POLLEN PRODUCTION, FERTILITY AND COMPATIBILITY STUDIES IN SHOE FLOWER (*Hibiscus rosa-sinensis* L.)

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Pollen production, fertility and compatibility studies in shoe flower (*Hibiscus rosa-sinensis* L.)" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "Pollen production, fertility and compatibility studies in shoe flower (*Hibiscus rosa-sinensis* L.)" is a record of research work done independently by Miss Baby Lissy Markose, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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We, the undersigned, members of the Advisory Committee of Miss Baby Lissy Markose, a candidate for the degree of Master of Science in Horticulture with major in Horticulture, agree that the thesis entitled "Pollen production, fertility and compatibility studies in shoe flower (*Hibiscus rosa-sinensis* L.)" may be submitted by Miss Baby Lissy Markose, in partial fulfilment of the requirements for the degree.

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Introduction

INTRODUCTION

Among the four botanical species of Hibiscus viz., Hibiscus rosa-sinensis L., H. schizopetalus Hook., H. mutabilis L. and H. syriacus L. grown all over the tropics and subtropics for ornamental purposes, Hibiscus rosa-sinensis L. is the most important. Several of the new varieties evolved mainly through hybridisation in the past are known for their large conspicuous flowers with magnificient shades. Shoe flower with its easiness for propagation and management is widely accepted as an ornamental species in all types of gardens. The climatic conditions prevailing in south India is particularly suitable for growing all types and species of Hibiscus.

The shoe flower, perhaps is one of the earliest shrubs grown in Kerala for ornamental as well as for medicinal purposes. Ornamental home gardening on a sophisticated basis is of more recent origin in our state and therefore the probable reason for growing Hibiscus on a wider basis in Kerala homes in the past might be because of its medicinal value. A large number of types are found freely growing in Kerala and most of these types are hardy and tolerant to drought conditions compared to the Hawaiian varieties. These types also produce large

number of flowers in contrast to Hawaiian hybrids. Morphological descriptions of most of these types, however, have not been made and therefore they do not possess distinct varietal names. There appeared great possibility to utilise the different types of Hibiscus existing in Kerala for hybridization purpose so as to combine their hardiness with the large beautiful flowers of Hawaiian hybrids. Detailed morphological descriptions of the available types were necessary in the absence of any reference in this regard.

The programme of crop improvement involves systematic hybridization, the pre-requisite of which is the correct knowledge on the compatibility between parents concerned. The pollen output, viability and receptivity of stigma are some of the important factors to be studied in detail before taking up any systematic hybridization programme.

The present studies were taken up with the following objectives.

1. To make detailed morphological descriptions of the types, varieties and species maintained in the germplasm collection in the College of Horticulture.

2. To study in detail the pollen morphology, pollen production, pollen fertility, pollen germinability and pollen storage of the different types/varieties and species.

3. To study the different aspects of compatibility like self, intervarietal and interspecific compatibility.

The results of the present study are expected to be useful for taking up detailed hybridization programme in shoe flower so as to evolve varieties suitable to humid tropical conditions.

Review of Literature

REVIEW OF LITERATURE

The genus *Hibiscus* contains about 300 species of different habitats viz., herbs, shrubs, trees and is widely distributed in the tropical and subtropical regions of the world. Out of this, about 20 species were reported to be of ornamental value (Bailey, 1949; Anon, 1959; Vasishta, 1972; Bhat, 1976; Srivastava, 1982). However, the four botanical species of *Hibiscus*, namely *H. rosa-sinensis* Linn., *H. syriacus*, *H. schizopetalus* Hook. and *H. mutabilis* L. are mainly used for ornamental purposes in the tropical and subtropical gardens (Bhat, 1976). Of these, *H. rosa-sinensis* Linn., popularly known as 'China rose' or 'shoe flower' is the most common and acclaimed as the most beautiful. The State of Hawaii in U.S.A. claimed to have the world's richest and finest varieties (Pal and Krishnamurthy, 1967). They bear unusually large flowers with wonderful bright colours.

1.1 Morphological description

The genus *Hibiscus* has been described by Bailey (1949); Anon (1959); Rendle (1971); Vasishta (1972); Bhatnagar (1975-76); Narayanaswami and Rao (1976) and Shukla and Misra (1979). Bailey (1949) gave brief

descriptions of Hibiscus rosa-sinensis, H. mutabilis, H. schizopetalus and H. syriacus. Though Hooker, 1945 as quoted by Shukla and Misra (1979) had given a species status for H. schizopetalus, Vilasini et al. (1966) considered this as a variety of shoe flower because of its very resemblance with the different varieties of shoe flower. A number of types, varieties and hybrids in Hibiscus rosa-sinensis are grown under different conditions. Vilasini et al. (1966) described 10 varieties of shoe flower grown in Kerala. Deviah (1968) reported that the flowers in hybrids were singles, flats or saucer shaped. A few cup shaped and some with petals re-curved and wavy also occurred.

Sundar (1971) reported that hybrid seedlings in Hibiscus bore most fascinating flowers and described ten such hybrids. Bhat (1976) described ten promising intervarietal hybrid seedlings evolved at IIHR, Hessaraghata. Bhat (1979) further described fourteen new varieties of Hibiscus rosa-sinensis evolved through hybridization at the IIHR. Bhat and Verma (1980) gave detailed morphological features of six hybrid seedlings.

Bailey (1949); Anon (1959); Pal and Krishnamurthy (1967) and Bose and Mukherjee (1972) reported that most of the varieties of Hibiscus rosa-sinensis and H. schizopetalus

blossomed almost throughout the year and more abundantly in the late rains. In H. mutabilis, flowering took place in profusion during September and October. Devaiah (1968) reported that hybrid seedlings of H. rosa-sinensis flowered profusely and bloomed continuously. Bhat (1976) observed that some of the H. rosa-sinensis cultivars like 'Cromwell', 'Battlion' and 'Snow Flake' flowered almost round the year under Bangalore conditions. The peak flowering was observed during the months of June to October, in majority of these varieties.

1.2 Anthesis and anther dehiscence

Vilasini et al. (1966) reported that in all the ten varieties of H. rosa-sinensis including Schizopetalus they observed, anthesis took place between 5 to 6 am although the time of dehiscence differed between varieties. In eight varieties it was between 7 and 10 am and in other two varieties, between 10 am and 1 pm and 3 and 5 pm. Bhat (1976) reported that anthesis and anther dehiscence took place during the morning hours.

The retentivity of the corolla was different in different varieties (Vilasini et al., 1966). In some varieties, the corolla was retained upto 24 hours, while in others, it lasted upto 60 hours. Devaiah (1968)

reported that in most of the hybrid seedlings the flowers remained on the plant for two to three days.

2. Pollen studies

Wodehouse (1935) was of opinion that the evaluation of palynological criteria should be critical and cautious in advanced investigations. The first use of the term 'palynology' was made by Hyde and Williams in 1845 as quoted by Bhojwani and Bhatnagar, 1974 and they referred the study of external morphological features of mature pollen grains as palynology. Palynology deals with the pre-tetrad and post-tetrad stages, the latter including pollen production, pollen morphology, anthesis, pollen dissemination, pollination, pollen germination and fertilization (Srivastava, 1982). The importance of palynology in plant taxonomy was stressed by Wodehouse (1935), Erdtman (1952) and Stanley and Linskens (1974).

King (1962) referred to such procedures as collection, drying, testing viability, storage and shipment particularly as those inclusive of the techniques of plant breeding as "Pollinicuration".

Literature pertaining to the pollen studies are numerous and some of the important works are reviewed here.

2.1 Pollen morphology

Morphological analysis of pollen has been used as a working base to throw light on the taxonomy, phylogeny and evolution of angiosperms (Nair, 1970). Gross morphology and surface topography of pollens, has been widely adapted to identify plants of divergent and of closely related taxa (Ahmedullah, 1983).

Among the procedures and terminology put forward by various workers, the ones proposed by Wodehouse (1935), the acetolysis process of Erdtman (1952), Nair (1960), Nair (1961) and NPC system of Bhojwani and Bhatnagar (1974) are widely followed.

Erdtman (1952) stated that the pollen grains possessed a unique form and performed a special and vital function. He observed that majority of pollen grains had an intine and an exine. Exine could be sculptured, reticulate or smooth, with or without spines, and with conspicuous apertures. Maurizio (1956) reported that morphological variations affecting different structures such as apertures, size, or exine pattern were the result of polyploidy. Thus the morphology of the pollen grains served as an index to the hybridity status of anyone individual even within a population of anyone crop plant.

Pollen morphology as an index to the chromosomal composition of the plant was indicated by Srivastava *et al.* (1974) in hybrid Amaranthus (*Amaranthus spinosus* x *A. dubius*).

Several workers like Fogle (1977), Maas (1977), Westwood and Challice (1978) and Martine and Fretz (1980) attempted to classify the clones and species of fruit trees based on pollen exine characteristics. Investigations on pollen morphology were conducted in several crops viz., citrus (Nair and Mehra, 1961), *Cocos nucifera* (Nair and Sharma, 1963), Annonas (Thakur and Singh, 1965), *Psidium guajava* (Singh and Sehgal, 1968), *Luffa cylindrica* (Nair and Deshpande, 1968), dioscorea (Jose and Magnoon, 1972), *Momordica charentia* (Saoji, 1975), *Bauhinia galpini* (Nalawadi *et al.*, 1975), nutmeg (Naseem, 1979), pineapple (Nair, 1982) and *Vitis vinifera* (Ahmedullah, 1983) in India.

In Malvaceae family, Wodehouse (1935), Lang (1937) and Erdtman (1954) reported that pollen grains of all members of Malvaceae were round and were provided with spines of varying shape and length. Erdtman (1952) described the pollen grains of Malvaceae as 3 to 4 colporate and polyforate, with a number of apertures, with diameter ranging from 30 µ as in *Plagianthus belutinus* to 190 µ

in Kokia kauiensis. Nair (1961), Prasad (1965) and Sharma and Rastogi (1965) reported that the pollen grains of Malvaceae, in general were pantoporate and spinous. In ornamental Hibiscus, Nair (1961) observed pollen variations in exine with regard to the spines. Vilasini et al. (1966) reported that the colour of the pollen grains of H. rosa-sinensis varied from light yellow to deep yellow but different varieties did not vary much in shape. They however, found that the pollen diameter for different varieties varied from 103.85 µ to 174.9 µ. Pollen grains of the genus Hibiscus were pantoporate and spinate (Nair and Kapoor, 1974). Srivastava (1982) described the pollen grains of Hibiscus hybrid (Hibiscus rosa-sinensis pentamerous x Hibiscus intermedium multipetalous) and found that the grains were pantoporate, spherioidal and spinose and the pollen size was 72 to 180 µ. He also recorded the occurrence of various abnormal types, including micrograins and pollenoid bodies in Hibiscus.

2.2 Pollen production

The relative quantity of pollen produced per blossom or per anther vary from variety to variety within a species. A precise measure of the amount of pollen produced by individual anthers, flowers or the plant itself is essential to evaluate the worth of a variety as a pollinator more accurately (Nair and Kapoor, 1974).

Different techniques have been adopted by various workers for the estimation of pollen production. Pohl, 1937 as quoted by Vilasini et al. (1966) computed the pollen output of some plants by emptying the thecae and suspending the grains in a fixed portion of suspension. Oberle and Geortzen (1952) demonstrated a method of determining the number of pollen grains per anther with the aid of haemocytometer, a technique adopted in the clinical field to count blood corpuscles. They used 2.5 ml water containing 0.25 per cent calgon as dispersing agent of pollen grains. They observed marked variation in the number of pollen grains produced by different species and different varieties of the same species. Pollen output in coconut was estimated by Gangolly et al. (1961), by a method more or less similar to that of Pohl (1937) and found that the mean production of pollen ranged from 11,678 to 26,245 per flower. The accuracy of haemocytometer in estimating the pollen production was further confirmed by the work of Rao and Khader (1962) in fruit crops like papaya, pomegranate and sapota and the method followed was similar to that of Oberle and Geortzen (1952). They used 2.5 ml of water containing 0.5 per cent teepol (detergent) for getting a uniform pollen dispersion. It was observed that the number of pollen grains per anther varied from 682 to 3,297 in sapota, 8950 to 12,465 in papaya and 15,982 to 23,170 in pomegranate.

Vilasini *et al*. (1966) found that 1.25 ml each of water and glycerine with teepol was the best suspension for even dispersion of *Hibiscus* pollen grains. They observed that pollen production ranged from 159 to 359 per anther in 10 varieties of *Hibiscus rosa-sinensis* studied. Investigations on this line as indicated above were also made in *Abelmoschus esculentus*, *Gossypium hirsutum* and *Althaea rosea* of Malvaceae family (Srivastava, 1982). Pollen production was 40-100 grains per anther in *A. esculentus*, 100-200 in *A. rosea* and 159 to 346 in *Gossypium hirsutum*. He observed higher pollen production in the middle period and least in the late periods. The pollen production per flower depended on the number of anthers in individual flower.

Brooks and Puri (1963) and Sharma and Singh (1970) reported variation in atmospheric conditions affecting pollen production. Sharma and Singh (1970) found that in mango, higher temperature and dry climate were associated with increased pollen production. In coconut, seasonal and diurnal variations in pollen yield were reported by Child (1974).

Lobanov (1950) obtained considerable evidence to show that pollination of fruit plants with larger amount of pollen resulted in greatest fertilization in

intra-varietal and inter-varietal crossings and in hybridisation of more distantly related forms. Sergreeva (1952) found that in gooseberries and currants, hybrids resulted from pollination with large amount of pollen were more vigorous and had more viable seeds than when small amount of pollen was used.

2.3 Pollen fertility

The functional potential of pollen, in the process of fertilization and production of seeds, was realised as early as the prehistoric period (Maheshwari, 1950). Stanley and Linskens (1974) emphasised the importance of pollen viability in hybridization and suggested various methods for testing the viability of pollen grains. Staining of pollen grain with dyes was often used to obtain viability indices. Staining technique to study the fertility of pollen grains was adopted by Zirkle, as early as in 1937, who introduced acetocarmine staining technique. He classified properly stained, plumpy and well developed pollen grains as viable and shrivelled ones as non-viable. Balasubramanyam (1959) in guava, Nirmalendumath and Randhawa (1959) in pomegranate, Singh (1961) in mango, Singh (1962 a) in litchi, Nalawadi *et al.* (1975) in Annona, Naseem (1979) in nutmeg, Remamenon (1980) in pepper, Nair (1982) in pineapple and

Tessy (1983) in jack, adopted acetocarmine test to find out the percentage pollen fertility. The staining properties of various other compounds on pollen fertility have also been reported. The chemicals used were iodine (Barnett and Carver, 1964), propinocarmine (Deshmukh et al., 1978), methyl green-glycerine jelly and aniline-oil gention violet (Gupta et al., 1979). The different stains possessed specific staining properties as explained by Stanley and Linskens (1974). According to them, acetocarmine stained essentially chromosomes, iodine starch, aniline blue in lactophenol callose, phloxin-methyl green both cytoplasm and cellulose.

Vietez (1952) found that the use of 2, 3, 5 triphenyl tetrazolium chloride was a quick and reliable method for determining the viability of maize pollen. But Oberle and Watson (1953) found that this technique was ineffective in the case of peach, apple, pear and grape pollen. Aslam et al. (1964) and Hauser and Morrison (1964) proposed the use of tetrazolium chloride for determining the pollen fertility.

Jacopini (1954) developed a rapid method of testing pollen viability using sodium biselenite. Viable pollen developed a pale yellow colour when stained with

two per cent sodium biselenite solution for half an hour to two hours in the case of stone and pome fruits. Non-viable ones showed no colour change. King (1960) described a test based on peroxidase reaction on agar medium. Viable pollen grains remained swollen and colourless whereas non-viable ones developed blue colour, but were not swollen. Heslop-Harrison and Heslop-Harrison (1970) introduced a fluorescence technique in which the viable grains produced bright fluorescence and was very promising in the case of members of graminae and compositae.

Stanley and Linskens (1974) opined that the use of stains was not sufficiently accurate when compared to germination tests, because the immature and aborted pollen grains also contained levels of constituent chemicals enough to yield positive results in stain tests.

2.4 Pollen germination

In vivo, the stigma provides a suitable site for germination. However, studies in vivo are not feasible because of the complications involving pistillate tissue. Germination tests were reported to be more accurate than stain tests which gave only a crude estimate of pollen viability (Stanley and Linskens, 1974).

It was Amici, 1824 as quoted by Srivastava (1982) who by his observations of pollen germination in Portulaca provided a scientific basis for demonstrating the functional importance of pollen grains. The use of artificial media for pollen grains dates back to the later half of 19th century. Adams, 1916 (cited from Johri *et al.*, 1977) reported good pollen germination at various concentrations of sugar for different fruit crops. Cane sugar was found to be better than any other source of carbohydrates tried for germination of pollen grains by many workers (East and Park, 1918; Auchtor, 1921 and O'Kelly and Joseph, 1955). Kobel, 1926 (cited from Johri *et al.*, 1977) germinated the pollen grains of certain fruit trees in sucrose solution and came to the conclusion that different species required varying concentrations of sucrose solutions for their germination. Generally two celled pollen grains required 10-20 per cent sucrose while three celled ones upto 60 per cent (Shivanna *et al.*, 1979).

Sucrose medium was used for *in vitro* germination of pollen of *Hibiscus* varieties by Vilasini *et al.* (1966) and they got 89.8 per cent germination and 125.6 μ tube growth in 45 per cent sucrose solution. Singh (1959) Randhawa and Sharma (1960), Randhwawa and Negi (1965)

found best germination in 20-25 per cent sucrose solution in fruit crops. Dubey (1969) reported that maximum germination and tube length of pollen was observed in 25 per cent sucrose in okra pollen grains. Pollen germination was satisfactory with 25 per cent sucrose for Annona (Nalawadi et al., 1975) and 15 per cent sucrose for Cocoa (Ravindran, 1977). Gupta et al. (1979) reported that rose pollen germinated best in 25 per cent sucrose. Srivastava (1982) noticed that in malvaceous crops, the pollen grains germinated in various concentrations of growth media, namely 15-50 per cent in Abelmoschus esculentus and 20-45 per cent in Gossypium hirsutum and Althae roses. Of these concentrations, 25, 30 and 35 per cent were found to be the most promising and as a standard, they suggested that 25 per cent growth media for germinating the malvaceous pollen grains.

