Journal of Plantation Crops 5(2): 109-111. *December* 1977.

IN VITRO GERMINATION AND GROWTH OF CACAO POLLEN*

P. N. RAVINDRAN

Central Plantation Crops Research Institute, Regional Station, Vittal 574 243, *Karnataka, India.*

ABSTRACT

This paper deals with pollen germination, pollen tube growth and pollen tube mitosis in cacao (*Theobroma cacao* L.). Good pollen germination and growth were obtained in a medium containing 15% sucrose, 100 ppm boric acid, and 100 ppm calcium nitrate. Boron or calcium was necessary for proper pollen germination and tube growth, and both, if present, enhanced tube growth considerably. No pollen germination was observed at low temperatures (10°C), and maximum germination and tube growth were obtained at 35°C. Further increase in temperature to 40°C affected adversely both germination and tube growth. Pollen tube mitosis began within 2½ hr of the initiation of pollen germination, and completed in 2½ hr. Pollen tube mitosis was normal in the cultivars studied.

INTRODUCTION

Our knowledge on *in vitro* germination of cacao (*Theobroma cacao* L.) pollen and factors affcting tube growth, pollen tube mitosis and sperm formation are far from complete. Jacob, Toxopeus, and Atanda (1969) reported germination of pollen in sucrose concentrations ranging from 0-30%. Varas (1962) found that pollen stored for a week in a desicator germinated better in a medium containing 10% sucrose and 2% agar. Martinson (1973) reported that the best germination was obtained in 5% sucrose-agar medium in shade and at a temperature of 28-31°C. Not much is known about the other factors influencing pollen germination and tube growth in cacao. Pollen tube mitosis and sperm formation in cacao also have not been studied.

MATERIALS AND METHODS

Cacao flowers of the variety Amazon Forastero were collected at 8.00 am and kept over calcium chloride for about 2hr. Germination and tube growth were studied in media containing 5-30% sucrose. Effects of boron (as boric acid) and calcium (as calcium nitrate) alone and in a 1:1 mixture were tested at concentrations from 50-300 ppm in a 15% sucrose solution. The use of agar was avoided because it is known to contain calcium and boron as contaminants (Moore and Jung, 1974). Effect of temperature on germination of pollen was studied over a range of 10-45°C. The pollen tube growth was measured in all cases after one hour unless otherwise mentioned. All germination counts and pollen tube measurements were made after killing and staining with phenol-aniline blue.

*Contribution No.151, Central Plantation Crops Research Institute, Regional Station, Vittal. Paper presented at the IV SABRAO Conference held in Canberra, Australia, 1977.

For studying pollen tube mitosis, pollen was dusted on cellophane bits floated on a nutrient solution containing 15% sucrose, 100 ppm boric acid, and 100 ppm calcium nitrate and with or without 0.05% colchicine. These bits were stained with 1% lactopropionic orcein after varying intervals of time.

Observations and Results

Pollen grains did not germinate in water. They, however, germinated and grew in sucrose solution. After one hour of incubation maximum germination was observed in 20% solution while tube growth was negligible, and hence no measurements were taken. Both germination and growth improved after 3-4 hr. The maximum germination was in 20% while tube growth was maximum in 10% (Table I). Growth was very slow when compared to an enriched medium.

Both boron and calcium enhanced germination and tube growth remarkably (Table II). With boric acid, 100 ppm in 15% sucrose solution appeared to be the best, while for calcium 50 and 100 ppm gave the best results. Pollen tube growth was more sensitive to changes in calcium and boron concentration.

Combination of boron and calcium brought about a remarkable enhancement in pollen tube growth. At all concentrations studied they increased tube growth by nearly two-thirds (Table II). Further, germination was very rapid and started within 15 min and pollen tubes grew rapidly. After two hours the tubes grew to such an extent that measurements became impossible.

Temperature had the expected effect and highest germination and maximum pollen tube length were obtained at 35°C (Table III). At this temperature germination was also very rapid and started in less than 10 min.

