INTROGRESSION OF YELLOW VEIN MOSAIC VIRUS RESISTANCE FROM *Abelmoschus caillei* (A. Cher.) Steveis INTO *A. esculentus* (L.) Moench.

By

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THESIS

submitted in partial fulfilment of the requirement for the degree of

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COLLEGE OF HORTICULTURE
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DECLARATION

I hereby declare that this thesis entitled " **Introgression of Yellow Vein Mosaic Virus resistance from *Abelmoschus caillei* (A.Cher.) Steveis into *A.esculentus* (L.) Moench"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled " **Introgression of Yellow Vein Mosaic Virus resistance from *Abelmoschus caillei* (A.Cher.) Steveis into *A.esculentus* (L.) Moench**" is a record of research work done independently by **Ms. V. Kousalya** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Dedicated to

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1. INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is one of the largest consuming vegetables, rich in vitamin A (86.67 µg/100g), riboflavin (0.10 mg/100g), vitamin C (18 mg/100g) and minerals like calcium (66 mg/100g), phosphorus, iodine , iron and potassium (Kale *et al.*, 1986). Average nutritive value (ANV) of okra is 3.21 per cent which is higher than tomato, brinjal and cucurbitaceous vegetables (Sharma and Arora, 1993).

It can be easily cultivated year round and it is one of the best adapted vegetables in the tropical condition. Early bearing, extended period of harvest, coupled with short life span in this crop are some of its plus points for vegetable growers. Okra exhibits a large extent of genetic variability. So an array of cultivars, adapted to different agroclimatic conditions are available in this crop, which display a wide spectrum of variation with respect to important economic and quality characters providing lot of scope for genetic improvement.

Virus diseases cause heavy loss in economically important plants. The extent of loss due to these diseases will vary greatly depending upon the value of crop and type of damage, which may be either qualitative or quantitative. In okra, Yellow Vein Mosaic Virus (YVMV) is the most destructive virus disease transmitted by vector white fly *(Bemisia tabaci)*. The reported yield reduction due to this disease infection is in the range of 50 to 90 per cent depending on the stage of the crop growth at which infection occurs (Sastry and Singh, 1974).

All the cultivars and land races of okra succumb to YVMV, indicating the absence of resistance to this virus in *A.esculentus*. Several varieties exhibited tolerance/ resistance to this virus at the time of release, but this tolerance / resistance has broken down with time.

Interspecific hybridization for YVMV resistance followed by selection in the segregating generations is an effective method for obtaining desirable recombinants.

Several wild species of cultivated okra showed high degree of resistance to YVMV. However resistant varieties developed by various research organizations by interspecific hybridization have started showing sign of susceptibility probably due to the arrival of new virus strains. Hence it is imperative to find diverse sources of resistance to YVMV and evolve YVMV resistant varieties by suitable gene introgression programmes.

In this context a semi wild okra species locally known as " Thamara Venda or Mara venda" *Abelmoschus caillei (A.*Cher) Steveis deserves importance. It adorns many remarkable traits such as resistance to YVMV, adaptability and perennial nature (Charrier, 1984; Chacko *et al.*, 1996). This species is a complex polyploid considered to be originated by contributing genomes of *A. esculentus* and *A.manihot* (Siemonsuma, 1982).

A. caillei variety Susthira has been developed in the Department of Olericulture, College of Horticulture, Kerala Agricultural University, Vellanikkara by selection from the existing variability expressed by this species. Although, Susthira is highly resistant to YVMV and high yielding, it is more suitable for kitchen garden due to its perennial nature, late bearing habit and less attractive pods. At the same time a popular *A. esculentus* variety Salkeerthi developed by the same department has a wide range of acceptability owing to its early bearing habit, excellent fruit quality, attractive light green pods and high yield. But it is susceptible to YVMV. Hence it can't be grown during summer when the disease is more prevalent. In this regard it will be a viable proposal to transfer YVMV resistance from Susthira (*A. caillei*) to Salkeerthi (*A.esculentus*).

Interspecific hybridization coupled with cytogenetical studies will offer basic information on cytotaxonomy, chromosome affinity between the species and probable gene transfer from wild to the cultivated species.

Biochemical markers are widely used for characterization and cataloguing of crop species and cultivars with respect to the their reaction to many diseases. Keeping the above facts into consideration, the present study was conducted with the following specific objectives.

1. To transfer YVMV resistance from *A.caillei* variety Susthira to *A.esculentus* variety Salkeerthi by interspecific hybridization.
2. To find the chromosome affinity between *A.esculentus* and *A.caillei.*
3. To test the level of YVMV resistance in the parental species, interspecific F_1 and its derivatives.
4. To assess the activity of Peroxidase (PO), Polyphenol Oxidase (PPO) and protein variation with respect to YVMV resistance in the species and their derivatives.

2. REVIEW OF LITERATURE

Okra (*Abelmoschus esculentus* (L.) Moench) is one of the popular vegetables relishes traditional food preparations and also furnishes rich amount of nutrient to our diet. Due to its high adaptability, it can be cultivated under a wide range of environmental conditions. But the susceptibility of most of the okra varieties to YVMV is the major problem limiting the cultivation of this crop. It is necessary to breed new okra varieties resistant to YVMV by introgressing resistant genes from diverse wild species of *Abelmoschus*.

The pertinent literature for the present study is reviewed under the following heads.

1.Yellow Vein Mosaic Virus(YVMV).
2. Cytological and Cytogenetical studies.
3. Activity of peroxidase, polyphenol oxidase and protein banding pattern studies.