Eventhough there are contradictory opinions on the endogenous and exogenous utilization of nutrients by pollen grains, there is a general unanimity of opinion on the role of sugar in controlling osmotic concentrations during germination of pollen (Visser, 1955; O'Kelly, 1955; Vasil, 1958).

Addition of substances like agar and gelatin promoted pollen germination (Agarwal *et al.*, 1957). Kubo (1960) pointed out that by adding agar or gelatin into the media, moisture could be supplied at a constant relative humidity. Stanley and Linskens (1974) emphasised that agar supplied moisture, carbohydrate and other nutrients.

Kuwada (1956) observed that the pollen of *Abelmoschus esculentus*, *Hibiscus manihot* and an amphidiploid between the two gave best germination in seven per cent agar and 20 per cent glucose. Datta (1958) obtained good results on pollen germination in different species of *Hibiscus* in four per cent sucrose and two per cent agar. Vilasini *et al.* (1966) also reported that by the addition of one per cent agar, the germination percentage was increased by two per cent and tube length by five μ in *Hibiscus rosa-sinensis*. In okra, seven per cent increase in germination and 60 μ increase in tube length was reported by the addition of 10 per cent agar-agar by Dubey (1969).

Singh (1959) found that papaya pollen gave 62.9 per cent germination in five per cent sucrose solution. By the addition of one per cent agar, a higher germination of 67.6 per cent was obtained. In mango

good germination of pollen was obtained in a medium containing 2.5 per cent sucrose and 0.5 per cent agar (Singh, 1961). Rao and Khader (1960) found that 16 per cent sucrose and 0.7 per cent agar was the best media for sapota pollen germination while Singh and Sehgal (1968) reported that 5 to 7.5 per cent sucrose and 0.5 per cent agar was best for guava. Tessy (1983) observed that 10 per cent sucrose with one per cent agar was best for jack pollen germination.

2.4.1. Effect of chemicals

2.4.1.1.Effect of boric acid

Schumucker (1935) first reported that boron stimulated pollen germination and tube growth in vitro and he found that one to 10 ppm boric acid stimulated both. He made this significant observation based on his studies on the occurrence of high levels of boron in the stigmatic fluids of Nymphaea. Thompson and Batjer (1950) observed that boron and boric acid at 25 to 40 ppm concentrations had stimulatory effect on pollen germination whereas at higher concentration boric acid had an inhibitory effect. Gausch and Dugger (1953) reported that borate ions reacted with sugar molecules to form an ionizable sucrose-borax complex which moved

through the cell readily than non-borated and non-ionizable sucrose molecule. Munzer (1960) found that 0.001 to 0.01 per cent boric acid had a stimulatory effect on germination and tube growth of pollen in more than 60 angiosperms. Vasil (1960) opined that boron helped in oxygen uptake in addition to synthesis of pectic substances required for the formation of wall of the germination tube. Johri and Vasil (1961) reported the effect of born as boric acid and borax in surpassing the effect of any known hormones, vitamins or other chemical substances. Linskens and Kroh (1970) opined that borunhad a role in the translocation and/or metabolism of carbohydrates.

Rao and Khader (1960) found that germination of sapota pollen could be enhanced appreciably by the addition of 100 ppm boric acid. Singh (1961) observed that 20 ppm boric acid gave better germination of mango pollen grains. Saoji (1975) reported 94 per cent germination in 100 ppm boric acid with 10 per cent sucrose in Momordica charentia.

Visser et al. (1977) reported that 15 per cent sucrose with 40 ppm boric acid increased the pollen germination in hybrid tea-rose. Ravindran (1977) found

that 100 ppm boric acid was optimum for cocoa pollen germination. In bitter gourd, Deshpande et al. (1970) observed good pollen germination with 200 ppm boric acid and in Jack, Tessy (1983) found 10 ppm the best.

In Hibiscus rosa-sinensis, Vilasini et al. (1966) reported that addition of 100 ppm boric acid increased pollen germination (93.02 per cent) and tube growth (138.60 μ) whereas in the absence of boric acid, only 89.80 per cent and 125.49 μ. They also reported that 200 ppm boric acid induced bursting of pollen grain and reduced pollen germination.

2.4.1.2 Effect of calcium nitrate

Lindforss, 1896 and Brink, 1924 as quoted by Tessy (1983) suggested that calcium nitrate was an inhibitor of pollen germination and tube growth. On the contrary, Brewbaker and Kwack (1963); Kwack and Brewbaker (1963); Kwack (1965); Jose and Magnoon (1972) and Ravindran (1977) realised the essential role of calcium in pollen germination and tube growth. Action of calcium appeared to be based on the non-metabolic incorporation of calcium into pectic substances of the pollen wall thus giving increased resistance against bursting of the pollen tubes. Weisenseel and Jaffe (1976)

reported that the role of calcium and potassium might be by maintaining the flow of currents through the pollen tubes. Govila and Rao (1969) and Govila (1970) observed that addition of Ca^{++} enhanced pollen germination in malvaceous crops. Rema Menon (1980) reported higher germination of pollen in pepper by the addition of 100 ppm Ca No_3. On the other hand, Tessy (1982) reported that Ca $N\hat{\theta}_3$ has an inhibitory action on jack pollen.

2.4.1.3 Effect of growth hormones

Smith (1939) found that 3-IAA had a favourable effect on pollen germination and rate of pollen tube elongation. Kato (1955) obtained increased growth of pollen tube, five times more than that of control, by the addition of 50 ppm GA in Lilium longifolium. In loquat pollen, Dikshit (1956) found that, NAA (5 ppm) and IAA (10 and 15 ppm) increased the percentage of germination and tube elongation. Konar (1958) studied the effect of IAA and kinetin in the pollen of Pinus roxburghii and found that addition of IAA increased the germination percentage and rate of tube elongation. Bose (1959) reported that GA did not affect pollen germination, but tube length was increased seven times

than that of control at 0.05 mg per litre (ppm) in Pisum sativum. Rao and Khader (1962) observed that both germination and tube growth were promoted by the addition of GA upto 100 ppm in sapota pollen.

Vilasini et al. (1967) tried three growth substances viz., 3-IAA, GA and 2,4-D at three different levels in Hibiscus and concluded that GA and 2,4-D inhibited pollen germination, whereas 3-IAA did not produce any such effect. All the above three growth regulators promoted pollen tube elongation, of which the effect of IAA was most pronounced.

Sinha (1973) observed that addition of 10 ppm IAA in the culture medium enhanced the pollen tube growth in jack fruit. Mc Leod (1975) observed that tomato pollen was almost completely insensitive to plant growth substances like auxins, gibberellins and abscissic acid.

According to Malik et al. (1977) auxins mobilized reserve food materials by enhancing the activity of hydrolysing enzymes and enhanced cell elongation. Tessy (1983) observed enhanced germination of 81.22 per cent by the addition of 10 ppm IAA rather than 77.65 per cent in sucrose-agar media.

2.4.2. Pollen tube growth

Amici, 1830 as quoted by Bhojwani and Bhatnagar (1974) the discoverer of pollen tube growth, recorded polysiphonous germination of the pollen grains in Hibiscus trionum and H. syriacus. In the latter some pollen grains gave rise to 20 to 30 tubes. Iyengar (1938) reported two tubes per pollen grain in Asiatic and in tetraploid American cotton and concluded that the frequency of pollen grain producing two tubes was greater in the tetraploid American type than in the diploid Asiatic ones. Purewall and Randhawa (1947) observed branching of the tubes both in culture media and on stigmatic surface of bhindi. Vilasini et al. (1966) reported polysiphonous germination in H. rosa-sinensis and Dubey (1969) in Abelmoschus esculentus. Nair et al. (1974) observed that Abelmoschus pollen grains were polysiphonous and that only one tube in anyone grain was functional while further growth of other tubes were arrested by the formation of callose plugs.

The time taken for pollen germination varied with species. Purewall and Randhawa (1947) found that

the pollen grains of H. esculentus germinated 30 minutes after they were placed in culture media. Vilasini et al. (1966) observed that pollen grains of Hibiscus rosa-sinensis commenced germination within 30 minutes of dusting and the rate of germination was highest during the first hour and no more germination was observable after the fourth hour. They also reported that the rate of tube elongation was highest from first hour upto eight hours. Dubey (1969) reported that germination of okra pollen started two hours after they were placed in sucrose solution of suitable concentration. In sucrose-agar medium, 89 per cent germinated within two hours.

2.5 Pollen storage

Methods of pollen collection from various crops for breeding purposes have been described by Barnett and Arisumi (1952), Singh et al. (1961) and Stanley and Linskens (1974). Critical external factors included relative humidity, temperature and atmosphere surrounding the pollen. Excellent reviews on pollen storage are available (Maheswari, 1944; Visser, 1955; Singh et al., 1961; and Shivanna et al., 1979).

2.5.1 Storage by controlling temperature and humidity

Pfundt, 1910 as quoted by Nair (1982) observed that most of the species maintained their pollen viability under low relative humidity. After detailed studies on the pollen storage requirements of 16 fruit trees, King and Hesse (1938) concluded that the optimum storage temperature for those pollen grains of tree crops was around 30°F. Nebel (1939) could successfully store pollen grains of apple, pear, peach, plum and apricot for 2 to 5½ years in a desiccator over sulphuric acid at 28°C temperature and 50 per cent relative humidity. Gollimick (1942) reported that grapes pollen could be stored alive for one year at 1°C under 40-50 per cent relative humidity. Varas (1962) found that cocoa pollen stored for a week in a desiccator germinated better in artificial medium. Singh (1962b) found that at room temperature the mango pollen remained alive for 12 to 20 days in desiccators and for eight days in petridish. At low temperature, the pollen stored in the desiccator exhibited longevity upto 7 to 10 months. Govila and Rao (1969) could store the pollen of cotton for two days only at 25 per cent RH.

Singha(1972) observed that jack fruit pollen could be stored alive upto eight months at 0°C and

and at a R.H. of 25 per cent or below. Coconut pollen dried at 40°C and kept over 43.4 per cent sulphuric acid in vacuum in sealed ampules at sub zero temperature, remained viable for more than one year (Child, 1974). Saoji (1975) reported that the pollen of Momordica charentia could be stored for five days in 9°C with four per cent R.H. Simmonds (1976) recorded that cocoa pollen could kept at 5°C in sealed tubes over calcium chloride for about one week.

2.5.2 Pollen storage by freezing

Griggs et al. (1953) were able to store the pollen of plum, peach, almond, apple, pear, cherry, citrus and walnut without loosing the viability for one to three years in a home freezer at -18°C. Better storage life of pollen grains of fruit crops in deep freezer has been reported by several workers (Singh, 1962 a; Singh, 1962 b; Shukla and Misra, 1975; Ekaratne and Senathirajah, 1983). Singh (1962 a) reported that litchi pollen would be stored under deep freeze condition (-23°C) for 31 months. Mango pollen gave a longevity of 14 months under deep freeze at -23°C (Singh, 1962 b). In citrus Sachan and Patro (1970) reported 50 per cent viability after 90 days storage in deep freeze. Shukla

and Misra (1975) reported 40 to 64 per cent fruit set with pollen stored in deep freeze for 15 days in kagzi lime. Ekaratne and Senathirajah (1983) reported that oven drying at 37°C for 2-8 hours followed by storage in a deep freezer proved to be the best method of storing the pollen of oil palm for 12 months.

2.5.3 Pollen storage by freeze drying

Lyophilization or freeze drying of pollen was reported to be one of the efficient methods of pollen storage by Stanley and Linskens (1974) and Nair (1977). Mathur (1969) fabricated a simple vacuum cum freeze drying apparatus for effective storing of pollen grains. Child (1974) reported that coconut pollen could be freeze dried and stored for long periods in sealed ampules for pollen exchange between distant countries.

2.5.4 Pollen storage in organic solvents

The efficiency of organic solvents like benzene, petroleum ether, ethanol, acetone and chloroform in storage of pollen grains was studied by Iwanami (1972 a,b; 1973, 1975) and Iwanami and Nakamura (1972). They pointed out that this method avoided the problem of maintaining a specific relative humidity and suggested that this technique might be a useful for transporting

pollen without refrigeration. Tessy (1983) reported that jack pollen could be stored upto 24 hours in petroleum ether and in acetone upto 12 hours and that the storage time could be extended upto 48 hours in 4°C. Mishra and Shivanna (1982) studied the efficacy of organic solvents for storing pollen grains of some leguminous taxa and reported that organic solvents were promising only for short term and not for long term storage.

3. Compatibility studies

Bailey (1949), Datta (1959), Anon (1959), Vasishta (1972), Bhat (1976) and Shukla and Misra (1979) reported that flowers of Hibisicus rosa-sinensis L., H. mutabilis L. and H. schizopetalusHook. were bisexual and generally cross pollinated and were entomophilous. Bhatnagar (1975-76) reported humming birds were the pollinating agents in Hibiscus. Vilasini et al. (1966) observed natural fruit set only in one variety out of the ten varieties studied. However, by artificial self pollination three varieties set seeds which indicated that the failure of setting seeds under natural conditions was due to the failure of pollination. They reported that some varieties of Hibiscus rosa-sinensis

were not capable of setting seeds by artificial self pollination and in some varieties the non seed setting nature was due to lack of pollen tube elongation beyond the region of stigmatic lobes. They did not get any seed set in Hibiscus schizopetalus and suggested that the early abscission of the style and stigma as possible reason for the absence of set. Bhat (1976) also reported that majority of the varieties did not set seeds naturally.

Patel (1933) reported that in cotton, hybridisation between Asiatic and new world types was a failure. Hutchinson et al. (1938) however, successfully crossed the Asiatic species of Gossypium arboreum and Gossypium herbacium with the wild African species Gossypium anomalum. They further reported that more closely related species crossed with each other, while others failed to cross or gave sterile hybrids. According to Silow (1941) the cause of interspecific incompatibility in the genus Gossypium as a whole was due to the lack of harmony between species. Sikka and Joshi (1960) enumerated several stages at which hybridization between Gossypium species might fail.

Tezima, 1930; Chizaki, 1934; Ustinova, 1937 as quoted by Nirmala (1982) and Arumugam et al. (1975) made

successful cross between Abelmoschus manihot and A. esculentus. Teszima reported that reciprocal crosses of the above species yielded no seeds. Teshima, 1933 as quoted by Nirmala (1982), Mamidwar et al. (1980), Nirmala (1982) observed fruit set when Abelmoschus esculentus was crossed with A. manihot. Abortive seed formation by reciprocal crosses of the above species was reported by Teshima.

Linskens (1975) reported that the interspecific incompatibility was heterogenic, i.e., controlled by more than one gene at different loci on the chromosomes.

Venkitaramani (1952), Joshi et al. (1958), Raman and Ramu (1962) and Datta and Premnath (1969) reported successful intervarietal crosses in different crops.

Gast (1971) reported that ornamental Hibiscus was a highly polymorphic cross-compatible group of species and complex hybrids. In India many new varieties of Hibiscus with wide array of contrasting flower colour combinations were produced by breeding (Devaiah, 1968; Sundar, 1971; Bhat, 1976, 1979; and Bhat and Verma, 1980). Devaiah (1968) reported that the Hibiscus seedlings evolved through hybridization from Hawaiian Hibiscus were of enormous size having vivid and

contrasting colour combinations. Sundar (1971) reported that hybrid Hibiscus bore most fascinating flowers with enormous size measuring 7 to 15 inches across and varied in colour combinations. Bhat (1976) cross pollinated nearly 700 flowers with 65 parental combinations and reported that there were slight variations with regard to number of days required for seed maturity (41 to 70 days) and number of seeds per capsule (2 to 15) in different intraspecific combinations. Bhat (1979) reported that fourteen new varieties of Hibiscus rosa-sinensis were bred at the Indian Institute of Horticultural Research, Hessarghatta after rigorous screening of the large population of Hibiscus hybrid seedlings. In IIHR, 1200 numbers of both interspecific and intervarietal hybrids were raised and from that six new varieties were released during the year 1980 (Bhat and Verma, 1980).

Materials and Methods

MATERIALS AND METHODS

The investigations on pollen morphology, pollen production, fertility and compatibility studies reported in the thesis were carried out during the period from May 1982 to September, 1983. The germplasm collection consisting of thirtyfour types/varieties of Hibiscus rosa-sinensis L. and two other species viz., Hibiscus mutabilis L. and Hibiscus schizopetalus Hook, maintained in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara were used for the present studies. The types/varieties/species were raised from cuttings/buddlings and planted in the year 1981. List of types/varieties and their sources are given below.

Accession number/Name			Source
1	Acc.1	(Quarrel star)	local collection from Kerala
2	Acc.2		local collection from Kerala
3	Acc.3		local collection from Kerala
4	Acc.4	(Shanti)	hybrid from IIHR Bangalore
5	Acc.5		local collection from Kerala
6	Acc.6		local collection from Kerala
7	Acc.7		local collection from Kerala

	Accession number/Name	Source
8	Acc. 8	local collection from Kerala
9	Acc. 9	local collection from Kerala
10	Acc.10	local collection from Kerala
11	Acc.11	local collection from Kerala
12	Acc.12	local collection from Bangalore
13	Acc.13	local collection from Kerala
14	Acc.14	local collection from Kerala
15	Acc.15	Lalbagh garden, Bangalore
16	Acc.16 (Nazneen)	hybrid from IIHR, Bangalore
17	Acc.17 (Narthaki)	hybrid from IIHR, Bangalore
18	Acc.18 (Hawaiian yellow)	hybrid from T.N.A.U.,Coimbatore
19	Acc.19	local collection from Bangalore
20	Acc.20 (Chitralekha)	hybrid from IIHR, Bangalore
21	Acc.21 (Tribal Queen)	hybrid from IIHR, Bangalore
22	Acc.22	local collection from Kerala
23	Acc.23	hybrid from IIHR, Bangalore
24	Acc.24	hybrid from IIHR, Bangalore
25	Acc.25	local collection from Kerala
26	Acc.26	local collection from Coimbatore
27	Acc.27 (Begnazeer)	hybrid from IIHR, Bangalore
28	Acc.28	Lalbagh garden, Bangalore
29	Acc.29	Lalbagh garden, Bangalore
30	Acc.30 (Smt.Indira Gandhi)	hybrid from IIHR,Bangalore

Accession number/Name		Source
31	Acc.31 (Thilakam)	hybrid from T.N.A.U., Coimbatore
32	Acc.32 (Yellow star)	T.N.A.U., Coimbatore
33	Acc.33	local collection from Kerala
34	Acc.34	local collection from Kerala
35	Hibiscus mutabilis (H.M.)	Local collection from Kerala
36	Hibiscus schizopetalus (H.S.)	Local collection from Kerala

1.1 Morphological description

Since all the types maintained did not possess distinct varietal names, detailed morphological descriptions of these types/varieties were made. A descriptive blank was prepared based on characters used for describing the types as adopted by Devaiah (1968), Sundar (1971), Bhat (1976, 1979), Bhat and Verma (1980) with suitable modifications. The blank used is presented in Appendix I. The colours of the flowers were compared by using the 'Dictionary of colour' by Maerz and Rea Paul (1950).

