INCOMPATIBILITY STUDIES ON SWEET POTATO (*Ipomoea batatas (L) Lam*)

By

PADMA KUMAR A.

THESIS

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DECLARATION

I hereby declare that this thesis entitled "Incompatibility studies on sweet potato (*Ipomoea batatas* (L.) Lam)" 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 award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani,
7.3.88

A. PADMA KUMAR

CERTIFICATE

Certified that this thesis, entitled "Incompatibility studies on sweet potato (*Ipomoea batatas* (L.) Lam)" is a record of research work done independently by Shri. A. PADMA KUMAR, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Chandramony. D

(D. CHANDRAMONY)
Chairman
Advisory Committee,
Assistant Professor of Botany,
College of Agriculture,
Vellayani.

Vellayani,
7.3.83.

APPROVED BY:

Chairman

Smt. D. CHANDRAMONY

Members

Dr. N. KRISHNAN NAIR

Smt. N. KAMALAM

Dr. James Mathew

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INTRODUCTION

INTRODUCTION

Of the three thousand and odd plant species used by man for food, about one hundred and fifty have entered world commerce and a dozen feed the majority of the human population. These dozen plants have emerged as the most important food crops because of their greater efficiencies. Four of these are root crops viz., sugarbeet, cassava, potato and sweet potato (Jones, 1970).

Sweet Potato (Ipomoea batatas (L) Lam.), the only economically important member of the family Convolvulaceae, is cultivated extensively throughout the tropics, subtropics and temperate regions of the world. They are grown from 40°N to 32°S of the equator and from sea level to 9000 ft. But, they grow best where the average temperature is 75°F or more with a well distributed annual rainfall of 30 - 50 inches and an abundance of sunshine. It can be grown on a wide range of soils, but a well drained sandy loam with a clay subsoil is considered ideal.

Sweet potatoes out rank most other carbohydrate foods as a source of vitamins, minerals and energy. On an average, sweet potatoes contain about 70 per cent water, 27 per cent carbohydrate (of which 3 - 6 per cent is sugar and the remainder mostly starch), 0.2 per cent fats, 1.5 - 2 per cent protein and 1.0 per cent crude fibres. Yellow fleshed tubers are rich in Vit. A.

The tuberous roots are eaten fresh (boiled or baked) or as canned or as dehydrated sweet potatoes. Some unmarketed grades are fed to live stock or even fermented to produce alcohol. Sweet potato starch is used for the manufacture of adhesives, textile and paper sizing, and for use in the confectionary and baking industries. Sweet potato leaf tips can be used as a good and cheap leafy vegetable. It is an important source of Vit. B_2, which is important for Asian diets. It also possesses unique medicinal properties as diuretics and saluretics, both of which are reported to help individuals suffering from high blood pressure (Villareal et al. 1979).

The area under sweet potato in Kerala is showing a decreasing trend, being 8400 hectares in 1955-56 and 4960 hectares in 1979-80. This declining trend in area is primarily due to the low productivity of this crop. Even though some increase in sweet potato production is observed due to improved agronomic practices, the per hectare production remains much below its potential. This happens mainly due to the use of poor yielding local varieties. At present, there is no improved variety evolved for this crop in Kerala. Better yield and higher area under this crop will result if improved varieties suitable for the different agro-climatic zones can be provided. Thus, evolution of superior clones holds tremendous scope in sweet potato cultivation.

The most important breeding method that can be successfully employed in this crop is heterosis breeding because it is very easy to maintain the novel types obtained by this method through vegetative propagation. The main obstacles for the implementation of this scheme are the following:

1. The shy flowering habit of the crop.
2. The high sterility observed in this crop due to its hexaploid nature.
3. The self and cross incompatibility mechanisms operating in this crop.

From the breeders point of view, sweet potato is termed as a problem crop due to the above reasons.

In sweet potato, some preliminary studies have been made by few earlier workers on the flowering habit, sterility, incompatibility mechanisms and methods to overcome the incompatibility barrier. However, no clear cut idea regarding the extent of incompatibility mechanism in our varieties and methods to overcome the incompatibility barrier is available. Hence, the present investigation was undertaken to assess the extent of self incompatibility mechanism present in sweet potato varieties and the relative merits of different methods to overcome the self incompatibility barrier.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Heterosis breeding plays an important role in the present day crop improvement programmes. For exploiting heterosis or hybrid vigour in any commercial crop improvement programme, it should satisfy two important factors. The foremost one is the presence of a mechanism which will ensure ways for easy hybridisation so that manual labour can be minimised, and the second one is the ability to produce selfed seeds for developing homozygous inbred lines. This is especially advantageous in seed crops which can also be propagated easily through vegetative means.

Failure of seed set is, however, noticed in several crop varieties. This may be due to many reasons. One among them is the incompatibility mechanism.

Flowering in sweet potato.

There are several contrasting reports about the flowering habit of sweet potato.

Kunjan (1957) quoted the reports of several earlier workers that the plant did not show any tendency to bloom and set seeds. This included the reports of Rosa (1926) and Miller (1937). The Encyclopedia Britanica (1962) gives an indication that the crop rarely produces flowers under common cultural conditions. Rosa (1926), quoted by Kunjan (1957), reported that, in the United States, he seldom observed flowering in sweet potato.

Clonal and/or environmental variations play an important role in the flowering habit of sweet potato, as reported by various workers. Good flowering in 66 varieties of this crop grown under Brazilian conditions was recorded by Menezes (1952). Clonal variation for flowering nature has also been reported by Vansch (1954). But, Fujise et al (1957) found that short day conditions favoured flowering in sweet potato. Artificial induction of flowering by 2, 4-D had been reported by Wittwer as quoted by Rao (1962).

Incompatibility studies

Incompatibility is the failure of plants with viable pollen and ovule to set seed due to some physiological hindrance, which prevents fertilization (Crane and Lawrence, 1952). This phenomenon has been reported in many angiosperms. Common causes attributed for such incompatibility are failure of pollen grains to germinate on the stigma, failure of pollen tube to grow down the style, degeneration of the embryo or endosperm, and various other genetic causes.

Incompatibility can be chiefly classified based on (a) floral morphology and (b) gene action. Based on floral morphology, it can be classified as homomorphic and heteromorphic systems. Incompatibility due to gene action include sporophytic and gametophytic types.

East and Mangelsdorf (1940) estimated that self incompatibility is present in more than 3000 species distributed among 20 families. Darlington and Mather (1949) reported the presence of incompatibility in half the species of flowering plants. According to Brewbaker (1957) incompatibility mechanism is in operation in members of 66 plant families.

Elliott (1958) reported that the presence of incompatibility mechanisms in a large number of flowering plants had been known for a long time, although satisfactory genetic analysis ofmany of them were not available.

A review of the reports regarding the presence of this mechanism in some of the important crop plants is presented below:

Cooper (1938) studied the various aspects of fruit set in apple and reported that all the varieties were self incompatible, of which many were also inter-incompatible. Irregular chromosomal duplications were found to be the cause of incompatibility and sterility in apple varieties which resulted in non-setting of fruits in this crop. It had also been reported by him that the system available in this crops was due to the presence of certain inhibitory substances of the pistil that considerably retarded the growth of pollen tubes.

Two types of incompatibility system, normal (N) and exceptional (M), had been reported in Nicotiana alata by Pandey (1964). Normal plants were reciprocally compatible with N. longsdorffi and exceptional plants were compatible only as males.

Thompson (1965) observed the presence of multiple series of S alleles for sporophytic self incompatibility in Brassica oleraceae. The 2, S alleles of a heterozygote may act independently or one may be dominant over the other. Dominance occurred more frequently in the pollen than in the stigma. In the five self incompatible selections of Kale, self compatibility was determined by genes which were independent of the S alleles.

Yaqub (1968) observed self incompatibility in Capsicum pubescens. All strains of C. cardenasii showed self incompatibility whilst all strains of C. frutescens and C. annuum tested were self compatible. Diallel analysis of interspecific cross between C. pubescens and C. cardenasii indicated a gametophytic incompatibility in Capsicum.

Brewbaker and Gorrez (1967) in their studies on the genetics of self incompatibility in the monocot genera Anahas and Gesteria, made the following observations. Genetic segregation for self incompatibility was noticed in both these cases. A single S locus with multiple alleles and gametophytic control of pollen phenotype was implicated in each genus.

Martin and Hered (1968) reported on the existence of ten incompatibility groups of intra-incompatible plants, in Ipomoea setifera. Further, from a genetic analysis of one set of related families, it was postulated that the genetic control of incompatibility in Ipomoea is based on a single locus sporophytically controlling system.

Incompatibility in sweet potato

Self incompatibility in sweet potato was first reported by Mexiodola in 1921 (Van schreven, 1953).

The Japanese scientist Terao (1934) was the first to classify sweet potato into different incompatibility groups. On the basis of percentage of fruit set, he classified 51 sweet potato varieties into three intra-incompatible but inter compatible groups.

Miller (1939) evaluated a number of linesof sweet potato on their fertility level when used as parents. Certain lines were found to be more fertile as female parents than others. Preliminary studies suggested that lack of fertility might be due to the style being deficient in an unknown substance which acts as an inhibitor to pollen germination. There were considerable differences in plants with regard to their ability to produce viable pollen. With the help of this information, planned crosses were made using very fertile female and good pollen producing plants as parents, increasing thereby the seed production.

Togari and Kawahara (1942) carried out studies on incompatibility relationships in sweet potato at the Tokyo Agricultural Experiment Station. Approximately 50 varieties were tested and classified into three groups, each of them was intra-sterile but inter-fertile. As shown by percentage of capsule set and seed number per capsule in compatible matings, differences in grade of compatibility were noted, in reciprocal cross between members of the three groups. The stimulating action of the pistil upon the pollen also varied. Germination of the pollen had been observed to be almost completely suppressed in selfing.

Taggart (1952) in his analysis on the fertility in numerous lines of sweet potato used for breeding purposes, observed that certain plants were better as female parents and some others were superior as male parents. Controlled crosses based on this inference had greatly increased the production of seeds.

Clonal variation for flower production was reported by Vansch (1954) in sweet potato. In incompatible intervarietal crosses, the pollen failed to germinate on the stigma. Heterostyly was not associated with incompatibility. The 61 varieties tested could be classified into at least 6 intra-sterile groups which were partially inter-fertile. Reciprocal differences in compatibility were marked. In a number of clones, self pollination had been found to occur in the bud itself.

