

**GENETIC IMPROVEMENT AND CYTOGENETICAL  
STUDIES IN THAMARAVENDA**  
[*Abelmoschus manihot* (L.)]

By  
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**THESIS**

Submitted in partial fulfillment of the  
requirement for the degree

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**DEPARTMENT OF OLERICULTURE**

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KERALA, INDIA

1996

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I hereby declare that this thesis entitled 'Genetic improvement and cytogenetical studies in Thamaravenda (*Abelmoschus manihot* (L.)) is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship or any other similar title, of any other university or society.

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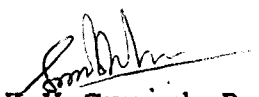
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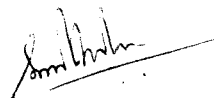
  
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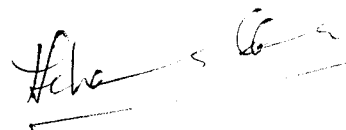
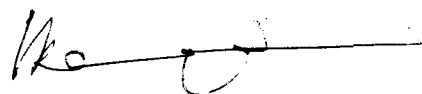
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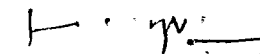
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Vellanikkara

10/1/96

*Reena Susan Chacko*

**REENA SUSAN CHACKO**

Dedicated to my beloved Father

was positively correlated with weight of the pod and seeds per pod.

Thirty hybrids from full diallel cross of six okra inbreds were evaluated by Sundhari et al., (1992). They observed significant and positive genotypic correlation (0.36) for internodal length and with number of pods per plant. Number of pods per plant, pod length and pod girth had positive and significant association with individual pod weight. Between pod length and pod girth the association was strong (0.38).

Mishra and Singh (1992) conducted a study on 18 cultivars of okra and observed positive and significant association of plant height with fruit length, fruits per plant, fruit weight and 1000 seed weight. However it had negative correlation with number of nodes per plant, days to 50 percent flowering, number of seeds per fruit, yellow vein mosaic resistance and yield per plant. The number of nodes per plant recorded a significant and positive association with fruits per plant at phenotypic as well as genotypic level. Days to 50 percent flowering showed a significant positive association with the seeds per fruit while fruit girth was negatively correlated with 1000 seed weight at both levels.

Studies conducted on ten okra inbreds revealed significant correlation of plant height with first flowering node (0.47 gcv), internodal length (0.63 gcv) and days to first flowering (0.49 gcv) (Patel et al., 1993).

#### 2.1.5 Path coefficient analysis.



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*Introduction*

## INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is an important vegetable crop grown throughout India for its tender pods. The average nutritional value of the pods is far superior in comparison with that of tomato and brinjal. Okra pods are rich source of vitamins, calcium, potassium, iodine and other mineral matters (Pal et al., 1952). Traditional cultivars of okra are highly susceptible to yellow vein mosaic virus (YVMV), shoot and fruit borer and generally they are annual having a short fruiting period. In this context an okra form locally known as "Thamaravenda", which was considered as *A. manihot* deserve importance, since it adorns many remarkable traits such as resistance to YVMV, tolerance to fruit and shoot borer, adaptability, good cooking quality and perennial nature suitable for ratooning. This form of okra is grown in Kerala to a very limited extent in the homesteads. Being an underexploited vegetable crop, no serious attempts have been made on its crop improvement, species affinity and cytotaxonomy.

Morphological features of this species form resembles those of *A. manihot* and *A. caillei* which are cultivated species of Papua New Guinea and West Africa. Probably this form of okra was introduced to this part of the country fairly early. During the course of evolution this species form has been showing some extent of variability in many plant characters. Collecting all the available genotypes and evaluating them based on the morphology, yield and yield contributing characters will help to select desirable forms of direct economic importance.

Cytogenetical studies on this species with regard to *A.esculentus* will offer basic information on cytotaxonomy, chromosome affinity between the species and possible gene introgression. Attempting the synthesis of the amphiploid between these edible species seems to be a viable proposition if the amphiploid combines itself with the desirable traits of both the parental species will be of great value.

Keeping the above points into consideration, the present study was conducted with the following specific objectives.

1. to collect the maximum available genotypes in the edible form of *Abelmoschus* sp. locally known as Thamaravenda,
2. to study the extent of genetic variability occurring in the genotypes collected, work out the selection parameters and to identify promising forms of direct economic importance,
3. to confirm the chromosome number of the species, make a detailed study on the morphological features and determine its species and taxonomical status,
4. to study the crossability and chromosome affinity with *A.esculentus*, and
5. to attempt the synthesise of amphiploid of *A.esculentus* and the species proposed for improvement.

*Review of literature*



## REVIEW OF LITERATURE

"Thamaravenda" was considered to be a form of *A. manihot* belonging to the family Malvaceae. This species forms were mainly cultivated in West Africa, Papua New Guinea and sparsely grown in the Indian subcontinent (Van Borssum Waalkes 1966). The plant is mainly cultivated for its tender pods for use as vegetable and for fibre and mucilage in industry. It is resistant to Yellow Vein Mosaic (Arumugham et al., 1975) Powdery Mildew (McLeod et al., 1983, Jambhale and Nerkar 1983, 1992) and Jassids (Nerkar, 1990) therefore being tried in interspecific hybridisation with the ultimate aim of developing a resistant cultivar.

As the volume of work done in *A. manihot* is less, literature on studies conducted in related species is also dealt with. The pertinent literature for the present study is reviewed under the following headings

1. Variability, biometrical and genetic divergence studies
2. Interspecific hybridisation and crossability studies
3. Cytological and cytogenetical studies
4. Induction of amphiploidy

### 2.1 Variability studies

Considerable amount of variability had been observed within different species of *Abelmoschus* in many plant characters except number of ridges per fruit (Vashista et al., 1982). Hamon and Charrier (1983) observed great variability especially in fruit shape in collected samples of *A. esculentus*. Isozyme analysis revealed a much greater

diversity among wild species *A.moschatus* and *A.manihot* than cultivated species *A.esculentus* and *A.caillei* (Hamon et al., 1991, Ariyo 1993).

Yield being a complex character affected by a number of component characters, information on the association of these characters was effective to study the relative importance of these characters in selection. A study on the coefficient of genotypic and phenotypic variance (gcv, pcv), heritability, genetic advance and correlation studies are useful guides for selection.

#### 2.1.2 Heritability, Genetic advance and Coefficient of variation.

Kaul et al., (1978) compared twenty genotypes of *A.esculentus* and reported high genotypic coefficient of variation (gcv) for pod yield per plant (31.14), seed yield per plant (24.73) and pods per plants (21.09). High heritability values were observed for pod yield per plant (0.94), days to first flower (0.89) and days to first harvest (0.87). In a three-season study with 18 cultivars of okra, gcv, heritability estimates and genetic advance were found to be high for yellow vein mosaic, branches per plant, pods per plant, seeds per pod, length of pod, plant height and moderate for pods per plant and pod weight. Days to 50 percent flowering, nodes per plant, pod girth and 1000 seed weight had low values (Mishra and Chhonkar, 1979).

Murthy and Bavagi (1982) conducted studies on six varieties of okra and reported high heritability for characters like plant height, days to flowering, pod length and yield. The highest heritability value (0.99) was shown

by pod length. Studies conducted on twenty five *A.esculentus* genotypes indicated high heritability and genetic advance values for fruits per plant, plant height and root length (Vashista et al., 1982). In another study by Palaniveluchamy et al., (1982), plant height showed the highest estimates of heritability and genetic advance.

Comparative study on eight cultivars of okra revealed that gcv and pcv were high for fruit yield per plant and plant height. A high genetic advance as percent of mean along with high heritability was observed for fruit yield per plant, number of branches and plant height (Reddy et al., 1985). Ariyo (1990) estimated gcv, pcv and heritability for 15 agronomic characters in okra for two seasons using 30 lines of diverse origin. High gcv and heritability estimates were recorded for plant height at flowering, edible pod length, final plant height, number of seeds per pod and length of mature pod indicating their likely effectiveness for selection.

An investigation on 23 okra genotypes for eight economic characters revealed high gcv and heritability estimates for yield per plant and number of fruits per plant. High genetic advance was shown by yield per plant (Venketesh, 1991).

### 2.1.3 Association of characters with yield

Studies conducted on 20 genotypes of okra showed that selection of plants having more primary branches per plant and plant height would result in selection of high yielding genotypes (Kaul et al., 1978). Fruit yield was found to have positive and significant genotypic association with number of fruits per plant, number of branches per plant, fruit

length and plant height (Singh and Singh, 1979 and Vashista et al., 1982).

Arumugham and Muthukrishnan (1981) conducted studies on okra variety Pusa sawani and F<sub>1</sub> hybrid of Col x A.manihot and reported that fruit yield was highly correlated with number, length and seed content of fruits and its lower degree with plant height and days to flowering.

Based on the studies conducted on six varieties of okra Murthy and Bavagi (1982) reported significant and positive correlation with number of fruits per plant and negative correlation with days to flowering. Reddy et al., (1985) found significant and positive correlation between fruit yield and days to flower, plant height, number of branches, fruit length, fruit girth and fruits per plant when they screened eight okra cultivars and their 28 F<sub>1</sub>'s. Yield was reported to have significant positive correlation with weight of pod and number of pods per plant by Mishra and Singh (1987) and Balakrishnan and Balakrishnan (1990).

Based on an investigation on 23 okra genotypes for eight economic characters Venkatesh, (1991) found that genotypic correlation of fruits per plant, fruit weight and plant height showed significant association with yield. Sundari et al., (1992) conducted a comparative study on 30 hybrids from full diallel cross of six okra inbreds. Characters like internodal length, number of pods per plant, pod length, pod girth and individual pod weight showed positive and significant association with yield. Correlation coefficient between yield and number of pods per plant was the highest (0.88). Similar results obtained except for internodal length by Mishra and Singh (1992) on evaluation of 18 cultivars of okra.

Plant height, number of pods per plant and days to first flowering were found to be the important yield contributing factors according to Patel et al., (1993) after evaluating ten okra hybrids.

2.1.4 Association between different components other than yield.

High correlation was observed between seed yield and pod yield when twenty genotypes of okra were evaluated by Kaul et al., (1978). High and positive correlation was found between node at which first fruit appeared and days to flowering, but with yield and fruit number, first fruiting node had negative correlation (Murthy and Bavagi 1982). El Maksoud et al., (1984) evaluated hybrids between the inbred lines of *A.esculentus* cultivars viz. Balady and Gold coast. In the F<sub>2</sub> generation, positive correlation was seen between plant height and each of fruits per plant, fruit weight and fruit length. Late flowering was positively associated with more fruits per plant and larger fruits. Comparative studies on eight cultivars showed that significant positive correlation was there for fruits per plant with days to flower, plant height, number of branches, fruit length and fruit width (Reddy et al., 1985).

Mishra et al., (1990) based on a study on 12 cultivars reported that plant height established a positive and significant correlation with nodes in main stem, pods per plant and pod weight, there was negative correlation between plant height with pod girth and seeds per pod. Branches per plant showed a positive significant correlation with seed per pod and negative correlation with pod length. Girth of pod

was positively correlated with weight of the pod and seeds per pod.

Thirty hybrids from full diallel cross of six okra inbreds were evaluated by Sundhari et al., (1992). They observed significant and positive genotypic correlation (0.36) for internodal length and with number of pods per plant. Number of pods per plant, pod length and pod girth had positive and significant association with individual pod weight. Between pod length and pod girth the association was strong (0.38).

Mishra and Singh (1992) conducted a study on 18 cultivars of okra and observed positive and significant association of plant height with fruit length, fruits per plant, fruit weight and 1000 seed weight. However it had negative correlation with number of nodes per plant, days to 50 percent flowering, number of seeds per fruit, yellow vein mosaic resistance and yield per plant. The number of nodes per plant recorded a significant and positive association with fruits per plant at phenotypic as well as genotypic level. Days to 50 percent flowering showed a significant positive association with the seeds per fruit while fruit girth was negatively correlated with 1000 seed weight at both levels.

Studies conducted on ten okra inbreds revealed significant correlation of plant height with first flowering node (0.47 gcv), internodal length (0.63 gcv) and days to first flowering (0.49 gcv) (Patel et al., 1993).

#### 2.1.5 Path coefficient analysis.

Path coefficient analysis facilitates the partitioning of the correlation coefficient of genetical parameters of crop plants into direct and indirect effects of various traits involving the yield per plant.

Path coefficient analysis carried out on twenty genotypes of okra by Kaul *et al.*, (1978) revealed that days to 50 percent germination had the maximum direct effect on days to first pod harvest and can be used as a marker character to select early plant types. It also indicated that primary branches per plant followed by pod yield per plant had the maximum direct effect on seed yield, which in turn had high correlation (0.65) with pod yield.

Singh and Singh (1979) conducted studies on thirty strains of okra and reported positive direct effect of internodal length, fruit width and number of fruits per plant towards yield. But number of branches per plant, days to flowering and fruit length had negative effect towards yield.

Six varieties of okra were tested by Murthy and Bavagi (1982) and their studies revealed direct and significant effect of number of fruits per plant (+1.2173) and days to flowering (+1.0658) on yield. But they had negative effect on yield through flowering and fruit number respectively.

Path analysis on  $F_2$  generation plants of seven okra genotypes showed that the number of fruits per plant followed by fruit weight had the highest direct effect on yield. (Balakrishnan and Balakrishnan 1990). Path analysis on cultivar 'Pusa sawani' by Singh *et al.*, (1990) revealed that number of fruits per plant, fruit weight, fruit length and fruit diameter were the important yield components of okra.

Similar results were obtained by Venkatesh (1991) in a study of 23 okra genotypes.

Fruit length and number of fruits per plant were considered as the most important variables for selection and hybridization programme for improvement of okra by Mishra and Singh (1992) based on a study on 18 cultivars of okra.

#### 2.1.6 Genetic divergence studies

$D^2$  and canonical analysis are used to assess the nature and magnitude of genetic divergence. It also helps in solving taxonomical problems in biological population of intervarietal or subspecies and species level. Invariably the application of  $D^2$  analysis is associated with the use of canonical variable analysis.

Based on Analysis conducted on thirty okra varieties using  $D^2$  statistics, the varieties were grouped into eight clusters by Singh and Singh (1979). Days to flowering, number of fruits per plant and fruit bearing branches were found to be important contributors to genetic divergence.

Pratap et al., (1980) employed  $D^2$  statistics on the data on yield and yield related characters to assess genetic divergence in seven diverse parents of *A.esculentus* and other 21  $F_1$  hybrids. The genotypes were divided into six clusters, cluster I and cluster II being the largest. Maximum genetic distance was shown between clusters III and V. Fruit length contributed most towards total divergence.

Suresh Babu (1987) conducted canonical variate analysis on 14 entries comprising of *A.esculentus* line 11 H R 20-31, *A.tetraphyllus* var *tetraphyllus* the inter specific hybrid, amphidiploid and nine advanced generation lines stabilized for resistance to yellow vein mosaic virus. The genotypes



would be grouped into four clusters. The recurrent parent, *A.esculentus* line 11 HR 20-31 and all the advanced generation lines formed one cluster. The solitary clusters were formed by the wild species and the amphidiploid. The reciprocal hybrids formed a separate cluster. The variable like fruits per plant, width of epicalyx segment, weight of fruit, days to flower and first fruiting node were the major contributing variables towards diversity of the entries.

Principle component analysis was used to analyze the variation pattern in 20 accessions of okra by Ariyo and Oduluja (1991). The studies revealed that the first three principal components viz. 1. life span, length of mature pod, length : breadth ratio, number of branches per plant, number of leaves per plant and flowering period; 2. stem pigmentation, colour of leaf veins, petiole pigmentation, pedicel length, final height and pod pigmentation, 3. pod colour, pod pigmentation, internodal length, stem diameter, pod length and leaf lobing accounted for 49.49 percent of the total variation among the 30 characters that described the 20 accessions. Venkatesh (1991) classified 23 genotypes of okra into five clusters based on principal component analysis.

