and directly differentiated into plantlets. Higher concentrations of IAA than 2 mgl^{-1} was found to be inhibitory for the growth and development of PLBs in *D. moschatum*. NAA had similar effect on PLB development of *D. moschatum*. Significant increase in fresh weight of PLBs and in growth index was recorded when 1 mg l^{-1} NAA was added to the medium. The PLBs were deep green in colour (Plate 8) and number and development of roots were optimum (Table 2.3.5).

BAP at 2 mgl^{-1} concentration was found to promote the growth of PLBs. Though the increase in fresh weight was not so sharp as observed in case of IAA or NAA, better regeneration was noted at this concentration of BAP. Higher concentrations than 3 mg l^{-1} was inhibitory and resulted in a sharp decrease in fresh weight of PLBs (Table 2.3.5). Kn, however, had no effect on seedling development of *D. moschatum*.

Effects of various growth regulators (either individually or in combinations) on growth, development and regeneration of callus in *G. densiflorum* was investigated and shown in the tables 2.3.6 and 2.3.7. The results clearly showed that IAA had a very little effect on growth and development of callus in *G. densiflorum*. No significant increase or decrease in fresh weight was noted when the calluses were inoculated in medium containing IAA. Lower concentration of NAA was found to promote the growth of callus. Nearly eightfold increase in fresh weight was recorded when 1 mgl^{-1} NAA was added in Peptone (2 gml^{-1}) enriched KnC medium. The percentage of callus frorming shoots was significantly high in it (Table 2.3.6). Higher concentrations of BAP like 2 mgl^{-1}or 3 mgl^{-1} were very effective in promoting the growth as well as the regeneration of callus in *G. densiflorum*.

