INTERSPECIFIC CROSS - COMPATIBILITY IN THE GENUS *Abelmoschus*

By

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THESIS

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I hereby declare that this thesis entitled "Interspecific cross-compatibility in the genus *Abelmoschus*" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Certified that this thesis entitled "Interspecific cross-compatibility in the genus *Abelmoschus*" is a record of research work done independently by Sindhu, S. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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INTRODUCTION

Introduction

Bhindi (*Abelmoschus esculentus* (L.) Moench) is one of the most important warm season fruit vegetables grown in the tropical and subtropical countries. The green tender fruits are rich in vitamins, protein, calcium, iron, magnesium and other minerals. As the fruits also contain iodine, they are recommended against goitre.

In India bhindi is extensively cultivated almost all over the country. The crop is cultivated throughout the year in south India. One of the major threats facing bhindi cultivation is yellow vein mosaic disease. This viral disease occurs throughout India wherever bhindi is grown especially in the rainy season. The loss in yield due to the virus ranges from 50 to 90 per cent depending on the stage of crop growth at which infection occurs (Sasthry and Singh, 1974). The virus is transmitted mainly by the whitefly, (*Bemesia tabaci*) and also by a leaf hopper (*Amrasca devastrans*). As the causal organism is virus the only control measure is the control of vectors using pesticides. The escalating cost of pesticides and the chemical residues which causes health hazards warrants alternative control methods, such as host-plant resistance. But varietal resistance to yellow vein mosaic in *Abelmoschus esculentus* is rare, whereas it is present in other species of *Abelmoschus*. It is in this juncture that interspecific hybridization in *Abelmoschus* assumes importance. In interspecific hybridization programme, the semi wild species like A. manihot and A. manihot subspecies tetraphyllus which have natural resistance for yellow vein mosaic are now being widely used for the transfer of resistance to the cultivated species, A. esculentus. Many workers have reported the incompatability between the semiwild species and the cultivated species, which is expressed through low seed set and low hybrid fertility in the interspecific crosses. This is a serious constraint in interspecific hybridization programme in the genus Abelmoschus. Hence the present study was aimed to probe into the reasons of low fruit and seed set in the interspecific crosses of Abelmoshcus and hybrid sterility.

Probing into such genetic mechanism will go a long way in developing suitable procedures to overcome the contraints and pave way for the development of new mosaic resistant bhindi varieties through interspecific recombination breeding.

REVIEW OF LITERATURE

2. Review of Literature

2.1 Interspecific hybridization

In crop improvement programmes, when the parents used in hybridization belong to two distinct species of the same genus it is known as interspecific hybridization.

Interspecific hybridization has been most commonly used for the transfer of specific characters as disease resistance to cultivated species. In crop plants, several characteristics of economic value, such as resistance to diseases and environmental stress, are present in wild and weedy relatives. There are many reports of such type of resistance in Bhindi and related crops.

Nariani and Seth (1958) reported that different species of *Abelmoschus* and *Hibiscus* were screened for their reaction to yellow vein mosaic virus by graft inoculation as well as by feeding viruliferous whiteflies. *Abelmoschus manihot* var. pungens, *A. crinitus*, *Hibiscus vitifolius* and *H. panduraeformis* could not be infected by either method and it indicated that they were immune to infection. Other species of *Abelmoschus* and *Hibiscus* which were infected with the virus showed great variation in symptoms from the typical mosaic to mild forms. Some species like *A. tuberculatus*, *A. manihot*, *A. angulosm*, *H. cannabinus* and *H. sabdariffa* carry the virus without showing the symptoms.

Sandhu et al. (1974) reported that five wild species of Abelmoschus were showing field resistance to yellow vein mosaic under conditions of heavy natural infection. H. manihot from Ghana, which was almost immune to yellow vein mosaic, could be considered as a good source to develop resistant lines. Two forms of Abelmoschus manihot introduced from Africa and Japan, proved to be highly resistant to the yellow vein mosaic (Arumugam et al., 1975).

Chelliah and Srinivasan (1983) found that resistance to yellow vein mosaic virus transmitted by Bemisia tabaci was present in A. manihot and A. manihot ssp. tetraphyllus. In the preliminary evaluation of Bhindi types under the research project on "Maintenance and evaluation of germplasm of crop plants" it was found that the semi-wild species, A. manihot was completely resistant to yellow vein mosaic disease while twenty other cultivars in the germplasm were severely affected by the disease (Anon, 1983).

Nerkar and Jambhale (1985) reported that the three related species, A. tetraphyllus, A. manihot and A. manihot ssp. manihot and their hybrids with A. esculentus cv. Pusa Sawani were free from disease symptoms under field conditions.

2.2 Achievements of interspecific hybridization in the genus Abelmoschus.

Jambhale and Nerkar (1986 a) reported that in trials of eight varieties under conditions of natural infection, four resistant lines derived from backcross of Abelmoschus (Hibiscus) esculentus x A. manihot showed only 4.09 - 19.37 per cent of plants affected with the virus. Parbhani kranti, derived from the same material, did not show any symptoms of virus infection and gave higher yields of marketable fruit than the susceptible A. esculentus varieties.

Parbhani kranti is an Abelmoschus esculentus variety derived from backcross of A. manihot to the okra pusa sawani. It carries resistance to yellow vein mosaic derived from A. manihot. Parbhani kranti out yielded Pusa sawani in trials at 3 sites over 3 years and produces slender, dark-green fruit 8-9 cm long (Jambhale and Nerkar, 1986b).

Two yellow vein mosaic resistant varieties Arka Anamika and Arka Abhay evolved through interspecific hybridization using A. manihot ssp. tetraphyllus var. tetraphyllus as a source of resistance have been released (Anon, 1991).

2.3 Comatibility in the genus Abelmoschus

Kuwada (1961) reported that the hybrid between A. esculentus and A. manihot was partially sterile and in 1974 he found that hybridization between A. tuberculatus and A. manihot was successful only when A. tuberculatus was the female parent but the hybrid was completely sterile.

During the course of investigations on interspecific hybridization in the genus Abelmoschus, it was observed that the hybrid embryo failed to grow beyond the heart or torpedo stage in certain cross combination. As a result of this post fertilization barrier, no viable seed could be obtained in crosses of A. esculetus with A. moschatus or A. ficulneus and of A. moschatus with A. ficulneus or A. tuberculatus (Gadwal et al., 1968).

Singh et al., (1975) reported that the hybrids of an accession from Gahana, which was identified as being immune to yellow vein mosaic, with Indian okra were only partially fertile while those between the Ghanian accession and A. tetraphyllus were completely sterile.

Interspecific hybrids of A. esculentus and A. ficulneus studied by Hussain and Chattopadhyay (1976) were resistant to yellow vein mosaic. But they were self sterile and produced many fruits without seeds or with only rudimentary seeds and resembled their wild parents in several morphological characters.

Mamidwar et al.. (1979) conducted crosses between A. esculentus and wild forms of A. manihot and A. tetraphyllus and found that fruit set was highest when A. esculentus was the female parent. The hybrids produced seedless fruit or fruits with shrivelled seeds.

Meshram and Dhabake (1981) reported that the hybrid between A. esculentus and A. tetraphyllus was spreading in habit and dwarf. The hybrid was highly male sterile and produced fruits without seeds. Meshram and Narkhede (1981) obtained interspecific hybrid between A. esculentus and A. radiatus which showed luxuriant vegetative growth and positive heterosis in flowering and fruiting. But the hybrid was highly pollen sterile and produced empty seeds.

The F_1 of the cross A. esculentus and A. manihot showed a seed fertility of only 7.1 per cent (Jambhale and Nerkar, 1982a).

Dhillon and Sharma (1982) produced interspecific hybrids of A. esculentus cultivars and a Ghanian accession of A. manihot ssp. manihot. They could realise appreciably good recovery of hybrids.

Jambhale and Nerkar (1982b) observed that the interspecific F_1 hybrid from the cross A. tetraphyllus and A. esculentus was sterile. Seventeen accessions of West African species of Abelmoschus was crossed with plants of A. esculentus. The F_1 interspecific hybrids were comparitively sterile, but a few produced germinable seeds (Siemonsma, 1982).

Pillai (1984) obtained hybrids with complete resistance to yellow vein mosaic by crossing Abelmoschus manihot with four susceptible cultivars of Abelmoschus esculentus. But none of them outyielded the highest yielding parent variety.

Tekale et al. (1985) studied eight hybrid lines derived from crosses between Abelmoschus esculentus and A. manihot and found that the pollen sterility and seed sterility in the lines ranged from 6.44 to 13.25 per cent and 24.96 to 52.14 per cent respectively.

In the F_2 population of a cross between A. esculentus and A. manihot, Mathews (1986) reported preponderance of low yielding yellow vein mosaic resistant plants similar to semi wild parents suggesting the presence of powerful genetic mechanism which restricts free recombination. Prabha (1986) reported high pollen sterility and low germination percentage in the interspecific hybrids of the above cross.

Embryo deterioration in ovules resulting from crosses between A. manihot and A. esculentus was investigated by serially sectioning hand pollinated ovules. Endosperm deterioration was first noted five days after pollination in the cross A. esculentus and A. manihot and was accompanied by a decrease in ovule weight (Bhargava, 1989).

Babu and Dutta (1990a) reported that the F_1 hybrids, (A. esculentus x A. tetraphyllus) resembled the wild parents and exhibited hybrid vigour. Its meiosis was abnormal showing more of univalents and less of bivalents and was completely sterile. The ampnidiploid (A. esculentus - tetraphyllus) obtained through colchicine treatment was fully fertile. It showed a low multivalent formation during meiosis and can survive as a new synthetic species.

Babu and Dutta (1990b) studied on fertility and germination of pollen grain from A. esculentus, A. tetraphyllus and their interspecific hybrid and found that the interspecific hybrid had the lowest pollen fertility (76.42%) and germination (58.2%).

Interspecific hybrids can be obtained between the two species of okra A. esculetus and A. caillei but the F_1 hybrid would be sterile (Hamon and Hamon, 1991).

2.4 Cytology of interspecific hybrids

In a cytogenetical study on an interspecific hybrid between A. esculentus and A. tetraphyllus Meshram and Dhapake (1981) reported that meiosis was abnormal and showed, on and average 37 bivalents and 55 univalents at metaphase. Anaphase separation was irregular and the hybrid was highly male sterile and produced fruits without seeds.

Meshram and Narkhede (1981) reported that meiosis in the interspecific hybrid between *A. esculentus* and *A. radiatus* was abnormal and showed 28 bivalents and 16 univalents. The anaphasic separation of chromosomes was irregular which led to the unequal distribution of chromosomes in gametes.

An amphidiploid produced from the F_1 of the cross *A. esculentus* and *A. manihot* by colchicine treatment differed from the F_1 in several growth, leaf, flower and fruit characteristics. Seed fertility of the amphidiploid was 88.1 per cent while that of the F_1 was 7.1 per cent. Chromosome configuration of the amphidiploid at metaphase was more or less regular forming 162 bivalents (Jambhale and Nerkar, 1982a).

Jambhale and Nerkar (1982b) reported that an amphidiploid was obtained by colchicine treatment of the sterile hybrid from the cross *A. tetraphyllus* and *A. esculentus* which was made to transfer resistance to yellow vein mosaic virus from the former to the latter. Chromosome pairing was normal in the amphidiploid with 129-134 bivalents per cell compared with 22-40 in the F_1. Dyads, triads and polyads were observed in the amphidiploid at telophase II.

2.5 Interspecific hybridization in cotton

Inorder to overcome sterility in F_1 hybrids Gossypium hirsutum x G. herbaceum, G. barbadense x G. thumbergii and G. hirsutum x G. harknessii, back crosses were combined with the use of stimulants and mentors (Eshankhodzhaev, 1977). As a result, seed set in the hybrids rose from 0.7 per cent to 1.5 per cent in the F_1 BC_1 to 15.1 per cent in the F_1BC_2 and to 50 per cent in the F_1BC_3.

In a study of morphological and other characters in F_1 and F_2 hybrids from the cross G. hirsutum ssp. mexicanum and G. mustelinum only 11 per cent seed set was recorded in the F_1. The F_1s were phenotypically intermediate between their parents, but pollen fertility was 56-89 per cent with a mean of 72.8 per cent (Omelchenko and Abdullaev, 1983).

Vlasova et al. (1983) observed deviation from the normal process of fertilization irrespective of whether the parents had the same chromosome number or different number. In the crosses, Gossypium wotschianum x G. trilobum, G. davidsonii x G. trilobum and G. davidsonii x G. barbadense ssp. peruvianum, fertilization was arrested at the progamic phase or at gametogenesis leading to dropping of the young fruits.

Liu *et al.* (1985) reported that irregular chromosome pairing and other meiotic abnormalities led to the abortion of virtually all the microspores in hybrids resulting from the crosses (*G. arboreum* x *G. herbaceum*) x *G. hirsutum* and autotetraploid *G. arboreum* x *G. hirsutum*.

Chiavegato *et al.* (1985) had done 245 crosses between chromosome doubled *G. herbaceum* var. africanum and four commercial varieties of *G. hirsutum* var. latifolium, which gave rise to thirteen completely sterile F_1 plants. However fertility was restored after first backcross.

In an interspecific cross between *G. herbaceum* and *G. arboreum* (Gennur *et al.*, 1986) recorded that pollen sterility ranged from 11.3 to 29.6 per cent.

Crosses were made between 4x cultivar of *G. hirsutum* and the wild 2x Mexican sp *G. aridum*. Abnormal meiosis resulted in no seed set with either by self or cross pollination. Seed was obtained, however following colchicine treatment (Shakhmedova *et al.*, 1988).

When *G. arboreum* was crossed with *G. davidsonii* fertilization was normal in cross pollinated ovules, but after 7 days of pollination the hybrid endosperm had developed many abnormalities. After 11 days the endosperm started degenerating and by the 16th day after pollination only few ovules had formed cell wall. Hybrid embryos were either undifferentiated or abnormally differentiated and aborted 11-22 days after pollination (He and Liang, 1989).

2.6. Interspecific hybridization in other vegetables

In a study on interspecific hybrids of genus Capsicum Malhova and Fileva (1969) found abnormal development of embryo and endosperm in crosses between C. pendulum and C. annuum and suggested this as one of the causes for their partial or complete sterility. Boukema (1982) reported male sterile hybrids between C. chacoense and C. annuum but the hybrids had larger leaves and were much vigorous than C. chacoense.

Kumar et al. (1988) reported that crosses between the wild species capsium chacoense and the cultivated species C. annuum, C. frutescens and C. chinense were successful when C. chacoense was the female parent, but the reciprocal crosses failed. C. chacoense x C annuum F_1 hybrids were partially fertile but the other two F_1s were completely sterile. The F_1 plants formed mostly bivalents at meiosis, with a few multivalents and univalents.

In a study on interspecific crosses of Lycopersicon involving L. esculentus and L. chilense, fruit set ranged from 20 to 59.6 per cent, although observations 20-40 days after fertilization revealed abnormal and restricted embryo development, leading to eventual embryo abortion (Gavrilenko and Surikov, 1988). Chen and Imanishi (1991) observed normal seeds in ovules of ripe hybrid fruit from crosses between four L. esculentum cultivars and two lines of L. chilense or L. peruvianum line. However only few ovules had the potential for germination.

Several interspecific hybrids involving S. melongena and the wild species S. indicum, S. integrifolium and S. surattense, were produced in the genus Solanum by Kirti et al. (1984) and found that formation of 12 bivalent per PMC was common (2n = 24), with some higher chromosome associations. Chiasma frequency was significantly lower in autotetraploids of S. melongena variety insanum, S. integrifolium and S. indicum than the doubled values of the corresponding diploids. Autotetraploids showed irregular chromosome distribution at anaphase I, and had pollen sterility up to 50 per cent. Amphidiploids were partially sterile despite regular chromosome behaviour.

S. melongena, S. khasianum and S. sisymbrifolium all (2n=24) were intercrossed, with reciprocals, and also self pollinated. Pollen germination after 24 hours was more than 95 per cent in all self pollinated, but ranged from 27.3 to 73.0 per cent in the interspecific crosses (Sharma et al., 1984).

Singh and Roy (1986) reported that hybrids were produced from the cross S. melongena cv Dorli x S. surattense only when the former was the female parent, while the cross cv Round Black x S. surattense was not successful in either direction. Meiotic analysis of hybrid plants revealed a higher univalent frequency than in the parents and the presence of laggards and chromosome bridges. However, the hybrid had good pollen fertility (76. 7%).

The meiotic behaviour of chromosomes was studied in interspecific hybrids of S. indicum with S. incanum and S. khasianum and of S. melongena with S. xanthocarpum by Narkhede et al. (1987) They observed that bivalents were mostly ring shaped in species but in the hybrids, the bivalents appeared to be ring, V and rod shaped.

Patil et al. (1990) reported that parents and F_1 hybrids between Solanum macrocarpon and S. melongena were subjected to cytological examination. It revealed that chromosome pairing was normal in both parents but in hybrids there was occurrence of quadrivalents and chromosome bridges in some cells.

Several crosses were made between different *S. aethiopicum* genotypes and tropical varieties of *S. melongena*. Despite sterility problem encountered in the F_1, a recurrent selection scheme involving two backcrosses to *S. melongena* was performed, yielding families with a high level of wilt resistance and a wide range of fruit shape and colour (Ano *et al.*, 1991).

MATERIALS AND METHODS

3. Materials and Methods

The present study was conducted at the Department of Plant breeding, College of Agriculture, Vellayani during the period August 1991 to January 1993.

3.1 Materials

An yellow vein mosaic susceptible local cultivar of Bhindi (*Abelmoschus esculentus* (L.) Moench) variety Anacomban, two semi wild species, resistant to yellow vein mosaic disease, *A. manihot* (L) Medik and *A. manihot* subspecies *tetraphyllus* were used as parents for the hybridization programme in the present study. The major distinguishing characters of the three parents are given in Table 1 and plant type provided in plate 1a to 1c.

Pure seeds of the three parents were collected from the germplasm of Bhindi maintained at the Department of Plant Breeding, College of Agriculture, Vellayani.

3.2. Experimental methods

3.2.1. Crossing Programme

Since Anacomban is having a shorter pre-flowering period compared to the other parents, phased planting was adopted for synchronisation of flowering. The following cross-combinations were attempted.

