Influence of growth regulators on asymbiotic germination and early seedling development of *Coelogyne punctulata* Lindl.

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Abstract The effects of various growth regulators, viz., indole-3-acetic acid (IAA), α-naphthalene acetic aid (NAA), 2, 4-dichlorophenoxy acetic acid (2, 4-D), 6-furfurylaminopurine (kinetin), and gibberellic acid (GA_3), in different concentrations (0.1–10 mg/l) and combinations in Kn C medium were studied on seed germination and seedling growth of *Coelogyne punctulata*. In another experiment, 90-day-old protocorms, developed on Kn C basal medium, were transferred to media containing growth regulators to study their influence on the seedling growth alone. IAA and 2, 4-D were inhibitory for both germination and seedling growth. However, IAA at 0.1 mg/l slightly promoted germination and seedling development. While NAA was stimulatory for seedling growth in protocorms developed on basal medium, its initial use did not significantly affect the germination and seedling growth. The influence of GA_3 was insignificant. Both germination and seedling growth were promoted by kinetin at lower concentrations. The best results were obtained when kinetin (1 mg/l) was used in conjunction with NAA (0.1 mg/l).

INTRODUCTION

The orchids produce many seeds but they require mycorrhizal association for germination in nature (Arditti, 1967). However, less than 5% of the seeds germinate in their natural environment. On the other hand, seeds sown in nursery beds require long periods of germination and any disturbance due to the soil or physical environment destroys the whole population (Northen, 1962). The propagation of orchids through *in vitro* germination of seeds has been

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emphasized by many workers (Arditti, 1967; Arditti *et al.*, 1981, 1982; Clement, 1973, 1981; Clement and Ellyard, 1979; Curtis, 1943; Ernst, 1975; Knudson, 1946, 1951; Mitra, 1971; Stoutamire, 1964, 1974, 1981). A number of media and their modifications in respect of macro- and micronutrient (Ziegler *et al.*, 1967; Arditti *et al.*, 1981; Henrich *et al.*, 1981), amino acid (Raghvan and Torrey, 1964; Fonnesbech, 1972), vitamin (Noggle and Wynd, 1943; Mead and Bulard, 1975, 1979), and carbohydrate (Ernst, 1967; Fonnesbech, 1972) contents have been used. To promote seed germination and seedling growth in orchids, many plant growth regulators have been tried (Hadley and Harvais, 1968; Goh, 1970; Hadley, 1970; Pierik and Steegmans, 1972; Harvais, 1973, 1982; Straus and Reisinger, 1976). The effects of the photoperiod, light intensity and/or quality, temperature, and pH on orchid seed germination are reviewed (Arditti, 1967). The physico-chemical requirements of orchid seed germination and seedling growth vary from species to species. Since very little critical experimental work seems to have been done, particularly with regard to distinguishing between effects of growth regulators on seed germination and subsequent growth and development of the seedling, the present study was conducted to fill this gap in information, using *Coelogyne punctulata* Lindl. Incidentally, this species with attractive flowers is becoming scarce in Meghalaya due primarily to its extensive collection and depleted natural habitats. In an attempt to promote its seed germination and seedling growth, a number of growth substances were tested during the present investigation.

MATERIAL AND METHODS

The plants of *Coelogyne punctulata* were collected from the forests in Meghalaya and grown in net houses. The flowering occurs in May-June. Immature capsules were sterilized in 7.5% sodium hypochlorite solution for 15 min and repeatedly rinsed with sterilized distilled water. The seeds were then extracted under aseptic conditions and sown in Kn C basal medium in one set and also in media containing different concentrations (0.1–10 mg/l) of growth regulators, viz., IAA, NAA, 2, 4-D, GA_3, and kinetin (kn), separately. Kinetin was also used in conjunction with NAA or IAA. The pH of each medium was adjusted to 5.2 before autoclaving at 1.06 kg/cm^2 for 15 min.

The seeds were germinated in dark for 9 weeks at 25°C $\pm$ 2°C and later transferred to continuous illumination of 3000 lux. The 90-day-old protocorms developed in Kn C basal medium were transferred to fresh medium having different growth regulators in the concentrations just mentioned. The percentage germination and seedling growth were recorded in the 9th and 26th week, respectively. For each treatment, 5 replicates were taken.

RESULTS

The effects of different growth regulators both on the germination and seedling development (Table I) and the seedling development alone (Table II) of *Coelogyne punctulata* are summarized. Some of the salient observations are as follows.