Data on the number of flowers produced from June 1982 to May 1983 were recorded. The number of flowers opened were counted daily and the total number of flowers produced in a month was computed.

1.2 Anthesis and anther dehiscence

In order to collect basic data on anthesis and anther dehiscence in the different types/varieties/species selected for the study, the following procedures were adopted.

Ten mature buds in each type/variety/species were tagged on the previous day of flower opening for observations on flower opening in different types/varieties/species selected for the studies. These flower buds were observed at half hourly intervals for recording their time of opening and repeated thrice.

For collecting data on the time of anther dehiscence, ten mature buds in each type/variety/species were tagged on the previous day of flower opening and they were observed at half hourly interval commencing from 6 pm and repeated thrice.

The time of the folding of corolla was also observed. For this purpose the opened flowers in each type/variety/species with stalks intact were collected in the evening and placed with their stalk ends dipped in water. These flowers were observed at hourly intervals and repeated thrice. The time taken for the folded corolla to fall down was also noted under field conditions. The closed flowers were observed at 12 hours interval until the corolla completely fell down.

2. Pollen studies

Pollen morphology, pollen production, pollen fertility, pollen germination and storage life of pollen were studied in thirty three types/varieties of Hibiscus rosa-sinensis and two other species viz., Hibiscus mutabilis and H. schizopetalus.

2.1 Pollen morphology

Flowers were collected soon after anther dehiscence and kept in a desiccator for one hour so as to collect the pollen grains needed for the study. Pollen morphology was studied after staining the pollen grains using acetocarmine.

2.1.1 Pollen size

Pollen grains were dusted in a drop of acetocarmine-glycerine medium on a clean microscopic slide and covered with zero coverglass for 30 minutes. Diameter of 10 normal well shaped, plumpy and well stained pollen grains from each type or variety or species as the case may be was measured at random using a standardized occular micrometer under low power of a microscope. The mean diameter was expressed in microns.

2.1.2 Pollen shape

The same slides were utilized to study the shape of pollen grains under high power magnification.

Colour of unstained, fresh pollen grains was studied under low power of a microscope.

2.2 Pollen production

A haemocytometer was used for estimating the number of pollen grains per anther. One hundred mature anthers were gathered from freshly opened flowers of each type/variety/species before anther dehiscence. Mature anthers of each type were taken in separate vials and stored in a desiccator for five hours for proper dehiscence. The method used by Vilasini et al. (1966) was followed which is detailed below.

After anther dehiscence, 1.25 ml of water containing 1.0 per cent teepol was added to the vials containing the anthers and the contents were shaken throughly. To this 1.25 ml of glycerine was added. A drop of the above suspension, drawn in a fine pipette, was transferred to each of the two counting chambers of a haemocytometer. Pollen grains in each of the four corner squares in both counting chambers were counted

and the mean number in eight corner squares were calculated. For each type/variety/species, five such estimates were made.

The number of pollen per anther was calculated as follows.

Let N = average number of pollen counted per corner square and X = number of pollen per anther

then N : X = 0.1 : 25

0.1 X = 25 N

X = 250 N

2.3 Pollen fertility

Fertility of the pollen grains was estimated by acetocarmine staining technique.

Pollen grains were dusted in a drop of acetocarmine-glycerine medium on a clean microscopic slide for 30 minutes for proper staining and examined under low power of a compound microscope. Pollen fertility was estimated by counting fertile and sterile pollen grains separately. Pollen grains which stained well, looked plumpy and well shaped were considered as fertile and those unstained, small or shrivelled as non-viable or sterile. The observations were made in ten different microscopic fields and the mean percentage of viable pollen grain was arrived at.

Three such estimates (slides) were prepared. Fertility of pollen grain was expressed as percentage.

2.4 Pollen germination

In vitro culturing of pollen grains was made in artificial media.

2.4.1 Standardisation of media

The media tried by Vilasini *et al*. (1966) did not give satisfactory germination of the pollen grain, which necessitated further detailed studies for standardising the media for pollen germination and tube growth.

The following three media were tried and the pollen grains from the normally seed setting type (Acc.2) in *H. rosa-sinensis* were utilised for standardisation.

1. Sucrose media

The varying concentrations of sucrose in distilled water namely, 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 per cent were made. Pollen grains were collected soon after anther dehiscence and the freshly collected pollens were dusted to a drop of the solution in cavity slides and allowed to rest as hanging drops. A humid environment was provided for the germination by placing

the slides in a desiccator containing water. Germinated and non-germinated pollen grains were counted and their tube length was measured after 24 hours, in 10 different microscopic fields by observing under the low power of microscope. Viability was expressed as percentage and tube length measured in microns (μ). The test was repeated for three times.

2. Sucrose with agar media

From the trials using sucrose media, the most suitable concentrations for maximum pollen germination and tube growth was arrived at. To these concentrations, agar at 0.5, 1.0 and 1.5 per cent was added. Sucrose alone in three concentrations (20, 25 and 30 per cent) and each in combination with concentrations of agar were also tried. Pollen germination and tube growth was assessed by hanging drop technique.

3. Sucrose-agar-boric acid media

From the previous studies, the suitable concentrations of sucrose-agar media were fixed (20, 25 and 30 per cent sucrose with one per cent agar). Sucrose-agar in the above concentrations and each in combination with three different concentrations of boric acid viz., 50, 100 and 150 ppm were prepared. The per cent viability

of pollen in the above media was studied by hanging drop technique.

To determine the optimum time required for pollen germination and tube growth, observations were made after 1, 2, 4, 6, 8 and 24 hours from dusting. The experiment was repeated thrice.

2.4.2 Pollen germination studies of different types/varieties/species

From the studies as detailed above, the best medium for pollen germination studies was fixed (20 per cent sucrose + 1 per cent agar + 100 ppm boric acid). This medium was used for further detailed studies on pollen germination and tube growth in 35 types/varieties of *Hibiscus rosa-sinensis* L. and in *H. mutabilis* L. and *H. schizopetalous* Hook. In all cases studies were repeated thrice.

The length of pollen tubes of ten grains selected at random from each slide was measured and mean tube growth was found out. As a number of pollen tubes emerged from a single grain, measurement were made only of the longest tubes. Three such measurements were made for each type and the data were statistically analysed.

2.5 Pollen storage

Five types/varieties viz., Acc.2, Acc.11, Acc.18, Acc.22 and Acc.26 of shoeflower were selected for the study. Flowers with dehisced anthers and fresh pollen grains as the case was used for pollen storage studies.

The different methods and conditions adopted are detailed below.

1. Flowers at room temperature (T_1)
2. Flowers at 4°C in refrigerator (T_2)
3. Flowers over calcium chloride at room temperature (T_3)
4. Flowers over calcium chloride at 4°C (T_4)
5. Pollen at room temperature (T_5)
6. Pollen at 4°C in refrigerator (T_6)
7. Pollen over calcium chloride at room temperature (T_7)
8. Pollen over calcium chloride at 4°C (T_8)
9. Pollen in petroleum ether at 4°C in refrigerator (T_9)
10. Pollen in acetone at 4°C in refrigerator (T_{10})

Pollen grains collected under different storage conditions were incubated in sucrose-agar-boric acid medium (20% + 1% + 100 ppm) and germination percentage were recorded at 12 hours interval upto 96 hours.

3. Compatibility studies

Seven types/varieties of *Hibiscus rosa-sinensis* L. (Acc.2, 5, 11, 16, 18, 22 and 26) and two other species namely *H. mutabilis* L. and *H. schizopetalus* Hook. were selected for the study.

3.1 Self compatibility

The above seven types/varieties of *H. rosa-sinensis*; *H. mutabilis* and *H. schizopetalus* were selfed.

3.2 Cross compatibility

3.2.1. Intraspecific crosses

The following were the female and male parents used.

Female parent	Male parent
Acc. 2	Acc. 2
Acc. 5	Acc. 11
Acc.11	Acc. 18
Acc.16	Acc. 22
Acc.18	Acc. 26
Acc.22	
Acc.26	

Cross combinations

2 x 11	5 x 2	11 x 2	16 x 2	18 x 2	22 x 2	26 x 2
2 x 18	5 x 11	11 x 18	16 x 11	18 x 11	22 x 11	26 x 11
2 x 22	5 x 18	11 x 22	16 x 18	18 x 22	22 x 18	26 x 18
2 x 26	5 x 22	11 x 26	16 x 22	18 x 26	22 x 26	26 x 22
	5 x 26		16 x 26			

3.2.2 Interspecific crosses

Three types/varieties of Hibiscus rosa-sinensis (Acc. 2, 18 and 26) and two other species viz., H. mutabilis (H.M.) and H. schizopetalus (H.S.) were used for crossing.

Cross combinations

2 x H.M.	18 x H.M.	26 x H.M.	H.M. x 2	H.S. x 2
2 x H.S.	18 x H.S.	26 x H.S.	H.M. x 18	H.S. x 18
			H.M. x 26	H.S. x 26
			H.M. x H.S.	H.S. x H.M.

3.2.3 Techniques of crossing and selfing

Mature buds of the female parent were emasculated on the evening prior to the expected date of flower opening. Emasculation was done by first removing the corolla by passing a knife without injuring the androecium and the style. The anther lobes were carefully scraped out leaving the pistil

with its stigma intact. The emasculated flowers were then bagged till the next morning. Pollen from the bagged flower of the desired parents, soon after dehiscence were brought along with the staminal column and slowly smeared on the stigmatic surface of the female parents. After pollination, the flowers were bagged and properly labelled. Swelling of the ovary was perceptible within 48 hours of pollination. The bags were removed after a week of pollination and young capsules were allowed to develop under natural conditions.

For selfing, the same procedure was followed. Pollen from the bagged flowers of the same plant was used.

Capsule development in each of the pollinated flowers was observed on the 5th day and 15th day after pollination. Wherever capsules were retained after 15 days they were observed till maturity.

For assessing the cross and self compatibility the capsules were harvested at full maturity, just in advance of dehiscence and the hybrid seeds were freshly sown in pots containing the potting mixture 1:1:1 (Soil: sand:cowdung).

The following observations were made.

(i) Number of crosses made and percentage of fruit set at 5th, 15th days and at maturity.

$$\text{Percentage of success} = \frac{\text{Number of fruit set} \times 100}{\text{Number of crosses made}} \quad (A)$$

(ii) Days to maturity

(iii) Number of seeds/fruit (capsule) (B)

(iv) Days to germination

(v) Percentage of germination of the seeds.

$$\frac{\text{Seeds germinated} \times 100}{\text{Number of seeds sown}} \quad (C)$$

(vi) Percentage of survival of the germinated seedlings (D)

$$\frac{\text{Seedlings survived} \times 100}{\text{Number of seedlings germinated}}$$

3.3 Crossability index

For successful crosses, a crossability index was used to measure the crossing affinity between each pair of parents. Crossability index was calculated as suggested by Rao, 1979.

$$\text{Crossability index} = \frac{\text{Crossing efficiency of the cross} \times 100}{\text{Selfing efficiency of female parent}}$$

$$= \frac{A^{c*} \times B^{c*} \times C^{c*} \times D^{c*}}{A^{s*} \times B^{s*} \times C^{s*} \times D^{s*}} \times 100$$

c* = crossed

s* = selfed

A = Percentage of fruit set

B = Average number of seeds per fruit

C = Percentage germination of the seeds

D = Percentage survival of the germinated seedlings

Statistical analysis

The data collected on different aspects were tabulated and analysed statistically. Transformations were done wherever needed and the data analysed by the analysis of variance technique. Significant results were compared after finding out the critical difference.

RESULTS

Results of the investigation on pollen morphology, pollen production, fertility and compatibility studies in Hibiscus rosa-sinensis L. and two other species viz., H. mutabilis L. and H. schizopetalus Hook. are presented below.

1.1 Morphological description

Morphological description of thirtyfour types/varieties of H. rosa-sinensis (H.R.); and H. mutabilis (H.M.) and H. schizopetalus (H.S.) are presented in Table 1. Photographs of all the types/varieties/species are presented in plates I, II, III, IV and V.

The petals of the local types were generally of single shade whereas most of the hybrids possessed different shades on the petals. The leaf shape varied widely between types/varieties/species ranging from deeply lobed to round or cordate ones. Generally the local types had ovate, serrate leaves, while the Hawaiian varieties and some hybrids had thick cordate-ovate crinkled leaves with almost entire or serrulate margin. The numerical arrangement of the floral parts viz., the number of epicalyx (5 to 9), number of lobes of calyx

Plate I - V. Types/varieties/species of Hibiscus

I.

1. Acc. 7
2. Acc. 19
3. Acc. 13
4. Acc. 14
5. Acc. 10
6. Acc. 22

II.

1. Acc. 17 (Narthaki)
2. Acc. 28
3. Acc. 16 (Nazneen)
4. Acc. 27 (Benazeer)
5. Acc. 23
6. Acc. 4 (Shanti)
7. Acc. 32 (Yellow star)
8. Acc. 24


1
2
3
4
5
6


1
2
3
4
5
6
7
8

Plate III.

1. Hibiscus mutabilis
2. Hibiscus schizopetalus
3. Acc.30 (Smt.Indira Gandhi)
4. Acc.29
5. Acc.20 (Chitralekha)
6. Acc.31 (Thilakam)

Plate IV.

1. Acc. 12
2. Acc. 15
3. Acc. 21 (Tribal Queen)
4. Acc. 18 (Hawaiian yellow)
5. Acc.33
6. Acc.11
7. Acc. 2
8. Acc.26
9. Acc. 1 (Quarrel star ?)


1
2
3
6
5
4


1
2
3
4
5
6
7
8
9

Plate V.

1. Acc. 5
2. Acc. 3
3. Acc. 6
4. Acc. 9
6. Acc. 25
7. Acc. 34
5. Acc. 8


1
2
3
4
5
6
7

(2 to 7), pedicel length, flower size, length of staminal column, number of anthers per flower (4 to 140) and length of style varied between species, varieties and types. Flowers of the Hawaiian varieties and hybrids in general were of large size. Double flowers had extremely short styles while the singles possessed long styles. The number of anthers were less in double flowers compared to singles. The hybrids had usually large sized anthers compared to local types. All the singles had monadelphous staminal column whereas in doubles, multiadelphous condition was observed. The staminal tube was seen dissected into two or more groups, each with varying numbers of anthers. The central group was more thicker and possessed more number of anthers compared to thinner tubes bearing lesser number of anthers in the laterals. In some types of doubles, the central monadelphous tube branched and the petals just surrounding the staminal tube got fused at the basal region of the tube. In most of the double flowers, petalody of the staminal column extended to the ovary. In Acc.3 alone pistil was rudimentary and in Acc. 34, stamens were absent.

1.1.1 Flower production

Data on the monthly flower production of the types/varieties/species are presented in Table 2.

Table 2. Flower production in different types/varieties/species of Hibiscus

Sl. No.	Types/ varieties/ species	1982							1983					Total
		June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	
1	Acc. 14	390	383	383	323	720	233	248	638	820	700	480	143	5461
2	Acc. 22	225	375	458	285	405	180	203	480	690	578	578	323	4780
3	Acc. 1	210	210	368	465	465	210	173	360	398	188	218	143	3408
4	Acc. 19	105	165	203	315	210	143	315	623	570	383	180	68	3280
5	Acc. 13	263	398	353	75	165	143	75	323	315	278	113	53	2554
6	Acc. 26	53	98	225	263	143	143	173	300	285	180	255	105	2223
7	Acc. 7	128	150	248	338	173	173	120	255	128	113	128	83	2037
8	Acc. 25	38	143	278	308	210	158	203	98	240	113	113	23	1925
9	Acc. 2	105	128	233	150	195	143	165	180	150	60	83	53	1645
10	Acc. 33	90	150	225	188	195	173	90	113	240	38	23	23	1548
11	Acc. 6	495	180	293	38	60	60	98	75	98	23	15	23	1558
12	Acc. 31	15	38	180	255	173	98	120	135	180	105	90	8	1397
13	Acc. 11	23	113	113	173	180	105	135	165	143	38	38	23	1249
14	H.M.	0	0	120	323	128	233	173	53	60	0	0	0	1090
15	Acc. 9	90	83	128	233	240	38	23	45	90	23	23	23	1039
16	Acc. 18	75	113	98	83	45	68	53	75	165	195	38	30	1038
17	Acc. 5	75	98	108	135	145	130	93	83	68	30	15	25	1005
18	Acc. 32	23	60	143	165	60	83	113	83	120	68	30	15	963
19	Acc. 3	113	218	165	98	153	45	30	30	23	15	30	8	925
20	Acc. 28	15	53	128	173	83	53	38	45	105	98	60	30	881

(Contd.)

Table 2. (Contd.)

Sl. No.	Types/ varieties/ species	1982						1983					Total
		June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	
21	Acc. 12	0	15	0	0	15	60	45	105	270	150	150	30	840
22	Acc. 29	45	83	105	45	68	45	98	90	68	45	38	15	745
23	Acc. 34	60	38	165	165	60	53	8	38	15	23	68	23	716
24	H.S.	30	53	143	150	90	60	75	53	23	12	8	0	697
25	Acc. 23	23	53	83	68	90	45	23	98	60	30	45	15	633
26	Acc. 10	133	105	158	23	58	15	8	10	30	10	0	0	550
27	Acc. 8	23	15	98	60	53	38	38	45	38	15	8	15	446
28	Acc. 21	23	38	45	30	30	15	8	23	30	23	23	8	296
29	Acc. 15	8	23	15	0	0	8	38	30	53	30	8	0	213
30	Acc. 27	8	8	0	23	30	15	15	0	30	30	23	15	197
31	Acc. 20	8	15	0	23	53	8	23	8	15	8	8	15	184
32	Acc. 16	23	38	38	8	15	15	15	0	8	0	0	0	160
33	Acc. 30	0	8	15	15	8	15	30	23	8	8	15	8	153
34	Acc. 17	0	0	0	8	45	23	23	0	8	0	15	15	137
35	Acc. 4	0	23	8	15	15	23	8	8	15	0	0	15	130
36	Acc. 24	0	0	15	8	15	8	8	23	8	15	0	0	100

Majority of the types/varieties of H.R. bloomed throughout the year. Among the two species, H.S. and H.M., the former flowered throughout the year except during May, while latter flowered only from August to February. It was observed that generally for all types/varieties/species August to October was the peak period of flowering followed by January to March while April-May was the lean period. Moderate flowering was noticed during June-July and November-December. Acc.14 showed the maximum flower production during the year followed by Acc.22 and the minimum was noticed in Acc.24. Sixteen types/varieties of H.R. and one species H.M. produced more than 1000 flowers per year.