2.1. YELLOW VEIN MOSAIC VIRUS

Yellow Vein Mosaic Virus (YVMV) of okra was first reported by Kulkarni (1924) in the Bombay region. Later it was studied by Uppal *et al*.. (1940) and Capoor and Varma (1950). The virus is neither sap nor seed transmissible, but it is readily transmitted through white fly (*Bemisia tabaci)* (Padda, 1968). The loss due to this disease is in the range of 50-90 per cent depending on the stage of the crop growth at which infection occur (Sasthri and Singh, 1974). The disease not only reduces yield adversely, but also affects marketability of the fruits (Pun and Doraisamy 1999). They also found the yield loss due to YVMV up to 95.7 per cent.

2.1.1. Incidence of YVMV

Chellaiah and Murukesan (1976) observed a significant increase in the incidence of YVMV and yield loss in okra sown in March-May compared with rest of the year. Goswami and Bhagavathy (1992) in their experiment observed the lowest disease incidence on okra

sown at beginning of October (16.7%) and highest incidence on crop sown in May and June (100%).

Mazumder *et al.* (1996) conducted experiment on the incidence of YVMV and its vector *Bemisia tabaci* in the cultivars Pusa Sawani, Parbhani Kranti and M-31. Lower disease incidence populations were recorded in crop sown between February 25 and March 20. Positive significant association was observed between disease incidence and white fly population, temperature, relative humidity (RH) of evening, rainfall and rainy days. Sangar (1997) in his experiment during rainy and summer seasons observed Arka Anamika was highly resistant, Arka Abhay resistant and Parbhani Kranti and V-6 were moderately resistant to YVMV. Nath *et al.* (1999) observed minimum disease incidence (4.44 %) in Parbhani Kranti and Arka Abhay at 90 days after sowing.

Pun *et al.* (1999) revealed effect of age of plants on susceptibility to YVMV. The greatest loss in the yield of fresh fruit was highest (95.7 %) when virus inoculated at one week old plants.

Pun *et al.*(2000) studied influence of weather factors on the incidence of YVMV. The meteorological factors were maximum and minimum temperature, morning and evening RH, Sun shine hours, wind velocity and total rainfall with the disease incidence.

Jose *et al.*(2003) annuciated okra YVMV in India is caused by association of a DNA beta satellite with a begomovirus. This disease is caused by a complex consisting of the monopartite begomovirus, Bhindi Yellow Vein Mosaic Virus (BYVMV) and a small satellite DNA beta component. BYVMV can systemically infect bhindi upon agioinoculation but produces only mild leaf curling in this host. DNA beta induces typical symptoms of YVMV when co-agroinculated with the begomovirus. The DNA beta component associated with BYVMV has a number of factors in common with those reputed for ageratum yellow vein disease and cotton leaf curl disease. BYVMV represents a new member of the emerging group of mono-partite begomoviruses requiring a satellite component for symptom induction.

2.1.2. Screening for resistance to YVMV.

Naraini and Seth (1958) in their screening experiment for YVMV inferred that, *Hibiscus manihot* var*: pungens, H. crinitus, H. vititolius and H. panduraetormis* were immune. Sandhu *et al.* (1974) in their screening test found that accession E 31830, ' Asuntem Koko' from Ghana was *A. manihot* (L) Medicus ssp. *manihot* was immune to YVMV.

Raghupathy *et al.* (2000) screened 12 okra cultivars, including the highly susceptible Pusa sawani and MDU-1. The disease was absent in the highly resistant cultivars BO-1 and HRB-55. Resistant cultivars were KS-404, HRB 9-2, Hy.8, P-7, Parbhani Kranti, Sel-10 and Sel-4, with a disease incidence of 0.5, 0.82, 1.26, 1.68, 2.87, 3.63 and 8.69 % respectively. BO-2 was susceptible (19.55%) and MDV-1 and Pusa Sawani recorded 90.83 and 91.53% respectively.

Ravisankar (2002) conducted screening experiment by grafting and vector transmission studies. He reported line AE-238 was free from YVMV. AE-265 x AE-190 also did not show disease symptoms in the field screening. Singh *et al.* (2002) revealed performance of different varities to YVMV out of the twelve cultivars were screened disease incidence varied from 0.7%(Arka Abhay) to 57.4% (Chhindwada Local).

2.1.3. Breeding for resistance to YVMV.

2.1.3.1.Selection

Joshi *et al.* (1960) used treatment line IC 1542 from West Bengal for developing the resistant varieties. Sureshbabu *et al.* (2002) reported a YVMV resistant edible perennial okra line AE 286 (*A.caillei*) through single plant selection.

2.1.3.2. Hybridization and selection

Singh (1962) bred "Pusa Sawani" from the cross between IC-1542 and Pusa Makhmali. Dhankhar (1996) developed a YVMV resistant okra "Varsha Uphar" out of the cross between Lam selection I x Parbhani Kranti. Fugro and Rajput (1999) using a partial diallel mating system involving nine genotypes, developed 36 F1 hybrids, of which Sel-4 x Parbhani Kranti, Pusa Sawani x Punjab-7, Sel-4 x BO-1, Sel-4 x Punjab-7 and Sel-4 x Sel-10 were free from YVMV. Rameshpathak *et al.* (1997) reported that the crosses Parbhani Kranti x HR-55 and Parbhani Kranti x EC 16511 were found to be resistant to YVMV.

Deo *et al.* (2000) annuciated that Parbhani Kranti and its hybrid Parbhani Kranti x HRB-9-2 were highly resistant to YVMV. Rattan and Bindal (2000) in their programme to develop okra hybrids resistant to YVMV found that lines 407, 409, 417, 430 were completely resistant.