Table 1. Distinguishing characters of parents

	A. esculentus var. Anacomban	*A. manihot*	*A. manihot* ssp. *tetraphyllus*
Source	College of Agriculture Vellayani	KAU, Vellanikkara	KAU, Vellanikkara
Habit	Annual herb	Perennial shrub	Perennial shrub
Stem	Thick, slightly rough, light green	Thick, slightly rough, light green	Thin, rough, purple
Leaves	Alternate, long stalked, cordate base, acute apex	Alternate, long stalked, cordate base, acute apex	Alternate, long stalked, cordate base, acute apex
Petiole	Long, light green	Long, ventral side sparsely hairy green, dorsal side deep purple	Ventral side sparsely hairy green, dorsal side deep purple
Flower	Solitary, axillary stalked, large buds, ovoid, acute	Solitary, axillary, stalked, large buds, ovoid acute	Solitary, axillary, stalked small buds, ovoid, acute.
Bracteoles	Long, narrow, hairy	Large with appressed stiff hairs	Large with appressed stiff hairs
Calyx	Hairy, light green	Companulate, obtuse, dentate at apex with red tinge	Spathaceous, green with purple tinge

Plate 1. Plant type of parents

a. *Abelmoschus esculentus* var. Anacomban

b. *A. manihot*

c. *A. manihot* ssp. *tetraphyllus*

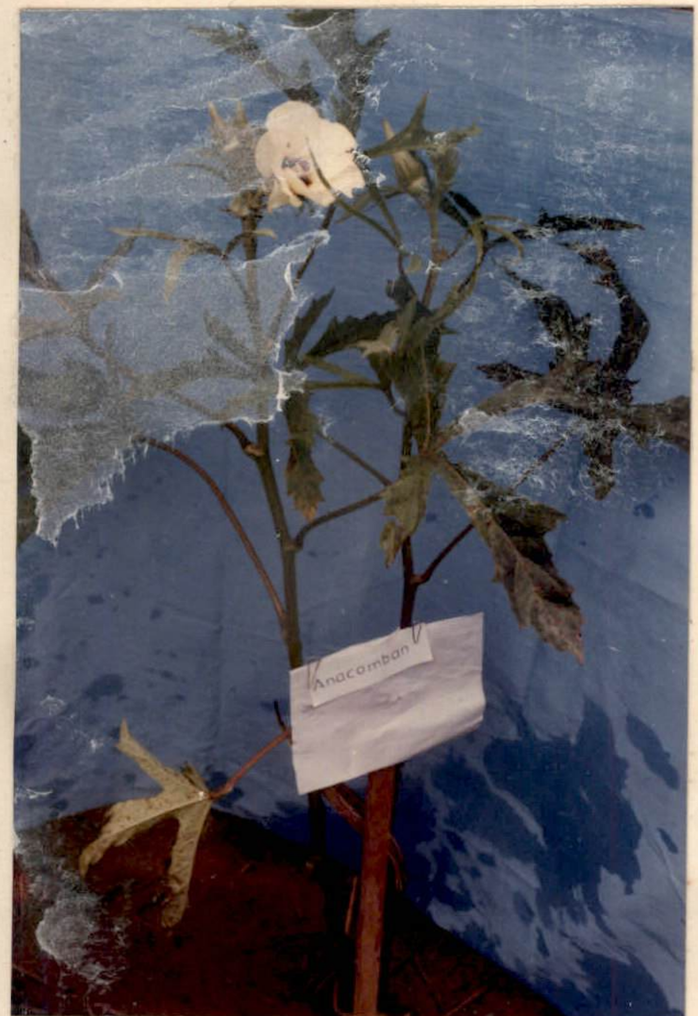


Plate 1a

Plate 1b

Plate 1c

1) *A. esculentus* variety Anacomban x *A. manihot*

2) *A. manihot* x *A. esculentus* variety Anacomban

3) *A. esculentus* variety Anacomban x *A. manihot* ssp. *tetraphyllus*

4) *A. manihot* ssp. *tetraphyllus* x *A. esculentus* variety Anacomban

3.2.1.1. Technique of crossing

The technique of crossing suggested by Giriraj and Rao (1973) was followed. The immature flower buds which would open the next day morning were selected in the previous evening. A shallow circular cut was made around the fused calyx, at about one centimetre from its base. The calyx cup along with corolla were removed as a hood exposing the stigma and staminal tube. The staminal tube was cut open at the base lengthwise without injuring the ovary or style, and removed carefully. The calyx cone which was removed earlier was used for protecting the emasculated flower. As an additional protection, it was covered with a butter paper cover also.

Mature flower buds of the pollen parent were protected by butter paper cover on the previous day of blooming. Pollination was done on the next day morning between 8 a.m. and 9.30 a.m. by rubbing the stigma of the emasculated flower with the staminal column collected from the male parent. The pollinated flowers were again protected by butter paper cover and labelled.

The mature dry fruits were collected thirty to fourty days after pollination and seeds extracted after sundrying the fruits for three days.

3.2.1.2 Percentage of fruit set

Total number of flowers crossed and the number of fruit set were recorded for each cross combination and percentage of fruit set calculated.

3.2.1.3 Mean fruit weight

Dry fruit weight of five fruits from each of three parents and four cross combinations were recorded and averaged.

3.2.1.4 Seed set

Number of normal and shrivelled seeds were counted in each crossed fruit and percentage of seed set was calculated.

3.2.1.5 Seed weight

The hundred seed weight of the three parents and four crosses were recorded.

3.2.1.6 Seed viability

The germinability of the seeds in three parents and four crosses was observed both under laboratory and field conditions. In the laboratory blotting paper method with four replications were used to assess the germinability.

3.2.2. Studies on hybrid fertility

Fifty F_1 plants from each of the crosses along with parents were grown in the field.

3.2.2.1 Pollen sterility

The acetocarmine staining technique (Zirkle, 1937) was used for assessing pollen sterility. Ten mature flowers were randomly picked from each treatment and pollen sterility was estimated as percentage of the number of sterile pollen grains to the total number of pollen grains scored.

3.2.2.2 Pollen viability

Since 25 per cent sucrose solution yielded good results the same was used for pollen viability studies.

Small drops of the 25 per cent sucrose solution were taken on clean glass slides on which the pollen grains were placed. The slides were then inverted and allowed to rest on two small glass rods placed in a petridish. Pollen grains were thus kept in hanging drops of sucrose solution. A humid environment was provided, by placing a wet filter paper at the bottom of the petridish. Counts of germinated and nongerminated pollen grains were recorded after twentyfour hours in three different microscopic fields.

3.2.2.3 Seed set

Number of normal and shrivelled seeds were counted in twentyfive F_1 fruits of each cross and percentage of seed set estimated.

3.2.2.4 Seed weight

Hundred seed weight of three parents and the four F_1 plants were recorded.

3.2.2.5 Seed viability

Blotting paper method with four replications was used to assess the seed viability.

3.2.2.6 Seed sterility

Seed sterility was estimated by immersing them in water and floating ones were considered as sterile.

RESULTS

4. Results

4.1 Crossing programme

4.1.1 Percentage of fruit set

The percentage of fruit set in parents, and in the two direct and two reciprocal crosses are presented in Table 2. The percentage of fruit set among the parents, A. esculentus var. Anacomban, A. manihot and A. manihot ssp. tetraphyllus were 96.00, 99.20 and 99.10, respectively. But in the crosses it ranged from 10.25 in A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus to 78.57 in the cross A. manihot x A. esculentus var. Anacomban. The percentage of fruit set was lower in direct crosses ie., 11.36 and 10.25. However, the reciprocal crosses showed a higher percentage of fruit set ie; 78.57 and 55.95. The percentage of fruit set is graphically represented in Figs.1(a) and 1(b).

The crossed fruits resembled the female parents in all crosses with respect to size and shape.

4.1.2 Fruit weight

The fruit weights of the parents and the four crosses are presented in Table 3. The mean dry fruit weight of A. esculentus var. Anacomban was 8.56 g, while A. manihot had 7.77 g and A. manihot ssp. tetraphyllus weighed 2.42 g. The fruit weight of the

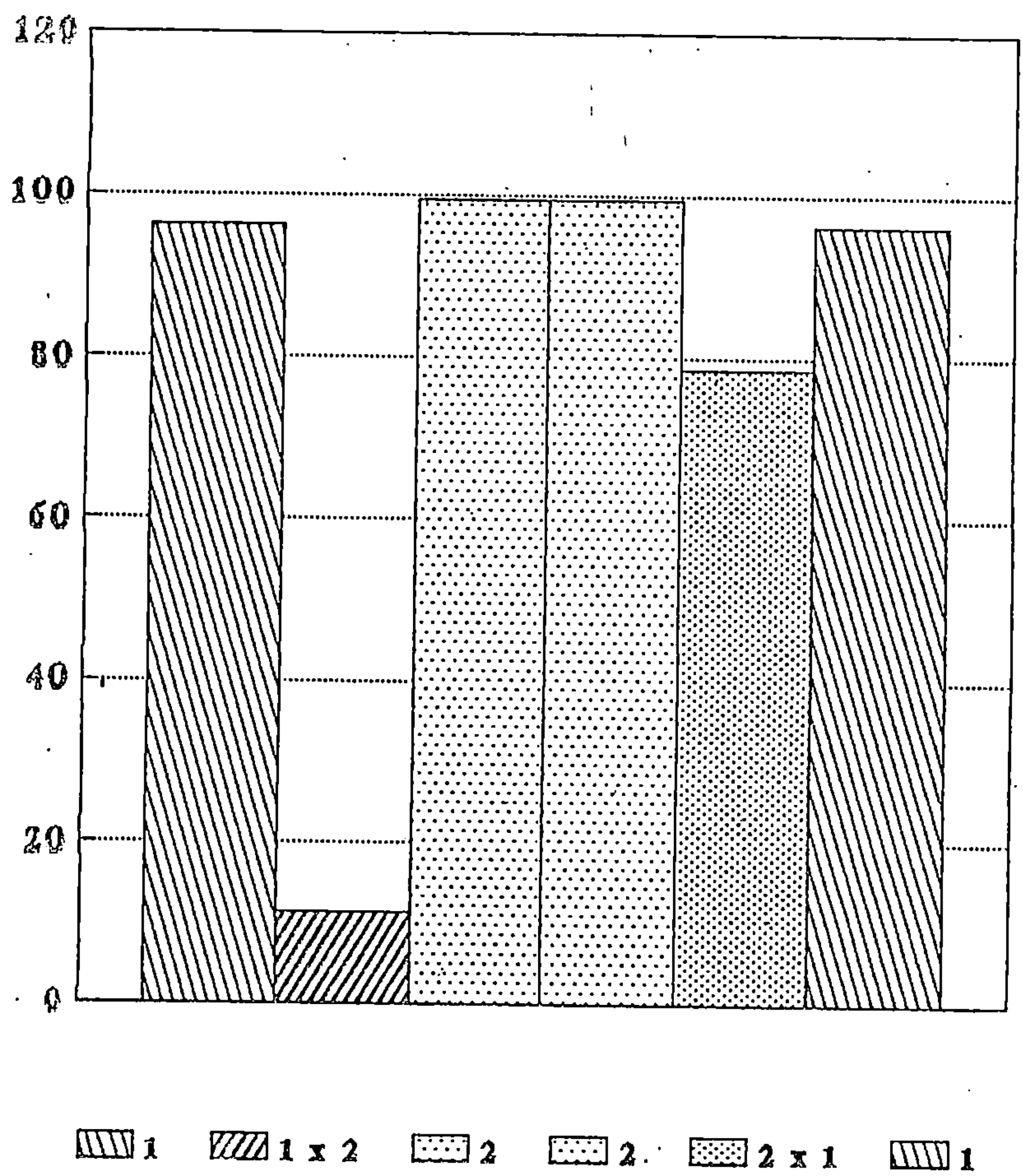
Table 2 - Percentage of Fruit set in parents and in crosses

Parent/crosses	Fruit set (%)
A. esculentus var. Anacomban	96.00
A. manihot	99.20
A. manihot ssp. *tetraphyllus*	99.10
A. esculentus var. Anacomban x *A. manihot*	11.36
A. manihot x *A. esculentus* var. Anacomban	78.57
A. esculentus var. Anacomban x *A. manihot* ssp. *tetraphyllus*	10.25
A. manihot ssp. *tetraphyllus* x *A. esculentus* var. Anacomban	55.95

Table 3 Fruit weight (dry weight) in parents and in crosses

Parent/crosses	Fruit weight (g)
A. esculentus var. Anacomban	8.56
A. manihot	7.77
A. manihot ssp. *tetraphyllus*	2.42
A. esculentus var. Anacomban x *A. manihot*	9.58
A. manihot x *A. esculentus* var. Anacomban	6.54
A. esculentus var. Anacomban x *A. manihot* ssp. *tetraphyllus*	8.89
A. manihot ssp *tetraphyllus* x *A. esculentus* var. Anacomban	0.93

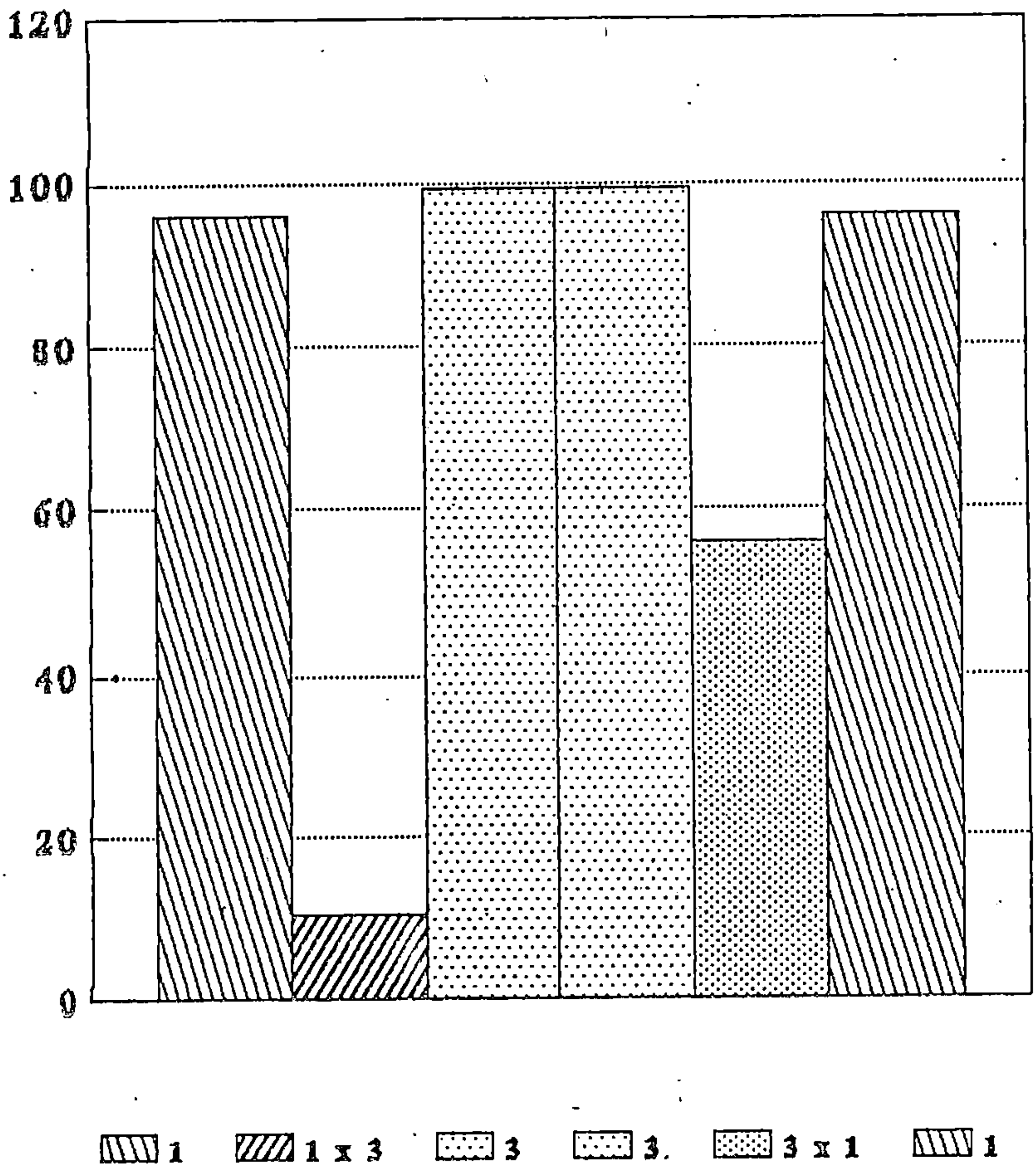
Fig. 1(a) Percentage of Fruit Set



1 A. esculentus var. Anacomban

2 A. manihot

Fig. 1(b) Percentage of Fruit Set



1 A. esculentus var. Anacomban

3 A. manihot ssp. tetraphyllus

cross A. esculentus var. Anacomban x A. manihot was 9.58 g and its reciprocal weighed 6.54 g. In direct crosses the mean fruit weight of crossed fruits transgressed the mean weight of parents. In reciprocal crosses the crossed fruits weighed lower than the mean fruit weight of the parents. In the cross A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus the crossed fruit weighed 8.89 g while the reciporcal weighed only 0.93 g. In this combination also the direct crossed fruit weighed more than the parental means. However, the reciprocal crossed fruit weighed less than the parental means. The variation in fruit weight among the parents and the four crosses is graphically represented in Figs. 2(a) and 2(b).

4.1.3 Seed set

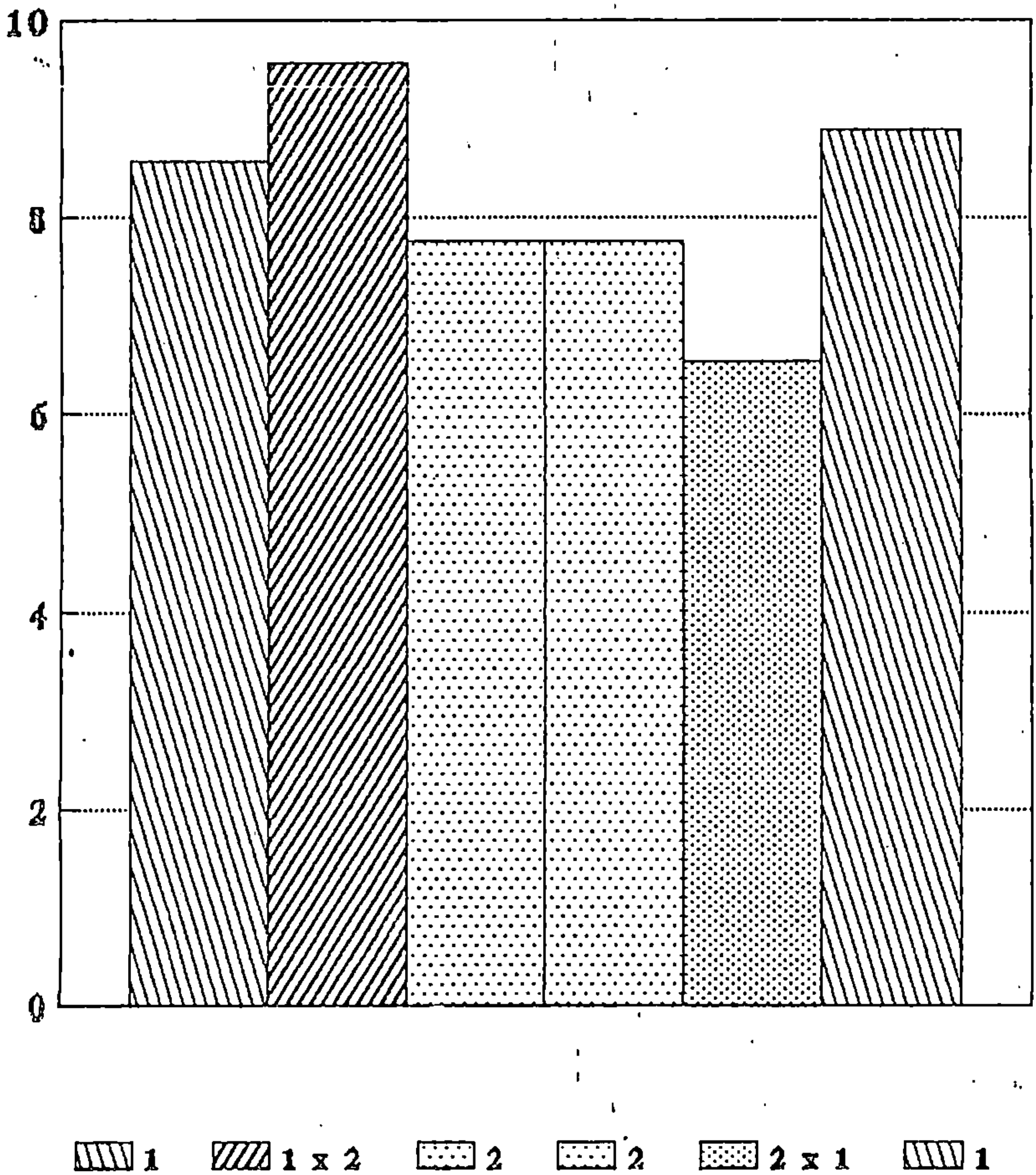
The percentage of seed set among the parents A. esculentus var. Anacomban, A. manihot and A. manihot ssp. tetraphyllus was 93.00, 94.02 and 97.05, respectively. The seed set among the three parents and the four crosses are presented in Table 4.