1.2 Anthesis and anther dehiscence

Results of the studies on anthesis are presented in Table 3. In all the types/varieties or species flower opening took place during the early morning hours. Majority of them opened between 5 to 6 am while Acc.10 and H.M. opened between 4 to 5 am. In Acc.2, 5, 6, 7, 13 and 27, flower opening took place between 6 to 7 am. But in most of the hybrids like Acc.4, 18, 20, 21, 23, 24, 28, 30 and 31, flower opening took place between 7 am and 8.30 am. There was little seasonal variation in this between types/varieties or species.

Table 3. Flower opening, anther dehiscence and corolla retention in different types/varieties/species of Hibiscus

Sl. No.	Types/ varieties/ species	Time of flower opening	Time of anther dehiscence	Time taken by the corolla to fold up (hrs.)	Time taken by the corolla to fall down (hrs)
1	Acc. 1	5.00 - 6.00 am	7.00 - 8.00 am	13 - 17	12 - 36
2	Acc. 2	6.30 - 7.00 am	6.30 - 7.30 am	15 - 18	24 - 36
3	Acc. 3	5.00 - 7.00 am	6.30 - 7.30 am	16 - 20	24 - 48
4	Acc. 4	7.00 - 8.00 am	8.00 - 9.00 am	20 - 28	36 - 60
5	Acc. 5	6.00 - 7.30 am	8.30 - 9.30 am	18 - 23	48 - 72
6	Acc. 6	7.00 - 7.30 am	7.00 - 7.30 am	14 - 16	12 - 36
7	Acc. 7	7.00 - 7.30 am	3.00 - 5.00 pm	16 - 19	36 - 60
8	Acc. 8	5.00 - 6.00 am	6.00 - 7.30 am	13 - 15	36 - 48
9	Acc. 9	5.00 - 6.00 am	7.00 - 8.00 am	18 - 20	12 - 36
10	Acc. 10	4.00 - 4.30 am	9.30 - 10.30 pm	14 - 19	48 - 60
11	Acc. 11	5.00 - 5.30 am	6.00 - 7.00 am	12 - 16	24 - 36
12	Acc. 12	5.00 - 5.30 am	10.00 - 1.00 pm	13 - 19	24 - 48
13	Acc. 13	7.00 - 7.30 am	2.00 - 5.00 pm	16 - 20	36 - 48
14	Acc. 14	5.00 - 6.00 am	9.00 - 1.00 pm	15 - 17	24 - 36
15	Acc. 15	5.00 - 6.00 am	7.00 - 8.00 am	13 - 15	48 - 72
16	Acc. 16	5.00 - 6.00 am	10.00 - 10.30 am	15 - 18	36 - 60
17	Acc. 17	5.00 - 5.30 am	9.30 - 10.30 am	16 - 19	36 - 60
18	Acc. 18	8.00 - 8.30 am	8.30 - 9.30 am	13 - 16	24 - 48

(Contd.)

Table 3. (Contd.)

Sl. No.	Types/ varieties/ species	Time of flower opening	Time of anther dehiscence	Time taken by the corolla to fold up (hrs)	Time taken by the corolla to fall down (hrs)
19	Acc. 19	5.00 - 6.00 am	7.30 - 8.00 am	14 - 16	24 - 48
20	Acc. 20	8.00 - 8.30 am	8.30 - 9.30 am	13 - 16	24 - 48
21	Acc. 21	7.30 - 8.30 am	9.00 - 9. 30 am	11 - 15	24 - 48
22	Acc. 22	5.00 - 5.30 am	5.00 - 6.30 am	13 - 18	24 - 48
23	Acc. 23	7.00 - 8.00 am	8.00 - 10.00 am	12 - 13	24 - 36
24	Acc. 24	8.00 - 8.30 am	9.00 - 10.00 am	13 - 16	12 - 24
25	Acc. 25	6.00 - 6.30 am	7.30 - 8.30 am	14 - 17	24 - 36
26	Acc. 26	5.00 - 6.00 am	6.30 - 8.30 am	15 - 21	24 - 48
27	Acc. 27	6.30 - 7.30 am	8.00 - 8.30 am	15 - 20	36 - 60
28	Acc. 28	7.00 - 7.30 am	8.00 - 9.00 am	13 - 16	12 - 36
29	Acc. 29	5.00 - 6.00 am	6.30 - 7.00 am	16 - 18	24 - 48
30	Acc. 30	7.30 - 8.00 am	8.30 - 9.30 am	14 - 20	24 - 48
31	Acc. 31	7.00 - 8.00 am	8.00 - 9. 00 am	12 - 17	12 - 24
32	Acc. 32	5.00 - 6.00 am	7.30 - 8.30 am	14 - 18	36 - 48
33	Acc. 33	5.00 - 5.30 am	7.00 - 8.00 am	13 - 15	24 - 48
34	Acc. 34	5.00 - 6.00 am	-	26 - 36	60 - 72
35	H.M.	4.00 - 5.30 am	5.30 - 6.30 am	12 - 15	24 - 36
36	H.S.	5.00 - 6.00 am	7.30 - 8.00 am	16 - 20	12 - 24

Table 3 represents the data on anther dehiscence. The result indicated that the anther dehiscence took place during the morning hours. In most of the types/varieties/species, anther dehiscence commenced soonafter the flower opening while in Acc.10, cleistogamy was observed i.e., the anther dehiscence took place before flower opening. In Acc.12 and Acc.14, anther dehiscence took place between 8.30 am to 1 pm and in Acc.7 and 13 it was between 2 to 5 pm. There was not much seasonal variation for anther dehiscence in most of the types/varieties/species. Anther dehiscence was generally earlier during winter and summer than on rainy periods. In Acc.16 and 21, structural sterility was noticed in summer i.e. anther dehiscence did not take place at that time.

Hibiscus flower lasted for a day, usually folding an hour or so after sundown. During cool months, they lasted for more time. Flowers of all the types/varieties/species folded between 12 to 18 hours after flower opening except in H.S. (16 to 20 hours), Acc.4 (20 to 28 hrs) and Acc. 34 (26 to 30 hrs). Generally the double petalled ones and the hybrid flowers lasted longer than others.

The retentivity of the corolla was different in different types/varieties/species (Table 3). In most of the types/varieties/species, the corolla fell down within 24 to 48 hours after flower opening. However, Acc.4, 7, 10, 16, 17 and 27 lasted upto 60 hours whereas, Acc. 5, 15 and 34 lasted upto 72 hours.

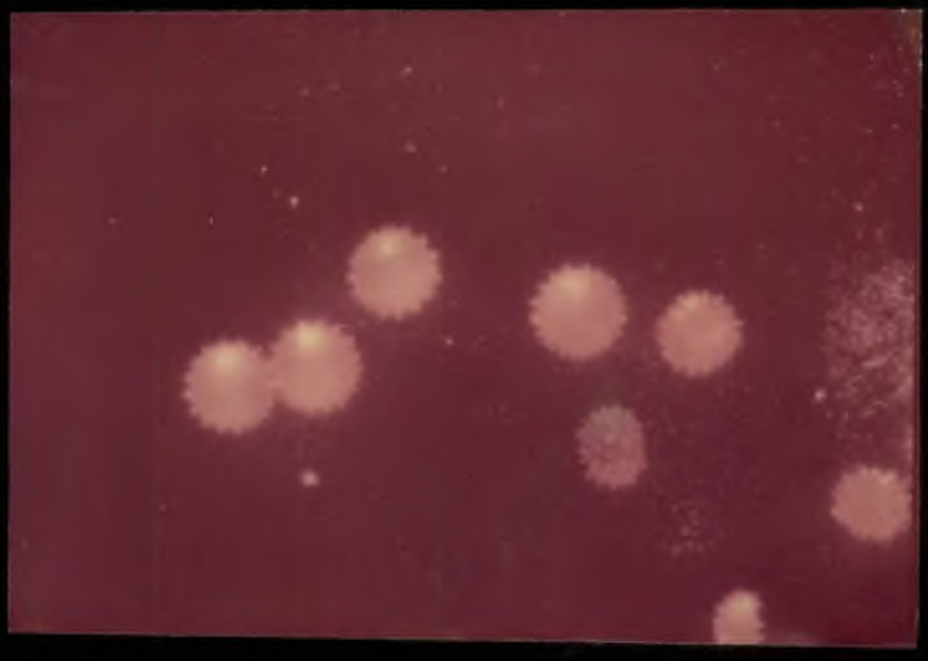
2. Pollen studies

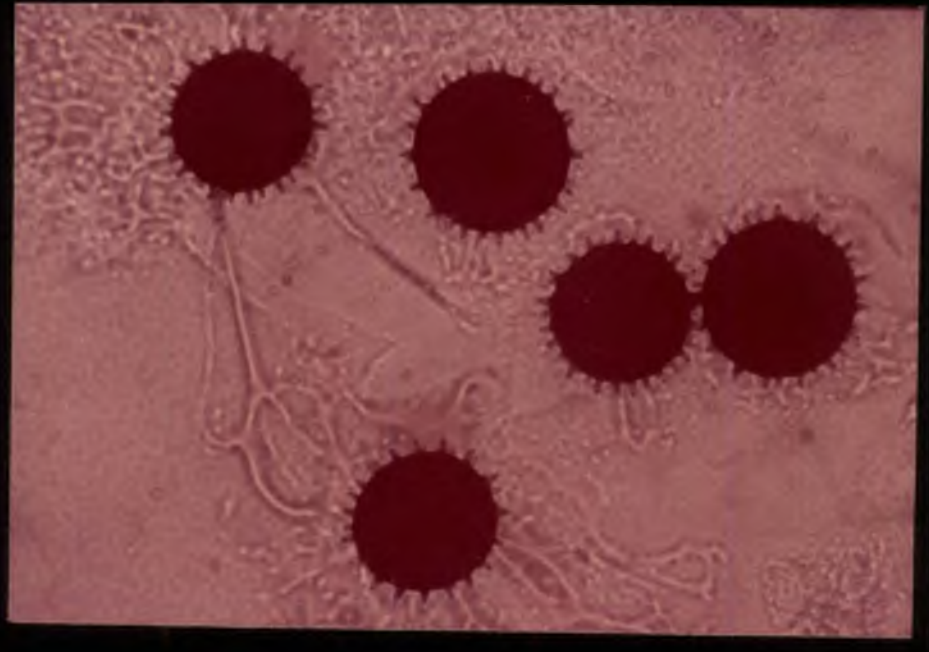
2.1. Pollen morphology

Pollen grains appeared as powdery mass to the naked eye and their colour varied from white in the case of Acc.10 to creamy-white in Acc.11, 26, 27 and Hibiscus mutabilis and yellow in all the remaining ones (Plate VI). The outer surface of the yellow pollen grains was found to be surrounded by a yellow substance under low power of a compound microscope. The amount of these substance varied with the types/varieties/species which resulted in varying shades of yellow.

2.1.1. Pollen shape

The pollen grains of different types/varieties/species were found to have more or less similar shape (Plate VII). Pollen grains were pantoporate, spheroidal and spinose. Along with normal pollen grains, abnormal





grains such as elongated ones and pollinoid bodies (antheridial bodies resembling the pollen grains) were also observed.

2.1.2 Pollen size

There was significant intervarietal variation for pollen size among the different types/varieties/species (Table 4). Size of the pollen grains varied from 198.58 μ in Acc.25 to 125.91 μ in Acc.29. The mean size was 160.67 u. Acc. numbers 25, 30, 11, 6, 20, 18, 21, 32, 2, 24, 4, 26, 5, 13 and 17 recorded a higher size than the mean, while the pollens of the remaining types/varieties/species were smaller. The pollen size was found to differ not only between types/varieties but also within them. Variation in size was found also between normal and abnormal grains and within the normal grains.

2.2 Pollen production

Table 4 gives an account of the results of the estimation of pollen production in thirtyfive types/varieties/species. The data when statistically analysed showed significant difference in pollen output among the types/varieties/species. The highest number of pollen per anther was recorded in H. mutabilis (500)

Table 4. Pollen size, production and fertility in different types/varieties/species of Hibiscus

Pollen size		Pollen production		Pollen fertility	
Types/ varieties/ species	Mean diameter of pollen (μ)	Types/ varieties/ species	Mean number of pollen grains/ anther	Types/ varieties/ species	Percentage fertility
Acc. 25	198.58	H.M.	500	H.M.	97.4 (80.76)
Acc. 30	186.24	Acc. 27	482	Acc.20	96.1 (78.58)
Acc. 11	174.24	Acc. 10	437	Acc. 1	94.2 (76.00)
Acc. 6	173.56	Acc. 33	375	Acc.10	91.2 (72.72)
Acc. 20	173.56	Acc. 6	362	Acc.22	89.6 (71.16)
Acc. 18	172.89	Acc. 32	357	Acc.28	88.5 (70.15)
Acc. 21	171.36	Acc. 23	350	Acc.29	88.2 (69.94)
Acc. 32	171.19	Acc. 2	345	Acc.11	87.6 (69.36)
Acc. 2	170.52	Acc. 22	345	H.S.	87.5 (69.26)
Acc. 24	170.18	Acc. 26	332	Acc.23	87.0 (68.87)
Acc. 4	166.63	H.S.	320	Acc. 6	86.7 (68.64)
Acc. 26	165.62	Acc. 24	312	Acc. 2	86.4 (68.34)
Acc. 5	164.61	Acc. 20	295	Acc.27	82.6 (65.34)
Acc. 13	163.59	Acc. 18	287	Acc.26	82.6 (65.32)
Acc. 17	163.08	Acc. 28	287	Acc.15	82.3 (65.12)
Acc. 27	160.58	Acc. 29	282	Acc.33	77.9 (61.96)
H.S.	159.95	Acc. 1	275	Acc.32	77.7 (61.85)
Acc. 8	159.87	Acc. 3	275	Acc. 3	76.5 (61.03)
Acc. 23	159.03	Acc. 15	275	Acc.18	74.4 (59.62)
Acc. 12	158.69	Acc. 25	270	Acc.24	67.5 (55.26)
Acc. 19	158.69	Acc. 31	270	Acc. 8	66.8 (54.83)

(Contd.)

Table 4. (Contd.)

Pollen size		Pollen production		Pollen fertility	
Types/ varieties/ species	Mean diameter of pollen (μ)	Types/ varieties/ species	Mean number of pollen grains/ anther	Types/ varieties/ species	Percentage fertility
Acc. 28	158.52	Acc. 30	257	Acc. 5	65.7 (54.17)
Acc. 10	157.75	Acc. 19	245	Acc.25	62.6 (52.31)
Acc. 9	157.50	Acc. 8	227	Acc.30	60.0 (50.75)
Acc. 1	157.00	Acc. 4	212	Acc.19	59.9 (50.69)
Acc. 3	156.66	Acc. 14	200	Acc. 4	58.9 (50.11)
Acc. 14	155.98	Acc. 17	182	Acc.31	58.9 (50.10)
H.M.	155.48	Acc. 9	170	Acc. 9	37.8 (37.93)
Acc. 33	153.28	Acc. 12	150	Acc.17	27.4 (31.59)
Acc. 22	146.02	Acc. 5	145	Acc.16	17.2 (24.52)
Acc. 31	141.20	Acc. 7	145	Acc.13	14.8 (22.62)
Acc. 16	139.76	Acc. 13	132	Acc.14	11.9 (20.14)
Acc. 7	139.28	Acc. 11	107	Acc.12	7.8 (16.24)
Acc. 15	136.42	Acc. 21	95	Acc.21	5.8 (13.94)
Acc. 29	125.91	Acc. 16	87	Acc. 7	4.6 (12.43)
Mean	160.67	Mean	268.46	-	-
C.D.	0.16	C.D.	0.17	C.D.	4.66

Note: The data are presented in the descending order.
Values in parentheses denote the means of the transformed data

and the lowest in Acc.16 (87). The average number of pollen per anther worked out to 268. From the table it was found that H.M., H.S. and 19 types/varieties of H.R. produced higher number of pollen grains than the mean while the remaining ones had a lesser pollen production.

2.3 Pollen fertility

The data presented in Table 4 gives the results of pollen fertility studies by acetocarmine staining technique. H. mutabilis and Acc.20 showed significantly higher percentage of fertility compared to all other types/varieties/species. The minimum pollen fertility was observed in Acc.7. The pollen fertility between types/varieties/species varied from 4.6 per cent in Acc.7 to 97.4 per cent in H.M.

2.4. Pollen germination

2.4.1.Standardisation of the culture medium

Results of the studies on the standardisation of medium for in vitro culture of pollen grains are presented in Table 5.

2.4.1.1. Sucrose solution alone

Table 5 a gives the result of the pollen germination and tube growth in different concentrations of sucrose. The germination percentage varied depending upon the sucrose concentration in the medium. Of the 13 sucrose concentrations tried, 20 per cent sucrose gave the highest percentage of germination (47.5) followed by 25, 30, 35, 40, 45, 15, 50 and 10 per cent. There was no significant difference between 20 and 25 per cent sucrose on pollen germination. It was also found that in concentrations below 5 per cent and above 55 per cent the pollen grains failed to germinate.

Maximum tube growth of pollen was observed in 20 per cent sucrose (137.33 μ) followed by 25 and 30 per cent which produced a tube growth of 131.5 μ and 124.97 μ respectively. There was no significant difference between these concentrations of sucrose on pollen tube elongation. Between the treatments 30 and 35 per cent, 40 and 45 per cent, 45 and 15 per cent, and 50 and 10 per cent, there was no difference.

In sucrose solutions of concentrations from 20 to 30 per cent, both percentage of germination and rate of tube elongation were found to be highest (Fig.1).