Factor, principal components and canonical analysis were used to study the extent of genetic diversity among 30 accessions of West African okra. Pigmentation of various parts of the accessions and fruit characters contributed significantly to the total variation observed. The first three canonical variables accounted for 100 percent of the total variance, the first variable primarily consisted of the number of pods per plant and pod weight whereas the second canonical variable was primarily loaded by number of

seed per plant and flower colour, the third canonical variable was comprised of 100 seed weight and number of epicalyx segments (Ariyo 1993).

## 2.2 Interspecific hybridization and Crossibility studies

Interspecific hybridization provides means to transfer a few of the valuable characters from wild or economically inferior species to the agronomically superior cultivated species. Interspecific hybridization was carried out in the genus *Abelmoschus* for the last half a century with different objectives.

The cross that had been most studied was that of two cultivated species *A. esculentus* and *A. manihot* (Teshima, 1933; Ustinova, 1949; Pal et al., 1952; and Kuwada 1969). Success although partial is most easily obtained if *A. esculentus*, the species with the highest chromosome number was used as the female parent. The F<sub>1</sub> hybrids were vigorous but not very fertile.

Pal et al., (1952) conducted studies on morphological characters and interspecific hybridization among the different species of *Abelmoschus* viz. *A. esculentus*, *A. tuberculatus*, *A. manihot*, *A. manihot* var *pungens* and *A. ficulneus*. Whereas the first four species readily crossed with each other and formed normal viable seeds, crosses with *A. ficulneus* resulted in only shriveled or empty seeds. The hybrid plants showed strong heterosis in growth and fruiting. They were also early in flowering. The studies revealed that *A. tuberculatus* was more related to *A. esculentus* than any other species.

Mamidwar *et al.*, (1980) while studying crosses between cultivars of *A.esculentus* and *A.manihot* found that fruit set was higher when *A.esculentus* was used as the female parent with 8.33 as the near value for percentage fruit set.

Hybridization work done between plant of *A.esculentus* and unnamed West African okra resulted in day length sensitive hybrids with shortened internodes (Martin 1982).

Interspecific crosses between two cultivars of *A.esculentus* and a cultivar of *A.manihot* and one of the reciprocals were successful. On comparison of parental  $F_1$ ,  $F_2$ ,  $BC_1$  and  $BC_2$  genotypes, heterosis over the better parent was observed for number of fruits per plant, plant height and number of branches (Dhillon and Sharma, 1982). Pillai (1984) produced interspecific hybrids between *A.manihot* and *A.esculentus* and found that the hybrids produced fruits but the seeds per fruit was less. Nerkar and Jambhale (1985) conducted hybridization between *A.esculentus* x *A.tetraphyllus* and *A.esculentus* x *A.manihot*. The hybrids exhibited heterosis for plant height, spread, number of branches, mean index of leaf and flower size.

Prabha (1986) conducted investigations to assess the extent of compatibility between *A.esculentus* and *A.manihot*. All the crosses were compatible and the hybrids exhibited vigour for several of the polygenically controlled characters.

### 2.2.1 Interspecific hybrid sterility.

Teshima (1933) observed that *A.esculentus* and *A.manihot* crossed only when the former was used as the female parent.

Chizaki (1934) also studied this cross and reported that the  $F_1$  hybrids were partially fertile.

Pal et al., (1952) conducted interspecific crosses between five species of *Abelmoschus* viz. *A.esculentus*, *A.tuberculatus*, *A.ficulneus*, *A.manihot* and *A.manihot* var *pungens* and reported that the crosses mostly resulted in shriveled or empty seeds. The various  $F_1$  hybrids studied were sterile. Pollen fertility ranged from 40 to 95 percent in different crosses, the highest being in the cross *A.esculentus* x *A.manihot*.

About 90 percent sterility was reported in interspecific hybrid between *A.esculentus* x *A.manihot* by Arumugham et al., (1975). In interspecific hybridization between different *Abelmoschus* species viable seeds could be obtained only in crosses between *A.manihot* ( $2n = 58, 68$ ) and *A.ficulneus* ( $2n = 72$ ) and *A.tuberculatus* ( $2n = 58$ ) resulting plants were sterile. (siemonsma 1982). Partial seed fertility of 5.9 percent and 7.1 percent were obtained in crosses *A.esculentus* x *A.manihot* and *A.esculentus* x *A.manihot* ssp *manihot* respectively by Jambhale and Nerkar (1985).

## 2.3 Cytological and cytogenetical studies.

### 2.3.1. chromosome number

The chromosome number of okra (*A.esculentus*) showed a wide range of variation. Teshima (1933) reported its chromosome number as  $2n = 72$ , Kuwada, (1966) confirmed it as  $2n = 124$ . The mostly prevailing chromosome number  $2n = 130$  had been reported by Joshi and Haridas (1976) and Joshi et al., (1974). The reported chromosome number for *A. manihot* was in the range of  $2n = 60$  to  $68$  (Teshima (1933) and Kuwada (1974)).

### 2.3.2 Ploidy levels in Abelmoschus

Charrier (1984) reported three ploidy levels in the genus *Abelmoschus* first ploidy level was having  $n = 28$  to  $36$  which included *A.angulosus* ( $n = 28$ ), *A.ficulneus* ( $n = 36$ ), *A.tuberculatus* ( $n = 29$ ), *A.manihot* ( $n = 30 - 34$ ) and *A.moschabus* ( $n = 34$ ). Ploidy level second had  $n = 62 - 69$  and it included species such as *A.crinatus* ( $n = 69$ ), *A.esculentus* ( $n = 62 - 65$ ), *A.tetraphyllus* var *tetraphyllus* ( $n = 65 - 69$ ) and *A. tetraphyllus* var *pungens* ( $n = 69$ ). The third ploidy level includes only *A.caillei* with  $n = 92 - 100$ .

### 2.3.3 Cytogenetical studies.

In order to identify the parental species of *A.esculentus* a number of interspecific crosses had been attempted. A considerable but incomplete pairing of chromosomes was observed in the cross *A.esculentus* ( $n = 65$ ) x *A.ficulneus* ( $n = 36$ ). Cytological studies of the PMCs revealed 27 bivalents and 46 univalents indicating good affinity of homologous chromosomes (Pal et al., 1952). Meiotic studies of the hybrid between *A.esculentus* ( $n = 65$ ) and *A.tuberculatus* ( $n = 29$ ) revealed almost perfect pairing of the genome of *A.tuberculatus* with 29 chromosomes of *A.esculentus*. The  $F_1$  hybrid gave 29 bivalents and 36 univalents (Joshi et al., 1974). *A.tuberculatus* was thus accepted as one of the progenators of *A.esculentus*.

Kuwada (1957, 1961) obtained sterile  $F_1$  hybrid of the cross between *A.esculentus*  $2n = 124$  and *A.manihot* ( $2n = 68$ ). The meiotic studies in the interspecific  $F_1$  showed little chromosome homology between the genome of the two species.

The amphidiploid ( $2n = 192$ ) obtained by colchicine treatment of the  $F_1$  hybrid was fertile and it was named as Nori-Asa.

Simonsuma (1982) proposed a hypothesis that *A.caillei* might be a natural amphiploid of *A.esculentus* and *A.manihot*. Kondiah *et al.*, (1990) made crosses between *A.caillei* and *A.tetraphyllum* and with two induced amphidiploids viz. *A.esculentus* - *manihot* and *A.esculentus* - *tetraphyllum*. Cytological studies in the cross between *A.tetraphyllum* x *A.caillei* gave 66.5 bivalents and 6.52 univalents whereas the cross between *A.esculentus* - *tetraphyllum* x *A.caillei*, 71.8 bivalents and 18.22 univalents were observed in addition to trivalents and tetravalents indicating high degree of homology between the genome of *A.tetraphyllum* and *A.caillei* which suggested that *A.tetraphyllum* would have contributed two genome to *A.caillei*. Chromosome pairing behavior in the  $F_1$  of *A.esculentus* - *manihot* x *A.caillei* gave 46 bivalents and 13 univalents. It was likely that out of 46 bivalents majority could be due to genome homology between *A.manihot* and *A.caillei*. It thus indicated indirectly that *A.manihot* might have contributed one genome and *A.tetraphyllum* two genomes to *A.caillei*.

#### 2.4 Induction of Amphidiploidy

Joshi *et al.*, (1974) reported sterile hybrid between *A.tuberculatus* ( $n = 29$ ) x *A.fisculneus* ( $n = 36$ ). The genomes of the two species showed very little homology. However the induced amphidiploid showed 65 bivalents. Although the amphidiploid was sterile the allotetraploid structure of *A.esculentus* could be reconstructed.

Jambhale and Nerkar (1982) synthesized an amphidiploid from the cross *A.esculentus* ( $2n = 130$ ) x *A.manihot* ssp *manihot* ( $2n = 194$ ) by colchicine treatment of the interspecific hybrid. The amphidiploid plants exhibited reduction in height and spread, number of primary branches, length of petiole, stipule, bracteole, and length of fruit. Fruit gigantism was observed for size of flower, breadth of bracteole and girth of fruit. Considerable increase in length of stomata, frequency of stomatal chloroplast and pollen diameter was observed in the amphidiploid while frequency of stomata per square millimeter was reduced, the chromosome configuration at metaphase - I in amphidiploid was more or less regular forming 162 bivalents.

Suresh Babu and Dutta (1987) induced an amphidiploid by colchicine treatment of the  $F_1$  hybrid of the cross *A.esculentus* ( $n = 65$ ) x *A.tetraphyllus* var *tetraphyllus* ( $n = 69$ ). The induced amphidiploid showed regular meiosis forming 134 bivalents and was highly fertile.

**Materials and method**



## MATERIALS AND METHODS

The present investigations were carried out in the College of Horticulture, Vellanikkara Thrissur during the year 1993 - '95. The study was carried out at the Vegetable Research Farm of the Department of Olericulture. The Vegetable Research Farm is located at an altitude of 22.5 m above MSL between  $10^{\circ} 32''$  N latitude and  $76^{\circ} 16''$  E longitude. The area enjoys a warm humid tropical climate.

The investigations were carried out under the following heads

1. Variability correlation, path coefficient and genetic divergence studies
2. Interspecific hybridization and crossability studies
3. Cytological and cytogenetical studies
4. Induction of amphiploidy

### 3.1 Materials

The materials comprised of twenty two diverse genotypes of "Thamaravenda" collected from different parts of the state and National Bureau of Plant Genetic Resources, Regional Station Vellanikkara varying in morphology and yield. List of genotypes and their sources are given in the table 1. For the cytogenetical studies *A.esculentus* line AE - 202 (plate 1) and a representative genotype of *A.manihot* AM - 4 (plate 2) were selected.

### 3.2 Methods

3.2.1 Variability, correlation, Path coefficient and Genetic divergence studies

Table 1. Morphological characters and place of collection of 22 genotypes of "THAMARAVENDA"

Genotype	Source	Stem Colour	Leaf Colour	Fruit Shape	Fruit Colour	Spines on fruit
AM1	Tripayar	b	e	Ridged broad long	b	Non spiny
AM2	Moovattupuzha	a	d	Deeply furrowed short	a	Non spiny
AM3	Manarkadu	c	e	Ridged, long broad	b	Non spiny
AM4	Ottapalam	c	e	Ridged, long broad	b	Non spiny
AM7	Mannuthy	c	e	Long, ridged broad	b	Non spiny
AM10	NBPGR Vellanikara	a	d	Smooth with typical constriction towards base	a	Non spiny
AM11	NBPGR Vellanikara	c	e	Deeply furrowed ridged long	b	Non spiny
AM12	NBPGR Vellanikara	a	d	Deeply furrowed, short	a	Non spiny
AM14	NBPGR Vellanikara	b	e	Long ridged, broad	b	Non spiny
AM18	NBPGR Vellanikara	b	e	Long ridged, broad	b	Non spiny
AM19	NBPGR Vellanikara	c	e	Long ridged, broad	b	Non spiny
AM20	NBPGR Vellanikara	c	e	Almost smooth short, slender	b	Spiny
AM21	NBPGR Vellanikara	c	e	Ridged oblong	b	Non spiny
AM23	NBPGR Vellanikara	b	e	Short, deeply furrowed	a	Non spiny
AM25	NBPGR Vellanikara	a	d	Shortest deeply furrowed	a	Spiny
AM27	NBPGR Vellanikara	a	d	Long slender and smooth	a	Non spiny
AM28	Mannuthy	a	d	Deeply furrowed, short broad	a	Spiny
AM31	Kaipamangalam	c	e	Ridged broad long	b	Non spiny
AM33	Trichur	b	e	Ridged broad	b	Non spiny
AM34	Mavelikkara	a	d	Smooth at the base and ridged at the end	a	Spiny towards Apex
AM35	Mavelikkara	c	e	Smooth at the base and ridged at the end	b	Spiny
AM36	Mavelikkara	b	e	Deeply furrowed oblong	b	Slightly spiny

a - Green

e - Deep pink

b - Predominantly green with pink tinge

c - Predominantly pink with green tinge

d - Light pink

Plate 1. *Abelmoschus esculentus* line AE - 202 selected for interspecific hybridisation.

Plate 1



Plate 2. AM - 4 the genotype of Thamaravenda selected for interspecific hybridisation

Plate 3. Variability in fruit shape and size observed in Thamaravenda

Plate 2



Plate 3

Under this programme the twenty two genotypes of *A. manihot* were raised in the field in a randomized complete block design with two replications. The spacing allotted was 60 cm between rows and 45 cm between plants. The treatments were raised in two equal rows of 5 m length which formed a plot in each replication. The crops received timely management and care as per the Package of Practices Recommendations of Kerala Agricultural University (1993).

#### 3.2.1.1 Observations recorded.

Five plants were randomly selected from each genotype per replication and observations were recorded on the different qualitative and quantitative characters. Observations on the following quantitative characters were taken and their averages worked out as treatment mean for further statistical analysis.

##### 1. Days to first flowering.

The number of days taken for the first plant to flower in an entry was recorded and termed as the date to first flowering.

##### 2. First flowering node.

The node at which first flower appeared in each of the observation plant was noted and their average was taken as first fruiting node.

##### 3. Plant height.

Plant height was recorded from the base to the tip of the plant after 120 days of sowing.

##### 4. Basal stem diameter.

Diameter of the stem at the base of the plant was measured in centimeter, 120 days after sowing.

5. Maximum number of internodes.

Total number of internodes on the main stem of each plant was taken after 120 days of sowing.

6. Internodal length.

The length of internode between sixth and seventh node of the plant taken, 120 days from the sowing.

7. Number of primary branches.

The number of primary branches per plant was counted 120 days after sowing.

8. Length of petiole.

The length of the petiole of seventh leaf of each plant was measured in centimeters 90 days after sowing.

9. Length of leaf.

The length of seventh leaf of each plant was measured in centimeters 90 days after sowing.

10. Length of main leaf lobe.

The length of main leaf lobe of seventh leaf from the tip of each plant was measured in centimeter 90 days after sowing.

11. Number of epicalyx segments.

The number of epicalyx segments of ripe flower bud in each genotype was counted 90 days after sowing.

12. Length of epicalyx segment.

Length of epicalyx segment of ripe flower bud of each genotype was recorded.

13. Width of epicalyx segment.

Width of epicalyx segment of the ripe flower bud of each entry was recorded.

14. Length of fruit.



Ten days after fruit set the length of the fruit was measured from the basal cap to the tip of fruit in an entry.

15. Girth of fruit

Ten days after the fruit set the diameter of the fruit was taken at the point of maximum bulging in an entry.

16. Single fruit weight.

Weight of a single fruit was taken ten days after fruitset from each of the observation plant

17. Number of ridges per fruit.

The number of ridges per fruit of each entry was noted, ten days after fruit set.

18. Number of fruits per plant.

Total number of fruits borne on the five observation plants were recorded in each entry and their mean was computed as number of fruits per plant.

19. Fruit yield per plant.

Weight of fruits harvested from the five randomly chosen observation plants were noted in each entry and their mean taken to get the fruit yield per plant.

