Studies on Pollen Morphology, Production and Viability in Different Varieties of 'Shoe Flower'

(Hibiscus rosa-sinesis L.)

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Introduction

The science of Palynology, though as old as any other branch of botanical science had been neglected till recently. The importance of the studies on various aspects of pollen has received much attention in recent years. A great deal of work has been done on the morphology, viability and storage longevity of pollen grains of temperate fruit trees in the U.S.A., U. S. S. R., U. K. and other scientifically advanced countries. But with regard to tropical and subtropical plants, information on these aspects is meagre.

'Shoe flower' (*Hibiscus rosa-sinensis* Linn. and *H. schizopetalus* Hook) is a common horticultural plant belonging to the natural order Malvaceae. Varieties of these species, different in colour, size and shape of flowers, are found to occur. Most of the varieties are non-seed-setting. Though a few produce seeds, the plants are usually propagated vegetatively. The non-seed-setting nature of many varieties may be due to the failure of either pollen germination on the stigma or pollen tube growth in the style.

The present investigation aims at studying the morphology, production and viability of pollen of ten varieties of 'shoe flower' maintained in the Agricultural College and Research Institute, Vellayani.

Review of Literature

1. *Pollen morphology*

Palynology—a term coined by Hyde and Williams (1944) meaning pollen and spore science—deals with the walls of the pollen grains, but not with their live interior. The close association between applied palynology and plant taxonomy has been emphasised by various workers. According to Wodehouse (1928), cited by Erdtman (1952), the forms of pollen grains are as useful as any other characteristic in the classification of plants. As a general rule, they serve best in distinguishing between and showing relationships among the higher

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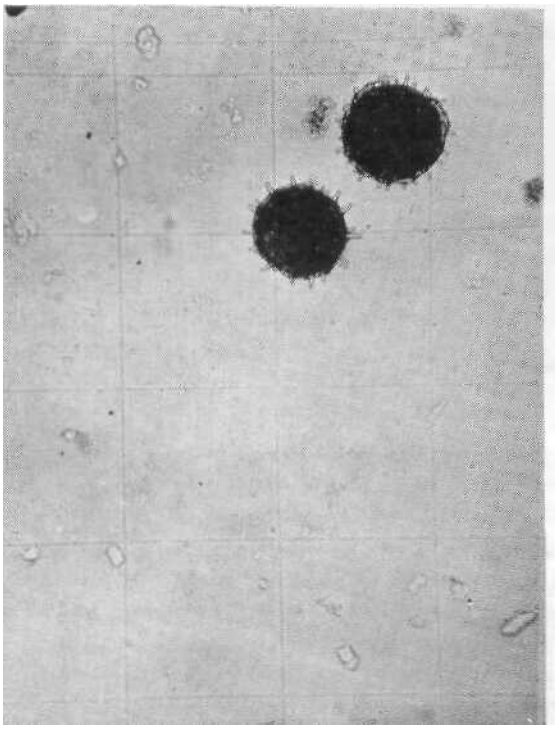

PLATE II. A corner square of the Spencer Bright line Haemocytometer with pollen grains of ' Australian single '

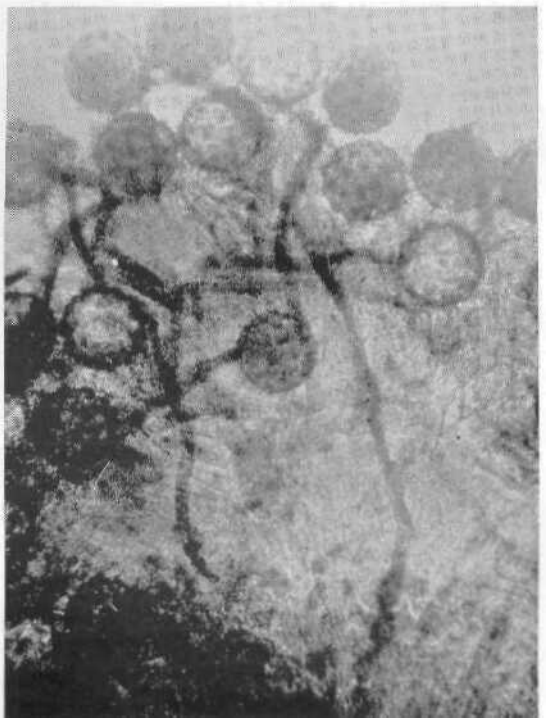

PLATE III. Pollen germination on the stigma in variety 'rose'

