HYBRIDISATION IN BANANA
CYTOMORPHOLOGICAL EVALUATION OF HYBRIDS AND EMBRYO CULTURE STUDIES

By

LEKSHMY M. L.

THESIS

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DECLARATION

I hereby declare that this thesis entitled "Hybridisation in banana - Cytomorphological evaluation of hybrids and embryo culture studies" 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.

Vellanikkara,
11 - 7 -1989

LEKSHMY, M.L.

CERTIFICATE

Certified that this thesis entitled "Hybridisation in banana - Cytomorphological evaluation of hybrids and embryo culture studues" is a record of research work done independently by Smt.Lekshmy, M.L., under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associate-ship to her.

Dr.(Mrs) P.K.Valsalakumari,
Chairman,
Advisory Committee
Associate Professor,
Department of Pomology and
Floriculture

Vellanikkara,
11 - 7 - 1989.

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LEKSHMY, M.L.

To my parents

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Introduction

INTRODUCTION

India occupies the second place in world banana production with an area of 281.6 thousand hecteres producing annually 4607.7 thousand tons of fruits. Kerala has 52.8 thousand hectares under banana cultivation with an annual production of 328.8 thousand tons of fruits. The productivity of banana in Kerala is very low compered to our neighbouring state, Tamil Nadu, which produces 911.6 thousand tons of fruits from an area of 32.6 thousand hecteres. During the last ten years the area under banana has not undergone considerable increase or decrease. But the productivity has shown a considerable decrease from 32.24 tons in 1977-78 to 6.22 tons in 1987-88.

Unlike other countries, banana cultivation in India, particularly in Kerala is polyclonal, with a number of varieties under cultivation. The important cultivars are, 'Nendran', 'Palayankodan' and 'Rasthali'. Nendran brings a premium price to the farmer and there is a personal liking for Nendran in our state. The systems of banana cultivation are also diverse ranging from raising of annually planted crops to semi perennial rainfed plantations. Nendran is always cultivated as an irrigated crop while other cultivars

are mostly raised as rainfed crops. Banana is often cultivated as an intercrop in coconut gardens and there is also a system of growing crops like ginger, yam, turmeric, colocasia and other vegetables in a banana based farming system. In each homestead in Kerala, a number of cultivars are under semi-perennial rainfed cultivation to meet the household needs.

The main constraints in banana production are poor yielding cultivars and diseases like leaf spot and bunchy top and pests like rhizome weevil. The yield of Nendran is only 10 to 12 t/ha. It is impossible to bring sufficient area under cavendish groups in Kerala which yields 50 to 55 t/ha because of the personal liking towards Nendran. There is a need to improve the productivity of 'Nendran', 'Palayankodan' and other cultivars, while restricting the cultivation to a few selected cultivars.

Exploring the possibility of improving the cultivars through hybridization, seems to be a desirable approach. In bananas, the possibility to bring the desirable qualities which are diversified in many varieties in a single variety is confronted with many obstacles as edible bananas are vegetatively parthenocarpic and

effectively seed-sterile. The breeders only hope is to exploit the partial female fertility met with in the horticultural varieties.

Breeding of banana was started in Trinidad in nineteen twenties. In the early stages, wild banana were used as pollen parents. The results of the hybridisation programme started recently in Tamil Nadu Agricultural University and in the Department of Pomology and Floriculture, College of Horticulture, Kerala Agricultural University with the main emphasis on the use of cultivars as female and male parents pointed to a shift in strategy that is needed in further breeding programmes. Studies in the Department of Pomology and Floriculture, College of Horticulture showed that clones belonging to different genomic groups could be used as female and male parents. Also, interclonal hybridisation in banana has proved its usefulness through the production of seedless hybrids in the F_1 generation. Seedlessness in banana, to some extent is related with ploidy level of the cultivars. Cytological analysis of the hybrids are useful in studying the ploidy level and morphological characters of the hybrids and to design further hybridisation programmes.

Though the important banana varieties of Kerala like Palayankodan and Nendran have been found to set seeds in the experiments conducted in the Department of Pomology and Floriculture, on artificial pollination (Karmacharya, 1984; Krishnakumar, 1987), the percentage of germination of seeds in the field is very low. Usually banana seeds possess a hard testa. Eventhough attempts have been made to soften the testa and to get easy germination, none of the methods could raise the germination percentage (Krishnakumar, 1987). Studies have also shown that as the excised embryo does not exhibit any dormancy, the factors affecting germination reside not in the embryo, but in any other part of the seed (Stotzky et al., 1962a). This knowledge led the workers to resort a new technique-excision of embryos and their culture in an aseptic medium. Though some results have been obtained in early studies it was in 1960 that Cox et al. developed an in vitro technique for germinating Musa balbisiana seeds. Rowe and Richardson (1975) used this technique to germinate hybrid seeds of banana and they could get 50 per cent germination of hybrid seeds. These results revealed that this in vitro technique can be used with increased success for the recovery of maximum number of hybrid seedlings in banana breeding programmes.

The present studies are in continuation of the previous works on hybridisation of banana in the Department of Pomology and Floriculture, College of Horticulture and aims at

1) Cytomorphological evaluation of hybrids evolved in the Department of Pomology and Floriculture.

2) Standardisation of embryo culture technique for the hybrid seeds of banana.

Review of Literature

REVIEW OF LITERATURE

In the improvement of banana through hybridisation, one limitation is the numerous pollinations that must be made onto some of the female parents in order to produce adequate quantities of seeds. Seedling production is further complicated by a characteristic 10 - 20 per cent germination rate. This erratic and generally low germination levels of seeds from banana hybrids have rendered the use of embryo culture very valuable. The coming part of this chapter deals with a brief review of the research done on banana breeding and the various aspects of embryo culture.

Banana breeding

Banana breeding was initiated at the Imperial College of Tropical Agriculture, Trinidad in 1922 and at the Department of Agriculture of Jamaica in 1924 (Dodds, 1950, 1958; Simmonds, 1966; Shepherd, 1968). The work was initiated to breed a variety resistant to panama disease which was a major problem of the Jamaican banana industry. The main aim at that time was to find a panama disease resistant substitute for Gros Michel and as much like it as possible in other characters (Simmonds, 1966). Breeding started both in Trinidad and Jamaica by crossing Gros Michel (AAA) as

female parent with wild seeded and disease resistant strains of Musa acuminata sub sp. malaccensis.

Among the resultant progenies selection was made from tetraploids as all other forms did not have any resemblance to Gros Michel (Cheesmen and Dodds, 1942). Among them, only two, IC-2 in Trinidad and S-19 in Jamaica had resemblence with Gros Michel and were resistant to panama disease and leaf spot, but possessed inferior bunch characters of the male parent like smaller fruits and occasional seed set (Cheesman, 1934; Dodds, 1950, Simmonds, 1966).

Later on male fertile diploid, Pisanglilin (AA) was introduced from Malaya (Simmonds, 1966) and was used as the male parent in crosses with the female parent Gros Michel. The new male parent used had somewhat longer fruits than the wild types, it was resistant to both panama disease and sigatoka. Also it transmitted a high degree of female sterility to the progenies (Simmonds, 1966). From this cross the clone Bodles Altafort (AAAA) was released to the growers in Jamaica (Osborne, 1962; Simmonds, 1966). But later it was found that some of the progenies from this cross were susceptible to panama disease. The parthenocarpic

tetraploids possessed poorly shaped bunches and often rather small fruits (Larter 1947; Dodds, 1958). This resulted in the rejection of Pisanglilin from the breeding programmes.

Dodds (1943) emphasised that the hope for banana breeding lay in first finding or breeding new male parents and then seeking commercial bananas among the primary tetraploids produced by them from Gros Michel. Male parent breeding made a sudden and dramatic progress in the late fifties as a result of material secured by the 1954-55 banana collecting expedition (Simmonds, 1956). Synthetic diploids with pendulous bunches and numerous long fruits became not uncommon.

In the recent years, the importance of Bodles Altafort declined because of the occurrence of short statured cavendish clone, Robusta, thus accommodating more plants/ hectare (Simmonds, 1966). Hence a semi-dwarf mutant of Gros Michel, namely, Highgate, which had potential advantages over the tall form in higher hand number as well as in sturdier habit, was used as a substitute for Gros Michel (Larter, 1947). The drawback in Highgate is the small size of fruits and this drew back could be overcome by the new male parents.

Thus many secondary triploids were produced from the cross between Highgate and advanced diploids at Jamaica (Osborne 1961-63, Simmonds, 1966). But many secondary triploids raised from backcross of primary tetraploids to seeded diploids were found unpromising also. (Dodds, 1943; Simmonds, 1966). This was due to the breakdown of highly selected and desirable Gros Michel combination in the secondary triploids.

In Honduras, breeding programme began with an extensive collecting expedition in the Western Pacific and South-East Asia from 1959 to 1961 (Rowe and Richardson, 1975). The programme began by a search for dwarfness in diploids. These were obtained by the improbable cross of triploid, Valery onto fully seeded Musa acuminata (AA). Although accessions with disease resistance were readily identified, there were no diploids with superior agronomic qualities and diseases resistance. Numerous crosses and selections among diploids resulted in the first diploid with superior agronomic features. This diploid, SH 2095 was derived from the crosses ('Sinwobogi' x 'Tjau legeda') x (Wild malaccensis x 'Guyod'). The vigorous parthenocarpic SH 2095 has up to 19 hands and weighs upto 30 kg. Fingers are long (20-23 cm) and do not drop from the

crown when ripe. The fruit does not ripen prematurily and remains firm after ripening. One defect of SH 2095 is poor pollen production which reduces its usefulness as a male parent. However, a few seeds are produced when used as a female parent. The good bunch characteristics of SH 2095 are readily transmitted to progenies.

To ensure higher levels of resistance to black sigatoka that spread throughout central America in the 1970s, progenies from the resistant Musa acuminata, sub sp. burmannica were introduced into the diploid breeding programme (Rowe, 1981). The burmannica progenies have very inferior bunch characteristics that are difficult to overcome. However one resistent hybrid from a population of 2500 progenies of burmannica had sufficiently adequate agronomic qualities. This hybrid SH 2969 is now being used as the burmannica source of resistence.

To induce burrowing nematode resistence in the diploids, bunches of 'Pisang jari buaya' were pollinated. One progeny SH 3142 - was found to be highly resistant to the burrowing nematode (Pinochet and Rowe, 1979) and had some resistance to black sigatoka. Several black sigatoka resistent diploid hybrids with advanced agronomic

features were selected from a segregating population of SH 2095 x SH 2989. One of the best was SH 3176 with 15 hands and longer and fuller fingers than the SH 2989 parent.

Diploid improvement continued in 1979-80 with the selection of SH 3248 from a cross between the nematode resistant SH 3142 and black sigatoka-resistant SH 2989; SH 3248 produced 16 hands with long fingers, good flavour and some resistance to black sigatoka. The diploid with the best agronomic characteristics developed in Honduras (SH 2095) had poor pollen thus limiting its usefulness as a male parent. However, a cross of SH 2095 x SH 2766 yielded the hybrid SH 3217 with bunch qualities similar to the SH 2095 parent with abundant pollen. These two advanced diploids (SH 3248, SH 3217) were the outstanding selections from a segregating population of 10,700 hybrids.

In 1982 P.R.Rowe selected a tetraploid with sufficient qualities to merit testing for possible commercial use (Stover and Simmonds, 1987). This selection, SH 3436 was from a cross of the burrowing nematode resistant diploid SH 3242 on to 'Highgate'. SH 3436 was highly resistant to black sigatoka and it had other desirable qualities of the bunch. Since it was derived from 'Highgate' the height is similar to Valery or Robusta.

Banana breeding in India

Banana breeding in India was started in 1949 at the Central Banana Research Station, Aduthurai in Tamil Nadu. The main aim of breeding was to evolve a wind resistant dwarf form of the variety 'Monthan' (Nair, 1953). The male parents used were all wild seeded diploids Musa acuminata, Musa balbisiana, Musa chiliocarpa and Musa coccinea. Commercial triploids of bispecific origin, viz., Poovan, Monthan, Rasthali, Peyan, Thote, Peykunnan, Rajavazhai and Nayvennan were used as female parents. In all the crosses, the fruit quality of hybrids was found to be far inferior to the female parents and fruits were seeded. A tetraploid hybrid 'Hybrid Sawai' evolved from cross between Nayvennan x Musa balbisiana was found promising in respect of yield and quality (Raman et al., 1978).

To produce nematode resistant hybrids in place of Matti (AA) it was crossed with nematode resistant clones. Of the different crosses only two, H-74 (Matti x Pisanglilin) and H-109 (Matti x Tongat) were found to be nematode resistant and retained the characters of Matti (TNAU, 1982). Recently a hybrid, Co-1 was derived from the cross involving Ladan (AAB) as female parent and the Musa balbisiana (BB) and Kadali (AA) as male parents (Athakiemenavelan et al., 1985).

This triploid hybrid produced phenotypically resembled the hill banana Virupakshi (AAB).

Banana Breeding in Kerala

In Kerala banana breeding was initiated only in 1982 (Karmacharya, 1984). Studies on cytotaxonomical aspects revealed the possibility of improving the cultivars by selection to meet the location specific requirements (Valsalakumari, 1984). The classification based on genetic divergence suggested the groups from which the parents could be conveniently selected for exploiting the wide variability existing in the crop. Studies have also shown that clones belonging to the different genomic groups could be used as male and female parents if they are compatible (Karmacharya, 1984; Valsalakumari, 1984). The results also indicated that several desirable cultivars such as Rasthali, Red banana and Palayankodan had viable pollen which could be utilised for the hybridisation programme.

Compatability studies by Karmacharya (1984) showed that out of the 27 combinations tried, 8 combinations, Agniswar x Pisanglilin, Palayankodan x Pisanglilin, Lacatan x Pisanglilin, Monnan x Pisanglilin, Nendravannan x Pisanglilin, Palayankodan x Sikuzani and Nendranx Sikuzani

were compatible. Among these four hybrids with Agniswar, Nendravannan, Nannen and Herichal as female parents and Pisangliilin as male parent produced bunches with promising features. Pollen viability studies revealed that pollen remained viable for 14 days when flowers along with the bract were stored in the open at room temperature. The storage life was increased to the maximum of 30 days when flowers along with bracts were stored in refrigerator (4°C at 10 per cent RH) (Karmacharya, 1984).

Clonal variation studies in Nendran (KAU Research Report 1984-85) revealed that out of the 144 clones collected, only two clones i.e. clone 35 and 123 can be populerised as the promising clones. Intraclonal variation studies in the cultivar Palayankodan (Rajeevan, 1985) identified two high yielding sub clones, 'Kalavoor' and 'Anchal' from the 24 accessions studied.

Studies on pollen fertility and pollen production of selected male parents (Krishnakumar, 1987) viz. Pisangliiin, Tongat and Sannachenkadali pointed out that out of the three, Pisangliiin had highest pollen production and pollen fertility. Pollen fertility was found to be maximum between 25th and 30th nodes standardisation of

media for pollen germination and tube growth indicated a medium containing 35 per cent sucrose was the best. Three compatable combinations of cultivars involving Palayankodan, Rasthali and Nendran as female parents and Pisanglilin as male parent were also suggested by Krishnakumar (1987). Seed production was found to be maximum in Palayankodan (102.96 seeds per bunch) followed by Nendran (13.65 seeds per bunch) and Rasthali (10.98 seeds per bunch). The various seed treatments tried were not effective in enhancing seed germination or getting early germination. However, two seeds in acid treatment from the cross Palayankodan x Pisanglilin germinated.

Evaluation of hybrids

In any crop, hybridisation is done in order to combine the qualities present in the parents in a single plant. Once a hybrid is produced it is important that it is evaluated for its acceptability over the parents. Thus in hybridisation work evaluation plays an important role, the superior ones are accepted and inferior ones are rejected. In banana the early hybrids from the cross Gros Michel x Musa acuminata, namely I-C-2 and 8-19, though possessed the predominance of characters of Gros Michel, were inferior in bunch characters (Cheesman, 1931; 1932, 1934; Cheesman and Larter 1935, Larter, 1935).

The tetraploid hybrid, Bodles Altafort produced from the Gros Michel x Pisanglilin (Osborne, 1962) was found resistant to Panama disease, leafspot and nematodes. In India the tetraploid hybrid evolved from the cross Poovan x Musa acuminata produced fruits of superior quality over the female parent. But the hybrid was lacking in good bunch, grade and fruit size. The tetraploid hybrid 'Hybrid Sawai' was evolved from the cross Nayvannan x Musa balbisiana clone. 'Sawai' was medium tall in stature, sturdy in appearance and yielded a heavy bunch with good round shaped fruits while the female parent had angular fruits.

Bhaktavathsalu et al. (1968) conducted a comparative study on Klue Teparod, a natural tetraploid (ABBB) and synthetic tetraploid hybrid, Hybrid Sawai (ABBB). Both were sturdy with 355 and 328 days respectively between shooting to harvest. The fruits of the former were medium sized while those of the latter were large and plump. Azhakiamanavalan and Rao (1982) made another comparative study of hybrid 135 and Virupakshi bananas. Hybrid 135 was bred in Tamil Nadu by using pollen from Musa balbisiana (2n = 22, BB) on Ladan (2nd = 33, AAB) to give a F_1 (2n = 22, AB) which was pollinated by Kadali (2n = 22, AA) to give the hybrid (2n = 33, AAB). There was a strong

morphological resemblance between hybrid 135 and Virupakshi (AAB). A comparison which involved 19 characteristics showed the former to be shorter and earlier and to produce more fruit of better quality.

In another study the hybrid Co.1 was compared with Virupakshi (AAB) (Ashokiamanovalen et al., 1983). Co-I was found to be similar to Virupakshi, but of shorter stature. It had more leaves and produced heavier bunches of heavier fruit (160.5 g) which had greater content of sugars and total soluble solids. The crop duration of CO-1 was 14-15.5 months and was found to be quite promising in hills and plains retaining its flavour.

In Kerala Agricultural University, three hybrids evolved from the cross, Agniswar x Pisanglilin were compared among themselves and also with the female parent (Krishnakumar, 1987). The hybrids were all found to be triploids (2n = 33, AAB). Morphological description of hybrids showed that Hybrid No.1 and Hybrid No.III resembled the female parent. In almost all the characters studied Hybrid No.III was found to be superior. Hybrid No.I was intermediate in characters and Hybrid No.II was inferior in all aspects.

Problems in banana breeding

The edible cultivars do not produce seeds when grown in pure stands, some of them are entirely female sterile, others will produce an occasional seed when a source of viable pollen is available (Purseglove, 1975).

Sterility in cultivated banana is due to a combination of gametic sterility resulting from structural hybridity of chromosomes and polyploidy and zygotic sterility resulting from genetic control of male sterility and parthenocarpy (Dodds, 1958). Sterility is mainly due to meiotic abnormalities and parthenocarpy is due to three complementary genes derived from the wild Musa acuminata (Dodds and Simmonds, 1948; Simmonds, 1953, 1962; Delange, 1969).

Recent studies have shown that many of the edible cultivated bananes are female fertile to some extent when artificial pollination is done (Sathiamoorthy, 1973; Purseglove, 1975; Karmacharya, 1984; Krishnakumar, 1987). Thus on artificial pollination seeds could be obtained. But these seeds show poor germination percentage and recovery of hybrid seedlings is low.

Little is known about factors which affect seed germination in the Genus Musa except that germination is

extremely variable and relatively difficult to obtain under artificial conditions. The use of seedling banana plants as research tools and the increased emphasis on banana breeding programmehas necessitated elucidation of factors effecting germination of these seeds. The seed yield of hybrids is usually low and for a successful breeding programme, it is imperative that a maximum number of these seeds be germinated.

The anatomy of the seed of Musa balbisiana has been described by McGahan (1961). Usually seeds are irregularly globose in shape and frequently have somewhat flattened sides. Seeds average 3-5 mm in diameter. The seeds are greyish brown and a scarious membrane covers the rugose seed coat. The endosperm of the mature seed is powdery in texture and incompletely fills the seed cavity (Mc Gahan, 1961). The embyyo is situated just below the micropylar cap and it is present below the embryo. The chalasal mass is an annular area of thin walled cells densely packed with a red brown substance and is gelatinous (Figure 2).

In order to improve the germination percentage several seed treatments were tried by several workers.