20. Days to first harvest.

The number of days taken to harvest the first fruit in the five observation plants in each genotype was taken and their average was taken to get the days to first harvest.

21. Days to final harvest.

The days in which the five observation plants of each genotype was finally harvested was treated as the days to final harvest.

22. Number of seeds per fruit.

Number of seeds per fruit of ten randomly selected pods was noted and the mean was worked out.

### 23. Hundred seed weight.

Weight of hundred seeds in each genotype was taken. Sample size was three.

#### 3.2.1.2 Statistical analysis

The mean of the values recorded on observations of five randomly selected plants were taken for statistical analysis. Data on different characters were subjected to statistical analysis at the computer centre, Department of Agricultural Statistics, Kerala Agricultural University. The analytical method suggested by Fisher (1954) was employed for estimation of various genetic parameters.

##### 3.2.1.2.1 Phenotypic, Genotypic and Environmental variances.

The variance components were estimated using the formula suggested by Burton (1952).

$$\text{Phenotypic variance (Vp)} = \text{Vg} + \text{Ve}$$

where,

$$\text{Vg} = \text{Genotypic variance}$$

$$\text{Ve} = \text{Environmental variance}$$

$$\text{Genotypic variance (Vg)} = (\text{Vt} - \text{VE})/\text{N}$$

where,

$$\text{Vt} = \text{Mean sum of squares due to treatments}$$

$$\text{VE} = \text{Mean sum of squares due to error}$$

$$\text{N} = \text{Number of replications}$$

$$\text{Environmental variance VE}$$

##### 3.2.1.2.2 Phenotypic and Genotypic coefficient of variation.

The phenotypic and genotypic coefficient of variation were calculated by the formula suggested by Burton and Devane (1953).

Phenotypic coefficient of variation (pcv) =  $(V_p/X) \times 100$

where,

$V_p$  = Phenotypic variance

$X$  = Mean of character under study.

Genotypic coefficient of variation (gcv) =  $(V_g/X) \times 100$

where,

$V_g$  = Genotypic variance

$X$  = Mean of character under study.

#### 3.2.1.2.3 Heritability

Heritability in the broad sense was estimated by the formula suggested by Burton and Devane (1953).

Heritability in the broad sense,  $H = (V_g / V_p) \times 100$

where,

$V_g$  = Genotypic variance

$V_p$  = Phenotypic variance

#### 3.2.1.2.4 Expected genetic advance

The genetic advance expected for the genotypes at five percent selection pressure was calculated using the formula suggested by Lush (1949) and Johnson et al., (1955) with value of the constant  $K$  as 2.06 as given by Allard (1960)

Expected genetic advance  $G_a = (V_g/V_p) \times K$

where,

$V_g$  = Genotypic variance

$V_p$  = Phenotypic variance

$K$  = Selection differential

### 3.2.1.2.5. Genetic gain (Genetic advance as percentage of mean)

Genetic advance (GA) calculated by the above method was used for estimation of genetic gain

$$\text{Genetic gain, (GA)} = (\text{GA}/\text{X}) \times 100$$

where,

GA = Genetic advance

X = Mean of character under study

### 3.2.1.2.6 Phenotypic, Genotypic and Environmental correlation coefficients

The phenotypic, genotypic and environmental correlation coefficients were worked out to study the extent of association between the characters. The phenotypic, genotypic and environmental variances were worked out in the same way as the variances were calculated. Mean product expectations of the covariance analysis are analogous to the mean square expectations of the analysis of variance. The different covariance estimates were calculated by the method suggested by Fisher (1954)

Phenotypic covariance between two characters 1 and 2

$$(\text{CoVp } 1, 2) = \text{CoVg } 1,2 + \text{CoVe } 1, 2$$

CoVg 1, 2 = Genotypic covariance between characters 1 and 2

CoVe 1, 2 = Environmental covariance between characters 1 and 2

Genotypic covariance between characters 1 and 2

$$\text{Covg } 1, 2 = (\text{Mt1, 2} - \text{Me1, 2}) / N$$

where,

Mt1, 2 = Mean sum of products due to treatment between

characters 1 and 2

$M_{e1, 2}$  = Mean sum of products due to error between characters

1 and 2

$N$  = Number of replications.

$V_{e1}$  = Environmental variance of character 1

$V_{e2}$  = Environmental variance of character 2

The phenotypic, genotypic and environmental correlation were adopted for the analysis using the formula by Dewey and Lu (1959). The characters which showed significant correlation with yield at one percent level of significance alone were considered for path coefficient analysis.

#### 3.1.2.2.7 Path coefficient analysis

In path coefficient analysis the correlation among cause and effect are partitioned into direct and indirect effects of causal factors on effect factor. The principles and techniques were suggested by Wright (1921) and Li (1955) for cause and effect.

#### 3.2.1.2.7 Genetic divergence studies.

The genetic divergence among 22 genotypes of *A. manihot* was calculated using all the 22 characters of variability analysis. The method suggested by Mahalanobis (1928) was used to estimate the genetic divergence.

Grouping of genotypes to clusters was done by Tocher's method (Rao, 1952). Coefficient among the various factors were worked out in all possible combinations according to the formula suggested by Johnson *et al.*, (1955).

### 3.2.2.1 Interspecific hybridisation.

Under this programme the genotypes AE - 202 and AM - 4 were crossed reciprocally. For crossing ripe flower buds of the male parent were selected and bagged in butter paper bags. On the female parent flowers were emasculated one day prior to their opening. A slight ring like incision was made at the base of the ripe flower bud as deep as to remove the corolla and calyx as one unit , exposing the staminal tube and stigma. Thus the undehisid anthers were removed carefully using a pair of forceps and the emasculated flowers were bagged. Next day morning, before 9.a.m., the butter paper bags were removed and pollination was done by the pollen of the bagged flower from the male parent. After pollination the flowers were again bagged and properly tagged. The bags were removed three days after pollination. When the pods were dried the seeds were collected.

### 3.2.2.2. Crossability and morphological studies.

Interspecific crossing was done between AE - 202 and AM - 4 in both directions. Altogether 35 crosses were made in each direction. The number of fruits set and the number of seeds per fruit were noted to work out the setting percentage and crossability index. Further the parental species and the interspecific  $F_1$  plants were raised in the field for comparison of their morphology as per IBPGR guide lines.

### 3.2.3. Cytological and cytogenetical studies

Cytological studies on the species *A.manihot* and *A.esculentus* were conducted by studying their meiosis. In order to observe the cytogenetic relationship between these

species, a detailed meiotic study on the interspecific  $F_1$  was also made. Elaborate pollen and microspore studies were also made on the species and their interspecific hybrid.

#### 3.2.3.1. Fixation of flower buds.

For this flower buds were collected from AE - 202, AM - 4 and their interspecific hybrid between 5.15 a.m. to 6.30 a.m. when the maximum number of pollen mother cells (PMCs) were in the stages of division. The collected buds were cut half way longitudinally and put immediately in Carnoy's B fixative consisting of six parts absolute alcohol, three parts chloroform and one part glacial acetic acid for 24 hours. Then the buds were transferred to another fixative containing three parts of ethyl alcohol and one part of acetic acid, the acetic acid part was saturated with ferric acetate which served as a mordant. After another 24 hours of fixing in the second fixative, meiotic preparations were made. For later use the flower buds in the fixative were stored in a refrigerator in 70 percent alcohol.

#### 3.2.3.2. Preparation of slides.

For the preparation of slides two or three anthers from a flower bud were taken at a time on the slide. Acetocarmine stain of 0.5 percent concentration was added and a coverslip was placed over it. Then it was subjected to heat near boiling. For obtaining the desirable spread of chromosomes pressure was applied on the coverslips by folds of blotting paper and was observed under a microscope to study the chromosome number and the association of chromosomes. The

slides were temporarily sealed with paraffin wax and could be stored for about three to four days without deterioration of the PMCs. Microphotographs were taken from temporary preparations using Leitz Biomed microscope attached with an automatic camera.

#### 3.2.3.3. Microspore and pollen fertility studies.

For studying the microspores immature anthers were placed on a slide and a drop of Alexander stain was added. This stain was prepared as per the procedure given by Alexander (1980). Later it was covered with a coverslip and slightly warmed. Subsequently slight pressure was applied so that the microspores were ejected out of the anther. Observations were made on the percent of tetrads, diads, triads, pentads etc.

Pollen fertility was assessed by staining in Alexander stain. Then the percent of stained and unstained pollen and their diameter were worked out.

#### 3.2.4. Induction of Amphiploidy.

For the induction of amphiploidy a method suggested by Suresh Babu and Dutta (1987) was followed. The amphiploids were primarily identified by studying the chloroplasts of the guard cells and subsequently confirmed by cytological studies.

##### 3.2.4.1. Chloroplast preparation.



For this study a method suggested by Jambhale and Nerkar (1980) for staining the guard cell of the adaxial epidermis of okra leaves was followed. Number of stomata per unit area, number of chloroplasts per stomata and average length of guard cells were noted.

*Results*

## RESULTS

The present investigation included variability, biometrical and divergence studies involving twenty two genotypes of *A. manihot*. Under this programme a detailed cytogenetical study on *A. manihot* in relation with *A. esculentus* was also made, Regarding the cytological observations, pre-metaphase-I stages were not easily amenable for critical analysis while chromosomes could be clearly observed in the early and late metaphase - I stages onwards. The results pertaining to the above aspects have been described under appropriate headings in this chapter.

- 4.1 Variability, Biometrical and Genetic divergence studies.
  - 4.1.1. Variability, heritability and genetic advance.

The general analysis of variance of the 22 genotypes of *A. manihot* showed significant differences for all the nineteen characters studied (Table 3). The genotypic mean and population mean are given in table 2. Range, genotypic and phenotypic coefficients of variation, heritability, genetic advance and genotypic gain for all the 19 characters are given in tables 4 and 5.

### 1. Plant height

There was significant difference among the genotypes for height of the plant. AM - 23 was the tallest and AM - 10 the dwarfest. The value ranged from 70.5 cm to 159.4 cm. The general mean for this character was 108.7cm. The phenotypic and genotypic coefficients of variation were 26.15 and 25.04 respectively. This character showed values of 0.91, 53.7

Table 2. Mean value of 19 biometric characters in 22 genotypes of "THAMARAVENDA"

Genotype	Height of the plant (cm)	No: of branches	Main stem diameter (cm)	No: of internodes on main stem	Inter nodal length (cm)
AM1	74.75	2.26	9.15	27.72	2.43
AM2	151.30	2.71	10.68	28.01	3.31
AM3	86.8	3.32	11.66	31.45	3.01
AM4	91.8	2.80	10.75	29.5	3.05
AM7	92.45	3.00	10.24	25.30	3.00
AM10	70.53	3.26	8.89	28.96	3.54
AM11	93.4	2.44	11.43	26.78	2.61
AM12	156.05	2.85	10.82	31.10	5.29
AM14	95.03	3.25	12.02	33.75	2.66
AM18	140.40	2.00	10.85	32.10	2.65
AM19	87.39	2.05	10.23	24.70	2.38
AM20	137.80	2.92	11.20	37.43	2.74
AM21	96.07	1.95	9.56	29.03	2.20
AM23	159.40	2.75	12.24	35.10	4.16
AM25	119.96	3.00	10.33	31.03	4.85
AM27	135.96	2.63	10.66	40.90	2.09
AM28	136.56	3.30	12.38	29.41	3.31
AM31	102.41	2.35	13.95	31.67	3.93
AM33	85.76	2.81	8.84	23.32	2.95
AM34	102.90	4.40	11.35	40.85	2.90
AM35	87.00	2.85	9.25	30.90	2.70
AM36	88.53	2.58	10.48	25.40	2.28
Grand mean	108.7375	2.79	10.77	30.66	3.09
CD	17.10 (5%)	0.77 (5%)	1.60 (5%)	3.184 (5%)	0.77 (5%)



Table 2 (Contd.)

Genotype	Fruit ridges	Single fruit weight (g)	Fruits / plant	Yield / plant (g)	Seeds / fruit	Hundred seed weight (g)
AM1	6.20	26.42	6.82	168.95	51.84	6.14
AM2	8.10	23.90	11.92	285.02	61.25	6.46
AM3	6.00	25.50	6.19	149.84	69.96	6.38
AM4	7.52	29.12	10.14	282.11	52.25	6.57
AM7	6.22	26.76	5.87	145.05	46.25	6.26
AM10	7.70	17.03	17.05	266.64	66.45	5.53
AM11	8.60	25.00	12.36	303.20	53.35	6.94
AM12	5.75	20.40	7.09	135.94	39.65	7.79
AM14	5.53	29.50	11.10	310.54	55.45	8.27
AM18	6.70	33.60	16.48	546.57	64.60	5.35
AM19	6.18	31.39	7.10	206.95	45.50	4.32
AM20	8.45	21.45	16.24	329.41	40.72	6.55
AM21	7.50	29.80	10.12	291.75	44.08	6.77
AM23	9.15	26.54	11.91	297.65	65.31	7.22
AM25	7.85	21.10	4.29	85.45	45.35	7.34
AM27	0.00	28.97	25.56	721.27	48.50	5.62
AM28	7.86	27.43	9.05	232.29	32.10	6.22
AM31	6.39	32.40	8.91	275.45	47.20	7.60
AM33	5.25	30.60	17.23	512.29	46.85	6.47
AM34	5.00	21.50	15.27	313.01	41.92	5.32
AM35	5.75	23.35	9.29	203.51	40.28	6.55
AM36	5.89	30.50	7.68	219.07	45.29	6.76
Grandmean	6.53	26.47	1.26	285.54	50.20	6.47
CD	0.453(5%)	2.52(5%)	1.88(5%)	53.5(5%)	6.29(5%)	0.11(5%)

Table 3. General Analysis of Variance for 19 characters in 22 genotypes of "THAMARAVENDA".

Source	DF	Ht of the plant	No. of branches	Main stem diameter	No. of internodes on mainstem	Internodal length	Petiole length	Length of leaf
Replication	1	1635.9	0.14	6.85	52.19	0.31	11.77	00.35
Treatment	21	1550.1*	0.59 *	3.12 *	45.03*	1.37 *	42.40*	10.33*
Error	21	0067.5	0.14	0.59	02.34	0.14	03.59	02.08
Source	DF	Length of basal leaf lobe	First flowering	First flowering node	Pedicel length	Fruit length	Fruit width	Fruit ridges
Replication	1	0.002	3.55	0.07	0.009	0.008	0.97	0.02
Treatment	21	25.30 *	59.85 *	1.04 *	0.280 *	7.080*	2.81 *	7.05 *
Error	21	1.75	2.75	0.05	0.020	0.720	0.27	0.05
Source	DF	Single fruit weight	Fruit/plant	Yield / plant	Seeds / Fruit	100 seed weight		
Replication	1	0.37	7.74	5558	2.09	0.007		
Treatment	21	38.30 *	50.58 *	42667.2 *	199.69 *	1.62 *		
Error	21	1.47	0.82	661.52	9.14	0.003		

\* Significant at 1 % level

and 48.38 for heritability, genetic advance and genetic gain respectively.

## 2. Number of branches.

The highest number of branches was produced by AM - 34 and lowest by AM - 21. The range was 1.95 to 4.40.  $\bar{g}cv$  and  $\bar{p}cv$  were 17.08 and 21.64 respectively. Compared to other characters studied, this had the lowest heritability of 0.62. The genetic advance and genetic gain were 0.78 and 27.95 respectively. The general mean recorded was 2.79.

## 3. Main stem diameter.

For this character the values ranged from 8.84 cm in AM - 33 to 13.95 cm in AM - 31. The general mean was 10.77 cm. The heritability was 0.68. Genetic advance, genetic gain,  $\bar{p}cv$  and  $\bar{g}cv$  were 1.91, 17.73, 12.65 and 10.44 respectively.

## 4. Number of internodes on main stem.

The genotypes showed significant difference for this character. The general mean for this character was 30.66. The highest number of internodes were produced by AM - 27 (4.90) and the lowest by AM - 19 (24.70). The genetic gain was 29.45 and heritability 0.90. The  $\bar{g}cv$  and  $\bar{p}cv$  were 15.07 and 15.88 respectively.