Fujise et al (1957) studied the flowering, capsule set and number of seeds per capsule in a collection of Japanese, Chinese and American varieties of sweet potato. According to them, marked differences in capsule set between the various combinations of the intra-sterile cross compatible groups were not, however, obtained.

Fujise (1964) classified 254 varieties of sweet potato into eight incompatibility groups.

Hernandez and Miller (1964) reported that thirty parents fell in six groups, five incompatibe and one self compatible. Most of those in each of the former groups were incompatible within the group, but cross compatible with parents of other groups. The inheritance of incompatibility factors appeared to be similar to that in Nicotiana, as reported by East (1925).

Williams (1964), in his work on sweet potato breeding, concluded that the widespread prevalence of cross and self incompatibility in sweet potato varieties is a major hindrance to the breeding programmes. However, he noticed that some varieties were partially self compatible.

Wang (1964 and 1964 a) classified 76 varieties and seedlings collected from various sources into five intra-incompatible, inter compatible groups and one intra-inter-compatible group. The five intra-incompatible groups were assigned genes S_1 to S_5 of a multiple allelomorphic series representing incompatibility groups I, II, III, IV and V. The varieties in group VI were postulated to possess a fertility restoring factor (Sf).

Martin (1965), in his review on the incompatibility in sweet potato, reported a homomorphic system for Ipomoea batatas.

He interpreted the failure of the pollen germination on the stigma after incompatible matings as due to the presence of unilateral incompatibilities. The compatible reactions between the parent and offsprings provided evidences for the existence of sporophytic system of incompatibility.

Wedderburn (1967) attempted hybridisation between <u>Ipomoea batatas</u> and <u>Ipomoea trichocarpa</u> or <u>Ipomoea gracilis</u> and found it to be fairly successful. A high degree of self incompatibility was noticed in all the three species. In all the cases, pollen could germinate well, but in incompatible pollination, pollen tube growth was abnormal.

On an analysis of 22 varieties of sweet potato for incompatibility reactions, Nair (1970) reported varietal variations. Some of them were distinguished as self incompatible and some as cross incompatible. These findings showed that incompatible strains occurred along with compatible strains. The insufficient length of the pollen tube and the presence of some sort of inhibitory factor at the stylar region were assumed to be the causes of incompatibility in this crop.

Pillai (1974) used 140 varieties of sweet potato for his study. Three strains were selected as female parents and ten as male parents. The female parents viz., 169-16, 172-03 and 172-04 were self incompatible. Cross incompatibility occurred in the crosses 172-03 x 172-02 and 172-04 x 169-16. Fruit set varied from 1.95 to 71.42 per cent in other crosses.

Joseph (1979) reported self incompatibility in all the eight varieties of sweet potato studied by him.

Breaking of incompatibility barrier

A number of methods have been tried by various workers to induce fertility by breaking the incompatibility barrier in crop plants. They include surgical techniques, bud pollination, hormonal treatment, polyploidy, irradiation etc.

Mangelsdorf and Reeve (1931) obtained hybrid seed from a cross between two genera of Zea and Tripsacum by employing the technique of cutting off portions of the style before pollination. Davis (1957) used the technique of cutting off portions of the styles before pollination and crossed two incompatible species of Lathyrus. Singh (1958) studied self incompatibility in Toria. When the stigma was scratched before selfing, there was an increase in pod and seed formation. In both of these plants, removal of upper third portion of the style caused greater increase in pod and seed formation. Goud et al (1970) observed that incompatibility in Capsicum could be overcome by amputation of the style followed by pollination. Charles et al (1974) investigated the nature of the incompatibility mechanism in two strains of the wild species of Ipomoea trichocarpa and eight cultivars of I. batatas. The incompatibility barrier was found to be located in the stigma and, hence, its removal resulted in successful seed set in Ipomoea trichocarpa. No such inhibition was found in

I. batatas and, hence, removal of the stigma failed to break the incompatibility barrier. Radhakrishnan (1976) pointed out that cross incompatibility in *Capsicum* was due to the inability of pollen tubes to grow beyond the upper region of the style. This was overcome by amputating the upper part of the style along with the stigma and pollinating the cut surface of the style after application of 5 per cent sucrose solution.

Togeri and Kawahara (1942), in their effort to induce pseudofertility in sweet potato by means of bud pollination, observed that since the stigma of the sweet potato flower would become receptive only a few hours before anthesis, bud pollination offered little prospect in overcoming self incompatibility. Van Schreven (1953) reported an increase in pollen germination after self pollination of sweet potato flowers in the bud stage. Haruta (1966) reported details regarding the use of bud pollination in *Brassica* and *Raphanus*.

According to Brewbaker (1957) stigma is the site of incompatibility for species with trinucleate pollen grains (sporophytic system) which remain viable only for a short period of time and do not germinate readily in vitro. The physiological explanation for this phenomenon was later given by Heslop-Harrison et al (1975). According to them, the pollen is inhibited in the stigma for the simple reason that some kind of stimulus from the stigma which is essential for pollen germination is blocked by the incompatibility reaction.

Majid (1964) observed that interspecific hybridisation attempted in Lycopersicon without the aid of techniques such as mixed pollination or application of Indole Acetic Acid to pedicels or grafting followed by pollinations with irradiated pollen, were unsuccessful. Chaudhari (1965) lengthened the life of arrow root flowers by applying P-chloro phenoxy acetic acid to pedicels and was able to attain successful seed set in crosses. In an attempt to detect a possible effect of 3-indole acetic acid on the incompatibility reaction, Henny and Ascher (1973) injected the auxin into the styles of Lilium at different time intervals before pollination with compatible and incompatible pollen. The findings suggested basic differences in the metabolic sensitiveness of compatible and incompatible tubes, and appeared to demonstrate that production of selfed seeds after applications of NAA and IAA resulted essentially from the prevention of floral abscission processes and did not stimulate the incompatible pollen tubes. Charles et al (1974) reported the suppression of floral abscission by the application of 2, 4-D (Con 100 ppm) to the pedicels, which resulted in successful seed set. They reasoned that the application of 2, 4-D gave adequate time for the pollen tube to penetrate the incompatible style and resulted in fertilization and seed set. So also, suppression of floral abscission by 2, 4-D was thought to be another reason for the successful seed set.

Allard (1960) reported that end of season pollination was found to be effective in breaking the incompatibility barrier in crops like tobacco. He observed contrasting reports regarding the success of this technique.

Szteyn (1965) reported that the use of a tetraploid instead a diploid tomato as pistillate parent in crosses with Lycopersicon peruvianum and L. glandulosum, increased the seed set in hybrids about 50 times. According to Malhova (1966), incompatibility in the inter specific cross between Capsicum annuum and C. pubescens could be overcome by using tetraploid form of C. annuum as female parent. Irikura (1969) pointed out that cross incompatibility in potato could be overcome by the use of ployploidy.

Pandey (1964) conducted self incompatibility studies in Nicotiana alata using pollen from X-irradiated buds in selfing with the self incompatible parental genotype S_1 S_3. Seventy five per cent of the X_1 plants showed pollen part mutation (mutation occurring in that part of the S-gene which governs the incompatibility reaction of the pollen).

Pre-treatment of the styles at 50°C for six minutes, resulted in fruit set following self pollination in Lilium longiflorum (Hooper et al 1967).

The studies of Kiss (1970) revealed that in Capsicum, good pollen germination and pollen tube growth were promoted by sucrose medium.

Reoggen (1970) reported a technique called Electric Aided Pollination (EAP) to break down self incompatibility in Brassica oleracese. A direct potential difference of 100 V was applied between the pollen and the stigma during pollination to break down the self incompatibility barrier. Increased seed production resulted in Brussel's sprout and Savoy cabbage following this technique. The advantage of this method over bud pollination was the saving in time to get the same quantity of seed.

Pollen tube growth

Inhibition of pollen tube growth or its abnormaly slow development inside the stylar column was reported to be one cause of incompatibility in angiosperms by Sears (1936).

Togari and Kawahara (1942) observed complete suppression of pollen germination after selfing in sweet potato.

The preliminary studies conducted by Miller (1943) in sweet potato revealed that lack of fertility might be due to the style being deficient in an unknown substance which might initiate pollen germination or it may contain some substances which act as inhibitor to pollen germination.

Martin (1959) reported the use of fluorescent technique to classify pollen germination behaviour on sweet potato stigmas following compatible and incompatible crosses.

The cytoembryological studies of Bannikova (1965) revealed that in an inter specific cross between *Nicotiana glutinosa* and *N. rustica*, not even a single pollen had reached the embryosac upto 12 days of pollination, and the embryosac finally degenerated.

Martin and Cabanillas (1966) in their observations on post pollen germination barriers to set seed, came to the conclusion that, in three cross combinations selected for good pollen germination, pollen tube failed to pass from the stigma to the style. Besides the incompatibility barrier inhibiting the pollen germination, a physiological barrier between stigma and style was also hypothesised.

Martin and Ortiz (1966) made observations on the germination of pollen in relation to incompatibility and sterility in sweet potato. Using a fluorescent technique, it was found that the pollen in incompatible crosses failed to germinate entirely, in compatible crosses germinated well and in partially incompatible crosses germinated occasionally.

Wadderburn (1967) fairly succeeded in crossing two highly self incompatible varieties of *Ipomoea batatas* and *I. trichocarpa*. He observed good germination of pollen in all the pollinations, both compatible and incompatible. However, the pollen tube growth in incompatible matings was found to be abnormal.

Hogenboon (1972) reported abnormaly slow growth of Lycopersicon esculentum pollen in styles of self compatible L.peruvianum and the hybrids of such crosses showed embryo abortion.

Nettancourt et al (1973) studied the ultrastructural aspects of self incompatibility mechanism in Lycopersicon peruvianum and reported that the incompatible pollen tubes were not only slow in their growth through the style, but were destroyed of the inner wall and lysis of the tube occurred.

Heslop - Harrison et al (1975) concluded from their studies in interspecific incompatibility system of the sporophytic type that the inhibition of pollen or pollen tube was taking place on the stigmatic surface.

Shigemura cited by Wang (1965) observed two types of pollen tubes in the style, the normal type and the other an abnormal type with swollen tip. The abnormal part stained more deeply (with lactophenol cotton blue) than other parts of the tube and the growth rate of the abnormal typo was less.

Radhakrishnan (1976) reported that the degree of germination of pollen grains was remarkably high when five per cent sucrose was applied on the cut end of the style before pollination in Capsicum.