Simmonds (1952) noticed that presowing treatments such as chipping of the teste, soaking seeds in sulphuric

acid, soaking in water and the application of temperature shocks are usually deleterious and often lethal - Stotzky and Cox (1962) used alternating temperatures for the germination of banana seeds. They found that alternating temperature differentials optimal for germination in soil were depended upon both the high and low temperature and range from 8 - 23°C. Germination is maximum when the seeds are held 6 - 12 hours at high (27-35°C) and 12-16 hours at the low (12-18°C) temperatures. Some germination can be induced by short exposures to alternating temperatures followed by constant high temperature, but continuous exposure to alternating temperatures is necessary for maximum germination.

Stotzky et al. (1962) obtained 80 per cent germination in banana seeds under aseptic condition. He removed a chip from the lateral portion of the seed coat and the time required for germination in sterile culture was shortened from 3-6 weeks required for intact seeds in soil to 6-10 days. It was also proven that as the excised embryo exhibits no dormancy, the factors delaying germination reside in other portions of the seed.

Krishnakumar (1987) used different methods such as treatment with concentrated sulphuric acid, quick dip of seeds in boiling water, treatment with gibberellic acid at 250 and 500 ppm and chipping the testas of seeds, to enhance

the germination percentage of banana seeds. He could not get positive results in any of the treatments.

Embryo culture

Embryo culture provides a way to get seed germination that is not possible with ordinary methods (Khanna, 1986). The underlying principle of the method of embryo culture is the aseptic excision of the embryo and its transfer to a suitable medium for development under optimum culture conditions. Selection of proper nutrient medium is an important aspect in embryo culture which varies widely in composition (Raghavan, 1977). In general, the younger the embryo, the more complex is its nutrient requirement after the growing embryo is transferred from one medium to another for continued optimum growth. After the embryo has grown into a plantlet in vitro, it is transferred to sterile soil or vermiculite and grown to maturity in a green house.

Excision of embryos and culture in aseptic medium was utilised by Tukey to germinate the seeds of early ripening sweet cherry and peach varieties (Kester and Hesse, 1955). Honma (1955) explains it as a useful tool in securing difficult combinations of genetic characters.

It is also been used to shorten the life cycle by overcoming the seed dormancy that occur in various plant materials. In some types of seeds the dormancy can be overcome by excision and culture of embryos. In still other types of seeds, whose dormancy is broken by specific light or temperature treatments, embryo culture has helped to localise the endogenous promoters or inhibitors of germination which maintain the seeds in a dormant or nondormant state (Raghavan, 1977).

Applications of embryo culture

Work with embryos of different species of Iris has established that seed dormancy is due to the presence of stable inhibitors of embryo growth present in the endosperm (Randolph and Cox, 1943), embryo (Lenz, 1955) or the seed coat (Lenz, 1955). Dormancy has been overcome in the seeds of other plants also by the embryo culture method, which has lead to the recognition of the primary role of endogenous inhibitors in the process (Cox et al., 1955). Culture of embryos of dormant seeds of wild oat (Avena fatua) at different intervals after harvest has shown that the defect of completely isolated dormant embryos is overcome by addition of gibberellic acid to the medium (Simpson, 1965).

Dormancy exhibited by orchid seeds is mainly due to immaturity of the embryo (Knudson, 1922). Morphological development of embryo and its subsequent germination take place in the soil in association with a micorrhizal fungus. Knudson (1922) succeeded in germinating orchid embryos into plantlets in the absence of the symbiotic fungus by growing them in a nutrient medium containing suger.

Natural sterility barrier in the seed could be overcome by resorting to culture of embryos (Abraham and Ramachandran, 1960). By resorting this method, seedlings of crop plants which are traditionally propagated by vegetative means were obtained in the case of banana (Cox et al., 1960) and tuber crops like colocasia (Abraham and Ramachandran, 1960).

In breeding programmes when dormancy of seeds and slow growth of seedlings necessitate long breeding seasons, embryo culture helps in reducing the breeding cycle of new varieties. The value of embryo culture in circumventing the slow germination of the seed and the slow growth of the seedlings is illustrated in weeping crab apple (Nickels, 1951) and in rose (Lammerts, 1946; Asen, 1948). The production of barley monoploids by the

selective elimination of chromosome involves crossing Hordeum vulgare with Hordeum bulbosum. The young embryo which invariably aborts in nature is dissected out and cultured. The chromosome of Hordeum bulbosum are eliminated during culture to produce a monoploid (Jensen, 1977). This is called bulbosum technique.

In seed testing trials, embryo culture method has figured as a rapid means of determining the viability of particular lots of seeds (Tukey, 1944). From a practical point of view, nursery men could use this as a quick test for predicting the viability of current season's supply of seeds for specific planting dates and thereby eliminate planting failures resulting from the use of seeds of low viability and expediate commercial movement of seeds of known viability (Raghavan, 1977).

Embryo culture method has also been used to study some fundamental problems in experimental embryogenesis (Narayanaswamy and Norstog, 1964; Maheswari and Rangaswamy, 1965; Wardlaw, 1965; Raghavan, 1966) to study host pathogen interaction (Padmanabhan, 1967) and to evaluate the mutagenic ability of irradiated substrates on living tissues (Natarajan and Swaminathe, 1958).

Embryoculture and plant breeding

Embryo culture has found its application both in applied and basic researches. In relation to crop improvement, the embryo culture has been quite commonly used for the recovery of hybrids between distinct taxa. Hybridisation between cultivated species and related wild species has been of great value for cultivar improvement (Khanna, 1986). Advances in embryo culture method have served to open the way effectively to obtain plants from inviable hybrids, the seeds of which are traditionally discarded due to their inability to germinate under normal conditions (Raghavan, 1977).

The composition of the culture medium is an important factor in the successful establishment of a tissue culture. Culture conditions favouring callus growth may not be suitable for organ differentiation. Each tissue type requires a different formulation depending on whether the objective is to obtain optimum growth rate or induce organogenesis. Several media have been developed by various workers to suit particular requirements of a cultured tissue. A standard or basal medium consists of a balanced mixture of macronutrient and micro nutrient elements, vitamins, a carbon source, organic growth factors, a source of reduced nitrogen supply and plant hormones.

Embryo abortion occurs quite frequently as a result of unsuccessful crosses in breeding. A major cause of early embryo abortion is the failure of endosperm to develop properly. Aseptically culturing the embryo in a nutrient medium can often overcome this problem (Bose, 1986). This technique is used to produce interspecific hybrids in cotton (Gill and Bajaj, 1984) and in peanuts (Halward and Stalker, 1987) where incompatability barrier like embryo or endosperm abortion exists and Sandhu (1984) used two different media to raise hybrid seedlings in cotton from immature hybrid embryos and ovules. The cultures were maintained at 25 ± 2°C and 55-65 per cent relative humidity under diffused light. Murashige and skoog medium supplemented with IAA (2 mg/l + Kinetin (0.5 mg/l) produced best results for embryo culture whereas B_5 medium supplemented with 2,4-D (2 mg/l) resulted *in* excessive callus growth from ovules.

Germination failures are common when plant breeders hybridise two widely divergent races. In other instances the embryo may partially or fully form after fertilisation but no seed is formed. In that situation which is common with interspecific and intergeneric crosses, direct culture of embryo may result in plantlet formation

(Khanna, 1986). In rice embryo culture have been applied to propagate hybrids capable of withstanding unfavourable environmental conditions often resistant to pest and diseases. Niles (1951) found that when kernels from crosses between cultivated varieties of rice were planted aseptically on a solidified mineral salt medium, embryos grew into transplantable seedlings. Difficulties encountered in rearing plants from interspecific crosses within the genus were also overcome by culturing the embryo (Butany, 1958; Nakajima and Morishima, 1959; Bouharmount, 1961).

For the regeneration of interspecific hybrids and allopolyploids also embryo culture method has been widely used. By the use of embryo culture fertile allopolyploids were regenerated, from crosses between triticale and Triticum monococum. The hybrids produce more grains per ear, more tillers and grains were of smooth appearance with high protein (Skiebe and Neumann, 1980). Interspecific hybrids were also recovered through embryo culture in crosses between barley x wheat (Zhou et al., 1979) wheat x rye (Zhu, 1979) Barley x rye (Cooper et al., 1978). Cooper et al. used Norstog's B II medium in which a white callus was formed from the embryos. This callus was sub cultured monthly and twelve plantlets were regenerated from callus which were grown to maturity.

Bajaj et al. (1970) cultured hexaploid triticale embryo on Murashige and Skoog medium supplemented with various substances. Best growth was shown by 16-18 days old embryos cultured on medium supplemented with IAA, kinetin, and macerated endosperm from young grains of Triticum durum. Dahmen and Mock (1972) used basal and complex agar sucrose nutrient media for comparing growth of mature intact seeds and excised embryos of maize. Complex medium increased root growth of seedlings from excised embryos. There were no nutrient media effects on shoot growth. No absolute requirement by cultured maize embryos for externally supplemented auxin was demonstrated. In the basal medium auxin inhibited root growth.

Balkanjieva (1985) could get 22 per cent regeneration of autotriploids of barley when embryos were cultured on a modified White's medium. Plants were regenerated from callus cultures initiated from immature embryos of barley when cultured on a modified Murashige and Skoog medium supplemented with 1.5 mg 2.4-D and 6.5 mg IAA/1.30 per cent callus initiation occurred within five days demonstrated a method by which incompatability between barley x Rye could be overcome by resorting to culture of embryos. Here the incompatability is due to early abortion of endosperm and the embryo. The hybrid embryos could be successfully grown

on Murashige and Skoog medium + casein hydrolysate (500 mg/l) + IAA (1 mg/l) + kinetin (0.5 mg/l).

Early in the development of embryo culture as a research tool, Laibach (1925, 1929) demonstrated that embryos of nonviable seeds of Linum perenne x Linum austraticum could be cultured in a nutrient medium and reared to maturity. This study set the stage for a series of later investigations to surmount barrier to crossability in plants whose embryos aborted in the seeds before they germinated or whose seeds were unable to support development of embryos to maturity.

To recover F_1 hybrids from Iris pseudacorus x Iris ensata cross, Yabuja (1985) used a special method of overcoming sterility by the production of amphidiploids by colchicine treatment of culture embryos. Effect of different growth regulators on growth of Iris embryos were studied by Stoltz (1977). Slatter (1950) used a method of embryo culture of certain bearded iris hybrids on nutrient agar. This method reduces the time interval between seed sowing and flowering and also ensures higher percentage of germination. Studies on the growth of mature iris embryos on White's medium with various agar concentrations revealed an inhibitory

role of higher agar concentration on the growth of excised embryos (Stoltz, 1971).

Custers (1986) used embryo culture method with some success to overcome the problem of abnormal development or abortion in interspecific crosses in Lilium and Tulipa. In another study embryos (0.3 - 0.4 mm long) of hybrids between distantly related Lilium spp were cultured on normal endosperm from interclonal crosses maintained on Murashige and Skoog medium with a success rate of 60 per cent.

In almost all fruit crops embryo culture technique has been used for rearing either normal embryos or hybrid embryos. Attempts were made to grow embryos of interspecific crosses of horticultural varieties of deciduous trees which ripen early, but have low yield of viable seeds, which nearly opened up the field of fruit tree breeding for exploitation and study (Tukey, 1934; Blake, 1939, Skirm, 1942; Lammerts, 1942). Lammerts (1942) used embryo culture as an effective technique for shortening the breeding cycle of deciduous trees and increasing the germination of hybrids. Early ripening forms of sweet cherry, peach and pear were obtained through this method (Zdruikovskaya - Rikhter, 1979). Generally seeds of apple, peach, pear and plum which contain abortive embryos do not respond to the usual after ripening

treatment at low temperature and in such cases embryo culture is the most promising method to preserve progenies which may have characteristics of horticultural value.

In Cerasus vulgaris x Cerasus tomentosa and Ribernigrum x Grossularia reclinata hybrids, it was possible to raise a second generation of plants from seeds which do not normally germinate by growing the aborted embryos under aspectic conditions (Kravtsov and Kazyanova, 1968). By means of invitro embryo culture a large group of hybrids were obtained from interspecific and intergeneric crosses in stone and pome fruits (Kurakov, 1979). Thus embryo culture might help in commercial fruit breeding programmes and in wide hybridisation between unrelated fruit trees by providing a route for seedling selection and by giving a higher percentage of seed germination.

In mango Iyer and Subramanyam (1972) advocated culturing of hybrid embryos obtained from hanging of freshly fallen fruits. This method could overcome the excessive fruit drop which is a major problem in Mango hybridisation. In papaya hybrid embryos which generally abort during the developmental stage between 70 to 90 days after pollination were successfully cultured in White's medium containing 0.5 ppm kinetin with or without 0.1 ppm each of GA_3 and IAA (Phadnis et al., 1970).

From interspecific crosses, embryos were excised at an early stage of development and cultured to produce viable hybrids in citrus (Chen and Wang, 1986; Starmantino, 1986). In pome and stone fruits, in vitro embryo culture was used to improve the efficacy of distant crosses (Kursakov, 1978). From nonviable early peach seeds, increased percentage of germination was achieved through this technique (Davidson, 1933; 1934; Blake, 1939). Hammarslag and Bauhan (1983) got plant regeneration from callus induced from immature embryos of peach.

In sour and sweet cherries, the poor germination of the seeds of very early varieties and of seeds produced through distant hybridisation was circumvented by the culture of embryos on a standard medium with GA_3 and myoinositol added of embryos removed from fruits when the first hint of red skin colour appeared (Spitsyn, 1972; Ivanika and Bakse, 1981). Embryo culture as a technique of producing hybrids which are otherwise inviable is also demonstrated in Rubus (Gellette and Puryear, 1983; Fiola and Swartz, 1985). Eugenia (Litz, 1984) persimon and almond (Zdruikovskaya-Rikhter, 1980, 1981, 1985).

In breeding work with crop plants embryo culture method is useful in the production of hybrids endowed with

desireble disease resistant quelities. To impart resistance to virus, molds and nematodes in cultivated tomato (Lycopereicon esculentum), it was crossed with the wild species Lycopersicon peruvianum which exhibits varying degrees of resistance to these agents. Although fruit development is normal in the hybrids, seeds often harbour underdeveloped embryos which often do not germinate. Hybrid seedlings have been raised and nurtured to the stage of flowering from such seeds by embryo culture (Smith, 1944; Choudhury, 1955; Alexander, 1956).

Vorobeva and Prikhod (1980) could raise both intergeneric and interspecific hybrids from tomato by culturing the embryos on White's medium with 2 per cent sucrose, 500 mg/l casein hydrolysate and 500 mg/l yeast extract. Hormonal regulation of growth and development of tomato embryos *in vitro* was studied by Neal and Topalaski (1985). They found that kinetin in combination with either GA_3 (10^{-7} or 10^{-8} M) or IAA (10^{-9} M) showed greatest potential for inducing development and growth of those embryos excised before differentiation has occurred.

In the breeding of capsicum, use of embryo culture in raising interspecific hybrids and in accelerating the breeding process to give 3-4 generations a year is advocated

(Pari, 1985). Embryo abortion which occurs in the interspecific crosses in Cucumis species could be overcome by resorting to embryo culture (Norton, 1980; Custer, 1982). Murashige and Skoog medium supplemented with Casein hydrolysate, Difco Bacto Agar, IAA (0.01 mg/l) and various concentrations of sucrose and kinetin was used for the purpose.

Marikis et al. (1981) used embryo culture for securing the embryos resulting from inter specific hybridisation in Phaseolus sp. In Allium sp. culture of immature embryos on BDS medium supplemented with auxins resulted in the production of plants from crosses between diploid Allium cepa and polyploids Allium schenoprasum and Allium nutans. The same technique was used to overcome the sterility in the interspecific crosses (Yurieva and Titova, 1984).

Embryo rescue technique was widely used for the recovery of interspecific hybrid in Brassica sp. (Bajaj et al., 1986; Ayotte et al.,1987; Mohapatra and Bajaj, 1987; Ayotte et al., 1985). They observed that interspecific embryo abortion is due to abnormal endosperm development.

In coconut embryo culture technique was used by many workers for the recovery of plants from normal embryos (Karunanatho et al., 1985; Tsherdi and Warga Dalem, 1982) and from hybrid embryos (Bah, 1986). Ackerman (1971) studied the genetic and cytological characters of interspecific and intergeneric crosses in Camellia by culturing partly developed embryos.

Embryo culture in banana breeding

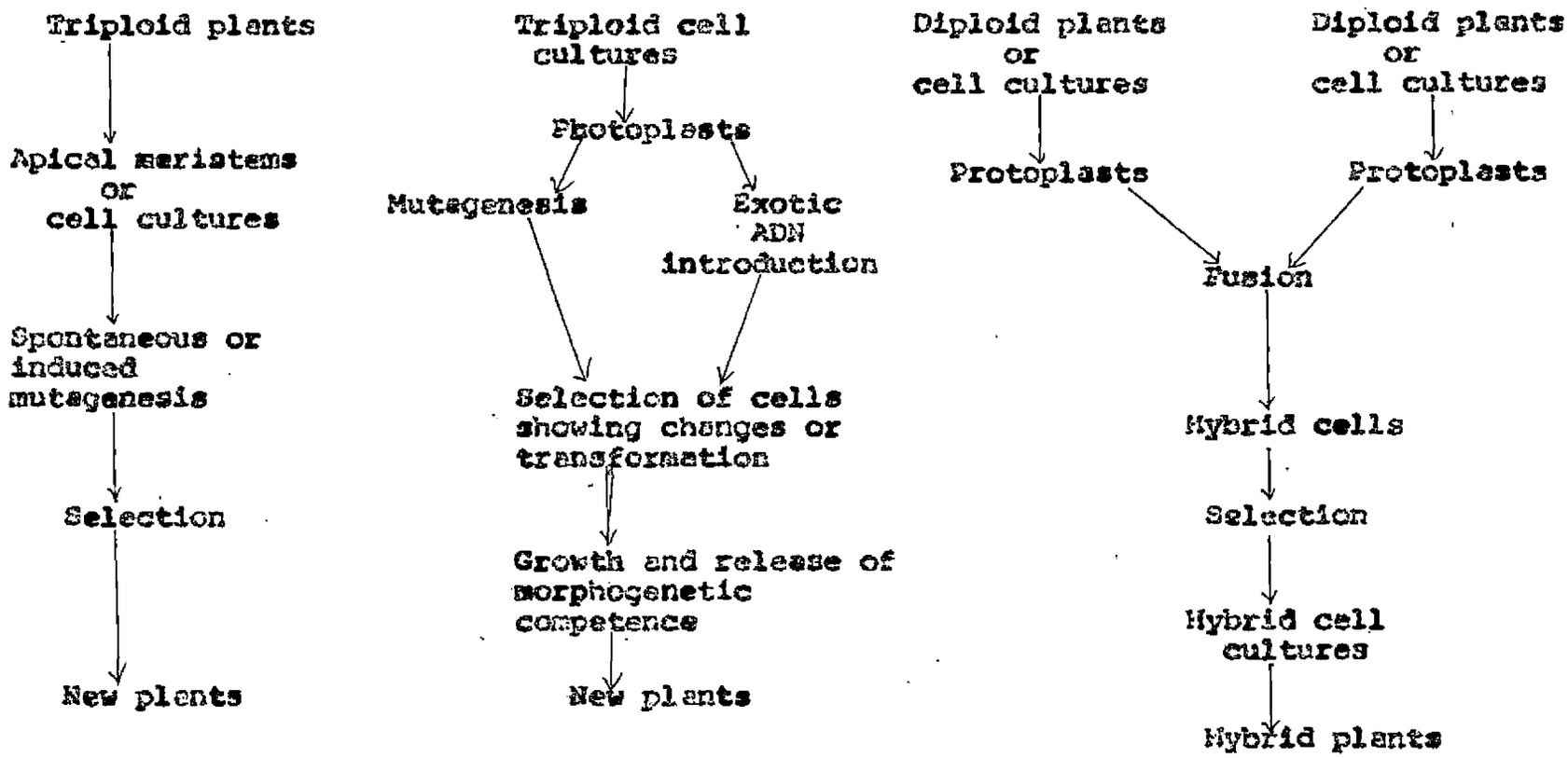
As pointed out by Rowe and Richardson (1975) one of the major limitations of banana breeding is the low rate of germination of hybrid seeds. The erratic and generally low germination levels of seeds from many Musa clones has rendered the use of embryo culture very valuable in hybridisation programme.

Early studies in the field has given only two per cent germination of hybrid seeds (Simmonds, 1958). In 1960 Cox et al. developed an in vitro technique for germinating Musa balbisiana seeds. They used modified Knudson's medium or Randolph and Cox (1943) medium containing 0.12 M sucrose (but without growth regulators) solidified with agar (0.5-0.7 per cent) to rear young plantlets from tiny embryos excised from banana seeds until they were large enough to be placed in the soil.

