POLLEN STUDIES IN COCONUT (*COCOS NUCIFERA* L.) WITH SPECIAL REFERENCE TO A SAMPLING PROCEDURE

K. U. K. NAMPOOTHIRI

Central Coconut Research Station, Kasaragod, Kerala

Received: August 18, 1969

The pollen studies of cultivated plants as a whole have attracted the attention of research workers in recent years, owing to its great significance in floral biology and its index value in interpreting taxonomic relationships and the hybridity status of cultivars (Nair, 1968).

The pollen-grains of coconut (*Cocos nucifera* L.) were studied by Nair and Sharma (1963) and Gangolly *et al.* (1961), and the pollen-grains of arecanut (*Areca catechu* L.) were studied by Raghavan and Baruah (1956) and Nair (1965). Nair and Sharma (1963) reported the occurrence of pollen variations, viz. the trichotomocolpate, porate and operculate forms, apart from the 1-furrowed ones, in coconut. They suggested that a statistical analysis of these pollen variations would be useful in determining the interrelationship of 8 varieties of coconut studied by them.

The present study is aimed at formulating a satisfactory sampling procedure for the determination of pollen sterility and pollen germination, to evaluate the reliability of using the universally employed stains triphenyl tetrazolium chloride and acetocarmine in determining pollen sterility, and to ascertain the correlation between pollen sterility and pollen germination.

MATERIAL AND METHODS

Twelve varieties of coconut, viz. 'Tall', 'Dwarf Orange'; 'Dwarf Green', 'Gangabondam', 'Laccadive', 'Andaman Dwarf', 'Andaman Giant', 'S. S. Apricot', 'S. S. Green', 'Philippines', 'New Guinea' and 'Seychelles', were taken up for the present studies. In each variety 20 individuals were selected for the study of pollen germination. Flowers of each individual were collected at random from 3 distinct parts, viz. the distal 8 cm and proximal 8 cm of the topmost spike and from the distal 8 cm of the lowest spike (these parts are here referred to as A, B and C respectively).

Pollen-grains were germinated at room temperature in an artificial solid medium containing 8 per cent sucrose, 2 per cent gelatin and 2 per cent agar, dissolved in distilled water. For each germination experiment 3,000 grains were counted at random from 6 slides and the percentage of germination was calculated.

T.T.C. (2, 3, 5-triphenyl tetrazolium chloride) and acetocarmine were used for determining pollen-sterility. From preliminary observations the best concentration of T.T.C. was fixed as 2 per cent and was mixed with equal parts of glycerine. Acetocarmine (0·5 per cent) was mixed with equal parts of glycerine. The preparation was sampled thoroughly, as the small and empty, shrivelled grains tended to collect at the edges of the cover-slip.

In 'Tall', 'Laccadive', 'Philippines' and 'Dwarf' varieties the incidence of male flowers at the 3 positions of the inflorescence was assessed and the ratios were worked out by using a sample of not less than 17 trees in each group.

Analyses of variance were set up to test the significance of the differences observed in the germination percentage of the pollen-grains taken from the A, B and C positions. The heterogeneity in the incidence ratios was tested by the χ^2 method.

RESULTS

Pollen sterility was significantly and negatively correlated with pollen germination (Table 1). The 'Z' test showed that the difference in the correlation coefficients was not significant, indicating that both the stains are more or less equally reliable. However, fresh as well as preserved pollen-grains—irrespective of viability—stained alike with acetocarmine, whereas old pollen-grains did not stain with T.T.C.

TABLE 1. SIMPLE CORRELATION COEFFICIENT BETWEEN STERILITY AND GERMINATION OF POLLEN

Stain used	Correlation coefficient	Level of significance (%)
Acetocarmine+glycerine	-0·83	0·1
2% T.T.C.+glycerine	-0·71	0·1

Analysis of the data on pollen germination showed that the overall difference between the germination percentage of pollen-grains taken from 3 different positions of the inflorescence was significant, pollen from the flowers at the top of the distal panicle giving the highest percentage of germination and that at the proximal end giving the lowest. The percentage of germination of pollen from the flowers at the lower part of the distal panicle was in between the above two values (Table 2).

A significant difference was noticed in the sterility of male flowers taken from the different spikes. When the inflorescence was divided into 3 equal parts, the flowers at the distal end were the least sterile (4·08 per cent), followed successively by those at the middle (4·53 per cent) and the proximal portions (4·80 per cent). However, when each spike was considered separately, pollen-sterility from the distal to the proximal portion did not show any definite trend of increase.

Owing to a marked difference in the percentage of germination of pollen from flowers taken from the 3 different positions of an inflorescence, the proportion of the number of male flowers on a fixed length at these positions were estimated in 'Tall', 'Laccadive', 'Philippines' and 'Dwarf' varieties. (The other varieties could not be studied because a sufficient number of trees were not available.) The χ^2 test indicated that the proportion of flowers at the 3 positions was constant from tree to tree except in 'Dwarf' trees. Although no difference was found in the proportion of male flowers between 'Tall' and 'Laccadive', the heterogeneity χ^2 for the incidence of male flowers in 'Tall', 'Laccadive' and 'Philippines' varieties was significant (Table 3).