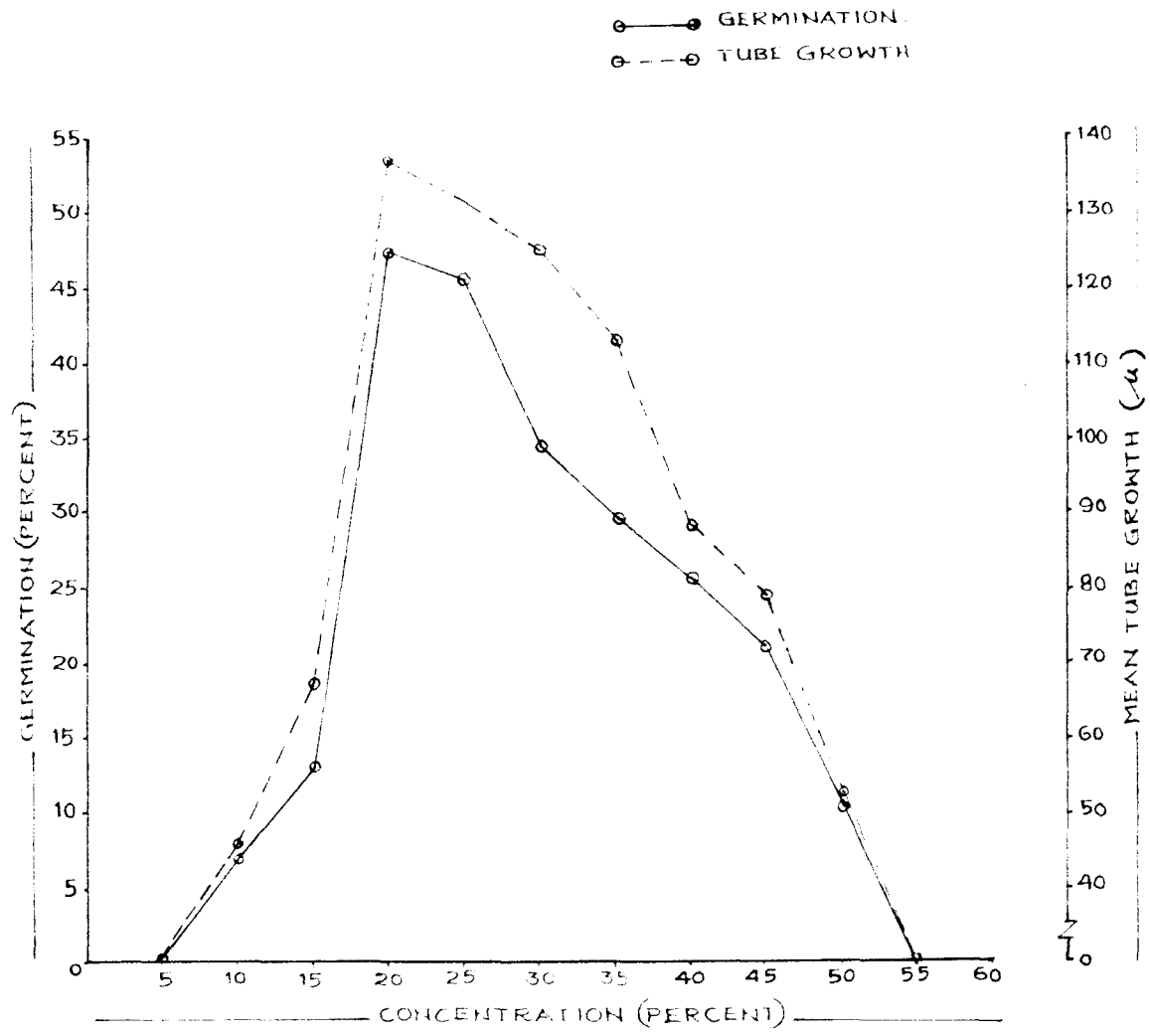
Table 5. Standardisation of media

a) Germination and tube elongation of pollen grains in sucrose medium.

Conc. of sucrose soln. %	Mean germination (%)	Mean pollen tube growth (μ)
0	0	0
5	0	0
10	6.7 (14.98)	44.63
15	13.2 (21.32)	67.29
20	47.5 (43.55)	137.33
25	45.8 (42.60)	131.50
30	34.3 (35.86)	124.97
35	29.6 (32.94)	112.96
40	25.5 (30.33)	87.89
45	20.9 (27.17)	78.97
50	10.4 (18.81)	52.19
55	0	-
60	0	-
C.D.	2.85	12.54

Values in parentheses denote the means of the transformed data.

Fig. 1 - EFFECT OF SUCROSE ON POLLEN GERMINATION AND TUBE GROWTH



Concentrations higher than 30 per cent were found to have retarding effect both on germination and tube growth. From these studies, three concentrations of sucrose solutions viz. 20, 25, and 30 per cent were found to be the best.

2.4.1.2. Sucrose with agar

Results of pollen germination and tube growth in different sucrose and agar concentrations are given in Table 5b. Among the three concentrations tried, 20 per cent sucrose solution gave the maximum germination of pollen grains.

Agar concentration of the medium also influenced the germination percentage and tube elongation. A concentration of one per cent agar was found to be significantly superior to other concentrations for pollen germination as well as for pollen tube growth. Agar concentrations 1.5 per cent and 0.5 per cent were statistically on par, and gave lower germination when compared to one per cent. The control (0% agar) recorded lowest germination percentage. From the results,it was found that 20 per cent sucrose and one per cent agar was the best medium for pollen germination and for pollen tube growth.

Table 5 b.

Germination and tube elongation in sucrose-agar medium.

Treatments		Mean germination (%)		Mean tube growth (μ)
Sucrose conc. %	Agar conc.%			
20	0.0	47.5	(43.55)	137.33
	0.5	78.3	(62.20)	490.62
	1.0	80.5	(63.80)	504.36
	1.5	79.0	(62.71)	382.47
25	0.0	45.8	(42.60)	131.49
	0.5	73.6	(59.11)	334.06
	1.0	76.4	(60.92)	351.92
	1.5	74.0	(59.31)	315.18
30	0.0	34.3	(35.86)	124.97
	0.5	62.6	(52.27)	250.63
	1.0	65.2	(53.87)	259.22
	1.5	60.8	(51.25)	238.27
C.D.		2.61		35.38

Values in parentheses denote the means of the transformed data.

2.4.1.3. Sucrose in combination with agar and boric acid

Table 5c represents the result of the pollen germination and tube growth in different sucrose-agar-boric acid media.

Addition of boric acid to sucrose-agar media increased the percentage germination and tube elongation. Among the four doses of boric acid tried, 100 ppm was found to be significantly superior than the rest. Concentrations above this resulted in bursting of pollen grains besides reducing the pollen germination. In the absence of boric acid in sucrose-agar media, there was significant reduction in pollen tube growth.

From the results it was found that a medium consisting of 20 per cent sucrose, one per cent agar and 100 ppm boric acid was the best for pollen germination (83.8%) and tube growth (962.48 μ). The lowest germination (50.5%) was in the media containing 30 per cent sucrose with one per cent agar and 150 ppm boric acid while the lowest pollen tube growth was noticed in 30 per cent sucrose with one per cent agar and without boric acid.

Observations on the germination of pollen grains in the best medium showed that the pollen grains commenced

Table 5 c.

Germination and tube elongation in sucrose-agar boric acid medium.

Treatments		Mean germination (%)		Mean tube growth (μ)
Sucrose with 1% agar (%)	Boric acid conc. (ppm)			
20	0	80.5	(63.80)	504.36
	50	81.8	(64.78)	819.91
	100	83.8	(65.69)	962.48
	150	69.2	(56.27)	642.89
25	0	76.4	(60.92)	351.92
	50	78.4	(62.33)	730.34
	100	80.2	(63.55)	823.09
	150	55.8	(48.31)	558.62
30	0	65.2	(53.87)	259.22
	50	72.0	(58.02)	555.97
	100	74.0	(59.25)	607.88
	150	50.5	(45.30)	436.19
C.D.		1.88		63.05

Values in parentheses denote the means of transformed data.

Table 6. Incubation period for pollen germination and tube elongation in sucrose-agar-boric acid media.

Hours after pollen planting	Percentage germination	Mean pollen tube length (μ)
1	68.6 (55.94)	447.32
2	74.8 (59.89)	668.33
4	81.8 (64.77)	884.04
6	82.8 (65.53)	904.18
8	83.0 (65.69)	960.83
24	83.0 (65.69)	962.48
C.D.	2.44	80.12

Note: Values in parentheses denote the means of the transformed data

germination within 30 minutes of dusting at room temperature in a humid chamber. The data presented in Table 6 showed that the rate of germination and tube elongation was highest during the first hour, which progressively decreased till eight hours, after which there was no further germination or tube elongation. The germination percentage although reached a maximum in eight hours after planting, there was no significant difference between 8 hours and 4 hours.

2.4.2. Pollen germination in different types/varieties/species

Results of the studies on pollen germination and tube growth of 33 types/varieties of H. rosa-sinensis and two other species namely H. mutabilis and H. schizopetalus are presented in Table 7.

Variations in pollen germination were significant between types/varieties/species. Pollen grains of Acc.11 gave the highest percentage of germination (85.8%) followed by H.M., Acc.2, 26, 27 and 22 which were on par. The percentage germination was below 20 per cent in four types. In Acc.3, 7, 9, 12, 13, 14, 16, 17, 19, 21, 28 and 31, the pollen grains failed to germinate.

Table 7. Pollen germination and tube elongation of different types/varieties/species of Hibiscus.

Pollen germination			Pollen tube growth	
Types/ varieties/ species	Mean per cent of germination		Types/ varieties/ species	Mean tube length (μ)
Acc. 11	85.8	(67.86)	Acc. 2	962.48
H.M.	84.5	(66.80)	Acc.11	961.42
Acc. 2	83.0	(65.69)	H.M.	940.22
Acc. 26	81.5	(64.56)	Acc.22	892.52
Acc. 27	79.6	(63.17)	Acc.26	862.84
Acc. 22	79.3	(62.92)	Acc.25	734.58
H.S.	74.6	(59.74)	Acc.27	718.15
Acc.15	72.9	(58.63)	H.S.	645.01
Acc. 33	71.3	(57.59)	Acc.18	621.16
Acc. 18	68.1	(55.63)	Acc.32	607.38
Acc. 5	63.6	(52.88)	Acc.30	589.89
Acc. 8	61.2	(51.47)	Acc. 4	576.11
Acc. 32	59.1	(50.21)	Acc. 8	544.84
Acc. 4	51.5	(45.85)	Acc.33	541.66
Acc. 25	46.3	(42.87)	Acc. 5	535.30
Acc. 30	44.2	(41.66)	Acc.15	520.99
Acc. 24	37.6	(37.84)	Acc.23	443.61
Acc. 1	37.5	(37.75)	Acc. 1	435.13
Acc. 23	28.1	(32.03)	Acc.29	427.18
Acc. 10	18.3	(25.34)	Acc.24	395.91
Acc. 29	15.7	(23.35)	Acc.20	299.45
Acc. 20	13.8	(21.77)	Acc.10	254.93
Acc. 6	2.5	(8.98)	Acc. 6	84.8
C.D.	5.23			109.05

Note: Values in parentheses denote the means of transformed data.

The germinating pollen grains in a majority were polysiphonous (Plate VIII and IX). The number of pollen tubes varied from 2 to 14 in the different types/ varieties/species. In some cases individual pollen tubes were found to be branched. It was further noticed that all the tubes except one stopped elongation. The pollen tubes usually were long and straight except for few terminal or intermediary enlargements called vesicles, where the cytoplasm was dense (Plate X). Formation of callose plugs was also observed which nearly or completely blocked the passage for cytoplasm (Plate XI).

Pollen tube length was found to vary among the different types/varieties studied. Greatest tube length was observed in Acc.2 (962.48 µ) followed by Acc.11, H.M., Acc.22 and 26 which were on par. The least tube length was observed in Acc.6 (84.80 µ) which was significantly inferior to all others.

2.5 Pollen storage

Results of the investigation conducted on storage capacity of pollen grains of the five types/varieties of Hibiscus rosa-sinensis under different conditions recorded at 12 hours intervals are presented in Table 8a

Plate VIII & IX

Pollen grains germinated in sucrose-agar-boric acid medium (X 100)

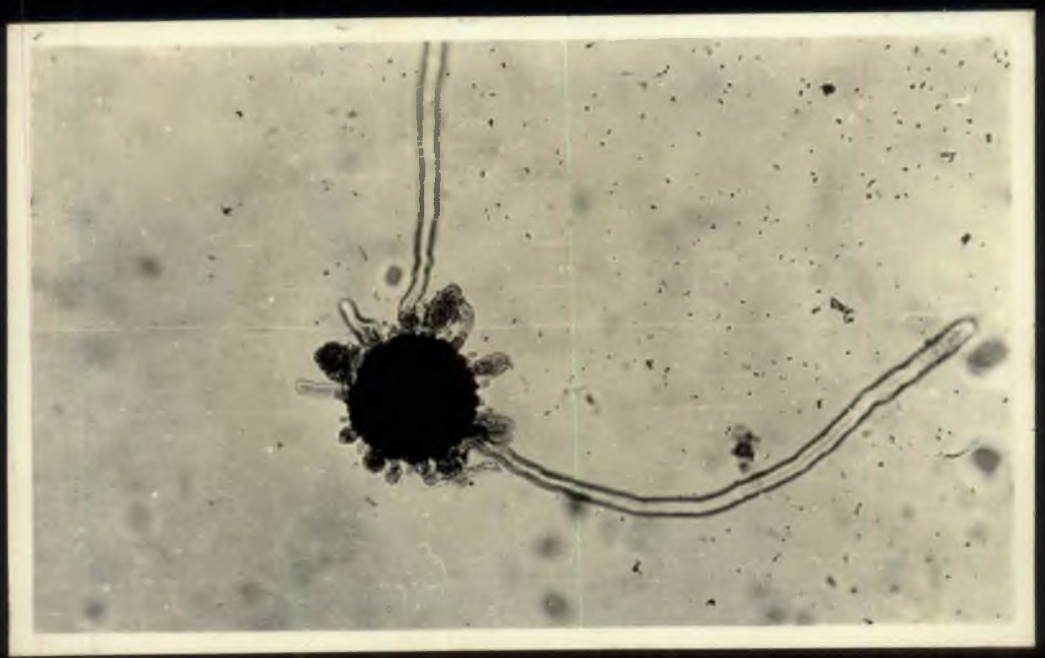
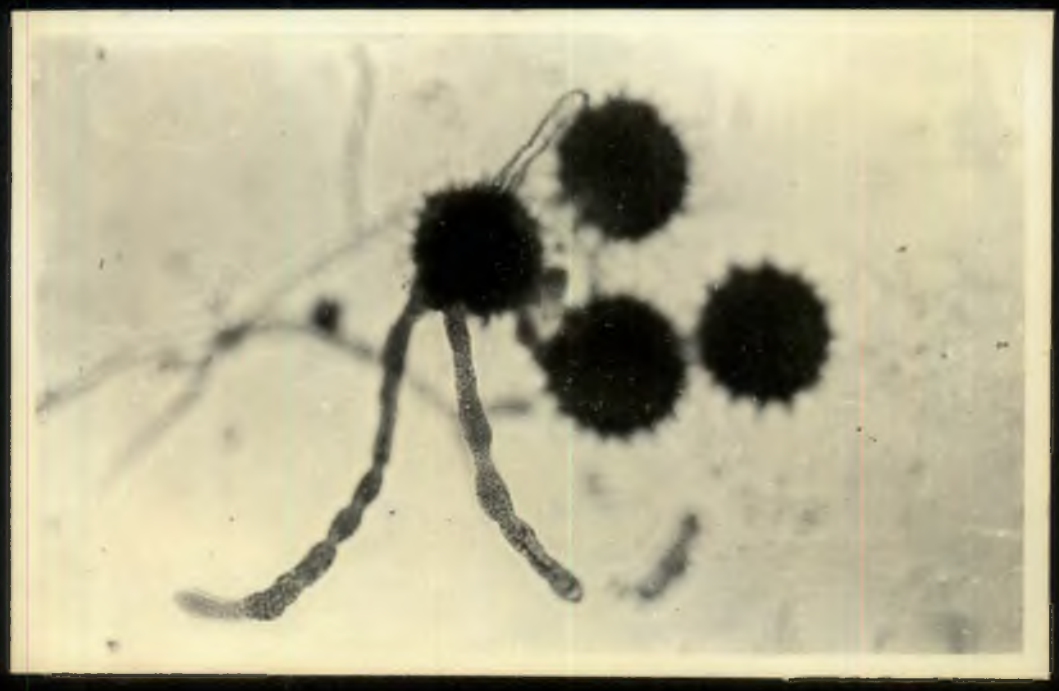
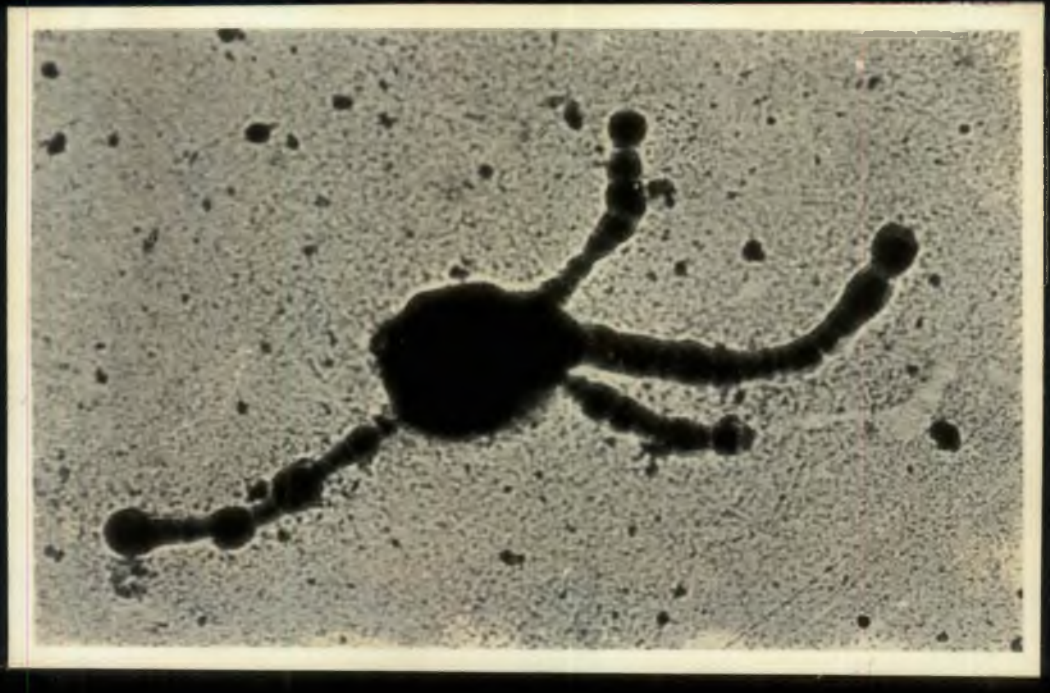


Plate X & XI

Germinated pollen grains showing vesicles and callose plugs (X 110)





and 8b. The tables have been arranged in the order of the storage life of pollen grains.