Shepherd (1968) raised hybrid seedlings of banana in Jamaica, by extracting embryos and growing them on a nutrient agar medium based on Knudson's solution. First in the Jamaica breeding programme and subsequently in Honduras, embryo culturing of seeds greatly increased germination rate, often upto 50 per cent (Rowe and Richardson, 1975). They used mature hybrid seeds from fully ripe bunches for embryo culture. The seeds were surface sterilised with 1 per cent $AgNO_3$ for 10 minutes before extracting the embryos. The extracted embryoswere placed on a modified Knudson's solution in test tubes and grown to a size suitable for transplanting directly to bags of soil. This method resulted in 50 per cent germination of hybrid seeds.

Krikorian and Croensuer (1983) have discussed the need and use of free cell and protoplast cultures for producing new bananas or plantains. They have outlined three ways that cell cultures might be used to produce new banana and plantain plants (Table 3). This scheme can be successful only if a capacity to produce or isolate competent cells and protoplasts and to regenerate protoplasts in large numbers has been developed (Krikorian, 1987).

Table 3. Possible approaches to producing new plants from the culture of meristems photoplasts and cells of banana and plantains



Source: Krikorian and Cronauer (1983)

These studies suggests embryo culture technique as the most effective technique for the recovery of hybrid seedlings in interspecific and intraspecific crosses. In order to achieve a good percentage of germination of hybrid seeds, the medium suggested by Rowe and Richardson (1975) will be used in the present study.

Materials and Methods

MATERIALS AND METHODS

The investigations were carried out in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, Trichur during 1987-88.

The studies consisted of two main aspects.

A. Cytomorphological evaluation of banana hybrids evolved in the Kerala Agricultural University.

B. Standardisation of embryo culture technique for hybrid seeds of banana.

A. Cytomorphological evaluation of hybrids

Three banana hybrids evolved in the Kerala Agricultural University were evaluated along with their parents. The hybrids were

1. Agniswar x Pisanglilin
2. Mannan x Pisanglilin
3. Vennan x Pisanglilin

The suckers of the hybrids and parents were planted at a spacing of 2 x 2 metres. The recommended package of practices were followed (Kerala Agricultural University, 1986).

The biometrical observations of the hybrids and parents were taken at monthly interval from two months after planting up to harvest. The following observations were recorded.

I. Plant characters

a. Plant height (cm)

The height of the plant was measured from the ground level to the axil of the youngest leaf.

b. Plant girth (cm)

The girth of the pseudostem was measured at 20 cm above the ground level.

c. Functional leaves per plant

The number of fully opened functional leaves (more than 50 per cent green) present at the time of observation were recorded.

d. Total leaf area per plant (m^2)

Length of lamina was measured from the base to the tip and breadth was measured at the broadest point in the middle. The leaf area was calculated by applying the formula, leaf area = length x breadth x 0.8 (Murray, 1960).

e. Petiole length (cm)

The length of the petiole was measured from the base to the point of emergence of lamina.

f. Duration (days)

(i) Planting to flowering interval (days)

The number of days from planting to flowering was recorded.

(ii) Flowering to harvest interval (days)

The number of days taken from bunch emergence to harvest was computed noting the date of emergence and date of harvest. The time of harvest was determined when the angularity of skin disappeared, that is, at the stage of round full (Simmonds, 1960).

Total duration of plant was computed by adding (i) and (ii).

(iii) Female phase (days)

The number of days taken from the opening of first bract containing female flowers to the opening of last bract containing female flowers was taken as female phase.

(iv) Male phase (days)

The number of days taken from the opening of first bract containing male flowers to the opening of the last bract containing male flowers was taken as male phase.

2. Bunch characters

a. Bunch weight (kg)

The bunch was weighed with 10 cm length of peduncle above the first hand and five cm length of the male axis below the last hand.

b. Hand weight (g)

The weight of the second hand was taken as the average weight of a hand (Gottreich et al., 1964).

c. Number of hands

The number of hands in each bunch was recorded.

d. Number of fingers

The total number of fingers in each bunch was recorded.

e. Number of fingers per hand

The mean number of fingers in a hand was obtained by dividing the total number of fingers in each bunch with the number of hands.

3. Finger characters

The middle finger in the top row of the second hand was chosen as representative finger (Gottreich at al., 1964) for recording physical characters of the finger.

a. Pedicel length (cm)

Pedicel was split longitudinally and the distance from base of pedicel up to pulp region was measured.

b. Finger length (cm)

The length of finger was measured from base of pedicel to apex along dorsal curve using a fine non-elastic thread and scale.

c. Finger girth (cm)

Circumference of finger was measured at the middle using a non-elastic thread and scale.

d. Finger weight (g)

The weight of finger was recorded in grem.

e. Pulp-peel ratio

Weight of pulp and peel was recorded and pulp/peel ratio was calculated on weight basis.

4. Quality characters

The fruits collected from well ripe bunches were taken for quality analysis. The middle fruit in the top row of second hand was selected as the representative sample (Gottreich et al., 1964). Samples were taken from three positions viz., top, middle and bottom and these samples were then pooled and macerated in a warring blender. Triplicate samples from these were used for analysis of different constituents as described below.

a. Total soluble solids (TSS per cent)

Total soluble solids were found out using an Erma pocket refractometer and was expressed as per cent.

b. Sugars (per cent)

Total reducing and non-reducing sugars in the samples were determined as per the method described by Association of official Agricultural Chemists (1960).

c. Acidity (per cent)

The macerated sample (10 g) was mixed with distilled water and made up to a known volume. 20 ml of the filtered solution was titrated against 0.1 N NaOH using phenolphthalein as indicator. The acidity was expressed

as per cent of citric acid (Association of official Agricultural Chemists, 1960).

d. Sugar-acid ratio

The sugar acid ratio was determined by dividing the total sugars with titratable acidity.

5. Pollen studies

The pollen studies consisted of the estimation of pollen fertility and pollen grain size in different nodes of male axis of the hybrids and the male parent, Pisanglilin.

Pollen grains were collected by scraping the anthers which were about to dehise using a blunt needle passing transversely along the lobe of the anther caring not to scrape the tissue (Karmacharya, 1984).

a. Pollen fertility

Pollen fertility was estimated by mounting pollen grains on glass slides in acetocarmine stain.

The collected pollen grains were dusted in a drop of acetocarmine stain (Alexander, 1980) on a clean microscopic slide and kept for staining and examined under the low power

of a microscope (10 x 10). Pollen fertility was estimated by counting both fertile and sterile pollen. Pollen grains which were well stained, normal and plumpy were considered as fertile, while those which were unstained and shrivelled were taken as sterile. For each node, three such microscopic slides were prepared and five fields from each slide were observed and values averaged. Fertility was expressed as per cent of the total number observed.

b. Pollen size

The extracted pollen grains were mounted on glass slides and stained with 0.5 per cent acetocarmine. A few drops of glycerin were added and pollen grains were covered with clean zero cover glass. The slides were kept as such for 30 minutes. Diameter of 30 well developed normal pollen grains were measured using a standardised occular micrometer under low power of the microscope (10 x 10). The mean diameter of pollen grains was expressed in microns.

6. Female fertility

In order to find out whether the hybrids are female fertile or not, they were crossed with Pisanglilin as male parent and total number of seeds produced per bunch was noted.

7. Taxonomic scoring at flowering

The hybrids were scored based on the fifteen morphological characters diagnostic of Musa acuminata and Musa balbisiana as suggested by Simmonds and Shepherd (1955). The fifteen morphological characters of the hybrids were examined and respective scores were given, finally the scores were added and the ploidy level was determined. Fifteen characters used for scoring and the key to the genomic groups are given in Table 1 and illustrated in Figure 1.

8. Cytological studies

Cytological studies consisted of counting the somatic chromosome of hybrids in root tips. Tips of freshly emerging roots were collected at 9-30 to 10 am so as to get the maximum number of cells in metaphase. Collected root tips were washed in water and fixed in 1:3 fresh acetic alcohol for reducing the staining of the cytoplasm. For best results the duration of fixing was found to be 8 to 10 hours.

After 8 to 10 hours of fixation in acetic alcohol the root tips were washed in water and hydrolysed in

Table 1. Characters used in taxonomic scoring of banana cultivars (Simmonds and Shepherd, 1955)

Characters	Musa acuminata	Musa balbisiana
Pseudostem colour	More or less heavily marked with brown or black blotches	Blotches slight or absent
Petiolar canal	Margin erect or spreading with scarious wings below, not clasping pseudostem	Margin inclosed, not winged below, clasping pseudostem
Peduncle	Usually downy or hairy	Glabrous
Pedicels	Short	Long
Ovules	Two regular rows in each loculus	Four irregular rows in each loculus
Bract shoulder	Usually high (ratio 0.28)	Usually low (ratio 0.30)
Bract curling	Bracts reflex and roll back after opening	Bracts lift but do not roll.
Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder	Broadly ovate, not tapering sharply
Bract apex	Acute	Obtuse
Bract colour	Red, dull purple or yellow outside, pink, dull purple or yellow inside	Distinctive brownish purple outside, bright crimson inside

(Contd.)

Table 1 (Contd.)

Characters	Musa ecuminata	Musa balbisiana
Colour fading	Inside bract colour fades to yellow towards the base	Inside bract colour continuous to base
Bract scars	Prominent	Scarcely prominent
Free tepal of male flower	Variably corrugated below tip	Rarely corrugated
Male flower colour	Creamy white	Variably flushed with pink
Stigma colour	Orange or rich yellow	Cream, pale yellow or pale pink

Key to the groups of edible bananas

1. Score 15-23 (Accuminata cultivars)
    1. Diploid - AA
    2. Triploid - AAA
    3. Tetraploid - AAAA

2. Score 26 or more (hybrid cultivars)
    1. Score 26-46 triploid - AAB
    2. Score about 49 diploid - AB
    3. Score 59-63 triploid - ABB
    4. Score about 67 tetraploid -ABBB

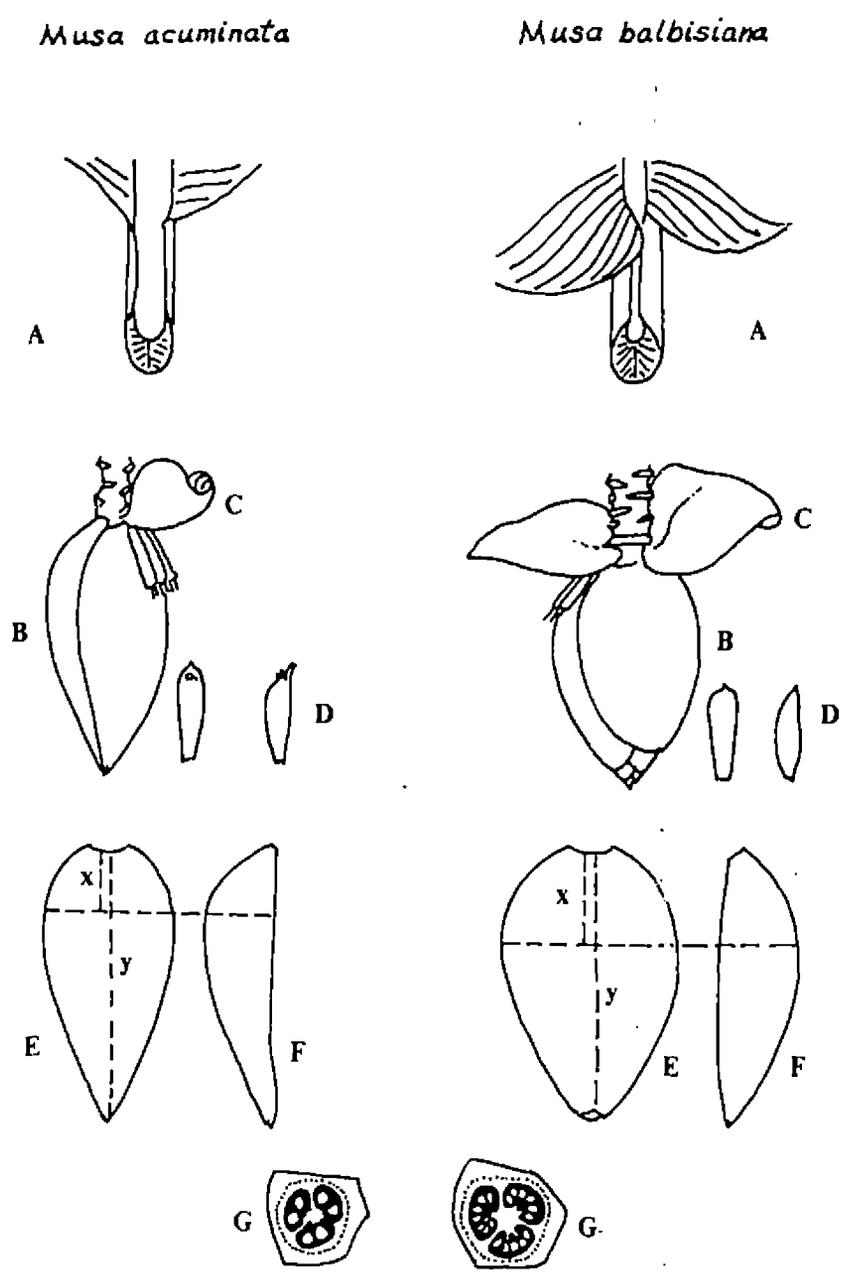


FIG. 1. PETIOLE, BRACT AND OVULE CHARACTERS USED IN THE TAXONOMIC SCORING SYSTEM.

A. PETIOLE, B. BRACT SCARS, C. BRACT CURLING, D. FREE-TEPAL OF MALE FLOWER, E. BRACT SHOULDER (RATIO = x/y) F. BRACT SHAPE, G. OVULE ARRANGEMENT.

1N Hcl at 60°C for 10 minutes, washed in several changes of water and stained in Leuco-basic fuchsin for 30 minutes. The stained portion of root tips were cut and placed on a slide with a drop of distilled water. The cover slip was placed and pressure was applied under several thickness of blotting paper so as to squash the material. Then the slide was placed under high power of a microscope and chromosome counts were taken (Hillary, 1939, 1940; Battaglia, 1957; Darlington and La Cour, 1976).

B. Embryo culture studies

1. Hybridisation

Hybridisation was done using the female parents Paleyankodan and Nendran and the common male parent, Pisanglilin (Plates 1 to 3) which had the highest pollen fertility and compatability with the female parents (Karmacharya, 1984).

Technique of crossing

The inflorescences were bagged two to three days before the anticipated opening of the first bract (Plate 4). Muslin cloth bags (0.5 x 10 m) were used for this purpose.

From the flowers of male parents opened on the day of crossing, anthers were collected just prior to

Plates 1 to 3. Parents used in hybridisation

Plate 1. Musa (AAB) 'Palayankodan'

Plate 2. Musa (AAB) 'Nendran'

Plate . 1

Plate . 2.

Plate 3. Musa (AA) 'Pisanglilin'

Plate 3.

Plate 4. A bunch ready for bagging

Plate. 4.

dehiscence. Crosses were made between 7.00 am to 10.00 am. Since the anthers did not dehisce properly, they were twisted and forced to dehisce. Pollen grains were taken out using 'No.1' camel hair brush. The cloth bags were opened and the inflorescences were examined to see if the bracts containing female flowers were opened. The stigma of female flowers were tested for their receptivity, by finger touch. The stickiness of the stigma indicated receptivity. The pollen grains taken out with the help of a camel hair brush were smeared over the receptive stigma of the female flowers. In some cases in which receptivity was doubtful on the first day, hand pollination was repeated the next morning also. The stigma which had lost receptivity turned bluish brown. The inflorescences were rebagged after pollination in order to prevent any possible cross pollination by insects or wind. The details of crossings were tagged on to the female parents.

The following observations were recorded.

1. Number of seeds per bunch.
2. Number of seeds in each hand.

2. Collection of seeds

The fully mature bunches were harvested and ripened in the room. The ripe fingers were longitudinally cut with

the help of a knife and examined for seeds. The seeds when present were extracted carefully and washed in tap water to remove the pulp. Seeds were rubbed with sand for easy removal of the pulp. These seeds were immediately used for inoculation or kept in distilled water for three days and then used for inoculation, or they could be even stored for a few days without losing the viability (Purseglove, 1975).

3. Surface sterilization of seeds

Thoroughly washed seeds were surface sterilized with 1 per cent $AgNO_3$ for 10 minutes. Then they were washed with sterile distilled water three or four times.

4. Excision of embryo

Sterilised seeds were carefully cut along the sides using a scalpel to remove the seedcoat. The embryo is seen embedded in the endosperm at the micropylar end (Figure 2). It was carefully removed without any damage. Only mature seeds from ripe bunches were used for the study.

5. Preparation of culture medium and inoculation

Culture medium used consisted of a modified Knudson's medium with the Berthelots salts containing

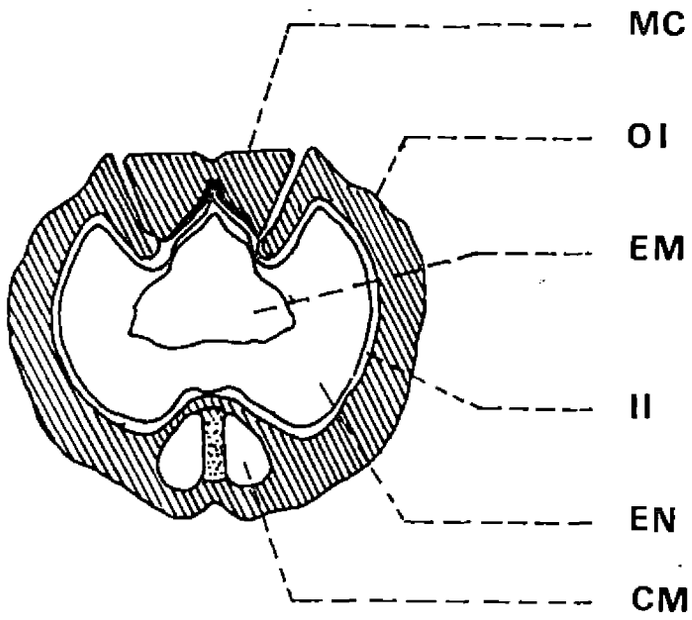


FIG. 2. DIAGRAMATIC LONGITUDINAL SECTION OF BANANA SEED.

MC - MICROPYLAR CAP
OI - OUTER INTEGUMENT
EM - EMBRYO
II - INNER INTEGUMENT
EN - ENDOSPERM
CM - CHALAZAL MASS

four per cent sucrose. Both solid and liquid media were used. The composition of the media used is given in Table 2. Double glass distilled water was used for the preperation of the media. The stock solutions were prepared and stored at 5°C in a refrigerator.

For preparing one litre of the culture medium, the required volume of the stock solutions were pipetted into an one litre beaker. Sucrose was added directly. The volume was increased to 950 ml by adding distilled water. The pH of the solution was adjusted to 5.6 to 5.8 using IN NaOH. Agar was added and the volume was made up to one litre in a volumetric flask. The solution was transferred to one 1000 ml conical flask and heated for melting the agar. Fifteen to twenty millilitre of the medium was immediately added to culture tubes. The culture tubes were plugged with cotton and were autoclaved for 20 to 25 minutes at a pressure of 15 psi. The culture tubes were then stored at a low temperature.

The excised embryos were immediately inoculated in the culture medium. The inoculation was carried out inside a laminar air flow chamber. The forceps and scalpels used for effecting the inoculation were sterilized by dipping in

Table 2. Composition of Knudson's medium (modified) (Rowe and Richardson, 1975)

Solution	Constituents	Quantity	Volume made upto stock	Volume pipetted
	$Ca(NO_3)_4 H_2O$	10 g		
A	$(NH_4)_2 SO_4$	5 g	1000 ml	100 ml
	KH_2PO_4	2.5 g		
	$MgSO_4 7H_2O$	2.5 g		
	Berthelots solution			
	$MnSO_4 7H_2O$	1 g		
	H_2BO_3	25 mg		
B	KI	250 mg	500 ml	0.5 ml
	$NiCl_2$	25 mg		
	$CoCl_2$	25 mg		
	$ZnSO_4$	50 mg		
	$CuSO_4$	25 mg		
	H_2SO_4	0.5 ml		
	Iron solution			
C	$FeSO_4 7H_2O$	2.785 g	500 ml	5 ml
	Na_2 EDTA	3.725 g		
	Thiamine solution			
D	Thiamine Hcl	250 mg	250 ml	1 ml
	Sucrose	40 g		
	Agar	5 g		
	pH	5.6-5.8		

95 per cent ethyl alcohol followed by flaming. After inoculation the culture tubes were incubated at 25°C in a culture room at 16 hours light and at an intensity of 2000 lux produced by cool white flourescent tubes.