In the direct cross of A. esculentus var. Anacomban x A. manihot the mean seed set was 64.62 per cent while its reciprocal cross recorded 77.33 per cent. The seed set was lower in both direct and reciprocal crosses when compared to parents. In the cross between A. esculentus var. Anacomban and A. manihot the

Table 4 Seed set in parents and in crossed fruits

Parents/crosses	Seed set (%)
A. esculentus var. Anacomban	93.00
A. manihot	94.02
A. manihot ssp. *tetraphyllus*	97.05
A. esculentus var. Anacomban x *A. manihot*	64.42
A. manihot x *A. esculentus* var. Anacomban	77.33
A. esculentus var. Anacomban x *A. manihot* ssp. *tetraphyllus*	66.32
A. manihot ssp. *tetraphyllus* x *A. esculentus* var. Anacomban	54.17

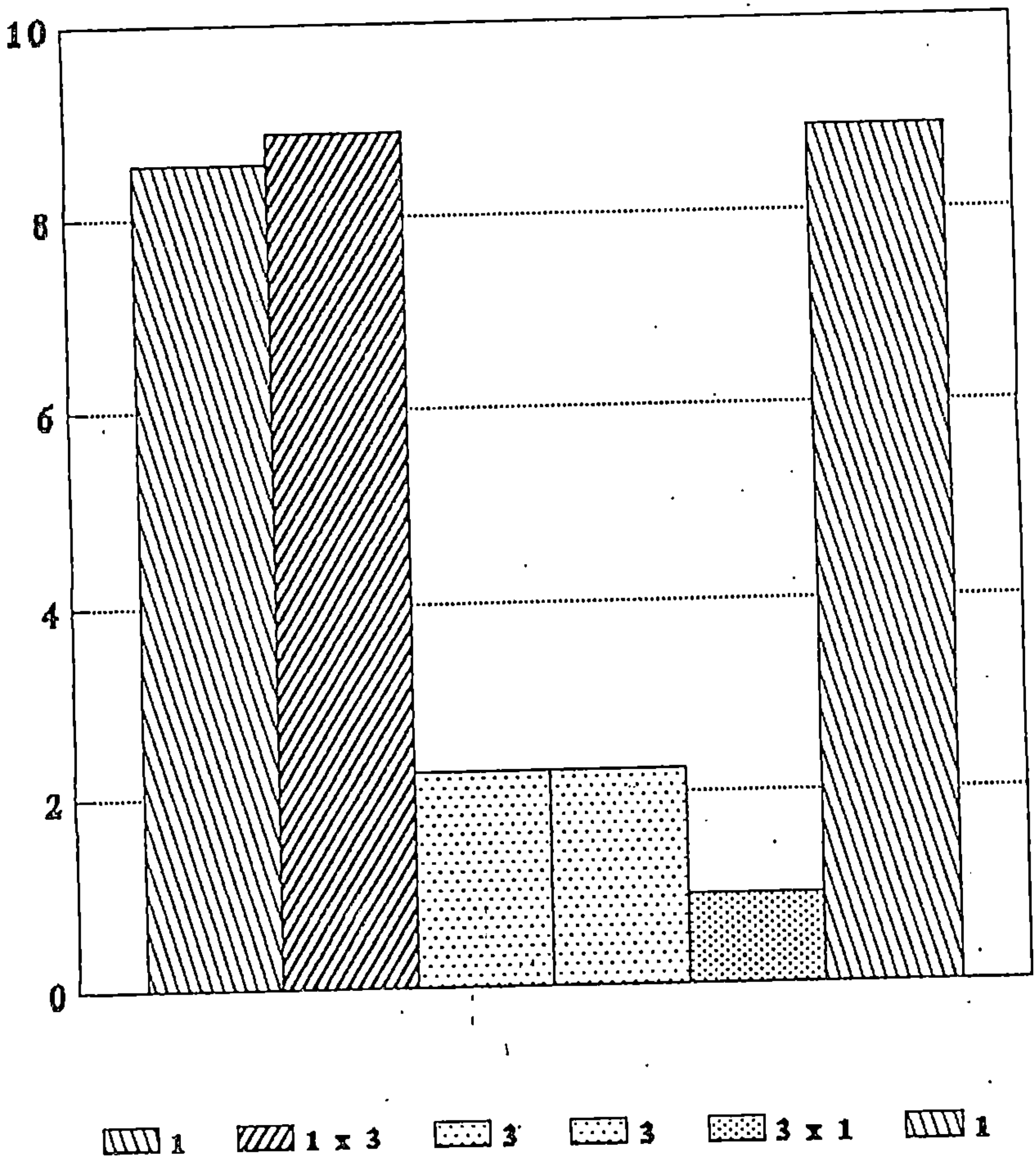
Fig. 2(a) Fruit weight (grams)



1 A. esculentus var. Anacomban

2 A. manihot

Fig. 2(b) Fruit weight (grams)



1 A. esculentus var. Anacomban

3 A. manihot ssp. tetraphyllus

seed set was higher in reciprocal cross when compared to the direct cross which is diagrammatically represented in Fig. 3(a).

The seed set between the cross A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus was 66.32 per cent and 54.17 per cent in the direct and reciprocal crosses, respectively. The percentage of seed set was lower in crossed fruits when compared to parents. In the direct cross between A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus the seed set was higher ie, 66.32 per cent while its reciprocal cross registered only 54.17 per cent which is diagrammatically represented in Fig. 3(b).

4.1.4 Seed weight

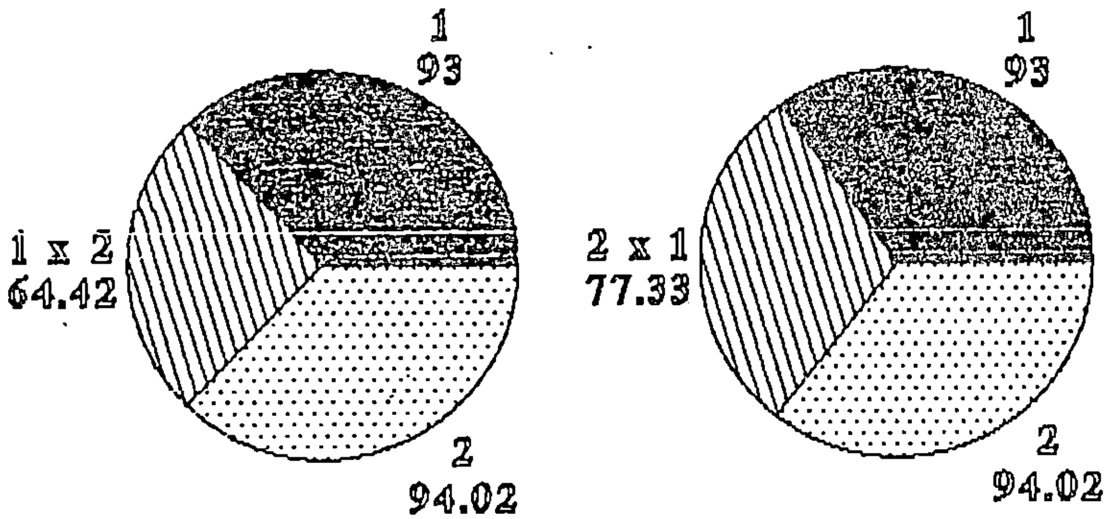
The data presented in Table 5 reveals the mean seed weights of the parents and four crosses. The mean seed weight of the three parents A. esculentus var. Anacomban, A. manihot and A. manihot ssp. tetraphyllus were 6.61 g, 5.70 g and 2.35 g respectively. The weight of the crossed seed ranged from 2.30 g to 6.54 g.

As regards the mean weight of the crossed seed, between A. esculentus var. Anacomban and A. manihot it was 5.65 g while its reciprocal cross weighed 5.04 g. The weight of the crossed seed was lower than the weight of the female parent in each of the

Table 5 Mean seed weight in parents and crosses

Parents/crosses	Hundred seed wt. (g)
A. esculentus var. Anacomban	6.61
A. manihot	5.70
A. manihot ssp. *tetraphyllus*	2.35
A. esculentus var. Anacomban x *A. manihot*	5.65
A. manihot x *A. esculentus* var. Anacomban	5.04
A. esculentus var. Anacomban x *A. manihot* ssp. *tetraphyllus*	6.54
A. manihot ssp. *tetraphyllus* x *A. esculentus* var. Anacomban	2.30

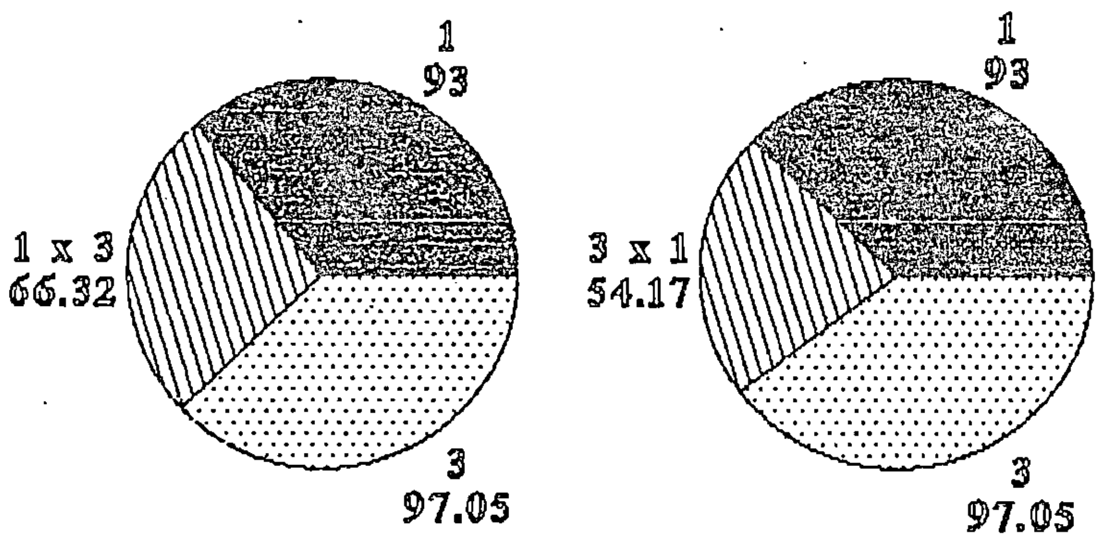
Fig. 3(a) Seed set in crossed fruits (percentage)



1 *A. esculentus* var. Anacomban

2 *A. manihot*

Fig. 3(b) Seed set in crossed fruits (percentage)



1 _A. esculentus_ var. Anacomban

3 _A. manihot_ ssp. _tetraphyllus_

crosses. In the direct cross it was 5.65 g which is less than the weight of A. esculentus var. Anacomban (6.61 g). Likewise in the reciprocal cross it was 5.04 g which is less than the weight of A. manihot (5.7 g). The mean weight of the crossed seed of A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus was 6.54 g and that of its reciprocal cross was only 2.3 g. The weight of the reciprocal cross seed was less than the weight of the direct cross and the female parent. In direct cross the weight of the crossed seed was less than the seed weight of the female parent. In the reciprocal cross also the seed weight (2.30 g) was less than that of the female parent namely A. manihot ssp. tetraphyllus (2.35 g). The crossed seeds in all the crosses resembled the female parents with respect to size and shape. The variation in seed weight among the parents and the crosses is graphically represented in Figs. 4(a) and 4(b).

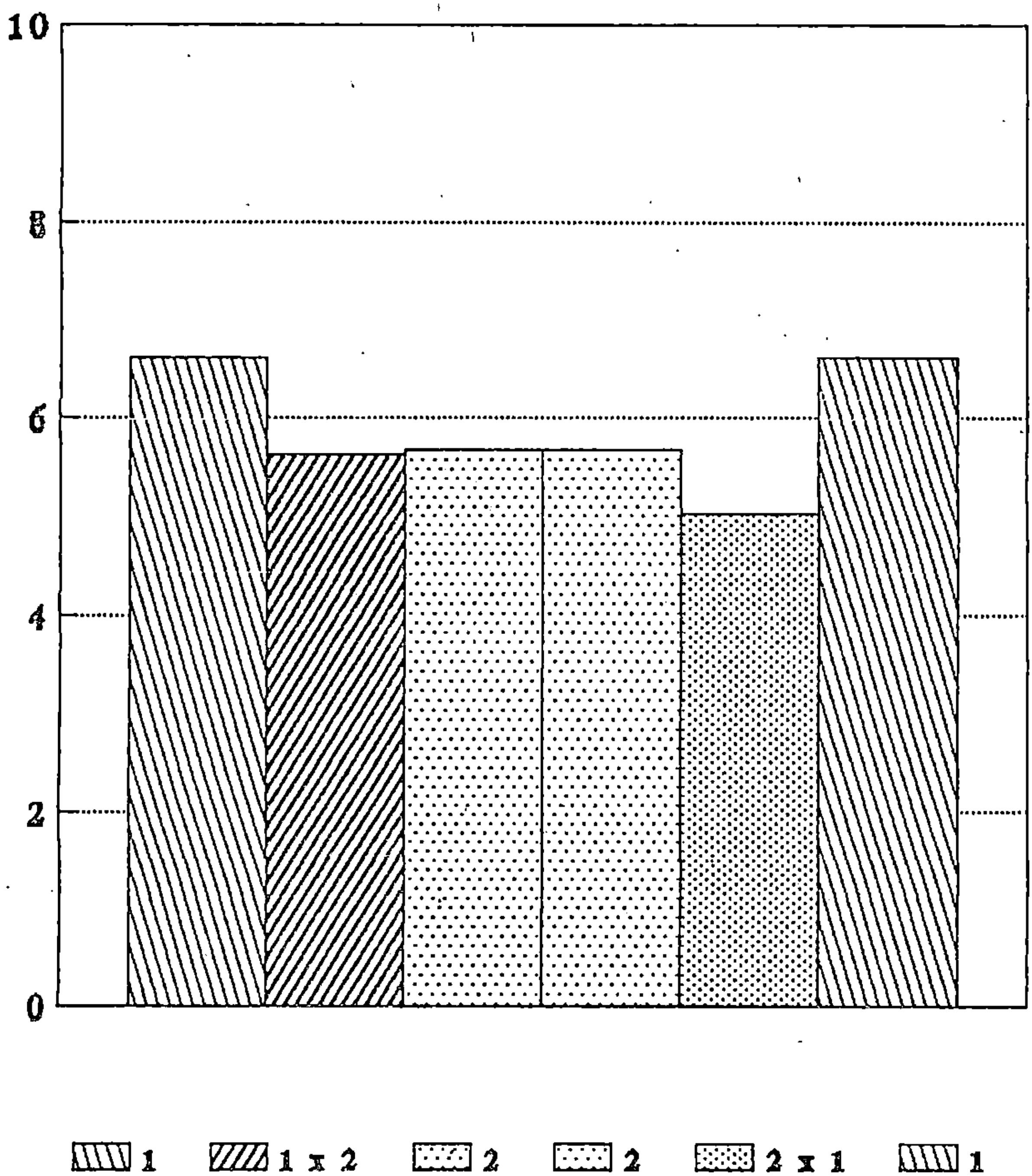
4.1.5 Seed viability

The seed viability of the parents and crossed seeds is presented in Table 6. The parents, A. esculentus var. Anacomban, A. manihot and A. manihot ssp. tetraphyllus registered a germination percentage of 100, 90 and 70 respectively. In crosses, germination percentage ranged from 35 in the cross A. manihot ssp. tetraphyllus x A. esculentus var. Anacomban to 100 in the cross A. manihot x A. esculentus var. Anacomban, which is diagrammatically represented in Figs. 5(a) and 5(b).

Table 6 Seed viability in parents and hybrids

Parents/crosses	Laboratory germination (%)	Field germination (%)
A. esculentus var. Anacomban	100	79
A. manihot	90	85
A. manihot ssp. *tetraphyllus*	70	48
A. esculentus var. Anacomban x *A. manihot*	70	88
A. manihot x *A. esculentus* var. Anacomban	100	81
A. esculentus var. Anacomban x *A. manihot* ssp. *tetraphyllus*	95	90
A. manihot ssp. *tetraphyllus* x *A. esculentus* var. Anacomban	35	26

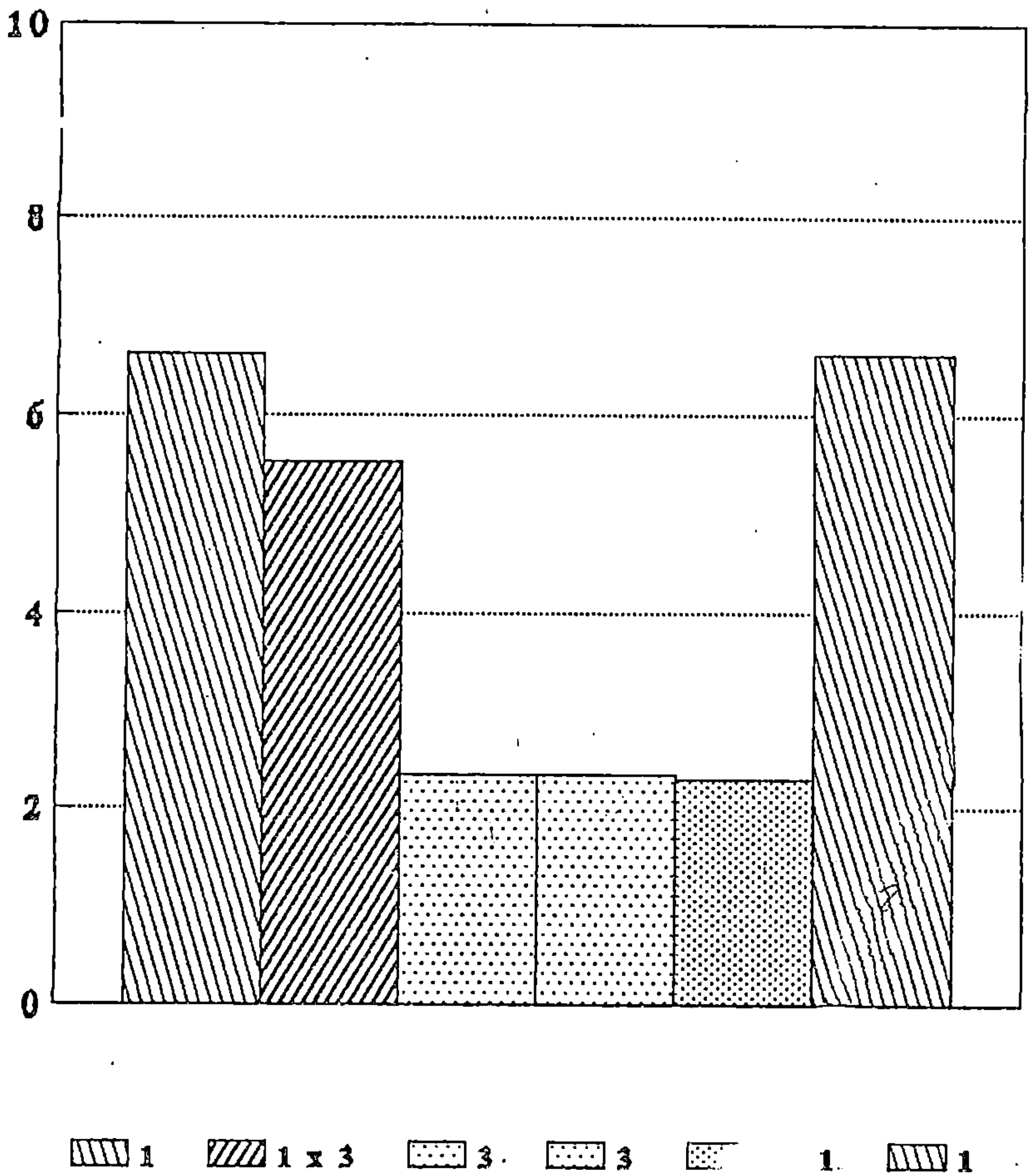
Fig. 4(a) Mean seed weight in parents and crosses