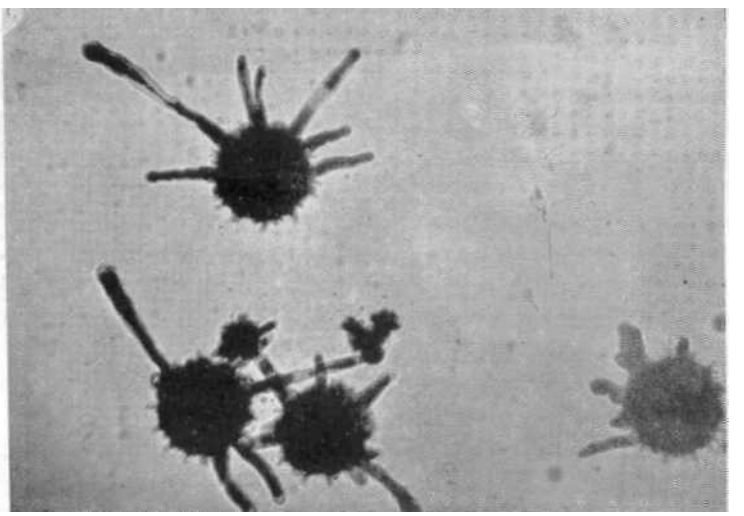

PLATE IV. Polysiphonous germination of pollen grains in variety 'rose'

PLATE I (b). Varieties of ***H. rosa-sinensis***
6. Achania malvaviscus 7. **Flosopleno** 8. Schizopetalus
9. Sunset 10. **Juno**

PLATE I (a). Varieties of *H. rosa-sinensis*
1. Cooperi 2. Australian single 3. **Splendens**
4. Rose 5. **Albus**

groups of plants such as families, tribes, genera and sometimes species. According to Wodehouse (1928) the pollen grains would reveal relationships which are obscure and difficult to demonstrate by other means.

But Wodehouse (1928) is of opinion that the evaluation of palynological criterion should be critical and cautious in advanced investigations.

A great deal of work has been done on the morphology of pollen grains by investigators all over the world. Some had described the pollen grains with reference to their morphological characters, while others suggested various systems of classification. Among them the names of Wodehouse, Faegri and Iversen, Selling and Erdtman are worth mentioning. Erdtman in his book on "Pollen Morphology and Plant Taxonomy", published in 1952, reviewed the work done by various investigators. The investigations carried out in the field of pollen morphology are so numerous that an exhaustive review is not relevant in this context. An attempt has been made to review the literature available on the morphology of pollen grains, with special reference to Malvaceae, to which 'shoe flower' belongs.

Erdtman (1943) reported that the tricolpate grains are usually found in the class Dicotyledons, monocolpate ones in Monocotyledons and acolpate ones in Gymnosperms, as well as in monocots and dicots. According to Wodehouse (1935), Lang (1937) and Erdtman (1954) the pollen grains of all members of Malvaceae are round and are provided with spines of varying shapes and lengths. Erdtman (1952) had described the pollen grains of Malvaceae as 34 colporate and polyforate, provided with a number of apertures, with diameter ranging from 30," as in *Plagianthus belutinus* to 190μ in *Kokia kauiensis.* The pollen grains are nearly always provided with spines varying in length from 1.5μ as in *Howittia triloculus* to 33μ as in *Hibiscus tiliaceus.* The spines in *H. Rosa sinensis*, *Malvaviscus penduliflorus* are occasionally branched; some like the grains of *Goethia cauliflora* lacked spines.

2. *Pollen Production.*

Different techniques have been adopted by various workers for the estimation of pollen production. Though these techniques differed in details, the fundamental idea of all these was more or less the same.

Pohl (1937a) computed the pollen output of some plants by emptying the thecae and suspending the grains in a fixed portion of the suspension, He found the number of pollen produced by each anther to be ranging from 500 in *Calluna vulgaris* to 30,000 in *Rumex acetosa.* The method followed by Erdtman (1938) was a little different. He macerated the flowers, collected immediately before anther dehiscence, by means of suitable chemicals dissolving all but the exines of the pollen grains. The exines were then counted using a counting chamber. Oberle and Georgtzen (1952) demonstrated a new method for determining the number of pollen grains per anther in grape vines, with the aid of a Haemocytometer. They observed a marked variation in the number of pollen grains produced by different species and different varieties of the same species. Even within the same variety, considerable variation in pollen production between two years had been noted.

The pollen output in coconut had been estimated by Gangolly *et al.*, (1961) by a method more or less similar to that of Pohl's and found that the mean production

of pollen ranged from 11,678 to 26,245 per flower. Madhava Rao and Abdulkhader (1962) had estimated the pollen production in papaya, pomegranate and sapota. The method followed was similar to that of Oberle and Georgtzen (1952). It was observed that the number of pollen grains per anther varied from 682 to 3,297 in sapota ; 8,950 to 12,465 in papaya and from 15,982 to 23,170 in pomegranate.

Lobanov (1950) obtained considerable evidence to show that pollination of fruit plants with large amount of pollen (completely covering the stigma) resulted in the greatest fertilisation in intra-varietal and inter-varietal crossings and in hybridisation of more distantly related forms. Positive results of this action was observed not only in seed production, but also in the development of the pericarp, particularly in plants with many seeded fruits, and progenies from such heavy pollination showed great evenness and viability. Sergreeva (1952) found that in gooseberries and currants hybrids resulting from pollination with large amount of pollen are more vigorous and have more viable seeds than when small amount of pollen is used. Tkacenko (1960) studied the influence of the quantity of pollen on fruit setting in vines. Among the three different levels of artificial pollination, in five varieties, the highest level of pollination gave the greatest fruit set.