The results indicated that the storage life of pollen grains was significantly influenced by the different methods of storage tried. In all the methods, the storage life as indicated by pollen germination rapidly decreased reaching the minimum within 48 to 96 hours. Storage of flowers with anther column intact at $4^{o}C$ in a desiccator over calcium chloride (T_4) gave significantly superior storage life of pollen. Pollen grains stored under the above method gave 64.7 per cent of germination at 12 hours of storage and lost their viability after 84 hours of storage. The next best storage methods were keeping the flowers at $4^{o}C$ in refrigerator (T_2), storage of flower over calcium chloride at room temperature (T_3) and storage of pollen over calcium chloride at $4^{o}C$ (T_8). When either flowers or pollen were stored at room temperature (T_1 and T_5), the pollen grains lost their viability within 48 hours. Storage of pollen in organic solvents, namely petroleum ether and acetone resulted in instantaneous loss of viability.

The results thus indicated the following.

1) Storage under low temperature increased the storage life and resulted in higher percentage of

Table 8a. Effect of storage conditions in pollen longevity

Treatments	Percentage of pollen germination							
	Hours after collection							
	12	24	36	48	60	72	84	96
1. Flowers over calcium chloride at 4°C (T_4)	64.70 (53.55)	48.6 (44.18)	30.6 (33.59)	20.3 (26.76)	11.85 (20.13)	4.35 (12.05)	0.58 (4.37)	0
2. Flowers at 4°C (T_2)	53.3 (46.89)	41.4 (40.03)	21.1 (27.36)	11.5 (19.80)	3.2 (10.35)	0.62 (4.52)	0	
3. Flowers over $CaCl_2$ at room temperature (T_3)	51.8 (46.01)	39.9 (39.20)	18.9 (25.75)	9.4 (17.86)	2.15 (8.46)	0.13 (2. 10)	0	
4. Pollen over $CaCl_2$ at 4°C (T_8)	57.8 (49.51)	38.5 (38.35)	21.0 (27.28)	11.0 (19.35)	1.2 (6.34)	0.08 (1.64)	0	
5. Pollen at 4°C (T_6)	40.65 (39.61)	30.7 (33.65)	11.2 (19.51)	3.7 (11.09)	0.11 (1.93)	0		
6. Pollen over CaCl2 at room temperature (T_7)	38.2 (38.16)	28.8 (32.49)	8.9 (17.38)	2.75 (9.63)	0.06 (1.38)	0		
7. Flowers at room temperature (T_1)	34.0 (35.67)	20.7 (27.03)	4.7 (12.57)	0.57 (4.34)	0			
8. Pollen at room temperature (T_5)	27.1 (31.36)	16.0 (23.59)	1.85 (7.80)	0.04 (1.13)	0			
9. Pollen in Petroleum ether at 4°C (T_9)	0							
10. Pollen in acetone at 4°C (T_{10})	0							
C.D.	1.36	1.43	2.24	1.96	1.79			

Note: Values in parentheses denote the means of the transformed data

Table 8b. Pollen longevity in five types/varieties

Types/varieties	Percentage germination							
	Hours after collection							
	12	24	36	48	60	72	84	96
Acc. 26	53.4 (46.94)	40.5 (39.52)	21.8 (27.86)	11.2 (19.53)	3.4 (10.58)	0.54 (4.20)	0.07 (1.46)	0
Acc. 11	55.4 (48.09)	41.9 (40.32)	14.85 (22.65)	8.05 (16.49)	1.45 (6.93)	0.52 (4.13)	0.05 (1.27)	0
Acc. 22	54.3 (47.45)	37.2 (37.56)	18.3 (25.34)	6.7 (14.96)	1.2 (6.22)	0.12 (1.96)	0	
Acc. 2	45.2 (42.27)	31.7 (34.24)	11.4 (19.70)	4.4 (12.10)	0.9 (5.45)	0.11 (1.89)	0	
Acc. 18	22.4 (28.82)	14.6 (22.44)	3.95 (11.45)	0.95 (5.59)	0.04 (1.18)	0		
E.D.	1.08	1.13	1.77	1.55	1.39			

Note: Values in parentheses denote the means of transformed data.

germination of pollen grains throughout the period of storage life.

2) Storage over calcium chloride both at room temperature and 4°C helped to increase the storage life.

3) Flowers with anther column intact were found to be better for increasing the life of pollen under storage than pollen grains as such.

The result of the analysis showed significant difference between varieties/types with respect to the time taken by the pollen grains to loose their viability (Table 8b). Acc.26 and 11 were found to have a significantly higher longevity period as compared to all other types or varieties. It was followed by Acc.22 and 2. Acc.18 was found to have the lowest storage life.

3. Compatibility studies

H. schizopetalus and all the varieties/types of H. rosa-sinensis except Acc.2 failed to set fruits naturally, while H. mutabilis produced fruits in abundance. In Acc.2, fruit set was occasional and occurred mainly during the peak flowering season (August to October).

3.1. Self compatibility

Results of the studies on the self compatibility of the seven types/varieties of Hibiscus rosa-sinensis (H.R.) and then other species H. mutabilis (H.M.) and H. schizopetalus (H.S.) are presented in Table 9. More than 75 per cent fruit set was observed in five varieties/types of H.R. and in H.M. after 5 days of pollination. But the percentage was reduced to 48 to 82.6 after 15 days of selfing. At maturity, H.M. gave the maximum percentage of capsule set (82.6%) followed by Acc.2 (62%) and Acc.26 (60.87%). Acc.5 gave the minimum (36%). Two types of H.R. viz., Acc.11 and Acc.16 and the species H.S. gave no fruit set when selfed. Number of days taken for maturity varied for different types/varieties/species. The variety Acc.18 took maximum time (38 days) while Acc.2, the least (28 days).

Mean number of seeds per capsule also differed in different types/varieties/species. In H. mutabilis, 154 to 218 seeds per capsule were found, whereas, in H. rosa-sinensis the number of seeds per capsule varied from 1 to 20.

With respect to germination of seeds, Acc.18 showed maximum percentage of germination (69.77%) followed

Table 9. Self compatibility in different types/varieties/species of Hibiscus (Percentage of capsule set, days for maturation and seed germination)

Sl. No.	Genotype	No. of flowers pollinated	Capsule set at 5 days %	Capsule set at 15 days %	Capsule set at maturity % (A^{s*})	Days to maturity	Average No. of seeds per capsule (B^{s*})	Days to germination	Germination percentage (C^{s*})	Survival of germinated seedlings (D^{s*})
1.	Acc. 2	50	80.00	64.00	62.00	30	12.20	17	45.90	100.00
2.	Acc. 5	25	76.00	48.00	36.00	31	8.50	18	65.12	96.43
3.	Acc.11	50	-	-	-	-	-	-	-	-
4.	Acc.16	10	-	-	-	-	-	-	-	-
5.	Acc.18	13	76.92	61.54	46.15	38	8.50	14	69.77	96.67
6.	Acc.22	21	76.19	57.14	42.86	30	12.00	14	56.67	97.06
7.	Acc.26	23	82.61	60.87	60.87	28	10.67	12	67.92	100.00
8.	H.M.	20	90.00	82.61	82.61	31	192.50	10	3.33	100.00
9.	H.S.	25	-	-	-	-	-	-	-	-

by Acc.26 and Acc.5 while H.M. showed the least (3.33%). Acc.5 took maximum time for germination (18 days) while H.M. took the least (10 days). All the types/varieties/species showed more than 95 per cent survival of germinated seedlings.

3.2 Cross compatibility

3.2.1. Intraspecific crosses

Cross compatibility among the seven types/varieties of *H. rosa-sinensis* was studied. Data relating to the percentage of capsule set (after 5 days, 15 days and at maturity), number of days required for seed maturity, and number of seeds per capsule, number of days required for seed germination, germination percentage of the hybrid seeds and the survival percentage of germinated seedlings are presented in Table 10.

The results of the crosses (Fig.2) showed that wherever Acc.11 was used as the female parent, there was no fruitset. This type was also self incompatible. In crosses where Acc.16 was the maternal parent, fruit set ranged from 15.79 to 38.88 per cent. In case where the female parent was Acc.5, the range of fruit set was from 16.22 to 37.84 per cent. Acc.22 when used as the female parent gave a fruit set ranging from 28.57 per cent to

50 per cent. The percentage of fruit set varied from 38.89 to 53.19 when Acc.2 was the pistillate parent. When Acc.26 was the material parent, percentage of fruit set ranged from 33.33 to 57.14.

With respect to seed set,it was found that in crosses where Acc.2, 5 and 26 were the maternal parent, the seeds set per fruit was high than their respective selfed maternal parent which ranged from 10.8 to 17.4, 8 to 13.8 and 6.5 to 13.6 seeds per capsule respectively. In crosses where the materal parents were Acc.#8 and 22, the mean number of seeds per fruit produced was between 5 to 8.67 and 8 to 12.5 respectively which was comparable to the seed production in their selfed maternal parent. The mean seed set per fruit was comparatively less when Acc.16 was used as the pistillate parent (3.25 to 7.00).

The germination percentage of the hybrid seeds was high in crosses where Acc.2, 22 and 26 were used as the female parent except in crosses 22 x 18 and 26 x 18. But it was low in crosses where Acc.5 and 18 were used as the maternal parent except in crosses 5 x 11 and 18 x 11. The percentage of germination of seeds obtained from cross combinations where Acc.16 was used as the pistillate parent, was very low as compared to other crosses.

Table 10. Intraspecific cross compatibility between different types/varieties of *Hibiscus rosa-sinensis* L.

Sl. No.	Genotype	No. of crosses made	Capsule set after 5 days %	Capsule set after 15 days %	Capsule set at maturity % (A^{c*})	Days to maturity	Average number of seeds/ fruit (B^{c*})	Days to germination	Germination percentage (E^{c*})	Survival of seedling percentage (D^{c*})	Crossability index % (C.I.)
1	2 x 11	47	70.60	63.82	53.19	28	16.2	13	76.54	100.00	189.96
2	2 x 18	36	72.22	55.56	38.89	27	17.4	11	56.32	97.96	107.53
3	2 x 22	30	80.00	63.33	40.00	28	12.2	12	73.77	95.56	99.09
4	2 x 26	55	76.36	65.45	50.94	28	10.8	14	70.37	100.00	111.51
5	5 x 2	49	75.51	38.78	20.49	27	13.8	16	62.32	100.00	91.71
6	5 x 11	44	75.00	47.73	20.45	29	11.0	16	74.55	100.00	87.27
7	5 x 18	33	69.70	51.52	36.36	30	8.0	15	55.00	95.45	79.47
8	5 x 22	37	67.57	43.24	16.22	31	9.25	11	65.22	96.67	49.23
9	5 x 26	37	81.08	54.05	30.84	31	8.75	14	59.09	100.00	82.98
10	11 x 2	50	-	-	-	-	-	-	-	-	-
11	11 x18	47	-	-	-	-	-	-	-	-	-
12	11 x22	49	-	-	-	-	-	-	-	-	-
13	11 x26	49	-	-	-	-	-	-	-	-	-
14	16 x 2	18	66.67	50.00	38.88	28	4.33	12	45.45	90.00	-
15	16 x11	20	70.00	45.00	25.00	29	3.67	14	33.33	100.00	-
16	16 x18	14	71.43	42.86	21.43	30	7.00	13	45.71	93.75	-
17	16 x 22	19	63.15	47.37	15.79	31	3.25	15	43.75	85.71	-
18	16 x 26	15	73.33	60.00	33.33	30	3.25	13	31.25	100.00	-

(Contd.)

Table 10. (Contd.)

Sl. No.	Genotype	No.of crosses made	Capsule set after 5 days %	Capsule set after 15 days %	Capsule set at maturity % (A^{c*})	Days to maturity	Average number of seeds/ fruit (B^{c*})	Days to germination	Germination percentage (C^{c*})	Survival of seedling percentage (D^{c*})	Crossability index % (C.I.)
19	18 x 2	33	78.78	60.61	48.49	37	8.67	12	55.81	100.00	88.68
20	18 x 11	24	70.83	58.33	50.00	35	7.33	9	61.08	100.00	112.31
21	18 x 22	21	66.67	47.62	28.57	36	5.00	11	56.00	92. 86	28.08
22	18 x 26	20	75.00	45.00	45.00	36	8.33	12	57.14	100.00	80.96
23	22 x 2	26	76.92	38.46	23.07	28	12.00	12	57.89	93.94	53.22
24	22 x 11	26	80.77	57.69	42.31	27	12.50	11	71.43	97.78	130.57
25	22 x 18	24	79.17	50.00	20.83	28	10.75	12	55.56	96. 67	42.51
26	22 x 26	29	72.41	48.20	31.03	28	8.50	9	67.40	100.00	62.88
27	26 x 2	50	80.00	64.00	56.00	27	11.20	14	71.43	100.00	101.56
28	26 x 11	49	77.55	67.35	57.14	28	13.60	15	77.94	100.00	137.30
29	26 x 18	24	70.83	45.83	37.50	26	10.50	15	56.60	100.00	50.52
30	26 x 22	24	66.67	37.50	33.33	27	6.50	8	75.75	92.00	35.71

Fig.2 - INTRASPECIFIC CROSSES AMONG DIFFERENT TYPES/VARIETIES OF HIBISCUS ROSA-SINENSIS.

♀ \ ♂	Acc.2	Acc.11	Acc.18	Acc.22	Acc.26
Acc.2	Ⓢ	⊗	⊗	⊗	⊗
Acc.5	⊗	⊗	⊗	⊗	⊗
Acc.11	O	O	O	O	O
Acc.16	⊗	⊗	⊗	⊗	⊗
Acc.18	⊗	⊗	Ⓢ	⊗	⊗
Acc.22	⊗	⊗	⊗	Ⓢ	⊗
Acc.26	⊗	⊗	⊗	⊗	Ⓢ

Ⓢ NORMAL SEED SET ON SELFING.

⊗ NORMAL SEED SET ON CROSSING.

O CROSSES UNSUCCESSFUL (NO FRUIT SET)

The percentage survival of the hybrid seedlings was more than 95 per cent in all the crosses except 16 x 2, 16 x 18, 16 x 22, 18 x 22 and 26 x 22.

There were only slight variations with respect to number of days required to seed maturity and seed germination in different cross combinations. In all the crosses the capsules took comparatively lesser time to attain its maturity than their selfed maternal parent. Germination of the hybrid seeds was earlier than their selfed maternal seeds except in the case of Acc.26.

3.2.2. Interspecific crosses

The results of all the crosses of three Hibiscus species are shown in Table 11 and Fig.3. The crosses between Acc.2 x H.S. and Acc.26 x H.S. although produced seeds, they were inviable. The fruits produced out of the cross Acc.18 x H.S. dropped after 15 days without attaining maturity. The results indicated certain degree of incompatibility between H. rosa-sinensis and H. schizopetalus.

The crosses between H. rosa-sinensis and H.mutabilis showed capsule set only after five days of crossing but in about 15 days the capsules fell down. Crosses using

Table 11. Interspecific cross compatibility between three species of Hibiscus

Sl. No.	Genotype	No. of crosses made	Capsule set after 5 days %	Capsule set after 15 days %	Capsule set at maturity %	Days to maturity	Average No. of seeds/ fruit	Germination percentage (%)
1	2 x H.M.	49	16.33	-	-	-	-	-
2	2 x H.S.	39	74.36	41.02	5.13	27	8.4	-
3	18 x H.M.	40	15.00	-	-	-	-	-
4	18 x H.S.	22	54.35	31.82	-	-	7.0	-
5	26 x H.M.	22	40.91	-	-	-	-	-
6	26 x H.S.	31	70.96	35.48	6.45	26	10.0	-
7	H.M. x 2	31	-	-	-	-	-	-
8	H.M. x 18	18	-	-	-	-	-	-
9	H.M. x 26	22	-	-	-	-	-	-
10	H.M. x H.S.	24	-	-	-	-	-	-
11	H.S x 2	20	-	-	-	-	-	-
12	H.S. x 18	24	-	-	-	-	-	-
13	H.S. x 26	21	-	-	-	-	-	-
14	H.S. x H.M.	28	-	-	-	-	-	-

Fig.3- INTERSPECIFIC CROSSES AMONG THREE SPECIES OF HIBISCUS

♀ \ ♂	Acc.2	Acc.18	Acc.26	H.M.	H.S.
Acc.2	Ⓢ	⊗	⊗	Ⓥ	⊘
Acc.18	⊗	Ⓢ	⊗	Ⓥ	Ⓥ
Acc.26	⊗	⊗	Ⓢ	Ⓥ	⊘
H.M.	O	O	O	Ⓢ	O
H.S.	O	O	O	O	O

Ⓢ NORMAL SEED SET ON SELFING.
⊗ NORMAL SEED SET WHICH GREW WELL.
⊘ NORMAL SEEDS BUT DID NOT GROW.
Ⓥ FRUIT SET, BUT FELL AFTER 15 DAYS.
Ⓥ FRUIT SET, BUT FELL AFTER 5 DAYS
O CROSS UNSUCCESSFULL (NO FRUIT SET)

Plate XII.

Capsules of *Hibiscus rosa-sinensis*

1. Acc. 26
2. Acc. 2
3. Acc. 16

Plate XIII.

