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FACTORS AFFECTING *IN VITRO* GERMINATION AND STORAGE OF COCONUT POLLEN

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ABSTRACT

Germination and storage trials were carried out with pollen from West Coast Tall cultivar. The effect of various chemicals like sucrose, boric acid, potassium nitrate and calcium chloride upon *in vitro* germination of pollen was studied. The highest percentage of germination (68.8%) was achieved with a concentration of 10% sucrose and 10 ppm boric acid. Germination was not noticed in distilled water, tap water or in potassium nitrate solution. The pollen tubes reached a length of 266μm after 3 hours with a diameter of 42.6 μm. The viability of pollen was maintained when it was stored at 70 and 50% relative humidity (R.H.) for six days (with 45% germination) and eight days (with 56.8% germination) respectively. The pollen could be stored upto 12 days (with 53.4% germination) in a freezer compartment (at sub-zero temperature) for 90 days retaining a minimum - 50% pollen germination.

INTRODUCTION

Coconut (*Cocos nucifera* L.) is the most versatile palm species that provides food, drink, fuel and shelter to millions in the tropical belt. Crop improvement in coconut is carried out by mother palm selection and exploitation of hybrid vigour. As a prerequisite for hybridisation work, it is essential to test the germination of pollen *in vitro* and to maintain the viability of pollen. Harland (1957) has rightly pointed out the necessity of accelerating pollen studies in coconut to identify the most efficient prepotent male transmitters.

In vitro germination was used as one of the tests for viability. Different germination media for *in vitro* pollen germination in coconut have been developed by various workers. Aldaba (1921) used 5 to 30% sucrose solution. Patel (1938) and Liyanage (1949) advocated lower concentrations of sugar and gelatine for better results (5 to 10% sucrose with 2% gelatine).

As for coconut pollen longevity, Patel (1938) maintained the viability for about 14 days when stored in a desiccator at about 50% RH. By the combined action of low temperature (O°C) and 50 to 65% relative humidity, pollen can remain viable for a year and more (Manthriratne, 1965). Whitehead (1963) reported that freeze dried coconut pollen could be stored for prolonged periods at deep freeze temperatures. Nuce de Lamothe *et al.* (1980) reported that in order to increase the storage of pollen its moisture content should be reduced to 50%.

The study has been taken up to find out the best medium for *in vitro* germination and to find out the effect of relative humidity and low temperature on viability of coconut pollen.

MATERIALS AND METHODS

Pollen samples were collected from the West Cost Tall coconut palms at the Central Plantation Crops Research Institute, Kasaragod, Kerala. The pollen was extracted by cutting the spikelets about 5 cm above the female flower after 5 to 6 days of the opening of the spathes. The male flowers were separated and crushed using a roller

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Table 1 : ***Effect of sucrose, boric acid and calcium chloride on pollen germination.***

Treatment	Sucrose (%)	Germination (%)	Boric acid (ppm)	Germination (%)	Calcium Chloride(ppm)	Germination (%)
1	5	3.2	5	21.7	10	6.6
2	6	4.9	10	32.4	20	16.2
3	7	6.8	15	31.2	30	10.2
4	8	10.4	20	20.1	40	6.1
5	10	14.2	25	20.0	50	6.0
6	12	14.7	30	9.4	60	2.5
7	14	15.8	40	8.1	70	2.1
8	16	17.9	50	4.3	80	1.6
9	18	16.2	60	2.5		
10	20	8.1	80	1.9		
11	24	2.0				
C.D. at 5%		3.41		9.27		4.15

pin. The crushed male flowers were kept in an oven at 40°C for 24 hours and the debris were removed from the pollen by sieving through a 0.2 mm mesh.

The following media/solutions were used for testing pollen germination: i) Tap water, ii) distilled water, iii) Potassium nitrate solutions varying from 5 to 80 ppm, iv) Calcium chloride solutions varying from 5 to 80 ppm, v) Sucrose solutions varying from 5 to 24%, vi) Boric acid solutions varying from 5-80 ppm. and vii) Sucrose + Boric acid mixture in different combinations.

a) Sucrose - 5, 6, 7, 8, 10, 12 and 14%.

b) Boric acid - 5, 10, 15, 20 and 25 ppm.