1 A. esculentus var. Anacomban

2 A. manihot .

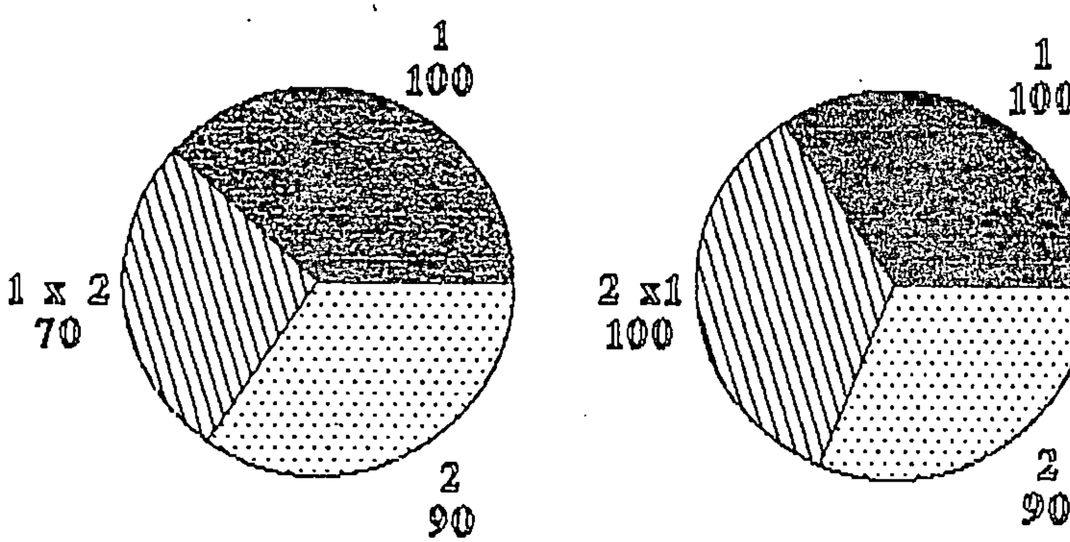
Fig. 4(b) Mean seed weight in parents and crosses



1 *A. esculentus* var. Anacomban

3 *A. manihot* ssp. *tetraphyllus*

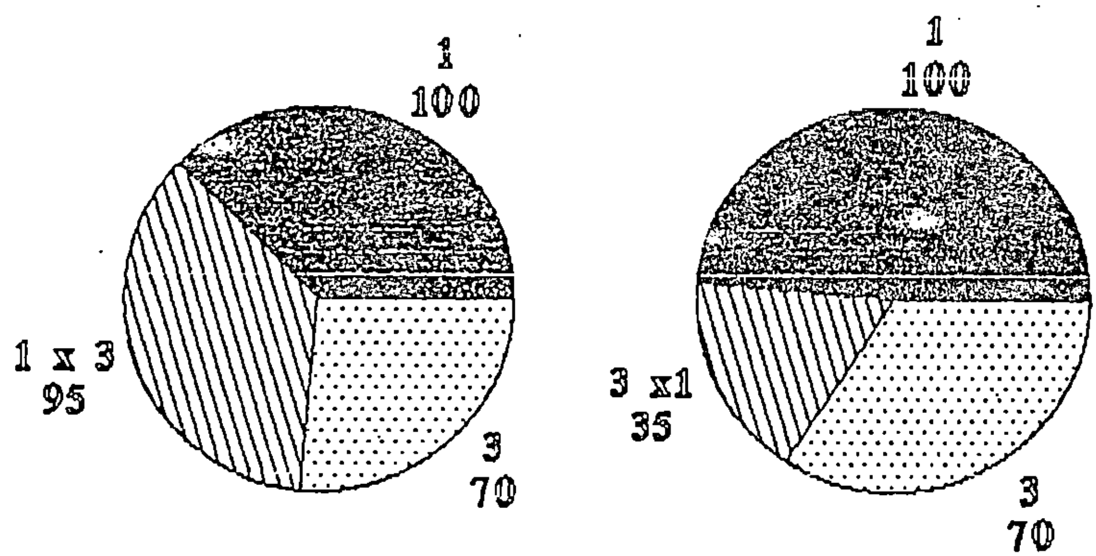
Fig. 5(a) Seed viability (percentage)



1 *A. esculentus* var. Anacomban

2 *A. manihot*

Fig. 5(b) Seed viability (percentage)



1 A. esculentus var. Anacomban

3 A. manihot ssp. tetraphyllus

In the cross A. esculentus var. Anacomban x A. manihot the crossed seed recorded a germination percentage of 70, while the seeds of its reciprocal cross registered 100 per cent germination. In the cross A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus the crossed seeds showed a higher germination percentage (95) while the seeds of its reciprocal cross recorded a low germination percentage (35).

In the field conditions except for the cross A. esculentus var. Anacomban x A. manihot, all the parents and crosses recorded a reduced germination percentage than in the laboratory conditions. The four hybrid plants are depicted in Plates 2a to 2d.

4.2 Hybrid sterility

4.2.1 Pollen sterility

Pollen sterility among parents and hybrids is depicted in Table 7. All the hybrids recorded a higher percentage of pollen sterility than the parents. The pollen sterility in A. esculentus var. Anacomban, A. manihot and A. manihot ssp. tetraphyllus were 3.12 per cent, 7.61 per cent and 5.56 per cent, respectively. In the hybrids the pollen sterility ranged from 16.48 per cent in A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus to 58.77 per cent in A. manihot x A. esculentus var. Anacomban. The hybrid of the cross A. esculentus var. Anacomban x A. manihot exhibited

Table 7 Pollen sterility in parents and hybrids

Parents/crosses	Pollen sterility (%)
A. esculentus var. Anacomban	3.12
A. manihot	7.61
A. manihot ssp. *tetraphyllus*	5.56
A. esculentus var. Anacomban x *A. manihot*	46.80
A. manihot x *A. esculentus* var. Anacomban	58.77
A. esculentus var. Anacomban x *A. manihot* ssp. *tetraphyllus*	16.48
A. manihot ssp. *tetraphyllus* x *A. esculentus* var. Anacomban	25.41

Plate 2 Plant type of hybrids

a. *A. esculentus* var. Anacomban x *A. manihot*

b. *A. manihot* x *A. esculentus* var. Anacomban

c. *A. esculentus* var. Anacomban x *A. manihot* ssp. *tetraphyllus*

d. *A. manihot* ssp. *tetraphyllus* x *A. esculentus* var. Anacomban.

Plate 2a

Plate 2b

plate 2c

plate 2d

46.8 per cent pollen sterility and its reciprocal exhibited pollen sterility of 58.77 per cent. The hybrid of the cross A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus manifested 16.48 per cent pollen sterility while its reciprocal cross exhibited 25.4 per cent pollen sterility. In both cases pollen sterility was higher in reciprocal crosses than direct crosses. The pollen sterility exhibited by the parents and hybrids is graphically represented in Figs. 6(a) and 6(b). Plates 3a to 3g presents the pollen sterility among parents and hybrids.

4.2.2 Pollen viability

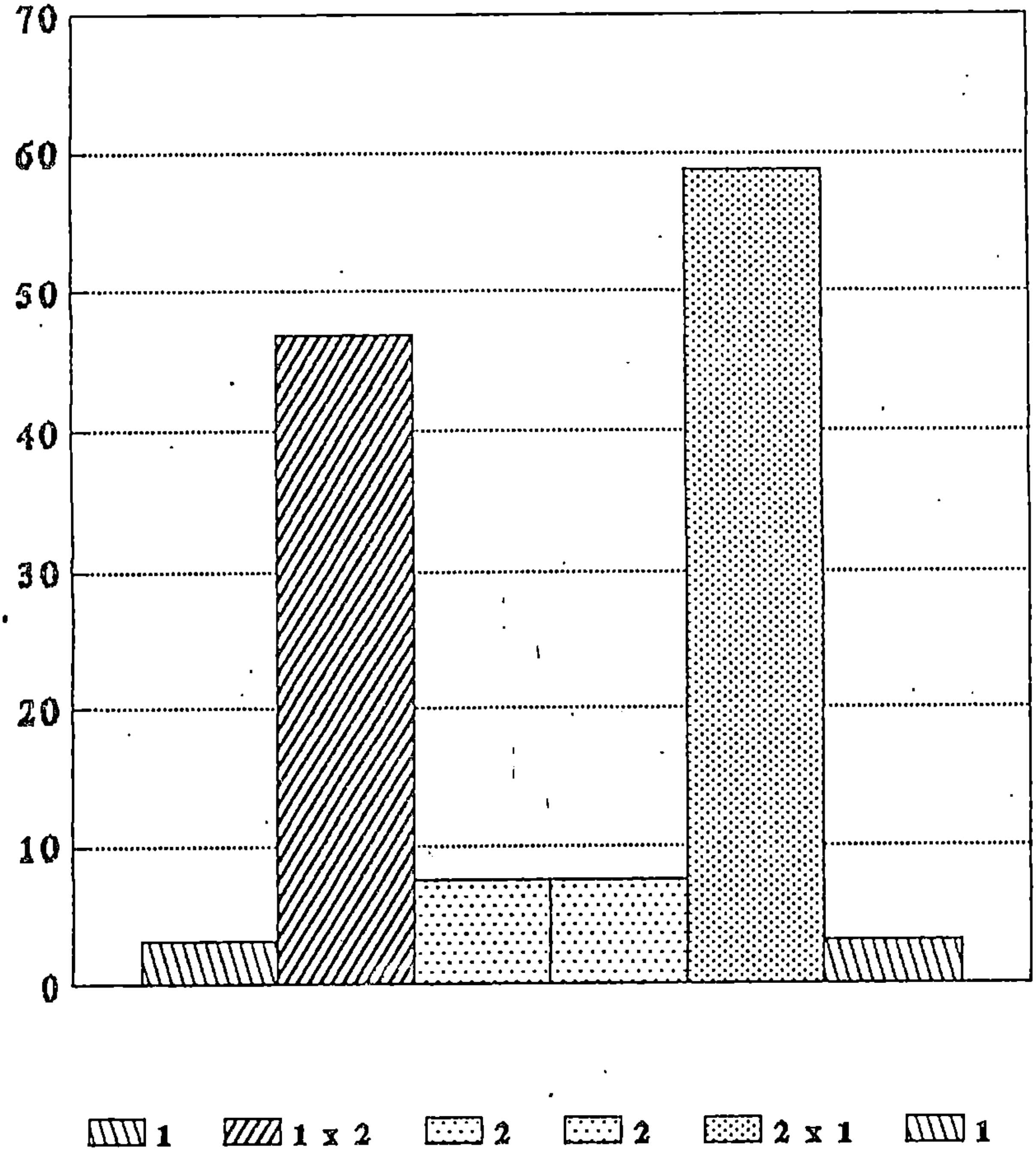
Pollen viability of the parents and hybrids are presented in Table 8. A. esculentus var. Anacomban, A. manihot and A. manihot ssp. tetraphyllus recorded 91.50, 86.70 and 88.00 per cent pollen sterility respectively.

Among the hybrids A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus showed highest percentage of pollen viability viz., 76.8 per cent and A. manihot x A. esculentus var. Anacomban manifested lowest being 28 per cent. The variation in pollen viability is represented graphically in Figs. 7(a) an. 7(b). The pollen viability was directly proportional to the pollen fertility. In crosses where the pollen sterility, was high the viability was low. The hybrids between the cross A. esculentus

Table 8 Pollen Viability in parents and hybrids

Parents/crosses	Pollen viability (%)
A. esculentus var. Anacomban	91.50
A. manihot	86.70
A. manihot ssp. *tetraphyllus*	88.00
A. esculentus var. Anacomban x *A. manihot*	40.00
A. manihot x *A. esculentus* var. Anacomban	28.00
A. esculentus var. Anacomban x *A. manihot* ssp. *tetraphyllus*	76.80
A. manihot ssp. *tetraphyllus* x *A. esculentus* var. Anacomban	74.35

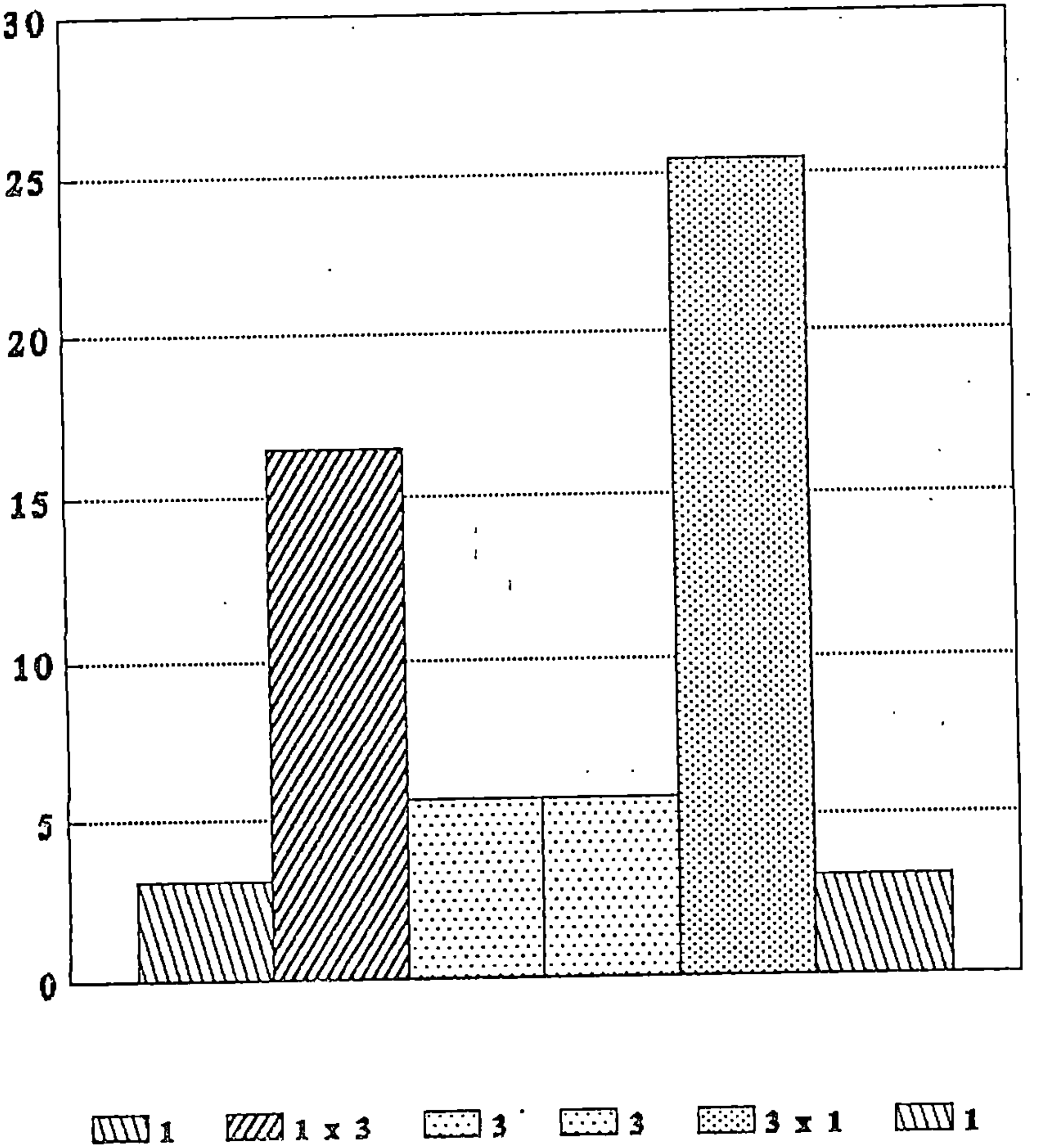
Fig. 6(a) Pollen sterility in parents and hybrids (Percentage)



1 A. esculentus var. Anacomban

2 A. manihot

Fig. 6(b) Pollen sterility in parents and hybrids (percentage)



1 *A. esculentus* var. Anacomban

3 *A. manihot* ssp. *tetraphyllus*

Plate 3 Pollen sterility in parents and hybrids

a. *A. esculentus* var. Anacomban

b. *A. manihot*

c. *A. manihot* ssp. *tetraphyllus*

d. *A. esculentus* var. Anacomban x *A. manihot*

e. *A. manihot* x *A. esculentus* var. Anacomban

f. *A. esculentus* var. Anacomban x *A. manihot* ssp. *tetraphyllus.*

g. *A. manihot* ssp. *tetraphyllus* x *A. esculentus* var. Anacomban

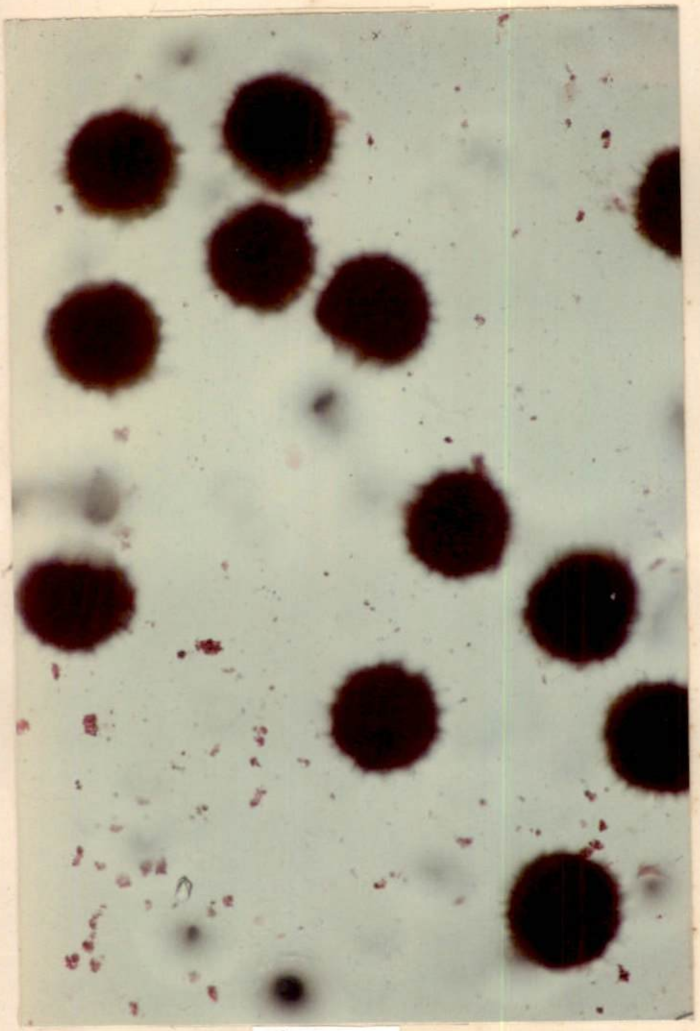


plate 3a

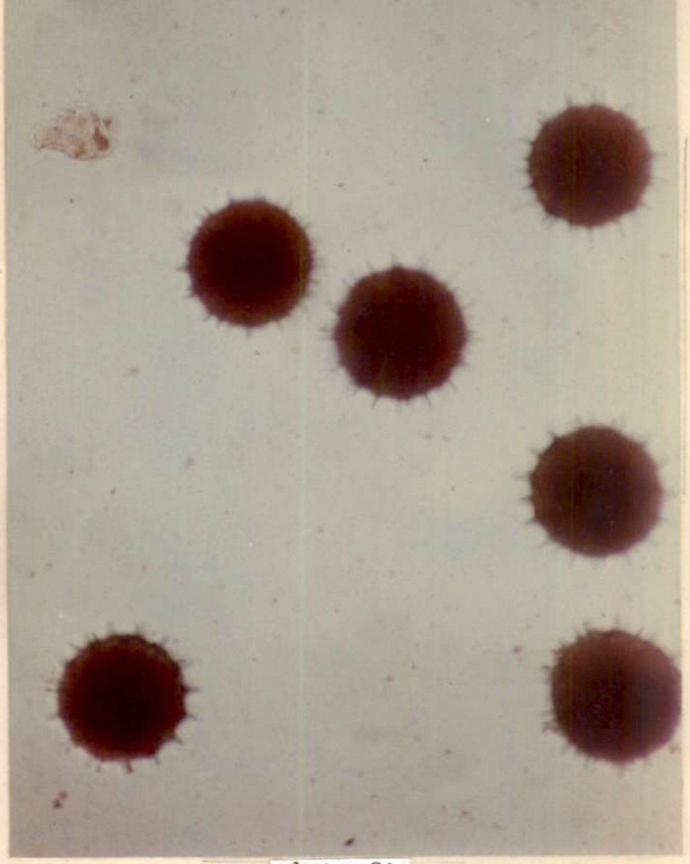
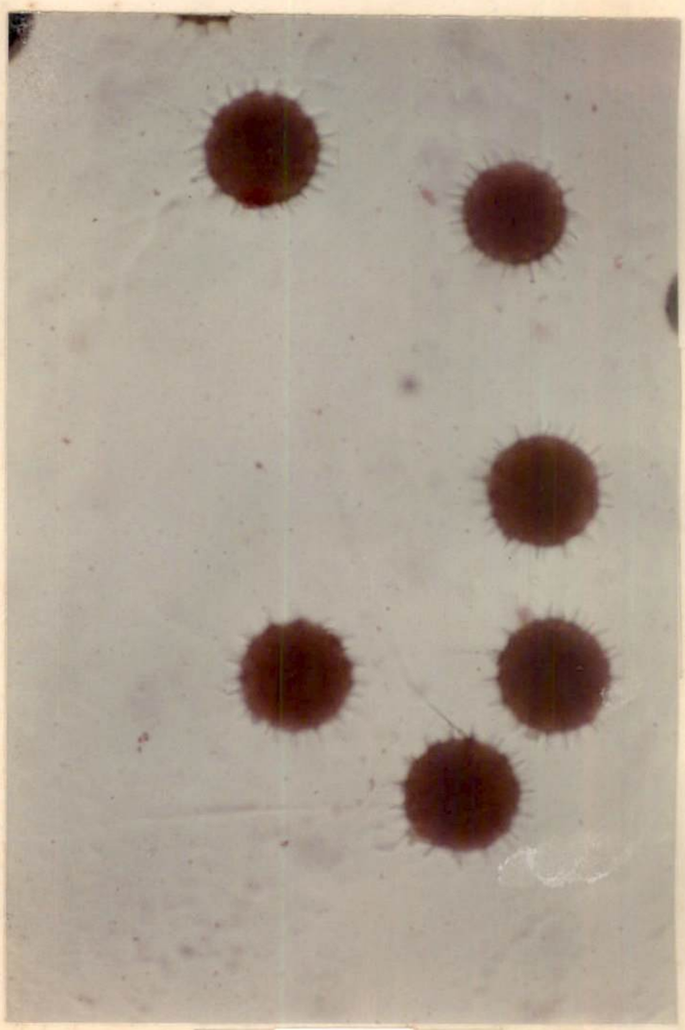