Capsules of *Hibiscus rosa-sinensis*

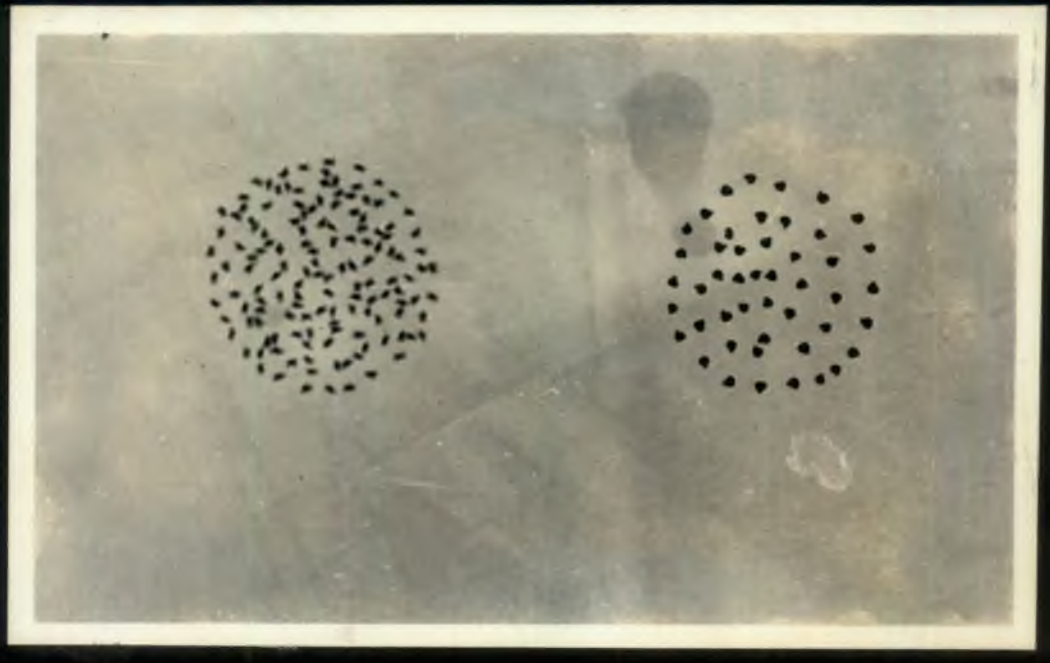
1. Acc. 22
2. Acc. 18
3. Acc. 5

Plate XIV.

Capsule and seeds of *H. mutabilis*

Plate XV.

Seeds of *H. mutabilis* and *H. rosa-sinensis*



H.M. and H.S. as the maternal parents did not produce any ovary stimulation, while on selfing, H.M. gave 82.6 per cent capsule set.

3.3 Crossability index

Crossability index was calculated (Table 10) and it was found to be the highest for Acc.2 x 11 and the lowest for 18 x 22.

3.4. Fruit and seed characters

Variation in fruit shape was noticed in different types/varieties/species (Plate XII and XIII). The shape of capsules in Fo was similar to that obtained by selfing the female parent. The capsules of different types/varieties of H. rosa-sinensis were of ovoid, oblong or subglobose. They were beaked or without beak, with acute, flat or emarginate. The selfed capsules of H. mutabilis were globose and hairy. (Plate XIV)

The seeds of different types/varieties of H. rosa-sinensis were found to have more or less similar shape. They were globose and black. The seeds of H. mutabilis were reniform and hairy (Plate X V).

Discussion

DISCUSSION

Among the three species of Hibiscus grown for flowers viz., Hibiscus rosa-sinensis L., H. mutabilis L. and H. schizopetalus Hook, the species H. rosa-sinensis is grown widely under varied climatic and soil conditions. Humid tropical conditions of Kerala provide excellent environment for growing a large number of Hibiscus varieties. Although shoeflower has been grown in Kerala from time immemorial especially in the homestead for ornamental purposes, attempt has not so far been made to make a comprehensive analysis of the several types available in this part of the country. Several of the types grown possess conspicuous magnifient flowers. The types grown in our state are more hardy and can withstand drought conditions.

The present investigations were mainly aimed at to make a detailed morphological description of the existing types as well as to study in detail pollen morphology, pollen production, pollen fertility, pollen germination and compatibility studies so as to arrive at possible conclusions for utilising these types for breeding purposes.

The 36 types/varieties/species maintained in the Department of Pomology and Floriculture, College of Horticulture were utilised for the study.

1. Morphological description

The natural variation that exists in Hibiscus is considerable. Morphological descriptions of most of the local types have not been made so far. Morphological description of the 36 types/varieties/species clearly showed that several of them are different from the varieties or types already described by Bailey (1949), Rendle (1971), Sundar (1971), Bhat (1976, 1979) and Bhat and Verma (1980). The growth habits, leaf shape, nature of flower and size, pedicel length, number of lobes in the calyx, colour of corolla, length of staminal column, number of anthers per flower, length of style and colour of stigma appeared to be the diagnostic characters that could be accounted for classification of shoeflower. The fruit shape and size varied between species and also among types/varieties. They were ovoid, oblong or subglobose and possessed ridges with varying intensities (Plate XII and XIII). The fruit characters also might be useful in the identification of varieties.

The present study also revealed that under Kerala conditions, H. rosa-sinensis and H. schizopetalus flowered throughout the year. However, two peaks of flowering viz. one in August-October and the other in January-March were observed. In H. mutabilis, flowering took place from August to February with a peak in September. Similar reports were made by Bailey (1949); Anon (1959); Pal and Krishnamurthy (1967); Devaiah (1968); and Bhat (1976). Anon (1959) reported that in Hibiscus rosa-sinensis, the peak period of flowering was during the period of late rains and in H. mutabilis, it was in September and October. Pal and Krishnamurthy (1967) reported that hot months were the peak season of flowering in H. rosa-sinensis. However, the peak flowering season under Bangalore condition was reported to be from June to October by Bhat (1976). The low rate of flower production noticed in April-May in the present study might be due to unusual heavy drought that occurred during the period. In general, flower production was high in local types compared to hybrids and exotic varieties (Table 2).

The present study also indicated that in all the types/varieties/species flower opening took place during the early morning hours with little seasonal variations which is in conformity with the reports of Vilasini et al. (1966) and Bhat (1976). The anther dehiscence commenced

soonafter the flower opening. Influence of climatic condition in anther dehiscence was also observed in the present study. It was observed that generally the double petalled ones and hybrid flowers lasted for more time without folding than others (Table 3). The retentivity of corolla varied greatly (12-72 hours) which was in agreement with the observations made by Vilasini et al. (1966) and Devi (1968).

2. Pollen studies

2.1. Morphology of pollen

Hibiscus pollen grains appeared as powdery mass to the naked eye and variation in the colour of pollen grains was also observed. Individual pollen grain was pantoporate, spheroidal and spinose. Occurrence of various abnormal types was also noticed. Nair and Kapoor (1974) and Srivastava (1982) also observed such variations. Pollen diameter varied and the mean pollen diameter in the types studied ranged from 125.91 to 198.58 µ. Varietal variation in pollen size in shoe flower was reported by Vilasini et al. (1966). Though there was variation in colour and size, the pollen grains of different types/varieties/species had similar shape (Plate VI and VII).

2.2. Pollen production

There was significant variation for the pollen output per anther among different types/varieties/species (Table 4) and it ranged from 87 to 500. Such variation in pollen production has been reported by Oberle and Geortzen (1952) in grapes, Rao and Khader (1962) in fruit crops like sapota (682 to 3,297), papaya (8,950 to 12,465) and pomegranate (15,982 to 23,170); Vilasini et al. (1966) in Hibiscus (159 to 359) and Srivastava (1982) in malvaceous crops like Abelmoschus esculentus (40-100), Althaea roses (100 to 200) and Gossypium hirsutum (159 to 346). The large size of the pollen grains might be the reason for fewer number of pollen grains per anther. The pollen production per flower depended on the number of anthers per flower and it was observed that it ranged from 4 to 140 (Table 1) in different types/varieties/species.

2.3. Pollen fertility

Estimation of pollen fertility by acetocarmine staining technique revealed significant variation in different types/varieties/species (Table 4). It was found that Hibiscus mutabilis (H.M.) had the highest number of fertile pollen (97.4%) followed by Acc.20 (96.1%) whereas, Acc.7 recorded the least pollen fertility

(4.6%) (Table 4). Vilasini et al. (1966) reported that the pollen fertility ranged from 4 to 97 per cent, which is in general agreement with the results of the present study.

2.4. Pollen germination

2.4.1. Standardisation of the culture medium

The pollen germination studies utilising the medium as reported by Vilasini et al. (1966) did not give desirable germination of pollen grains, which necessitated standardisation of the proper medium for the best germination of pollen grains. Pollen germination studies showed that sucrose, agar and boric acid content in the growth media had profound influence on germination and tube elongation of Hibiscus pollen. There was no germination in distilled water. The inability of the pollen grains to grow in distilled water might be due to the lack of food reserves in the pollen grains as reported by Ehlers (1951). Pollen germination was tried in different media viz., sucrose alone, sucrose + agar and sucrose + agar + boric acid in different concentrations. From the studies it was found that maximum germination of pollen grains was obtained in 20 per cent

sucrose + one per cent agar + 100 ppm boric acid followed by 25 per cent sucrose + one per cent agar + 100 ppm boric acid. The results thus clearly showed that a complete medium consisting of sucrose-agar-boric acid is necessary for proper germination of pollen grains in Hibiscus.

The effect of sucrose on the germination of pollen grains was noteworthy. Pollen germination and tube length improved with the increasing concentration upto 20-25 per cent, thereafter recording a downward trend (Fig.1).

The effect of sucrose in pollen germination could be attributed to factors like nutrition, osmotic or turgour phenomena (Visser, 1955, Johri and Vasil, 1961) or it might be due to a combination of various factors as postulated by O'Kelley (1955).

Pollen germination and tube elongation of Hibiscus were stimulated by agar-agar present in the media. The germination and tube length reached 80.5 per cent and 504.36 μ in one per cent agar mixed with 20 per cent sucrose, whereas in sucrose (20 per cent) alone, it was only 47.5 per cent and 137.33 μ (Table 5b). Influence of agar and gelatin present in the culture medium on germination

of pollen grains was reported by Agarwal *et al*(1957). The stimulatory effect of agar-agar on pollen tube growth was observed by Dubey (1969). Combined effect of sucrose and agar on pollen germination was observed by several workers; Kuwada (1956) in *Abelmoschus esculentus* and *Hibiscus manihot*; Vilasini *et al*. (1966) in *H. rosa-sinensis*; Singh and Sehgal (1968) in guava and Nair (1982) in pineapple, thus supporting the present findings.

The beneficial effects of agar might be attributed to the supply of moisture, carbohydrate and other nutrients as suggested by Stanley and Linskens (1974).

Pollen germination and tube growth were further increased by the addition of 100 ppm boric acid in the medium containing 20 per cent sucrose and one per cent agar (Table 5c). The stimulatory effect of boric acid on pollen germination and tube growth has been reported by various workers in a wide range of plants like sapota (Rao and Khader, 1960); mango (Singh, 1961); *Hibiscus* (Vilasini *et al*., 1966); cocoa (Ravindran, 1977) and tea rose (Visser *et al*., 1977). In the present study, it was observed that upto a concentration 100 ppm boric acid, germination and tube growth increased. At 150 ppm

pollen grains burst which resulted in poor pollen germination.

Pollen is generally considered to be deficient in boron and obviously addition of boron could increase the pollen germination and tube elongation (O'Kelley, 1955). Boron is reported to occur in a relatively higher concentration in pistillate tissues and Brewbaker (1959) suggested that borate supplied to the pollen had a vital control on the bursting of pollen tube at the time of germination either by controlling the water uptake or by structural enforcement by distending the intine. It has also been suggested that boron helped in oxygen uptake in addition to the synthesis of pectic substance required for the formation of the germination tube walls (Vasil, 1960). The beneficial effects of boron in pollen germination is also attributed to the promotion of sugar absorption, translocation and/or its metabolism (Gausch and Dugger, 1953; Linskens and Kroh, 1970).

The pollen grains commenced germination within 30 minutes of dusting in the best medium viz. 20 per cent sucrose + one per cent agar + 100 ppm boric acid and the rate of germination and tube elongation were highest during the first hour. After 8 hours the germination totally ceased. Thus the optimum incubation period for

germination and tube growth was found to be 4 to 8 hours after pollen planting (Table 6). This is in agreement with the reports of Vilasini *et al.* (1966).

2.4.2. Pollen germination in different types/varieties/species

There was significant difference in germination of pollen grains and tube growth between types/varieties/species (Table 7). In 12 of them, the pollen grains failed to germinate. The results showed that certain types/varieties which gave high fertility in acetocarmine test, gave either poor or no germination *in vitro*. The necessity for *in vitro* culture of pollen grains to assess the viability is thus emphasised. Stanley and Linskens (1974) opined that the use of stains was less accurate as compared to germination tests.

As per the statement of Johri *et al.* (1977), viability should be considered as quite distinct from the germinability of pollen i.e., the self or cross compatible pollen, though viable, may or may not germinate on the stigma. Also in an artificial medium, the viable pollen may fail to germinate due to lack of certain essential factors. But the viable pollen should give a higher percentage of germination and produce sufficiently long tubes and affect fertilization following compatible pollination.

The germinating pollen grains in a majority of cases were polysiphonous (Plate VIII and IX) and a single grain produced as many as 14 pollen tubes. Different types/varieties/species were found to differ in the length of their pollen tubes (Table 7). Acc.2 produced the greatest tube length 962.48 µ followed by Acc.11 and H.M. (961.42 µ and 940.22 µ). This observation is in agreement with reports of polysiphonous germination in different species of Malvaceae (Purewall and Randhawa, 1947; Vilasini et al. 1966; Dubey, 1969 and Srivastava, 1982). Occurrence of branched tubes, terminal or intermediary vesicles and callose plugs were observed in the present study. Dubey (1969) also observed callose plugs in 23 per cent pollen tubes of Okra. Tupy (cited from Panda and Datta, 1961) also observed callose plugs in apple and found that sugar consumption was same in the pollen tubes with and without callose plugs. He further explained that glycopyranose was responsible for the formation of tube walls and its limited use during respiration of pollen tubes restricted the pollen tube growth. Patel and Datta (1960); Panda and Datta (1961) opined that callose formation was due to incompatibility reaction. However, detailed investigations appear to be necessary on the occurrence of callose plugs, branched tubes and vesicles in Hibiscus.

2.5 Pollen storage

Use of stored pollen in hybridization becomes necessary especially when the species or varieties flower in different seasons. Trials on pollen storage revealed that storage capacity of pollen grains of H. rosa-sinensis was very low under room temperature (Table 8a). The viability was lost within 48 hours after dehiscence. Storage of flowers with anther column intact at 4°C in a desiccator over calcium chloride gave best results followed by storing them at 4°C devoid of calcium chloride. Pollen could be stored upto 84 hours but, there was rapid deterioration in pollen germination with time of storage. Storage in organic solvents resulted in instantaneous loss of viability (Table 8a).

The results thus indicated that storage under low temperature especially over calcium chloride at 4°C increased the storage life and resulted in higher percentage of germination of pollen grains. The effect of low temperature on pollen storage is thus confirmed in the present study also. It was also found that storage with calcium chloride was beneficial. Evidently the reduction in humidity when stored over calcium chloride has ultimately resulted in a better storage life. Methods of

efficient storage of pollen have been worked out in several crops. King and Hesse (1938) found that controlling temperature and humidity increased the storage life of pollen grains of fruit crops. Nebel (1939) could store pollen grains of apple, pear, plum, peach and apricot for 2 to 5½ years at 28°C and 50 per cent relative humidity. Singh (1962a), Singh (1962 b), Singha (1973), Child (1974) and Simmonds (1976) found that controlled temperature and humidity increased the storage life of several fruits and plantation crops. Griggs et al. (1953) and Nixon (1959) reported that storage of pollen grains in air tight containers at -18°C increased the storage life of pollen of temperate fruits like peach, plum, apple, cherry and olive. Similar findings were reported by Singh (1962 a) in litchi where a low temperature of -23°C could prolong the storage life of pollen. The resistance or tolerance to damage of pollen in the controlled temperature and humidity was due to the reduction of metabolic process and reduced respiration rate due to low water content. The biochemical reaction is inhibited due to low water content and reduced enzyme activity due to dehydration of protein (Shivanna et al., 1979).

The efficacy of organic solvents like petroleum ether, benzene, acetone, ethanol and chloroform in

prolonging the storage of pollen grains reported by Iwanami (1972, 1973 and 1975) and Iwanami and Nakamura (1972), Mishra and Shivanna (1982), Nair (1982) and Tessy (1983) does not hold good in the case of Hibiscus. The pollen grains lost their viability instantaneously when stored in organic solvents. The observed loss of viability during storage might be due to concurrent changes in enzyme activities, respiration rates and activities of vitamins and endogenous growth hormones (Nilsson, 1956; Nebel and Ruttle, 1957; Stanley and Poostchi, 1962 and Ganeshan and Sulladmath, 1983).

The methods of storage employed in the present study were not effective for long term storage of pollen grains, since viability was lost rapidly beyond three days of storage. Further studies with more efficient methods of storage is therefore necessary. However, in Hibiscus the storage of pollen may not be a problem under our conditions since there is great synchronisation in flowering amongst the types/varieties or species.

The nature of floral parts also influenced the storage life. The flowers with anther column intact possessed better storage life than pollen grains as such, indicating that the longevity of pollen when once detached from the anther column decreased considerably. It might be due to the extreme delicacy of pollen grains.

The effect of varieties on the pollen germination was also significant. Of the five types/ varieties tried, Acc. 26 and Acc. 11 were found to have significant higher longevity period as compared to all other varieties (Table 8b).

3. Compatibility studies

H. schizopetalus and all the types/varieties of H. rosa-sinensis except Acc.2 failed to set fruits naturally. H. mutabilis also produced fruits in abundance. In Acc.2, fruit set was occasional and occurred mainly during the peak flowering season (August-October). Vilasini et al. (1966), Pal and Krishnamurthy (9972) and Bhat (1976) had also reported that only very few varieties set fruits under natural conditions. The failure of natural fruit set might be due to problems like lack of pollination, fertilization, self sterility etc.

Studies on self compatibility showed that out of the seven types/varieties and two species self pollinated, five types/varieties of H. rosa-sinensis viz., Acc.2, 5, 18, 22 and 26 and H. mutabilis were found to be self compatible (Table 9). It could be presumed that failure to set fruits under natural conditions

might be due to factors other than self sterility. It was interesting to note that Acc.11 and *H. schizopetalus* which had a high percentage of pollen germination and tube growth *in vitro* conditions, failed to set fruits on selfing. This might perhaps be due to the maternal influence like cytoplasmic incompatibility or inhibition of pollen germination on stigmatic surface. In the case of self incompatible type Acc.16, pollen germination was totally absent *in vitro*. Brewbacker (1959) reported homomorphic self incompatibility in malvaceous crops. Self incompatibility mechanism has been noted by several workers in different crops like Easter lily (Emsweiler and Stuart, 1948), mango (Singh *et al.*, 1962), *Jasminum* (Veluswamy, 1981) and in *Hibiscus* by Vilasini *et al.*(1966).