Pollen sterility

Wang (1964) found that the anthers of some varieties of sweet potato degenerated and failed to dehisce. But, certain varieties had 50 per cent or more sterile pollen and this contributed to very low fruit set when they were used as pollen parent.

Martin (1967) observed that the incompatibility relationships in sweet potato were masked by a more powerful and generally occurring sterility system. According to him, sterility was associated with the hexaploid nature of the crop. So the gametes might not carry a well balanced set of chromosome material. Such weaknesses of gamete would be expressed during the critical growth phases of the pollen tube.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation was carried out in the Department of Agricultural Botany, College of Agriculture, Vellayani, Trivandrum during the period 1980-'82. The experiment was laid out in the main season extending from November to March. An additional crop was also taken during April - August.

Materials

Thirty one varieties of Sweet Potato (Ipomoea batatas (L) Lam) were used for screening the incompatibility reactions. They included varieties collected from Central Tuber Crops Research Institute, Sreekaryam, Trivandrum, in addition to the varieties available in the Department of Agricultural Botany, College of Agriculture, Vellayani. Details of varieties used with their source are presented in Table I. The experiment was laid out in the dry lands of the Instructional farm attached to the College of Agriculture, Vellayani. Each variety was represented by nine mounds having three plants each.

PART A

Methods

Screening for self compatibility/Incompatibility reactions

Techniques of selfing

The time of anthesis was found to be between 5.30 and 9.30 A.M. under Vellayani condition. In order to study the

Table - I

Names and Sources of sweet potato varieties used for the study.

Sl.No.	Name of Variety	Source
1.	S 391	C.T.C.R.I., Trivandrum
2.	S 157	"
3.	S 124	"
4.	S 378	"
5.	H 42	"
6.	Kanjangadu local	"
7.	H 633	"
8.	H 620	"
9.	B 137	"
10.	S 214	"
11.	S 187	"
12.	H 2416	Department of Agricultural Botany, College of Agriculture, Trivandrum.
13.	SP 1	"
14.	Kottaramchumala	"
15.	H 2742	"
16.	H 4025	"
17.	H 3032	"
18.	H 2425	"
19.	H 2421	"
20.	H 4125	"

Table - I (Continued)

Sl.No.	Name of Variety	Source
21.	H 2662	Department of Agricultural Botany, College of Agriculture, Trivandrum.
22.	H 2412	"
23.	Pichivella	"
24.	H 3045	"
25.	H 4126	"
26.	Bhadrakalichumala	"
27.	H 4330	"
28.	H 4024	"
29.	H 2648	"
30.	H 2743	"
31.	H 4340	"

incompatibility reaction, well developed flower buds that would open on the next day were selected and covered with butter paper bags in the evening. They were pollinated on the next day morning (6 - 9 A.M.) with their own pollen. For effecting assisted pollination, one or two anthers were taken from the flower using a forceps. The pollen grains contained in the anthers were dusted on the stigmatic surface, with the help of a Camel's hair brush. Pollination was done between 6 and 9 A.M. The flowers were labelled and again bagged after pollination. The bags were retained for one day to avoid the chance of any contamination.

Another simple method of selfing tried consisted of using paper cones made up of drawing sheets, for covering the mature flower buds. They were fitted as caps on the buds, and thus the opening of the flowers were prevented in the next day morning. Removing the paper cones, artificial pollen transfer was done at the anthesis time. The selfed flowers as in the previous case were then labelled and covered with butter paper bags.

PART B

Methods used for overcoming the self incompatibility barrier.

(1) Bud pollination.

The twenty one self incompatible varieties identified were bud pollinated, since it had been reported by various workers including East (1934) that the inhibitory substances causing incompatibility was not observed in young buds.

Flower buds that would open on the next day morning were selected and the corolla was split open using a blade. Pollen from the opened flowers of the same plant was then transferred to the exposed stigma, with the help of a Camel's hair brush. The pollinated flowers were bagged with butter paper bags and labelled properly.

(ii) Pollination after cutting the stigma.

Brewbaker (1957) had reported that inhibition of pollen tubes at the stigmatic region was associated with sporophytic incompatibility. So, attempts were also made to effect pollination after cutting the stigma.

Flower buds that would open on the next day were selected and bagged using butter paper bags. On the next day morning the stigmatic region was carefully cut and removed using a sharp blade. Pollen collected from the same flower was dusted immediately on the cut surface of the style using a Camel's hair brush. The pollinated flowers were bagged and labelled properly.

(iii) Removal of the stigma and pollination with the help of sucrose solution.

The cut end of the style is not as suitable for pollen germination as the stigma. So, as a modification of the amputation methods, sucrose solution was used on the cut stylar surface as a nutrient medium to facilitate the pollen germination.

Surcrose solutions of various concentrations viz., 0.5, 1.0 and 5.0 per cent were tried.

The flower buds which would open on the next day were selected, and bagged. On the next day morning, just prior to pollination, the stigmatic region was cut and removed. A drop of the sucrose solution was placed on the cut surface of the style with the help of a glass filler. Freshly collected pollen from the same flower was carefully dusted on the drop of sucrose placed on the cut end of the style. Utmost care was taken to see that a drop of sucrose solution along with the dusted pollen remained adhered to the amputed surface of the style. The pollinated flowers were properly bagged and labelled.

(iv) Pollination after removal of the stigma and a part of style.

Here also, the flower buds that would open on the next day were selected, bagged and labelled first. On the next day morning, the style of the selected flower was held gently and, with a sterile and sharp blade the top portion of the style, about 2 - 3 m.m length, along with the stigma was cut and removed, without causing injury to the other floral parts. Pollen from the same flower was dusted on the cut surface before they got dried up.

(v) Amputation of the Stigma and part of the style and pollination with the help of sucrose solution.

The method was similar to method three and method four. Here, after removing the stigma along with the top portion of the style, a drop of sucrose solution 0.5% was applied on the cut surface. Pollen from the same flower was applied on the cut surface without disturbance.

(vi) Pollination with germinated pollen grains.

Pollen germination studies were undertaken in the laboratory, using 0.5, 1.0, 2.0 and 5.0 per cent sucrose solutions independently and in combination with boric acid at 100 and 200 ppm concentration.

One drop of the solution was placed on a clean coverglass and pollen collected afresh from the opened flowers were placed on the drop of the solution without disturbing it. This cover slip was then placed upside down on a cavity slide and the slide was examined at intervals of 2, 4, 6, 8, 10 and 12 hours under a microscope. But, it was found that these laboratory treatments were not effective in promoting pollen germination. Hence, this technique was not tried in the field.

(vii) End-of-Season Pollination.

Pollination at the end of the growth season was reported to be effective for seed setting in normally self incompatible species (Allard, 1960). Hence, this treatment was also undertaken.

The month of March was fixed as the late stage of the season. The method adopted was same as that used for screening the incompatibility reaction.

Flower buds that would open on the next day were selected bagged and labelled. Next day morning, pollen from the same flower was dusted on the stigmatic surface. Cover was retained for that day to avoid contamination by any foreign pollen.

(viii) Application of 2, 4 Dichloro phenoxy acetic acid (2,4-D)

Charles et al (1974) reported that some chemicals like IAA, NAA, 2, 4-D etc. were useful in breaking the self incompatibility barrier in sweet potato. Hence, this method was tried.

Flower buds that would open on the next day morning were selected, bagged and labelled. In the next morning, pollen from the same flower was transferred to its stigma. They were again bagged. A small bit of cotton dipped in the 2, 4-D solution was applied on the pedicel of the flowers immediately after pollination. The 2, 4-D solution was so carefully used that all other parts except pedicels were virtually unaffected.

2, 4-D solutions of varying concentrations viz. 50, 100, 150 ppm were tried.

PART C

a. Pollen sterility

Pollen sterility in fourteen sweet potato varieties was also studied. The pollen grains from freshly opened flowers were collected and mounted in glycerine acetocarmine medium on a clean slide.

The slides were examined under the low power of a compound microscope. Well filled and uniformly stained pollen grains were taken as fertile and the rest as sterile. 500 pollen grains from different microscopic fields were scored for each variety from a slide.

b. In vivo germination of pollen grains and pollen tube growth

In order to study the germination of pollen grains on the stigmatic surface and the extent of pollen tube growth on the stylar column, the ovaries along with the intact styles were collected at intervals of 2, 4, 6, 8 10 and 12 hours after assisted self pollination. The ovary alongwith the style was taken off and used for later studies. Pistils from the pollinated flowers obtained by adopting the following techniques were collected, fixed and preserved in 70 per cent alcohol.

1. Selfing
2. Bud pollination
3. Pollination after removal of stigma and style
4. Delayed pollination
5. Pollination followed by 2, 4-D treatment

The pistils were fixed in Carnoy's fluid (1:3 Acetic Alcohol). Fresh materials as well as fixed materials were tried. The material was taken in a watch glass with a few drops of IN HCl. Then it was kept in the oven for a period of 10 minutes at 60°C for hydrolysis. This was stained in Lactophenol cotton blue. In order to facilitate better staining of pollen grains and pollen tube, the pistils were kept in the stain for a minimum period of three hours. Then the pistil was taken off and placed on a drop of glycerin kept on a slide. A coverglass was put on it and gently pressed so as to ensure uniform spreading of the tissues. The slides were examined under a microscope.

RESULTS

RESULTS

The following observations were made during the course of this investigation.

Flowering in sweet potato.

Sweet potato was reported to be a non-bloomer by many workers. But, in the present investigation, majority of the varieties tested produced flowers indicating that Vellayani condition are congenial for flowering of sweet potato. But, there were differences among the varieties for the habit of flowering and also for the flower production capacity. The details of flowering habit are presented in Table II and III.

Of the 31 varieties tested, Kotteramchumala failed to produce any flowers. Nine varities viz., S157, Kanjangadu local, H 2416, S 137, H 2425, H 2412, H 4126, S 391 and S 187 were found to produce flowers in very low proportion. The five varieties S 124, S 214, H 4125, H 2648 and H 2743 produced flowers in fair proportion. All the other 16 varieties flowered profusely.

The observations regarding the time taken for flowering was found to be highly variable from variety to variety and also in between different plants of the same variety. As a general rule, it was found that 21 varieties flowered early. The 9 varieties which are classified as late flowering types include S 157, S 214, S 391, S 187, S 124 Kangangadu local, H 4025, H 2425 and H 4340.

Table - II

Early flowering varieties (those flowered between 40 - 60 days after planting) and the total number of flowers recorded/day on the peak flowering time from five mounds.