IAA AND NAA

IAA at 0.1 mg/l slightly promoted seed germination and seedling growth. However, its higher concentrations were inhibitory (Table I). Except at 0.1 mg/l, where the leaf size and number

Table I Effect of growth regulators supplemented in Kn C medium on seed germination and seedling growth of *Coelogyne punctulata*

Treatment	Concentration (mg/l)	Germination after 9 weeks (%)	Seedling characteristics after 26 weeks of culture						
			Fresh weight (mg)	Leaf		Root		Colour	Development
				Size (mm)	Number	Size (mm)	Number		
Control		64	18	3	2	2	1	Green	Poor
IAA	0.1	68	24	3	2	2	1	Green	Good
	0.5	62	23	2	2	1	2	Green	Poor
	1.0	60	22	1	1			Yellowish	Poor
	5.0	58	19					Brown	Inhibited
	10.0	40	18					Brown	Inhibited
NAA	0.1	64	24	4	2	2	1	Green	Best
	0.5	60	20	3	2	1	2	Green	Good
	1.0	60	21	2	1	1	1	Green	Poor
	5.0	54	20					Yellowish	Inhibited
	10.0	52	16					Yellowish	Inhibited
2, 4-D	0.1	52	18	2	2			Brownish	Poor
	0.5	40	20					Brown	
	1.0	38	22					Brown	
	5.0	30	20					Brown	
	10.0								Inhibited
GA_3	0.1	62	18	2	1	1	2	Green	Poor
	0.5	63	24	3	2	2	2	Green	Good
	1.0	58	22	3	2	1	2	Yellowish	Poor
	5.0	54	20	2	2	1	1	Yellowish	Poor
	10.0	52	20					Yellowish	

Table I **Effect of growth regulators supplemented in Kn C medium on seed germination and seedling growth of *Coelogyne punctulata*** (cont.)

Treatment	Concentration (mg/l)	Germination after 9 weeks (%)	Seedling characteristics after 26 weeks of culture						
			Fresh weight (mg)	Leaf		Root		Colour	Development
				Size (mm)	Number	Size (mm)	Number		
kn	0.1	86	26	5	2	2	2	Dark green	Best
	0.5	82	22	3	2	2	1	Dark green	Good
	1.0	58	22	2	1			Dark green	Inhibited
	5.0	50	20					Green	Inhibited
	10.0	45	18					Green	Inhibited
kn+NAA	1.0 + 0.1	88	27	5	3	3	2	Green	Best
IAA	1.0 + 0.1	71	22	3	2	1	2	Light green	Good

Table II Effect of growth regulators on *Coelogyne punctulata* protocorms developed on Kn C medium

Treatment	Concentration (mg/l)	Seedling characteristics after 26 weeks of culture						
		Fresh weight (mg)	Leaf		Root		Colour	Development
			Size (mm)	Number	Size (mm)	Number		
Control		16	1	2			Green	Poor
IAA	0.1	24	3	3			Green	Poor
	0.5	22	3	2			Yellowish	Poor
	1.0	20	1	2			Brownish	Inhibited
	5.0	20					Brown	Inhibited
	10.0	19					Brown	Inhibited
NAA	0.1	30	8	3	7	2	Green	Best
	0.5	26	5	2	3	2	Green	Good
	1.0	23	3	2	2	1	Yellowish	Poor
	5.0	22					Brown	Inhibited
	10.0	21					Brown	Inhibited
2, 4-D	0.1	21					Yellowish	Inhibited
	0.5	22					Brownish	Inhibited
	1.0	22	1	1	1	1	Brown	Poor
	5.0	26					Brown	Inhibited
	10.0	23					Brownish	Inhibited
GA_3	0.1	20					Green	Inhibited
	0.5	21					Green	Inhibited
	1.0	23	2	1			Yellowish	Poor
	5.0	26	3	3			Yellowish	Good
	10.0	22	2	2			Yellowish	Poor

Table II Effect of growth regulators on *Coelogyne punctulata* protocorms developed on Kn C medium (cont.)

Treatment	Concentration (mg/l)	Seedling characteristics after 26 weeks of culture						
		Fresh weight (mg)	Leaf		Root		Colour	Development
			Size (mm)	Number	Size (mm)	Number		
kn	0.1	26	3	2	2	1	Dark green	Good
	0.5	30	7	3	5	4	Dark green	Best
	1.0	24	3	2	2	1	Green	Poor
	5.0	22	1	2			Green	Inhibited
	10.0	20					Green	Inhibited
kn+NAA	1.0 + 0.1	33	8	4	6	3	Dark green	Best
kn+IAA	1.0 + 0.1	24	6	2	5	2	Light green	Good