Table I. *Effect of sucrose on pollen germination*

Concentration of sucrose	Germination (%)		Tube growth (in μ) after 4 hr
	After 1 hr	After 4 hr	
5%	0	18.0	163.3
10%	8.1	26.7	204.0
15%	8.1	47.9	142.0
20%	44.6	60.3	167.3
30%	30.7	48.1	141.0

Table II. *Effect of boric acid and calcium on pollen germination and tube growth*

Concentration	Boric acid		Calcium nitrate		Boron+Calcium	
	Germination (%)	Mean tube growth in (μ)	Germination (%)	Mean tube growth in (μ)	Germination (%)	Mean tube growth (in μ)
50 ppm	62.7	158	96.4	237	96.8	407
100 ppm	87.8	251	88.6	247	94.3	476
200 ppm	91.2	190	91.3	227	96.1	412
300 ppm	57.9	122	85.5	197	89.6	444

Table III. *Effect of temperature on pollen germination and tube growth*

Temperature	% germination	Mean tube growth (in μ)
10°C	0	0
25°C	86.0	246.0
35°C	92.7	447.4
> 40°C	0	0

In cacao the division of the generative nucleus into the two sperm nuclei takes place within the pollen tube. As soon as the pollen tube growth commenced the generative and vegetative nuclei moved towards it and subsequently entered it. The generative nucleus stains deeply while the vegetative nucleus does not. The division of the generative nucleus began within 2-2½ hr of the initiation of germination. The nuclear behaviour was normal. There were ten chromosomes and these were in most cases arranged in a chain-like manner. The chromosomes were highly condensed and deeply stained. The nuclear division was completed in 2-2½ hr and two sperm nuclei were formed. By this time no trace of the generative nucleus was usually visible and the tube contained two sperm nuclei. These were in most cases found moving a little distance behind the growing tip.

Discussion

The optimum sugar concentration required for pollen germination differes considerably with species (Johri and Vasil, 1961). Martinson (1973) reported that 5% sugar in 2% agar was optimum for cacao pollen germination. In the present study the maximum germination and tube growth were obtained in 15-20% sucrose solution and in presence of 100 ppm each of born and calcium. The germination and tube growth were very much limited at 5% sucrose level, and for optimum growth calcium and boron were found necessary. Agar has been reported to contain calcium and boron as contaminants (More and Jung, 1974) and this may account for the different results obtained by Martinson (1973).

The process of nuclear division leading to sperm formation took place in the pollen tube as in most other angiosperms. The behaviour of the nuclei in the pollen grains was similar to that reported for other species (Vasil, 1962). The generative nucleus divides to form the sperm nuclei while the vegetative nucleus disintegrates gradually. The whole process from pollen germination to sperm formation took about 5 hr.

In conclusion, we may say that the ideal sugar concetnration for cacao pollen germination and growth ranged from 15-20%. The maximum efficiency in both germination and tube growth were obtained in presence of 100 ppm solutions each of boric acid and calcium nitrate. The maximum tube growth was at 35°C. The pollen tube mitosis started within 2½ hr of germination and was completed in 2½ hr.

References

1. JACOB, V.J., TOXOPEUS, H., AND ATANDA, O. 1969. *In Report of Cocoa Res. Insti., Nigeia.* p. 126.
2. JOHRI, B. M. AND VASIL, I. K. 1961. Physiology of pollen. *Bot. Rev.* **27**: 325-381.
3. MARTINSON, V. A. 1973. *In Ann. Report of Cocoa Res. Insti., Ghana* (1971–'72). p. 178-79.
4. MOORE, P. H. AND JUNG, W. L. 1974. Studies in sugarcane pollen, I. *In vitro* germination of pollen. *Phyton* **32**: 147-157.
5. *VARAS, J. 1962. Factors affecting the germination of cacao pollen *in vitro*. *Turrialba* **12**: 220-222.
6. VASIL, I. K. 1962. Formation of male gametes in pollen tubes of some crop plants. *In* Plant Embryology, A Symposium. pp. 254-260. C.S.I.R., New Delhi.

*Original not seen