## 5. Internodal length.

For internodal length also the genotypes showed significant difference. The general mean for this character was 3.09 cm. The highest value of 5.29 cm was recorded by AM - 12 and the lowest 2.09 cm by AM - 27. The heritability



gcv, pcv, genetic advance and genetic gain were 0.82, 25.40, 28.11, 1.46 and 47.25 respectively.

#### 6. Petiole length.

The grand mean for this character was 22.8 cm. The values ranged from 31.35 cm for AM - 31 to 10.30 cm for AM - 10. The gcv was 19.27 and pcv was 20.97. The values of 0.84, 8.34 and 36.47 were recorded for heritability, genetic advance and genetic gain respectively.

#### 7. Length of leaf.

The largest leaf was produced by AM - 34 and smallest leaf by AM - 1. The range of observations was 23.75 cm to 13.52 cm. The general mean was 18.22 cm, gcv and pcv were 11.15 and 13.68 respectively. Heritability, genetic advance and genetic gain were 0.66, 3.41 and 18.72 respectively.

#### 8. Length of basal leaf lobe.

Significant difference was recorded regarding this character by different genotypes. The largest value was recorded by AM - 31 and the smallest by AM - 10. The grand mean for this character was 26.19 and the values ranged from 17.15 to 32.9 cm. The heritability was 0.87 pcv and gcv were 14.04 and 13.10 respectively. Genetic gain was noted to be 25.20.

#### 9. Days to flowering.

The general mean recorded by this character was 60.11 days. The earliest flowering genotype was AM - 10 (42.43 days) and the latest flowering genotype was AM - 1 (67 days).

The gcv and pcv for this character were 8.89 and 9.31 respectively and the heritability was 0.91.

10. First flowering node.

The genotype AM - 20 flowered at the lowest node (6.25th node) whereas the genotype AM - 1 flowered at the highest node (9.08th node). The overall mean recorded was 7.67. The heritability for the character was 0.92. The gcv and pcv were 9.2 and 9.62 respectively.

11. Pedicel length.

The heritability and genetic advance recorded by this character were 0.87 and 0.69. The grand mean was 2.41 cm. The genotype AM - 12 had the longest pedicel (3.57 cm) and AM - 25 had the shortest pedicel (1.85 cm).

12. Fruit length.

Significant difference was recorded by the genotypes for this character (plate 3). The genotype AM - 27(plate 4) produced the longest fruit of 17.89 cm. The smallest fruit had a length of 9.45 cm produced by AM - 25. The grand mean for this character was 12.5 cm. The heritability, genetic advance, pcv and gcv were 0.82, 3.32, 14.27 and 15.81 respectively.

13. Fruit girth.

The values for this character ranged from 12.75 cm for AM - 23 to 7.47 cm for AM - 27. The general mean was 10.67 cm. Heritability and genetic gain were 0.83 and 58.65 percent respectively. The gcv and pcv were 10.62 and 11.68 respectively.

#### 14. Fruit ridges.

Large and significant difference was recorded by this character among the genotypes. It had a high heritability value of 0.99. There was a genotype having smooth fruit AM - 27 and another with fruits having ridges as high as 9.15. The gcv and pcv for these characters were 10.62 and 11.68 respectively.

#### 15. Single fruit weight.

The average fruit weight of 22 genotypes also showed significant difference. The values ranged from 17.03 g for AM - 10 to 33.60 g for AM - 18. The heritability for the character was 0.93 and the genetic gain was 32.15 percent. gcv was 16.22 and pcv was 16.85.

#### 16. Fruits per plant

There was significant difference among the 22 genotypes for this character also. The minimum number of fruits was observed for the genotype AM - 25 (4.29) and the maximum number fruits was produced by AM - 27 (25.56). The heritability observed was 0.97. Genetic gain had a high value of 89.79 percent. The general mean for this character was 11.26. The gcv was 44.31 and pcv was 45.04.

#### 17. Yield per plant.

This character had the highest values for gcv , pcv and genetic advance. The treatment mean ranged from 85.45 g for AM - 25 to 721.27 g for AM - 27. The general mean for this character was 285.54. Heritability value was 0.97. The gcv, pcv and genetic advance values were 50.75, 51.55 and 102.95 respectively.

Plate 4. AM - 27 a superior genotype of Thamaravenda

Plate 5. The interspecific hybrid of AM - 4 x AE - 202

Plate 4



Plate 5

Table 4. Range , Coefficient of variation, gcv, pcv and Heritability as a percentage of mean for 19 characters in "THAMARAVENDA"

Character	Range	Coeff of variation	Genotypic coeff of variation	Phenotypic coef f of variation	Herita-bility
Height of plant	70.53-159.4	7.56	25.04	26.15	0.917
No of branches	1.95-4.40	13.29	17.08	21.64	0.623
Mainstem diameter	8.84-13.95	7.14	10.44	12.65	0.681
No of internodes on mainstem	23.32-40.90	4.99	15.07	15.88	0.901
Internodal length	2.09-5.29	12.04	25.40	28.11	0.817
Petiole length	10.30-31.35	8.29	19.27	20.97	0.844
Length of leaf	13.52-23.75	7.92	11.15	13.68	0.664
Length of main leaf lobe	17.15-32.90	5.05	13.10	14.04	0.871
First flowering	42.43-67.40	2.76	8.89	9.31	0.912
First flowering node	6.25-9.08	2.79	9.20	9.62	0.916
Pediceal length	1.85-3.57	5.78	14.97	16.05	0.870
Fruit length	9.45-12.75	6.80	14.27	15.81	0.815
Fruit girth	7.47-12.75	4.85	10.62	11.68	0.827
Fruit ridges	5.00-9.15	3.34	28.66	28.86	0.987
Single fruit weight	17.03-33.60	4.58	16.22	16.85	0.926
Fruits / plant	4.29-25.56	8.04	44.31	45.04	0.968
Yield / plant	85.45-721.3	9.00	50.75	51.55	0.968
Seeds / fruit	32.10-69.96	6.02	19.44	20.36	0.913
100 seed weight	4.32-8.27	0.79	13.90	13.92	0.997

Table 5. Genetic advance and Genetic gain as percentage of mean for 19 characters in "THAMARAVENDA."

Character	Genetic advance	Genetic gain	Mean +- SE
Height of the plant	53.70	49.38	108.74 +- 8.22
No: of branches	0.78	27.96	2.79+-0.37
Main stem diameter	1.91	17.73	10.77+- 0.77
No: of internodes on main stem	9.03	29.45	30.66+- 1.53
Internodal length	1.46	47.25	3.09+- 0.37
Petiole length	8.34	36.47	22.87+- 1.9
Length of leaf	3.41	18.72	18.22+-1.44
Length of main leaf lobe	6.60	25.20	26.19+-1.32
First flowering	10.51	17.48	60.11+- 1.66
First flowering node	1.39	18.27	7.67+- 2.79
Pedicel length	0.69	28.63	2.41+- 5.78
Fruit length	3.32	26.56	12.50+- 0.85
Fruit girth	2.11	19.85	10.63+- 0.52
Fruit ridges	3.83	58.65	6.53+- 3.34
Single fruit weight	8.51	32.15	26.47+- 1.21
Fruits / plant	10.11	89.79	11.26+- 0.91
Yield / plant	293.95	102.95	285.54+- 25.72
Seeds / fruit	19.21	38.27	50.2+- 3.02
100 seed weight	1.85	28.59	6.47+- 0.05

#### 18. Seeds per fruit.

The highest number of seeds per fruit was produced by AM - 3 (69.96) and the lowest seed number per fruit was in AM - 28 (32.10). The general mean for this character was 50.20. Heritability, genetic gain, gcv and pcv were 0.91, 38.27 percent, 6.02 and 19.44 respectively.

#### 19. Hundred seed weight.

This character had the highest heritability value of 0.99. Significant difference was recorded by the genotypes for this character also. The lowest hundred seed weight was for AM -19 (4.32g) and highest for AM - 12 (7.79 g). The gcv was 13.90 and pcv was 13.92. The general mean recorded for the character was 6.47 g.

#### 4.1.2 Correlation studies.

The genotypic and phenotypic correlation ( $r_g$  and  $r_p$ ) of the different characters with the yield was worked out. The results are given in table 6 and 7. The characters fruit length, fruit girth, fruit ridges and fruits per plant showed significant phenotypic and genotypic correlation with yield. But for internodal length, the genotypic correlation alone was significant. The character fruits per plant showed the highest and significant positive correlation with yield ( $r_g = 0.91$  and  $r_p = 0.91$ ). The next significant positive correlation was shown by fruit length ( $r_g = 0.67$  and  $r_p = 0.6$ ). Number of fruit ridges showed the highest negative and significant correlation with yield ( $r_g = -0.52$  and  $r_p = 0.51$ ) followed by fruit girth ( $r_g = -0.49$  and  $r_p = -0.44$ ).

##### 4.1.2.1 Inter-correlation among different characters





Almost all the delete characters studied showed significant correlation among themselves. Plant height showed significant and positive correlation with number of internodes in main stem ( $r_g$  and  $r_p = 0.46$ ) and internodal length ( $r_g = 0.46$  and  $r_p = 0.43$ ). Number of branches per plant had significant and positive correlation with number of internodes on mainstem ( $r_g = 0.52$ ) and significant and negative correlation with single fruit weight. Main stem diameter had significant and positive correlation with petiole length, leaf length and length of basal leaf lobe ( $r_g = 5.02, 0.66$  and  $0.78$  respectively).

Number of internodes per stem had significant positive correlation with fruits per plant ( $r_g = 0.51$ ) and significant negative correlation with fruit girth ( $0.42$ ). Internodal length had significant negative correlation with fruit length ( $r_g = 0.56$ ), single fruit weight ( $r_g = 0.43$ ) and hundred seed weight ( $r_g = 0.51$ ) but positive correlation with fruit girth ( $r_g = -51$ ).

First flowering day had significant and positive correlation with first flowering node ( $r_g = 0.58$ ), fruit length ( $r_g = 0.45$ ) and single fruit weight ( $r_g = 0.50$ ). There was significant positive correlation for fruit length with single fruit weight ( $r_g = 0.48$ ), fruits per plant ( $r_g = 0.53$ ), negative significant correlation with fruit girth ( $r_g = -0.64$ ) and fruit ridges ( $r_g = -0.83$ ). With seeds per fruit, fruit girth showed a high positive correlation ( $r_g = 0.59$ ) whereas with fruits per plant its correlation was significant and negative ( $r_g = -0.49$ ).

Fruit ridges showed significant negative correlation with fruits per plant ( $r_g = -0.43$ ).



#### 4.1.3 Path-coefficient analysis.

The direct and indirect contribution of the different yield components were found out by partitioning the correlation between yield and their components into direct and indirect effect. Only those characters which showed significant correlation either with yield or yield contributing characters were considered for this analysis. These characters were plant height, number of internodes, internodal length, days to flower, fruit length, fruit girth, fruit ridges and fruits per plant.

Positive direct effects on yield were shown by the plant height, days to flower, fruit length, fruit girth and fruits per plant whereas number of internodes, internodal length and fruit ridges showed negative direct effect on yield. The highest positive direct effect was shown by fruits per plant (0.8463) followed by plant height and highest in direct effect by number of internodes (table 8).

Though plant height showed a positive direct effect on yield, its indirect effect on yield through all the yield contributing factors were negative except for fruits per plant (0.19). The direct effect of number of internodes per plant on yield was negative (-0.21). But it had positive indirect effect on yield through plant height (0.12), fruit length (0.03), fruit ridges (0.03) and fruits per plant (0.43). A negative indirect effect on yield was shown by number of internodes through internodal length (-0.007), days to flower (-0.005) and fruit girth (-0.009) though its correlation with yield was positive (0.39) (Fig. 1).

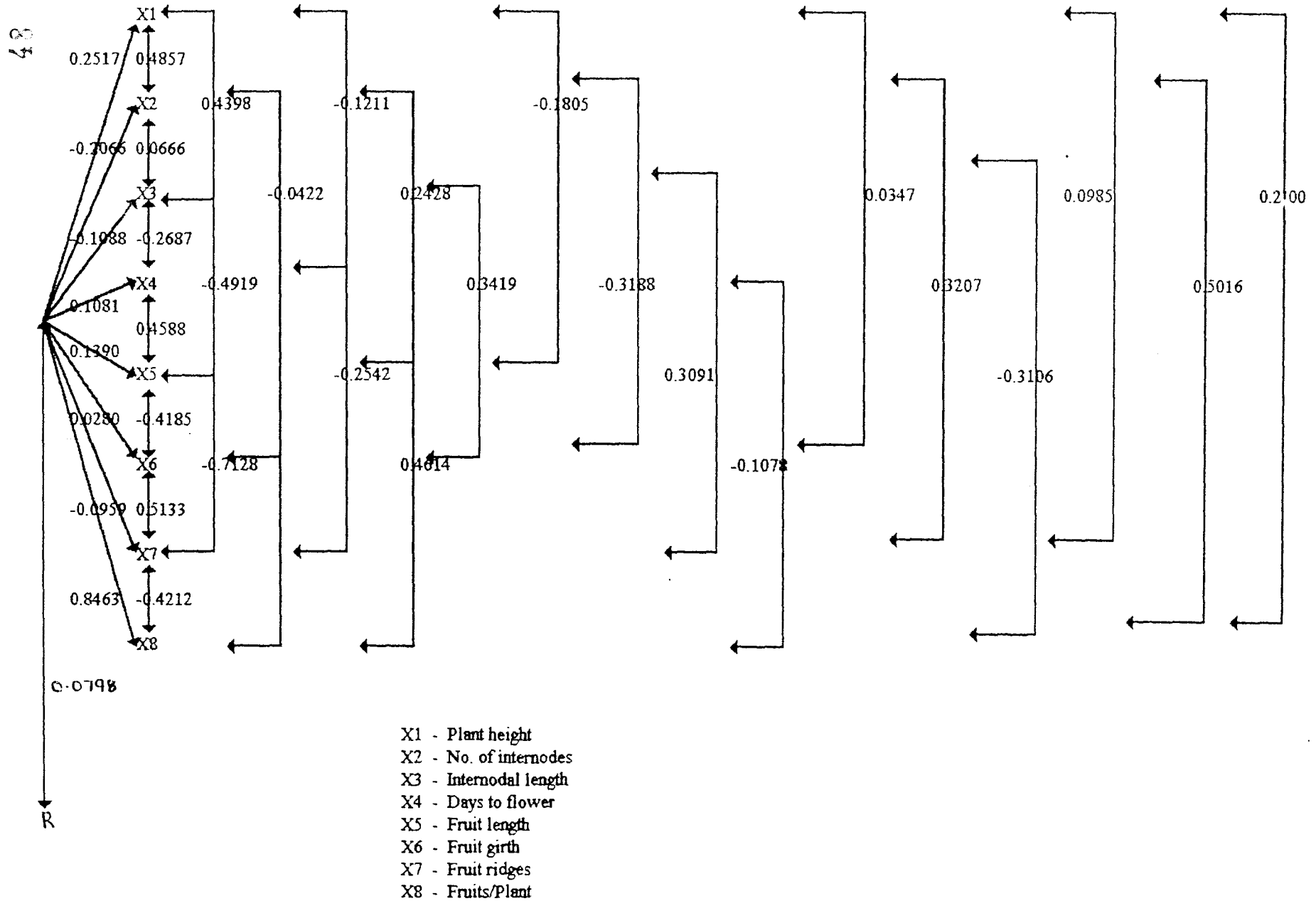
Table 8. Direct and Indirect effect of yield components on fruit yield in "THAMARAVENDA"

Sl no	Plant height	No of internodes	Internodal length	Days to flower	Fruit length	Fruit girth	Fruit ridges	Fruits/plant	Correlation with yield
Plant height	<u>0.252</u>	-0.100	-0.048	-0.013	-0.025	-0.001	-0.009	0.195	0.229
No of internodes	0.122	<u>-0.207</u>	-0.007	-0.005	0.034	-0.009	0.031	0.425	0.385
Internodal length	0.111	-0.014	<u>-0.109</u>	-0.029	-0.068	0.009	-0.029	-0.263	-0.453
Days to flower	-0.031	0.009	0.029	<u>0.108</u>	0.064	-0.006	0.024	-0.145	0.043
Fruit length	-0.045	-0.050	0.054	0.0496	<u>0.139</u>	-0.012	0.068	0.391	0.671
Fruit girth	-0.009	0.066	0.037	-0.023	-0.058	<u>0.028</u>	-0.049	-0.347	-0.494
Fruit ridges	-0.025	0.066	-0.037	-0.028	-0.99	0.014	<u>-0.096</u>	-0.357	-0.519
Fruits/plant	0.058	-0.104	0.034	-0.019	0.064	-0.012	0.040	<u>0.846</u>	0.911

Underlined diagonal values indicate direct effect

Residual 0.0798.