Ravisankar (2002)crossed fifteen lines with two testers, AE-285 and AE-190 (Parbhani Kranti) in a line x tester mating design to produce 30 hybrids. Among them a parent AE-238 and two hybrids were free from YVMV.

2.1.3.3. Interspecific hybridization

Interspecific hybridization followed by selection in the segregating generations is an effective method for obtaining YVMV resistant recombinants.

Arumughan *et al.*(1975) reported that several exotic accessions of *A.manihot* were resistant to YVMV and the crosses made between *A. esculentus* and *A. manihot* yielded viable F_1 seeds. However, up to 40 percent sterility appeared in F_2 generation.

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

Interspecific crosses between two cultivars of *A.esculentus* and a cultivar of YVMV resistant *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).

Nirmaladevi (1982) reported that *A.manihot* L. was crossable with *A.esculentus* (L.) Moench. The interspecific F_1 hybrid exhibited significant heterobeltiosis for many of the economic characters and resistance to YVMV . She observed significant genetic distance between *A. manihot* L. and *A. esculentus* (L.) Moench

Pillai (1984) produced interspecific hybrids from *A. manihot* and four YVMV susceptible *A.esculentus* cultivars. Though the hybrids produced fruits, number of seeds per fruit count was very much low (8 to 12) as against that of the parents (50 to 90). Further, the hybrids were found to be resistant to the YVMV disease. A decrease in percentage of pollen fertility in the hybrids (28.7 – 57.4) as against the parents (98 – 99) is presumably the reason for scanty hybrid seed recovery.

Nerkar and Jambhale (1985) crossed *A. tetraphyllus*, *A.manihot* and *A. caillei* with *A.esculentus* var. Pusa Sawani. Though most of the F_1 hybrids exhibited partial to complete sterility, the amphidiploids of the hybrids developed through colchicine treatment were fertile and resistant to YVMV with good agronomic attributes.

Prabha (1986) crossed the yellow vein Mosaic disease susceptible varieties of YVMV. with resistant wild / semi wild species, *A. manihot*. The first generation hybrids, though did not produce viable seeds profusely, were not totally sterile. The scanty viable seed recovery from the hybrids against the parents was suspected to be due to chromosomal differentiation that could have taken place during speciation in the genes.

Prakash (1986) also studied the cross compatibility between *A.esculentus* and *A.manihot*, Wild species like *A. tetraphyllus*, *A.manihot* ssp. *tetraphyllus*, *A.ficulneus*,

A.moschatus and *Hibiscus huegetic* were resistant to yellow vein mosaic virus. interspecific hybridization was done in the above resistant wild species with the locally adapted high yielding variety, Kiran. Normal fruits and seeds are produced in the cross combinations involving *A.tetraphyllus* and *A.manihot*. Among the cultivated varieties, Arka Anamika, Parbhani Kranti and Vijay F_1 hybrid were found promising in the southern region of Kerala.

Sheela (1986) attempted to induce desirable genetic recombinants combining resistance to YVMV in the interspecific crosses of *A.esculentus* x *A. moschatus, A.esculentus* x *A. caillei* and *A. esculentus* x *A. tetraphyllus*. Fruit set in the interspecific crosses was found to be less (28% -42%) compared to the female parents (81%-97.81%). It was high when cultivated varieties were used as female parents. The crossed fruits resembled the fruits of female parent in all the crosses irrespective of the pollen source. In the crosses with *A.moschatus*, 100 seed weight was categorically low compared to other crosses. All the crossed seeds showed good germination percentage except in crosses with *A. moschatus*. Crosses with *A. moschatus* provided shriveled and non-viable seeds. Eleven days old embryos of the crosses *A.moschastus* x *A.esculentus* cv."Anakomban" were found to be brown at the time of excision. Ten days old embryo of the same crosses showed no response in ½ MS, MS and MS + BA 0.5 mg/lit + CW 150 mg/lit. media. Embryos less than 10 days old could not be cultured *in vitro*, as excision was difficult. In the irradiated crosses, *A.caillei* x "Anakomban" (*A.esculentus*) and *A.caillei* x " Eani Venda " were identified as better crosses for isolating recombinants.

Pushparajan (1986) reported reproductive isolation of *A.moschatus* from all other species of the genes *Abelmoschus*. Cheriyan (1986) reported that there was no reciprocal difference in the crossability index in crosses of *A. esculentus* with *A. manihot* and *A. tetraphyllus*.

Madhusoodanan and Nazeer (1986) carried out successful intercrossing between a 'Saudanien' and a 'Guinean' type of okra, of which the later is reported to be immune to YVMV disease. However, due to difference in chromosome number of the parents the hybrid

exhibited abnormal meiosis leading to sterility and thereby hinders fruitful incorporation of the disease resistant gene to the former one. In the F_4 generation of interspecific hybrids between *Abelmoschus esculentus* and *A. manihot*, evaluated by Alex (1988), the genotypic coefficient of variation was maximum for number of branches per plant and minimum for first fruiting node.

Sureshbabu and Dutta (1990) obtained heterotic hybrids from crosses of *A.esculentus* with *A. tetraphyllus*. Meiosis was abnormal in hybrids leading to hybrid sterility. They produced fully fertile amphidiploid *A.esculentus* x *A. tetraphyllus* by colchicine treatment, resembling the F_1 plants with YVMV resistance and larger fruits. They also reported that progenies from the back cross of the amphidiploid of the cross between *A.esculentus* and *A.tetraphyllus* with the cultivated parent was readily feasible and they combined the YVMV resistance of the wild species and the desirable fruit characters of the cultivated species. Failure of seed formation in interspecific hybrids may be due to slow pollen tube growth, abnormal pollen tube, abortion of fertilized ovules or sparsity of pollen grains.