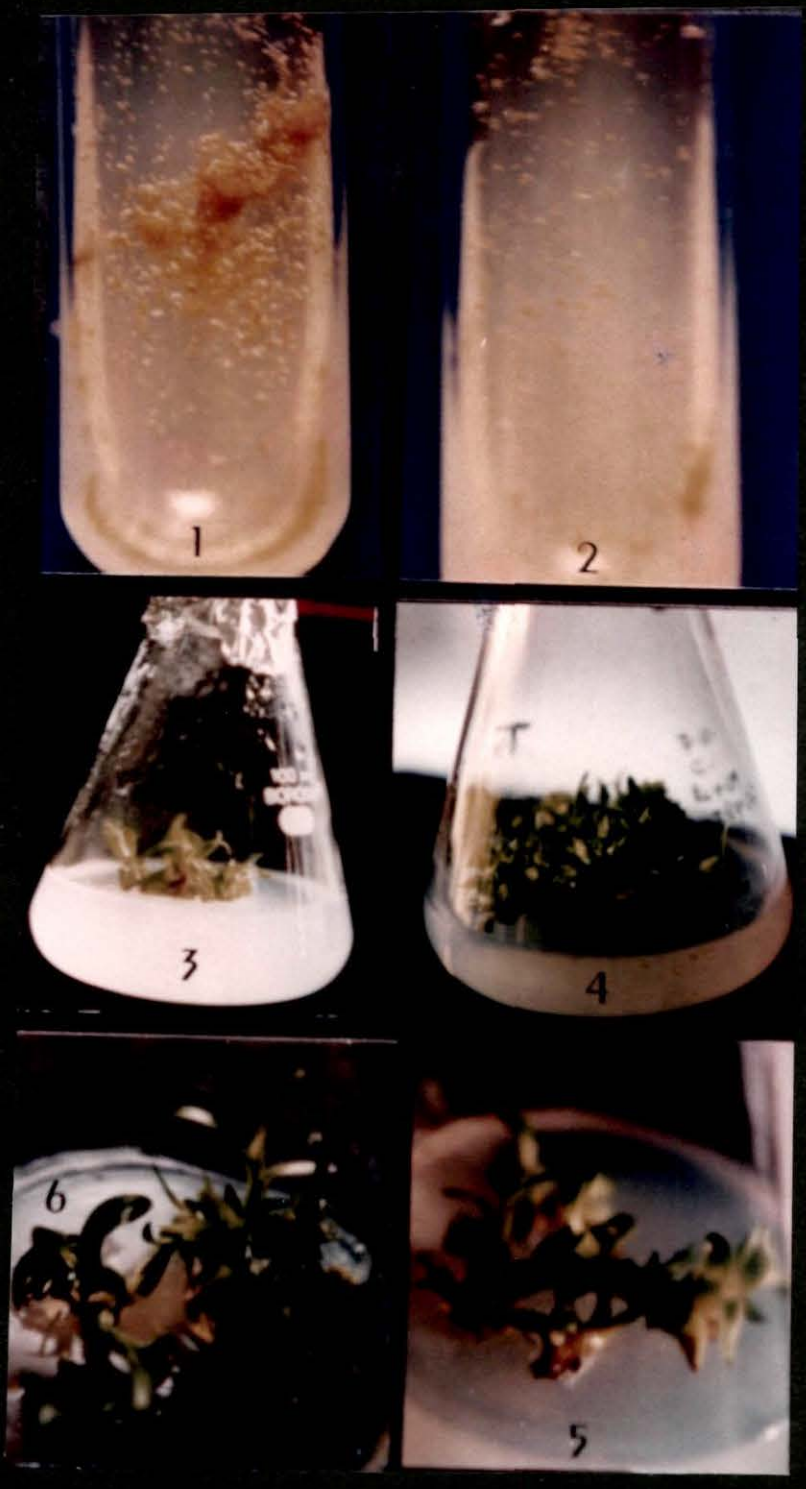

Plate 8

Table 2.3.6 Effect of growth regulators on growth, development and regeneration of callus in *G. densiflorum*. Data were collected after4 and 8 weeks following inoculation.

Treatment	Concentration (mg l^{-1})	Weight (Wt) of Callus: Initial Wt.# of callus (gm)	Wt after# 8 weeks (gm)	Growth index	% of callus forming shoots
Control	0.0	0.0048	0.018± 0.43	2.75	6
(KnC + PEP 2 gm l^{-1})	0.5	0.0048	0.020 ± 0.20	4.00	6
	1.0	0.0048	0.019± 0.80	2.90	14
IAA	2.0	0.0048	0.017 ±1.00	2.52	9
	3.0	0.0048	0.011 ± 0.20	1.29	17
	4.0	0.0048	0.009 ± 0.67	0.87	---
	0.5	0.0048	0.034***± 0.30	5.40	37
	1.0	0.0048	0.038***± 0.66	6.91	44
NAA	2.0	0.0048	0.024*± 0.90	4.00	26
	3.0	0.0048	0.019 ± 0.16	2.95	17
	4.0	0.0048	0.014 ± 0.43	1.91	---
	0.5	0.0048	0.025**± 0.37	4.20	21
	1.0	0.0048	0.033***±0.90	5.87	39
BAP	2.0	0.0048	0.047***± 0.10	8.79	47
	3.0	0.0048	0.053***± 0.76	10.04	42
	4.0	0.0048	0.027* ± 0.33	4.62	40
	0.5	0.0048	0.014± 0.39	1.91	--
	1.0	0.0048	0.014 ± 0.60	1.91	12
Kn	2.0	0.0048	0.016± 1.00	2.33	6
	3.0	0.0048	0.012***± 0.80	1.50	--
	4.0	0.0048	0.009***± 0.47	0.87	--

Values followed by asterisks in each organic additive treatment within the same column are significantly different from the control (growth regulators) using Students t-test at *5% level; ** 1% level and *** 0.1% level.

Mean of 5 samples.

Table 2.3.7 Effect of various combinations of BAP and NAA on growth, development and regeneration of callus in G. densiflorum.