The following observations were recorded.

Time taken for germination

Time taken for the excised embryo, to initiate swelling was noted. Time taken for the production of roots and shoots was also noted.

Percentage of germination

This was worked out by dividing the number of embryos germinated by the total number inoculated and was expressed in percentage.

Height of seedlings

The length of the shoot from the swelled embryo to the base of youngest leaf is taken as the height of the seedlings.

Number of leaves per plant

This is the total number of fully opened leaves at the time of observation.

Hardening of the plantlets

When the seedlings attained five to six centimetre height with three to four leaves they were taken out from culture tubes and were thoroughly washed in tap water to remove the ager and nutrient medium. Two procedures were followed for hardening the plants.

In the first method the seedlings were kept in test tubes containing distilled water for four days and covered with a fibre glass cover. The plants were then removed from distilled water and planted in small polythene bags (11 cm x 8 cm), filled with vermiculite and watered daily. After three weeks the plants were transferred to larger polythene bags (21 cm x 16 cm) containing potting mixture (sand, soil and cowdung in 1:1:1 ratio).

In the second method, the seedlings were put in test tubes containing nutrient solution (1/10 concentration of Knudson's mineral salts) for two weeks. Mature blackened roots were cut and removed before keeping the plants in this solution. The test tubes were kept inside a fibre glass cover and frequently sprayed with cold water during day time. After two weeks, the plants with new roots were transferred to small polythene bags (11 cm x 8 cm) containing powdered sand and vermiculite in 1:1 ratio. After two weeks the plants were transferred to larger polythene bags (11 cm x 8 cm) containing powdered sand and vermiculite in 1:1 ratio. After

two weeks the plants were transferred to larger polythene bags (21 cm x 16 cm) containing a potting mixture of sand, vermiculite and powdered cowdung in 2:1:2 ratio.

After keeping the plants in large polythene bags for two weeks they were transferred to mudpots containing potting mixture (sand, soil and cowdung in 1:1:1 ratio) and observed for further growth. During the hardening period observations on height of the plants and number of leaves produced were recorded at three week intervals.

C. Statistical analysis

The data recorded were statistically analysed following methods outlined by Snedecor and Cochran (1967).

Results

RESULTS

Results of the evaluation of the banana hybrids and embryo culture studies of hybrid seeds are presented in this chapter.

A. Evaluation of hybrids

Three hybrids viz., 'Agniswar' x 'Pisanglilin', 'Mannan' x 'Pisanglilin' and 'Vannan' x 'Pisanglilin' were morphologically described. The ploidy level of hybrids were determined using Taxonomic scoring (Table 4) and cytological studies. The photographs of the hybrids are shown in Plates.

1. Morphological description

Agniswar x Pisanglilin (AAB, 2n = 33)

The plant is 228.33 cm tall at flowering with a circumference of 57.67 cm at the base. It takes 183 days for flowering, 108 days for flowering to harvest with a total duration of 292.67 days.

Pseudostem: Green with black blotches

Leaves: Petiole 43.34 cm long, clasping the pseudostem loosely, margins of petiole erect, red coloured, lamina

190 cm long, 62 cm broad, base of lamina unequal, number of leaves 29.33.

Inflorescence: With basal female and distal male flowers, female axis semipendulous, male axis positively geotropic, male flowers deciduous, peduncle medium long and hairy.

Bract: Deciduous, shoulder low, outside colour dull purple, inside purple, inside colour continuous to base, broadly ovate, roll back after opening, apex obtuse, bract scars prominent.

Female flowers: Arranged in two rows, united tepal 4.3 cm long, 2.3 cm broad, cream coloured, lobes 3+2, ovate, free tepal 3.3 cm long, 3.7 cm broad, colour cream, below tip corrugated, stemens not fertile, staminodes 5, filament length 1.6 cm, anther lobe 0.7 cm long, cream coloured, pistil 4.5 cm long, stigma cream coloured 2.5 cm in circumference, with 3 lobes, ovary 8.3 cm long, 7.5 cm in circumference, colour light green, ovules arranged in two regular rows in each loculus.

Male flowers: Arranged in two rows united tepal 4.8 cm long 1.9 cm broad, below tip corrugated, stemens 5, all fertile, filament 2.2 cm long, colour white, anther lobe 2.3 cm long cream coloured, pistillode 4.5 cm long, stigma colour orange yellow, 0.5 cm in circumference, ovary white 1.4 cm long and 0.6 cm broad.

Plates 5 to 7. Hybrids used in the study

Plate 5. 'Agniswer' x 'Pisenglilin'

Plate. 5.

Bunch: Position of mature bunch 30-50° to the horizontal, bunch weight 11.03 kg, number of hands 6, number of fingers 74.33, fingers in a hand arranged loosely.

Finger: 16.77 cm long and 13.37 cm in circumference, finger weight 87.14 g.

Ripe fruit: Yellow, loosely attached in the hand, rind thick, pulp white coloured, fit for consumption, sweet taste, T.S.S. 24 per cent, total sugars 13 per cent, reducing sugars 9.78 per cent, non-reducing sugars 3.22 per cent, sugar/acid ratio 25.82, keeping quality good.

Mannan x Pisanglilin (AAB, 2n = 33)

The plant is 277.5 cm tall at flowering with a circumference of 54.33 cm at the base. It takes 218.33 days for flowering, 117.33 days from flowering to harvest with a total duration of 335.67 days.

Pseudostem: Green with black blotches.

Leaves: Petiole length 53.45 cm, not clasping the pseudostem, margins of petiole erect, black coloured, lamina 220 cm long, 54 cm broad, base of lamina unequal, number of leaves 27.33.

Inflorescence With basal female flowers and distal male flowers, female axis semipendulous, male axis positively

Plate. 5.

Bunch: Position of mature bunch 30-50° to the horizontal, bunch weight 11.83 kg, number of hands 6, number of fingers 74.33, fingers in a hand arranged loosely.

Fingers: 16.77 cm long and 13.37 cm in circumference, finger weight 87.14 g.

Ripe fruit: Yellow, loosely attached in the hand, rind thick, pulp white coloured, fit for consumption, sweet taste, T.S.S. 24 per cent, total sugars 13 per cent, reducing sugars 9.78 per cent, non-reducing sugars 3.22 per cent, sugar/acid ratio 25.82, keeping quality good.

Mannan x Pisanglilin (AAB, 2n = 33)

The plant is 277.5 cm tall at flowering with a circumference of 54.33 cm at the base. It takes 218.33 days for flowering, 117.33 days from flowering to harvest with a total duration of 335.67 days.

Pseudostem: Green with black blotches.

Leaves: Petiole length 53.45 cm, not clasping the pseudostem, margins of petiole erect, black coloured, lamina 220 cm long, 54 cm broad, base of lamina unequal, number of leaves 27.33.

Inflorescence With basal female flowers and distal male flowers, female axis semipendulous, male axis positively

geotropic, male flowers deciduous, peduncle short and glabrous.

Bract: Deciduous, shoulder low (ratio 0.5) outside colour dull purple, inside colour dull purple, inside colour continuous to base, broadly ovate, roll back after opening, apex obtuse, bract scars prominent.

Female flowers: Arranged in two rows, united tepal 7 cm long, 2.7 cm broad cream coloured, lobes 3+2 acute, free tepal 3.2 cm long, 3 cm broad, cream coloured, below tip corrugated stamens not fertile, staminodes 5, filament length 1.8 cm anther lobe 1.2 cm long cream coloured, pistil 4 cm long, stigma cream coloured, 1.8 cm in circumference with 3 lobes, ovary 8.2 cm long, 6.4 cm in circumference, colour light green ovules arranged in two regular rows in each loculus.

Male flowers: Arranged in two rows, united tepal 4.6 cm long, 1.8 cm broad, below tip corrugated, stamens 5, all fertile, filament 2.5 cm long, colour white, anther lobe 2.2 cm long, cream coloured, pistillode 4.8 cm long, stigma colour orange yellow, 0.6 cm in circumference, ovary white, with reddish tinge at the base, 1.8 cm long and 0.6 cm broad.

Plate 6. 'Mannan' x 'Pisanglilin'

Plate 7. 'Vannan' x 'Pisanglilin'

Plate. 6.

Plate. 7.

Bunch: Position of mature bunch 35-40° to the horizontal bunch weight 6.79 kg, number of hands 5.33, fingers 59.67. Fingers in a hand arranged loosely.

Fingers: 15.32 cm long, 13.03 cm circumference, round in shape, finger weight 114.55 g.

Ripe fruit: Yellow loosely attached to the hand, rind thick, flesh white coloured, fit for consumption, sweet taste, T.S.S. 22.67 per cent, total sugars 16.79 per cent, reducing sugars 15.38 per cent, non-reducing sugars 1.41 per cent, sugar acid ratio 28.25, keeping quality good.

Vannan x Pisanglilin (AAB, 2n = 33)

The plant is 255.67cm tall at flowering with a circumference of 55 cm at the base. It takes 197 days for flowering, 101.33 days from flowering to harvest with a total duration of 297 days.

Pseudostem is green with black blotches

Leaves: Petiole 51.1 cm long, clasping pseudostem loosely, margins of petiole erect and black coloured, lamina 195 cm long 56 cm broad, base of lamina equal, number of leaves 28.5.

Inflorescence with basal female and distal male flowers female axis semipendulous, male axis positively geotropic, male flowers deciduous, peduncle long and glabrous.

Bract: Deciduous, shoulder low (ratios 0.42) outside colour dark purple, inside dull purple, inside colour continuous to base, broadly ovate, roll back after opening, apex obtuse, bract scars prominent.

Female flowers: Arranged in two rows, united tepal 4.6 cm long, 2.5 cm broad, cream coloured, lobes 3+2, free tepal 3.7 cm long, 3.4 cm broad, cream coloured, below tip corrugated, stamens not fertile, staminodes 5, filament 1.6 cm long, anther lobe 1.1 cm long, cream coloured, pistil 4.2 cm long, stigma cream coloured, 2 cm in circumference with 3 lobes, ovary 7 cm long, 7 cm in circumference, colour light green, ovules arranged in two rows in each loculus.

Male flowers: Arranged in two rows, united tepal 6.7 cm long, 2 cm broad, below tip corrugated, stamens 5, all fertile, filament 2.5 cm long, white coloured, anther lobe 2.4 cm long, cream coloured, pistillode 5.4 cm long, stigma colour orange yellow, 0.6 cm in circumference, ovary white with greenish tinge at the top, 1.7 cm long and 0.6 cm broad.

Bunch: Position of mature bunch 60-65° to the horizontal, bunch weight 11.78 kg, number of hands 8, fingers 117.67, fingers in a hand arranged tightly.

Finger: 12.57 cm long, 12.1 cm circumference, round in shape, finger weight 90.89 g.

Ripe fruit: Yellow, tightly attached to the hand, rind thin, not peeled easily, flesh yellow coloured, fit for consumption, sweet taste, T.S.S. 21 per cent, total sugars 15.64 per cent, reducing sugar 12.30 per cent, non reducing sugars 3.33 per cent, sugar acid ratio 32.58, keeping quality good.

Taxonomic scoring

Taxonomic scoring at flowering based on fifteen morphological characters (Table 4 and Fig.1) revealed that all the three hybrids were triploids. Scores obtained for the hybrids, Agniswar x Pisanglilin, Nennen x Pisanglilin and Vannan x Pisanglilin were 35, 40, 39 respectively (Table 4).

Quantitative and quality characters

The mean values of the 26 characters of the hybrids and parents are given in Tables 6 to 10. The analysis of variance of the characters are given in appendices (i to v). The following comparisons were made.

1. The hybrids were compared with the parents.
2. The hybrids were compared among themselves.

Table 4. Taxonomic scoring and chromosome number of hybrids

Hybrids	Pseudostem colour	Petiolar canal	Peduncle	Pedicel	Ovule	Bract shoulder	Bract curling	Bract shape	Bract apex	Bract colour	Bract colour fading	Bract scars	Free tepal of female flower	Male flower colour	Stigma colour	Total scores	Chromosome number (2n)	Genomic group
Agniswar x Pisanglilin	3	1	2	3	2	3	1	3	4	4	4	1	2	1	1	35	33	AAB
Mannen x Pisanglilin	3	4	3	3	2	3	1	2	4	4	3	1	2	3	2	40	33	AAB
Vannen x Pisanglilin	2	3	4	4	2	3	1	3	4	4	4	1	2	1	1	39	33	AAB

a. Growth parameters

The mean values of the growth parameters viz., height, girth, number of functional leaves, leaf area per plant and petiole length of the hybrids and their parents recorded at flowering stage are given in Table 6. Growth models of the hybrids are given in Table 5 and Figure 3. Linear growth curves were fitted for describing the variations in height and girth (Figure 3.1 and 3.2). Quadratic growth curves were fitted for characters like number of functional leaves, leaf area and petiole length (Figure 3.3 to 3.5).

(i) Height

The hybrid Agniswar x Pisanglilin (228.33) was significantly shorter than its female parent Agniswar (284.67 cm) (Table 6). Hybrids, Mannan x Pisanglilin (277.5 cm) and Vannan x Pisanglilin (255.67 cm) were significantly taller than both the parents. Parents Mannan, Vannan and Pisanglilin recorded 212.5 cm , 227 cm and 160.33 cm heights respectively.

Among the hybrids, there was significant variation in terms of height. Hybrid Agniswar x Pisanglilin was the shortest among the three (228.33 cm). The hybrid Mannan x Pisanglilin recorded the maximum height (277.5 cm).

Table 5. Growth models of hybrids

	Hybrid	Equation	R^2 values
Height	A x P	$Y = 44.88 + 22.41\ x$	0.79
	M x P	$Y = 28.67 + 26.83\ x$	0.82
	V x P	$Y = 34.71 + 26.52\ x$	0.84
Girth	A x P	$Y = 17.55 + 4.15\ x$	0.77
	M x P	$Y = 12.49 + 4.65\ x$	0.85
	V x P	$Y = 17.73 + 4.57$	0.75
Petiole length	A x P	$Y = -20.39 + 18.09\ x - 1.43\ x^2$	87.27
	M x P	$Y = -29.75 + 21.89\ x - 1.59\ x^2$	66.89
	V x P	$Y = -36.44 + 25.00\ x - 1.89\ x^2$	89.69
Functional leaves	V x P	$Y = -2.03 + 4.05\ x - 0.35\ x^2$	96.21
	M x P	$Y = -2.29 + 3.59\ x - 0.28\ x^2$	85.10
	V x P	$Y = -2.07 + 3.96\ x - 1.89\ x^2$	88220
Leaf area	A x P	$Y = -7.49 + 4.70\ x - 0.37\ x^2$	91.24
	M x P	$Y = -6.22 + 4.04\ x - 0.30\ x^2$	92.48
	V x P	$Y = -6.29 + 4.16\ x - 0.32\ x^2$	90.60

Table 6. Mean values of growth parameters of the hybrids and parents

Hybrids/parents	Growth parameters				
	Height (cm)	Girth (cm)	Functional leaves (no)	Leaf area per plant (m^2)	Petiole length (cm)
Agniswar x Pisanglilin	226.33	51.67	12.00	8.07	43.34
Mannan x Pisanglilin	277.50	54.33	11.33	8.43	53.45
Vannan x Pisanglilin	255.67	55.00	10.00	8.10	51.10
Agniswar	284.67	64.33	9.33	8.06	42.33
Mannan	212.50	52.50	9.33	9.18	32.65
Vannan	227.00	52.50	8.67	9.46	32.75
Pisanglilin	160.33	38.33	5.67	2.63	39.67
CD (0.05)	14.77	5.10	0.88	0.60	3.82

FIG. 3.-GROWTH MODELS OF HYBRIDS.

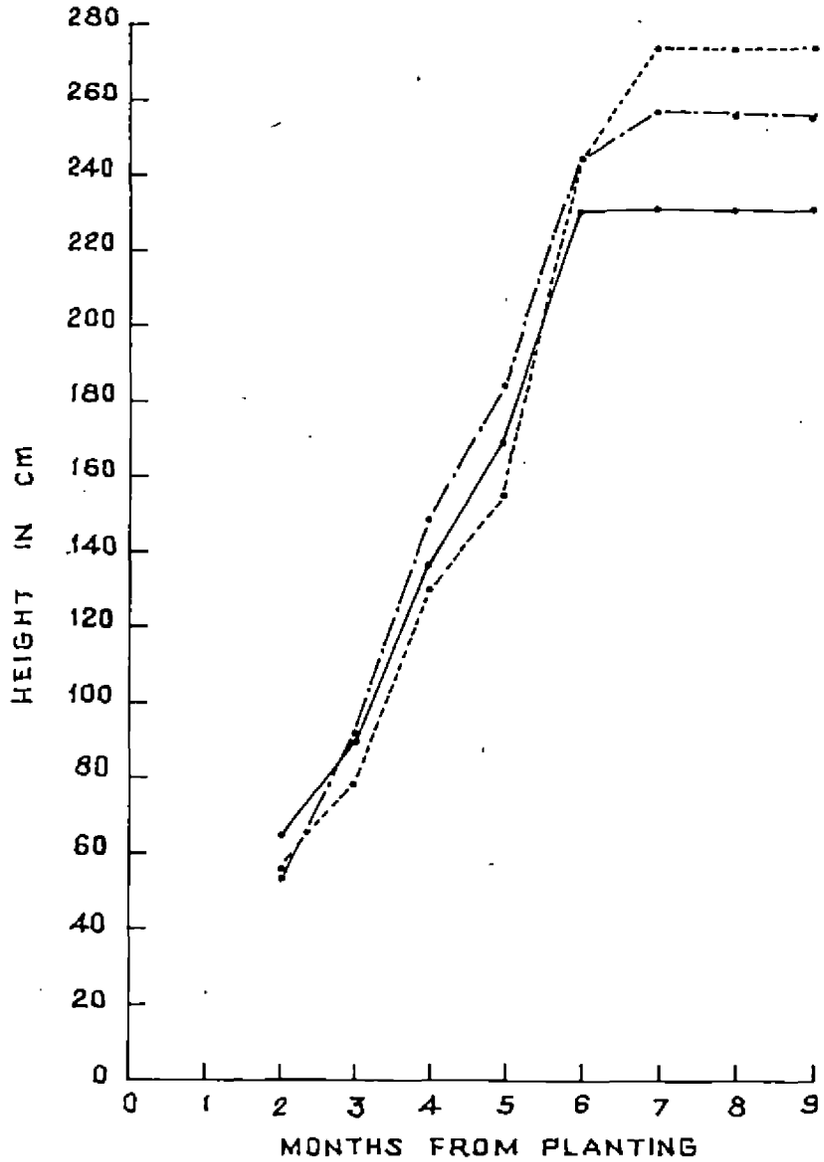


FIG. 3·1. HEIGHT.

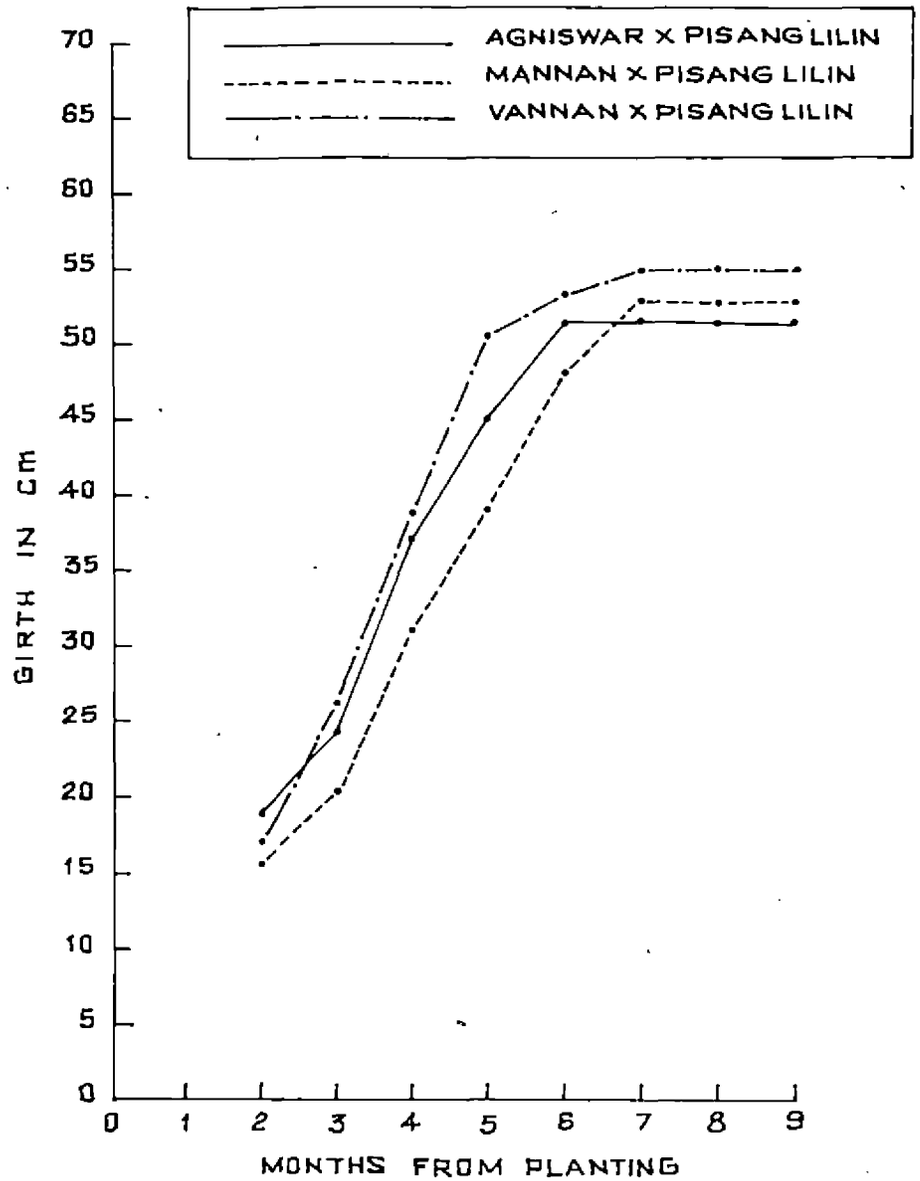


FIG. 3·2. GIRTH.