3. Pollen Viability.

(a) *By staining methods.* Zirkle (1937) had described a method for mounting pollen grains in acetocarmine. The grains which stained well and looked plump and normal were taken as viable and the unstained, shrivelled ones as non-viable. Balasubramanyam (1959) in guava, Nirmalandunath and Randhawa (1959) in pomegranate and jSngh (1961) in mango followed this technique for testing the viability of pollen.

Vietez (1952) found that the use of 2, 3, 5-triphenyl tetrazolium chloride was quick and reliable method for determining the viability of maize pollen. This test was carried out at 30° C using a 2% solution and gave the best results. But Oberle and Watson (1953) found that this technique was ineffective in the case of peach, apple, pear and grape pollen. Jacopini (1954) has recommended sodium biselenite as a rapid indicator of pollen viability. He found that treatment of pollen grains with 2% sodium biselenite for periods ranging from ½ to 2 hours, according to species, proved a rapid and reliable means of determining pollen viability in stone and pome fruit trees. Grains with full germinative power turned pale yellow while non-viable grains did not change colour.

A new viability test was described by King (1959) for Irish potato pollen. This was based on the peroxidase reaction on agar medium. Viable grains could be recognised by their colourless swollen appearance. Non-functional grains became blue and did not swell.

Ostapenko (1956) questioned the validity of the various staining methods in determining pollen viability. Lower values were obtained from germinating pollen grains of various fruit trees in sucrose solution than by acetocarmine colouration or by the method of estimation by peroxidase test, especially in the case of pollen that had been stored for several months. So these methods might be regarded as only of relative value in determining pollen viability.

(b) By germination on artificial medium.

There are accumulated evidences to show that pollen grains can germinate in artificial

media containing sugars like sucrose, lactose and glucose and that sucrose medium gives best results in most cases.

Adams (1916) during his investigations on germination of pollen grains of apple and other fruit trees found that best germination was obtained in apple, in 2.5-10% cane sugar solution, in pear 4-8%, in straw-berry 8% and and in long-berry 4%. In rasp-berry and black currants best germination was obtained in 16% sugar solution. Kobel (1926) studied, the pollen viability of certain fruit trees in sucrose solution and reported an optimum concentration of 5 per cent for quinces, 10% for peaches and apricots, 10-15% for plums and pears, 5-15% for apples and 15% for cherries. Dikshit (1956) found that loquet pollen germinated successfully in 1% sucrose solution. Singh (1959) obtained results similar to that of Kobel with peach's pollen, but found that there was a verietal response to pollen germination tests, among the ten varieties studied.

Schumucker (1935) discovered that boron as borate was a stimulant to pollen germination and tube growth in many species. The element was found to occur in the tissues of the pistil of the species studied. Thompson and Batjer (1950) found that boron in low concentrations viz., 2.5-40 ppm stimulated pollen germination and pollen tube growth in different species of fruit trees. They also observed that higher concentrations inhibited pollen germination and tube growth. Raghavan and Baruah (1956) reported that pollen germination and tube growth were considerably stimulated by boric acid in arecanut pollen grains, cultured in sucrose medium. Resnik (1956, 1958) obtained increased pollen germination in citrus by addition of boric acid. Better results were obtained at concentrations ranging from 10 to 100 ppm. Munzner (1960) found that 0.001 to 0.01% of boric acid had a stimulating effect on pollen germination and tube growth in more than 60 angiosperm species. Singh (1960) recorded that 20 ppm of boric acid and boron increased pollen germination and tube elongation in mango. Vasil (1960) obtained 62% germination in 30% sucrose solution in *Pennisetum typhoidium*; but when boric acid (0.01) was added, 78% germination was obtained in 25% sucrose solution. Madhava Rao and Abdul Khader (1960) have reported that germination of pollen in sapota could be enhanced appreciably by the addition of 100 ppm boric acid to the sucrose-agar medium. It was shown that without the stimulant the germination in the control was only 8%.

Addition of substances like agar and gelatin can promote pollen germination. Agarwal, Khanna and Singh (1957) found that 1% agar medium containing 6% sucrose gave best results with the pollen of *Momordica charantia*. Singh (1959) found that papaya pollen gave 62.9% germination in 5% sucrose solution. When 1% agar was added to the sucrose solution, higher germination of 67.6% was obtained. In grapes good germination was obtained with a medium containing 5% sucrose and 2% agar. Nirmalendu Nath and Randhawa (1959) studied the viability of pollen of pomegranate and obtained satisfactory pollen germination in 12.5% sugar solution and 12.5% sugar-agar media. Randhawa and Ramakrishnan Nair (1960) found that plum pollen gave good germination in 20% sucrose and 1.5% gelatin. But Singh (1961) found that highest mean pollen germination was obtained in 25% sucrose solution. Addition of 0.5 percent agar to sucrose solution increased pollen germination to some extent but not markedly. Kuwada (1956) observed that the pollen of *Abelmo-*

schus esculentus and *Hibiscus manihot* and an amphidploid between the two gave best germination in 7 percent agar and 20 per cent glucose. Datta (1958) in his studies on pollen germination in different species of *Hibiscus,* used a medium containing 2 percent agar and 4 percent sucrose and obtained good results.