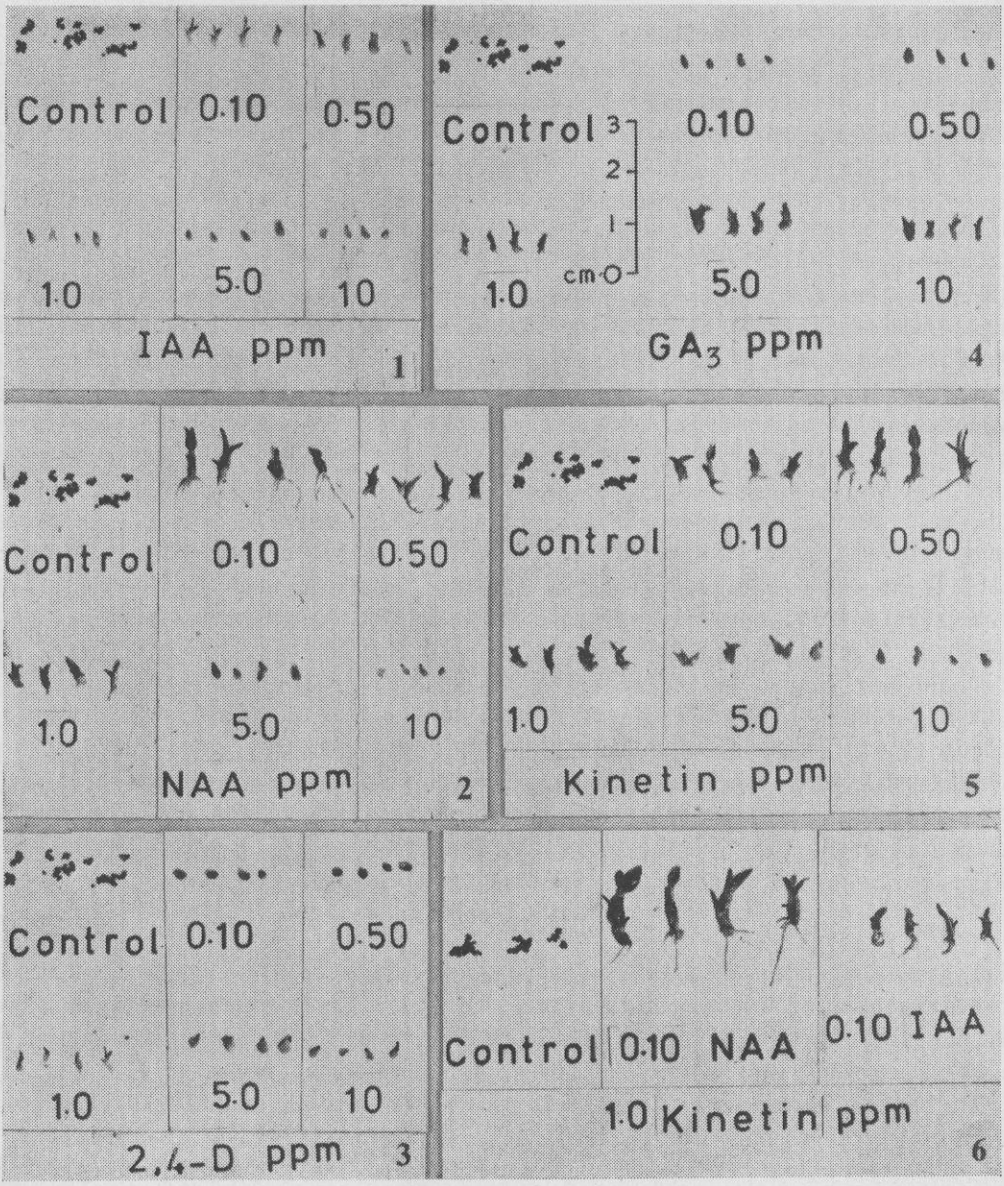

Fig. I *Coelogyne punctulata* seedlings. 1–6, effect of growth regulators: 1, IAA; 2, NAA; 3, 2, 4-D; 4, GA_3; 5, kinetin; 6, kinetin + NAA, kinetin + IAA.

increased, the influence of IAA on seedling development alone was inhibitory and the roots did not develop at any of the concentrations of this auxin in the medium (Table II, Fig. I 1). Though NAA did not significantly influence seed germination and seedling growth at lower concentrations (Table I), it markedly enhanced seedling development at 0.1 mg/l concentration (Table II, Fig. I 2).

2, 4-D

Both germination and seedling development were inhibited by 2, 4-D at all concentrations (Tables I, II). In the experiment on seedling development alone, concentrations higher than 0.1 mg/l resulted in callus formation (Fig. I 3). The only exception was the concentration 1 mg/l of 2, 4-D at which single leaf and root initiation were recorded.