Hormone Concentration (mgl^{-1})		Weight (Wt) of callus		Growth index	% of callus forming shoots
BAP	NAA	Initial Wt.* (gm)	Wt* after 8 weeks (gm)		
0.0	0.0	0.0048	0.037 ± 0.76	6.70	28.0
0.5	0.5	0.0048	0.047** ± 0.33	8.79	47.0
1.0	0.5	0.0048	0.050** ± 0.20	9.41	63.0
2.0	0.5	0.0048	0.057*** ± 0.65	10.87	72.0
3.0	0.5	0.0048	0.057*** ± 0.50	9.41	59.0
0.5	1.0	0.0048	0.058*** ± 0.43	11.08	52.0
1.0	1.0	0.0048	0.059*** ±.080	11.29	58.0
2.0	1.0	0.0048	0.064*** ±1.06	12.33	63.0
3.0	1.0	0.0048	0.041 ± 0.87	7.54	49.0
0.5	2.0	0.0048	0.042* ± 0.10	7.75	50.0
1.0	2.0	0.0048	0.057*** ± 0.73	10.87	52.0
2.0	2.0	0.0048	0.049** ± 0.60	9.20	46.0
3.0	2.0	0.0048	0.042 ± 0.80	7.75	49.0
0.5	3.0	0.0048	0.030 * ± 1.00	5.25	32.0
1.0	3.0	0.0048	0.021*** ± 0.67	3.37	26.0
2.0	3.0	0.0048	0.018*** ± 0.54	2.75	21.0
3.0	3.0	0.0048	0.013*** ± 0.30	1.70	17.0

Values followed by asterisks in each organic additive treatment within the same column are significantly different from the control (no growth regulators) using Students t-test at *5% level; ** 1% level and *** 0.1% level.

Significant and manyfold increase in fresh weight of callus was achieved and 47 percent calluses were found to develop shoot when 2 mg l^{-1} or 3 mg l^{-1} BAP was added to peptone supplemented KnC medium. The calluses were green, hard and compact in nature (Plate 9). Higher concentrations of BAP than 3 mg l^{-1} retarded the growth of callus and thus inhibitory for the process. However, the regeneration percentage expressed as the percentage of callus forming shoot, was not satisfactory when BAP or NAA was used individually. Kn had no effect either on growth or regeneration of callus in *G. densiflorum*.

Various combinations of BAP and NAA was tested to obtain better growth and regeneration of callus. Optimum development and regeneration (Plate 10) was achieved when 2 mg l^{-1} BAP and 1 mg l-1 NAA was added together with peptone 2 gm l^{-1} supplemented moified KnC medium. The growth index obtained in the said combination was very high (Table 2.3.7) and 72% calluses were found to regenerate. But when higher concentrations of BAP was combined with higher concentrations of NAA, growth of callus was inhibited (Table 2.3.7).