Fig 1. Path diagram showing direct and indirect effects of the components of yield in "Thamaravenda"



Internodal length had a direct negative effect on yield (-0.1088) and its indirect effects on yield through number of internodes, days to flower, fruit length, fruit ridges and fruits per plant were also negative. Positive indirect effect on yield through plant height (0.1107) and fruit girth (0.0096) was shown by this character. Its correlation with yield was significant and negative (- 0.453).

Positive direct effect on yield (0.1081) was shown by days to flower and positive indirect effect on yield was shown by this character through number of internodes, internodal length, fruit length and fruit ridges. Its effects on yield through plant height, fruit girth and fruits per plant were negative.

Fruit length showed direct positive effect (0.1390) and indirect positive effect through internodal length, days to flower, fruit ridges and fruits per plant on yield. It had a high positive correlation with yield. But its indirect effect on yield through plant height, number of internodes and fruit girth was negative.

Though fruit girth showed a positive direct effect on yield (0.0280), its correlation with yield was negative and significant (- 0.494). Except through number of internodes (0.0659) through all other characters taken in this study its indirect effect on yield was negative. It showed the greatest negative indirect effect through fruits per plant.

Both direct effect on yield (0.0959) and correlation with yield were negative (-0.519) for fruit ridges. Positive

indirect effect on yield was shown by this character through plant height (0.0248), number of internodes (0.0663) and fruit girth(0.0144). Though other characters like internodal length, days to flower, fruit length and fruits per plant the effect on yield was negative for these characters.

Fruits per plant showed the greatest direct effect on yield (0.8463) and correlation with yield (0.911). It also acted positively on yield through plant height, internodal length, fruit length and fruit ridges. However its effect on yield through number of internodes and days to flower and fruit girth were negative.

#### 4.1.4 Genetic divergence studies.

On grouping the 22 different genotypes, three clusters were obtained(table 9).

Cluster I had the maximum number of genotypes (15). It was followed by cluster III having six genotypes. Cluster II had only a single genotype.

Fruits of all the genotypes in cluster I were moderately ridged with medium length though their yield varied widely. All of them gave moderate yields. The plants were comparatively of lesser height with medium sized leaves. The genotypes in this cluster were AM - 1, AM - 3, AM - 4, AM - 7, AM - 10, AM - 11, AM - 14, AM - 18, AM - 19, AM - 20, AM - 21, AM - 33, AM - 34, AM - 35 and AM - 36.

Cluster II consisted of AM - 27 alone. This gave highest yield and had the longest fruits which were smooth



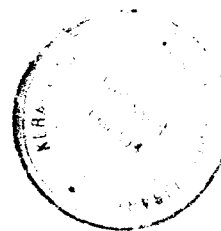


TABLE 9. Clustering pattern in 22 Genotypes of "THAMARAVENDA".

Cluster number	Number of genotypes in each cluster	Genotypes
I	15	AM1, AM3, AM4, AM7, AM10, AM11, AM14, AM18, AM19, AM20, AM21, AM33, AM34, AM35, AM36
II	1	AM27
III	6	AM2, 1M12, AM23, AM25, AM28, AM31

and the thinnest. The plants were very tall and had the maximum number of internodes among genotypes.

Genotypes yielding fruits with deeper ridges were included in cluster III. They were AM - 2, AM - 12, AM - 23, AM - 25, AM - 28 and AM - 32. They were tall and comparatively with longer internodes. These plants flowered comparatively earlier and gave shorter fruits and lower yield.

The maximum intra cluster distance of 3.707 shown by cluster I. Cluster III showed an intracluster distance of 2.914 and for cluster II the intracluster distance was zero since it contained a single genotype (table 10).

The intercluster distance between cluster II and cluster III was the maximum (8.95) followed by that between cluster II and cluster I (7.56). The intercluster distance between cluster I and cluster III was minimum (3.374).

A diagrammatic representation of clustering of genotypes is given in figure 2.

#### 4.2 Cytological, cytogenetical and crossability studies

A detailed observation of meiosis and salient features of the external morphology were expounded in the parental species, interspecific hybrid and the amphiploid. The observations pertaining to the above aspects have been described under appropriate heading in this chapter.

##### 4.2.1 Meiosis in *A.esculentus*

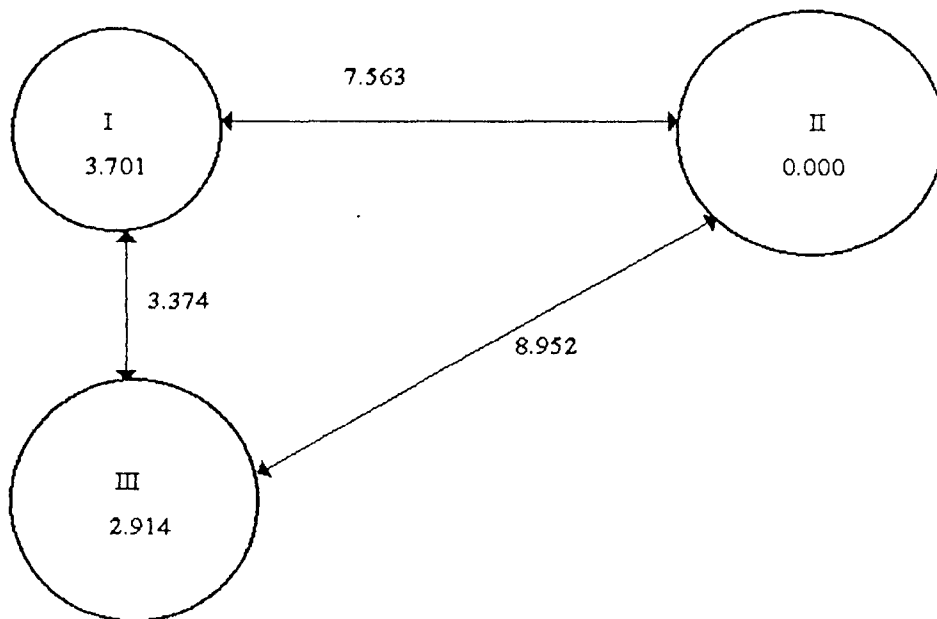
Cytological studies confirmed the chromosome number of *A.esculentus* line AE 202 as  $2n = 130$ . The chromosome configuration at metaphase I was regular forming 65 bivalents (Plate 6). A few pollen mother cells (PMC's) having upto

TABLE 10. Inter and Intra cluster distances among three clusters from 22 Genotypes of "THAMARAVENDA".

Cluster	I	II	III
I	<u>3.701</u>		
	-----		
II	7.563	0.000	
		-----	
III	3.374	8.952	2.914
			-----

Underlined diagonal figures indicate intracluster distance

Fig2. Diagrammatic representation of clustering of 22 genotypes in THAMARAVENDA



CLUSTER DIAGRAM

four univalents were also observed due to the precautions separation of some bivalents at this stage (Table 11). At anaphase I disjunction of chromosome was regular without any abnormalities. Distribution of chromosomes to the poles was equal at anaphase I and II. After telophase II four separate cells, microspores forming the tetrads were observed (plate 14).

Pollen stainability was 96.15 percent (Plate 11). The mean diameter of fertile pollen grain was 0.0594  $\mu\text{m}$  and the range was 0.0531 to 0.0708  $\mu\text{m}$ .

#### 4.2.2. Meiosis in *A. manihot*.

The course of meiosis in *A. manihot* line AM - 4 was studied and the chromosome number  $2n = 184$  was confirmed for this species. At metaphase I the chromosomal association consisted of 92 bivalents (Plate 7). Due to the early separation of some bivalents a few cells showing upto six univalents were also observed (Table 12). At anaphase I and II normal disjunction of chromosomes to the poles was observed in all the PMC's studied. At telophase I and II, two and four groups were formed respectively. At tetrad stage four microspores were observed.

The average pollen stainability was 97.08 percent and mean fertile pollen diameter was 0.0593  $\mu\text{m}$  (Plate 12).

#### 4.2.3 Interspecific hybridization.

Interspecific hybridization between *A. esculentus* and *A. manihot* were carried out in both directions. The crosses were successful only when *A. manihot* was kept as the female parent (plate 5). The fruit set in the successful direction

Plate 6. Metaphase I in AE - 202 showing 65 bivalents x 1000

Plate 7. Metaphase I in AM - 4 showing 92 bivalents x 1000

Plate 6

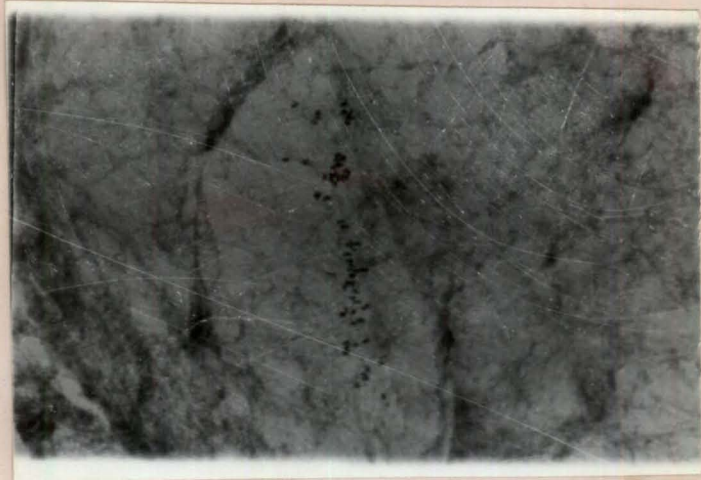


Plate 7



Table 11 . Chromosome association in metaphase 1 in ~~procs~~ of *A. esculentus* line AE - 202

IV	III	II	I	FREQUENCY NO	FREQUENCY %
-	-	64	2	2	40
-	-	65	-	2	40
-	-	63	4	1	20
RANGE				TOTAL NO OF	
-	-	63 - 65	2 - 4	CELLS = 5	
MEAN /					
CELL					
-	-	64.0	2.4		



Table 12. Chromosome association at Metaphase 1 in PMCs of THAMARAVENDA line AM - 4

IV	III	II	I	FREQUENCY NO	FREQUENCY %
-	-	90	4	2	40
-	-	92	-	1	20
-	-	91	2	1	20
-	-	89	6	1	20
RANGE				TOTAL NO OF	
-	-	89 - 92	2 - 6	CELLS = 5	
MEAN /					
CELL					
-	-	90.5	3.0		

of crosses was 62.85 percent and crossability was worked out as 40.6percent.

#### 4.2.4 Meiosis in the interspecific hybrid.

Meiosis in the interspecific hybrid (Plate 8) was critically observed and the chromosome number was ascertained as  $2n = 157$ . Chromosomal association in metaphase I consisted of bivalents and univalents (Table 13) in the PMC's examined. The number of bivalents ranged from 63 to 65 and the maximum number of bivalents were observed in 60 percent of the PMC's. In general the bivalents were oriented in the equatorial plane while the univalents were scattered in the cytoplasm. In the later stages of meiosis some disjunctional abnormalities like laggards, unequal distribution of chromosomes, multipolar spindle formation and micronuclei were noticed. The highly irregular meiosis resulted in the formation of diads, triads, pentads and hexads in varying proportion in addition to the normal tetrads. (Plate 15 and Table 16). Subsequently pollen grains produced were mostly sterile (Plate 13). Pollen stainability observed was only 18.26 percent. The mean diameter of fertile pollen grains was  $0.062 \mu\text{m}$  and that of sterile pollen grains was  $0.0363 \mu\text{m}$ . It produced an average 20.42 seeds per pod.

#### 4.2.5 Induction<sup>of</sup> amphiploidy.

Amphiploidy was induced by treating 0.1 percent colchicine on the vegetative buds. Only one bud out of 45 buds of the interspecific hybrid of *A.manihot* x *A.esculentus* resulted in amphiploidy. This could be easily made out by vigorous shoot with thick leaves put forward by the buds effected by colchicine treatment. Amphiploidy was further

Table 13 . Chromosome association at Metaphase 1 in PMCs of interspecific hybrid AM - 4 x  
AE - 202

IV	III	II	I	FREQUENCY NO	FREQUENCY %
-	-	65	27	3	60
-	-	64	29	1	20
-	-	63	31	1	20
RANGE				TOTAL NO OF	
-	-	63 - 65	27 - 31	CELLS = 5	
MEAN /					
CELL					
-	-	64.0	29.0		

identified by studying the stomata and guard cell chloroplast of the adaxial leaf epidermis, subsequently confirmed by meiotic studies.

#### 4.2.6 Identification of amphiploidy by stomatal studies.

Stomata and guard cell chloroplast of parental species and the amphiploid induced were studied. It was found that the length of stomatal opening ( $0.0592 \mu\text{m}$ ) and the number of chloroplasts in the guard cells (20) of the amphiploid was markedly more in comparison with the progenitor species, whereas intensity of stomata per millimeter (13.5) was lower in the induced amphiploid.

#### 4.2.7 Meiosis in the amphiploid.

Meiosis in the  $C_1$  generation amphiploid was studied. Its chromosome number was confirmed as  $2n = 314$ . The chromosome configuration at the metaphase I was more or less regular forming 157 bivalents (Plate 9 and 10) in the 40 percent of the PMC's studied. At this stage bivalents ranged from 155 to 157, univalents 0 to 4 (Table 14). Subsequent stages of meiosis were regular. At anaphase I and II disjunction of chromosomes to the poles was equal. Normal tetrads having four microspores were found at the end of meiosis. Pollen stainability observed in the amphiploid was 58.33 percent. The mean diameter of fertile pollen grains was  $0.071 \mu\text{m}$ . Amphiploid produced an average of 42.5 seeds per fruit.

#### 4.2.8 Morphological studies.

Observation recorded on the morphological characters of *A.esculentus*, *A.manihot* and the interspecific hybrid are presented in the table 15.