Sl.No.	Name of the Variety	Total number of flowers/day
1.	S 378	99
2.	H 42	71
3.	H 620	48
4.	H 633	36
5.	H 2743	45
6.	H 2648	18
7.	SP 1	26
8.	H 4024	10
9.	H 4330	52
10.	H 4126	11
11.	H 3045	44
12.	Pichivella	28
13.	H 2421	39
14.	H 4125	18
15.	H 2662	19
16.	H 2412	6
17.	H 2742	38

Table - III

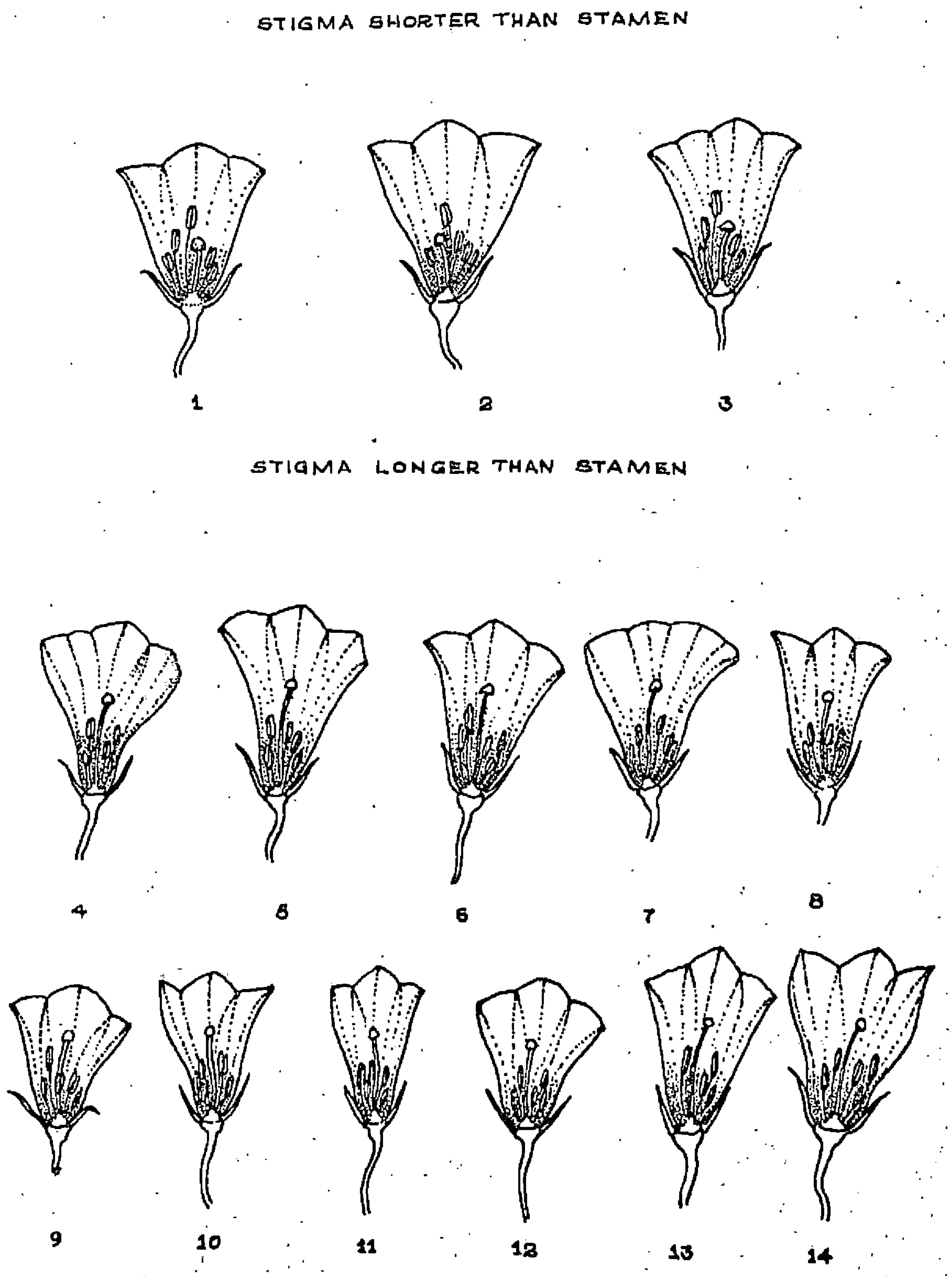
Late flowering varieties (those flowered after 60 days of planting and total number of flowers recorded/day on the peak flowering time from five mounds.

Sl.No.	Name of the variety	Total number of flowers/day
1.	S 391	11
2.	S 157	7
3.	S 187	22
4.	S 124	48
5.	Kanjangadu local	13
6.	H 2416	5
7.	S 137	2
8.	S 214	26
9.	H 4026	74
10.	H 3032	76
11.	H 2425	5
12.	H 4340	102
13.	Bhadrakalichumala	108

Floral morphology

The thirty varieties which produced flowers carried clusters of 2 - 10 flowers in axillary cymes. The flowers bloomed in acropetal succession. The colour of the flowers varied from deep purple to white.

The flowers of sweet potato carried 5 stamens which exhibited considerable variation in length among themselves and also in relation to the length of the pistil. In all the thirty varieties, the stamens exhibited a more or less uniform pattern of difference in their lengths, one long, two medium and two small. The style was long, slender and thread like and carried a large globular stigma at the tip. Considerable difference in length of style was noticed in the different varieties and it was longer than the longest stamen in eleven varieties viz., S 157, S 187, S 378, H 42, H 3045, H 2648, H 2742, H 4024, S 214, H 2416 and Pichivella. The sixteen varieties viz., H 620, H 633, S 124, H 4126, H 4330, SP 1, H 4025, H 3032, H 2412, H 2662, H 4125, H 2743, H 4340, H 2421, Bhadrakalichumala and Kanjangadu local had their style length more or less the same as that of the longest stamen. In the other three varieties viz., S 391, S 137, and H 2425 the style was slightly lower in length than the longest stamen. Slight variations were noticed even within the same variety.


STIGMA SHORTER THAN STAMEN
1
2
3
STIGMA LONGER THAN STAMEN
4
5
6
7
8
9
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12
13
14

FIG: 1

STIGMA AND STAMEN ARE OF SAME HEIGHT

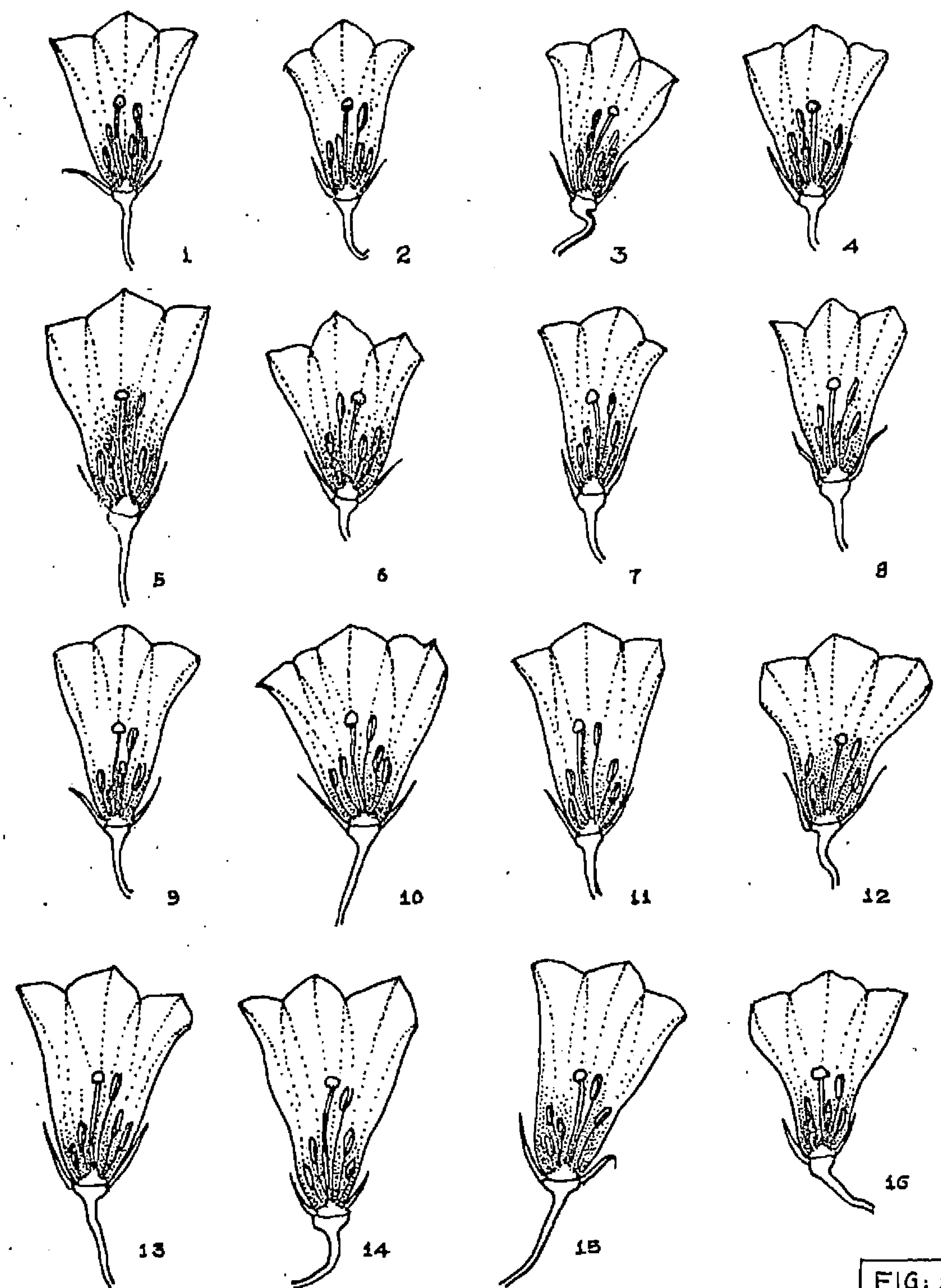


FIG: 2

Thickness of the Pedicel

Seven varieties viz., S 391, H 42, H 633, SP 1, H 2742, H 3032 and H 2412 possessed very thin pedicels. Varieties S 378, S 214, H 2743 and H 3045 showed very thick and strong pedicels. All the other varieties tested were intermediate in pedicel thickness.