For germination test, liquid media were used. On a drop of above mentioned media in a microscope slides pollen was dusted with the help of a camel hair brush and were kept in a moist chamber to assess the germination at the end of 2 hours. The pollen grains with pollen tube length equal to or greater than the pollen diameter was considered as germinated. One hundred pollen grains were counted under the L. P. (10 X) of a microscope for germination test for each treatment, which was replicated thrice in a Completely Randomised Block Design. The pollen length was measured under a Zeiss research microscope using a calibrated occular micrometer. For this, Agar gelatin media (8% sucrose + 10 ppm boric acid + 2 % agar + 1% gelatin were used in order to facilitate the measurement of pollen tubes.

For pollen storage studies, the pollen samples were packed separately in 4 butter paper packets and were stored as follows:

i) Desiccator with anhydrous calcium chloride (O% RH)

ii) Desiccator with conc. sulphuric acid and water (1:4) (50% RH)

iii) Desiccator with 50% glycerol (70% RH)

iv) Laboratory condition (85%-95% RH)

Another sample to be stored at sub-zero temperature in a refrigerator (freezer compartment) was placed in a screwcapped vial. This bottle was sealed in a double polythene bag to ensure that the pollen grains were not exposed to high humidity.

RESULTS AND DISCUSSION

In vitro pollen germination

Effect of chemicals on pollen germination

Germination was not observed in plain water or distilled water alone or in potassium nitrate solution. Among the different levels of sucrose, boric acid and calcium chloride, the

germination percentage was significantly higher in 12, 14, 16 and 18% sucrose solution, and 10 and 15 ppm concentration of boric acid and 20 ppm of calcium chloride solutions. The maximum germination was obtained with 16% sucrose (17.9%), 10 ppm boric acid (32.4%) and 20 ppm calcium chloride (16.2%) (Table 1), respectively. The combined effect of sucrose and boric acid was highly pronounced as compared to either of them used individually. A combination of 10% sucrose + 10 ppm boric acid was the best with 68.8% pollen germination (Table 2).

A wide range of variation on the percentage of germination of pollen collected from different palms was observed in the present investigation. This may be due to the sucrose content or type of sugar present in the pollen at the time of shedding or certain genetic factors. According to O'Kelley (1955) and Vasil (1960a, 1960b) sugars play major roles in pollen germination namely (i) osmotic regulation and (ii) nutrition in pollen germination and tube growth. Linskens and Kroh (1970) had opioned that sucrose is probably the best and commonly used source of carbohydrate energy for pollen. It was seen that 10 ppm boric acid is ideal for *in vitro* germination of coconut pollen. Boron stimulates pollen germination and tube growth as discovered for the first time by Schmucker (1935) who suggested that boron is involved in the synthesis of pectic substances for the growing pollen tube walls. This was further supported by studies of Spurr (1957), O'Kelley 1957, Raghavan and Baruah (1959) and Young *et al.* (1966). Boron occurs in the pollen at about 0.7 ug/mg dry weight, while the stigma may contain 10 times that level of boron (Stanley 1971). So stigmatic surface helps in pollen germination by providing necessary boron supplement.

Calcium chloride also plays a major role in pollen germination. In coconut, the germination was optimum at 20 ppm of calcium choloride. Cook and Walden (1965) observed that calcium of any of three different salts, namely calcium chloride, calcium nitrate or calcium sulphate improved germination four to five fold in maize. However, in coconut calcium nitrate had deleterious effect on germination. Kwack (1967) found that calcium protects the pollen tubes against the growth inhibitory action of may substances by binding into the pectic regions of the tube wall and thereby increasing the rigidity and decreasing the permeability of the wall. Thus, calcium helps in the regulated uptake of solutes and prevents the bursting of pollen tubes (Kwack 1967). Dickinson (1967) found that carbohydrates leak out of pollen if calcium is not present.

Combination of 10% sucrose and 10 ppm boric acid gave very good germination.

Table 2 : *Combined effect of sucrose and boric acid on pollen germination* (%)

Sucrose (%)	Boric acid (ppm) 5	10	15	20	25	
5	49.7	51.4	48.4	38.2	36.1	
6	53.4	58.9	47.2	39.7	40.2	
7	60.1	63.5	59.5	54.8	49.2	
8	62.2	68.3	60.4	55.5	52.4	
10	62.5	68.8	63.5	61.7	61.0	
12	60.9	64.0	64.9	63.3	64.0	
14	60.5	62.5	68.0	68.7	64.8	

Figures mentioned in the table are mean values.