Amici (1830), the discoverer of pollen tube, recorded poly-siphonous germination of the pollen grain in *Hibiscus trionum* and *H. syriacus.* In the latter species, he observed that some grains gave rise to 20 to 30 tubes. But Guignard (1904), after 'in-vivo' studies of *H. trionum* found that only one tube played a part in fertilisation. Stenar (1925) found that in *Althea rosea* ten tubes per grain and in *Malva neglecta* fourteen. Lang (937) observed that in *Anodo cristata* and *Lavertia caehmeriana,* the pollen grains produced five to ten tubes, when cultured 'in-vitro'. Iyengar (1938) reported two tubes per grain in Asiatic cotton and in tetraploid American cotton and concluded the frequency of pollen grains producing two tubes is greater in the tetraploid American types than in the diploid Asiatic ones. The same author observed poly-siphonous germination in *Hibiscus vitifolius* also. Purewall and Randhawa (1947) found that the pollen grains of *H. esculentus* germinated thirty minutes after they were placed in culture media. Branching of the tubes was found to occur both in culture media and on stigmatic surfaces. Datta (1958) confirmed that pollen tubes are produced from more than one aperture of the pollen grain in *Hibiscus* species, *Abutilon avicennae* and *Sida rhombifolia.*

Materials and Methods

Selection of Material

Ten varieties of shoe flower (Nine varieties of *H. Rosa-sinensis* Linn. and *H. schizopetalus* Hook.) of persistent flowering habit, selected from the collection maintained in the Agricultural College garden, Vellayani, formed the material for this investigation. Photographs of the flowers of the ten varieties are presented in Plate-1.

Experimental Techniques

1. Pollen morphology.

Morphology of pollen grains was studied in all the ten selected varieties, with reference to such characters as pollen shape and pollen size. The following procedures were adopted in studying these characters. Flowers were collected soon after anther dehiscence and kept in desiccator for one hour to obtain pollen grains for the study.

(a) Pollen shape: Pollen shape of all the varieties was determined by examination of pollen grains mounted in acetocarmine under the high power (63x) objective of a compound microscope. The pollen grains were placed on a clean slide, a drop of acetocarmine placed over them and covered with zero cover glass.

(b) Pollen size: The pollen grains being spherical, their diameter was taken to represent the size. Pollen grains of each variety were mounted in acetocarmine-glycerine mixture and covered with a zero cover glass. Diameter measurements of 100 grains taken at random, for each variety, were made using a standardised ocular micrometer. The data were tabulated and analysed adopting the analysis of variance method.

2. Pollen production

The procedure followed for estimation of pollen production was more or less similar

to that adopted by Madhava Rao and Abdul Khader (1962) which was a modification of the method adopted by Oberle and Geortzen (1952). In the present study, the larger and heavier pollen grains of shoeflower posed a special problem, in that they failed to disperse evenly in the watery medium and tended to settle down soon. The procedure was therefore modified to the extent as described below to suit the present material of study.

Hundred anthers were gathered from freshely opened flowers of each variety, collected before anther dehiscence. The anthers of each variety were taken in separate vials and stored in a desiccator. After anther dehiscence, 1.25 ml of water containing 1.0% teepol was added and the contents shaken thoroughly. Glycerine, when added, helped to give a more even dispersion of pollen grains. A mixture with equal parts of water and glycerine (1.25 ml each) was found to be the best suspension medium for the material.

A drop of the above suspension, drawn in a fine pipette, was transferred to each of the two counting chambers of a Spencer Brightline Haemocytometer. Each chamber has an area of nine square millimetres. Each of the four corner square millimetre area is divided into sixteen areas, while the other five square millimetres are ruled into small divisions. This facilitates easy counting of pollen grains. The counting chambers are 0.1 mm in depth so that the volume of the solution over a square millimetre is 0.1 cubic millimetre. Thus by using the Haemocytometer, the average number of pollen grains in 0.1 c. mm. of the suspension can be accurately determined. From this, the number of pollen grains per anther can be estimated. The contents of 100 anthers were suspended in 2.5 ml of the solution. Thus the contents of 100 anthers were suspended in 0.025 ml or 25 c. mm. of the solution.

By using the low power objective of the microscope the pollen grains in each of the four corner squares of each counting chamber were counted and the mean number of pollen grains in four corner squares was calculated. For each of the 10 varieties, 10 such estimates were made so that the number of anthers studied, in each variety was 1,000. The data were tabulated and analysed using the analysis of variance technique. (Plate—11)

3. *Pollen viability.*

Viability of pollen grains was assessed by three methods:—

(a) Acetocarmine staining method.

(b) Germinating pollen grains in artificial media, and

(c) Pollen germination on the stigma.

(a) Pollen grains of each variety mounted in acetocarmine were examined after 30 minutes. The pollen grains, which appeared normal, plump and well stained were taken as viable and the unstained and shrivelled ones as nonviable. Observations were made in 10 different microscopic fields and the mean percentage of viable grains was calculated. The data were tabulated and analysed statistically using analysis of variance technique.