Dutta (1991) reported the development of okra lines having high yield, quality and resistance to YVMV by interspecific hybridization between *A.esculentus* and YVMV resistant wild species *A.manihot* subsp *tetraphyllus*. Nerkar (1991) revealed the use of wild relations (*Abelmoschus spp.*) of okra with resistance to okra YVMV, powdery mildew (*Erysiphe cichoracearum*), jassids (*Empoasca* spp.) in breeding programmes to develop pest and disease resistant varieties.

Sindhu (1993) observed that the fruit set in direct crosses of *A.esculentus* cv Anakomban x *A.manihot* and *A.esculentus* cv. Anakomban x *A. manihot* ssp. *tetraphyllus* was very low compared to the reciprocal crosses, exhibiting partial incompatibility consequent on the slow pollen tube growth of *A.manihot* and *A.manihot* ssp. *tetraphyllus*. Seed set was low in the crossed fruits and there was recovery of shriveled seeds which may be attributed to the poor endosperm development. The crossed seeds exhibited good viability. Four hybrids recorded pollen sterility which may be attributed to meiotic abnormalities due to difference in

chromosome number between the species, seed set in the hybrids was very low and was inversely proportional to pollen sterility. Recovery of empty seeds which appeared normal may be ascribed to endosperm degeneration viability of F_2 seeds was very low.

Sheela (1994) attempted combining the economic attributes of cultivars and yellow vein mosaic disease resistance of wild relatives. Varietal difference in compatibility of *A.esculentus* with the donor parents, *A.caillei* and *A. tetraphyllus* was noticed. Reciprocal crosses registered higher compatibility than the direct crosses. Natural crossing of *A.tetraphyllis* with *A. essculentus* and *A. caillei* was also observed. She further observed a higher proportion of low yielding YVMV resistant types similar to the wild types in F_2 and F_2M_2 populations indicating strong genetic mechanisms preventing recombinations. However, more recombinants appeared in the F_2M_2 generation indicating the breakage of undesirable linkage through irradiation.

Manjuchandran (1996) successfully cultured 12 and 15 days old interspecific embryos of *A.esculentus* var. Kiran x *A.moschatus* and *A. esculentus* cv. "Anakomban" x *A.moschatus* in MS medium, supplemented with BA 0.5 mg/lit and CW 150 ml/lit. Twelve days old embryos gain lesser germination percentage than fifteen days old embryos. For the multiplication of interspecific hybrids produced by embryo culture, MS medium supplemented with BA 1 mg/lit and CW 150 ml/lit was found optimum for enhanced release of axillary buds. In this medium, the number of multiple shoots per culture was 7.80 and 5.16 respectively for *A.esculentus* var. Kiran x *A.moschatus* and *A.esculentus* cv. "Anakomban" x *A.moschatus*. *Ex-vitro* survival per cent was 36.0 and 30.0 for *A.esculentus* var. Kiran x *A.moschantus* and *A.esculentus* cv. "Anakomban" x *A. moschatus* respectively.

Chandran *et al.* (1996) reported that transplantable seedlings could be obtained by embryo rescue technique through culturing twelve and fifteen days old embryos of the crosses between *A.esculentus* and *A.moschatus*. This suggested the potential of tissue culture methods to overcome the post –zygotic incompatibility barriers in interspecific crosses.

Chandran and Rajamony (1997) reported that when cultivated types were used as the female parent in hybridization using wild donors, maximum fruit set was obtained in crosses where *A.manihot* used as pollen source. The percentage of viable seeds obtained was also maximum in crosses involving *A.manihot.*

John (1997) estimated the extent of variability in the F_2M_2 and F_3M_3 generations as a result of hybridization and irradiation of the interspecific hybrids between *A.esculentus* and *A.manihot.* In the F_2M_2 generation, the irradiated treatments were late flowering and had more number of leaves, flowers and fruits. Irradiation increased pollen sterility and was maximum at 10 KR. Seed set was lower in the irradiated treatments.

The families in the F_4M_4 generation of inter specific hybrids resistant to YVMV were late flowering (Philip,1998). He recorded higher mean value for number of leaves, branches, flowers and fruits compared to the cultivated parent. The fruit yield per plant was higher than the parents inspite of the reduction noticed in average fruit weight. In the F_5M_5 generation, the families were early flowering and recorded increase in leaf area, pollen sterility and number of branches / plant. The fruit yield / plant, average fruit weight and number of seeds / fruit were higher in the F_5M_5 families.

2.1.3.4. Interspecific hybrid sterility

Teshima (1933) observed that *A.esculentus* and *A. manihot* crossed only when the former was used as the female parent. He also studied this cross and reported that the F_1 hybrids were partially fertile. The interspecific hybrid sterility may be genic or chromosomal (Stebbins, 1950). Genic sterility is typically due to the genetic constitution of organism and so is diplontic (Dobzhansky, 1951). He pointed out that the only practical criteria to distinguish between genic and chromosomal sterility is provided by effects of doubling the chromosome number. The chromosomal sterility results from structural differences between chromosomes which cause deficiency, duplication and other disharmonious combinations of chromosome segment to be distributed to the gametes.