Plate 8. Metaphase I in AM - 4 x AE - 202 with  $2n = 157$   
having 65  $\pi^s$  and 27  $I^s$  x 1000

Plate 9. Metaphase I in the synthetic amphiploid AM - 4 x  
AE - 202 with  $2n = 314$  x 1000

Plate 10. Chromosome configuration during Metaphase I showing  
157 bivalents in the synthetic amphiploid drawn  
using camera Lucida x 1000



Plate 8

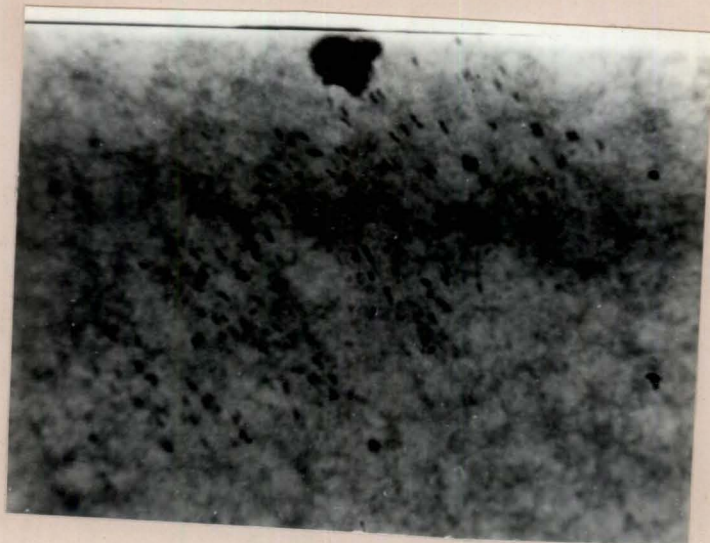


Plate 9

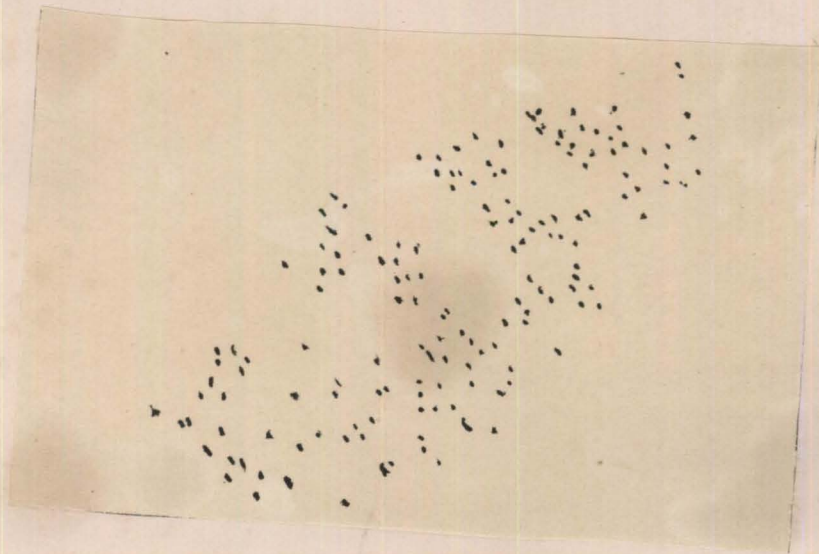


Plate 10

Table 14 . Chromosome association at Metaphase 1 in PMCs of synthetic amphiploid involving  
AE - 202 AND AM - 4

IV	III	II	I	FREQUENCY NO	FREQUENCY %
-	-	157	-	2	40
-	-	155	-	1	20
-	-	156	2	1	20
-	-	155	4	1	20
RANGE				TOTAL NO OF	
-	-	155 - 157	2 - 4	CELLS = 5	
MEAN /					
CELL					
-	-	155.75	1.5		

Plate 11. Pollen of AE - 202 x 400

Plate 12. Pollen of AM - 4 x 400

Plate 13. Sterile and fertile pollen of the cross AM - 4 x AE  
- 202 x 400



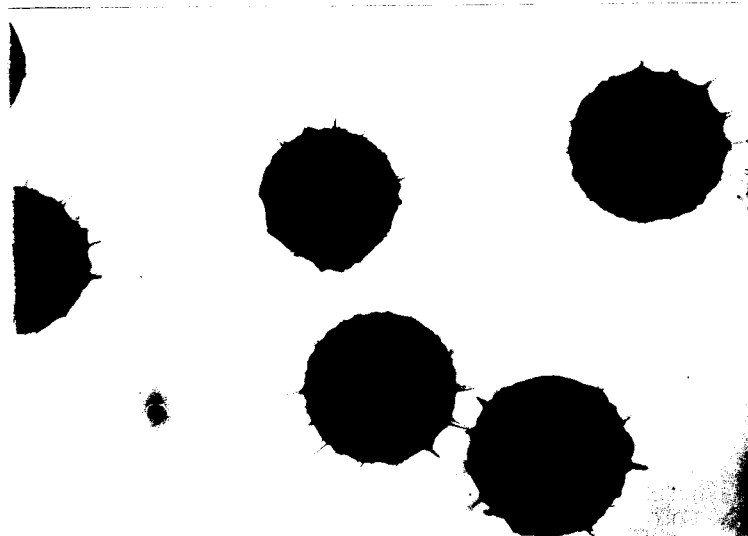


Plate 11

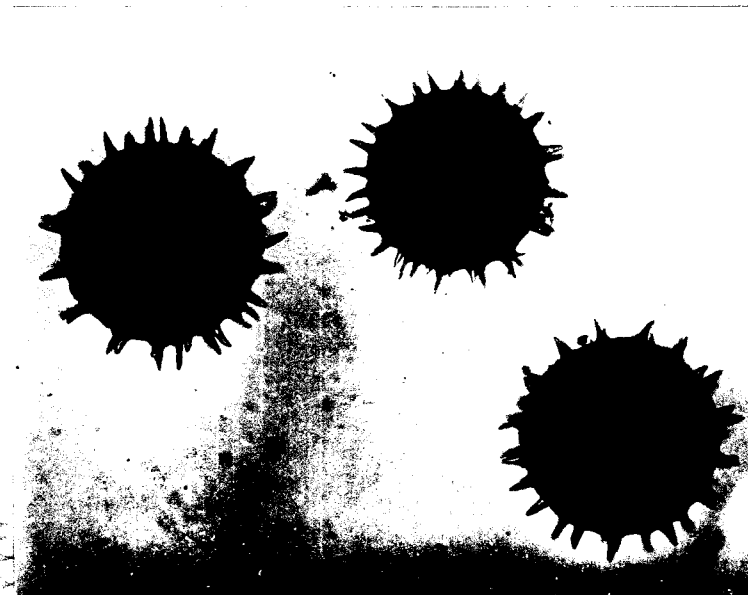


Plate 12



Plate 13

Plate 14. Normal tetrads seen in AE - 202 x 400

Plate 15. Dyads, triads, tetrads, pentads and hexads seen in  
the F<sub>1</sub> (AM - 4 x AE - 202) x 400

Plate 14

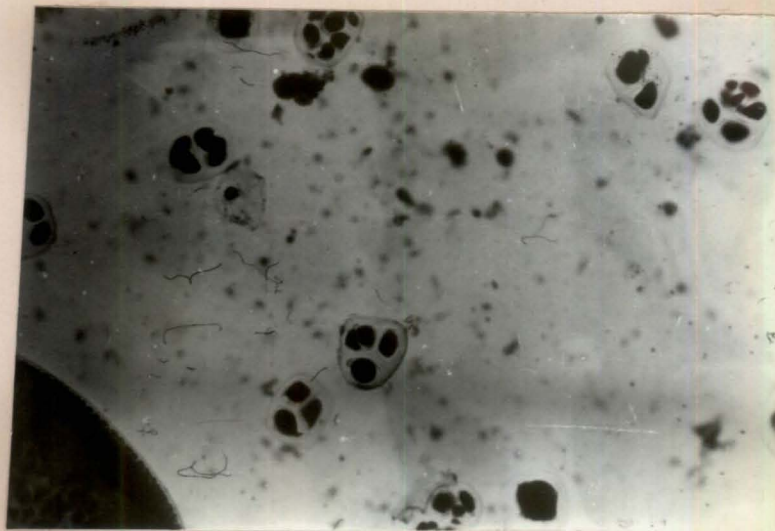
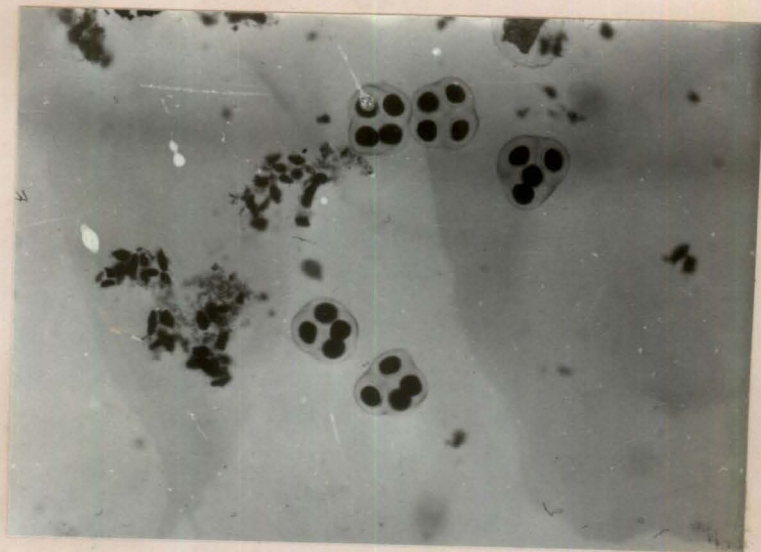


Plate 15

Table 15. Comparison of characters of parents (AE - 202 and AM - 4 ) and their interspecific hybrid

CHARACTERS	AE - 202	AM - 4	F <sub>1</sub>
Plant height	84.80	101.52	161.67
Days to flowering	44.67	61.06	52.67
First flowering node	6.33	7.52	7.46
Number of primary branches	2.3	3.23.2	4.1
Number of internodes	25.34	24.13	36.67
Internode length	4.1	2.3	7.4
Stem colour	Pale green	Green with purple patches	Green with purple patches
Petiole length(cm)	17.56	28.98	30.24
Shape of leaf	Iregular palmate	Iregular palmate	Regular palmate
Basal lobes of leaf	Not overlapping	Overlapping	Overlapping
Size of leaf	Small	Medium	Large
Colour of leaf	Green	Green with pink veins	Green with pink veins
Length of stomatal opening	0.0374	0.0503	0.0413
Range	0.0295 -0.0472	0.0354 - 0.0531	0.0354 - 0.0472
Number of stomata per mm	25.8	25.92	32.4
Number of stomatal chloroplasts	11.38	10.9	12.5
Flower colour	Yellow	Yellow	Yellow
Red colouration of petal base	Present on both sides	Only on one side	Present on both sides
Flower size	Small	Medium	Large
Number of epicalyx segments	10	7.52	6

(Contd.)

Table 15. (Contd.)

CHARACTERS	AE - 202	AM - 4	F <sub>1</sub>
Length of epicalyx segment	4.6	3.05	2.4
Width of epicalyx segment	0.16	0.95	0.65
Persistence of epicalyx segment	not persistent	Not persistent	Not persistent
Length of fruit(cm)	28.29	14.23	19.73
Girth of fruit(cm)	6.5	9	10.7
Fruit shape	Long slender	Oblong	Semi - oblong
Number of fruit ridges	5	7.8	8.1
Fruit colour	Pale green	Green with purple tinge	Green with purple tinge
Number of seeds per fruit	58.87	52.25	20.42
Number of fruits per plant	11.2	10.14	12.26
Single fruit weight	31.16	29.12	20.67
Field reaction to YVMV	susceptible	Resistant	Resistant
Fertility of pollen	96.15	97.08	18.26
Pollen size	0.0594	0.0593	Fertile: 0.062 Sterile: 0.0363
Range(mm)	0.0531 -0.0708	0.059 -0.0649	0.059 - 0.0708

Parental differences was seen in almost all the characters studied. These included characters such as plant height, days to flower, number of primary branches, number of internodes, internodal length, shape of leaf, leaf size, flower size, number of epicalyx segments, length and girth of fruits, number of fruit ridges and fruit colour.

Morphologically all the plants of the interspecific hybrid looked alike. Plants were robust and vigorous and represented more towards the semi-wild species (Plate 5). In the interspecific hybrid the mean values were higher for the characters like plant height, internodal length, number of internodes, petiole length, size of leaf, number of stomata per square millimeter, number of stomatal chloroplasts, girth of fruits, number of fruits per plant and number of fruit ridges. The  $F_1$  hybrid recorded 115.7 percent heterosis with respect to fruits per plant over its cultivated parent AE - 202. Intermediate mean values were recorded for characters such as days to flowering, first flowering node, length of stomatal opening, number of epicalyx segments, width of epicalyx segments and length of fruit.

The induced amphiploid grossly resembled the  $F_1$  hybrid for most of the morphological characters. The characters such as girth of stem, leaf shape, primary branches, width of epicalyx segments, length of fruit and field reaction to YVMV were similar to those of the  $F_1$  hybrids. Unlike the interspecific hybrid the amphiploid was more fertile and produced an average 42.5 seeds per pod.

Table 16. Frequency of dyads, triads, tetrads, pentads and hexads during the formation of microspores in AM - 4, AE - 202 and their interspecific hybrid

FREQUENCY %	DYADS	TRIADS	TETRADS	PENTADS	HEXADS
AE 202	-	-	100	-	-
AM 4	-	-	100	-	-
F1(AE202 * AM 4)	12.9	28.5	34.1	16.3	8.3

*Discussion*



## DISCUSSION.

The present study was aimed at the genetic improvement and cytogenetical studies on Thamaravenda which was considered as a form of *A.manihot*. Thus the study explored the variability occurring in the species proposed for improvement and pinpointed different characters to be considered for further development. It also elucidate chromosome affinity of the species with *A.esculentus*. These information provide the basic biometrical and cytological edifice for further breeding programme in "Thamaravenda". Based on these, the present study was carried out and the results obtained are discussed below.

### 5.1 Variability studies.

The success of crop improvement largely depend on the magnitude of variability and the extent of heritability exhibited by the desirable characters. In ~~the~~ okra great variability in qualitative and quantitative characters had been observed by many workers like Vashista et. al., (1982). Hamon and Charrier (1983) Hamon et. al., (1991) and Arigo (1993).

In the present study the twenty two genotypes showed significant difference among themselves in all the 19 characters studied namely plant height, number of branches, main stem diameter, number of internodes of main stem, internodal length, petiole length, length of leaf, length of basal leaf lobe, days to first flowering, first flowering node, pedicel length, fruit length, fruit girth, fruit ridges, single fruit weight, fruits per plant, yield per plant, seeds per fruit and hundred seed weight. This

revealed considerable scope for improvement of these characters.

The yield per plant, length of fruit, number of fruits per plant was highest for AM - 27. It also had the largest number of internodes on the main stem (40.9). The internodal length was least for this genotype (3.09 cm). AM - 23 was the tallest genotype (159.4 cm). The earliest flowering genotype was AM - 10 which flowered in 42, 43 days and the genotype AM - 20 flowered at the lowest node (6.25). Fruit girth was highest for AM - 23 (12.75 cm) and lowest for AM - 27 (7.47 cm). The heaviest fruits were produced by genotype AM - 18 (33.6 g). The number of seeds per fruit was highest for AM - 3 (69.96) and the hundred seed weight highest for AM - 12 (7.779g). The genotypic coefficient of variation was of high magnitude for yield per plant, fruits per plant, seed yield per plant, internodal length and height of plant resulting in high heritability. This indicated that these characters were least affected by environment. Kaul et. al., (1978), Mishra and Chhonkar (1979), Murthy and Bavagi (1982), Ariego (1990) and Venketesh (1991) have also reported a high genotypic coefficient of variation for the above characters coupled with a high heritability. The characters like main stem diameter, length of leaf, days to first flowering, first flowering node and fruit girth had comparatively lower gcv indicating that these characters are highly influenced by environment. The effectiveness of selection depends upon the heritability and genetic advance of the character studied. High heritability along with high genetic gain was shown by height of the plant, yield per plant, fruits per plant and fruit ridges, This indicates the presence of additive genes and

shows that these characters can be improved by selection. Such results were also obtained by Mishra and Chhorkar (1979), Murthy and Bavagi (1982), Reddy et al., 1985 and Ariyo (1990). Eventhough heritability was high for days to first flowering and first flowering node, these characters showed a low genetic gain indicating the action of non-additive gene action for the expression of these characters. The present study also indicates that high heritability is not always coupled with high genetic gain as reported by Johnson et al., (1995). Therefore direct selection has only limited scope for improving these characters.

## 5.2. Correlation studies

A thorough knowledge of the relationship between yield and its component characters will help to produce a simultaneous improvement of yield and yield contributing characters. In the present study also the characters fruit length and fruits per plant showed significant positive phenotypic and genotypic correlation with yield. Such results were also obtained by Singh and Singh (1979), Arumugham and Muthukrishnan (1981), Vashishta et al., (1982), Reddy et al., (1985), Mishra and Singh (1987), Singh et al., (1990) and Venketesh (1991). Therefore an improvement in these characters will produce a simultaneous improvement in yield. The highest yielding genotype AM - 27 had the maximum length for the fruit which directly influenced the yield. It had the maximum number of fruits per plant and the fruits had no ridges (Plate 4).