plate 3b



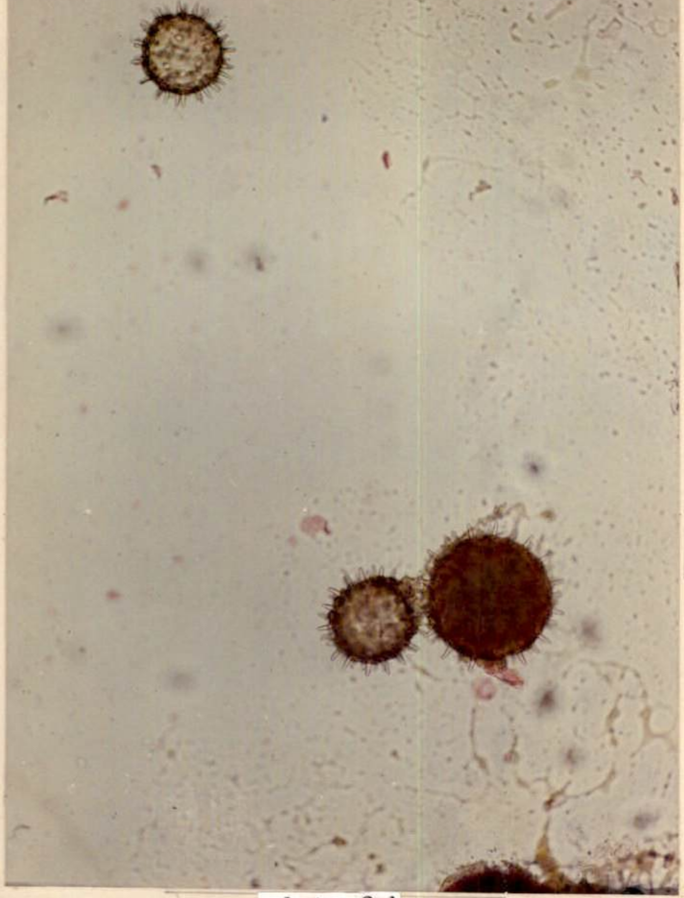


plate 3d

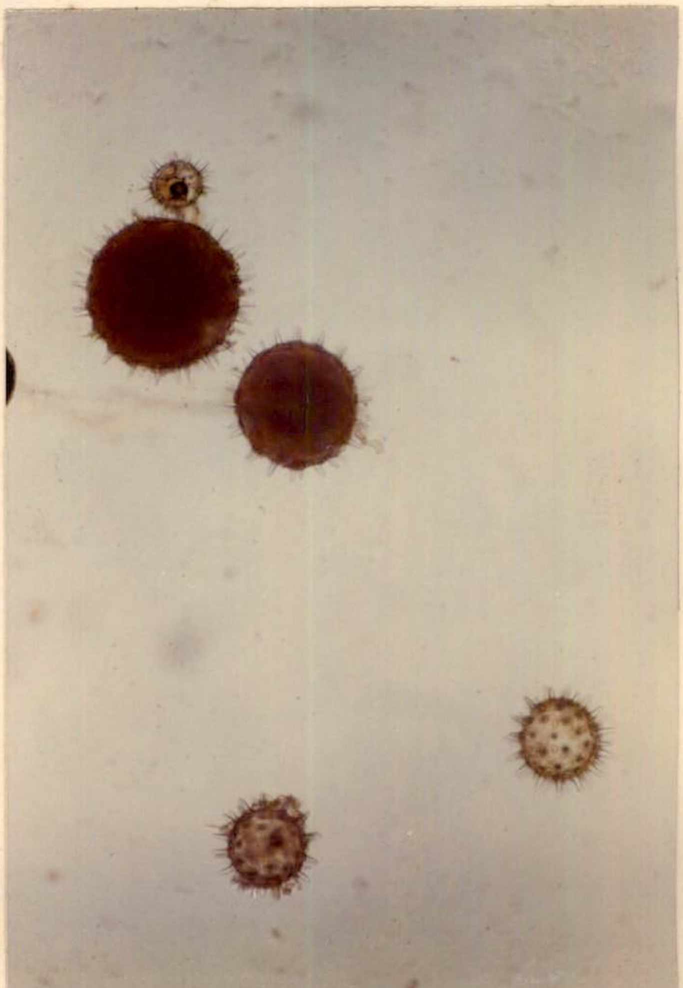


plate 3e

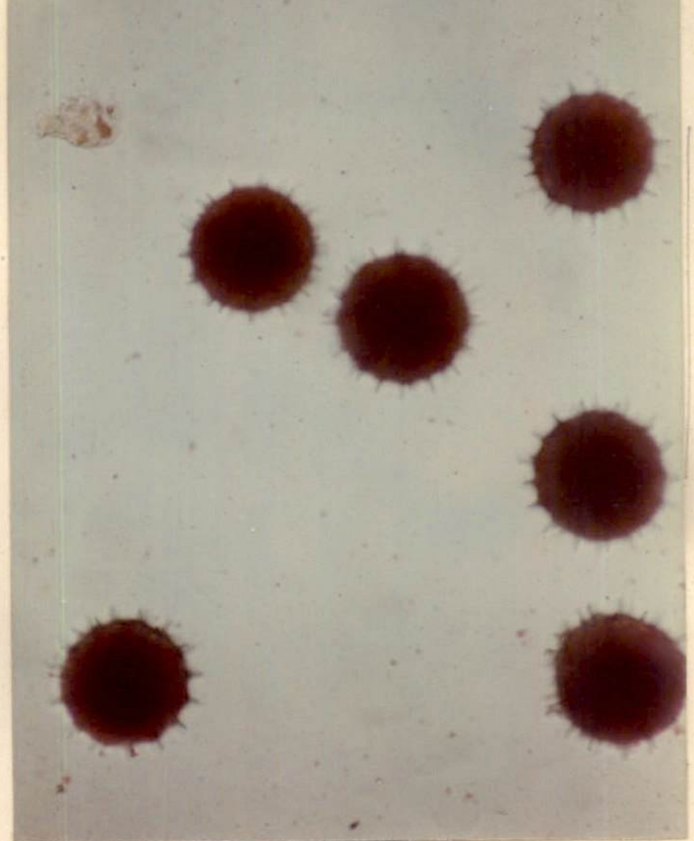


plate 3b

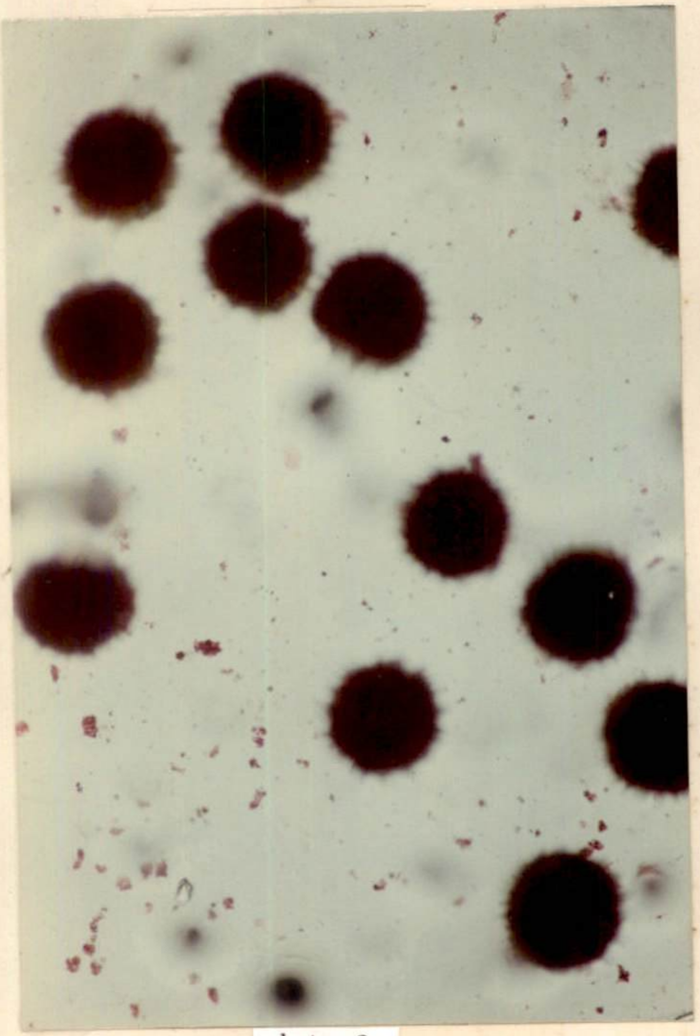


plate 3a

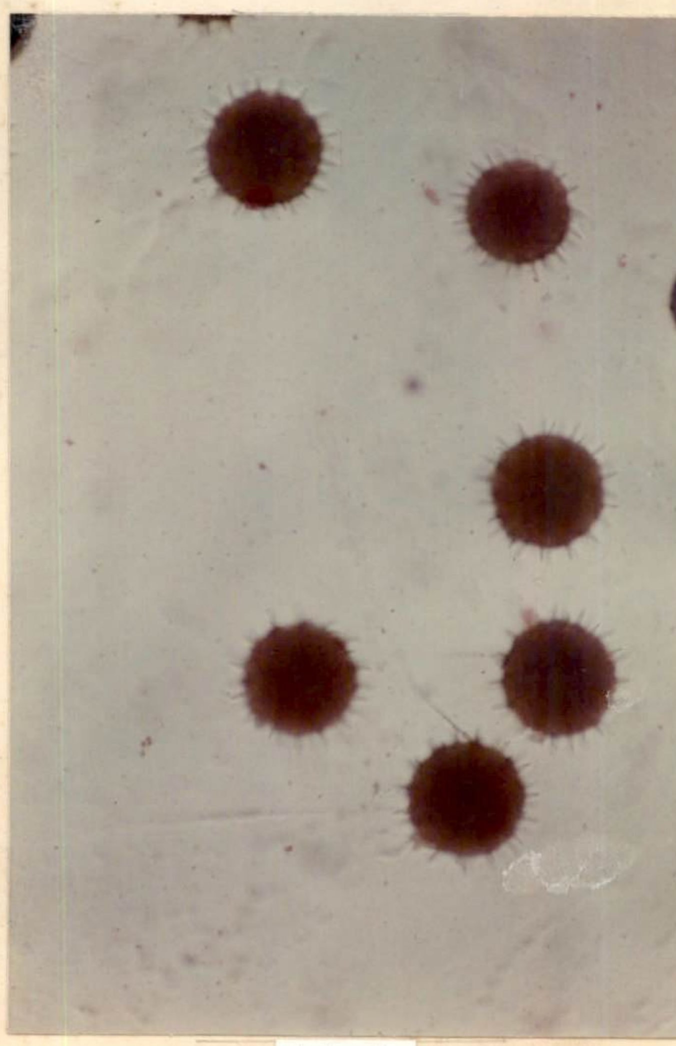
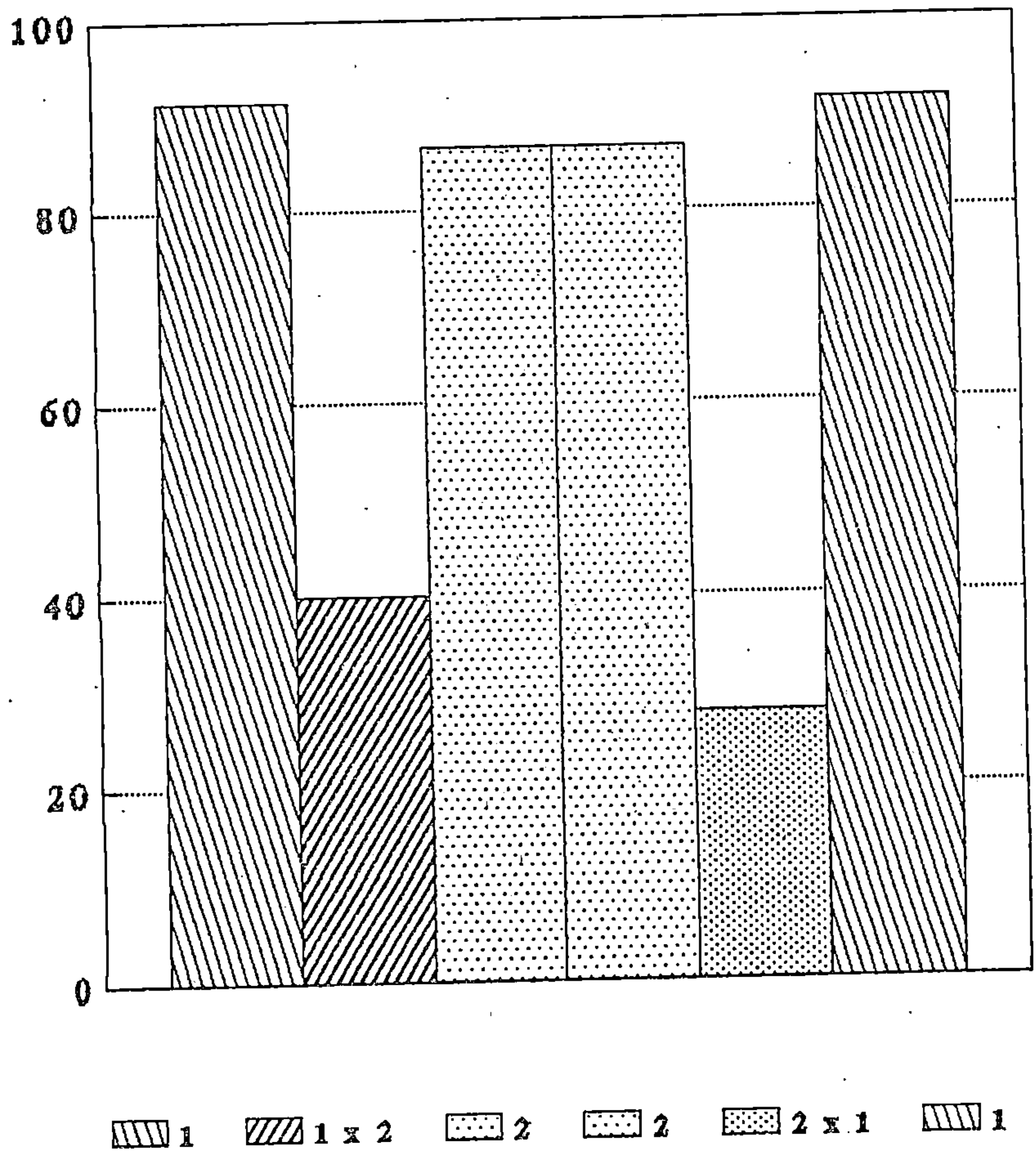


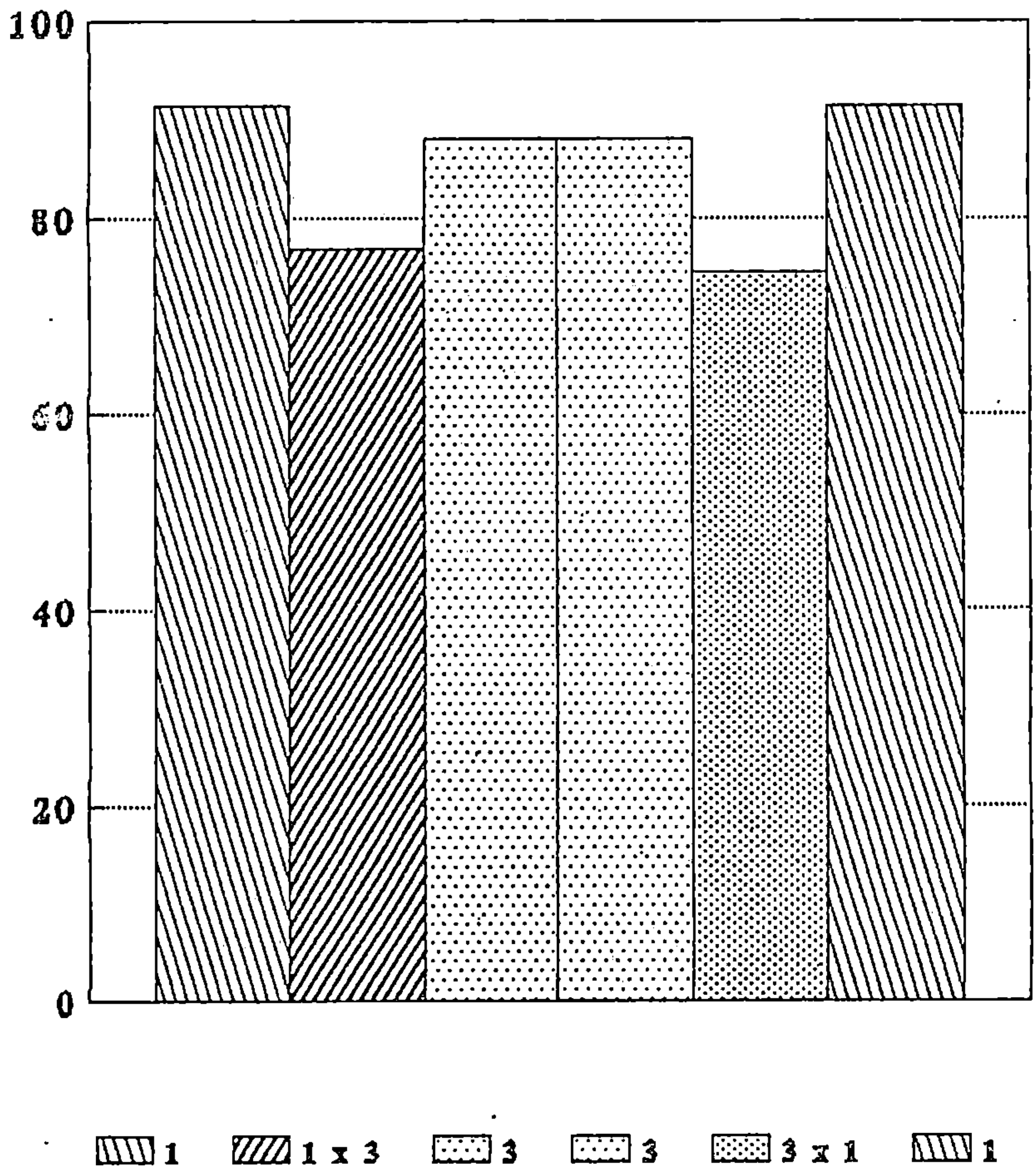
Fig. 7(a) Pollen viability (percentage)



1 <u>A. esculentus</u> var. Anacomban

2 <u>A. manihot</u>

Fig. 7(b) Pollen viability (percentage)



1 *A. esculentus* var. Anacomban

3 *A. manihot* ssp. *tetraphyllus*

var. Anacomban x *A. manihot* exhibited 46.8 per cent pollen sterility while its reciprocal exhibited 58.77 per cent pollen sterility. The pollen viability in above case was comparitively low which exhibited 40 and 28 per cent respectively. The pollen sterility in the hybrids of the cross *A. esculentus* var. Anacomban x *A. manihot* ssp. *tetraphyllus* was 16.48 per cent as against its reciprocal which was 25.41 per cent. The pollen viability in the above case was 76.80 and 74.35 per cent respectively.