Floral abscission

Observations regarding the time of floral abscission showed uniformity in all the varieties. Unpollinated flowers and flowers of incompatible varieties that were artificially pollinated, were found to abscise one week after flower opening.

Natural fruit and seed setting

Natural fruit set was noticed in almost all the varieties which produced flowers. Further, it was observed that the four varieties viz., S 378, S 124, H 3045 and Pichivella produced fruits abundantly. Even though the local variety 'Bhadrakalichumala' produced flowers in abundance, its fruit set was very low. All the open pollinated seeds were found to be viable in germination tests.

Screening for Compatibility/Incompatibility reactions

All the thirty varieties were tested for their incompatibility reactions. The results are presented in Table IV. It was indicated that S 378 was the only one variety that could set fruit among the thirty varieties tested.

Table - IV

Results of screening for compatibility/incompatibility reactions.

Sl. No.	Name of the Variety	Percentage of fruit set	Mean number of seeds	Percentage seed germination
1.	S 157	0	-	-
2.	S 187	0	-	-
3.	S 378	36.0	1.33	41.67
4.	H 42	0	-	-
5.	H 3045	0	-	-
6.	H 2648	0	-	-
7.	H 2742	0	-	-
8.	Pichivella	0	-	-
9.	H 4025	0	-	-
10.	B 214	0	-	-
11.	H 2416	0	-	-
12.	H 620	0	-	-
13.	H 633	0	-	-
14.	S 124	0	-	-
15.	H 4126	0	-	-

Table - IV (Continued)

Sl. No.	Name of the Variety	Percentage of fruit set	Mean number of seeds	Percentage seed germination
16.	H 4330	0	-	-
17.	SP 1	0	-	-
18.	H 4024	0	-	-
19.	H 3032	0	-	-
20.	H 2412	0	-	-
21.	H 2662	0	-	-
22.	H 4125	0	-	-
23.	Bhadrakelichumala	0	-	-
24.	H 2743	0	-	-
25.	H 4340	0	-	-
26.	H 2421	0	-	-
27.	Kanjangadu local	0	-	-
28.	S 391	0	-	-
29.	S 137	0	-	-
30.	H 2425	0	-	-

There was 36 per cent fruit set for this variety. All the other varieties were found to be self incompatible. Germination tests were conducted using the selfed seeds and it was found that there was 41.62 per cent germination. The germinated seeds produced healthy seedlings.

Varietal reactions to different techniques of breaking incompatibility.

The different techniques were tried in 21 varieties including 20 self incompatible and one self compatible varieties. Fifty flowers of each variety were subjected to each of the different techniques and the percentage of fruit set and seed set were assessed.

(a) Bud Pollination.

The results of bud pollination are summarised in Table V. There was no fruit set in any of the incompatible varieties. However, the compatible variety S 378 recorded 49.2 per cent fruit set. Germination tests conducted showed that there was 48 per cent germination.

(b) End-of-season pollination.

Table VI gives the results of this method which was done at the end of the growth season. Here also, 20 incompatible varieties and the one compatible variety S 378 were tested.

H 3045 recorded 12.5 per cent fruit set and 42.3 per cent seed germination. H 2468 recorded 10 per cent fruit set and 62.4 per cent seed germination. The compatible variety S 378

Table - V

Effect of bud pollination on fruit set.

Sl. No.	Name of the variety	Percentage of fruit set	Mean number of seeds	Percentage seed germination
1.	S 214 (incompatible)	0	-	-
2.	S 124 (")	0	-	-
3.	H 4125(")	0	-	-
4.	H 2468(")	0	-	-
5.	H 2743(")	0	-	-
6.	S 378 (compatible)	49.2	1.52	48.0
7.	H 42 (in compatible)	0	-	-
8.	H 3045(")	0	-	-
9.	S 2742(")	0	-	-
10.	Pichivella ")	0	-	-
11.	H 4025 ")	0	-	-
12.	H 620 ")	0	-	-
13.	H 633 ")	0	-	-
14.	H 4330 ")	0	-	-
15.	SP 1 ")	0	-	-
16.	H 4126 ")	0	-	-
17.	H 3032 ")	0	-	-
18.	H 2662 ")	0	-	-
19.	Bhadrakalichumala)	0	-	-
20.	H 4340 ")	0	-	-
21.	H 4024 ")	0	-	-

Table - VI

Effect of end-of-season pollination.

Sl. No.	Name of the variety	Percentage of fruit set	Mean number of seed	Percentage seed germination
1.	S 214 (Incompatible)	0	-	-
2.	S 124 (")	0	-	-
3.	H 4125(")	0	-	-
4.	H 2468(")	10	1.0	62.4
5.	H 2743(")	0	-	-
6.	S 378 (Compatible)	38	1.41	52.0
7.	H 42 (Incompatible)	0	-	-
8.	H 3045(")	42.5	1.0	42.3
9.	H 2742(")	0	-	-
10.	Pichivella ")	0	-	-
11.	H 4025 ")	0	-	-
12.	H 620 ")	0	-	-
13.	H 633 ")	0	-	-
14.	H 4330 ")	0	-	-
15.	SP 1 ")	0	-	-
16.	H 4126 ")	0	-	-
17.	H 3032 ")	0	-	-
18.	H 2662 ")	0	-	-
19.	Bhadrakalichumala)	0	-	-
20.	H 4340 ")	0	-	-
21.	H 4024 ")	0	-	-

recorded 38 per cent fruits set and 52 per cent germination. All the other varieties failed to produce any fruits.

(c) Pollination after amputating the stigma

The results are summarised in Table VII. Here also, as in the case of bud pollination, none of the varieties produced any fruit in response to this technique. Even the variety S 378 which was normally compatible set no fruit

(d) Pollination after amputation of the stigma and a part of the style

The results are presented in Table VII. None of the 21 varieties produced any fruit in response to this technique.

(e) Pollination after amputating the stigma and application of 0.5 per cent sucrose solution

The results were same as above. No fruit set was observed by the application of this method.

(f) Pollination after amputating the stigma along with a part of the style and application of 0.5 per cent sucrose solution

The results were same as shown in Table VII.

(g) Pollination with germinated pollen grains

Pollen germination was studied in vitro using different concentrations of sugar solution independently as well as in combination with boric acid. Pollen from the variety S 378 was subjected to the different treatments. The results

Table - VII

Effect of self pollination after cutting the stigma/stigma and a part of the style.

Sl. No.	Name of the variety	Percentage of fruit set	Mean number of seeds	Percentage seed germination
1.	S 214 (Incompatible)	0	-	-
2.	S 124 (")	0	-	-
3.	H 4125(")	0	-	-
4.	H 2468(")	0	-	-
5.	H 2743(")	0	-	-
6.	S 378 (Compatible)	0	-	-
7.	H 42 (Incompatible)	0	-	-
8.	H 3045(")	0	-	-
9.	H 2742(")	0	-	-
10.	Pichivella ")	0	-	-
11.	H 4025(")	0	-	-
12.	H 620 (")	0	-	-
13.	H 633 (")	0	-	-
14.	H 4330(")	0	-	-
15.	SP 1 (")	0	-	-
16.	H 4126(")	0	-	-
17.	H 3032(")	0	-	-
18.	H 2662(")	0	-	-
19.	Bhadrakalichumala)	0	-	-
20.	H 4340(")	0	-	-
21.	H 4024(")	0	-	-

are given in Table VIII. None of the treatments induced pollen germination in vitro and, hence, the pollination using germinated pollen grains could not be performed.

(h) Pollination after application of 2, 4-D solution

The results of the preliminary observations are shown in Table IX. Three different concentrations of 2, 4-D (50, 100 and 150 ppm) were tried on five varieties. In the compatible variety S 378, 2 4-D application promoted the fruit set. Of the other four incompatible varieties, H 42 showed no response to the treatment. The variety H 4340 showed a positive effect to 2, 4-D at 100 and 150 ppm concentrations with 60 and 10 por cent fruit set, respectively. The other two varieties H 620 and H 4025 recorded 20 and 10 per cent fruit set at the concentration of 150 ppm. In general, it was shown that application of 2 4-D at 100 ppm was most effective in producing maximum percentage of fruits in sweet potato varieties which were originally self incompatible.

Results of the application of 2, 4-D at 100 ppm concentration in 21 varieties of sweet potato are given in Table X. This method induced fruit set in 13 varieties, including 12 incompatible and one compatible variety, S.378. Eight varieties did not show any response for fruit set.

The results indicated an increase in the percentage of fruit set in the self compatible variety S 378. Another observation recorded in connection with this treatment was

Table - VIII

Pollen germination in sweet potato.

Name of the variety used - S 378 (Self compatible)

Sl. No.	Treatments		Percentage of pollen germination
1.	Sugar solution	0.5%	0
2.	Sugar solution	1.0%	0
3.	Sugar solution	2.0%	0
4.	Sugar solution	5.0%	0
5.	Sugar solution	0.5% + 100 ppm Boric acid	0
6.	Sugar solution	1.0% + 100 ppm Boric acid	0
7.	Sugar solution	2.0% + 100 ppm Boric acid	0
8.	Sugar solution	5.0% + 100 ppm Boric acid	0
9.	Sugar solution	0.5% + 200 ppm Boric acid	0
10.	Sugar solution	1.0% + 200 ppm Boric acid	0
11.	Sugar solution	2.0% + 200 ppm Boric acid	0
12.	Sugar solution	5.0% + 200 ppm Boric acid	0

Table - IX

Effect of 2, 4-D to break the self incompatibility barrier.

Concentrations of 2, 4-D solution	Percentage of fruit set				
	S 378	H 42	H 620	H 4340	H 4025
50 ppm	60	0	0	0	0
100 ppm	80	0	0	60	0
150 ppm	40	0	20	0	10

Table - X

Effect of 2, 4-D (100 ppm) in fruit set.

Sl. No.	Name of the variety	Percentage of fruit set	Mean number of seeds	Percentage of seed germination
1.	S 214 (Incompatible)	46.0	-	-
2.	S 124 (")	58.5	-	-
3.	H 4125(")	78.0	-	-
4.	H 2468(")	75.6	-	-
5.	H 2743(")	86.3	-	-
6.	S 378 (Compatible)	79.4	-	-
7.	H 42 (Incompatible)	0	-	-
8.	H 3045(")	67.1	-	-
9.	H 2742(")	0	-	-
10.	Pichivella ")	49.2	-	-
11.	H 4025(")	0	-	-
12.	H 620 (")	0	-	-
13.	H 633 (")	0	-	-
14.	H 4330(")	44.5	-	-
15.	SP 1 (")	0	-	-
16.	H 4126(")	60.0	-	-
17.	H 3032(")	0	-	-
18.	H 2662(")	81.2	-	-
19.	Bhadrakalichumala)	0	-	-
20.	H 4340(")	63.1	-	-
21.	H 4024(")	48.5	-	-

that all flowers treated remained on the plant for more than one week (normal time for abscission). So also, all the treated pedicels showed various kinds of crackings and swellings.