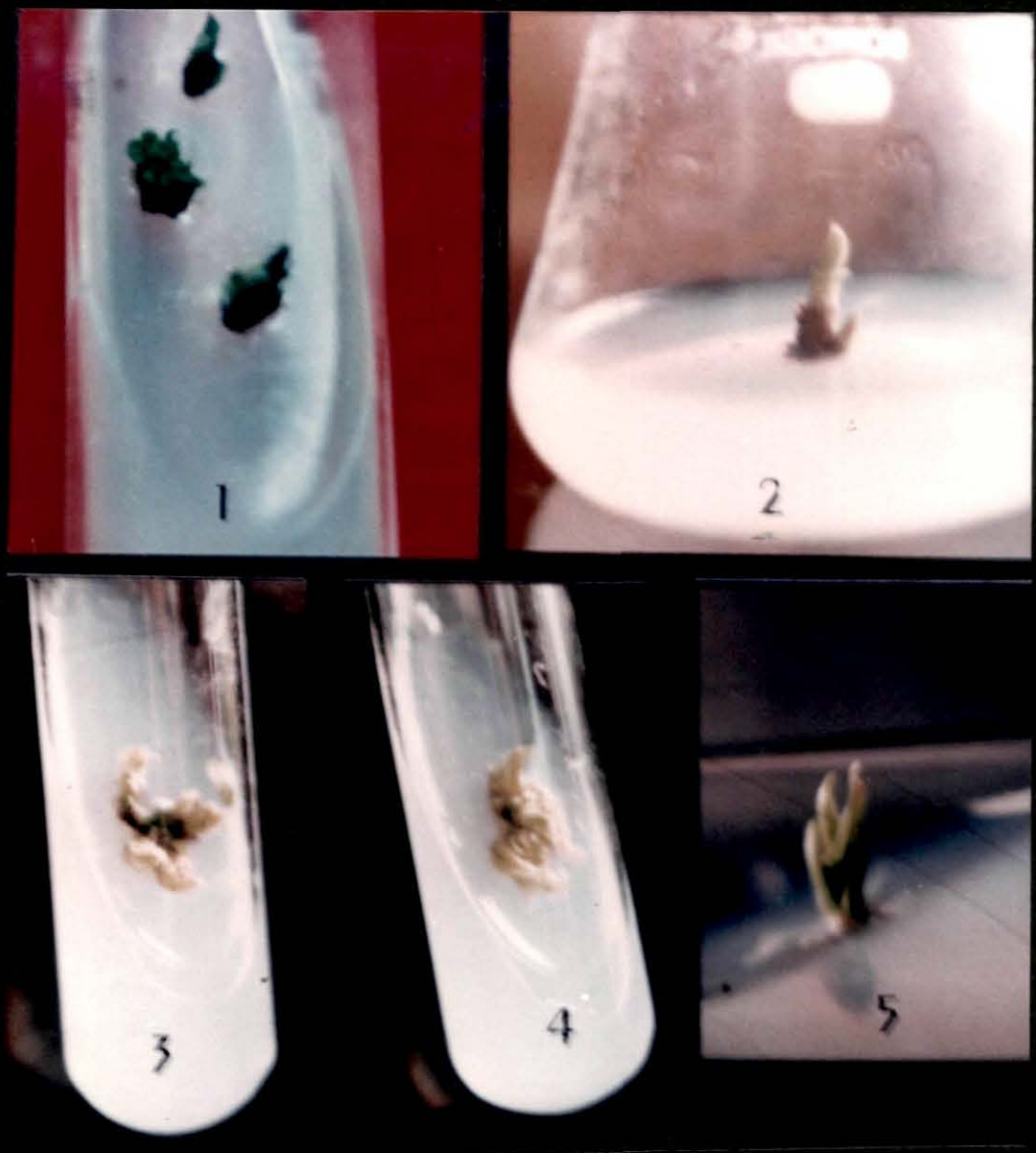

Plate 10

2.4 DISCUSSION

The present investigation deals with the effects of various growth regulators on seed germination; growth, development and regeneration of PLBs in three critically endangered orchids of North Eastern Himalayas, namely, *Arundina graminifolia, Dendrobium moschatum* and *Geodorum densiflorum*. Though during the last three decades tissue culture techniques have been extensively exploited for micropropagation of orchids (Prakash *et al* 1995, Park *et al* 1996), and huge literature has been accumulated, few of them were directed to find out the proper nutritional and hormonal requirement for optimum germination, better growth and development and regeneration. Meagre information is available in this regard in *A. graminifolia* and *D. moschatum* while such information is completely lacking in *G. densiflorum*. Due to either lack of or inadequacy of information in this regard in the three investigated orchid taxa, the present work was directed to find out suitable growth regulators for seed germination; better growth, development and regeneration of PLBs/ calluses in the orchids concerned.