Doubling of the chromosome number results in the presence of meiosis a complet homology for every chromosome in the organism, pairing and segregation are therefore usually regular and no chromosomal imbalance results. If the sterility is genic and diplontic the imbalance in the somatic tissue of the diploid is retained in the corresponding tissue of tetraploid, hence the sterility persist.

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 per cent in different crosses, the highest being in the cross *A.esculentus* x *A. manihot.*

According to Stebbins, (1958) the immediate cause of failure of embryo development in interspecific hybrid can be grouped into three categories viz. the disharmony may reside entirely or chiefly in the chromosomes and genes of the two parental species as they are combined in the hybrid nuclei. There may be disharmonious interaction between the chromosomes or genes of one species and the cytoplasm of other as it has been contributed by egg of maternal parent. The hybrid embryo may be perfectly capable of developing so for as its on constitution is concerned, but may be inhibited action of maternal tissue surrounds it, or in higher plants by the endosperm which nourishes it.

Stebbins (1958) reported if the failure of embryo development is due to the incompatibility between parental chromosomes and genes, reciprocal hybrids between the two parents will be equally weak or inviable, thus the removal of the embryo from it maternal surrounding will not help to develop.

About 90 per cent 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 (Simonsuma; 1982). Partial seed fertility of 5.9 per cent and 7.1 per cent were obtained in crosses *A.esculentus* x *A.manihot* and *A.esculentus* x *A.manihot* ssp. *manihot* respectively by Jambhale and Nerkar (1985). Bhargava (1989) observed embryo deterioration in ovules resulting from crosses between *A.manihot* and *A. esculentus*. Endosperm deterioration was first noted five days after pollination in the cross *A.esculentus* x *A. manihot* and was accompanied by a decrease in ovule weight. Cell division at this stage was random and within six days embryos had formed an undifferentiated cell mass surrounded by multiple layers of endothelium.

2.2 CYTOLOGICAL AND CYTOGENETICAL STUDIES.

2..2.1. Chromosome number

The chromosome number of okra *A.esculetus* exhibited 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 et al. (1974) and (Joshi and Haridas 1976). The reported chromosome number for *A.manihot* was in the range of 2n = 60 to 68 (Teshima (1933) and Kuwada (1974).

Sureshbabu and Dutta (1994) studied the chromosome polymorphism in nine okra lines which were resistant to YVMV because of the genes introgressed from *A.tetraphyllus*. The chromosome number of the lines ranged from 2n = 120 to 144. All the lines showed regular meiosis. Results confirmed the existence of complex polyploidy in *A.esculentus* which can sustain considerable chromosome addition and deletion.

Chacko *et al.* (1996) made detailed cytological studies on a semi wild okra *A.caillei* line AM-4 and reported its chromosome number as 2n = 184. Sureshbabu *et al.* (2002) made elaborated cytological studies on an YVMV resistant underexploited okra type of Kerala

locally known as "Thamaravenda" (*A.caillei* line AE.286) and reported its chromosome number as 2n = 184.

2.2.2. Ploidy levels in *Abelmoschus*

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

2.2.3. 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 Pollen Mother Cells (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 showed 29 bivalents and 36 univalents during meiosis (Joshi *et al.*, 1974). *A.tuberculatus* was thus accepted as one the pregenerators of *A.esculentus*.

Kuwada (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 F1 hybrid was fertile and it was named as Nori-Asa.

Joshi *et al.*(1974) reported sterile hybrid between *A.tuberculatus* (n=29) x *A.ficulneus* (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. Configuration at metaphase-I in amphidiploid was more or less regular forming 162 bivalents. Simonsuma (1982) reported that *A.caillei* might be a natural amphidiploid of *A.esculentus* and *A.manihot*.

Sureshbabu (1987) obtained F_1 hybrid between *A.esculentus* and *A.manihot* ssp. *tetraphyllus* var. *tetraphyllus* with 36 bivalents and 62 univalents which suggested that one genome was common for *A.esculentus* and *A.manihot* ssp. *tetraphyllus* var. *tetraphyllus*. Sterility in the hybrid was attributed to the failure of development of female gamete. The induced amphidiploid showed regular meiosis forming 34 bivalents owing to preferential pairing of chromosomes. The pollen stainability of amphidiploid was 94 per cent.

Kondiah *et al.* (1990) made crosses between *A.caillei* and *A.tetraphyllus* and with two induced amphidiploids viz. *A.esculentus –manihot* and *A.esculentus – tetraphyllus*. Cytological studies in the cross between *A.tetraphyllus* x *A.caillei* gave 66.5 bivalents and 6.52 univalents whereas the cross between *A.esculetnus – tetraphyllus* 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.tetraphyllus* and *A. caillei* which suggested that *A.tetraphyllus* would have contributed two genome to *A.caillei*. Chromosome pairing behaviour 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. tetraphyllus* two genomes to *A.caillei*.

Chacko *et al.* (1996) reported the chromosome number of *A.esculentus* line AE-202 and Thamaravenda type AM-4 as 2n=130 and 2n=184 respectively. These observations indicated that AE-202 belonged to tetraploid group of *A.esculentus* and that AM-4 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 homologous counter parts of AM-4 forming 65 bivalents and 27 univalents showing a good amount of chromosome affinity. The synthetic amphidiploid produced from the interspecific hybrid showed a lower frequency of multivalents and its chromosome number was 2n=314. It recorded more fertility than F_1 hybrids. The *A.esculentus* line AE-202 and Thamaravenda line AM-4 were crossable only when AM-4 was used as female parent.