It was also observed that even though the 12 varieties produced fruits, they were devoid of any seeds. Immature small seeds in the developing stage were found in mature fruits of the varieties S 378 and H 620.

The varieties which failed to produce any fruit were found to be injuriously affected by the 2, 4-D solution. So also, the pedicels of these varieties were found to be very thin compared to other varieties.

Pollen tube growth

Pistils were separated from the flowers by bi-hourly intervals after normal anthesis. They were fixed, hydrolysed and pollen tube growth on the stigma was studied. The results revealed the following.

After 2 hours of anthesis pollen tube growth was not found in the 21 varieties tried. After 4 hours, only the pollen grains of the variety S 378 produced sufficiently long pollen tube. After 10 hours, pollen grains of twelve more varieties showed signs of germination. They include S 214, S 124, H 4125, H 2468, H 2743, H 3045, Pichivella, H 4330, H 4126, H 2662, H 4340 and H 4024. The eight varieties which failed to show any sign of pollen germination include H 41, H 2742, H 4025, H 620, H 633, SP 1, H 3032 and Bhadrakalichumala. After 10 hours of normal anthesis, the style and stigma were shrivelled and, hence no observation was made.

Results of pollen tube growth after bud pollination revealed that this method did not promote germination of pollen grains on the stigma of the 20 self incompatible varieties and the one self compatible variety S 378.

The effect of pollination after cutting the stigma alone and cutting the stigma with a part of the style was observed under the microscope. It was observed that very few pollen grains were fouhd to stick on the cut surface of the style than on the stigmatic surface. Pollen germination on the cut surface was also not observed.

Pollen germination was observed in the 13 varieties which produced fruits following 2, 4-D treatment and pollen germination was not observed in the eight varieties which failed to produce fruits.

It was observed that the rate of pollen tube growth was same as that observed after normal self pollination and 2, 4-D treatment had no effect in enhancing the rate of pollen tube growth.

Pollen sterility

The results are presented in Table XI. Pollen sterility in sweet potato was found to vary from 54.10 to 6.01 per cent. The maximum sterility was recorded by Bhadrakalichumala with 54.10 per cent, closely followed by H 620. The minimum values were given by H 2743 and S 378 (6.6 and 6.01 per cent, respectively).

Table - XI

Pollen sterility in sweet potato.

Sl.No.	Name of the variety	Pollen sterility (percentage)
1.	H 2662	11.60
2.	H 2416	26.70
3.	S 187	31.90
4.	H 2742	19.10
5.	Bhadrakalichumala	34.10
6.	H 2612	12.70
7.	H 2421	31.40
8.	H 42	28.60
9.	H 2743	6.60
10.	H 633	15.90
11.	H 4126	8.80
12.	S 378	6.01
13.	SP 1	12.10
14.	H 620	37.60

DISCUSSION

DISCUSSION

The observations made during the course of the present study are discussed as follows.

Flowering habit in sweet potato.

Sweet potato, a vegetatively propagated root crop, has often been reported as a shy bloomer (Woda, (1925), Rosa, (1926) and Miller (1935), cited by Kunjan (1957)). In the present investigation majority of the varieties tested showed a tendency for flowering. This is in line with the reports of Thompson (1925) and Charles et al (1974) that good flowering in sweet potato can be obtained under tropical conditions. The tropical condition prevalent in Kerala accounts for the flower production in sweet potato varieties tested.

Out of the 31 varieties tried, only one variety viz., Kottaramchumala failed to produce any flower while varieties S 157, Kanjangadu local, H 2416, S 137, H 2425, H 2412, H 4126, S 391 and S 187 produced flowers in very low proportion. Other five varieties viz., S 124, S 214, H 4125, H 2648 and H 2743 produced flowers in fair proportion. The varieties which flowered profusely include S 378, H 42, H 3045, H 2742, Pichivella, H 4025, H 620, H 633, H 4330, SP 1, H 4024, H 3032, H 2662, Bhadrakalichumala, H 4340 and H 2421. Thus, from the results it is clear that flowering habit in this crop is very much influenced by the varietal difference as reported by Tioutine (1935) Miller (1938) Richharia and Gosh (1950), cited by Rao (1962) and Vansch (1954).

Flowering in sweet potato was also reported to be influenced by various environmental factors like rainfall, relative humidity, sunshine hours, photoperiodism, temperature, wind velocity etc. In the present study, it was noted that the flowering habit varied depending on seasons. The flowering nature observed in this crop during April-June was different from that in December-March. Kottaramchumala was the only one variety that did not produce flowers during the season December-March. But, during April-June period, in addition to Kottaramchumala varieties like Kanjangadu local S 214, S 124, H 2412, S 137, S 157, S 187, H 2648 and H 4340 also remained as non-bloomers. So also, flower production during this period (April-June) was found to be very low in certain profusely flowering types of December-March season. Thus, the present study supports the observations made by the earlier workers (Miller, (1937, 1938), Shigemara et al (1939), Fujise et al (1957), Ogorodnikov (1969), and Joseph (1979) that climatic factors have got a profound influence in the flowering habit of sweet potato varieties.

From the observations on the number of days taken for first flowering by each variety, no consistent indication was

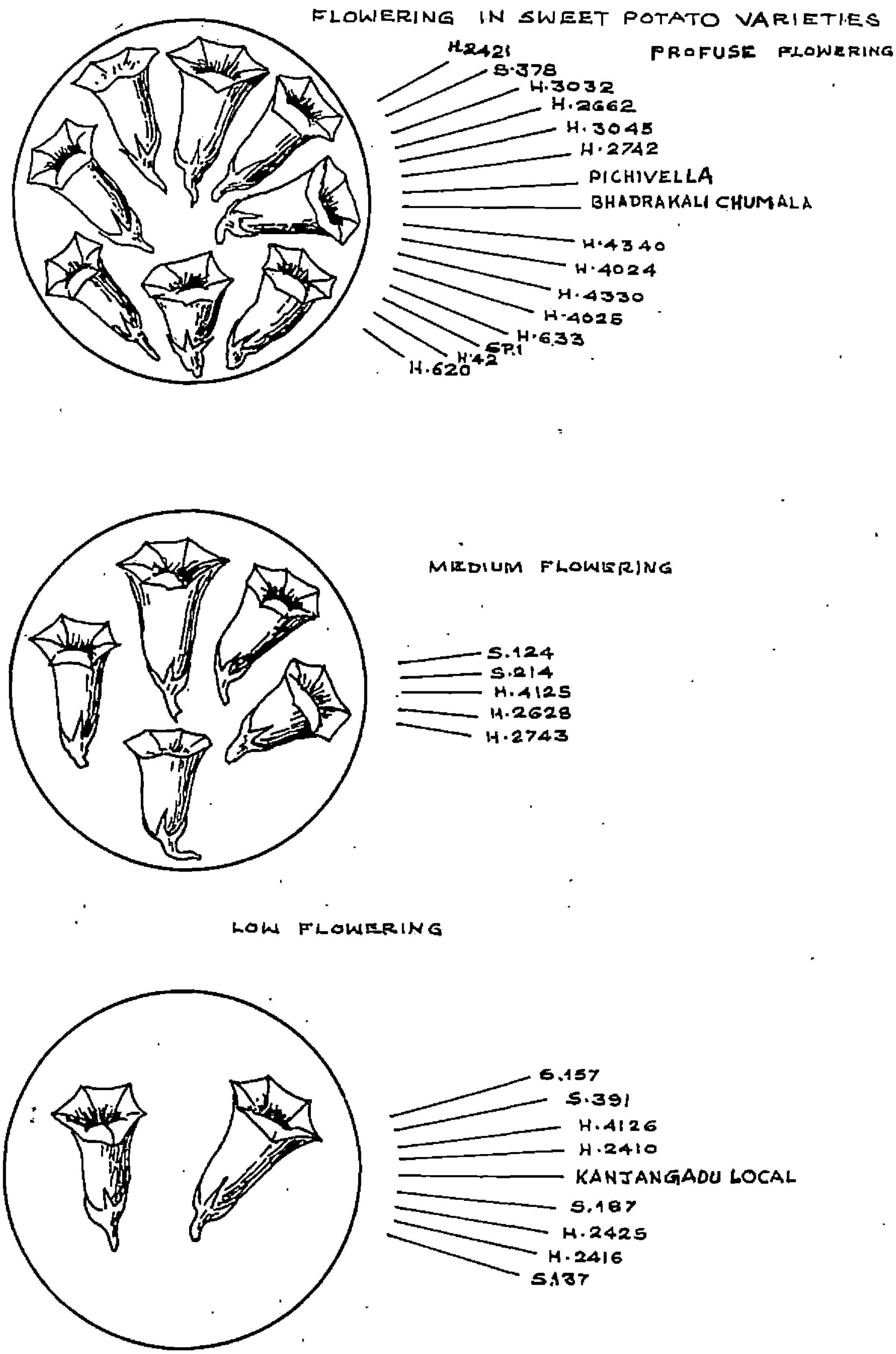

FLOWERING IN SWEET POTATO VARIETIES
PROFUSE FLOWERING
H2421
S·378
H·3032
H·2662
H·3045
H·2742
PICHIVELLA
BHADRAKALI CHUMALA
H·4340
H·4024
H·4330
H·4025
H·633
SP.1
H·42
H·620
MEDIUM FLOWERING
S.124
S.214
H·4125
H·2628
H·2743
LOW FLOWERING
6.157
S·391
H·4126
H·2410
KANJANGADU LOCAL
S.187
H·2425
H·2416
S.137

FIG: 4

obtained. There was wide variation among the plants of the same variety. Even though majority of the varieties flowered early, S 378, and H 42 can be considered as very early types. Varieties S 157, S 214, S 391, S 187, S 124, Ranjangadu local, H 4025, H 2425 and H 4340 could be considered as late flowering types. Nair (1970) obtained similar results and he explained that the early and late flowering habit depends upon the maturity of the planting material used. He added that the mature vines at the preflowering stage produced progenies which showed earliness in flowering.