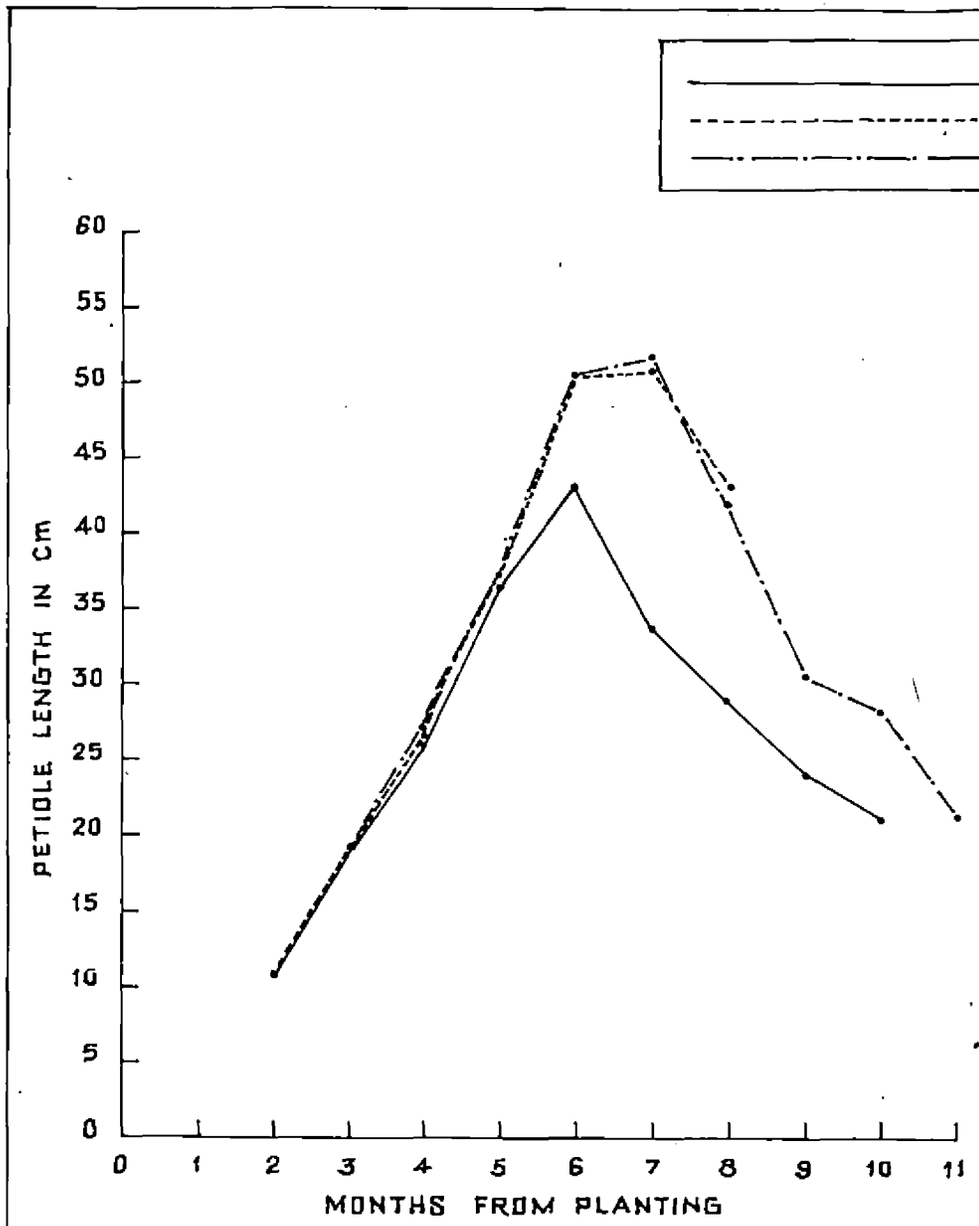


FIG. 3·3. PETIOLE LENGTH.

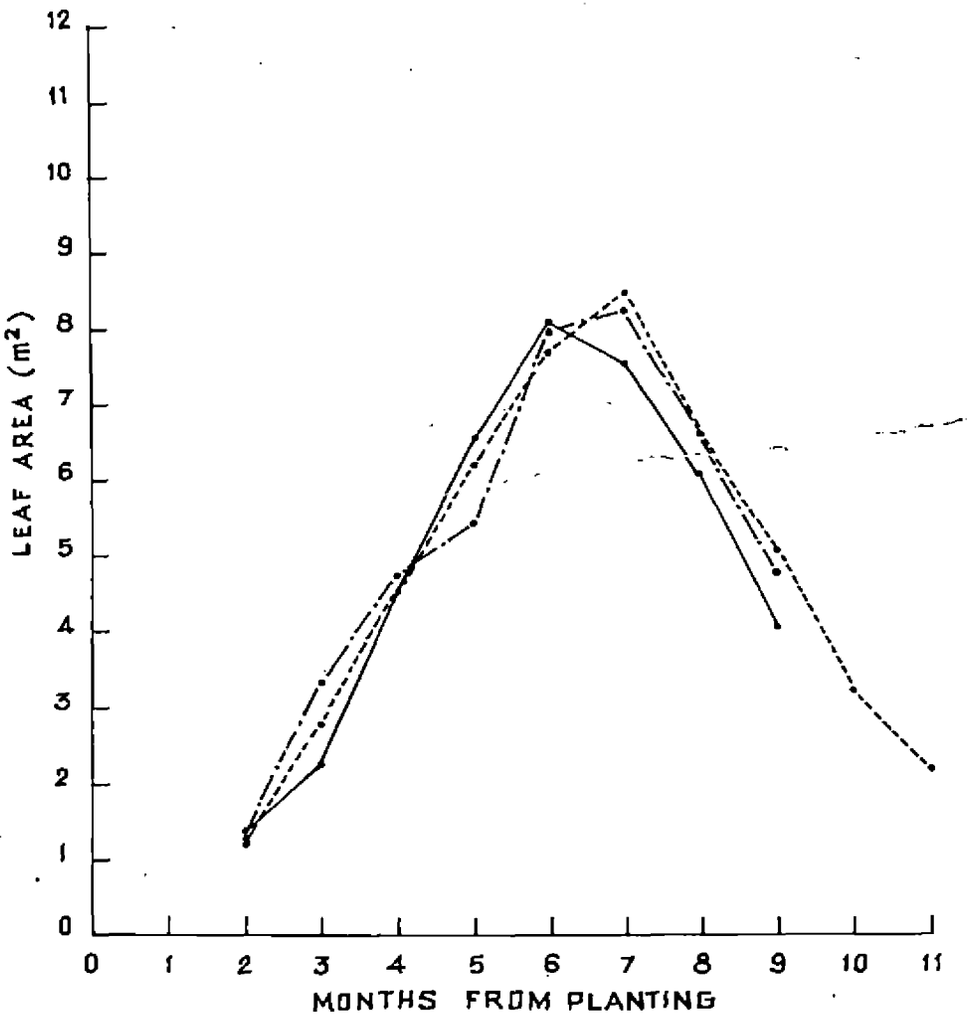


FIG. 3·4. LEAF AREA.

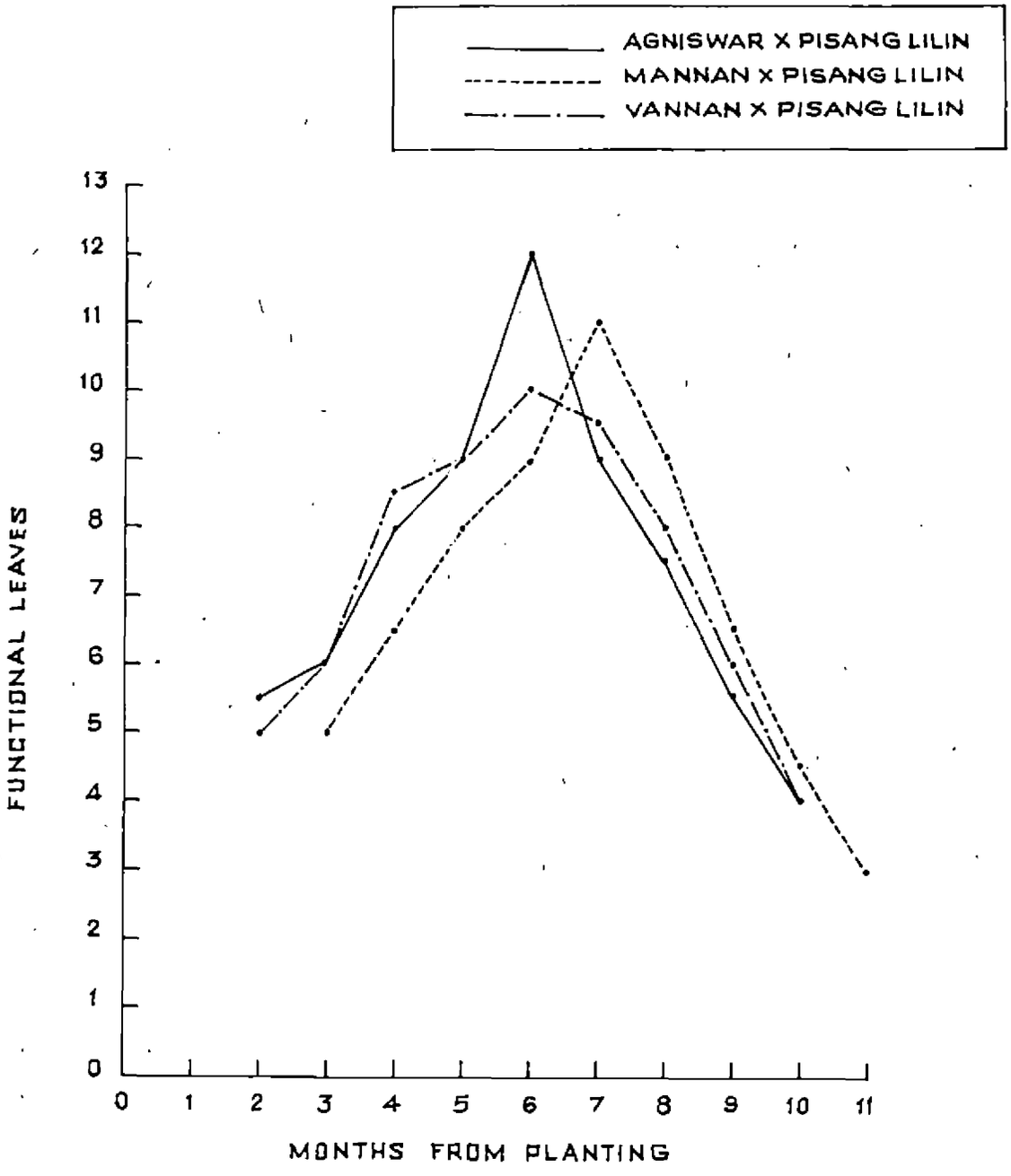


FIG. 3·5. FUNCTIONAL LEAVES.

(ii) Girth

The hybrid Agniswar x Pisanglilin showed significant difference in girth (51.67 cm) from its parents, Agniswar (64.33 cm) and Pisanglilin (38.33 cm) while the other two hybrids were not significantly different from their female parents. Hybrid from the cross, Mannan x Pisanglilin recorded a girth of 54.33 cm while its female parent had 52.5cm girth. The hybrid Vannan x Pisanglilin recorded a girth of 55 cm whereas its female parent had 52.5 cm girth.

Among the hybrids the variation in girth was not significant (Table 6). Vannan x Pisanglilin recorded a girth of 55 cm, Mannan x Pisanglilin 54.33 cm and Agniswar x Pisanglilin 51.67 cm.

(iii) Number of functional leaves

The hybrids had significantly larger number of functional leaves than their respective parents (Table 6). The hybrid Agniswar x Pisanglilin recorded the maximum number of functional leaves (12) which was significantly superior to Vannan x Pisanglilin (10). Mannan x Pisanglilin had 11.3 functional leaves. The parents Agniswar, Mannan,

Vannan and Pisanglilin had 9.33, 9.33, 8.67 and 5.67 functional leaves respectively.

(iv) Leaf area per plant

Except the hybrid Agniswar x Pisanglilin the other two hybrids had lower leaf area than the respective female parent. However all of them possessed large leaf area than the male parent Pisanglilin (2.63 m^2). The hybrid Agniswar x Pisanglilin recorded a leaf area of 8.07 m^2 whereas its female parent recorded a leaf area of 8.06 m^2.

Among the hybrids there was no significant variation in leaf area (Table 6). The hybrid Mannan x Pisanglilin recorded the maximum leaf area of 8.43 m^2 which was on par with the other two hybrids.

(v) Petiole length

The hybrids Mannan x Pisanglilin and Vannan x Pisanglilin were significantly superior to their parents in petiole length. The hybrid Agniswar x Pisanglilin recorded a petiole length of 43.34 cm which was on par with the female parent Agniswar (42.33 cm) as well as the male parent Pisanglilin which recorded a petiole length of 39.67 cm.

Among the hybrids maximum petiole length of 53.45 was recorded by Mannan x Pisanglilin followed by Vannan x Pisanglilin (51.1 cm) and Agniswar x Pisanglilin (43.34 cm).

b) Duration

The duration aspects such as planting to flowering interval, flowering to harvest interval and duration of male and female phases of hybrids and parents are given in Table 7.

(i) Planting to flowering interval

Hybrids took longer duration from planting to flowering than the parents (Table 7). The hybrid, 'Agniswar x Pisanglilin' took 183 days from planting to flowering and did not differ significantly from that of their parents. Hybrids Mannan x Pisanglilin and Vannan x Pisanglilin took 261.33 days and 197 days and were significantly higher than their parents. Female parents Agniswar, Mannan and Vannan took 171.33 days, 171 days and 174 days respectively. Male parent, Pisanglilin took 177.67 days from planting to flowering.

Among hybrids 'Mannan x Pisanglilin' took maximum number of days from planting to flowering (218.33) and

Table 7. Mean values of the duration of the hybrids and parents

Hybrids/parents	Duration (days)				
	Planting to flowering	Flowering to harvest	Planting to harvest	Male phase	Female phase
Agniswar x Pisanglilin	183.00	108.00	292.67	102.67	5.33
Mannan x Pisanglilin	218.33	117.33	335.67	111.67	4.33
Vannan x Pisanglilin	197.00	101.33	297.00	92.67	7.00
Agniswar	171.33	139.00	310.00	132.33	5.33
Mannan	171.00	86.00	257.00	77.00	4.67
Vannan	174.00	70.00	244.00	63.00	5.67
Pisanglilin	177.67	107.67	284.00	93.67	7.67
CD (0.05)	14.46	9.90	14.59	6.98	1.03

and Agniswar x Pisanglilin took the least number of days (163.00).

(ii) Flowering to harvest

Hybrid Agniswar x Pisanglilin took significantly lesser time from flowering to harvest (108 days) than the female parent, Agniswar (139 days). However it was on par with the male parent, Pisanglilin (107 days). The other two hybrids took significantly more times than their respective female parents (Table 7).

Among hybrids a duration 117.33 days was recorded by Mannan x Pisanglilin which was on par with Agniswar x Pisanglilin (108 days) and was significantly higher than that of Vannan x Pisanglilin (103.33 days).

(iii) Planting to harvest - Total duration

The hybrid Agniswar x Pisanglilin (292.67 days) was earlier than its female parent Agniswar (310 days). Other two hybrids had longer duration than the parents. The male parent recorded a total duration of 284 days.

Among the hybrids Mannan x Pisanglilin took maximum number of days from planting to harvest (335.67 days) followed by Vannan x Pisanglilin (297 days) which was on par with Agniswar x Pisanglilin (292.67 days).

(iv) Female phase

The hybrids Agniswar x Pisanglilin and Mannan x Pisanglilin did not differ significantly from their respective female parents in terms of female phase but showed significant variation from the male parent. Vannan x Pisanglilin showed significant difference from its female parent, Vannan (5.67 days). The male parent Pisanglilin took 7.67 days to complete the female phase.

Among hybrids, Vannan x Pisanglilin took more days to complete the female phase (7 days) whereas Agniswar x Pisanglilin and Mannan x Pisanglilin took 5.33 days and 4.33 days respectively.

(v) Male phase

There was significant variation among the hybrids and parents in terms of male phase (Table 7). The hybrid Agniswar x Pisanglilin took less time to complete the male phase (102.67 days) than its female parent (132.33 days) whereas the other two hybrids took more days for the completion of male phase (Table 7). The male parent Pisanglilin recorded 93.67days as male phase which was on par with the hybrid Vannan x Pisanglilin (92.67).

Among the hybrids Mannan x Pisanglilin recorded maximum male phase (111.67 days) followed by Agniswar x Pisanglilin (102.67 days).

C. Bunch characters

The mean values of bunch characters viz., bunch weight, number of hands and fingers, hand weight and number of fingers per hand are summarised in Table 8. With respect to bunch characters such as bunch weight, hand weight and number of hands, hybrids were found to be superior to the parents. Among hybrids, Vannan x Pisanglilin was found to be significantly superior to the other two hybrids.

Bunch weight

With respect to bunch weight, the hybrids, Agniswar x Pisanglilin (11.33 kg) and Vannan x Pisanglilin (11.78 kg) behaved significantly superior to the parents. The hybrid Mannan x Pisanglilin (6.79 kg) recorded higher values than its parents with respect to bunch weight. The parents, Agniswar, Mannan, Vannan and Pisanglilin recorded bunch weight, 5.28 kg, 6.25 kg, 3.75 kg and 2.3 kg respectively.

Among the hybrids, Agniswar x Pisanglilin recorded maximum bunch weight (11.83 kg) which was on par with

Table 8. Mean values of the bunch characters of the hybrids and parents

Hybrid	Bunch characters				
	Bunch weight (kg)	Hand weight (g)	Number of hands	Number of fingers	Number of fingers/ hand
Agniswar x Pisanglilin	11.33	1341.67	6.00	74.33	12.39
Nannan x Pisanglilin	6.79	1066.00	5.33	69.67	11.38
Vannan x Pisanglilin	11.78	1324.17	8.00	117.67	14.63
Agniswar	5.28	790.00	5.67	75.00	13.25
Nannan	6.25	785.00	7.33	73.50	9.33
Vannan	3.75	520.00	5.67	80.50	14.00
Pisanglilin	2.30	650.00	4.00	50.00	12.50
CD (0.05)	2.16	160.64	1.04	16.23	1.43

Vennan x Pisanglilin (11.78 kg) followed by Mannen x Pisanglilin (6.79 kg).

Hand weight

All the three hybrids were significantly superior to the parents with respect to hand weight (Table 8). The hand weights recorded by the parents Agniswar, Mannan, Vannan and Pisanglilin were 790 g, 785 g, 520 g, and 650 g respectively. Among the hybrids, Agniswar x Pisanglilin recorded maximum hand weight (1341.67 g) which was on par with Vennan x Pisanglilin (1324.17 g). The hybrid 'Mannan' x 'Pisanglilin' was significantly inferior to the other two hybrids (1066 g).