4.2.3 Seed set

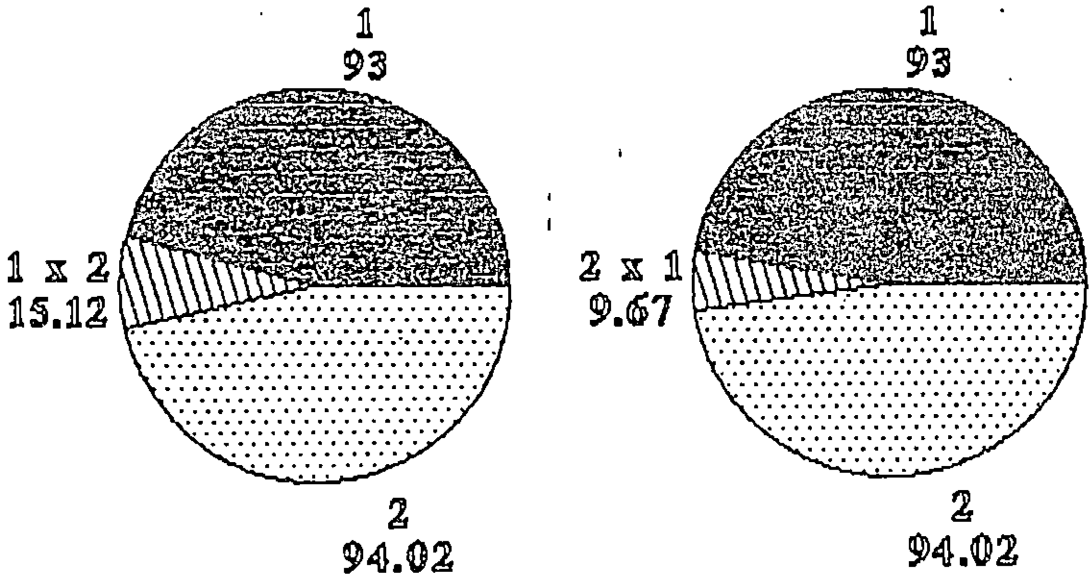
The seed set in parents and the hybrids expressed as percentage is presented in Table 9. The seed set in the parents, *A. esculentus* var. Anacomban, *A. manihot* and *A. manihot* spp. *tetraphyllus* was 93.00, 94.02 and 97.05 per cent respectively.

The seed set was 15.13 per cent in the hybrid of the *A. esculentus* var. Anacomban x *A. manihot* and 9.68 per cent in its reciprocal. Here the seed set was higher in direct crosses compared to reciprocals. The seed set was 58.22 per cent in the hybrid of the cross *A. esculentus* var. Anacomban x *A. manihot* ssp. *tetraphyllus* and 60.17 per cent in its reciprocal. In this cross the seed set was higher in reciprocal cross compared to direct cross. In general the seed set was low in hybrids as depicted in Plates 4 a to 4 d. The variation in seed set among the parents and the hybrids is diagrammatically represented in Figs. 8(a) to 8(b).

Table 9 Seed set in parents and F_1 plants

Parents/crosses	Seed set (%)
A. esculentus var. Anacomban	93.00
A. manihot	94.02
A. manihot ssp. *tetraphyllus*	97.02
A. esculentus var. Anacomban x *A. manihot*	15.13
A. manihot x *A. esculentus* var. Anacomban	9.68
A. esculentus var. Anacomban x *A. manihot* ssp. *tetraphyllus*	58.22
A. manihot ssp. *tetraphyllus* x *A. esculentus* var. Anacomban	60.16

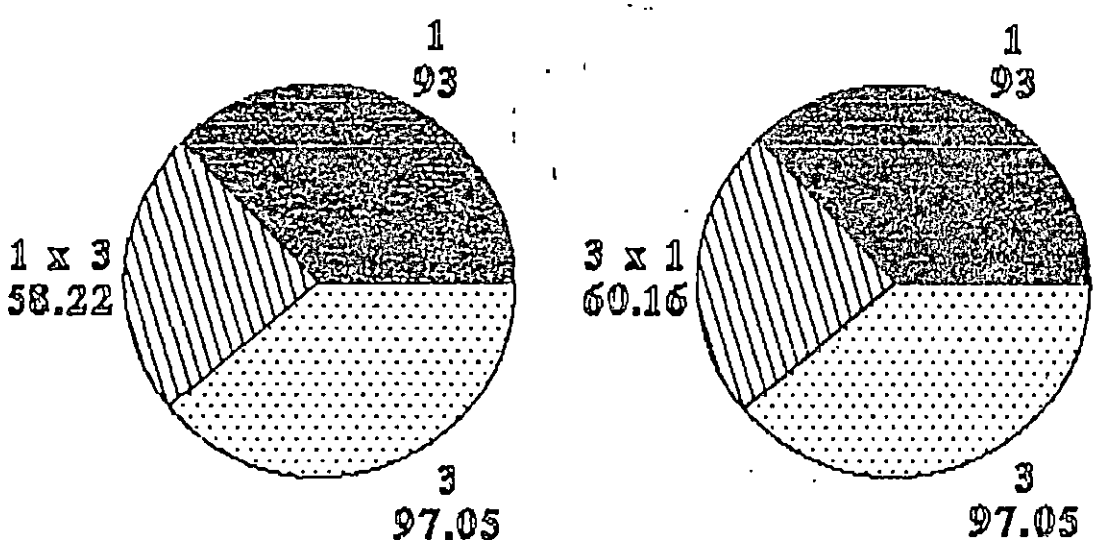
Fig. 8(a) Seed set in parents and F_1 plants (percentage)



1 *A. esculentus* var. Anacomban

2 *A. manihot*

Fig. 8(b) Seed set in parents and F_1 plants (percentage)



1 *A. esculentus* var. Anacomban

3 *A. manihot* ssp. *tetraphyllus*

Plate 4 Seed set in F_1 Plants

a. *A. esculentus* var. Anacomban x *A. manihot*

b. *A. manihot* x *A. esculentus* var. Anacomban

c. *A. esculentus* var. Anacomban x *A. manihot* ssp. *tetraphyllus*.

d. *A. manihot* ssp. *tetraphyllus* x *A. esculentus* var. Anacomban

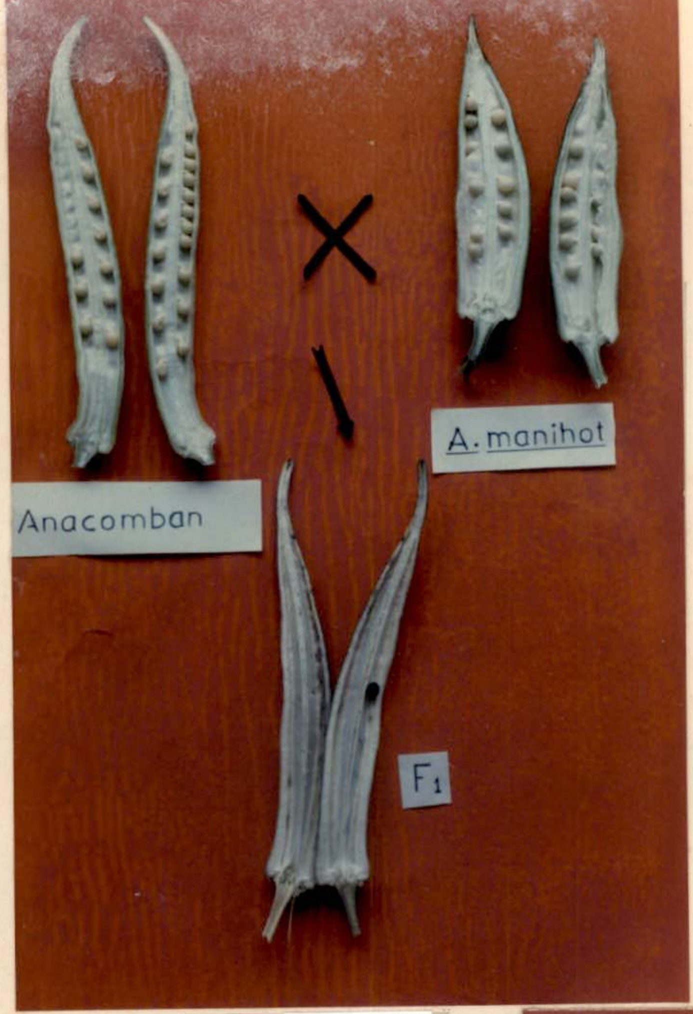
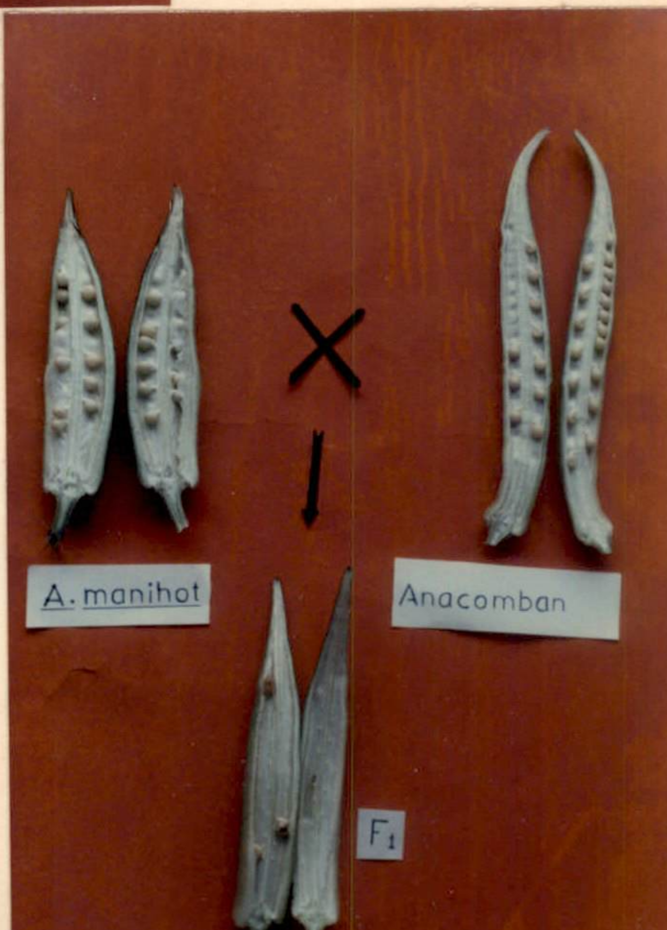


plate 4a



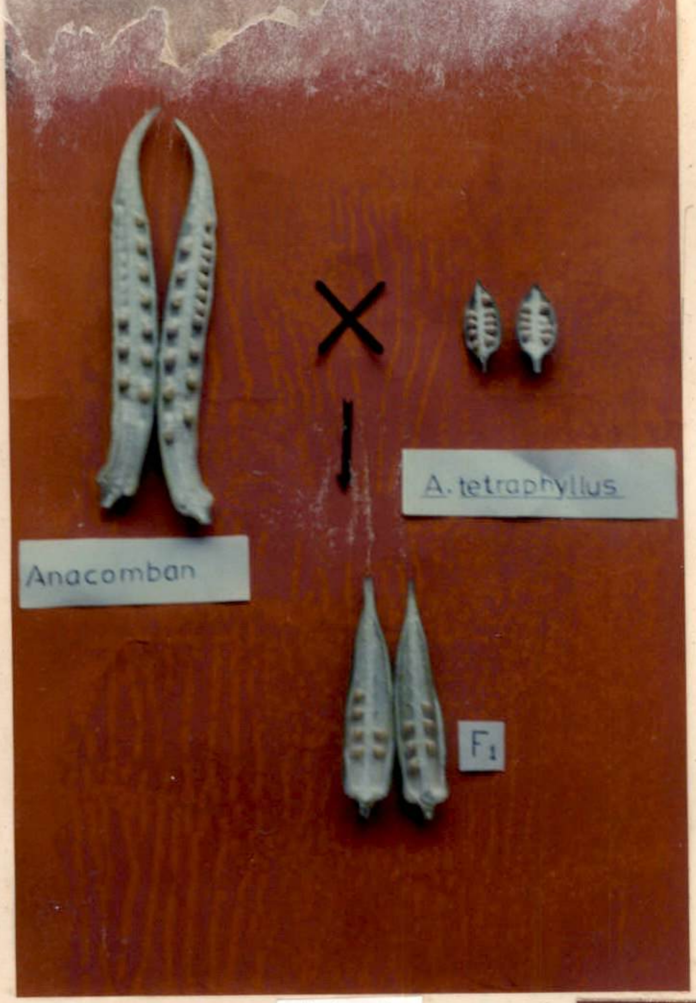


plate 4c

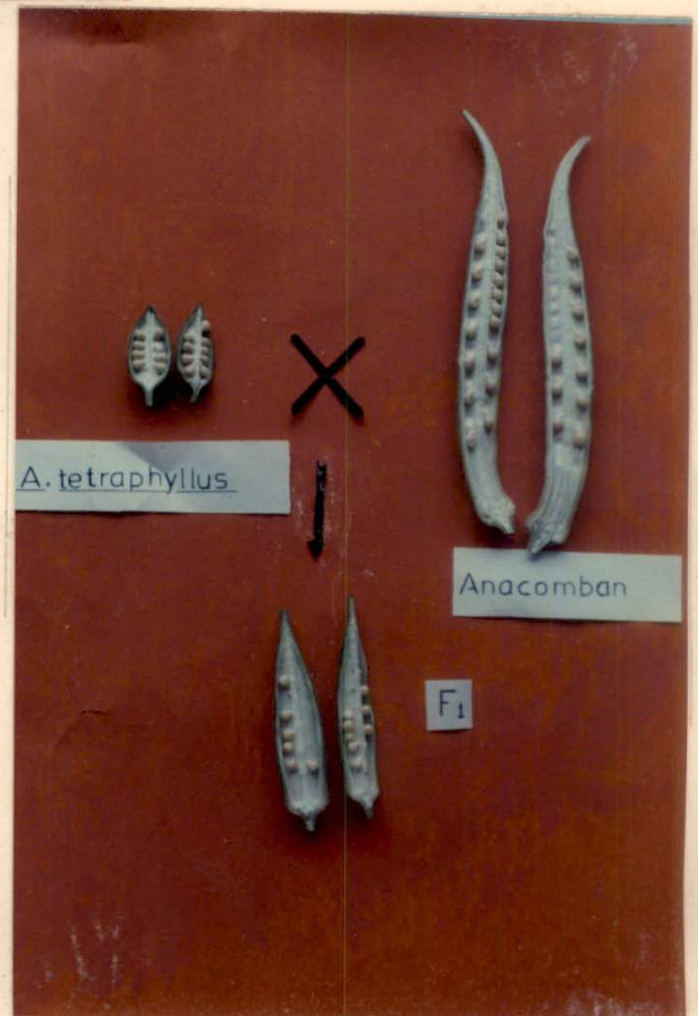


plate 4d

4.2.4 Seed weight

The seed weight of the three parents and four F_1 plants seeds is presented in Table 10. The mean seed weight of the parents being, A. esculentus var. Anacomban 6.61 g, A. manihot 5.7 g and A. manihot ssp. tetraphyllus 2.35 g. The F_2 seeds of A. esculentus var. Anacomban x A. manihot weighed 5.21 g while its reciprocal weighed 4.51 g. In direct and reciprocal crosses, the F_2 seeds weighed less than both the parents. The F_2 seeds of the cross A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus weighed 2.61 g while its reciprocal weighed 1.61 g only. Here also the F_2 seeds weighed less than both the parents. The variation in seed weight among the parents and the F_1 plants are graphically represented in Fig. 9(a) and 9(b).

4.2.5 Seed Viability

The seed viability in the parents and the F_1 plants is presented in Table 11. The parents, A. esculentus var. Anacomban, A. manihot, and A. manihot ssp. tetraphyllus manifested higher germination percentage (100, 90 and 70) respectively. The F_2 seeds showed very low germination percentage. In the cross A. esculentus var. Anacomban x A. manihot the F_2 seeds showed a germination percentage of 12 and its reciprocal cross showed only 4 per cent germination. The reciprocal cross recorded very low germination percentage as compared to direct cross. In the cross A. esculentus

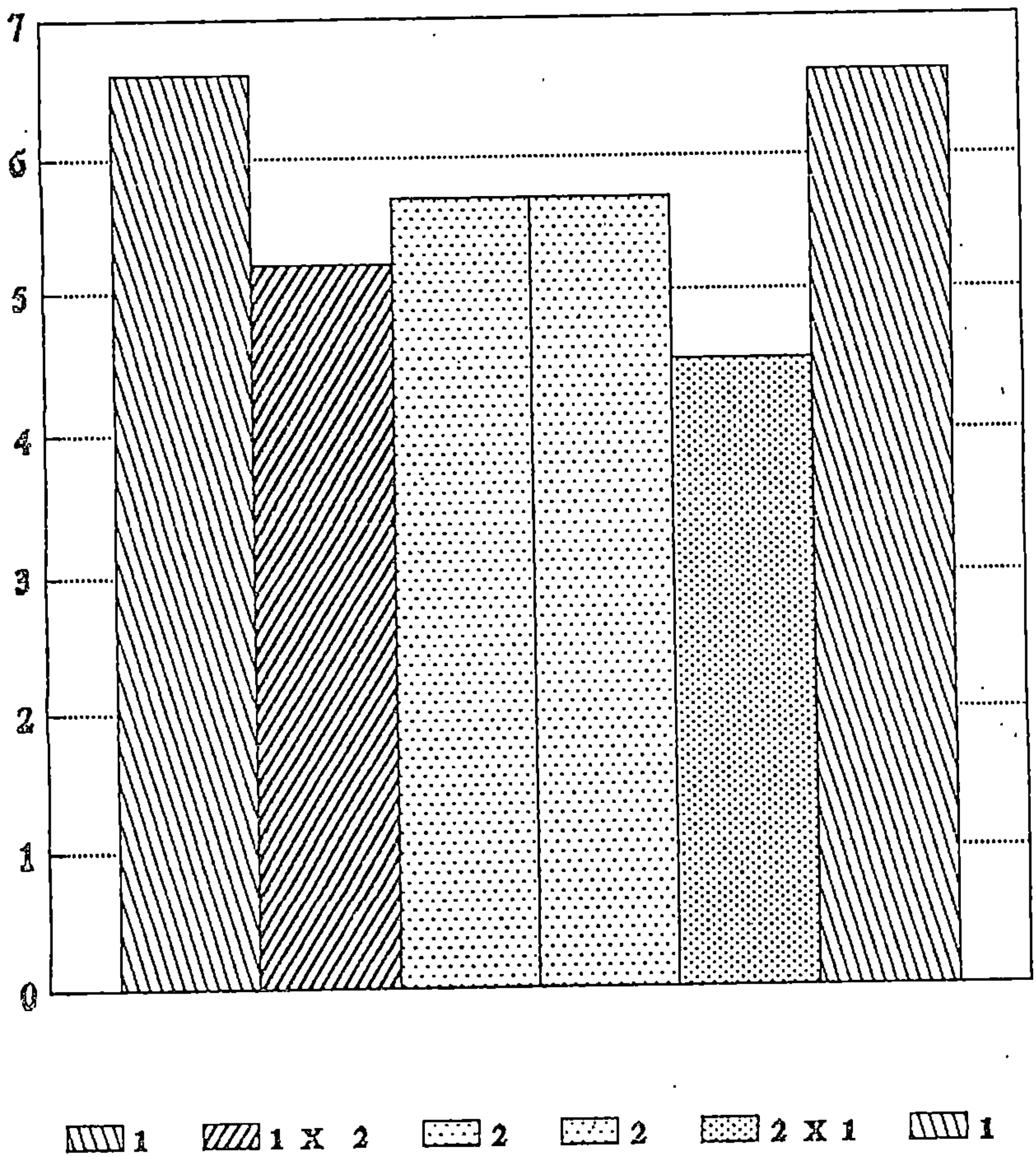
Table 10 Seed weight in parents and F_1 plants