Natural setting of fruits and seeds

Natural setting of fruits and seeds in sweet potato had been reported to take place in almost all agroclimatic conditions. (Thompson 1925, Encyclopedia Britanica 1962).

Natural seed set was observed in all the varieties which produced flowers. The present investigation also showed that natural seed set can be obtained in both the seasons viz., December-March and April-June. The seed set observed in 'Bhadrakalichumala' was very low even though they produced sufficient flowers in both seasons.

Sampath and Bhanumurthy (1948) observed free setting of fruits and seeds at Coimbatore and Bapatla. The optimum conditions for setting of fruits and seeds were those existed during December-January-February. They were of the opinion that temperature was the most important factor in this connection.

Similar observations had been made by growers of this crop in Hawaii, who in addition found that during the moist seasons of the year, especially when the foliage exhibit a tendency to develop luxuriantly, seed set was considerably decreased. In the present study also similar results were observed. Even though the crop set seeds in both the dry and wet seasons, it was found to be low during the rainy season. The observations clearly indicated that the conditions prevailing at this area could be considered as ideal for flowering and setting of fruits and seeds in sweet potato.

Screening for compatibility/Incompatibility reactions

The data obtained during course of the present investigation (Table II) revealed failure of seed set in 29 out of the 30 flowering varieties screened. Only the variety S 378 produced fruits and seeds on assisted self pollination. The seed setting ability of S 378 was earlier reported by Venkiteswaralu (1980). Failure of seed set in sweet potato is reported to be due to incompatibility mechanisms. Reports related to this phenomenon are numerous.

Allard (1960) reported some self sterile or self incompatible strains in sweet potato. Both self and cross incompatibility had been reported in this crop by Williams (1964) and Martin (1965). Fairly high and varying degrees of self incompatibility reactions had been accounted in this crop for failure of seed set by various workers including Woda (1935), Sampath and Bhanumurthy (1948), Abraham (1957), Kunjan (1957) and Wang (1965, 1966). It was also reported that self incompatibility rather than self compatibility was the rule in this crop which had seriously limited the selfing programmes of sweet potato breeders (Joseph, 1979). Thus, the failure of seed set in all the twenty nine varieties of sweet potato noted in the present investigation can be attributed to the presence of self incompatibility mechanism operating in this crop.

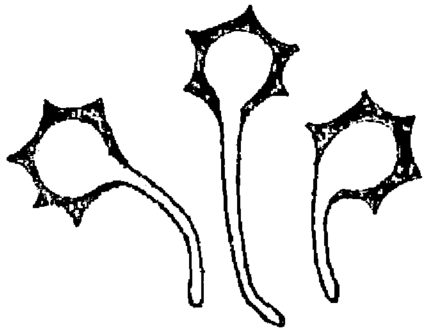
Germination of pollen grains on the stigma was noted six hours after pollination in the variety S 378. All the other 20 varieties tried by assisted selfing failed to show any pollen germination in the stigmatic region after four hours. Signs of pollen germination after ten hours were observed in 12 out of the 21 varieties tried. They include, Pichivella, H 3045, H 4126, H 4330, H 4024, H 2662 H 2468, H 2743, H 4340, H 4125, S 214 and S 124. Pollen grains failed to germinate in the stigma of other eight varieties viz. H 41, H 2742, H 3032, SP 1, H 633, H 620, H 4020 and Bhadrakalichumala even after ten hours. The pollen germination could not be observed after ten hours due to the shrivelling of the style and stigma.

Williams and Cope (1967) found that fewer pollen grains were retained on incompatible stigmas than on compatible ones in Ipomoea trichocarpa and attributed this phenomenon to lack of mechanical anchorage. Similar observations were

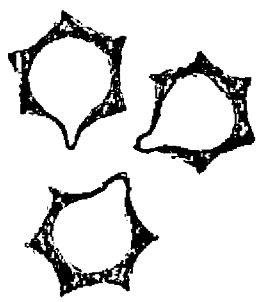
IN VIVO POLLEN GERMINATION IN SWEET POTATO

VARIETIES SHOWING POLLEN GERMINATION

AFTER 4 HOURS



AFTER 10 HOURS



VARIETIES SHOWING NO POLLEN GERMINATION

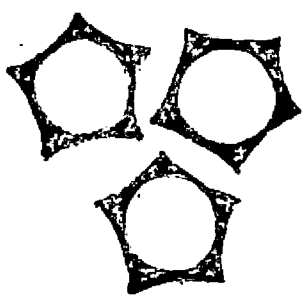


FIG: 5

recorded by Charles et al (1974). But, the present investigation showed contradictory results in Ipomoea batatas. Almost equal number of pollen grains were found to be retained after compatible and incompatible matings.

Failure of pollen germination was observed in eight varieties listed above and very slow pollen tube growth after ten hours was observed in another twelve varieties. The pollen grains were found to germinate sufficiently earlier in the only one self-fertile variety S 378.

The primary site of self incompatibility mechanism in Ipomoea batatas was considered to be the stigmatic surface which was inhibiting the pollen germination (Togari and Kawahara (1942), Van Schreven (1953), Martin and Ortiz (1966), and Martin and Cabanillas (1965). Failure of pollen germination in the stigma did the post pollen germination barriers could be considered as reasons for the failure of fruit and seed set in the above varieties. In any case, stigmatic region could be considered as the site of the incompatibility mechanism as reported by Tagari and Kawahara (1942).

Failure of pollen germination was attributed to the lack of growth substances necessary to stimulate the germination of incompatible pollen (Fujise, 1964). Complete germination failure was ascribed by Martin and Cabanillas (1965) to a strong incompatibility reaction resulting from the interaction of substances diffusing from the pollen or stigma or both. Martin (1967) reported disorientation of pollen tube in incompatible styles in Ipomoea batatas. The disorientation of the pollen tube had been caused by the evasion of substances inhibitory to tube development, present in the central core of the style. Mechanical retardation in tube growth could have resulted from the nature and arrangement of cells in the region of tube penetration as reported by Martin and Ortiz (1967).

From the present study, it can be concluded that the incompatibility may be due to the lack of some growth promoting factors required for pollen germination on the stigma. So also, the rate of pollen tube growth may not be sufficient to effect fertilization and seed development at the proper time.

The variety S 378 was found to be self compatible, but the percentage of fruit and seed set was very low. It was indicated that along with incompatibility barrier some other factors might also be there, influencing the fruit and seed set in this crop. Climatic factors and time of pollination had much influence on fruit and seed set as reported by Miller (1938), Monteclaro and Miller (1951), and Srinivasan (1977).

Joseph (1979) reported that the total contribution of weather elements alone on fruit and seed set in sweet potato amounted to 40 per cent and 32.5 per cent, respectively. Time of pollination was also found to have a decisive role in the percentage of fruit and seed set as reported by Hernandez et al (1967) and Srinivasan (1977).

Thus the low percentage of fruit and seed set observed in the self compatible variety S 378 can be attributed to the influence of the above factors.

The selfed seeds obtained from the above variety were found to be germinating well. The seedlings showed good vigour indicating that there was no after effect for the incompatibility mechanism.

Methods of breaking the self-incompatibility barrier

The results of various methods tried for breaking the self-incompatibility barrier along with the results obtained by the in vivo pollen germination studies are discussed as follows.

In the present study, bud pollination was found to be quite ineffective in breaking the self incompatibility barrier. This is in line with the reports made by Togari and Kawahara (1942) and Fujise (1957).

Togari (1942) explained the reason for the failure of the method saying that the stigma of sweet potato become receptive only a few hours before anthesis, by which time the pollen fails to germinate on the stigma. Lack of stigma receptivity during the bud stage coupled with the absence of certain growth promoting substances can be considered as the cause for the failure of this method.

The technique used by Mangelsdorf and Reeves (1931) and Davis (1957) in trimming styles before pollination was tried in this crop during the present study to break the self-incompatibility. But, the result showed that the method was quite ineffective to overcome the incompatibility barrier. Here, even the variety S 378 which was normally self compatible also failed to produce any fruit when this method was tried.

The observations recorded in the present study regarding pollen germination revealed that the number of pollen grains retained on the cut end of the style after self pollination was very low when compared to that on the stigma and there was no sign of pollen germination. These results supported the findings of Mangelsdorf and Reeves (1937) and Davis (1957). Large reduction in pollen germination was recorded by them when stigma and style were removed before pollination. Martin and Cabanillas (1965) recorded that the germination of pollen completely failed at the style-ovary junction. Charles et al (1974) reported that the incompatibility reaction was greater when the site of pollen germination was nearer to the ovary.

Sucrose was found to be a favourable medium for pollen germination in many crop plants as reported by Kiss (1970) and Radhakrishnan (1976). But, contradictory results were obtained in sweet potato in the present study, which showed that application of sucrose solution on the cut surface of the style was not at all favourable for pollen germination and seed set.

Martin and Telek (1971) after analysing the stigmatic secretion of sweet potato revealed that it contained only traces of sugar and a lot of other compounds. Thus, it could be assumed that in addition to sugar, some other growth promoting substances were present in the stigmatic secretion, which was essential for pollen germination.

Attempts on artificial germination of pollen in different concentrations of sucrose and sucrose + boric acid solutions had also been made with a view to pollinate the flowers with artificially germinated pollen grains. But even the pollen grains from the compatible variety did not germinate in the artificial media.

Brewbaker (1957, 59) observed that the trinucleate pollen grains of a sporophytic system would not germinate readily in vitro. Martin and Ortiz (1966) reported that pollen of sweet potato and related species failed to germinate in artificial media. The failure of pollen germination in sucrose solution at various concentrations and sucrose boric acid mixture may be due to some deficiency of the media for the germination of sweet potato pollen grains.

The fruit and seed set observed for the end-of-season pollination indicated that the incompatibility reaction was reduced at the end of the growth season. This may be due to the inadequate quantity of the inhibiting substance on the stigma, confirming the earlier report made by Allard (1960). The results also indicated that varietal difference did exist in the expression of this quality.

Regarding the 2, 4-D treatment which resulted in fruit development in 13 varieties, it was found that the rate of the pollen tube growth was not at all enhanced by its application. Here along with the self compatible variety S 378, the other 12 self incompatible varieties which showed signs of pollen germination on the stigma following self pollination produced fruits. But, the other 8 varieties which did not show signs of pollen germination on the stigma even after 10 hours of self pollination, failed to produce any fruit. From these results, it was indicated that some factors other than that which enhances the rate of pollen tube growth were also involved in the fruit development.