(iii) Number of hands

The hybrid Agniswar x Pisanglilin did not differ significantly from female parent with respect to number of hands in the bunch while the other two hybrids showed significant variation from female parent. Female parents Agniswar, Mannan and Vannan had 5.67, 7.33 and 5.67 hands per bunch while the male parent Pisanglilin produced the least number of hands per bunch (4).

Among hybrids Vennan x Pisanglilin had maximum number of hands per bunch (8) followed by Agniswar x

Plates 8 to 10 Hands of hybrids

Plate 8. 'Agniswar' x 'Pisanglilin'

Plate 8.

Plate 9. 'Mannan' x 'Pisenglilin'

Plate 10. 'Vannan' x 'Pisenglilin'

Plate 9.

Plate 10

Pisanglilin (6) which was on par with Mannan x Pisanglilin (5.33).

(iv) Number of fingers

With respect to number of fingers per bunch the hybrid Vannan x Pisanglilin recorded 117.67 fingers per bunch which was significantly superior to its parents Vannan and Pisanglilin (80.5 and 50 respectively). The hybrid Agniswar x Pisanglilin produced 74.33 fingers per hand which was on par with its female parent (75). The hybrid Mannan x Pisanglilin produced 59.67 fingers per bunch which was lesser than its female parent (73.5). The hybrid Vannan x Pisanglilin produced 117.67 fingers per bunch which was significantly superior to its female parent (80.5). The male parent, Pisanglilin produced 50 fingers per bunch.

The hybrids showed significant variation in terms of number of fingers in the bunch. Maximum number was recorded by Vannan x Pisanglilin (117.67) followed by Agniswar x Pisanglilin (74.33) and Mannan x Pisanglilin (59.67).

(v) Number of fingers/hand

Among hybrids, Mannan x Pisanglilin recorded significantly higher values for number of fingers per hand (11.38) than its female parent (9.33) while the hybrids Vannan x Pisanglilin (14.63) and Agniswar x Pisanglilin (13.25) did not show significant variation from their female parents (14 and 13.25 respectively), when compared with the male parent Pisanglilin (12.5) the hybrid, Vannan x Pisanglilin had significantly higher number of fingers per hand.

Among hybrids, Vannan x Pisanglilin had significantly higher values than the other two hybrids and it recorded maximum number of fingers per hand followed by Agniswar x Pisanglilin and Mannan x Pisanglilin.

d. Finger characters

Mean values of finger characters such as pedicel length, finger length, finger girth, finger weight and pulp/peel ratio are summarised in Table9.

(1) Pedicel length

Hybrids were significantly inferior to the parents with respect to pedicel length (Table 9). There was significant variation among hybrids. The hybrid Agniswar x

Table 9. Mean values of finger characters of the hybrids and parents

Hybrids/parents	Pedicel length (cm)	Finger length (cm)	Finger girth (cm)	Finger weight (g)	Pulp/peel ratio
Agniswar x Pisanglilin	1.87	16.77	12.37	87.14	1.60
Nennan x Pisanglilin	1.47	15.32	13.03	114.55	2.14
Vannan x Pisanglilin	1.20	12.57	12.10	90.89	4.80
Agniswar	2.10	12.00	7.50	45.00	2.72
Nennan	2.15	11.38	8.75	58.73	1.52
Vannan	3.00	10.48	10.25	45.50	1.70
Pisanglilin	1.67	15.25	6.16	38.50	1.66
CD (0.05)	0.26	0.86	0.44	8.23	0.22

Pisenglilin had maximum pedicel length (1.87 cm) followed by Mannan x Pisenglilin (1.47 cm) and Vannan x Pisenglilin (1.2 cm).

(ii) Finger length

The hybrids were significantly superior to the female parents with respect to finger length. When compared to the male parent Pisenglilin (15.25 cm), the hybrid Vannan x Pisenglilin had shorter fingers. Within the hybrids also variation in finger length was significant. The hybrid Agniswar x Pisenglilin had longer fruits (16.77 cm) followed by Mannan x Pisenglilin (15.32 cm) and Vannan x Pisenglilin (12.57 cm).

(iii) Finger girth

The hybrids were found to be superior to both parents with respect to finger girth (Table 9). Parents Agniswar, Mannan, Vannan and Pisenglilin recorded finger girths 7.5 cm, 8.75 cm, 10.25 cm and 6.16 cm respectively. Within the hybrids maximum finger girth was recorded by Mannan x Pisenglilin (13.03 cm). Hybrids Agniswar x Pisenglilin (12.31 cm) and Vannan x Pisenglilin (12.1 cm) were on par.

(iv) Finger weight

Hyorids were significantly superior to perents with respect to finger weight (Table 9). Perents Agniswar, Mennan, Vennan and Pisanglilin had finger weights 45 g, 58.73 g, 45.5 g and 39.5 g respectively. Among the hybrids Mennan x Pisanglilin had produced fingers weighing 144.55 g which was significantly superior to Agniswar x Pisanglilin (87.14 g) and Vennan x Pisanglilin (90.89 g) which were on par.

(v)Pulp/peel ratio

Except the hybrid Agniswar x Pisanglilin other two hybrids were significantly superior to the perents with respect to pulp peel ratio. The hybrid Agniswar x Pisanglilin recorded a pulp/peel ratio of 1.61 while its perents, Agniswar and Pisanglilin recorded pulp/peel ratios 2.72 and 1.66 respectively. The perents Mennan and Vennan recorded pulp peel ratios 1.52 and 1.7 respectively.

There was significant difference among hybrids in terms of pulp peel ratio. Maximum pulp peel ratio was recorded by Vennan x Pisanglilin (4.8) followed by Mennan x Pisanglilin (2.14) and the least by Agniswar x Pisanglilin (1.6).

e. Quality characters

The data pertaining to quality characters such as total soluble solids (TSS) acidity, reducing sugars, non-reducing sugars, total sugars and sugar acid ratio are given in Table 10. The hybrids were superior to the parents with respect to TSS, acidity and non-reducing sugars while with respect to reducing sugars, total sugars and sugar acid ratio parents were superior to the hybrids Male parent Pisanglilin was inferior to all others in all the quality characters.

(i) Total soluble solids

Except the hybrid Vannan x Pisanglilin, other two hybrids were superior to the parents. Vannan x Pisanglilin recorded 21 per cent TSS which was on par with the female parent Vannan (21.5 per cent). Female parents Agniswar and Mannan recorded TSS values 21.33 and 21.5 and male parent Pisanglilin recorded 19 per cent.

There was significant variation among hybrids with respect to TSS. Agniswar x Pisanglilin recorded maximum TSS (24 per cent) followed by Mannan x Pisanglilin (22.67 per cent).

(ii) Acidity

Except the hybrid Vannan x Pisanglilin other hybrids showed significant variation in acidity when compared with

Table 10. Mean values of quality characters of the hybrids and parents

Hybrids/parents	T.S.S. (Per cent)	Acidity (per cent)	Reducing sugars (per cent)	Non-reducing sugars (per cent)	Total sugars (per cent)	Sugar acid ratio
Agniswar x Pisanglilin	24.00	0.51	9.78	3.22	13.00	25.82
Mannan x Pisanglilin	22.67	0.67	15.38	1.41	16.79	25.25
Vannan x Pisanglilin	21.00	0.48	12.30	3.33	15.64	32.58
Agniswar	21.33	0.44	11.70	1.39	13.09	30.28
Mannan	21.50	0.45	16.59	1.09	17.62	39.10
Vannan	21.50	0.46	14.50	2.28	16.78	37.15
Pisanglilin	19.00	0.24	7.38	0.97	8.35	34.32
CD (0.05)	0.75	0.04	1.2	0.4	1.19	2.14

parents. The hybrid Vannan x Pisanglilin recorded an acidity of 0.48 per cent where as its female parent recorded 0.46 per cent acidity. The parents Agniswar, Mannan and Pisanglilin recorded acidity values 0.44 per cent, 0.45 per cent and 0.24 per cent respectively.

Among the hybrids also significant variation in the percentage of acidity was observed. Maximum percentage of acidity was recorded by Mannan x Pisanglilin (0.67) followed by Agniswar x Pisanglilin (0.51).

(iii) Reducing sugars

With respect to reducing sugar content hybrids were inferior to the female parents, but superior to the male parent, Pisanglilin (7.38 per cent). Female parents Agniswar, Mannan and Vannan had reducing sugar contents 11.7 per cent, 16.59 per cent and 14.5 per cent respectively.

There was significant variation among hybrids in terms of reducing sugar content. The hybrid Mannan x Pisanglilin recorded maximum reducing sugar content of 15.38 followed by Vannan x Pisanglilin (12.3) and Agniswar x Pisanglilin (9.78).

(iv) Non-reducing sugars

The hybrids Agniswar x Pisanglilin and Vannan x Pisanglilin were significantly superior to the parents in

non-reducing sugar content (Table 10) whereas in the case of Mannan x Pisanglilin, the difference in non-reducing sugar content was not significant. The parents, Agniswar, Mannan, Vannan and Pisanglilin recorded non-reducing sugar contents of 1.39 per cent, 1.09 per cent, 2.28 per cent and 0.97 per cent respectively. Among the hybrids, Vannan x Pisanglilin recorded a value of 3.33 per cent which was on par with Agniswar x Pisanglilin (3.22 per cent) and least value was recorded by Mannan x Pisanglilin (1.41 per cent).

(v) Total sugars

With respect to total sugar content, hybrids were on par with the female parents and superior to the male parent Pisanglilin (8.35 per cent) and also differed significantly among themselves. Female parents, Agniswar, Mannan and Vannan had total sugar values 13.09 per cent, 17.62 per cent and 16.78 per cent respectively. The hybrid Mannan x Pisanglilin recorded the maximum value (16.79 per cent) which was on par with Vannan x Pisanglilin (15.64 per cent) and the least value was recorded by Agniswar x Pisanglilin (13.0 per cent).

(vi) Sugar acid ratio

Hybrids were inferior to the parents with respect to sugar acid ratio. The parents Agniswar, Mannan, Vannan

and Pisanglilin recorded higher values for sugar acid ratios, 30.28, 39.1, 37.15 and 34.32 respectively. Among hybrids Vannan x Pisanglilin recorded the highest value (32.58) which was significantly superior to other two hybrids, Agniswar x Pisanglilin (25.02) and Mannan x Pisanglilin (25.25) which were on par.

4. Male fertility

All the three hybrids were found to produce abundant pollen in their male flowers. The pollen of the hybrids were compared with the pollen of male parent 'Pisanglilin'. Female parents 'Agniswar', Mannan and Vannan were found to be non polliniferous, the enther lobes of which were black and dry.

Table 11 gives an account of results of pollen studies viz., pollen size and pollen fertility of hybrids and the male parent.

a. Pollen morphology

The pollen grains of the hybrids and the male parent, Pisanglilin were found to have similar morphological features. The grains appeared as creamy white powdery mass to the naked eye. The grains were spherical in shape.

The intine and exine were clearly visible. The exine were smooth and uniform in thickness (Plates 11 to 14).

b. Pollen size

The pollen grains of all the three hybrids were bigger than that of the male parent (Table 11). Among the hybrids Vannan x Pisanglilin had pollen grains of biggest size (130.91 μ) followed by Mannan x Pisanglilin (126.31 μ) and Agniswar x Pisanglilin (124.22 μ). The pollen grains of Pisanglilin were 119.96 μ in diameter.

c. Pollen fertility

The results of the studies on pollen fertility by acetocarmine staining technique of the hybrids and the male parent are given in Table 11. Among the hybrids, hybrid Agniswar x Pisanglilin had the highest fertility of 69.99 per cent followed by Mannan x Pisanglilin (56.85 per cent) and Vannan x Pisanglilin (54.63 per cent). The male parent Pisanglilin had a fertility of 63.95 per cent which was significantly higher than that of the hybrids.

5. Female fertility

To assess the fertility status, the hybrids were crossed with Pisanglilin as male parent. The details of

Plates 11 to 14. Pollen grains of hybrids and male parent stained in acetocarmine (x 1000)

Plate 11. 'Agniswar' x 'Pisanglilin'

Plate 12. 'Mennen' x 'Pisanglilin'

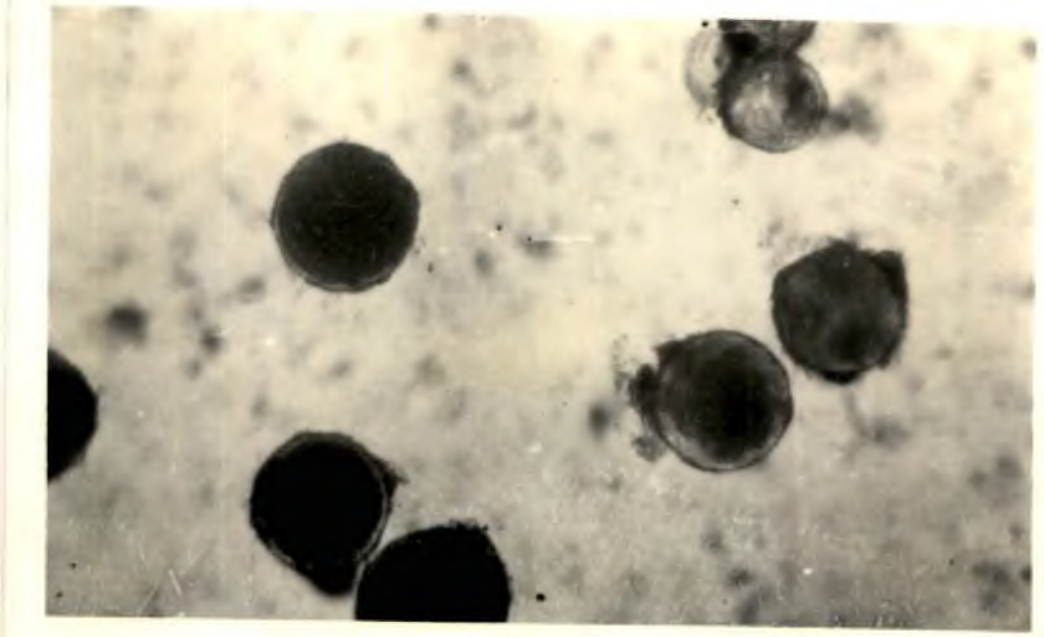


Plate 11

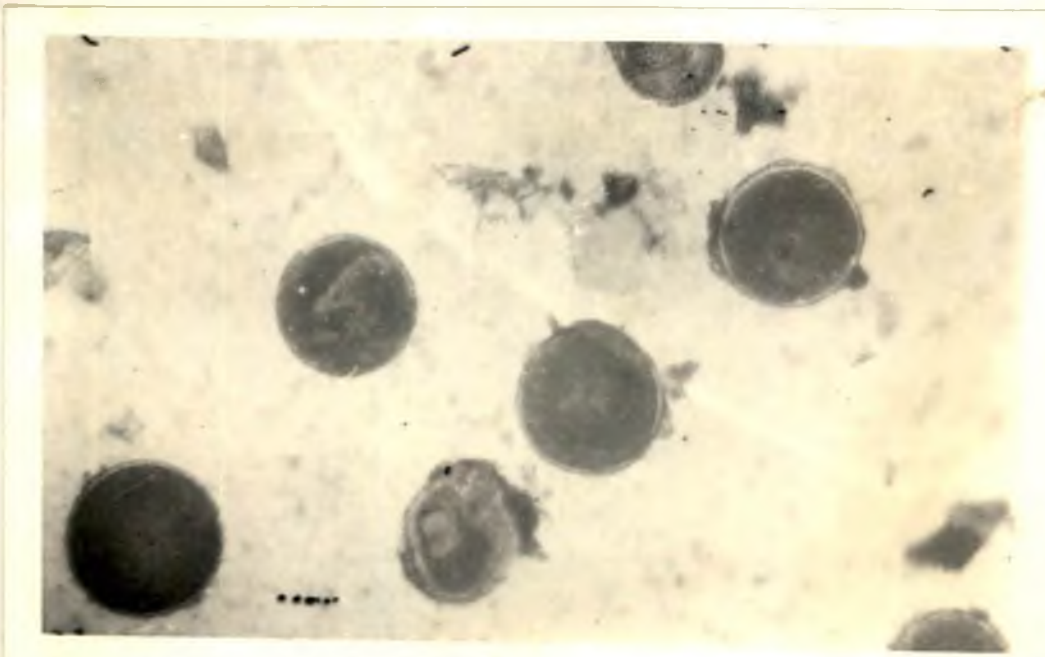


Plate 12

Plate 13. 'Vannen' x 'Pisanglilin'

Plate 14. 'Pisanglilin'

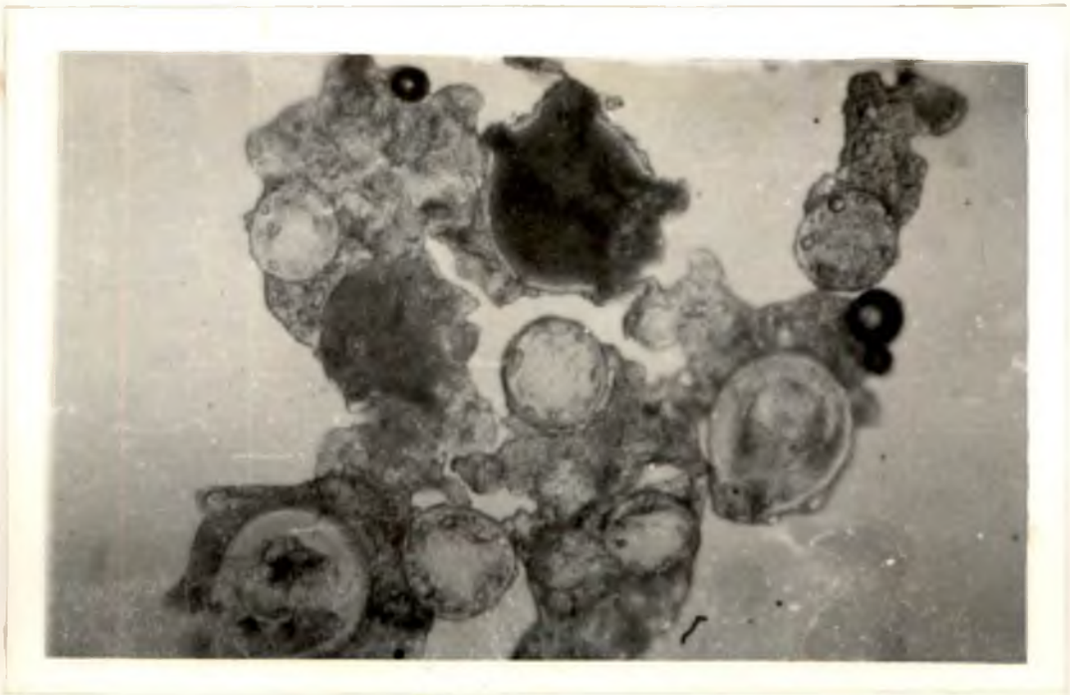


Plate. 13.

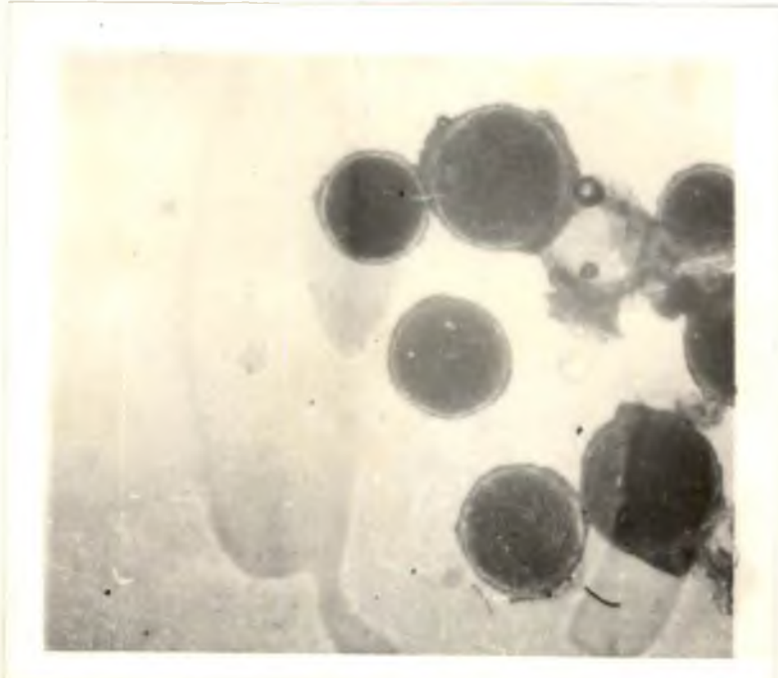


Plate . 14.