(b) To standardise the media for testing pollen germination and pollen tube growth, preliminary trials were conducted in stages with the pollen grains of the normally seed testing variety *(H. Rosa-sinensis,* rose).

First stage: Sucrose solutians of different concentrations, such as 0.5, 10 etc. up to 60% were made and small drops of the solution were placed on clean glass slides on which the pollen grains from one flower were 'planted'. The slides were then inverted and allowed to rest on two small glass rods placed in a petridish. Pollen grains were thus kept in hanging drops of sucrose solution. A humid environment was provided, by placing a wet filter paper at the bottom of the petridish. Counts of germinated and non-germinated pollen grains were made, after 24 hours, in ten different microscopic fields. Three concentrations which gave higher germination were used for the next stage.

Second stage: Two series of similar trials were conducted.

(1) Sucrose alone in three concentrations viz. 35, 40 and 45%.

(2) Sucrose, in the above three concentrations, each in combination with three different concentrations of boric acid viz., 50, 100 and 200 ppm.

Third stage: The combination of sucrose + boric acid which gave the best result was tried, in combination with 0.5 g., 1.0 g., 1.5 g. and 2.0 g. of agar, and the best combination of sucrose + boric acid + agar was used for conducting germination tests of pollen of all the ten varieties.

Comparative study of varieties: The standard medium was takeu in clean, sterile petridishes and the pollen grains of different varieties were dusted on to their surface. Separate plates were used for studying different varieties with a replication of five plates for each variety. Observations were made after 24 hours for determining the percentage of germination and length of the pollen tube.

After 24 hours each petridish was examined under the low power of the microscope. The number of germinated and non-germinated pollen grains in a microscopic field was counted. In each petridish, 10 such counts were made at random, and the percentage of germination was determined. The data were analysed statistically by the analysis of variance technique.

The length of pollen tubes of ten grains, selected at random from each petridish, was measured using a standardised ocular microscope and the mean tube length was found out. As a number of pollen tubes emerged from a single grain, measurements were made only of the longest tube. Five such estimations were made for each variety and the data analysed using analysis of variance technique.

(c) Pollen germination on the stigma was studied "in-vivo'. For this study, flowerbuds were emasculated and bagged in the evening. On the following day, they were pollinated with pollen from fresh flowers of the same variety. On the day after pollination, the styles were fixed in 1:1 acetic alcohol After keeping the material in the fixative overnight, they were transferred through alcohol series to 70% alcohol for storage. At the time of examination, the styles were boiled for a short period in lacto-phenol, cooled, stained with cotton blue and squashed on clean slides. The slides were then examined in a microscope for pollen germination on the stigma and pollen tube and the data tabulated and presented.

(Plate—III)

Results.

The results obtained during the present investigation are furnished below.

1. *Pollen Morphology.*

The colour of the pollen grains was found to vary from light yellow to deep yellow in most of the varieties studied. In *'achania malvaviscus'* the pollen grains were light brown in colour. The outer surface of the pollen grains was found to be surrounded by a yellow substance. The amount of this substance varied with the different varieties and resulted in various shades of yellow. This yellow substance was found to occur in large amounts in 'cooperi', albus', 'schizopetalus' and 'australian single.

a) Pollen Shape.

The pollen grains of the different varieties were found to have more or less similar shape. Following Erdtman's (1952) classification, they may be described as spherical, 34 colporate and polyforate. In all the 10 varieties, it was observed that the pollen grains were provided with spines.

(b) *Pollen size.*

Statistical analysis of the data showed that the inter-varietal variation in pollen diameter was highly significant. The mean size of the pollen grains in the different varieties was found to vary from 103.35μ to 174.9μ. The greatest mean size was observed in 'achania malvaviscus' (174.9 μ) followed by 'juno' and 'australian single'. Though their size difference was not significant, the pollen grains of 'juno' and 'australian single' were found to be singnificantly larger in size than those

TABLE I

Pllen Size

Sl. No.	Variety	Size in μ				
		Normal		Sterile		Mean
		Min.	Max.	Min.	Max.	
1	*H. rosa-sinensis* var. cooperi.	135	150	60	150	103.35
2	*H.* ,, ,, australian single	150	180	60	150	159.3
3	*H.* ,, ,, splendens	135	180	105	150	152.4
4	*H.* ,, ,, rose	135	165	60	150	156.15
5	*H.* ,, ,, albus	135	150	60	150	105.0
6	*H.* ,, ,, achania, malvavicus	150	225	75	150	174.9
7	*H.* ,, ,, flosopleno	135	165	90	165	139.65
8	*H.* ,, ,, schizopetalus	135	165	105	150	146.55
9	*H.* ,, ,, sunset	150	210	60	165	154.05
10	*H.* ,, ,, juno	150	180	45	165	161.1

of the remaining seven varieties. The pollen grains of 'rose' were larger than those of 'splendens' 'schizopetalus' 'flosopleno' and 'albus' The smallest mean size was observed in 'cooperi' (103.35 μ). The mean pollen size of the different varieties is given in Table I.