2.2.4. Segregating progenies of species hybrid

Mathews (1986) evaluated the F_2 populations of interspecific cross of *A. manihot* x *A.esculentus* along with the parents and F_1s. A preponderance of low yielding yellow vein mosaic resistant plants similar to the semi-wild parents was observed among the F_2 populations, suggesting the presence of powerful genetic mechanisms which restrict free recombinations.

According to Kalloo (1988) two species of genes involved in hybridization have enormous distinction for many characteristics. As a consequence such species hybrids should produce an F_2 population with a wide range of variation. Also, extreme recombinants are expected in F_2 but in practice, the possibility of appearance of such individuals is remote. Widely or semiwild parental types appear frequently and recombinants produced in F_2 exhibit rather a narrow range. Usually, F_2 populations show a pronounced shift towards wild parent especially when one is cultivated and other is wild. This situation is extensively demonstrated in the F_2 segregants of the crosses like *Lycopersicon esculentum x L. peruvilianum, L.esculentum x L. pimpinellitolium, L.esculentum x L.hirsutum, Cucumis melo x C.metuliferus and Cucurbita maxima x Cucurbita lundelliana.* The most probable for this one sided

segregation may be reduced pairing, chromosome differentiation, restricted recombination linkage, gametic and zygotic elimination.

3.1. PEROXIDASE, POLYPHENOL OXIDASE ACTIVITY AND PROTEIN BANDING PATTERN STUDIES

The expression of genes is known to be influenced by gene-interaction, developmental stage, temperature and background genotype of the cultivar.). A positive correlation between peroxidase and polyphenol oxidase activity and resistance to different plant pathogens has been observed Reuveni and Ferreira (1955), and Jennings *et al.*(1969). Phenols as active resistant factors in defence mechanism have been established in some cases (Sharma *et al.*, 1983). The quantitative change in peroxidase activity may be responsible for determining resistance or susceptibility to given plant pathogen.

Mohan and Khanna (1988) reported that peroxidase and polyphenol oxidase in relation to brown rust of wheat showed greater activity in disease resistant cultivars. Aboutaleb *et al.* (1988) reported Interaction of wilt resistant and susceptible cotton plants with three isolates of *Fusarium oxyporum* f.sp. *vasinfectuum* and one isolate of *F.monliforme* showed that both inoculated and resistant plants possessed a significantly higher amount of total soluble protein than non-inoculated and susceptible plants. Total soluble proteins, peroxidase and polyphenol oxidase activities increased gradually with time following inoculation.

Vecchi and Matta (1989) annuciated cytochemical evaluations of peroxidase, polyphenol oxidase and phenols in compatible and incompatible combinations of tomato and *Fusauim oxyporum*. Enhancement of phenolic metabolism in susceptible plants infected with a non pathogenic race and in resistant plants infected with a pathogenic race confirmed their role in resistance mechanism.

Yu and Wang (1990) soaked seedlings of 11 watermelon cultivars for 88 h. in a 50 per cent toxin solution containing mainly fusaric acid from liquid culture of *Fusarum oxyporum* f. sp.*niveum*. Peroxidase isoenzyme activity and banding patterns were determined before and after treatment. Results showed that resistance to the toxin was correlated with resistance of the cultivars having one or two more isoenzyme bands than those of resistant cultivars, it was suggested this factors could be used for early screening.

Peroxidase activity was positively correlated with induced resistance against Tobacco Mosaic Virus (TMV) and Tobacco blue mould (*Peronespora tabaciana*) in cultivar KY 14 in which the stem was injected with *Peronospora tabaciana* and the leaf was inoculated with TMV. The increase was evident in cytosol, intercellular fluid and cell wall fractions. The isozyme pattern of peroxidases of isoelectric focusing gels showed an increase of two anionic peroxidases. Both peroxidases were positively correlated with induced resistance (Ye *et al.*, 1990).

Peroxidase activity was used as a biochemical marker for the resistance of muskmelon (*Cucumis melo*) to *Pseudoperonospora cubensie*. The activity of the infected plants was higher than that of the uninfected plants (Reuveni *et al.*, 1991). Li *et al.* (1991) Phenylalanine ammonia lyase (PAL), peroxidase and Polyphenol oxidase levels were analysed in five groundnut cultivars before and after infection by *Puccinia arachidis*. PAL activity was positively related to resistance and could only be detected after infection. PO activity was higher in infected plants than in healthy plants of most cultivars at 12, 24 and 48 hours after infection. PPO activity in healthy plants was related to resistance but no relationship was found between its activity and resistance in infected tissues.

The activity of peroxidase, polyphenol oxidase and phenylalamine ammonia lyase in cucumber leaves was markedly reinforced after inoculation with *Colletotrichum gloeosporioides* to induce resistance to *C.lagenarium*. Polyacrylamide Gel and Electrophoresis (PAGE) showed that the enzyme band number of peroxidase isoenzymes

wider and darker. The phenol and lignin contents of the leaves also increased after inoculation. (Li *et al.* 1993).

Ahmed *et al.* (1994) reported total phenols orthodihydroxy phenols, flavonoids, total proteins and soluble proteins were high in virus free plants of cultivars resistant to okra YVMV while the enzymes peroxidase and polyphenol oxidase showed no significant differences between virus free susceptible and resistant cultivars. However, changes in these constituents were induced by inoculation with the virus. Infection generally resulted an increase in total protein in both resistant and susceptible cultivars to a greater extent. In resistant lines accompanied by an increase in peroxidase and polyphenol oxidase activity, whereas this was almost reversed in susceptible lines. Higher amounts of phenols and their oxidation products such as quinines, formed by increased peroxidase and polyphenol oxidase, may be responsible for a reduction in virus multiplication in the resistant lines P7, Ghana and their F1 hybrid.

