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Control of Germination and Tube Elongation in *Theobroma cacao* L. Pollen*

The role of various growth regulators in pollen germination and tube growth has been studied in recent years (Sondheimer and Linskens, 1974; Balasimha and Tewari, 1977). The germination and tube extension are accompanied by initiation of protein synthesis and rapid formation of polysomes (Linskens, Schrauwen and Konings, 1970; Mascarenhas and Bell, 1969). The basic requirements for the *in vitro* germination of cacao pollen has been standardised (Ravindran, 1977). The role of growth regulators in pollen germination and tube growth in cacao is described in this paper.

Freshly opened flowers of *Theobroma cacao* L. var Amazon Forastero were collected at 08.00 hr. The pollen grains were dusted on 1.5 cm^2 cellophane papers and floated on liquid medium of Brewbaker and Kwack (1963) containing 15% sucrose with or without test chemicals. The cellophane paper was removed after 1, 2 or 3 hr incubation, killed and stained with phenolaniline blue. Germination and

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tube lengths were recorded and statistically analysed.

For studying the effect of actinomycin D and p–flourophenylalanine during pre–anthesis period, the flower buds were forced open about 12–15 hr before anthesis and the chemicals were sprayed using a syringe. Distilled water was sprayed as control. After anthesis pollen grains were collected for germination studies.

The effect of growth regulators and inhibitors on germination and growth of pollen tube is presented in Table I. Gibberellic acid, IAA and BAP enhanced tube elongation significantly though the percent germination was unaffected except in case of BAP. The role of growth hormones and cyclic–AMP in enhancing pollen tube growth has been reported in *Calotropis procera* (Balasimha and Tewari, 1977). In cacao pollen also cyclic–AMP increased tube growth at optimal concentrations (Table I). However, auxin, cytokinin or GA_3 had no effect on pollen tube extension in *Petunia hybrida* (Sondheimer and Linskens, 1974). On the contrary, they reported small but reproducible promotive effect of ABA. Our results on the promotive effect of ABA in cacao pollen is in agreement with reports of Sondheimer and Linskens (1974) and those of Shukla and Tewari (1973). In *Calotropis procera* low

Table I. *Effect of pollen germination and growth*

Treatment	Tube length (μ m)		Per cent germination (3 hr)
	1 hr	3 hr	
Experiment 1			
Control	64.28	165.53	53.7
ABA (5)+	101.27*	208.30*	43.7
ABA (25)	89.10	135.43*	37.9
GA_3 (10)	133.98**	229.42**	46.6
IAA (10)	76.56**	237.47**	62.8
BAP (5)	107.45**	160.64	25.8
Experiment 2			
Control	140.05	266.00	51.0
Cyclic AMP (10)	213.75*	280.62*	60.0
Cyclic AMP (20)	175.00*	272.25	75.0
Actinomycin D (0.5)	147.50	272.50	50.0
Actinomycin D (1.0)	132.50	265.50	53.0
Experiment 3			
Control	45.41	89.76	60.2
p–flourophenylalanine (10)	38.81	66.00	24.8
p–flourophenylalanine (20)	48.05	51.88	15.8
Chloramphenicol (10)	43.96	97.94	51.8
Chloramphenicol (20)	59.14	102.70	50.2

\+ Numbers in parentheses indicate concentration in ppm
* Significant at 5% level
** Significant at 1% level

Table II. *Effect of actinomycin D and p-flourophenylalanine when sprayed 12–15 hr before anthesis*

Treatment		Tube length (μ m) (2 hr)	% germination (2 hr)
Control		214.00	61.49
Actinomycin D	1 ppm	166.00**	32.19
Actinomycin D	10 ppm	158.62**	36.03
p-flourophenylalanine	10 ppm	138.00**	28.74
p-flourophenylalanine	20 ppm	185.55**	34.21

** Significant at 1% level

concentrations of ABA enhanced tube growth markedly (Shukla and Tewari, 1973) which is much contrary to the generally inhibitory role of this natural growth inhibitor.

None of the inhibitors *viz.*, actinomycin D, chloramphenicol and p-flourophenylalanine had any significant effect on pollen tube growth. However, p-flourophenylalanine inhibited the germination. This suggested neither mRNA nor protein synthesis was affected possibly because sufficient long lived mRNA and proteins were available for these processes. Furthermore, data obtained with chloramphenicol show that new synthesis of mitochondria is also not required. To test that mRNA and protein synthesis occur before anthesis, actinomycin D and p-flourophenylalanine were sprayed 12-15 hr, before anthesis and later examined for germination and growth of pollen taken from these treated flowers after opening. The result shows that there was marked and significant inhibition not only in tube extension but in germination also (Table II). These data indicated that RNA and protein synthesis occurred during this pre-anthesis period and that is the reason why pollen tube growth was not affected *in vitro* by these inhibitors during post-anthesis period. Growth regulators like GA3, IAA, BAP and ABA are limiting in these processes though their precise mechanism of action remains to be known.

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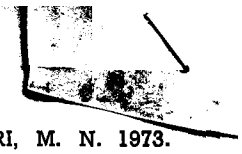

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Colchicine Induced Tetraploids in Ginger *(Zingiber officinale* Rosc.)*

The crop improvement work in ginger (*Zingiber officinale* Rosc.) at present is confined to collection of accessions from different growing areas, their comparative yield evaluation and selection. Evaluation of newer varieties through hybridisation is not feasible since majority of the cultivars do not flower and none of them set seed either by selfing or by cross pollination (Ratnambal, 1979; Nair, Nambiar and Ratnambal, 1982). Cytological evidences indicate that the sterility in the genus *Zingiber* is likely to be chromosomal (Ratnambal, 1979). The present attempt to synthesise tetraploids in ginger was undertaken to explore the possibilities of obtaining regular flowering and seed set.

Tetraploids were induced in ginger cultivar Rio de Janeiro through colchicine treatment during 1977–78 and comparative morphological and anatomical characteristics of diploids and induced tetraploids are presented in this communication.

Fresh rhizomes of ginger cultivar Rio de Janeiro were washed in water, dried with cotton wad and separated into single buds. These buds were treated with 0.1, 0.25, 0.5, 0.6 and 1% aqueous colchicine or colchicine in agar paste for 2, 4, 6, 8, 12 and 24 hr duration. The aqueous colchicine was applied either by wetting the growing point by means of cotton or by keeping germinated rhizomes inverted in vials containing the solution.

Colchicine in agar was applied to the growing points with a camel-hair brush. In control, the rhizomes were treated with distilled water. The treated rhizomes were thoroughly washed in running water and planted in sand along with the control. Germinated rhizomes were transplanted after one month to earthen pots containing potting mixture.

Out of 300 single buds treated with various concentrations of colchicine,

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