The pollen size was found to differ not only between varieties but within varieties also. In all the varieties variation was observed between normal and sterile grains as is evident from the above table. Besides this the size of the normal grains varied among themselves. Greatest variation was seen in 'achania malvaviscus' ie. 150 to 225 μ.

c. *Pollen production.*

The data when statistically analysed showed that the varieties are significantly different in their pollen output.

TABLE II

Pollen Production

Variety No.	Mean no of pollen grains/4 corner sqs.	Mean no. of pollen grains/ anther-column 2 x 62·5	$ E of	C. D. 0 05)
2	5.75	359		
4	5-6	350		
9	5.35	334		
8	5.2	325		
10	4.55	284		
3	4.05	253	22.983	64.875
1	3.3	206		
6	3.2	200		
5	3.05	190		
7	2.55	159		

The mean number of pollen grains per anther was found to vary among the different varieties studied. The highest number of pollen per anther has been recorded in 'australian single'. The lowest number was noted in 'flosopleno' The variation in pollen output per anther as observed between 'australian single' and 'rose', 'sunset', or 'schizopetalus' was not significant, but it was significant with the rest of the six varieties. The mean number of pollen grains per anther of the 10 varieties is presented in Table 11 above.

Pollen Viability.

(a) Acetocarmine staining method

The data on statistical analysis indicated that varietal variation in pollen viability was highly significant.

Significant difference in the viability of pollen between varieties was noted.' Rose' showed highest percentage of viability followed by 'schizopetalus', 'australian single' and 'achania malviscus'. A decrease in viability percentage was noted in 'juno', 'splendens' and 'sunset'. 'Flosopleno' 'cooperi' and 'albus' showed viability percentage less than 15. The range of variation in the mean percentage of viability was found to be great, ie., from 4% in 'albus' to 97 04% in 'rose . (Table III).

TABLE III

Pollen Viability (a)

Variety No.	Mean (%)	S. E. of Means	C. D. (P = 0.05)
4	97.04		
8	89.70		
2	86.82		
6	83.70		
10	69.32	0687	1.395
3	68.66		
9	53.90		
7	12.42		
1	6.65		
5	4.00		

(*b*) *Germinating pollen grains in artificial media*

1. Sucrose solution alone:

Among the various concentrations tried, it was found that from 0 to 20% the pollen grains failed to germinate Bursting of the pollen grains was observed a few minutes after 'planting' in the hanging droplets. From 20% onwards, pollen germination was observed, but percentage of germination was found to be very low and the pollen tubes failed to elongate. In sucrose solution of concentrations varying from 35 to 45%. both percentage of germination and rate of tube elongation were found to be high. Concentrations higher than 45% were found to have retarding effects both on germination and tube growth. In 60% sucrose solution no germination was observed.

Though the pollen grains of 'rose' gave good percentage of germination and tube elongation in 35-45% sucrose solution, other varieties gave poor results. So the studies were carried forward to the second stage. Concentrations of sucrose solution selected were 35, 40 and 45 per cent.

The results obtained during the study on germination of pollen grains in sucrose solution are presented in Table IV,

TABLE IV

Standardisation of media on sucrose alone

Concentration of sucrose solution per cent	Germination per cent (Mean)	Mean pollen tube length
0		
5		
10		
15		
20	10.5	33.0
25	23.06	47.31
30	41.6	69.16
35	76.39	92.6
40	83.46	113.75
45	89.8	125.6
50	69.28	89.06
55	41.92	39.6
60	- -	. .

2. Sucrose solution with boric acid:

Boric acid was found to increase the percentage of germination and tube elongation. 45% sucrose solution with boric acid gave best results. In low concentrations of boric acid (50 ppm) the increase in percentage of germination was not marked. At higher concentrations (200 ppm) bursting of the pollen grains was noted,

The pollen grains of the other varieties, when tested in 45% sucrose solution with 100 ppm boric acid, gave fairly good germination and tube elongation.

In Table V the means of observations recorded during the study of pollen germination and tube growth in sucrose solution with boric acid are presented.

TABLE V.

Sucrose solution with boric acid

Concentration	Germination percent (Mean)	Pollen tube length *(ft)*
35% Sucrose	76.41	92.45
40% ,,	84.20	114.02
45% ,,	89.80	125.49
35% ,, + 50 ppm. boric acid	79.03	99.00
40% ,, + 50 ppm. ,,	86.40	121.90
45% ,, + 50 ppm. ,,	90.61	130.20
35% ,, + 100 ppm. ,,	81.52	112.56
40% ,, + 100 ppm. ,,	87.90	127.00
45% ,, + 100 ppm. ,,	93.02	138.60
35% ,, + 200 ppm. ,,	39.73	90.40
40% ,, + 200 ppm. ,,	49.00	115.61
45% ,, + 200 ppm. ,,	52.70	124.12

3. Sucrose solution in combination with boric acid and agar:

Among the four doses of agar used, 1 g agar with 45% sucrose solution and 100 ppm boric acid was found to be the best combination.

The sucrose-boric acid-agar medium was used for studying the germinability of pollen grains of the ten varieties.

The results obtained are presented in Table VI.