Table 11. Pollen size and fertility of hybrids and male parent

Hybrids/parents	Pollen size (diameter in μ)	Pollen fertility (per cent)
Agniswar x Pisanglilin	124.22	59.99
Mennen x Pisanglilin	126.31	56.85
Vannan x Pisanglilin	130.91	54.63
Pisanglilin	119.96	63.95

crosses and seed set obtained are furnished in Table 12. Female fertility of the hybrids was compared with their female parents. Table 13 and 14 show the pattern of fertility of hybrids and the female parents when crossed with Pisanglilin.

The results of the crosses showed that, all the three hybrids were fertile and compatible with Pisanglilin. Among the three, the hybrid Agniswar x Pisanglilin was more fertile followed by Nannan x Pisanglilin and Vannan x Pisanglilin.

With respect to seed yield per bunch female parent, Agniswar recorded a seed set of 20.50 per bunch whereas the fertile hybrid Agniswar x Pisanglilin produced 13.5 seeds per bunch. Agniswar produced seeds upto sixth hand with maximum number of seeds in third and fourth hands (4.50). The hybrid Agniswar x Pisanglilin produced seeds upto fifth hand with the maximum number of seeds in the second hand (5.5).

Female parent Nannan recorded a seed set of 2.5 per bunch whereas the hybrid Nannan x Pisanglilin produced 1.5 seeds per bunch. Nannan produced seeds upto sixth hand with maximum number of seeds in third and sixth hands. The hybrid Nannan x Pisanglilin produced seeds upto second hand with maximum number of seeds in the second hand.

Table 12. Seed set in crosses between the hybrids and male parent Pisanglilin

Sl. No.	Hybrids	Number of flowers pollinated			Number of seeds obtained		
		Ist cross	IInd cross	Total	Ist cross	IInd cross	Total
1	Agniswar x Pisanglilin	70	75	145	12	15	27
2	Mannan x Pisanglilin	63	79	142	1	2	3
3	Vennan x Pisanglilin	113	123	236	2	1	3

Table 13. Pattern of fertility of hybrids with respect to position of hands

Sl. No.	Hybrids	Position of hand	Number of seeds in hand			Mean No. of seeds/ hand	Mean No.of seeds/ bunch
			Ist Cross	II Cross	Total		
1	Agniswar x Pisanglilin	1	2	4	6	3.0	13.5
		2	5	6	11	5.5	
		3	3	2	5	2.5	
		4	1	2	3	1.5	
		5	1	1	2	1.0	
	Total		12	15	27		
2	Mannan x Pisanglilin	1	-	-	-	-	1.5
		2	1	2	3	1.5	
		3- 6	-	-	-		
	Total		1	2	3		
3	Vannan x Pisanglilin	1	-	-	-	-	
		2	2	1	3	1.5	1.5
		3 - 8	-	-	-		
	Total		2	1	3		

Table 14. Pattern of fertility in female parents with respect to position of hands

Sl. No.	Female parents	Position of hand	Number of seeds in hand			Mean No. of seeds/ hand	Mean No.of seeds/ bunch
			Ist Cross	II Cross	Total		
1	Agniswar	1	3	4	7	3.5	
		2	3	4	7	3.5	20.5
		3	4	5	9	4.5	
		4	4	5	9	4.5	
		6	3	3	6	3.0	
		7	-	-	-	2.5	
			19	22	41		
2	Mannan	1 - 2	-	-	-	-	
		3	2	-	2	1.0	
		4 - 5	-	-	-	-	2.5
		6	2	1	3	1.5	
		7	-	-	-		
			4	1	5		
3	Vannan	1	-	-	-	-	
		2	2	1	3	1.5	
		3	1	1	2	1.0	2.5
		4-5	-	-	-		
			3	2	5		

Female parent Vannan recorded a seed set of 2.5 per bunch whereas the hybrid Vannan x Pisenglilin produced 1.5 seeds per bunch. Vannan produced seeds upto third hand with maximum number of seeds in second hand. The hybrid Vannan x Pisenglilin produced 1.5 seeds per bunch with maximum number of seeds in second hand.

B. Embryo culture studies

To get seeds for embryo culture, Palayankodan and Nendran as female parents were crossed with Pisenglilin the male parent. A total of 47 seeds were obtained from the cross 'Palayankodan x Pisanglilin'. From the cross Nendran x Pisanglilin, 11 seeds were obtained (Table 15) which were flat and without endosperm. Seeds from the cross Palayankodan x Pisanglilin were used for embryo culture studies. Seeds were collected from well ripe bunches. From immature bunches embryos were destroyed during excision.

Pattern of seed set in the two crosses are given in Table 16. Comparison of seed set in different hands showed that basal hands were more fertile than the distal ones. Palayankodan produced seeds upto the sixth hand with the maximum seed set in second hand (5.66) and the least in the sixth hand (1). In Nendran seed set was noticed in the

Table 15. Seed set in crosses of Palayankodan x Pisanglilin and Nendran x Pisanglilin

Sl. No.	Crosses	Number of flowers pollinated				Number of seeds obtained			
		I Cross	II Cross	III Cross	Total	I Cross	II Cross	III Cross	Total
1	Palayankodan x Pisanglilin	161	142	126	429	20	16	11	47
2	Nendran x Pisanglilin	45	43	49	137	4	3	4	11

Table 16. Pattern of fertility with respect to position of hands

Sl. No.	Parents	Position of hand	Number of seeds in hands				Mean No. of seeds/ hand	Mean No. of seeds/ bunch
			I	II	III	Total		
1.	Palayenkodan x Pisanglilin	1	3	2	1	6	2.00	15.66
		2	7	6	4	17	5.66	
		3	5	2	3	11	3.66	
		4	2	2	1	5	1.66	
		5	2	2	1	5	1.66	
		6	1	1	1	3	1.00	
		7 - 10	-	-	-	-	-	
	Total		20	16	11	47		
2.	Nendran x Pisanglilin	1	1	1	1	3	1.00	3.66
		2	2	2	2	6	2.00	
		3	1	-	1	2	1.66	
		4	-	-	-	-	-	
		5	-	-	-	-	-	
	Total		4	3	4	11		

first and second hands with the maximum of seeds in the second hand (2).

2. Embryo culture

Seeds were surface steriliaed with 1 per cent $AgNO_3$ for 10 minutes for the sterilisation of embryos. A modified Knudson's media containing Berthelots salts was used for culturing the embryos. Compsotion of the media used is given in Table 2. Germination occurred as a growth of white mass of cells called callus (Plate 15). The callus tissue was covered with very small white hair like structures. From this callus roots and shoots were produced. Shoots appeared as small greenish tubular outgrowths from the callus tissue (Plates 16 to 17). From one centimeter long tubular outgrowth first leaf separated.

In solid media within one week of culturing callus tissue was produced and in another one week roots were produced shoot formation occurred within three weeks of culturing.

Embryos cultured in the liquid media (modified Knudson's media without agar) germinated earlier than the embryos cultured in solid media. In liquid media callus and root formation occurred within one week of culturing.

Plates 15 to 17. Response of cultured embryos in liquid media.

Plate 15. Enlargement of embryo into callus (Four days after inoculation)

Plate 15.

Plate 16. Formation of shoot from the callus
(14 days after inoculation)

Plate 17. Plantlet with single leaf
(21 days after inoculation)

Plate. 16

Plate 17.

Table 17. Response of cultured embryos in the media

Number of embryos inoculated	Number of callus formed	Root formation	Shoot formation	% of germination
30	24	24	24	80

Table 18. Response of embryos to different culture medias

	Time taken for germination		
	To produce callus	To produce roots	To produce shoots
Solid media (Modified Knudson's C media with agar)	7	14	21
Liquid media (Modified Knudson's C media without agar)	4	7	14

Culture tubes maintained at 16 hours light at 25°C produced normal plantlets. 80 per cent of the cultured embryos germinated and produced transplantable plantlets.

Growth of the plantlets at different intervals are shown in Table 19. The mean shoot length recorded was 1.27 cm after 10 days of germination with a mean number of leaves 0.8. An average of 7.93 roots were produced per plantlets. Seventy days after germination, the shoot length recorded was 5.43 cm with a mean number of leaves 4.55. Average number of roots per plant was 13.25. At this stage the plantlets were taken out of test tubes for hardening (Plate 18)

Hardening of the plantlets

In the first method of hardening, where the plantlets were kept in test tubes containing distilled water for four days and then planted in small polythene bags containing vermiculite, the plantlets showed poor growth. But in the second method, where the plantlets were kept in nutrient solution and then removed to polythene bags containing powdered sand and vermiculite plantlets showed normal growth (Table 20).

During the hardening period observations were recorded onthe morphological characters such as shoot length,

Plate 18. Plantlets during hardening
(Three months after germination)

Plate . 18.

Table 19. Growth of plantlets from embryo culture at monthly intervals

	Shoot length (in cm)	Number of leaves	Number of roots
10 days after germination	1.27	0.80	7.93
40 days after germination	3.13	1.86	11.33
70 days after germination	5.43	4.55	13.25

	Shoot length in cm	Number of leaves	Girth
100 days after germination	7.61	6.28	1.33
130 days after germination	13.56	9.00	2.10
160 days after germination	17.56	10.94	3.34
190 days after germination	20.13	12.32	4.26

Table 20. Growth of plantlets from embryo culture media during different methods of hardening

	Shoot length (cm)		Number of leaves	
	I method	II method	Ist method	II method
100 days after germination	6.0 (a)	7.61(b)	5.00(a)	6.29(b)
130 days after germination	7.9 (a)	13.56(b)	6.00(a)	9.00(b)
160 days after germination	11.25(a)	17.56(b)	7.33(a)	10.94(b)
190 days after germination	13.43(a)	20.13(b)	9.50(a)	12.32(b)

(a) Average of 4 values

(b) Average of 10 values

number of leaves and girth of plants (Table 19). Seedlings of 190 days age had 12.32 leaves and were 20.13 cm in height (Plate 19).

4. Planting out

Seedlings of 190 days age were transplanted to mud pots containing potting mixture (sterilized using 0.1 per cent Emisan) in the ratio 1:1:1 sand, soil and cowdung. There was 100 per cent survival of the plantlets.

Plate 19. Plantlets ready for transplanting
(Six months after germination)

Plate. 19.

Discussion

DISCUSSION

In the present studies on the evaluation of hybrids, three hybrids evolved in the Kerala Agricultural University viz. Agniswar x Pisanglilin, Mannan x Pisanglilin and Vannan x Pisanglilin were evaluated along with the parents. The various characters like growth parameters, duration aspects, bunch and finger characters, quality characters, fertility aspects and ploidy level of the hybrids were studied in deteil and were compered with the parents.

In banana, naturally a vegetatively propagated crop, the main limitations in hybridisation programme is the low seed set obtained in many cases. The breading work has to be carried out on a large scale to produce adequate number of seeds. In many cases seed yield is about 1-10 seeds per bunch, the lower numbers being most frequent and often a bunch may yield no seed at all. Further more seed germination is very difficult to obtain under natural conditions. Simmonds (1952); Stotzky et al. (1962 a, 1962 b); and Krishnakumar (1987) have tried different seed treatment methods to enhance the germination of banana seeds.

The different methods were found to be deleterious and often lethal. Thus the need for a new technique for enhancing the germination percentage of hybrid seeds of banana has been envisaged by many workers (Cox et al., 1960; Stotsky et al., 1962a, 1962 b; Shepherad, 1968; Rowe and Richardson, 1975, Krishnakumar, 1987).

Among the various edible diploids employed in banana hybridisation at the various centres of the world, Pisanglilin is the important one. According to Simmonds (1966) it was the first edible diploid to be intensively studied cytologically and the first also to be used in banana breeding and has contributed to the constitution of nearly all the male parents bred for use in banana breeding. High male fertility status of Pisanglilin also increases its usefulness as a potential male parent in hybridisation programme. Among the three male parents used in hybridisation programmes by Krishnakumar (1987), namely 'Pisanglilin', 'Sanne chenkadali' and 'Tongat' only Pisanglilin was found to be compatible. Female fertility of South Indian Bananas, Palayankoden, Nendran, Agniswar, Mannan, Vannan and Rasthali has been reported by Karmacharya (1984) and Krishnakumar (1987). Among them Palayankodan recorded highest fertility when crossed with Pisenglilin followed by Nendran (Krishnakumar, 1987).

He recorded a seed yield of 102.96 per bunch from the cross Palayankodan x Pisanglilin and 13.65 from the cross Nendran x Pisanglilin. But the germination percentage of these seeds were found to be very poor even when they were subjected to various seed treatments. Thus a new method for increasing the germination percentage of the hybrid seeds for the recovery of hybrid plants was found necessary. With this idea in the present study embryo culture method was tried for the hybrid seeds. Seeds from the cross Nendran x Pisanglilin were found to be flat and without endosperm. Hence seeds from the cross Palayankodan x Pisanglilin were used for the study.

Studies on hybrids

The three hybrids, viz., 'Agniswar' x 'Pisanglilin', 'Mannan' x 'Pisanglilin' and 'Vannan' x 'Pisanglilin' were found to be triploids (2n = 33) with AAB genomic group on cytological studies and taxonomic scoring at flowering as suggested by Simmonds and Shepherd (1955). Cytological studies have shown that the female parents Agniswar and Vannan are diploids (2n = 22) of AB genome and the male parent Pisanglilin is diploid (2n = 22) of AA genome. Since these two hybrids are triploids, it is clear that the maternal parent does not undergo normal meiosis, instead produces unreduced diploid gametes. The paternal parent

undergoes normal meiosis and produce haploid gametes. The progenies of Agniswar x Pisanglilin and Vennan x Pisanglilin are thus triploids. The earlier breeding investigations also obtained similar results in cytological study (Cheesman, 1931, 1934; Cheesman and Larter, 1935; Larter, 1935). Larter (1935) had explained the production of tetraploid from the cross Gros Michel x Robusta, where the progenies arise from triploid Gros Michel eggs and haploid Robusta pollen.

The female parent, Mannan is triploid (2n = 33) with AAB genome (Velsalakumari, 1984). The hybrid from the cross 'Mannan' x'Pisanglilin' was found to be a triploid which may be due to the fact that the tetraploid parent during meiosis may produce diploid gametes also which when combines with haploid gamete from male parents, Pisanglilin results in the production of a triploid hybrid plant which is in agreement with Cheesman's studies (1932 b). In Cheesman's studies both tetraploids and triploids were obtained when Mysore (AAB) was crossed with Musa malaccensis.

Taxonomic scoring at flowering based on fifteen morphological characters (Table 1 and Figure 1) as suggested by Simmonds and Shepherd (1955) revealed that all the three hybrids were triploids. Scores obtained for the hybrids,

Agniswar x Pisanglilin, Mannan x Pisanglilin and Vannan x Pisanglilin were 35, 40, 39 respectively (Table 4). There was significent variation among hybrids for morphological characters such as height and petiole length. The hybrid Agniswar x Pisanglilin was the shortest among the three hybrids while Mannan x Pisanglilin was the tallest. The pseudostem of the hybrids were marked with dark brown blotches. Male flowers of the hybrid Mannan x Pisanglilin could be easily distinguished by the reddish tinge at the base of the ovary.

The hybrid Agniswar x Pisanglilin was the shortest among the three and it was shorter than the female parent, Agniswar, but it had maximum number of functional leaves at flowering. Morphological description of banana hybrids has been given by Cheesman (1932 a, 1932 b, 1934, 1949); Larter (1935); Nair (1953); Raman (1976) and Krishnakumar (1987). Krishnakumar (1987) has given the morphological description of hybrids of same parentage. Among the hybrids obtained from the cross Agniswar x Pisanglilin, Hybrid No.I and Hybrid No.III were similar and resembled female parent whereas the hybrid No.II showed similarity with male parent with respect to morphological characters. All the hybrids had more or less similar floral characters. Male flowers of

the three hybrids in the present study had similar floral characters except for a reddish tinge at the base of ovary of the male flowers of the hybrid Mannan x Pisanglilin.

Growth models were fitted for describing the variations in morphological characters of the hybrids (Table 5 and Figures 3.1-3.5). Linear growth curves were fitted for describing the variations in height and girth (Figures 3.1 and 3.2). The values for these characters goes on increasing upto certain stage i.e. upto flowering and later there is no change in the values. Quadratic curves were fitted for characters like number of functional leaves, leaf area and petiole length (Figures 3.3 - 3.5). The values for these characters goes on increasing upto certain stage and later they show a decreasing trend. Krishnakumar (1987) had also used such growth models for assessing the variation in morphological characters of the hybrids.

Considering the growth parameters the hybrids were superior to the parents with respect to height, girth, number functional leaves and petiole length. Hybrids were inferior to the parents as far as leaf area is concerned. But the hybrid Agniswar x Pisanglilin was found to be intermediate to both parents in height (228.32 cm) and girth (51.67 cm). In terms of leaf area per plant also the hybrids were intermediate

to parents. But the hybrid, Agniswar x Pisanglilin (8.07 m^2) showed slightly higher values than the parents. These results are in general agreement with the studies by Nair (1953) and Raman (1976) who noticed expression of intermediary characters in banana hybrids. The hybrid produced at the Central Banana Research Station, Aduthurai from the cross, Monthan x Musa coccinea was found to be intermediate in terms of height which could resist winds better without reducing the total yield as a result of the possibility of addition of more plant per acre (Nair, 1953).

The hybrid Mannan x Pisanglilin was taller than the parents (277.5 cm) and was superior in terms of girth (54.33 cm), number of functional leaves (11.33) and petiole length (53.45 cm). Similarly the hybrid Vannan x Pisanglilin was taller than both its parents (255.67 cm) and was superior to parents with respect to girth (55 cm), number of functional leaves (10) and petiole length (51.10 cm). They recorded a leaf area of 8.43 m^2 and 8.1 m^2 respectively which is intermediate to both the parents. The hybrid Agniswar x Pisanglilin recorded superiority over the parents in terms of functional leaves (12) leaf area (8.07 m^2) and petiole length (43.340 cm). Such a superiority of hybrids over parents has also been revealed by early workers (Rao et al., 1963; Singh, 1963; Kheder et al., 1977).

Among the hybrids 'Mannan x Pisanglilin' recorded maximum height (277.5 cm) leaf area (8.43 m^2) and petiole length (53.45 cm) whereas the hybrid Agniswar x Pisanglilin recorded maximum number of functional leaves (12). The hybrid 'Vannan' x 'Pisanglilin' recorded maximum girth (55 cm) among the three hybrids.

The hybrid Agniswar x Pisanglilin was earlier than the female parents as far as total duration of the plant is concerned which is a desirable character (Table 7). But the hybrids Mannan x Pisanglilin (335.67 days) and Vannan x Pisanglilin (297 days) took more time from planting to harvest than the female parents Mannan (257 days) and Vannan (244 days).

With respect to bunch and fruit characters, the hybrids Agniswar x Pisanglilin and Vannan x Pisanglilin were superior to their parents. This indicates the expression of heterosis in banana hybrids. Heterosis with respect to bunch yield and quality has been reported by Raman (1976) in banana hybrids. Among the hybrids Agniswar x Pisanglilin recorded the highest values for average bunch weight (11.83 kg) and handweight (1341.67 g) followed by Vannan x Pisanglilin, 11.78 kg and 1324.17 g respectively. The hybrid Mannan x Pisanglilin recorded the least values for bunch characters.

Considering the quality aspects the hybrid Vennan x Pisanglilin produced superior quality fruits in terms of sugar acid ratio (32.58) and non-reducing sugars (3.33 per cent) Mannan x Pisanglilin though had higher acidity (0.67 per cent) reducing sugars (15.38 per cent) and total sugars (16.79 per cent) was inferior in non-reducing sugar content (1.41 per cent) and sugar acid ratio (25.25).

Among the hybrids only Agniswar x Pisanglilin was shorter than the female parent while the hybrids, Mannan x Pisanglilin and Vennan x Pisanglilin were significantly taller than their female parents. All the three hybrids were significantly taller than the male parent Pisanglilin. Taxonomic scoring at the time of flowering revealed that all the three hybrids were triploids with scores 35, 40 and 39 respectively for Agniswar x Pisanglilin, Mannan x Pisanglilin and Vennan x Pisanglilin. All of them belonged to AAB genomic group (Simmonds and Shepherd, 1955).