Table 3 : ***Effect of relative humidity (RH) on pollen germination (%)***

Sl. No.	RH (%)	Pollen germination (days after preservation) 0	2	4	6	8	10	12	14	16	18
1	0	68.5	68.4	64.2	64.1	62.5	56.9	53.4	46.2	21.4	-
2	50	68.5	67.5	64.0	61.6	56.8	38.4	24.1	-	-	-
3	70	68.5	62.4	45.7	45.0	23.3	12.4	-	-	-	-
4	90 (control)	68.5	59.3	31.6	18.3	6.0	0.4	-	-	-	-

Table 4 : ***Effect of low temperature on germination (%) of pollen***

Sl. No.	Methods of preservation	Pollen germination (days after preservation) 1	10	15	30	45	60	75	90	105	120	135	150
1	Freezer compartment of refrigerator (Sub-zero temp.)	68.5	68.1	67.0	65.5	66.0	61.8	60.0	52.8	42.6	16.9	10.5	8.9
2	Control (room temp.)	68.5	0.4	-	-	-	-	-	-	-	-	-	-

The germination was poor in 10% sucrose and 10 ppm boric acid separately. Gauch and Durgger (1953) postulated that boron-sucrose complex is formed which traverses the cell membrane more rapidly than does sucrose alone. O'Kelley (1957) observed an increase in the absorption of both sucrose and glucose by pollen upon the addition of boron in the proper concentration. Under the laboratory condition i.e, at 28°C pollen starts germinating after 45 minutes of incubation. After one hour, the mean tube length was 90.3µm and it reached 236.3µm after two hours. After three hours the mean length was 266.1µm and there was no further tube growth beyond three hours. The increase in the tube length from first to second hour was 161% as compared to only 12.6% increase between second and third hour. Branched pollen tubes were observed rarely (Plate 1).

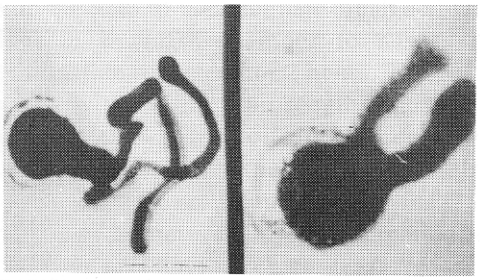

Plate 1. Branched pollen tube

Pollen storage

Effect of relative humidity on storage/ viability of pollen The main objective of pollen storage is its subsequent use for pollination to obtain fruit set. The viability of fresh and stored pollen is best determined by germination test. Coconut pollen could be stored for six days (with 45% germination) and eight days (with 56.8% germination) at 70 and 50%, RH, respectively. However, in a desiccator containing fused calcium chloride (0% RH), the pollen could be stored even upto 12 days giving germination of 53.4% (Table 3). This result is in agreement with that of Ekaratne and Senathirajah (1983) in Oil palm *(Elaeis quineensis)*. They found that pollen could not be stored for long at 70% RH. But when the humidity is 0%, pollen could be stored for many days. Khosh Khui *et al.* (1979) obtained best results with regard to viability of date palm pollen when stored at 20-40% RH over 2-3 weeks. It is

ecessary to control RH during storage so hat the pollen will not get rehydrated.

ffect of temperature on storage/viability of pollen Temperature also plays a major ole in the preservation of pollen. Coconut ollen which was stored in a room emperature has recorded 68.5% of ermination, which decreased to 0.4% in the ext 10 days. The pollen could be uccessfully stored at sub-zero temperature n a refrigerator (freezer compartment) for 0 days retaining at least 50% pollen ermination (Table 4). Whitehead (1963) eported that freeze dried coconut pollen ould be stored for prolonged period at deep reeze temperatures. There are many vidences for the long term preservation of pollen of many species at very low temperature as indicated by Barnabas and Rajki (1981), Eeink (1983), Filippova (1986) and Hanna *et al.* (1986).

Thus, the present study shows that storage of pollen at 0% RH and sub-zero temperature would be ideal for storing the coconut pollen for long period without much loss in viability.

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