TABLE VI

Sucrose and boric acid in combination with agar

Agar in combination with 45% sucrose and 100 ppm boric acid.	Germination per cent (Mean)	Mean tube length in (μ)
0.5 g	92.91	139.40
1.0 g	95.06	143.93
1.5g	93.82	129.08
2.0 g	86.17	93.12

Comparative study of varieties

Observations made on pollen germination and pollen tube growth are dealt with separately.

(a) *Pollen germination*

The analysis of the data shows that the intervarietal variation in the percentage of germination is hignly significant.

Pollen grains of 'rose' gave the highest percentage of germination, followed by 'schizopetalus' 'juno' and 'australian single.' The percentage of germination is below 5% in 'flosopleno' and 'splendens' and the difference between the two is not significant. In 'cooperi', 'albus' and 'malvaviscus', the pollen grains failed to germinate. Thus a wide range in the germinability of pollen of the different varieties was met with.

Polysiphonous germination was observed in all the varieties in which the pollen grains germinated. The number of pollen tubes varied from 2 to 10 in the different varieties. (Plate IV)

(b) *Pollen tube elongation*

Pollen tube length was found to vary among the different varieties studied. Greatest tube length was observed in 'sunset', followed by 'schizopetalus', 'juno,' 'rose' and 'australian single.' The difference between the tube length in 'australian single' and 'flosopleno' is not significant. The least tube length was observed in 'splendens'.

(c) *Pollen germination on the stigma*

In 'rose', 'sunset', 'juno', 'australian' single', 'splendens' and 'schizopetalus' pollen grains germinated on the stigma. But pollen tube elongation in the style was observed only in 'rose', 'sunset', 'juno' and 'schizopetalus'. In 'australian single and 'splendens' much tube elongation was not observed, beyond the region of stigmatic lobes. It was also observed that in 'schizopetalus', the coralla with staminal column and style falls off at about 24 hours after anthesis.

TABLE VII

Pollen germination (Comparative study of varieties)

Variety	Mean % of germination	S. E. of Means	C. D. (P=0.05)
4	95.72		
8	74.36		
10	41.00		
9	32.24	0.902	1.862
2	10.98		
3	4.60		
7	2.80		

TABLE VIII

Pollen tube elongation (Comparative study of varieties)

Variety	Mean tube length in μ	S. E. of Means	C. D. (P=0.05)
9	281.62		
It!	228,88		
8	205.02		
4	143.22	2.14	4.417
i	93.61		
7	93.60		
2	86.20		

Discussion

Pollen morphology

In the present investigation, the pollen grains of the ten different varieties of shoe flower are found to be spherical, 3-4 colporate, polyforate and provided with spines. This observation is in agreement with the general description given by Wodehouse (1935), Lang (1937) and Erdtman (1952) for the pollen grains of *Malvaceae*. Though there is slight variation in colour, the pollen grains of the different varieties have more or less similar shape.

The size of the pollen grains in different varieties of the shoe flower varies from 103.35 μ as in 'cooperi' to 174.9 μ as in 'achania malvaviscus'. Even within the same variety the size of the pollen grains tends to vary. Variation is observed not only between normal and sterile grains but also within the normal grains.

Pollen production

In the different varieties of shoe flower studied, the pollen output per anther is found to vary from 159 in 'flosopleno' to 359 in 'australian single'. Inter-varietal variation in pollen output has been reported by Oberle and Geortzen (1952) in grape vine and also by Madhava Rao and Abdul Khader (1962) in papaya, pomegranate and sapota.

The number of pollen grains per anther of the different varieties of shoe flower is very small and this may possibly be due to the larger size of the grains and the larger number of anthers (25 to 90) in each flower.

Pollen viability

The acetocarmine staining method revealed a marked variation in pollen viability in the different varieties. It is found to vary from 4% in 'albus' to 97% in 'rose'.

The pollen grains of the different varieties show great variation in germination in nutrient media also. The range of variation is from zero in 'albus', 'cooperi' and 'achania malvaviscus' to 95.72% in 'rose'. But it is seen that the variety 'australian single' which gave 86.82% viability as per acetocarmine test gives only 10.98% in germination test in nutrient media. Also 'achania malvaviscus' which gives 83.7% in the acetocarmine test, gives no germination at all in the latter test. These results indicate that certain varieties which give high viability in acetocarmine test give either poor or no germination. But none of the varieties gives an increase in pollen viability in the germi-

nation test than that obtained by the aceto-carmine method. This result is in agreement with the views of Ostapenko (1956) that the staining methods are only of relative value in determining pollen viability; for lower values were obtained by germinating pollen grains in sugar-agar medium than by acetocarmine.

Germination trials of pollen grains in artificial medium reveal that a medium containing 45% sucrose, 100 ppm boric acid and one g agar is most suitable for many varieties of shoe flower. Among the different concentrations of sucrose solution tried, good pollen germination is obtained in 35 to 45%. In lower concentrations the pollen grains tend to burst and in higher concentrations they fail to germinate. But pollen grains of all the varieties do not germinate successfully in this concentration. This may be due to a difference between the varieties in their specific requirements.