A detailed and systematic investigation has been done to elucidate the effects of various growth regulators on seed germination; growth, development and regeneration of PLBs/ calluses in *A. graminifolia, D. moschatum* and *G. densiflorum*. It has been observed that plant growth regulators elicit different responses in seed germination and growth, development and regeneration of PLBs and calluses depending on the nature of growth regulators and their concentrations used. Lower concentrations of IAA and NAA promoted the seed germination and growth and development of PLBs in *A. graminifolia* and *D. moschatum*. 86% seed germination was recorded in *A. graminifolia* when 1 mgl^{-1} IAA was added to CM enriched VW media. Further increase in germination was achieved when IAA was replaced by NAA. 92% seeds were germinated when the seeds were inoculated in medium containing NAA. However higher concentrations of both IAA and NAA were found inhibitory to seed germination. IAA and NAA had almost similar effects on seed germination of *D. moschatum*. 81% and 84% germination was recorded in *D. moschatum* when 2mg l^{-1} IAA and 1 mg l^{-1} NAA was added respectively to CM supplemented KnC medium.

However, higher concentrations of IAA and NAA had been inhibitory to seed germination in *D. moschatum*. That IAA promotes seed germination in many orchids was reported earlier by several workers (Arditti 1967, Blowers 1958, Ernst 1967, Ichihasi and Kako 1973, Kano 1968, Mathews and Rao 1980, Mitra 1975, Straus and Resinger 1976). The results obtained in the present study in *A. graminifolia* and *G. densiflorum* find support in these earlier reports. Although inhibitory effects of IAA and NAA on seed germination were reported in a few orchids (Hadley and Harvais 1968, Hadley 1970), findings of the present investigation in *A. graminifolia* and *D. moschatum* do not support that.

BAP and Kn at any concentration was proved inhibitory to seed germination in *A. graminifolia.* Significant decrease in seed germination percentage was noted when BAP or Kn was added to the medium. But in *D. moschatum*, BAP slightly promoted the seed germination. However, Kn had inhibitory effect on seed germination of *D. moschatum.* The inhibitory effect of Kn on seed germination was earlier reported in a few orchids like *Dendrobium* and *Laeliocattleya* by Kano (1965) which finds support in the present investigation.

Apart from promoting seed germination, both IAA and NAA had been very effective for growth and development of PLBs in *A graminifolia* and *D. moschatum.* Sharp and significant increase in fresh weight of PLBs and higher growth indexes were obtained when IAA and NAA were used as growth regulators. A perusal of earlier literature reveals that IAA and NAA promoted seedling growth in a number of orchids (Boesmann 1962, Blowers 1968, Hayes 1969, Israel 1963, Mayer and Pelloux 1948). The results of the present investigation in *A. graminifolia* and *D. moschatum* find support in these earlier reports. BAP was found inhibitory for *A. graminifolia* but promoted seedling development and optimum root and leaf development in *D. moschatum.*

Plant growth regulators used either individually or in combination elicit different results in seed germination and growth, development and regeneration of callus in *G. densiflorum.* IAA had no effect on seed germination or on seedling growth. NAA and BAP when used individually with Peptone enriched KnC medium promoted seed germination in *G. densiflorum.*

They were also very effective in growth and development of callus. Both BAP and NAA resulted in a sharp increase in fresh weight of callus when used individually. But, none of them alone could yield optimum regeneration, expressed as percentage of callus producing shoots. Optimum callus growth and regeneration was achieved when BAP and NAA was used in combination. Sharp and manifold increase in fresh weight of PLB and higher regeneration percentage was achieved when 2 mg l-1 BAP and 1 mg l^{-1} NAA was added to peptone enriched modified KnC medium. The interacting effect of auxin - cytokinin is well known in orchids (Hadley and Harvais 1968, Harvais 1982, Pierik and Steegmans 1972, Rao 1977). The present study in *G. densiflorum* confirms the earlier reports. Moreover, it has lead to the introduction of current idea that reactions between auxin and cytokinin provide better mechanism for regulation of all types of morphogenetic phenomena observed in *in vitro* raised plants. In *A. graminifolia* and *D. moschatum* where CM was very effective in seed germination, IAA or NAA alone was very effective in both seed germination and growth and regeneration of PLBs. This may be due to the fact that CM might have acted as a source of cytokinin which ultimately lead to a proper auxin-cytokinin interaction and promoted better growth and regeneration. However, Kn was found inhibitory to seed germination for all the three taxa included in the present investigation.