Sarma *et al.*(1995) reported okra YVMV infection reduced the chemical constituents of okra leaves, such as chlorophyll, reducing sugar, phosphorus and potassium content whereas total phenol, total sugar, non-reducing sugar, nitrogen and protein contents increased. The extent of increase or decrease of these constituents varied with the different stages of plant growth. In green fruits, total sugar, reducing sugar, non-reducing sugar, nitrogen, protein, phosphorus and potassium content were decreased by infection.

Gupta *et al.* (1995) studied the levels of total phenol, polyphenol oxidase and peroxidase in leaves of *Alternaria* leaf blight resistant and susceptible cultivars of *Brassica* spp. They reported an increased level of total phenol and more number of bands for polyphenol oxidase in resistant cultivars.

Solorzano *et al.* (1996) levels of enzymatic activity were studied in tomato leaves treated with NaH2PO4, used as an elicitor of induced resistance, and inoculated with *Alternaria solani* at different stages of the experiment. Between days 10 and 15, an increase

in enzyme specific activities was obtained for both peroxidase and polyphenol oxidase compared with the untreated control, followed by a decrease 45 days after the treatment. The results confirmed that a relationship exists between the enzymes and resistance in the tomato plants.

Bhagat (1997) revealed the carbohydrate content of leaf tissues of three cultivars of bhindi in relation to infection by okra YVMV. Healthy and inoculated leaf samples of the cultivars Parbhani Kranti (resistant), Vaisali Vadhu(susceptible) and Pusa Sawani (highly susceptible) was analysed for reducing and non-reducing sugars. Healthy leaves of susceptible and highly susceptible cultivars showed a higher content of reducing and non-reducing sugars. Healthy leaves of susceptible and highly susceptible cultivars showed a higher content of reducing, non-reducing and total sugar than the resistant one. In diseased leaves their amount increased in all cultivars compared with uninoculated leaves. The increase being greater in susceptible cultivars than resistant.

Gupta *et al.* (1997) compared peroxidase isozymes patterns in leaf extracts from *Capsicum annum* F_1 hybrids and their pollinator parents. The variation in number and intensity of isozyme profiles within parental cultivars was less than that in F_1 hybrids. It was concluded that the study of isozyme profiles was useful to detect genetic variability between parents and their corresponding hybrids.

Hossain *et al.* (1998) annuciated, particularly biochemical changes in leaf constituents due to okra YVMV infection. BARI –1 (resistant) had the lowest percentage leaf infection among the cultivars, while the highest disease incidence was observed in Pusa Sawani. Total sugar, reducing sugar, non-reducing sugar and total chlorophyll were lower in virus infected leaves than healthy leaves, but total phenol, orthodihydroxy phenol and carotene contents were higher in infected leaves. the reduction in sugar and chlorophyll synthesis was higher in susceptible cultivars compared to resistant cultivars.

Peroxidase activity was used to understand the susceptible and resistant interactions between Cassava (*Manihot esculenta)* and *Xanthomonas axonopodis* pv. *manihotis* and X. *cassavae.* It was found that peroxidase activity in the resistant interactions was two fold higher than that of control or the susceptible interactions, which may be related to lignin deposition (Pereira et al., 2000).

Pal *et al.* (2002) studied the various biochemical parameters such as oil, sugar, phenol content and total soluble protein content along with its different fractions in seeds of four cultivars of okra and two wild species (*A.ficulneus and A.moschatus*). All the six genotypes differed significantly in terms of all the biochemical parameters except prolamin content. SDS-PAGE (Sodium dodecyl sulphate – PAGE) of soluble seed portion revealed some differences among the species in terms of the number of position and intensity of bands, while varietal discrimination based on SDS – PAGE could not be done. Similarly index calculated based on band homology established more divergence of the cultivated species with *A.moschatua* compared with *A.ficulneus*.

3. MATERIALS AND METHODS

This research work was conducted in the Department of Olericulture, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur. The experimental site is located at an altitude of 22.5 m above MSL. The research field experiences a typical warm humid tropical climate. The experiment was conducted from April 2003 to September 2004. The weather data during the period of investigation were presented in the Appendix. I .

The investigations were carried out under the following heads,

A. Interspecific hybridization and crossability studies.
B. Screening for resistance to YVMV.
C. Cytological and Cytogentical Studies.
D. Activity of Peroxidase, Polyphenol oxidase, and Protein banding pattern.

3.1. Materials.

Materials for this study comprised of *A.esculentus* variety Salkeerthi (Plate 1) susceptible to YVMV and *A.caillei* variety Susthira (Plate 2) highly resistant to the same virus. These species were used to raise F_1 and F_2 generations. The source information of the parental species is given in Table 1.

3.2. METHODS

3.2.1 Interspecific hybridization

Under this programme *Abelmoschus esculentus* variety Salkeerthi and *A.caillei* variety Susthira were raised in the field during April 2003 and crossed reciprocally. For crossing ripe flower buds of both parents were selected and bagged with 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 buds as deep as to remove the corolla and calyx as one unit, exposing the staminal tube and stigma. The undehised

Table 1. Source information of parental species

SNo.	Source information	Parent 1	Parent 2
1	Species	*A.esculentus*(L.)Moench	*A.caillei* (A.Cher.) Steveis
2	Varietal name	Salkeerthi	Susthira
3	Acc.No.	AE-202	AE-286-1
4	Source	KAU, Vellanikkara	KAU, Vellanikkara
5	Reported chromosome number	2n = 130	2n = 184
6	Crop duration	Annual type	Perennial type
7	Pod colour	Light green	Green
8	Length of pod	25-28cm	15-18 cm
9	Nature of epicalyx segment	Lanceolate	Winged
10	Reaction to YVMV	Susceptible	Resistant

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 developed pods were dried the seeds were collected.