Fruit setting could be seen in most of the selfed types or varieties within five days after pollination. However all those set were not carried to maturity. Only 36 to 70 per cent matured indicating that fruit shedding is a conspicuous problem in *Hibiscus*. The causes of fruit drop have been attributed to factors like physiological and other inherent factors.(Addicott and Lynch, 1955; Chadha and Singh, 1963; Randhawa, 1971 and Bardwaj, 1975). In addition, it is possible that the drop is also influenced by climatic and other environmental factors.

It was observed in the present study that the success or failure of fruit set was also influenced by season. Compared to January to March, fruit setting was high in August to October. For hybridisation work in Hibiscus the favourable period could be exploited for better fruit set.

3.2 Cross compatibility

Intervarietal and interspecific crosses were attempted. The crossability was assessed on the basis of percentage of fruit set, average seeds per fruit, percentage germination of seeds and percentage survival of the germinated seedlings. Out of the 30 intervarietal crosses made, only four were failure (Table 10). When Acc.11 was used as maternal parent fruit set could not be observed in all crosses. This perhaps indicated that certain varieties were not suitable as female parent. A complete analysis of the varieties will be necessary before launching large scale hybridization programme.

In the remaining 26 crosses, the percentage of capsule set was high after five days of pollination, but later their percentage reduced considerably (Table 10). The capsule set in those crosses indicated cross compatible nature between them. The percentage of capsule set at maturity was maximum in crosses where Acc.2 and 26 were

used as the pistillate parent. It further indicated that cross compatibility was decided to a great extent by the varieties selected.

With respect to seed set, in general it was found that in crosses where Acc.2, 5, 22 and 26 were the maternal parents seed set per fruit was higher than their selfed maternal parent (Table 10). This confirmed that intraspecific cross incompatibility is only very little in Hibiscus. The possibility of evolving new varieties through intervarietal crosses is thus very high in shoe flower. New varieties of Hibiscus were evolved through breeding by Deviah (1968), Sundar (1971), Bhat (1976, 1979) and Bhat and Verma (1980). In the successful crosses the number of seeds ranged from 3.25 to 17.6 seeds per capsule. Bhat (1976) reported 2 to 15 seeds per capsule in some crosses.

The germination percentage of hybrid seeds varied between 31.25 to 77.94. The germination was well over 50 per cent in all the successful crosses, except where Acc.16 was used as the pistillate parent. This indicated the absence of hybrid inviability between the types/varieties of H.rosa-sinensis. The low germination percentage of seeds in crosses where Acc.16 was used as female parent, could be attributed to hybrid inviability.

The survival percentage of germinated seedlings varied from 90 to 100 and this showed that hybrid inviability was not a problem in Hibiscus. In all the crosses the capsules took comparatively lesser time (26-37 days) to attain maturity than their selfed maternal parent (28-38 days). Germination of hybrid seeds were also earlier by 8 to 16 days than the seeds obtained from their selfed maternal parent (12-18 days). Bhat (1976) in IIHR reported that the capsules took 41 to 70 days for seed maturity under Bangalore conditions. In the present study only 26 to 37 days were required for seed maturity under Kerala conditions. The influence of climate on seed maturity is thus clearly established.

The crossability being a function of percentage of fruit set, number of seeds per fruit, percentage germination of seeds, percentage survival of germinated seedlings both in crossed and selfed maternal parent, the measure of crossability affinities between any two species is reflected in hybrids produced (Rao, 1979). There was a significant reciprocal effect on over all crossability index indicating that the maternal parent had an influence on crossability index. Veluswamy (1981) reported that although strong interspecific cross incompatibility as well as strong self incompatibility

system existed in Jasminum, crosses were successful within species and the crossed seeds germinated well.

The results of the interspecific cross compatibility study indicated the existence of a cross incompatibility barrier among the three species of Hibiscus (vide Table 11). Efforts to produce hybrids between the three species ended in complete failure. Whatever fruits that initially set either dropped soon or in those which were carried to maturity, the seeds did not germinate. The observations made by Gast (1971) that ornamental Hibiscus was highly polymorphic cross-compatible, needs further confirmation by detailed studies.

In the present study, crosses were failure where H. mutabilis and H. schizopetalus were used as the female parent. Whenever H. rosa-sinensis was used as the maternal parent, there was better fruit set. In the case of H. rosa-sinensis x H. mutabilis fruits that were initially set did not come to maturity while in H. rosa-sinensis x H. schizopetalus, in fruits which were carried to maturity the seeds did not germinate.

In crosses where fruit drop occurred after five days of pollination, it indicated that the initial growth was mainly stimulatory caused by entry of pollen

tubes in the ovarian cavity. Subsequent degeneration of the ovarian tissues perhaps resulted in the shedding of those fruits that were set. The failure of seed germination might also be due to embryo and endosperm incompatibility. According to Rao (1979), the failure of crosses where there was no fruit set at all or only resulted in parthenocarpic fruit production or fruits with shrunken seeds, were presumed to be post-fertilisation phenomena. He further stated that the production of shrunken seeds was the result of genetic incompatibility between embryo and endosperm due to interspecific lethal genes. Muthukrishnan and Papiah (1980) and Veluswamy (1981) reported successful fruit set in interspecific hybrids of jasmine. The seeds however did not germinate.

According to Silow (1941), the cause of interspecific incompatibility in the genus Gossypium as a whole was due to lack of harmony between species. Linskens (1975) reported that interspecific incompatibility was heterogenic. Pushkarnath (1942) showed that the expression of self and cross incompatibility in several species of diploid potatoes were controlled by an incompatible gene possessing a number of alleles.

Summary

SUMMARY

The present investigations were carried out in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during the years 1981 to 1983. The summary of the work done and results obtained are given below.

1. Morphological description of 34 types/ varieties of H. rosa-sinensis and two other species viz., H. mutabilis and H. schizopetalus were made.

2. In H. rosa-sinensis and H. schizopetalus, flower production was found to be throughout the year with two peak seasons, viz., August to October and January to March while in H. mutabilis flowering took place from August to February with a peak in September.

3. In all the types/varieties or species, flower opening and anther dehiscence took place during the morning hours; former between 4 to 8.30 am and the latter soonafter flower opening, except in Acc.7, 10, 12, 13 and 14.

4. In all the types/varieties/species except in H. schizopetalus, Acc.4, and 34, corolla folded within 12 to 18 hours after flower opening. Generally, the double

petalled ones and hybrid flowers last longer. The retention of the corolla differed between types/ varieties and species from 24 to 72 hours.

5. Studies on pollen morphology showed that while colour of pollen grains varied, the shape was more or less constant in all types, varieties and species. Individual pollen grain was pantoporate, spheroidal and spinose. The pollen size was found to differ not only between types and varieties but within them also; the diameter ranged from 125.91 μ in Acc.29 to 198.58 μ in Acc.25.

6. The pollen output per anther ranged from 87 in Acc.16 to 500 in H. mutabilis and there was significant difference between the types, varieties and species.

7. Pollen fertility as indicated by the aceto-carmine staining technique ranged from 4.6 per cent in Acc.7 to 97.4 per cent in H. mutabilis.

8. Media for the pollen germination was standardised. A medium containing sucrose (20%), agar (1%) and boric acid (100 ppm) was found to be the best for pollen germination as well as for better pollen tube growth.

9. Pollen grains commenced germination within 30 minutes of dusting in the best medium in a humid chamber and the rate of germination and tube elongation were highest during the first hour. Germination and tube growth continued till eight hours of incubation at a decreased rate.

10. Significant variation in pollen germination and tube elongation was observed between types/varieties or species. Out of 35 types/varieties/species studied, only 23 of them showed germination in the best medium. Acc.11 showed maximum germination of 85.8 per cent followed by H. mutabilis and Acc.2, while the tube length was maximum in Acc.2 (962.48 µ) followed by Acc.11 and H. mutabilis. Acetocarmine staining technique was not a reliable method of estimating viability of pollen as all the grains stained in the acetocarmine technique did not germinate in vitro. Polysiphonus germination was observed in majority of cases.

11. Pollen storage studies indicated that the storage capacity of Hibiscus pollen was very low under room temperature. But pollen viability was retained upto 84 hours when the flowers were stored with anther column intact at 4°C in a desiccator over calcium chloride. Low temperature and low humidity had a significant

influence on the longevity of pollen grains. Acc.26 and 11 were found to have maximum storage life as compared to other types or varieties.

12. It was found that only Acc.2 and H. mutabilis set fruits naturally. Acc.2, 5, 18, 22, 26 and H. mutabilis were found to be self compatible. The fruits took 28 to 38 days to reach maturity. Mature seeds germinated within 10 to 18 days. The number of seeds per capsule ranged from 154 to 218 in H. mutabilis and 1 to 20 in H. rosa-sinensis. Seed germination ranged from 3.33 per cent in H. mutabilis to 69.77 per cent in Acc.18. The survival percentage of germinated seedlings were over 95 per cent.

13. Intraspecific cross compatibility was observed in all the crosses attempted in the species H. rosa-sinensis except in cases where Acc.11 was used as the maternal parent. In all the crosses, the capsules took comparatively lesser time to attain its maturity than their selfed maternal parent. In majority of the crosses, the germination of hybrid seeds was earlier than the seeds obtained from selfed maternal parent. The cross Acc.2 x 11 had the highest crossability index followed by Acc. 2 x 18, 2 x 26, 18 x 11, 22 x 11, 26 x 2 and 26 x 11.

14. Interspecific compatibility was practically absent. Only in the crosses between Acc. 2 x H.S., and Acc. 26 x H.S., fruit set was observed, but the seeds obtained from the fruits failed to germinate. Thus a strong barrier for crossability and hybrid inviability between the species was evident.

15. The capsules of different types or varieties of H. rosa-sinensis were of ovoid, oblong or subglobose and H. mutabilis were globose and hairy. The seeds of H. rosa-sinensis were globose and black and that of H. mutabilis were reniform, brown and hairy.

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* Originals not seen

Appendices

Appendix - I

Descriptive blank for Hibiscus

1. Habit - Vigorous/moderately vigorous/not vigorous
2. Lateral branches - Profused/few
   Erect/slightly drooping/drooping
3. Leaves
   Shape - Ovate/cordate/cordate-ovate/oval/obovate/oblong/linear
   Size - Length cm to cm
   Breadth cm to cm
   Margin - Entire/serrate/serrulate/dendate/dendiculate/lobed/divided/undulate/crenate/notched
   Apex - Acute/accuminate/obtuse/emargenate/mucronate
4. Flower
   Shape - Saucer/cup/ball/funnel/tubular
   Diameter - cm to cm
   Nature - Single/double
   Erect/slightly pendulous/pendulous
5. Pedicel - Jointed/not jointed
   From axil to joint cm
   From joint to involucre cm
   Total cm

6. Epicalyx

   Number -

   Shape - Linear/ovate/lance pointed

7. Calyx

   Shape - Shallow cup/linear cup/tubular/companulate

8. Corolla

   Nature - Crinkled/smooth/medium

   Entire/dissected

   No.of whorls - Single/double/tripple/multipple

   Colour - Upper side -

   Base -

   Tip -

   Boarder -

   Lower side -

9. Stamens

   Length - cm

   No. of anthers -

10. Pistil

    Length of style - cm

    Colour of stigma -

11. Whether naturally

    seed setting/not -

Appendix - II

Weather data for the period from May 1982 to September 1983

Month	Temperature (°C)		Relative humidity (%)	Total rainfall (mm)	Number of rainy days per month
	Maximum	Minimum			
1982					
May	33.80	24.50	79.9	113.5	8
June	30.60	23.10	79.8	657.6	26
July	29.10	22.92	87.5	600.9	26
August	28.90	24.30	85.0	575.4	27
September	30.98	24.00	78.9	67.4	10
October	32.04	23.13	77.0	277.8	18
November	31.40	23.93	71.9	98.4	7
December	31.93	23.19	58.4	52.0	1
1983					
January	33.25	21.64	51.3	Nil	Nil
February	34.46	22.70	64.0	Nil	Nil
March	36.15	23.76	65.0	Nil	Nil
April	36.20	25.80	66.0	Nil	Nil
May	35.10	25.50	69.0	37.4	3
June	31.90	24.50	79.0	387.2	19
July	29.70	23.70	87.0	580.6	21
August	29.10	23.80	87.0	754.7	26
September	29.50	23.40	84.0	494.6	24

Appendix - III

Analysis of variance for pollen size in different types/ varieties/species of Hibiscus.

Source	Degrees of freedom	Mean sum of squares
Between types/varieties/ species	34	56.51**
Within types/varieties/ species	3465	0.35
Total	3499	

** Significant at 1% level

Appendix - IV

Analysis of variance for pollen production in different types/varieties/species of Hibiscus

Source	Degrees of freedom	Mean sum of squares
Between types/varieties/ species	34	0.81**
Within types/varieties/ species	140	0.02
Total	174	

** Significant at 1% level

Appendix - V

Analysis of variance for pollen fertility in different types/varieties/species

Source	Degrees of freedom	Mean sum of squares
Between types/varieties/species	34	1167.82**
Within types/varieties/species	70	8.20
Total	104	

** Significant at 1% level

Appendix - VI

Analysis of variance for standardisation of sucrose medium for pollen germination and tube growth

Source	Degrees of freedom	Mean sum of squares	
		Pollen germination	Pollen tube growth
Treatments	8	307.06**	3658.82**
Error	18	2.77	3.40
Total	26		

** Significant at 1% level

Appendix - VII

Analysis of variance for standardisation of sucrose-agar and sucrose-agar-boric acid media for pollen germination and tube growth.

Source	Degrees of freedom	Mean squares			
		Sucrose-agar		Sucrose-agar-boric acid	
		Pollen germination	Pollen tube growth	Pollen germination	Pollen tube growth
Between sucrose levels	2	19.27**	78145.1**	217.23**	216238.96**
Between agar/boric acid levels	3	39.63**	111037.73**	309.84**	313190.08**
Interaction	6	433.73**	9362.45**	7.89**	4068.46**
Error	24	2.41	440.86	1.24	1399.94
Total	35				

** Significant at 1% level

Appendix - VIII

Analysis of variance for time of incubation for pollen germination and tube growth

Source	Degrees of freedom	Mean sum of squares	
		Pollen germination	Pollen tube growth
Treatment	5	50.11**	127067.4**
Error	12	1.88	2027.64
Total	17		

** Significant at 1% level

Appendix - IX

Analysis of variance for pollen germination and tube growth in different types/varieties/species of Hibiscus

Source	Degrees of freedom	Mean sum of squares	
		Pollen germination	Pollen tube growth
Between types/varieties/ species	22	830.77**	161010.36**
Within types/varieties/ species	46	10.11	4402.08
Total	68		

** Significant at 1% level

Appendix - X

Analysis of variance for pollen longevity of five types/varieties under different storage conditions at 12 hours interval

Source	Degrees of freedom	Mean sum of squares				
		12 hours	24 hours	36 hours	48 hours	60 hours
Types/varieties	4	1676.73**	1284.33**	963.70**	669.33**	271.96**
Treatments	7	857.87**	723.95**	114.52**	1132.59**	721.52**
Interaction	28	25.33**	20.40**	24.54**	23.60**	44.87**
Error	80	3.51	3.88	7.29	9.49	5.90
Total	119					

** Significant at 1% level

POLLEN PRODUCTION, FERTILITY AND COMPATIBILITY STUDIES IN SHOE FLOWER (*Hibiscus rosa-sinensis* L.)

By

BABY LISSY MARKOSE

ABSTRACT OF A THESIS

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the requirements for the degree of

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ABSTRACT

Investigations were conducted on the pollen morphology, production, fertility and compatibility in 34 types or varieties of *Hibiscus rosa-sinensis* L. and two other species viz., *H. mutabilis* L. and *H. schizopetalus* Hook. in the Department of Pomology and Floriculture, College of Horticulture, Kerala Agricultural University, during the year 1981-'83. Since the types maintained did not possess any distinct varietal names, detailed morphological descriptions were made for identification. Observations were also made on certain aspects of blossom biology like flower opening, anther dehiscence, folding of corolla and retention of corolla.

There was distinct variation in the morphology of flowers in the 36 types/varieties or species studied. Flower opening took place during the morning hours and in most of the types/varieties/species anther dehiscence commenced soon after flower opening. The time taken for the folding of the corolla ranged from 12 to 36 hours after flower opening and for the retention of corolla ranged from 24 to 72 hours.

Pollen grains of Hibiscus were pantoporate, spheroidal and spinose and were 125.91 to 198.58 microns (μ) in diameter. Pollen production per anther varied from 87 to 500 and percentage of pollen fertility ranged from 4.6 to 97.4. Standardization of media for pollen germination and tube growth indicated that a medium consisting of 20 per cent sucrose + one per cent agar + 100 ppm boric acid was the best. The pollen grains commenced germination within 30 minutes of dusting and gave satisfactory germination even after four hours of incubation in the best medium. Of the 35 types/varieties/species tested, only in 23 cases, pollen germinated in vitro. Acc.11 showed maximum germination of 85.8 per cent followed by H.mutabilis and Acc.2 while tube length was maximum in Acc.2 (962.48 μ) followed by Acc.11 and H. mutabilis. Polysiphonous germination was observed in majority of cases. Pollen grains could not be stored for more than three days in any of the methods employed in the present study. Of the different methods tried, storage of flowers with anther column intact at 4^{o}C over calcium chloride in a desiccator was found to be the best followed by storage of flowers at 4^{o}C without calcium chloride. Acc.26 and Acc.11 had the longest shelf life followed by Acc.2.

It was found that only Acc.2 and H. mutabilis set fruits naturally. Out of the seven types/varieties of

H. rosa-sinensis and two other species viz., H. mutabilis and H. schizopetalus selfed, only in five types or varieties of H. rosa-sinensis and in the species H. mutabilis self compatibility was noticed. The fruits matured in 28 to 38 days and germinated in 10 to 18 days. The number of seeds per capsule ranged from 8.5 to 192.5 and seed germination ranged from 3.33 to 69.77 per cent. Survival percentage of germinated seedlings was more than 95 per cent.

Out of the intraspecific cross combinations tried, compatibility was observed in all the crosses except in cases where Acc.11 was used as the female parent. The cross Acc.2 x 11 had the highest crossability index followed by Acc.2 x 18, 2 x 26, 18 x 11, 22 x 11, 26 x 2 and 26 x 11. Interspecific compatibility was practically absent.