Experiments with auxins and cytokinins either individually or in combination on orchid seed germination and seedling growth have given inconsistent and therefore inconclusive results (Arditti 1979). In the present study it has been observed that plant growth regulators markedly influenced seed germination and subsequent stages of growth and regeneration. This can be used for micropropagation of endangered orchids whose existence are threatened due to various reasons.

2.5 SUMMARY

This part of the present study deals with the effects of various growth regulators on seed germination and growth, development and regeneration of PLBs/calluses of three critically endangered orchids of North Eastern Himalayas, namely, *Arundina graminifolia, Dendrobium moschatum* and *Geodorum densiflorum*. Extensive and systematic investigation revealed a differential response of the growth regulators on different taxa.

Optimum seed germination and better growth of PLBs were obtained in *A. graminifolia* when the seeds were inoculated in medium containing lower concentrations of IAA or NAA. 86% seed germination was recorded when 1 mg l^{-1} IAA was added to CM (15%) enriched VW medium. Further increase in germination percentage was achieved when IAA was replaced by NAA. 92% seed germination was recorded when 1 mgl^{-1} was added to the CM supplemented VW medium. Higher concentrations of both were inhibitory to both seed germination and growth of PLBs. BAP and Kn at any concentration proved inhibitory to seed germination and growth of PLBs in *A. graminifolia*. Sharp decrease in germination percentage and fresh weight of PLBs was recorded when BAP or Kn was added to the medium.

IAA and NAA had similar effects on seed germination and seedling growth of *D. moschatum* when used with CM supplemented KnC medium. Both promoted seed germination and seedling growth in *D. moschatum*. 81% seed germination was recorded when 2 mgl^{-1} IAA was added to the medium. The growth index too was found very high when the said concentration of IAA was added. NAA at $1mgl^{-1}$ resulted in a significant increase in seed germination. The fresh weight of PLBs increased significantly when 1 mgl^{-1} NAA was added to the medium. Higher concentrations of both the auxins were inhibitoy to seed germination and seedling growth as significant decrease in both germination percentage as well as the fresh weight of PLBs were noted at those concentrations. BAP slightly promoted seed germination in *D. moschatum* and was found very effective for the growth and regeneration of PLBs. However, Kn was inhibitory to both seed germination and seedling growth of *D. moschatum*.

In *G. densiflorum*, IAA had no effect on seed germination and growth of callus. NAA on the other hand promoted seed germination. Significant increase in seed germination was recorded when 1 mgl^{-1} was added to Peptone supplemented KnC medium. Moreover, NAA was found very effective for callus growth. BAP too was very effective for seed germination and seedling growth of *G. densiflorum*. Shoot formation from callus was increased when BAP was added to the medium. However, Kn was found inhibitory to seed germination as well as for the growth of callus. Although both BAP and NAA alone was effective in promoting seed germination and growth of callus, the regeneration percentage (expressed as percentage of callus forming shoot) was not optimum. But BAP-NAA combination resulted in a sharp increase in fresh weight of callus and in regeneration percentage. 72% regeneration was recorded when 2 mgl^{-1} BAP and 1 mgl^{-1} NAA was added to Peptone supplemented KnC medium.

It has been observed that IAA promoted seed germination in *A. graminifolia* and in *D. moschatum* but had no effect on *G. densiflorum*. NAA was effective for both seed germination and growth, development of PLBs / calluses in all the three studied genera. BAP was very effective in seed germination and subsequent growth of PLBs /callus in *D. moschatum* and *G. densiflorum*. However, it was found inhibitory for *A. graminifolia*. Kn was inhibitory to seed germination and seedling growth of all the three orchids investigated.

It can be concluded that the growth regulators markedly influence seed germination and subsequent growth of PLB and callus of orchids. The subtle interraction of auxins and cytokinins could be beneficially utilized for micropropagation of orchids. This would be helpful in managing the existing orchid populations and reestablishing some of those whose existence have been threatened.