Different explanations were offered by earlier workers for the incompatibility mechanism. Martin and Cabanillas (1966) after studying the incompatibility mechanism in sweet potato varieties, reported that the growth of pollen tube was delayed at the style stigma junction. Under field conditions, this temporary arrest in the early phase of tube penetration might act to retard the tube extension to the ovary and prevent fertilization. Moreover, the style tend to shrivel shortly after flower opening on the same day. So the chance of slow growing pollen tubes to reach the ovary appeared to be less. In the present study, it was observed that 2, 4-D treatment was effective in breaking the incompatibility barrier, even though no enhancement of pollen tube growth was observed.

It was also observed that the flower life was increased, after the application of 2, 4-D solution. Normally the flowers will abscise after one week following incompatible matings. This was also reported by Joseph (1979). But here the flower remained for one month indicating that the flower life was increased by 2, 4-D application. Probably this may be helping to overcome the incompatibility barrier. It could be considered that here the slow growing pollen tube get more time to effect fertilization.

The failure of proper development of seeds (immature seeds) in the 2, 4-D treated flowers might be due to its severe burning effect on the pedicels, which is evident from the cracking and other malformations observed on the pedicels. Due to this the food supply to the developing embryos might have been prevented resulting in the formation of immature seeds.

The reason could also be due to the embryo abortion. Incompatibility between embryo and the somatic cell could result in improper development of fruits after successful fertilization as reported by Charles et al (1974). They had produced viable seeds after 2, 4-D treatment by embryo culture indicating that the embryos were viable and could produce good seedlings.

Fruit set and fertility of pollen

Wang (1964) reported that along with incompatibility, temperature, pollen sterility and meiotic abnormality were also responsible for failure of seed and fruit set. Martin (1967) also suggested that seed set failure might be due to certain sterility factors associated with gametic imbalances. The results of the present study also showed that pollen sterility was playing a significant role in the low seed set in sweet potato.

The reason for the poorly formed pollen grains were given by Warmke and Cruzado (1949). They were of the opinion that non-disjunction and other meiotic irregularities were common in this crop and could account for higher percentage of poorly formed pollen grains. Thus, the meiotic abnormalities due to the hexaploid nature of this crop might be the reason for the high percentage of pollen sterility in sweet potato.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

A study was conducted at the Department of Agricultural Botany, College of Agriculture, Vellayani, to study the possibility of improving sweet potato through heterosis breeding. During the course of this investigation, the flowering habit, natural fruit and seed set, extent of incompatibility and methods to break the incompatibility barrier were studied on thirty one varieties of sweet potato selected from different sources.

Flowering and natural fruit and seed set were found to exhibit considerable variability. Even though natural fruit set was observed throughout the year, the maximum expression was obtained during December-January-February.

Except one variety viz. S 378 all the other 29 varieties studied showed self incompatibility. Delay in pollen germination and slow growth of the pollen tube along with the short flower life can be attributed to be the reasons for the incompatibility mechanism observed in the above varieties of sweet potato.

Of the eight methods tried to break the incompatibility barrier, the 2, 4-D treatment was found to be the best. Another method which registered fruit and seed production was the end-of-season pollination. All the other methods failed to produce any fruit after incompatible matings.

The success of 2, 4-D treatment which resulted in fruit set in 13 varieties was considered to be due to its effect on increasing the flower life by preventing floral abscission. The failure of seed set in the fruits of 2, 4-D treated flowers may be due to the reduction in food supply to the developing embryos as a result of the injuries on the pedicels following 2, 4-D treatment.

Pollen sterility was observed in all tho varieties studied indicating that they were also playing a role in the low fruit and seed set in this crop.

Thus, the present study indicate good scope for heterosis breeding in this crop. The self incompatible varieties can be directly used as female parents in hybridisation programmes without resorting to genetic or cytoplasmic male sterility. So also, there is good scope for producing inbred lines by standardising the 2, 4-D treatment.

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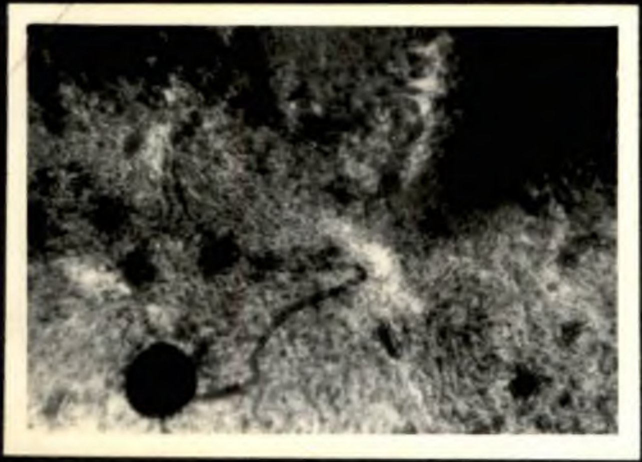
Plate I. A general view of the experimental plot.

Plate II. Sweet potato in bloom.

Plate IV. Fruit of sweet potato.

Plate VII. Pollen germination in S 378, after four hours.



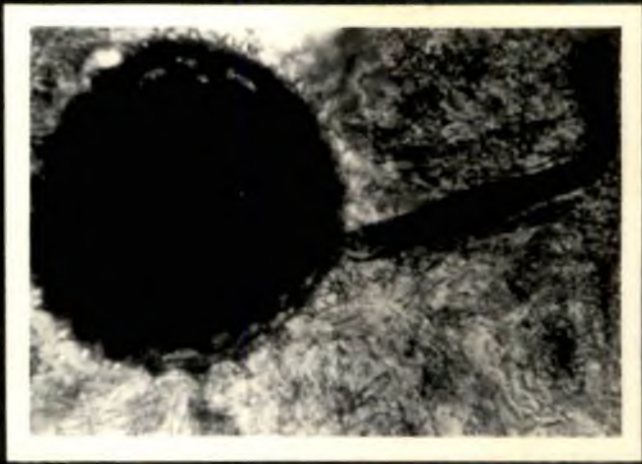
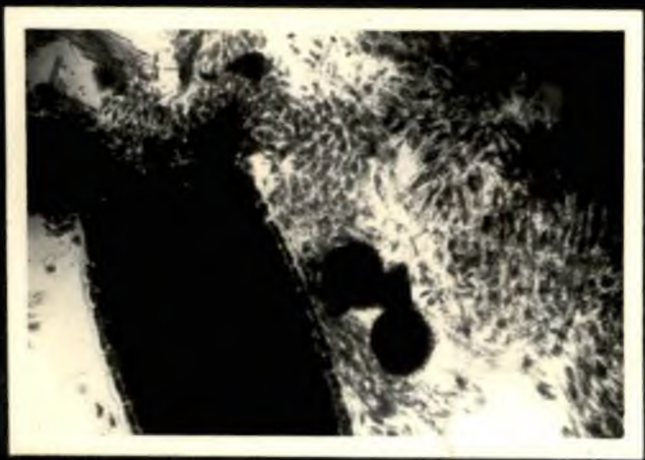


Plate VIII. Pollen germination in S 378, after four hours. (High power observation).

Plate IX. Pollen germination after ten hours (H 2662).



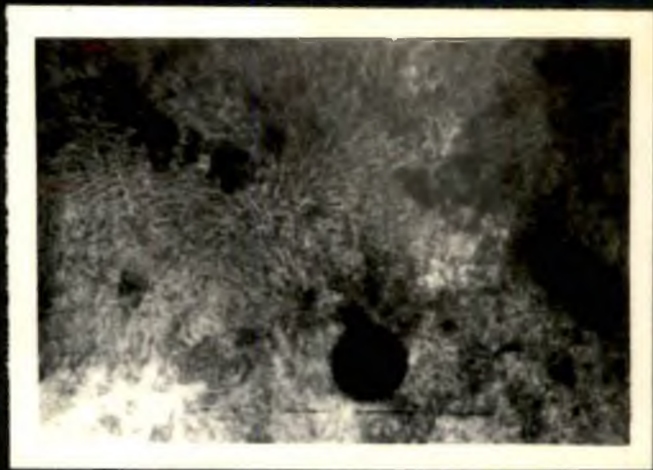


Plate X. Pollen germination after ten hours (H 2662, after applying 2, 4-D 100 ppm).

Plate XI. Failure of pollen germination (H 42) after ten hours.

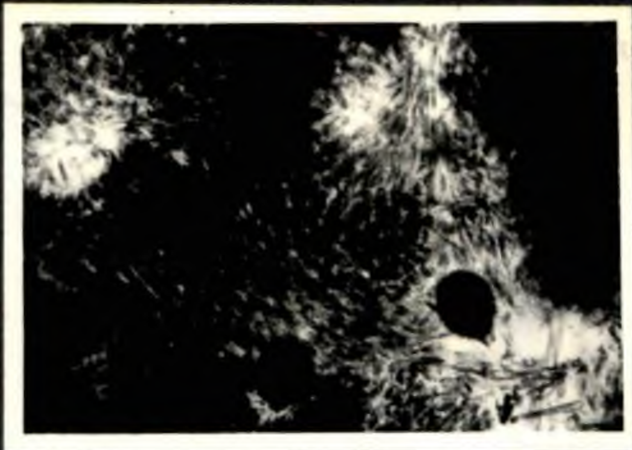


Plate ~~XII.~~ Failure of pollen germination (H 42) after ten hours (after applying 2, 4-D 100 ppm).

INCOMPATIBILITY STUDIES ON SWEET POTATO (*Ipomoea batatas (L) Lam*)

By

PADMA KUMAR A.

ABSTRACT OF A THESIS

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requirement of the degree

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1983.

ABSTRACT

In a study conducted on the flowering and natural fruit setting in sweet potato (*Ipomoea batatas* (L.) Lam), it was observed that varietal difference did exist for the above trait.

Of the thirty varieties screened for compatibility/incompatibility reactions, only one variety viz. S 378 was found to be self-compatible.

Except 2, 4-D treatment and end-of-season pollination, all the other methods including bud pollination, surgical techniques, application of sugar solution etc failed completely to break the self-incompatibility barrier in sweet potato.

The success of 2, 4-D treatment, which resulted in fruit set in 13 varieties might be due to its effect on increasing the flower life by preventing floral abscission.

The failure of seed set in the fruits obtained by 2, 4-D treatment could be due to the reduction in food supply to the developing embryos as a sequel to the injuries sustained on the pedicels following the application of 2, 4-D solution.

Pollen sterility studies confirmed its role in the low fruit and seed set in sweet potato.