Internodal length and main stem diameter showed negative correlation with yield. Negative correlation of internodal length with yield in *A.esculentus* was reported by Mishra and

Singh (1992). The internodal length was the minimum in AM - 27. High yielders are having the minimum internodal length is clearly substantiated by the fact that the internodal length is having a negative association with yield. Though negative correlation of the internodes per stem with fruits girth was observed, the yield was not substantially reduced as the number of internodes increased. The phenotypic correlation was smaller than genotypic correlation indicating that environment had smaller, but similar effect on these characters.

The above finding shows that plants producing long fruits with minimal internodal length as well as plants having more fruits per plant and minimum fruit girth may be selected for future breeding programmes.

### 5.3 Path coefficient analysis.

Path coefficient studies revealed that plant height, days to flower, fruit length, fruit girth and fruits per plant had a positive direct effect on yield whereas number of internodes, internodal length and fruit ridges showed negative direct effect on yield. Such results were also reported by Murthy and Bavagi (1982), Balakrishnan and Balakrishnan (1990), Singh et al., 1990, Venketesh (1991) and Mishra and Singh (1992).

Though plant height did not have a significant correlation with yield, the indirect effects of the same through the number of internodes, internodal length, days to flower, fruit length, fruit girth and fruit ridges was negative. The indirect effect of plant height through fruits per plant was minimum indicating that plant height is not an important yield determining factor. This is also justified

by the fact that the direct effect of internodal length on yield was also negative as against the result got by Singh and Singh (1979) in *A.esculentus*. Days to flower also did not have a significant effect on yield. Fruit length, with a significantly high correlation with yield, but not having much of the direct effect, did influence through the fruits per plant. This observations coincide with the findings of Singh et al., (1990) and Mishra and Singh (1990) in *A.esculentus*). Though the fruit girth did not have a negative direct effect, the correlation of the same with yield was significantly negative. Similar results as in the case of fruit girth hold good for fruit ridges also. A high direct effect of the fruits per plant on yield was observed. This is supported by the fact that the correlation of fruits per plant with yield was highly significant. Such an observation was earlier noted by many workers like Kaul et al., (1979), Singh and Singh (1979), Murthy and Bavagi (1982), Balakrishnan and Balakrishnan (1990), Singh et al., (1990) and Mishra and Singh (1992).

#### 5.4 Genetic divergence studies.

The non-hierarchical Euclidean cluster analysis was used to cluster genotypes. This has resulted in three clusters in the present study. The cluster II distances itself from clusters I and cluster III, but the distance between cluster I and cluster III was not to that extent. Similar results had been earlier reported by Pratap et al., (1980), Suresh Babu (1987), Ariyo and Odulaga (1991) and Ariyo (1993) in the genus *Abelmoschus*. In the cluster II fruits of all the genotypes were moderately ridged with medium length. Though the yield varied widely all of them gave moderate yields.

These plants were comparatively of lesser height with medium sized leaves. The genotypes in this cluster were AM - 1, AM - 3, AM - 4, AM - 7, AM - 10, AM - 11, AM - 14, AM - 18, AM - 19, AM - 20, AM - 21, AM - 33, AM - 34, AM - 35 and AM - 36.

Cluster II consisted of AM - 27 alone. This genotype gave highest yield and had the longest fruits which were smooth and slender. The plants were very tall and had the maximum number of internodes among the genotypes. These traits made this genotype quite distinct from others thus it formed a solitary cluster (Plate 2).

Genotypes yielding fruits with deeper ridges were included in cluster III. They were AM - 2, AM - 12, AM - 23, AM - 25, AM - 28, AM - 31. These plants were tall with comparatively longer internodes and flowered comparatively earlier and gave fruits with lower yield.

### 5.5 Cytogenetical studies.

The present cytogenetical studies between *A.esculentus* and *A.manihot* evaluate the interrelationship between the two species based on the chromosome number, chromosome homology and response to chromosome doubling of the interspecific hybrid.

#### 5.5.1 The Chromosome number of *A.esculentus* line AE - 202 and the *A.manihot* line AM - 4.

The somatic chromosome number of *A.esculentus* ranged from  $2n = 66$  to as high as  $2n = 144$  (Charrier 1984). Ford (1938) reported two kinds of okra, a diploid type with  $2n = 66$  and a tetraploid type with  $2n = 130$ . The variety of okra that Teshima (1933) used in his experiment was having  $2n =$

72. The somatic chromosome number of okra observed by several investigators except Teshima (1933) and Ford (1938) was always in the range of 120's and 130's. From their information it is possible to deduce that there might be two forms of okra, one in the range of 60's to 70's and the other 120's to 130's in their somatic chromosomes. It was widely believed that chromosome race with  $2n = 72$  realized by Teshima (1933) had disappeared. But this species form of okra was rediscovered by Ugale et al., (1976) and Kamalova (1977). The *A.esculentus* line AE - 202 used in the present study has  $2n = 130$  chromosome showing that this line belongs to the mostly prevailing tetraploid group.

Cytological studies on Thamaravenda which was considered to be a form of *A.manihot* evoke a considerable interest. It was established in the present study that its chromosome number  $n = 92$ . Charrier (1984) reported *A.manihot* as a diploid species of *Abelmoschus* having chromosome number in the range of  $n = 30 - 34$ . This indicated that Thamaravenda cannot be considered as a species form of *A.manihot* as its chromosome number realized in the present investigation as  $n = 92$  which tallies with gametic chromosome number of *A.caillei*. So from the present study it can be deduced that Thamaravenda stands apart from any form of *A.manihot*, but has strong evidence that it comes under the complex polyploid species *A.caillei*.

#### 5.5.2 Cytology of interspecific hybrid.

In the interspecific hybrid almost all the haplophase chromosomes ( $n = 65$ ) of *A.esculentus* were found to pair with the homeologous counterparts of the Thamaravenda which is now recognized as *A.caillei* forming mostly 65 bivalents and 27

univalents. This confirms the proposal of Siemonsuma (1982) that *A.caillei* might be a natural amphiploid of *A.esculentus* and *A.manihot*. The fertile amphiploid of "Nori-Asa" between these two species realized by Kuwada (1957, 1964) resembled *A.caillei* in morphological characters. According to Kondaiiah et. al., (1990) *A.manihot* might have contributed one genome to *A.caillei*. Joshi and Haridas (1976) had already confirmed the allopolyploid origin of *A.esculentus* constituting the genomes of *A.tuberculatus* and *A.ficulneus*. So the present study confirmed the complex polyploid origin of Thamaravenda which can be regarded as *A.caillei*. Further cytological studies on this species in relation with the basic genomes of *A.tuberculatus*, *A.ficulneus* and *A.manihot* will provide its true mode of origin. Having a high degree of chromosome affinity between *A.esculentus* and *A.caillei* confirmed in the present investigation shows that the gene introgression between these species is quite possible and this information can be utilized in the future breeding programmes.

The interspecific hybrid was found to be partially sterile. The cytological basis for the high sterility could be enquired by taking into consideration of the data available on the meiosis. In the pollen mother cells observed at metaphase I univalents ranged from 27 to 31. Further abnormalities were also recorded at the later stages and these included presence of lagging chromosomes, occurrence of micronuclei and multipolar spindle formation. Thus the occurrence of distributional abnormalities of chromosome at meiotic stages could be accounted for the 81.74 percent pollen sterility in the  $F_1$  hybrid.

### 5.5.3 Cytological studies in the synthetic amphiploid.



In the present study an amphiploid was induced by doubling the chromosome complement of the interspecific hybrid. The amphiploid expressed normal chromosome behavior, increased pollen fertility and seed set in comparison with the interspecific hybrid. Its chromosome number was established as  $n = 157$ .

In the amphiploid the frequency of multivalents was very low even though as high as 65 bivalents were observed in the interspecific  $F_1$  hybrid. This can be attributed to the preferential pairing of chromosomes coined by Darlington (1937). Presumably the parental chromosomes are similar enough to pair in the  $F_1$ , yet they differ in small segments that is the cryptic chromosomal differences (Stebbins 1950) of the species involved. Burnham (1962) observed that in genera having small chromosomes there would be low frequency of crossing over and hence no multivalent association might occur. So the extremely small chromosomes of the parental species can also be attributed to the regular chromosome behavior of the amphiploid. The detailed cytological studies in amphiploid synthesized in the genera *Hibiscus* and *Abelmoschus*, Kuwada (1964) and Jambhale and Nerkar (1982) showed that they can survive as stable and fertile synthetic species. The amphiploid synthesized in the present study can be a proof and infact an experimental evidence thereof.

#### 5.5.4 Crossability between the parental species.

The result of the crossability studies between the two species showed that crosses were successful only when Thamaravenda line AM - 4 was kept as female parent. The reciprocal crosses did not set any fruit. This unilateral cross incompatibility can be attributed to some pre or post

fertilization barrier as per Kalloo (1988). As the crosses are successful when the parental line having much higher chromosome number was kept as female parent shows that embryo endosperm balance might have a critical role in the seed set in this specific cross combination.

*Summary*

## SUMMARY

The present investigation on "Genetic improvement and cytogenetical studies" in Thamaravenda (*Abelmoschus manihot* L.) was conducted at the Vegetable Research Farm, Department of Olericulture, College of Horticulture, Vellanikkara during the years 1993 - 95. The major objectives of the study were to collect the maximum available genotypes of the edible form of *Abelmoschus* sp locally known as "Thamaravenda", study the extent of genetic variability and identify promising genotypes among them, confirm the chromosome number of the species, determine its species and taxonomic status, study its crossability and chromosome affinity with *A.esculentus* and attempt to synthesis an amphiploid of *A.esculentus* and the species proposed for improvement.

Genetic variability and divergence among 22 genotypes were assessed. The genotypes studied showed significant variation among themselves for all the characters namely plant height, number of primary branches, main stem diameter, number of internodes on mainstem, internodal length, petiole length, length of leaf, length of basal leaf lobe, days to first flowering, first flowering node, pedicel length, fruit length, fruit girth, number of fruit ridges, single fruit weight, fruit per plant, yield per plant, seeds per fruit and hundred seed weight. The existence of considerable variation indicate the scope for improving the population.

The genotype AM - 27 produced the highest number of fruits per plant (25.56) and the yield was also maximum for this genotype (721.27 gm per plant). Besides this, it produced smooth, long fruits and had maximum number of internodes.

These desirable qualities combined in this single genotype indicate its scope for use in further breeding programme. High value of genotypic coefficient of variation combined with high heritability was shown by characters like fruits per plant, yield per plant, fruit ridges and height of plant showed that these characters were least influenced by environment. High heritability along with high genetic gain was shown by height of the plant, yield per plant, fruits per plant and number of fruit ridges. This indicates the presence of additive genes and shows that these characters can be improved by selection.

Significant positive phenotypic and genotypic correlation with yield was shown by fruit length and fruits per plant indicating that an improvement of these characters will produce a simultaneous improvement in yield. Fruit girth, fruit ridges, internodal length and main stem diameter showed correlation with yield.

When path coefficient analysis on the genotypes was conducted it was seen that fruits per plant showed the highest positive direct effect on yield. Positive direct effects on yield were shown by plant height, days to flower, fruit length, fruit girth and fruits per plant. The residual effect was only 0.0798 indicating that 92.02 percent of the variation in yield was contributed by the component characters namely plant height, number of internodes, internodal length, days to flower, fruit length, fruit girth, fruit ridges and fruits per plant.

The 22 genotypes were grouped into three clusters with the aid of Euclidean cluster analysis. Cluster I had the maximum number of genotypes (15) followed by cluster III which had six genotypes. Cluster II had only a single

genotype AM - 27. Cluster I showed the highest intracluster distance followed by cluster III. Cluster I consisted of plant producing medium broad fruits while plants with broad short and deeply furrowed fruits were included in cluster III. The single genotype AM - 27 included in cluster II had the longest most slender and smooth fruits which made it quite distinct from the other genotypes.

The chromosome number of *A.esculentus* line AE - 202 and Thamaravenda type AM - 4 was confirmed to be  $2n = 130$  and  $2n = 184$  respectively. Both these species showed regular meiosis forming bivalents. Due to precarious separation of bivalents 4 - 6 univalents could also be observed. The chromosome number of  $2n = 130$  for AE - 202 showed that it belonged to the tetraploid group of *A.esculentus*. The somatic chromosome number  $2n = 184$  for AM - 4 indicated that it was not at all a form of *A.manihot*, but it came under the complex polyploid species *A.caillei*.

In the interspecific hybrid between AE - 202 and AM - 4, all the haplophase chromosomes ( $n = 65$ ) of *A.esculentus* were found to pair with the homeologous counter parts of AM - 4 forming mostly 65 bivalents and 27 univalents. This is indicative of the complex polyploid origin of Thamaravenda having all the genomes of *A.esculentus*.

The interspecific hybrid was found to be partially sterile producing on an average 20.42 seeds per fruit. The reason for this must be due to the meiotic irregularities such as more number of univalents at metaphase - I, presence of lagging chromosomes, occurrence of micronuclei and multipolar spindle formation.

An amphiploid was produced by colchicine treatment of the vegetative buds of interspecific hybrid, which had normal chromosome behavior and increased pollen fertility and seed set. The chromosome number of amphiploid was found to be  $2n = 314$ . The amphiploid showed lower frequency of multivalents owing to the preferential pairing of chromosomes.

Successful crosses between AE - 202 and AM - 4 was possible only when AM - 4 was used as female parent. The reciprocal crosses did not set any fruit. This showed the existence of unilateral cross compatibility between the two species.

## ***References***



## REFERENCES

- Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. *Stain Technol.* **55(1):13 - 18**
- Allard, R.W. 1960. Principles of Plant Breeding. John Wiley and Sons Inc. New York **p.89 -98**
- Ariyo, O.J. 1990. Variation and heritability of fifteen characters in okra (*Abelmoschus esculentus* (L.) Moench). *Tropical Agriculture* **67(3):213 - 216**
- Ariyo, O.J. 1993. Genetic diversity in West African Okra (*Abelmoschus caillei*). Stevels multivariate analysis of morphological and agronomic characteristics. *Genetic Resources and Crop Evolution* **40(1):25 - 32**
- Ariyo, O.J. and Odulaja, A. 1991. Numerical analysis of variation among accessions of okra (*Abelmoschus esculentus* (L) Moench) Malvaceae. *Annals of Botany* **67(6):527 -531**
- Arumugham, R., Chelliah, S. and Muthukrishnan, C.R. 1975. *Abelmoschus manihot* - A source of resistance to Bhendi Yellow vein Mosaic. *Madras Agric.J.* **62(5):310 - 312**
- Arumugham, R. and Muthukrishnan, C.R. 1981. Association of metric traits in bhindi. *South Indian Hort.* **29(1):1-3**
- Balakrishnan, S. and Balakrishnan, R. 1990. Association and path analysis in bhindi (*Abelmoschus esculentus*) L.Moench.) *South Indian Hort.* **38(5):274 -275**

- Bhargava, V.R. 1989. Interspecific hybrid incompatibility in okra. *Plant cell Incompatibility Newsletter*. (21):7 - 10
- Burton, G.W. 1952. Quantitative inheritance in grass. *6th Int. Grassld Cong. Proc.* 1:277 - 283
- Burnham, C.R. 1962. Discussions in cytogenetics - amphiploidy *Z. Zuchtung.* 47:21 - 30
- Burton, G.W. and Devane, E.H. 1953. Estimating heritability in tall fescue replicated clonal material., *Agron. J.* 45:378 - 478
- Charrier, A. 1984. Genetic resources of *Abelmoschus* (okra) *Report: AGPC:\BPGR\84\194 (Rome)* p.10 - 20
- Chizaki, Y. 1934. Breeding of new interspecific hybrid between *Hibiscus esculentus* (L.) and *Hibiscus manihot* (L.) *Proc. Crop Sci Soci Japan* 6:164 - 172
- Darlington, C.D. 1937. Recent Advances In Cytology. 2nd Ed. *Blakistons, Philadelphia* P.671
- Dewey, D.R. and Lu, K.M. 1959. A correlation and path coefficient analysis of components of crested <sup>wheat</sup> grass seed production. *Agron J.* 51:515 - 518
- Dhillon, T.S. and Sharma, B.R. 1982. Interspecific hybridisation in Okra (*Abelmoschus* sp) *Genetica Agrarria* 36 (3/4):247 - 255