Fertility aspects

With respect to fertility aspects all the three hybrids were found to be both male and female fertile on pollen studies and on artificial pollination. Male fertility of the hybrids were compared with that of the male parent 'Pisanglilin' as the female parents were non polleniferous

(Valsalakumari, 1984). The hybrids had larger sized pollen grains than the male parent, Pisanglilin, but had lower pollen production and fertility.

Among the hybrids, the hybrid Vannan x Pisanglilin had largest pollen grains (139.91 µ) but had the lowest fertility (54.63 per cent). The male parent Pisanglilin recorded highest fertility of 63.95 per cent but the pollen grains were smaller in size (119.96 µ). The other hybrids were inferior to the male parent in fertility aspect but had larger sized pollen grains. The presence of viable pollen in the male flowers of banana hybrids such as IC-1 and IC-2 has been reported by Cheesman (1932 a, 1932 b, 1934) and in the three hybrids evolved from the cross Agniswar x Pisanglilin by Krishnakumar (1987).

Under natural conditions the hybrids were all female sterile and no seeds were produced. Female fertility on artificial pollination with pollen from Pisanglilin was revealed in all the three hybrids. The seed yield per bunch on artificial pollination was lower than that of the parents in all the three cases. This low female fertility is a desirable character for the hybrids which increases their acceptability due to their edible nature (Dodds, 1950). The hybrid Agniswar x Pisanglilin recorded the maximum

seed set of 13.5 per bunch followed by Mannan x Pisanglilin (1.5) and Vannen x Pisanglilin (1.5). The presence of seeds in banana hybrids IC-1 and IC-2 on artificial pollination has been reported by Cheesman (1932 a, 1932 b, 1934) and in the two hybrids from the cross Agniswar x Pisanglilin viz., 'Hybrid No.I' and 'Hybrid No.III' (Krishnakumar, 1987).

Three main causes of female sterility in edible bananas have been envisaged by Shepherd (1954). They are failure of pollination and or fertilization, failure of embryosac development, failure of zygote development. In diploids, Dodds (1943) showed that all three causes operated. Thus a proportion of the ovules of a seeded diploid were not fertilised. Structural hybridity in the edible diploid, Pisanglilin lead to many genetically unbalanced embryosacs and even balanced zygotes of the same edible diploid failed in early development, apparently as a consequence of interaction with an unfavourable maternal environment. Pisanglilin, in consequence, though a diploid and gametically rather fertile, is almost entirely female sterile and sterility is largely caused by the genetic system responsible for parthenocarpy and sterility (Dodds and Simmonds, 1948; Simmonds, 1953).

The pattern of female fertility in different hands of the hybrids showed that basal hands were more fertile than

the distal ones. Within the fertile fruits, the seeds are localised at the styler end. In all the three hybrids studied, maximum number of seeds were obtained from the second hand. Such a type of seed distribution in the hands of a fertile bunch and within an individual fruit has been given by Shepherd (1954, 1960 b). Delange (1969) reported that in seeded bananas all the hands were equally fertile whereas in cultivated bananas, basal hands were more fertile than the others which is in general agreement with the results of the present study.

Crosses involving the female parents Palayankodan and Nendran and the male parent Pisanglilin also revealed similar type of fertility status between the different hands (Krishnakumar, 1987). Palayankodan has been found to be female fertile in many crosses (Nair, 1953; Sundaraj et al., 1957; Alexander, 1976; Raman 1976; Karmacharya, 1984 and Krishnakumar, 1987). The female fertility status of 'Nendran' was first reported by Karmacharya (1984). He obtained seeds when Nendran was crossed with 'Sikuzani'. Krishnakumar (1987) observed female fertility of Nendran when crossed with Pisanglilin. The results of the present study are in agreement with Krishnakumar's findings.

Edible diploids do not produce seeds when grown in pure stands, some of them are entirely female sterile

others will produce an occasional seed when a source of viable pollen is available. The seedset in cultivated bananas on artificial pollination has been reported by many workers (Cousins, 1927; Cheesman, 1934; 1949; Nair, 1953; Sunderaj et al. 1957; Borges 1971; Alexander, 1976; Raman, 1976; Karmacharya, 1984; Krishnakumar, 1987).

Embryo culture studies

Generally the cultivated bananas are seedless, on pollination only a few number of seeds could be obtained. Shepherd (1954, 1960a, 1960b) reported that even under most favourable conditions the fertility status of Gros Michel hardly exceeded three seeds per bunch. A considerable proportion of bunches is seedless. The highest recorded yield of seed from a bunch is 60 seeds from 40000 ovules available for fertilization. Variation in seed yield may be attributed to various factors like fruit size, time of pollination, pruning treatments, season, soil fertility and locality (Shepherd, 1954).

The banana seeds possess a hard testa and due to the presence of this hard seed coat, they show very poor percentage of germination (Dodds, 1950). Germination of hybrid seeds play an important role in breeding programmes.

Generally seed yield of hybrids is low and for a successful breeding programme, it is important that a maximum number of these seeds be germinated (Stotzky et al., 1962 a, 1962 b). In his early studies Cheesman (1932 a) found that artificial pollination of the sterile and parthenocarpic Gros Michel [illegible] (2n = 33) by a fertile Musa identified as a variety of Musa maleccensis (2n = 22) results in formation of occasional seeds, the average seed production being less than one per hundred flowers. Dodds (1956) also had reported the low yield of seeds in early breeding programmes as the main problem of breeding. In many cases the yield is about 1-10 seeds per bunch the lower numbers being the most frequent and often a bunch may yield no seed at all. In the present studies from the cross, Palayankodan x Pisangliilin, an average of 15.66 seeds per bunch only could be obtained where as from the cross Nendran x Pisangliilin the average seed yield per bunch was only 3.66 (Table 16).

Little is known about factors which affect seed germination on the genus Musa except that germination is extremely variable and relatively difficult to obtain under artificial conditions. The use of seedling banana plant as research tools and the increased emphasis on banana breeding

programmes has necessitated elucidation of factors affecting germination of these seeds. As in other crops various types of seed treatment methods have been tried with banana seeds. These treatments such as chipping of testa (Simmonds, 1952, Stotzky et al., 1962; Krishnakumar, 1987) soaking the seeds in sulphuric acid (Simmonds, 1952; Krishnakumar, 1987) soaking in water (Simmonds, 1952) application of temperature shocks (Simmonds, 1952; Stotzky and Cox, 1962), and treatment of the seeds with gibberellic acid (Krishnakumar, 1987)could not improve the germination percentage. This was explained by Dodds (1943 b) as the failure of seed germination was often due to lack of viable embryo and this difficulty could not be overcome by chemical means. Some fruitful results have been obtained in the past by few workers. Simmonds (1958) observed 21 per cent viability for Gros Michel seeds while Shepherd (1960 b) got 5 to 25 per cent for triploid clones. Sathiamoorthy (1973) noticed germination per cent ranging from 0.003-0.6 in banana seeds.

As the various seed treatment methods have failed in enhancing the seed germination, new methods like embryo culture have to be tried for the recovery of maximum hybrid seedlings. This technique has been widely used in many crops for the recovery of interspecific and intraspecific hybrids.

The underlying principle of the method is the aseptic excision of the embryo and its transfer to a suitable medium for development under optimum culture conditions. In general, it is relatively easy to obtain pathogen free embryos, since they are within the sterile environment of the ovule. Thus entire ovules, seeds or capsules containing ovules are sterilized and embryos aseptically separated from the surrounding tissue (Raghavan, 1977). In the present studies seeds from fully ripe bunches from the cross (Palayankodan x Pisangliiin) were extracted carefully and used for embryo culture studies.

The seeds were sterilized using 1% Ag NO_3 solution for 10 minutes. This resulted in complete sterilisation of hybrid seeds. Rowe and Richardson (1975) used the same method for the sterilization of hybrid banana seeds. They reported that surface sterilization with 10 per cent chlorax for 10 minutes resulted in injury to the embryos. Surface sterilisation with 1 per cent sodium hypochlorite for 20 minutes followed by washing in three changes of sterile distilled water was suggested by Litz (1984) for sterilizing nucellar callus explants of Mango.

The use of proper nutrient medium is an important aspect on the success of the work. Different nutrient media

have been tried by various workers for different purposes. For culturing triticale embryos a Murashige and Skoog medium supplemented with IAA and Kinetin and maccerated endosperm from young grains of Triticum durum was used by Bajaj et al. (1978). Cooper et al. (1978) used Norstog's B II medium for the recovery of seedlings barley x rye. In papaya hybrid embryos were successfully cultured in Whites medium containing 0.5 ppm kinetin with or without 0.1 ppm each of GA_3 and IAA (Iyer and Subramanyam, 1972). A White's medium containing 2 per cent sucrose, 500 mg/l casein hydrolysate and 500 mg/l yeast extract was used to raise both intergeneric and interspecific hybrids from tomato (Vorobeva and Prikhod, 1980).

In the present study, the hybrid embryos gave 80 per cent germination in modified Knudson's C.media. Cox et al. (1960) and Rowe and Richardson (1978) used Knudron's C media for culturing embryos containing 2 per cent sucrose and 1.2 per cent agar. A media containing 4 per cent sucrose and 0.5 per cent agar was used by Rowe and Richardson (1975) for culturing tiny hybrid embryos. They could get 50 per cent germination by this method.

In liquid media time taken for germination was much less compared to solid media and also there was a faster rate

of growth of seedlings which is in general agreement with the findings by Stoltz (1971). In solid media the slow germination and growth was due to the presence of agar which reduces the availability of water as a result of increased water binding by the agar (Stoltz, 1971). Agar is considered to have a brushpile structure with the intermeshing threads of the agar forming a solid phase. As the concentration of agar increased the interslices holding the water are reduced. Because of the hydrophilic nature of agar water is bound to the surface of the fibrils both by hydrogen bonding and imbibition process. As the agar concentration is increased a proportionately larger amount of water is in the bound condition and cannot be absorbed by the roots.

The optimum culture conditions found suitable for the germination and normal growth of plantlets was 16 hours photoperiod at 25°C. Under reduced light abnormal elongation of plantlets was observed. This is in accordance with the studies by Banks et al., (1979) and Litz (1984).

Hardening of the plantlets to make them adapt to the outside environment is a critical process due to anatomical and physiological peculiarities of the plantlets. From the leaves of the plantlets after transplanting, excessive water loss has been noticed due to improper development of cuticle

and slowness of stomatal response to water stress (Brainered and Fuchigami, 1981; Fabbri et al., 1984). A period of humidity acclimatization was necessary for the newly transferred plantlets to adapt to the outside environment during which the plantlets undergo morphological and physiological adaptation enabling them to develops typical terrestrial plant water control mechanism (Grout and Aston, 1977; Sutter et al., 1985). In the present studies high relative humidity (90 to 100 per cent) was maintained during the initial period of planting out with the help of microscope covers and intermitent water sprays. After two weeks the covers were removed at short intervals to get the plantlets hardened with respect to lower relative humidity. By this method they could be hardened for their land in the open, in about four to six weeks.

Brown and Sommer (1982) and Amerson et al. (1985) have specified in their studies that addition of inorganic nutrients to the potting mixture was essential for the normal growth of plants. This is in agreement with the present study where application of 5 ml nutrient solution containing 1/10 conc. of knudsons inorganic salts at weekly intervals enhanced the survival and promoted growth of the plantlets than those grown in distilled water. A high percentage of survival of the plantlets could be achieved through this method of planting out.

Summary

SUMMARY

The present investigations on cytomorphological evaluation of banana hybrids and embryo culture studies were carried out in the Department of Pomology and Floriculture, College of Horticulture during 1987-1988. The salient results of the study are summarised below.

The growth parameters, duration, bunch characters, finger characters and quality characters of three banana hybrids, viz. Agniswar x Pisanglilin, Mannan x Pisanglilin and Vannan x Pisanglilin were studied along with their parents. Male and female fertility of the hybrids were also estimated and compared with parents.

With respect to various growth parameters, duration aspects, bunch characters, finger characters and quality characters, the three hybrids differed significantly from parents and also among themselves. Among the three hybrids, Agniswar x Pisanglilin was the shortest (228.33 cm) and it produced maximum number of functional leaves (12) at flowering, while with respect to growth parameters, the hybrids Mannan x Pisanglilin and Vannan x Pisanglilin were taller than the parents (Mannan x Pisanglilin 277.50 cm and Vannan x Pisanglilin 255.67 cm) and had more girth (Mannan x Pisanglilin,

54.33 cm, Vennen x Pisanglilin 55 cm) more number of functional leaves (Mennan x Pisanglilin, 11.33 Vennan x Pisanglilin 11.33) and more petiole length (Mennan x Pisanglilin, 53.45 cm, Vennan x Pisanglilin, 51.10 cm). But the hybrid Agniswar x Pisanglilin showed superiority over the parents in terms of number of functional leaves (12) leaf area (8.07 m^2) and petiole length (43.34 cm).

With respect to duration aspects except the hybrid Agniswar x Pisanglilin (292.67 days) other two hybrids took more time from planting to harvest than the parents.

All the three hybrids were superior to their parents, in terms of bunch weight and hand weight. The hybrids Agniswar x Pisanglilin and Mennan x Pisanglilin and Vennan x Pisanglilin recorded bunch weights 11.33 kg, 6.79 kg and 11.78 kg and hand weights 1341.67 g, 1066 g and 1334.17 g respectively whereas the parents Agniswar, Mennan, Vennan and Pisanglilin recorded bunch weights 5.28 kg, 6.25 kg, 3.75 kg and 2.30 kg and hand weight 790 g, 785 g, 520 g and 650 g respectively.

In quality characters such as T.S.S., nonreducing sugars and acidity the hybrids showed superiority over the parents. The values for these characters were 24 per cent,

3.22 per cent and 0.51 per cent respectively for Agniswar x Pisanglilin, 22.67 per cent, 1.41 per cent and 0.67 per cent respectively for Mannan x Pisanglilin, 21 per cent, 3.33 per cent and 0.48 per cent respectively for Vannan x Pisanglilin.

All the three hybrids were found to be male fertile on pollen studies. Compared to the male parent, Pisanglilin, all the three hybrids produced bigger pollen grains and the hybrid Vannan x Pisanglilin produced pollen grains of the biggest size (130.91 u).

With respect to pollen fertility the hybrids had lower values when compared with Pisanglilin, the male parent (63.95 per cent). Among the hybrids, Agniswar x Pisanglilin recorded maximum fertility of 59.99 per cent.

The hybrids showed very low fertility when pollinated with pisanglilin. Among the three, Agniswar x Pisanglilin produced maximum number of seeds per bunch (13.5) and Mannan x Pisanglilin and Vannan x Pisanglilin produced 1.5 seeds per bunch. With respect to fertility pattern seed production was more on basal hands, the maximum being in the second hand.

Artificial pollination on Palayankodan by the pollen from Pisanglilin yielded 15.66 seeds per bunch on an average

while the seed yield from the cross, Nendran x Pisanglilin was only 3.66 per bunch.

The embryos were aseptically removed and cultured in modified Knudson's C medium at 16 hours light at 25°C. Eighty per cent of the embryos germinated within one week of culturing. When the embryos were cultured in liquid media germination was earlier than those cultured in solid media.

Growth of the plantlets was better in polythene bags containing powdered sand and vermiculite and with nutrient solution than those grown in vermiculite + distilled water alone, when the plantlets were taken out for hardening after two months of growth in culture tubes. Six month old seedlings when transplanted to mud pots containing potting mixture in the ratio 1:1:1 sand, soil and cowdung there was 100 per cent survival.

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*Originals not seen

Appendices

APPENDIX - I

Analysis of variance for growth parameters of hybrids and parents

Source	Degree of freedom	Mean sum of squares				
		Height (cm)	Girth (cm)	Functional leaves	Leaf area (M^2)	Petiole length (cm)
Block	2	8.75	2.48	0.19	0.02	0.18
Treatment	6	5444.92	175.42	12.65	15.98	196.96
Error	12	68.93	8.23	0.25	0.11	4.61
Total	20					

APPENDIX- II

Analysis of variance for duration of hybrids and parents

Source	Degree of freedom	Mean sum of squares				
		Planting to flowering (days)	Flowering to harvest (days)	Planting to harvest (days)	Male phase (days)	Female phase (days)
Block	2	109.91	5.77	340.06	0.43	0.0
Treatment	6	907.82	1457.41	2884.60	1538.21	4.38
Error	12	66.02	30.98	67.27	35.37	0.33
Total	20					

APPENDIX - III

Analysis of variance for bunch characters of hybrids and parents

Source	Degree of freedom	Mean sum of squares				
		Bunch weight (kg)	Hand weight (g)	Number of hands	Number of fingers	Number of fingers/ hand
Block	2	1.21	2319.00	1.29	116.33	1.81
Treatment	6	41.12	315150.69	5.22	1354.46	9.32
Error	12	1.48	8152.33	0.34	83.20	0.65
Total	20					

APPENDIX - IV

Analysis of variance for finger characters of hybrids and parents

Source	Degree of freedom	Mean sum of squares				
		Pedicle length (cm)	Finger length (cm)	Finger girth (cm)	Finger weight (g)	Pulp/peel ratio
Block	2	0.05	1.15	0.04	75.51	0.01
Treatment	6	1.02	16.85	20.92	2523.03	4.16
Error	12	0.02	0.23	0.06	21.40	0.02
Total	20					

APPENDIX - V

Analysis of variance for quality characters of hybrids and parents

Source	Degree of freedom	Mean sum of squares					
		T.S.S. (per cent)	Acidity (Per cent)	Reducing sugars (Per cent)	Non-reducing sugars (Per cent)	Total sugars (Per cent)	Sugar acid ratio
Block	2	0.11	0.00	0.45	0.03	0.04	0.21
Treatment	6	7.05	0.05	31.68	3.03	31.77	84.69
Error	12	0.18	0.00	0.46	0.05	0.45	1.52
Total	20						

HYBRIDISATION IN BANANA CYTOMORPHOLOGICAL EVALUATION OF HYBRIDS AND EMBRYO CULTURE STUDIES

By

LEKSHMY M. L.

ABSTRACT OF A THESIS

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ABSTRACT

Investigations on the evaluation of three banana hybrids evolved in Kerala Agricultural University viz. Agniswar x Pisanglilin, Mannan x Pisanglilin and Vannan x Pisanglilin and standardisation of embryo culture technique for hybrid seeds of banana were carried out in the Department of Pomology and Floriculture, College of Horticulture during the year 1987-88.

The hybrids were evaluated for their morphological characters, duration aspects, bunch characters, finger characters and quality aspects. Male and female fertility status of the hybrids were also studied. The three hybrids differed significantly for the characters studied. Among the hybrids, Agniswar x Pisanglilin was the dwarfest (228.33 cm) and had maximum number of functional leaves at flowering. The hybrid Mannan x Pisanglilin recorded highest values for total duration (218.33), duration of male phase (111.67 days) finger weight (114.55 g) acidity (0.67) reducing sugars (15.38 per cent) and total sugars (16.79 per cent). The hybrid Vannan x Pisanglilin recorded the highest values for duation of female phase (7 days)

bunch weight (11.78 kg) number of fingers (117.67) and pulp peel ratio (4.6).

The hybrids Mannan x Pisanglilin and Vannan x Pisanglilin were found to be taller than the parents (277.5 cm and 255.67 cm respectively). They also recorded higher values for girth (54.33 cm and 55 cm respectively), number of functional leaves (11.33 and 10 respectively) petiole length (53.45 cm and 51.1 cm respectively), bunch weight (6.79 kg and 11.78 kg respectively) hand weight (10.66 g and 1324.17 g respectively) nonreducing sugars (1.41 per cent and 3.33 per cent) and acidity (0.67 per cent and 0.48 per cent respectively). The hybrid Agniswar x Pisanglilin showed superiority over the parents with respect to the number of functional leaves (12) leaf area (8.07 m^2) petiole length (43.34 cm) bunch (11.33 kg) and hand weight (1341.67 g) T.S.S. (24 per cent) nonreducing sugars (3.22 per cent) and acidity (0.51 per cent).

All the hybrids showed very low female fertility on artificial pollination and were male fertile on pollen studies. Among the hybrids, Agniswar x Pisanglilin recorded maximum values for male and female fertility (59.99 per cent and 13.5 seeds per bunch respectively) with maximum number of seeds on second hand.

Seeds obtained from the cross Palayankodan x Pisanglilin were used for embryo culture studies using the modified Knudsm's C medium. Eighty per cent of the cultured embryos germinated within one week of culturing. In the liquid media germination and growth seedlings were found to be at a faster rate than the solid media. After two months of growth of the plantlets in culture tubes, they were taken out for hardening and were successfully transplanted to mud pots containing potting mixture. There was 100 per cent survival of the plants.