GA_3

Different concentrations of GA_3 showed little effect on seed germination and seedling growth (Table I). However, at 10 mg/l, leaf and root formations were completely inhibited. The lower concentrations of GA_3 (0.1 mg/l and 0.5 mg/l) completely inhibited seedling development (Table II, Fig. I 4). However, at higher concentrations, the leaf development alone was promoted.

KINETIN

The germination and seedling growth were much higher at 0.1 mg/l of kinetin in the medium (Table I). As shown in Table II and Fig. I 5, at 0.5 mg/l of kinetin, both leaf and root development were optimum. However, higher concentrations were inhibitory.

KINETIN WITH NAA OR IAA

Kinetin (1 mg/l) in conjunction with NAA (0.1 mg/l) was better as compared to kinetin (1 mg/l) and IAA (0.1 mg/l) for both seed germination and seedling growth (Table I). The same was true for their influence on seedling development (Table II, Fig. I 6). The interacting influence of kinetin and NAA showed optimum results.

DISCUSSION

Plant growth regulators elicit different responses in orchid seed germination and seedling growth depending on the concentrations used. The results of the present investigations reveal that the effect of growth regulators on seedling development alone is more pronounced than in a situation where both germination and seedling development occur.

The inhibitory effects of IAA have been reported on germination and seedling growth in *Orchis purpurella* (Hadley and Harvais, 1968), *Dactylorhiza purpurella*, *Coeloglossum viride*, and *Platanthera bifolia* (Hadley, 1970). On the other hand, IAA promoted seedling growth in *Laeliocattleya* (Kano, 1965) and *Phalaenopsis* (Ernst, 1967). In the present investigation, IAA at 0.1 mg/l slightly promoted seed germination and seedling growth as also leaf development in experiments on the effect of IAA on seedling development alone. However, higher concentrations were inhibitory. NAA at lower concentrations (0.1 mg/l and 0.5 mg/l) enhanced seedling development markedly as compared to its influence on seed germination and seedling growth. Though the optimum concentrations are not indicated, NAA stimulates germination and seedling growth in several species like *Cattleya warheri* (Mayer and Pelloux, 1948), *Epidendrum nocturnum* (Yates and Curtis, 1949), *Cattleya* (Withner, 1951), *Dendrobium* (Israel, 1963), *Cattleya aurantiaca*, *Cymbidium madidum*, *Bletilla* sp., and *Chondrorhyncha*

discolor (Straus and Reisinger, 1976). 2, 4-D was found to be inhibitory for both germination and seedling development of *C. punctulata*, though it promoted callus formation. Similar inhibitory effects have been reported by Goh (1970) in *Vanda* Miss Joaquim.

GA_3 did not show much difference in germination in *C. punctulata*. However, at lower concentrations (0.5–1.0 mg/l), it slightly enhanced seedling development. In the experiment on the influence of GA_3 on seedling development alone, a reverse picture was obtained where higher concentrations (5.0 mg/l, 10.0 mg/l) promoted only the leaf development and the root development did not occur. Higher concentration of GA_3 in *Bletilla striata*, *Dendrobium*, and *Brassolaeliocattleya* (Kano, 1965) resulted in inhibition of germination, leaf and root growth, and mortality of the seedling. On the other hand, Blowers (1958), Hirsh (1959), and Harvais (1982) observed that gibberellic acid was stimulatory for germination and/or seedling growth in *Cattleya* and *Cypripedium*, respectively.

In *C. punctulata*, kinetin showed a pronounced stimulatory effect on both germination and seedling growth. While kinetin has been reported to be stimulatory for germination and seedling growth in *Cattleya* (Pierik and Steegmans, 1972) and *Cypripedium reginae* (Harvais, 1982), its inhibitory role was observed in *Dendrobium* and *Laeliocattleya* (Kano, 1965).

From the results obtained in the present study, it is clear that kinetin at higher concentrations prevents rooting, whereas NAA and IAA at lower concentrations promote it. It was interesting to note the interacting effects of kinetin and auxin on seedling growth. The kinetin : NAA ratio of 10 : 1 resulted in best germination and subsequent growth in *C. punctulata*. This is in line with the studies of Harvais (1982) in *Cypripedium reginae*. The interacting influence of kinetin and auxin on shoot/root balance in orchids is very well known (Hadley and Harvais, 1968; Pierik and Steegmans, 1972; Rao, 1977; Harvais, 1982).

It may be concluded that the use of growth regulators markedly influences the germination and seedling growth of orchids. The subtle interaction of auxin and cytokinin could be beneficially utilized for raising seedling *in vitro*. This would be helpful in managing the existing orchid populations and in re-establishing some of those orchids that have disappeared from a particular region.

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