- Dutta, O.P. 1990. Okra germplasm utilization at 11 HR, Bangalore. *International Okra Plant Genetic Resources Workshop*. IBPGR/NBPGR, New Delhi, 8 - 12 Oct. 1990 **p.14**
- El-Maksoud, M.A., Helal, R.M. and Mohammed, M.H. 1984. Heritability estimates and correlation studies of six economic characters in okra. *Ann Agri Sci, Ain Shams University* **29 (1):439-452**
- Fisher, R.A. 1954. A fuller theory of "junctions in breeding". *Heredity*. **8:(187-197)**
- Ford, C.E. 1938. A contribution to a cytogenetical survey of the Malvaceae. *Genetica*. **20.431-452**
- V.R. Joshi;  
Gadwal, A.B. and Iyer, R.D. 1968. Interspecific hybrids of *Abelmoschus* through ovule and embryo culture *Indian J. Genet.* **28 (3):269-274**
- Hamon, S. 1987. Organization genetique due genre *Abelmoschus* (gombo). Co-evaluation de deux e species cultivees de en Afrique-detquest (*A. esculentus* and *A. caillei*). *These universite de Paris-Sud, Centre d'Orsay. Paris, France* **99-211**
- Hamon, S. and Charrier, A. 1983. Large variation of Okra collected in Benin and Togo. *Plant Genetic Resources Newst.* **(56):52 - 58**

- Hamon, S. and Yapo, A. 1986. Perturbation induced within the genus *Abelmoschus* by the discovery of a second edible okra species in West Africa. *Acta Horticulture* (182): **133-143**
- Hamon, S., Charrier, A., Koechlin, J. and Sloten D.H. Van: 1991. Potential improvement of Okra (*Abelmoschus* spp) through the study of its genetic resources. *Plant Genetic Resources News* (86): **9 -15**
- Jambhale, N.D. and Nerkar, Y.S. 1980. A technique for permanent chloroplast preparation. *Curr. Sci* **49** : **150**
- Jambhale N.D. and Nerkar, Y.S. 1981. Occurrence of spontaneous amphiploidy in an interspecific cross *Abelmoschus esculentus* x *Abelmoschus tetraphyllus*. *J Maharashtra Agric. Univ.* **6(2)** : **167**
- Jambhale, N.D. and Nerkar, Y.S. 1982. An induced amphiploid of *Abelmoschus*. *Indian J. Genet. Pl. Breed.* **42(3)** : **372 - 375**
- Jambhale, N.D. and Nerkar, Y.S. 1982. Induced amphiploid in the cross *Abelmoschus esculentus*(L.) Moench x *A. manihot* (L.) Medik ssp *manihot*. *Genetica Agraria* **36(1/2)** : **19 -22**
- Jambhale, N.D. and Nerkar, Y.S. 1983. Interspecific transfer of resistance to yellow vein mosaic disease in okra. *J. Maharashtra Agric. Univ.* **8(2)** : **197**
- Jambhale, N.D. and Nerkar, Y.S. 1985. An unstable gene controlling developmental variation in okra. *Theor. Appl. Genet* **71(1)** : **122 - 125**

- Jambhale, N.D. and Nerkar, Y.S. 1992. Screening of okra cultivars, related species and interspecific hybrid derivatives for resistance to powdery mildew. J. Maharashtra Agric. Univ. 17(1):53 - 54
- Johnson, H.W., Robinson, H.P. and Comstock, R.E. 1955. Estimates of genetical and environmental variability of soyabean. *Agron. J.* 47(10):314 - 318
- Johnson, H.W., Robinson, H.F. and Comstock, R.E. 1955 b. Genotypic and phenotypic studies in soyabean and their implications in selection. *Agron. J.* (10):477 - 482
- Joshi, A.B., Gadwal, V.R. and Haridas, M.W. 1974. Okra Evolutionary Studies in world crops diversity and changes in the Indian subcontinent (Hutchinson, J.B. (Ed Cambridge p.99 - 105.)
- Joshi, A.B. and Haridas, M.W. 1976. Evolution of Crop Plants. Longmans, London, p:194 - 195
- Kaloo, G. 1988 Distant hybridisation in vegetable crop species *Vegetable Breeding Vol II* p148 - 150 CRC Press Inc. Florida. p 143-150
- Kamalova, G.V. 1977. Cytogenetical studies of some species of the Malvacea. *Uzbekistan Biology* Ja.3:66 - 69
- Kerala Agricultural University 1993. Package of Practices Recommendations. Directorate of Extension, Thrissur, Kerala. 171 - 172

- Kaul, T., Lal, G. and Peter, K.V. 1978. Correlation and path coefficient analysis of components of earliness, pod yield and seed yield in okra. *Agne.Sci.* **48(8): 459 - 63**
- Kondaiah, G.M., Kulkarni, U.G. and Nerkar, Y.S. 1990. The evolution of West African cultivated Guinean okra. Further Evidence. *J of Maharashtra Agric. Univ.* **15(1):65 -68**
- Kuwada, H. 1957. Cross compatability in the reciprocal cross between amphiploid and its parents *A.esculentus* and *A.manihot* and the characters and meiotic division of the hybrids offered among them. *Jap J. Breed* **7:103-111**
- Kuwada, H. 1961. Studies on the interspecific crossing between *Abelmoschus esculentus* (L.) Moench and *A.manihot* (L.) Medikis and the various hybrids and polyploids derived from the above two species. *Fac. Agric Kagawa Univ. Mem.* **8:91**
- Kuwada, H. 1964. The newly artificially raised amphiploid plant named 'al-fungo (*Hibiscus mutamoschatus*), obtained from the progeny of *H.mutabilis* x *H.moschatus*. *Jap. J. Breed* **14:27-32**
- Kuwada, H. 1966. The new amphiploid plant named *Abelmoschus tuber-esculentus* obtained from progeny of the reciprocal crossing between *A.tuberculatus* and *A.esculentus*. *Jap. J. Breed.* **16(1):21 - 30**

- Li C.C.1955. Population Genetics. The University of Chicago Press London **p.144 -171**
- Lush,J.L.1949. Animal Breeding. Plans lown State university Press Annes. **p.473**
- Mahalanobis, P.C. 1928. A statistical study at Chinese head measurements. *J. Asiatic Soc Bengal* **25:301 - 377**
- Mamidwar, R.B., Nerkar, Y.S. and Jambhale, N.D. 1980. Cytogenetics of interspecific hybrids in the genus *Abelmoschus*.*Indian J. Hered.* **11(2):35-40**
- Martin,F.W. 1982. A second edible okra species and its hybrids with common okra. *Ann.Bot.* **50(2):277 - 283**
- McLeod,J.M., Witcher W., Epps, W.M. and Robbin, M.L. 1983. Resistance of Okra plant introductions to root knot nematode and fusarium wilt. *Hort Science* **18(2):249 - 250**
- Meshram, L.D. and Dhapke, D.K. 1981. Cytogenetical studies on an interspecific hybrid between *Abelmoschus esculentus* (L.) Moench x *A. tetraphyllus* (L.). In *Fourth International SABRAO Congress* 4 - 8 May 1981 **p:33**
- Mishra, R.S. and Chhonkar, V.S. 1979. Genetic divergence in okra. *Indian J. Agric. Sci* **49(4):247 - 9**
- Mishra,R.S. and Singh,D.N. 1987. Association and path coefficient analysis of some biometric characters in okra. *The Andhra Agric. J.* **34(4):369 -374**

- Mishra, R.S., Rath, U.K. and Sahu, G.S. 1990. Association of biometric characters in okra. *Orissa J. Agric Res* **3(1):6-8**.
- Mishra, R.S. and Singh, D.N. 1992. Interrelationships in some quantitative traits in okra. *Indian J. Agric Res.* **26(1):40 - 44**
- Murthy N.S. and Bavagi, J.N. 1982. Correlation and path coefficient analysis in bhindi. *South Indian Hort.* **28(4):35 - 38**
- Nair, P.G. and Kuriachan, P. 1976. A spontaneous hybrid between *Abelmoschus tuberculatus* and *A. esculentus* (L.) Moench. New Botanist **3:48 - 53**
- Nerkar Y.S. and Jhambhale N.D. 1985. Transfer of resistance to Yellow Vein Mosaic from related species into Okra (*Abelmoschus esculentus*). *Indian J. Genet.* **45(2):268 -70**
- Pal, B.P., Singh, H.B. and Swarup, V. 1952. Taxonomic relationships and breeding possibilities of species related to okra (*Abelmoschus esculentus*). *Bot. Gaz.* **113:455 - 464**
- Palaniveluchamy, K., Muthukrishnan, C.R. and Irulappan. I. 1982. Studies on heritability and genetic advance in bhindi (*Abelmoschus esculentus* (L.) Moench). *Madras Agric. J.* **69(9):597 - 598**
- Pratap, P.S., Dharkar, B.S., Pandit, M.L. and Dudi, B.I. 1980. Genetic divergence in parents and their hybrids in okra



(*Abelmoschus esculentus* (L.) Moench. *Genetica Agraria*  
**34 (3/4):323 - 330**

Patel, S.S., Kulkarni, U.G. and Nerkar, Y.S. 1993. Association studies in okra (*Abelmoschus Esculentus* (L.) Moench). *Gujarat Agric. Univ. Res. J.* **19(1):162 - 163**

Pillai, U.P.R. 1984. Evaluation of interspecific hybrids of bhendi with reference to yellow vein mosaic resistance and hetrosis. MSc(Ag) thesis, K.A.U. Thrissur **P.P.51**

Prabha, P. 1986. Cross-combatibility between *Abelmoschus esculentus* and *Abelmoschus manihot* and hybrid sterility. MSc thesis, Kerala Agricultural University **pp 49**

Rao, C.R. 1952. Advanced Statistical Method in Biometrical Research. *John Wiley and Sons, New York* **p:390**

Reddy, K.R., Singh, R.P. and Rai, A.K. 1985. Variability and association analysis in okra. *Madras Agric. J.* **72(8):478-480**

Siemonsuma, J.S. 1982 a. West African okra-morphological and cytological indications for the existance of a natural amphiploid of *Abelmoschus esculentus* (L). Moench and *A.manihot* (L) Medikus. *Euphytica* **31(1):241-252**

Siemonsuma, J.S. 1982.b. La culture due gambo (*Abelmoschus legume fruit tropical arec reference) speciale d'voire) theory waginger Agricultural Univ Netherlands* **pp 297**

- Singh, H.B. and Bhatnagar, A. 1975. Chromosome number in okra from Ghana. *Indian J. Genet. Plant. Breed.* **36:24 -27**
- Singh, H.P. and Singh, H.N. 1979. Path coefficient analysis for yield component in okra. *Indian J. Agric. Sci.* **49(4):244 -6**
- Singh H.P and Haridas, H.W. 1956. Allopolyploid nature of okra (*Abelmoschus esculentus* (L.) Moench. *Nature Lond* **178:1190**
- Stebbins, G.L. Jr. 1958. The inviability, weakness and sterility of interspecific hybrids. *Adv. Genet.* **9:147 - 203**
- Sundhari, S.S., Irulappan, I., Arumugham, R. and Jayasankar, S. 1992. Association analysis in okra. *Abelmoschus esculentus* (L.) Moench. *South Indian Hort.* **49(3):182 - 183**
- Suresh Babu, K.V. and Dutta, O.P. 1987. Cytogenetical studies in okra Ph D (Hort) Thesis University of Agricultural sciences Bangalore. **pp 166**
- Suresh Babu, K.V. and Dutta, O.P. 1990. Cytogenetic studies of the F<sub>1</sub> hybrid *Abelmoschus esculentus* x *A. tetraphyllus* and its amphiploid. *Agric Res. J. Kerala* **28:22 - 25**
- Teshima, T.k. 1933. Genetic and Cytogenetical studies in an interspecific Hybrid of *Hibiscus esculentus* and *H. manihot*. *J. Fec. Agri. Hokkaido Univ.* **34:1 - 155**

- Ugale, S.D., Patil, R.C. and Khuspe, S.S. 1976. Cytogenetic studies in the cross between *Abelmoschus esculentus* (L.) Moench and *A. tetraphyllus*. *J. Maharashtra Agric. Univ.* **1(2-6):106-110**
- Ustinova, E.I. 1949, A description of the interspecific hybrid of *Hibiscus esculentus* and *H. manihot*. *Genetica* **6:58 - 60**
- Van Borssum Waalke, J. 1996 Malesian Malvaceae revised. *Blumea* **14(1):1 -25**
- Vashishtha, R.N., Pandita, M.L. and Bhutani, R.D. 1982. Variability studies in okra (*Abelmoschus esculentus* (L.) Moench) under dry farming conditions. *Haryana J. Hort. Sci.* **11(1/2):117 - 121**
- Venkatesh, N. 1991. Studies on variability association and genetic divergence of economic characters in bhindi. (*Abelmoschus esculentus* (L.) Moench). MSc thesis Annamalai University, Annamalai Nagar, 608 002 **pp:113**
- Wright, S. 1921. Correlation and causation. *J. Agric. Res.* **20:557 -58**

**GENETIC IMPROVEMENT AND CYTOGENETICAL  
STUDIES IN THAMARAVENDA**  
[*Abelmoschus manihot* (L.)]

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**ABSTRACT OF A THESIS**  
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## ABSTRACT

The present investigation on "Genetic improvement and cytogenetical studies in Thamaravenda (*A.manihot* L.)" was conducted at the Vegetable Research Farm, Department of Olericulture, College of Horticulture, Vellanikkara during the year 1993 - 95. The major objectives were to collect maximum number of genotypes of the local Thamaravenda type, to assess their variability, identify promising genotype among them, confirm chromosome number of the species and its species affinity with *A.esculentus* and attempt the synthesis of an amphiploid of *A.esculentus* and the "Thamaravenda" type, AM - 4.

Twenty two genotypes of Thamaravenda collected from different parts of Kerala were raised in randomized block design in the field and statistical analysis was done on the various observations recorded namely plant height, number of primary branches, main stem diameter, number of internodes on mainstem, internodal length, fruit length, fruit girth, single fruit weight, fruits per plant, yield per plant, seeds per fruit and hundred ~~seed~~ weight. The genotypes expressed significant variation for all the characters studied. Genotype AM - 27 showed a combination of desirable characters like highest number of fruits per plant, high yield and long fruits. High value of genotypic coefficient of variation combined with high heritability was shown by characters like fruits per plant, yield per plant, number of fruit ridges and height of plant. Fruit length and fruits per plant showed significant positive correlation with yield. Fruits per plant showed the highest positive direct effect on yield. Using Euclidean cluster analysis the 22 genotypes

were grouped into three clusters each having fifteen, one and six genotypes respectively. The solitary cluster II consisted of AM - 27.

The chromosome number of *A.esculentus* line AE - 202 and Thamaravenda type AM - 4 was confirmed to be  $2n = 130$  and  $2n = 184$  respectively. These observations indicated that AE - 202 belonged to tetraploid group of *A.esculentus* and that AM - 4 actually came under *A.caillei*. In the interspecific hybrid between AE - 202 and AM - 4 all the haplophase chromosomes ( $n = 65$ ) of *A.esculentus* ~~were~~ found to pair with the homeologous counter parts of AM - 4 forming 65 bivalents and 27 univalents showing a good amount of chromosome affinity between the species. The interspecific hybrids was found to be partially sterile. The synthetic amphiploid produced from the interspecific hybrid showed a lower frequency of multivalents and its chromosome number was  $2n = 314$  and was more fertile than  $F_1$  hybrid. The *A.esculentus* lines AE - 202 and Thamaravenda line AM - 4 were crossable only when AM - 4 was used as female parent.