Parents/crosses	Seed weight (g)
A. esculentus var. Anacomban	6.61
A. manihot	5.70
A. manihot ssp. *tetraphyllus*	2.35
A. esculentus var. Anacomban x *A. manihot*	5.21
A. manihot x *A. esculentus* var. Anacomban	4.51
A. esculentus var. Anacomban x *A. manihot* ssp. *tetraphyllus*	2.61
A. manihot ssp. *tetraphyllus* x *A. esculentus* var. Anacomban	1.61

Table 11 Seed viability in parents and F_1 plants

Parents/crosses	Germination (%)
A. esculentus var. Anacomban	100.00
A. manihot	90.00
A. manihot ssp. *tetraphyllus*	70.00
A. esculentus var. Anacomban x *A. manihot*	12.00
A. manihot x *A. esculentus* var. Anacomban	4.00
A. esculentus var. Anacomban x *A. manihot* ssp. *tetraphyllus*	1.33
A. manihot ssp. *tetraphyllus* x *A. esculentus* var. Anacomban	1.00

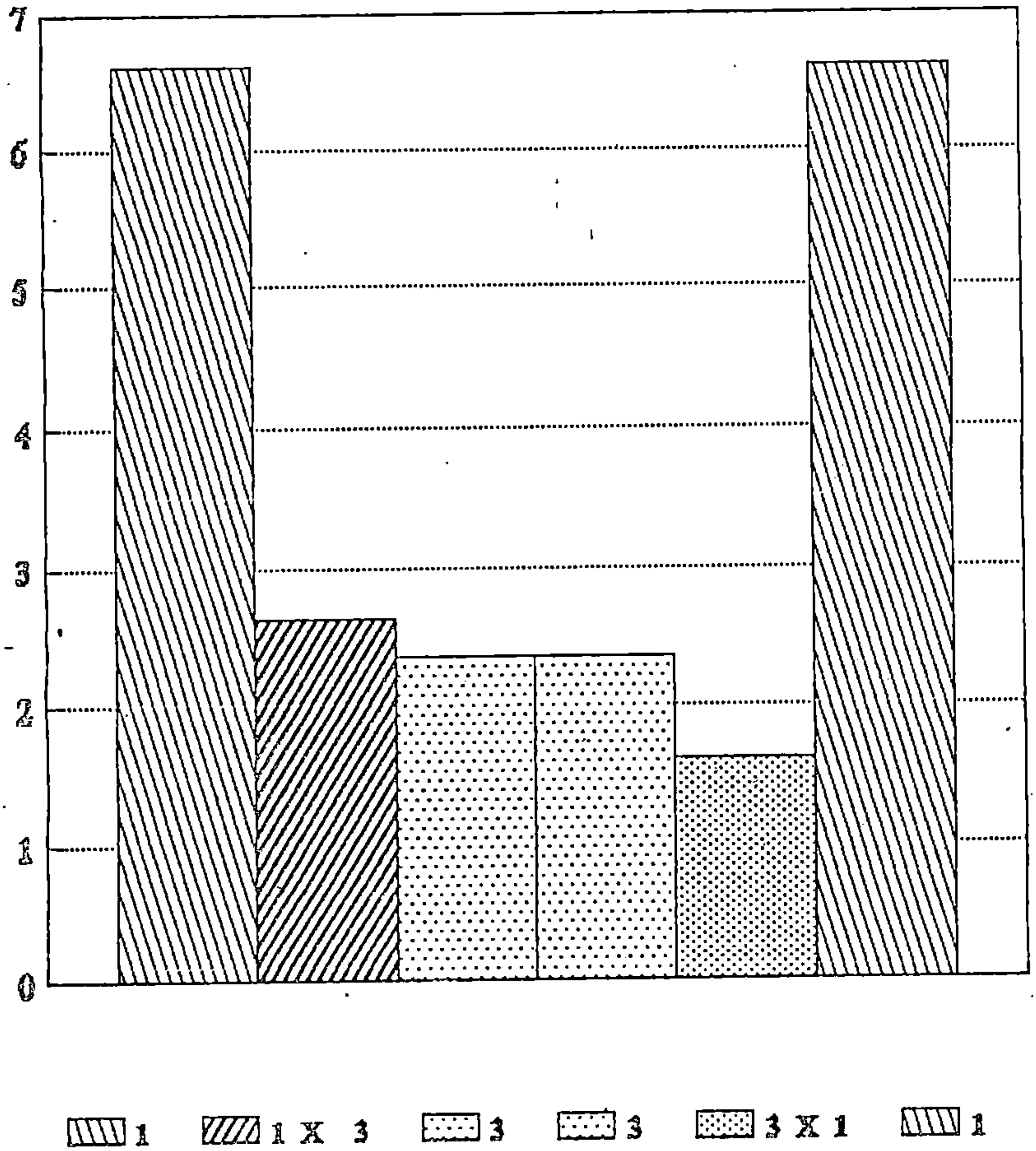
Fig. 9(a) Seed weight in parents and F_1 plants (grams)



1 A. esculentus var. Anacomban

2 A. manihot

Fig. 9(b) Seed weight in parents and F_1 plants (grams)



1 *A. esculentus* var. Anacomban

3 *A. manihot* ssp. *tetraphyllus*

var. Anacomban x A. manihot ssp. tetraphyllus the F_2 seeds showed very low germination percentage (1.33 %) while its reciprocal cross had only 1 percent germination. The variation in seed viability among the parents and F_1 plants is graphically represented in Fig.10(a) and 10(b).

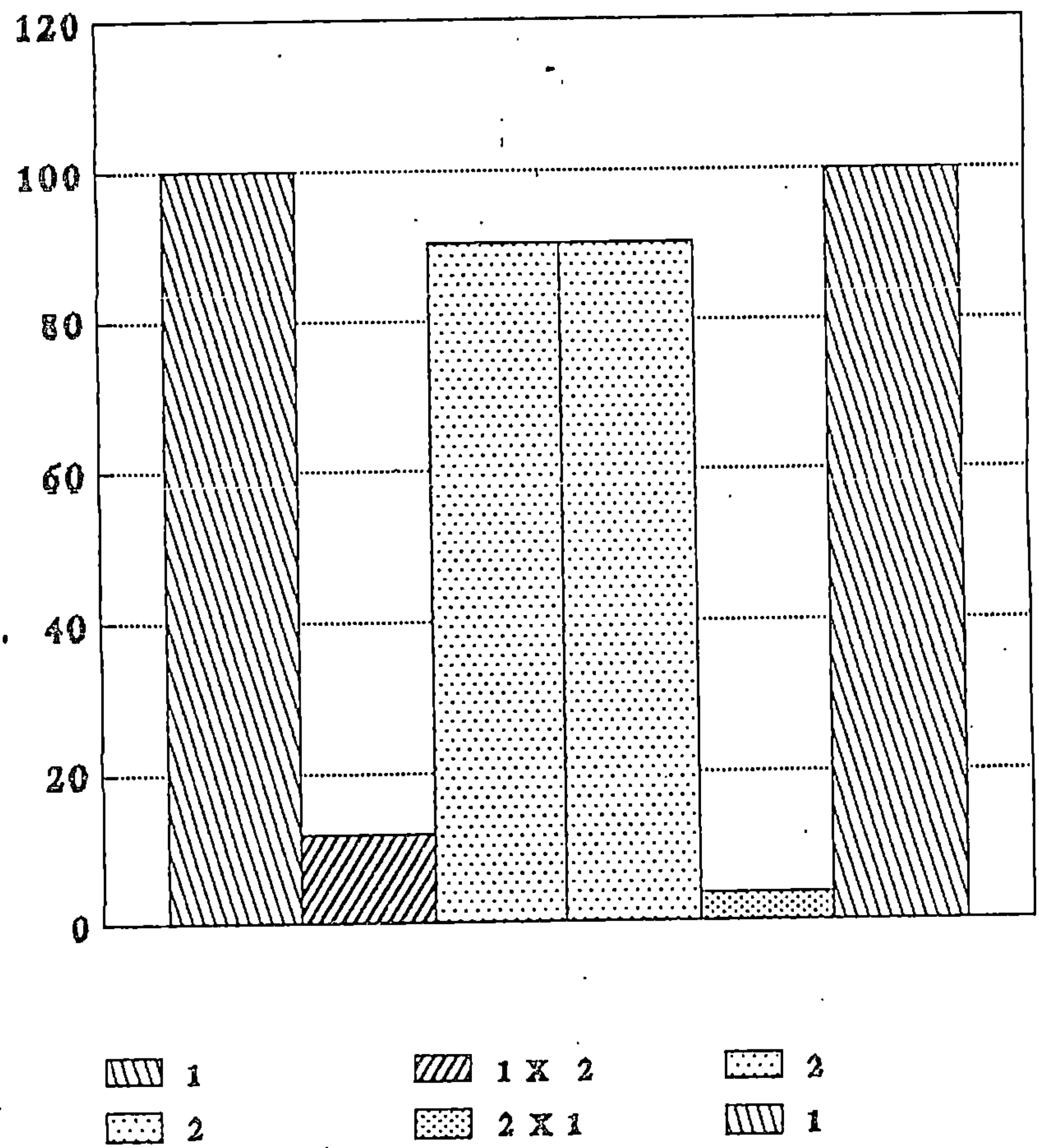
4.2.6 Seed sterility

The seed sterility in parents and F_1 plants is presented in Table 12. In parents the seed sterility was low. A. esculentus var. Anacomban recorded 0.54 per cent sterility while A.manihot and A.manihot ssp. tetraphyllus recorded 5.37 and 8.10 per cent seed sterility respectively. The F_2 seeds in four crosses showed high level of seed sterility. In the cross A. esculentus var. Anacomban x A. manihot the seed sterility was 81.40 per cent where as in its reciprocal cross it was 87.50 per cent. The seed sterility was higher in reciprocal cross. In the cross A.esculentus var. Anacomban x A.manihot ssp. tetraphyllus the F_2 seeds showed 98.4 per cent sterility whereas it was 98.70 per cent in its reciprocal cross.

Table 12 Seed sterility in parents and F_1 plants

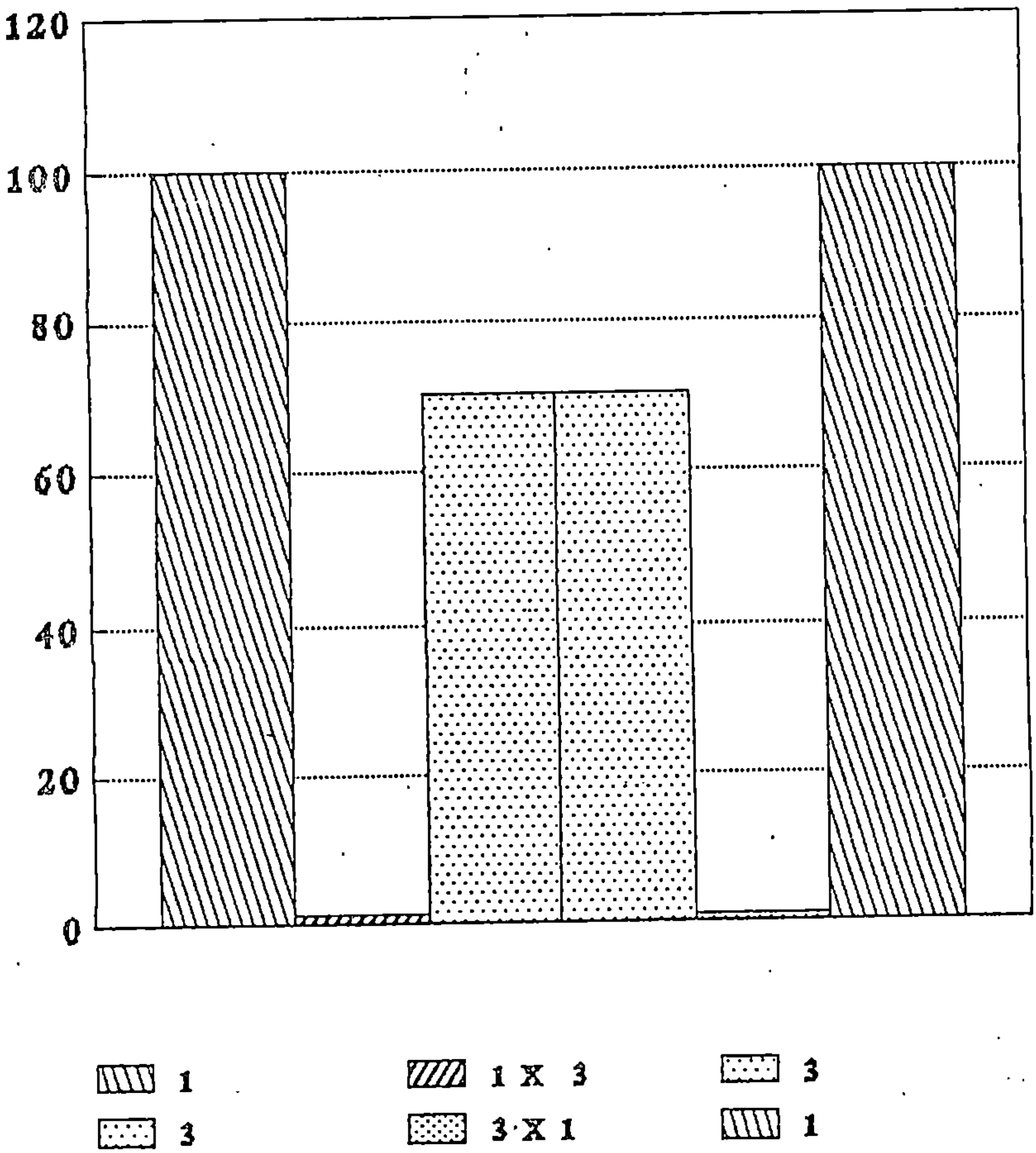
Parents/crosses	Seed sterility (%)
A. esculentus var. Anacomban	0.54
A. manihot	5.37
A. manihot ssp. *tetraphyllus*	8.10
A. esculentus var. Anacomban x *A. manihot*	81.40
A. manihot x *A. esculentus* var. Anacomban	87.50
A. esculentus var. Anacomban x *A. manihot* ssp. *tetraphyllus*	98.40
A. manihot ssp. *tetraphyllus* x *A. esculentus* var. Anacomban	98.70

Fig. 10(a) Seed viability in parents and F_1 plants (percentage)



1 *A. esculentus* var. Anacomban

Fig. 10(b) Seed viability in parents and F_1 plants (percentage)



1 _A. esculentus_ var. Anacomban

3 A. manihot ssp. tetraphyllus

DISCUSSION

5. Discussion

Studies on interspecific hybridization aims at transferring characters of economic value such as pest and disease resistance, drought resistance and allied stress tolerances from the wild relatives to the cultivated species. Interspecific hybridization involves the combination between two populations which have achieved specific rank due to genetic differentiation. When genetic differentiation has proceeded still further and the two populations have achieved specific rank, the hybrid usually acquires still another property, the property of reproductive incapacity or sterility in the F_1 generation. So interspecific hybrids more often, suffer a loss in reproductive capacity, with both F_1 and later generations showing a greater or lesser degree of hybrid sterility. Interspecific hybrids runs the gamut from complete fertility to complete sterility (Allard, 1990).

In interspecific hybridization recombination of two gene pools which have been separated in reproduction for many generations take place. This creates several isolation barriers in interspecific hybridization. There are several reviews on the barriers to interspecific hybridization (Dobzhansky, 1951; Stebbins, 1950, 1958; Briggs and Knowles, 1967; Allard, 1990; Singh 1990). Four major barriers identified by them are incompatibility, hybrid inviability, hybrid sterility and hybrid breakdown.

In incompatibility, the zygote formation is prevented by failure or ineffectiveness of pollen growth or failure of fertilization. The major causes of hybrid inviability given by Stebbins (1958) are noncompatibility of the parental chromosomes, cytoplasmic genic interactions and noncompatibility between embryo and the surrounding tissue called somatoplastic sterility. Hybrid sterility has been classified as gametic and zygotic by Renner (1929), haplontic and diplontic by Muntzing (1930), as genic and chromosomal by Dobzhansky (1951) and due to cryptic structural hybridity by Stebbins (1950). When segregants in the F_2 and later generations are sterile it is referred to as hybrid breakdown.

In Abelmoschus interspecific hybridization are mostly being done for the transfer of resistance to yellow vein mosaic disease from the wild species to the cultivated species. Many workers have reported that interspecific hybrids in the genus Abelmoschus were resistant to yellow vein mosaic. But the hybrids were partially or completely sterile (Kuwada, 1961; Singh et al., 1975; Meshram, 1981; Jambhale and Nerkar, 1982; Tekale et al., 1985; Hamon and Hamon, 1991).

The results of study on interspecific cross compatibility in the genus Abelmoschus and reasons for low fruit set, seed set and hybrid sterility are discussed here. The fruits resembled the female parent in size and shape in all the four crosses although the percentage of fruit set was low. The fruit set in the cross A. esculentus var. Anacomban x A. manihot was 11.36 per cent while its reciprocal cross recorded 78.57 per cent. In the other cross between A. esculentus var. Anacomban and A. manihot ssp. tetraphyllus the fruit set was 10.25 per cent and 55.95 per cent in direct and reciprocal crosses, respectively. Gavrilenko and Surikov (1988) in a study on interspecific crosses of Lycopersicon involving L. esculentus and L. chilense observed that the fruit set ranged from 20 to 59.6 per cent which is in agreement with the present study. Cherian (1988) recorded 80.00 to 94.80 per cent fruit set in the crosses between three accessions of A. manihot and A. esculentus which is very high compared to the present value.

The low fruit set may be attributed to the variation in chromosome number among the parental species. The somatic chromosome numbers of okra used by several workers, were always in the range of 120's to 130's. There might be two kinds of okra genotypes, one in the range of 60's to 70's (diploid group) and the other, 120's - 130's (tetraploid group) in their somatic chromosomes (Markose and Peter, 1990). The present cultivated Indian varieties possesses a somatic chromosome complement of 2n = 130. The chromosome behaviour during meiosis is regular forming bivalents.

Joshi and Hardas (1956) reported that A. esculentus (2n = 130) is an amphidiploid of A. tuberculatus (2n=58) and an unknown species (2n = 72) Charrier (1983) reported that A. esculentus is a polyploid species originated through amphidiploidy. In A. manihot and A. manihot ssp. tetraphyllus also there are varying reports of chromosome numbers as (2n = 60, 66 and 68) and (2n = 130, 138) respectively.

The percentage of fruit set was higher in reciprocal crosses (78.57 and 55.95) when compared to the direct crosses (11.36 and 10.25). Mamidwar et al.. (1979) conducted crosses between A. esculentus and A. tetraphyllus and recorded that the fruit set was highest when A. esculentus was used as the female parent. Cherian (1988) observed that the percentage of fruit set did not differ widely in direct and reciprocal crosses among the three accesssions of A. manihot and A. esculentus which are not in conformity with the findings of the present study. Abelmoschus manihot and A. manihot ssp. tetraphyllus are slow growing types, evidenced from the time taken for first blooming, fruit development and allied attributes. Hence based on these evidences the low fruit set in crosses involving A. manihot and A. manihot ssp tetraphyllus as pollen parents may be due to the slow pollen tube growth.

The seed set in the cross A. esculentus var. Anacomban x A. manihot was 64.42 per cent while its reciprocal cross recorded

77.33 per cent. In the cross A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus, the seed set in direct and reciprocal cross was 66.32. and 54.1 per cent respectively. The low seeds set and recovery of shrivelled seeds in the crossed fruits may be due to partial or complete failure of the endosperm owing to genetic imbalance (Riley, 1967; Allard, 1990; Singh, 1990). In certain hybrids the abnormal development of the endosperm will cause the hybrid seed fail to develop. The complete or partial failure of the endosperm in the hybrids usually interferes with nourishment of the developing embryo (Allard, 1990).