In the present studies, it is found that 100 ppm boric actd can increase pollen germination. 200 ppm induces bursting of pollen grains and reduces pollen germination. The result is in favour of the findings of previous workers that boric acid when added to a basic medium is capable of increasing pollen germination. Thompson and Batjer (1950) in different species of fruit trees, Raghavan and Baruah (1956) in arecanut and Resnik (1956, 1958) in citrus obtained results to show that boric acid enhances pollen germination. Munzner (1960) found that 10-100 ppm of boric acid had a stimulating effect on pollen germination. Mahava Rao and Abdul Khader (1961) have also reported that germination of pollen in sapota could be enhanced by the addition of 100 ppm boric acid to the medium.

Presence of 1% agar along with sucrose and boric acid in the medium is found to increase the percentage of germination. This is also comparable with the results obtained by many previous investigators on different plants.

In the different varieties in which the pollen grains germinated, it is found that a single grain produces as many as ten pollen tubes. This observation is in agreement with previous reports of polysiphonous germination in different species of Malvaceae by Amici (1830) in *Hibiscus trionum* and *H. syriacus*, Purewall and Randhawa (1947) in *H. esculentus*, Datta (1958) in *H. vitifolius*, *H. esculentus* *H. sabdariffa* and other species of *Hibiscus*, and by various other workers.

The different varieties are found to differ in the length of their pollen tubes. It is found that 100 ppm boric acid can enhance pollen tube elongation. This result is in agreement with the reports of many workers.

Pollen germination on the stigma

A close parallelism exists between germination of pollen grains in the artificial media and that on the stigma, in all the varieties except in ' flosopleno '. Only the pollen grains of those varieties which germinate in the nutrient media are found to germinate on the stigma. In the case of ' flosopleno', though the pollen grains germinate in the artificial medium no germination takes place on the stigma.

It is found that the varieties of shoe flower behave differently with regard to pollen germination on the stigma and pollen tube growth in the stylo after self-pollination. Another observation which is relevant in this context is that only one out of the ten varieties studied sets seeds under normal conditions. These results make it possible for a classification of the ten varieties of shoe flower as given below.

Varieties

↓

Pollen germination after self-pollination

No pollen germination after self-pollination.

Var. 1. Cooperi
2. Albus
3. Achania malva-viscus
4. Flosopleno

↓

Tube elongation in the style

No tube elongation in the style

Var. 1. Australian single
2. Sple

↓

Seed setting

Var. 1. Rose
2. Sunset
3. Juno.

No seed setting

1. Schizopetalus

The above classification reveals that varieties 'cooperi', 'albus', 'achania malvaviscus' and 'flosopleno' are not capable of setting seeds by artificial self-pollination. In the case of 'australian single' and 'splendens', the non-seedsetting nature is due to the lack of pollen tube elongation beyond the region of stigmatic lobes. Pollen tube elongation takes place in the style in 'rose', 'sunset', 'juno' and 'schizopetalus', but seed setting is recorded only in 'rose', 'sunset' and 'juno'. This indicates that the failure of setting seed in 'sunset' and 'juno' under natural conditions may be due to the failure in pollination. The non-seed-setting nature in 'schizopetalus', inspite of pollen tube elongation in the style requires further explanation. This may perhaps be due to the abscission of the corolla and staminal column with the style, 24 hours after anthesis, whereas in the seed setting varieties this takes place only after 48-60 hours. The non-seed-setting nature of 'schizopetalus' under natural conditions may therefore be due to the early abscission of the style and stigma

Summary and conclusions

Studies were undertaken on the morphology, production and viability of pollen of ten varieties of shoe flower maintained at the Agricutural College garden, Vellayani.

It was found that though there was slight variation in colour, the pollen grains of all the varieties had more or less similar shape. Variation in size of pollen grains was observed not only among varieties, but whithin varieties also.

In pollen production, the varieties were found to differ significantly. The number of pollen grains per anther was found to vary from 159 to 359. The large size of pollen grains and large number of anthers per flower might be the reason for the fewer number of grains per anther.

A marked variation in the viability of pollen was met with in the different varieties studied. In both the viability tests viz., the acetocarmine staining test and the germination test in artificial medium, highest pollen viability was recorded by [illegible] and lowest by 'albus'. A comparison of the results obtained in the two tests revealed that acetocarmine staining test was not reliable for estimation of pollen viability, because lower values were obtained when the pollen grains were germinated in artificial media.

A medium containing 45% sucrose, 100 ppm boric acid and one gram agar was found to be the best medium for pollen viability in most of the varieties of shoe flower studied.

Polysiphonous germination was observed in all the seven varieties in which pollen grains germinated.

The different varieties were found io differ in the length of their pollen tubes. It was found that 100 ppm. boric acid can enhance pollen tube elongation.

Pollen germination studies *in vivo* showed that the pollen grains of only six out of ten varieties germinated on the stigma. Among the above six varieties pollen tubes elongated only in four. Seed-setting after artificial self-pollination was recorded only in three varieties in which tube elongation in the style was observed. The non-seed-setting nature of 'juno' and 'sunset' under natural conditions can be attributed to the failure of pollination. The failure to set seed in 'schizopetalus' may be due to the early abscission of the style and stigma.

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