TABLE 2. MEAN VALUES OF THE PERCENTAGE OF GERMINATION OF POLLEN IN DIFFERENT VARIETIES

Variety	Percentage of germination			Grouping
	A	B	C	
'Tall'	90·37	89·40	85·78	$\overline{ABC}$
'Dwarf Orange'	82·52	80·42	79·74	$\overline{ABC}$
'Dwarf Green'	77·66	77·42	75·90	$\overline{ABC}$
'Gangabondam'	69·10	65·46	68·11	$\overline{ACB}$
'Laccadive'	91·64	88·07	85·08	ABC
'Andaman Dwarf'	89·80	87·94	86·78	$\overline{ABC}$
'Andaman Giant'	86·24	85·48	80·24	$\overline{ABC}$
'S. S. Apricot'	81·44	82·66	73·48	$\overline{BAC}$
'S. S. Green'	93·00	90·72	87·48	$\overline{ABC}$
'Philippines'	93·28	93·68	79·32	$\overline{BAC}$
New Guinea'	94·44	95·28	93·72	$\overline{BAC}$
Seychelles'	92·80	92·48	96·04	$\overline{CAB}$

TABLE 3. PROPORTION OF MALE FLOWERS AT THE 3 DIFFERENT PARTS OF THE INFLORESCENCE IN 4 VARIETIES AND THEIR HETEROGENEITY χ^2 VALUES

Variety	Proportion of male flowers			Heterogeneity χ^2 value	Significance
	A	B	C		
'Tall'	0·37	0·20	0·43	7·25	N.S.
'Laccadive'	0·40	0·19	0·41	23·06	N.S.
'Philippines'	0·35	0·25	0·40	29·85	N.S.
'Dwarf'				101·65	Sig. at 0·1%

Sig., significant; N.S., non-significant.

DISCUSSION

Pollen sterility assessed by acetocarmine or T.T.C. was negatively correlated with pollen germination *in vitro*. Acetocarmine and T.T.C. were equally reliable in determining pollen sterility. Trials with old pollen revealed that coconut pollen looses its stainability with T.T.C. when stored for a long time. But fresh as well as preserved pollen, irrespective of viability, stains alike with acetocarmine, because its staining capacity does not depend on the viability of the pollen but on its contents. The pollen of *Lolium perenne* L. taken from herbarium material stained normally, although its viability was lost even after a day (Vasil, 1958).

The germination percentages of the pollen in the flowers taken from 3 distinct positions (A, B and C) of the inflorescence were significantly different from each other. These results are in conformity with those of Raghavan and Baruah (1956) in arecanut, where the extent of sterility decreased in the anthers of the flowers from the proximal to the distal portion of the panicle. The proportion of male flowers at these positions was more or less constant in 'Tall', 'Laccadive' and 'Philippines' varieties, whereas in 'Dwarf' palms the proportion varied significantly from tree to tree.

As no definite trend was found in the increase in pollen sterility from the distal to the proximal portion of inflorescence, the technique of 'stratified' sampling may not give precise estimates. Moreover, erratic opening of male flowers at the different positions of an inflorescence also poses practical difficulties for 'stratified' sampling. Therefore simple random sampling of flowers is the most practical method for the determination of pollen sterility and pollen germination in coconut.

SUMMARY

Pollen sterility has a high negative correlation with pollen germination. Although acetocarmine and 2, 3, 5-triphenyl tetrazolium chloride are equally good for estimating pollen sterility, T.T.C. may be preferred when the pollen is not fresh. The germination percentage is highest in the male flowers at the upper portion of the topmost spike, followed by those at the lower portion of the topmost spike and those at the upper portion of the lowest spike. The proportion of male flowers at these 3 positions is constant in 'Tall', 'Laccadive' and 'Philippines' varieties, whereas in 'Dwarf' variety the incidence ratio varies considerably.

Although pollen sterility is more in the flowers at the proximal portion than that in flowers at the distal portion of the spike or the inflorescence, there is no definite trend in that increase, which makes it difficult to group the spikes into distinct homogeneous strata. This indicates that stratified sampling if adopted may not give precise estimates. Hence a simple random-sampling technique seems to be the most practical method for the determination of the pollen sterility and pollen germination in coconut.

ACKNOWLEDGEMENTS

Thanks are due to Mr M. C. Nambiar, Director, Central Coconut Research Station, Kasaragod, and to Mr K. Satyabalan, Botanist, for their constant encouragement and guidance. Grateful acknowledgement is made to Mr N. Sankar, former Statistician, for advice regarding the statistical analysis.

REFERENCES

GANGOLLY, S. R., KAMALAKARAN, A. K., BALAKRISHNAN, T. K. and PANDALAI, K. M. 1961. Studies on the pollen in the coconut (*Cocos nucifera* L.). *Indian Cocon. J.* **14**: 49–66.

NAIR, P. K. K. 1968. The growth of palynology as a new science. *Palynol. Bull.* **4**(1): 1–13.

NAIR, P. K. K. and SHARMA, M. 1963. Pollen grains of *Cocos nucifera* L. *Grana palynol.* **4**(3): 373–9.

NAIR, R. B. 1965. Pollen grains of *Areca catechu*. *Arecanut J.* **16**(3): 3–4.

RAGHAVAN, V. and BARUAH, H. K. 1956. On the factors influencing fruit set and sterility in arecanut. I. Studies on pollen grains. *Arecanut J.* **7**: 48–55.

VASIL, I. K. 1958. A criticism of Baj Pai and Lal's paper entitled "Storage experiments with pollen of cultivated fruit trees and vegetables". *Sci. Cult.* **24**(5): 233.