After double fertilization the endosperm normally begins to develop and apparently also secretes some growth promoting substances. But in the hybrids, endosperm development is rather slow and this secretion is probably reduced (Riley, 1967). In hybrids the megasporangium or nucellus begins to grow several cells thick as against one-celled in normal cases. The growth of the megasporangium in some cases is so pronounced that this tissue completely surrounds the endosperm. In normal cases a gap remains in the megasporangium leaving an opening from the endosperm to the integument. When the embryos are in the eight to sixteen cell stage all seeds in normal cases has this gap, against 75 per cent to 26 per cent in some hybrids (Riley, 1967).

The absence of gap cuts off the nutrients from the endosperm, with the result that the developing ovules collapse leading to the formation of shrivelled seeds. This type of abnormal growth of endosperm may be the cause of recovery of shrivelled seeds in the present study. Failure to obtain thrifty F_1 hybrid can result from genetic or from cytoplasmic incompatibilities that are expressed either in failure of fertilization or in death of the zygote at any stage between early cleavage division and maturity (Allard, 1990).

In the cross *A. manihot* and *A. esculentus* Bhargava (1989) observed endosperm deterioration five days after pollination. He and Liang (1989) reported normal fertilization between *Gossypium arborium* and *G. davidzonii*, but after seven days of pollination the bybrid endosperm developed many abnormalities.

The crossed fruits resembled the selfed fruits of female parent in size and shape which shows that fruit development in the interspecific cross is not influenced by difference in species. The mean fruit weight was lower in reciprocally crossed fruits than that of female parent. The mean fruit weight of the crossed fruits in the cross *A. manihot* x *A. esculentus* var. Anacomban was 6.54 g whereas its female parent recorded 7.77g. The mean fruit weight of crossed fruits in the cross *A. manihot* ssp. *tetraphyllus* x *A. esculentus* var. Anacomban was 0.93 g whereas its female parent recorded 2.42g. This may be due to reduced number of seeds in the crossed fruits. The crossed seeds resembled the female parents in appearance since seed coat being a purely maternal tissue.

In all four crosses the F_1 seeds showed a slight reduction in seed weight compared to that of the female parents. In the cross *A. esculentus* var. Anacomban x *A. manihot* the F_1 seed weighed 5.65 g and its female parent weighed 6.61g. The seed weight of *A. manihot* was 5.7 g while in the cross *A. manihot* x *A. esculentus* var. Anacomban the F_1 seeds weighed on an average 5.04g. The F_1 seed of the cross between *A. esculentus* var. Anacomban and *A. manihot* ssp. *tetraphyllus* was 6.54 g and that of *A. esculentus* var. Anacomban was 6.61g. The F_1 seed weight of *A. manihot* ssp. *tetraphyllus* x *A. esculentus* var. Anacomban was 2.30 g and that of its female parent was 2.35 g. Jawaharlal *et al*. (1988) reported that seed weight in *A. esculentus* was high when it had more mature embryo and contain adequate nutrient reserves. The reduction in weight of the F_1 seeds in all the crosses may be due to reduced amount of nutrient reserves.

The F_1 seeds has shown good seed viability both in laboratory and in field conditions except for the reciprocal cross of *A. esculentus* var. Anacomban x *A. manihot* ssp. *tetraphyllus*. In laboratory conditions the F_1 seeds of the cross A. esculentus var. Anacomban x A. manihot recorded 70 per cent germination while its reciprocal cross registered 100 per cent germination. In the cross A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus the F_1 seeds showed a germination percentage of 95 while the seeds of its reciprocal cross recorded a lower germination of 35 per cent.

Dhillon and sharma (1982) reported appreciably good recovery of hybrids from the crosses of A. esculentus cultivars and A. manihot ssp. manihot. Cherian (1988) has reported that the percentage of seed germination was less in interspecific hybrids of Abelmoschus than in parents except in A. esculentus x A. manihot ssp. tetraphyllus (93.33 per cent) and A. manihot x A. esculentus (81.33 percent) which is in agreement with the present study.

The four major barriers in interspecific hybridization are incompatibility, hybrid inviability, hybrid sterility and hybrid breakdown. When zygote formation is prevented by failure or ineffectiveness of pollen tube growth or failure of fertilization it is referred to as incompatibility (Allard, 1990). In the present study fertilization had taken place and crossed fruits were produced, 11.36 per cent in the cross A. esculentus var. Anacomban x A. manihot and 78.57 per cent in its reciprocal cross. In the cross A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus the percentage of fruit set was 10.25 and 55.95 in its reciprocal cross. The percentage of fruit set was very low in both the reciprocal crosses. This may be due to slow pollen tube growth when the semiwild species were used as the pollen parent. This points the possibility of partial incompatibility existing between A. esculentus var. Anacomban and the two semiwild species A. manihot and A. manihot ssp. tetraphyllus.

When the crossed seeds become inviable the phenomenon is attributed to hybrid inviability. In the present study all the crosses produced viable seeds and the viability of F_1 seeds ranged from 35 to 100 per cent. So the barrier in interspecific hybridization of Abelmoschus may not be due to hybrid inviability..

When raised in field all the four hybrids and the two semiwild parents exhibited field tolerance to yellow vein mosaic disease while A. esculentus var. Anacomban exhibited susceptibility to disease.

All the four hybrids exhibited pollen sterility. Pollen sterility in the cross A. esculentus var. Anacomban x A. manihot was 46.8 per cent while its reciprocal cross recorded 58.77 per cent, while in the other cross combination between A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus the percentage of pollen sterility was 16.48. The reciprocal cross of the above combination recorded 25.41 per cent pollen sterility. Tekale et al. (1985) found that the pollen sterility ranged from 6.44 to 13.24 per cent in the hybrid between A. esculentus and A. manihot which is very low when compared to the present value. Prabha (1986) reported 32.5 and 35.2 per cent pollen fertility in direct and reciprocal crosses between A. esculentus and A. manihot. Cherian (1988) in the cross A. esculentus and A. manihot observed that the pollen fertility was 19.0 per cent in the direct and 15.0 per cent in the reciprocal corss which is very low compared to the present value. Cherian (1988) reported high pollen fertility in the cross A. esculentus x A. tetraphyllus which is in agreement with the present value. Babu and Dutta (1990b) reported 76.42 per cent pollen fertility in the interspecific hybrid between A. esculentus and A. tetraphyllus which is in agreement with the present study.

Meshram and Dhapake (1981) reported high pollen sterility in the cross A. esculentus and A. tetraphyllus which is not in conformity with the findings of the present study. High pollen sterility was recorded in the cross between A. esculentus and A. radiatus (Meshram and Narkhede, 1981). In interspecific hybrids of Gossypium also high pollen sterility was reported (Omelchenko and Abdullaev, 1983; Gennur, et al., 1986).

The high pollen sterility in the hybrids may be due to meiotic abnormalities resulting from the difference in the number of chromosomes. The disorganized disjunction in structurally heterozygous hybrids usually causes less than a full complement of chromosomes to be partitioned to each gamete in the meiotic process, and as a result, many or all gametes are non functional (Allard, 1990). Meshram and Dhapake (1981) observed that meiosis was abnormal in the hybrid between A. esculentus and A. tetraphyllus. Anaphase separation was irregular and the hybrid was highly male sterile. Meshram and Narkhede (1981) reported that the high pollen sterility in the hybrid between A. esculentus and A. radiatus may be attributed to meiotic abnormalities like irregular distribution of chromosomes at anaphase-1. Babu and Dutta (1990a) reported that in the F_1 hybrids, among A. esculentus x A. tetraphyllus meiosis was abnormal showing more of univalents and less of bivalents.

The somatic chromosome number of A. esculentus ranges from 120 to 130 and that of A. manihot ssp. tetraphyllus ranges from 130 to 138 while A. manihot possesses a somatic chromosome complement of 2n = 60, 66. The pollen sterility between the crosses of A. esculentus and A. tetraphyllus was less (16.48 and 25.41%) in direct and reciprocal crosses. On the contrary in the cross A. esculentus and A. manihot the pollen sterility was as high as 46.8 and 58.77 per cent in direct and recirprocal crosses. This is a clear indication of difference in chromosome number affecting meiosis and thereby causing pollen sterility.

Seed set in the hybrid of the cross between A. esculentus var. Anacomban x A. manihot is 15.13 percent while its reciprocal recorded 9.68 per cent. In the cross A. esculentus var. Anacomban x A.manihot ssp. tetraphyllus seed set was 58.22 per cent while its reciprocal cross recorded 60.16 per cent. Prabha (1986) recorded 6.89 per cent seed set in the cross A. esculentus var. Anacomban x A. manihot and 12.65 per cent in its reciprocal cross which is not in agreement with the present study.

In all four crosses, the percentage of seed set was proportional to the pollen fertility. The higher the pollen sterility lower was the seed set. In the direct and reciprocal crosses among A. esculentus var. Anacomban and A. manihot pollen sterility was high (46.8 per cent and 58.77 per cent) and the seed set percentage was low (15.12 and 9.67). In the direct and reciprocal crosses among A. esculentus var. Anacomban and A. manihot ssp. tetraphyllus pollen sterility was low (16.48 and 25.41 per cent) and the seed set was found to be high (58.22 and 60.16 per cent).

The F_2 seeds showed a drastic reduction in weight. The seed weight of F_2 seeds was 5.21 g in the cross A. esculentus var. Anacomban x A. manihot compared to 6.61g in its female parent. The reciprocal cross of the above combination recorded 4.51g compared to 5.7 g of its female parent. In the cross A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus the seed weight was 2.61g compared to 6.61g of its female parent. The reciprocal cross of the above combination recorded seed weight of 1.61g compared to 2.35 gm in its female parent. Prabha (1986) recorded seed weight of 5 gm in the cross A. esculentus var. Anacomban x A. manihot and 4.41 g in its reciprocal cross which is in agreement with the present study.

The reduction in seed weight of F_2 seeds may be due to high seed sterility exhibited by the hybrids. The seed sterility in the hybrids ranged from 81.4 per cent to 98.7 per cent. Seed sterility was higher in the crosses with A.manihot ssp. tetraphyllus (98.4 and 98.7 per cent). Seed sterility was comparatively lower in crosses with A. manihot (81.4 and 87.5 per cent). Jambhale and Nerkar (1982a)reported 7.1 percent seed fertility in the F_1 of the cross A. esculentus and A. manihot which is low when compared to the present study. Tekale et al. (1985) reported 24.96 to 52.14 per cent seed sterility in hybrids between A. esculentus and A. manihot which is lower when compared to the present study. The high seed sterility exhibited by the F_2 seeds may be due to endosperm deterioration which in turn contributed to the recovery of empty seeds, although the seeds appeared normal.

The seed viability of the F_2 seeds was very poor. It was 12 per cent and 4 per cent in the direct and reciprocal crosses between

A. esculentus var. Anacomban and A. manihot. The seed viability in the seeds of the cross between A. esculentus var. Anacomban and A. manihot ssp. tetraphyllus was still less, being 1.33 per cent and 1 per cent in direct and reciprocal crosses respectively. Prabha (1986) recorded 25 per cent and 16.2 per cent geminability of the F_2 seeds in direct and reciprocal cross between A esculantus var. Anacomban and A. manihot which is higher than the present value. The low seed viability may be due to high degree of seed sterility exhibited by the hybrids.

Sterility that becomes apparent only at the time of the formation of the gametes or gametophytes is called hybrid sterility and can be divided into two types, chromosomal and genic (Allard, 1990). The demonstration that amphidiploids derived from certain hybrids become fertile once each chromosome has a fully homologous mate with which to pair shows that the sterility of the F_1 is sometimes due entirely to chromosomal sterility and not to genic imbalance (Allard, 1990). An amphidiploid produced from the F_1 of the cross A. esculentus and A. manihot by colchicine treatment recorded seed fertility of 88.1 per cent while that of the F_1 was 7.1 per cent (Jambhale and Nerkar, 1982 a). Jambhale and Nerkar (1982b) reported that an amphidiploid was obtained by colchicine treatment of the sterile hybrid from the cross A. tetraphyllus and A. esculentus in which chromosome pairing was normal compared with the F_1. Babu and Dutta

(1990a) reported that the amphidiploid obtained through colchicine treatment was fully fertile and showed a low multivalent formation during meiosis and can survive as a new synthetic species compared to its completely sterile F_1 hybrids.

Interspecific hybrids run the gamut from complete fertility to complete sterility (Allard, 1990).. In the present study the hybrids in all four crosses were almost completely sterile with only 12 and 4 per cent seed viability in the direct and reciprocal crosses, of *A. esculentus* var. Anacomban x *A. manihot*. In the cross *A. esculentus* var. Anacomban x *A. manihot* ssp. *tetraphyllus* the seed viability was still poor (1.33 per cent and 1 per cent) in direct and reciprocal crosses respectively. Hence these findings point towards the possibility of the presence of hybrid sterility as the barrier of interspecific hybridization in *Abelmoschus*.

There are many reports of regaining the fertility in interspecfic hybrids by conducting back crosses. Martin (1982) reported that the interspecific hybrids between a *Abelmoschus* sp. and *A. esculentus* were comparatively sterile, but a few produced germinable seeds, back crosses were more fertile, with almost complete fertility in BC_2. Back cross of *Abelmoschus esculentus* x *A. manihot* to the *A. esculentus* had seed fertility of 58.85 per cent.

Eshankhodzhaev (1977) reported that in order to overcome sterility in F_1 hybrids of Gossypium, back crosses were done. As a result, seed set in the hybrids increased from 0.7 per cent to 1.5 per cent in the F_1 BC_1 to 15.1 per cent in the F_1 BC_2 and to 50 per cent in the F_1 BC_3.

Chiavegato et al. (1985) reported that fertility was restored in completely sterile F_1 plants after the first back cross in Gossypium. Despite sterility problems encountered in F_1 of the interspecific cross between Solanum aethiopicum and S.melongena, a recurrent selection scheme involving two back crosses to S.melongena was performed, yielding families with wilt resistant fruits.

All the hybrids produced showed high field tolerance to yellow vein mosaic disease, while one of the parents A. esculentus var. Anacomban was infected by yellow vein mosaic. The two semi wild parents and all the hybrids remained tolerant to the disease. The F_1 fruit characters were not acceptable as they were small and hairy. Although the hybrids were almost sterile it was not completely sterile. The present study points that the pollen sterility may be one of the major causes for the hybrid sterility. Hence in order to utilize the field tolerence exhibited by the hybrids and to overcome hybrid sterility backcrossing may be resorted to. Backcrossing the F_1 as the seed parent to one or the other of the parental species overcome the male sterility expressed by the hybrid (Allard, 1990).

By resorting to backcrossing hybrids as the seed parent and A. esculentus var. Anacomban as the pollen parent, the fertility can be restored, thereby paving the way for the transfer of yellow vein mosaic disease resistance from semiwild species like A. manihot and A. manihot ssp. tetraphyllus to the otherwise desirable but yellow vein mosaic susceptible A. esculentus var. Anacomban, the popular cultivar.

SUMMARY

Summary

The study on Interspecific cross-compatibility in the Abelmoschus was undertaken during 1991-92. The objective of the study was to probe into the reasons of low fruit and seed set in the interspecific hybrids of Abelmoschus. Local cultivar Abelmoschus esculentus var. Anacomban and the two semi wild species A. manihot and A. manihot ssp. tetraphyllus were used as parents. The following crossing scheme was adopted,

A. esculentus var. Anacomban x A. manihot

A. esculentus var. Anacomban x A. manihot ssp. tetraphyllus.

Their reciprocal crosses were also made. The four cross combinations were utilized for the study.

The fruit set in direct crosses was very low when compared to the reciprocal crosses. This may be due to the partial incompatibility consequent on the slow pollen tube growth when A. manihot and A. manihot ssp. tetraphyllus were used as the pollen parent.

The crossed fruits resembled the female parent, but showed a slight reduction in weight which may be attributed to the reduced number of seeds in the crossed fruits.

The seed set in the crossed fruits was low. Further there was recovery of shrivelled seeds which may be ascribed to poor endosperm growth.

The hybrid seeds resembled the seeds of the female parents. The reduction in weight of the hybrid seeds when compared to the female parent may be due to reduced nutrient content. The hybrid seeds exhibited good viability in both laboratory and field conditions.

The hybrids exhibited field tolerance to yellow vein mosaic disease. All the four hybrids recorded pollen sterility. The crosses between A. esculentus var. Anacomban and A. manihot recorded higher pollen sterility than between A. esculentus var. Anacomban and A. manihot ssp. tetraphyllus. The reason for pollen sterility may be attributed to meiotic abnormalities, due to difference in chromosome number between the species.

The seed set in the hybrids was very low. From the observations it was seen that the seed set was inversely proportional to the pollen sterility. The lower the pollen sterility higher was the seed set. So the low seed set may be due to pollen sterility. All the F_2 seeds showed a drastic reduction in weight compared to the female parent. The reduction in weight may be due to high seed sterility. The seed sterility or the recovery of empty seeds may be due to the endosperm degeneration.

The seed viability of the F_2 seeds was very poor. The low seed viability may be due to high seed sterility.

The present study leads to the conclusion that the reason for low fruit and seed set in interspecific hybridization in Abelmoschus may be due to partial incompatibility between the species. The hybrid sterility may be due to meiotic abnormalities consequent on the difference in chromosome number between the species.

Although there was partial incompatibility and hybrid sterility the crossing of local cultivar with the semi-wild species of A. manihot and A. manihot ssp. tetraphyllus could be successful in transferring the disease resistance of the latter to the cultivated species. Further more all the hybrids exhibited field tolerance to the dreadful disease of bhindi namely yellow vein mosaic. This is indeed a positive aspect.

In order to maintain the field tolerance to yellow vein mosaic disease exhibited by the hybrid and to overcome the hybrid sterility back crossing may be resorted to. Back crossing the F_1 as the seed parent to the local cultivar Anacomban may overcome the hybrid sterility. There by paving way for the development of new mosaic resistant bhindi varieties.

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INTERSPECIFIC CROSS - COMPATIBILITY IN THE GENUS *Abelmoschus*

By

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Abstract

~~The study on~~ Interspecific cross-compatibility in the genus *Abelmoschus* was undertaken with the objective of probing into the reasons of low fruit and seed set in the interspecifc hybrids of *Abelmoschus* which may pave the way for the development of yellow vein mosaic resistant varieties through recombination breeding. Crossing was done between the local cultivar *Abelmoschus esculentus* var. *Anacomban* and two semi wild species (*A. manihot* and *A. manihot* ssp. *tetraphyllus*).

The fruit set in direct crosses of *A. esculentus* var. Anacomban x *A. manihot* and *A. esculentus* var. Anacomban x *A. manihot* ssp. *tetraphyllus* was very low compared to the reciprocal crosses, exhibiting partial incompatibility consequent on the slow pollen tube growth of *A. manihot* and *A. manihot* ssp. *tetraphyllus*. The seed set was low in crossed fruits and there was recovery of shrivelled seeds which may be attributed to the poor endosperm development. The crossed seeds exhibited good viability.

All the hybrids exhibited field tolerance to yellow vein mosaic disease. The four hybrids recorded pollen sterility which may be attributed to meiotic abnormalities, due to difference in chromosome number between the species. The seed set in the hybrids

was very low. The seed set was inversely proportional to pollen sterility. The lower the pollen sterility higher was the seed set. The F_2 seeds showed reduction in seed weight which may be due to high seed sterility exhibited by the hybrids. The recovery of empty seeds which appeared normal may be ascribed to endosperm degeneration. The seed viability of F_2 seeds was very low.

The present study leads to the conclusion that the reason for low fruit and seed set in interspecific hybridization in Abelmoschus may be due to partial incompatibility. The interspecific hybrids displayed hybrid sterility which may be attributed to the meiotic abnormalities consequent on the difference in the chromosome number between the parents. However, all the hybrids were not completely sterile. The hybrids exhibited field tolerance to yellow vein mosaic disease which is an added advantage. Back crossing the F_1 as the seed parent to the A. esculentus var. Anacomban may overcome the hybrid sterility and pave way for the development of varieties with yellow vein mosaic resistance coupled with economic attributes of the popular cultivar Anacomban.