3.2.2. Crossability and Morphological Studies.

Interspecific crossing was done betweens *A.esculentus* and *A.caillei* in both directions. The number of fruits set and the number of seeds per fruit were noted to work out the fruit setting percent and crossability index. Percent of fruit set and crossability index were worked out using the following formulae.

$$\text{Fruits set per cent} = \frac{\text{Number of fruits set}}{\text{Number of crosses made}} \times 100$$

$$\text{Crossability Index} = \frac{\text{Number of seeds in crossed fruits}}{\text{Number of seeds in selfed fruits}} \times 100$$

Further the parental species and the interspecific F_1 plants obtained by direct and reciprocal crosses were raised in the field during September 2003 for comparison of their morphology. The interspecific F_1 plants were selfed and the few seeds obtained were used to raise F_2 generation. Side by side interspecific F_1s were also crossed with the parental species *A.esculentus* and *A.caillei* to work out the possibility of raising backcross generations. Finally the parents and the resultant F_1 and F_2 generations were raised in the field during May 2004. The treatment genotypes received timely management and care as per the package of practice recommendation of Kerala Agricultural University (2002). For the comparison of morphological characters, guidelines as per the International Plant Genetic Resources Institute (IPGRI) were followed.

Highly susceptible okra line (AE-202) was raised all around the field. No plant protection measures were taken as it would reduce the vector population and there by hinder the natural epiphytotic condition.

3.2.2.1.Observations recorded

Five plants were randomly selected from each parental species and interspecific F_1 hybrids for taking the observations. In the case of F_2 generation 25 individual plants were selected and their morphological characters noted separately. The observations recorded on the different quantitative characters were analysed statistically.

3.2.2.1.1 Qualitative characters

1.	Plant habit	:	Branched/unbranched
2.	Pubescence		
	i) Stem	:	Smooth/Pubescent/warty
	ii) Lamina	:	Smooth/ Pubescent
	iii)Petiole	:	Smooth/Pubescent
3.	Pigmentation		
	i) Stem	:	Green/green with red tinge
	ii) Petiole	:	Green/ green with red tinge/ red with green tinge.
	iii) Vein	:	Prominent/not prominent, green/whitish green
	iv) Corolla throat	:	Present/absent
4.	Leaf size	:	Small/medium/large
5.	Leaf shape	:	Cordate/hastate/sagitate/others
6.	Laminal margin	:	Deeply fid/narrowly fid/serrated
7.	Leaf tip	:	Pointed/blunt
8.	Flower	:	
	i) Bud	:	Hairy/scaly/smooth/resinous
	ii) Corolla	:	Yellow/golden yellow, red throat/purple throat
	iii)Calyx	:	Hairy/smooth, fleshy/nonfleshy

iv)Stigma : Bifid/multifid purple/red/others, smooth/hairy

9. Fruits :

i) Immature fruit : Green/darkgreen/yellowish green/red/ deep red/others

ii)Size : Small/medium/long/extra long

iii)Shape : Round/angular/ridged/straight/curved

iv)Tip : Pointed/blunt

v)Dehiscence at maturity : Dehiscent/indehiscent

vi)Fruit hairiness : Present/absent, less hairy/ highly hairy, only on ridges/ entire fruit.

vii)Bending : Snaps/bends

10. Seeds :

i) Seediness : Low/medium/high

ii)Shape : Round/depressed

iii)Hairiness : Smooth/hairy

Leaf characters like colour of the leaf base, colour of the leaf vein and leaf lobing were recorded from seventh leaf of each observation plant. Flower characters such as flower colour and purple throat at corolla were noted at the time of anthesis. Fruit characters such as fruit pubescence and fruit colour were recorded at the time of harvest.

3.2.2.1.2 Quantitative characters

1) Plant height (cm)

   The height of the plant was measured from the base of the plant to tip at 90 days after sowing.

2) Internodal length (cm)

   The length of the internode between sixth and seventh node of the plant was measured at 90 days after sowing.

3) The number of primary branches

The number of primary branches per plant was counted at 90 days after sowing

4) Flower size

The length and width of the fully opened flower was recorded at 60 days after sowing

5) Length of epicalyx segment

Length of epicalyx segment of the ripe flower bud of each genotype was recorded at 60 days after sowing.

6) Width of epicalyx segment

Width of epicalyx segment of the ripe flower bud of each genotype was recorded at 60 days after sowing

7) Petiole length (cm)

Length of petiole of seventh leaf of each plant was recorded at 60 days after sowing.

8) Days to flowering

In each observation plant date of opening of the first flower was recorded and the number of days from sowing to flowering was worked out

9) Days to first harvest

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

10) First fruiting node

The node at which first fruit was formed and expressed in numbers.

11) Length of fruit (cm)

Three fruits were harvested from each observation plant at seven days after flowering and the fruit length was measured from basal cap to the tip of fruit.

12) Girth of fruit (cm)

Three fruits were harvested from each observation plant at seven days after flowering and the circumstance of the fruit was recorded at the point of maximum bulging.

13) Locules per pod

Three fruits were harvested from each observation plant at seven days after flowering and the number of locules per pod was recorded by taking cross section of the pod.

14) Number of ridges per pod

The number of ridges per pod of each genotype was noted.

15)Number of fruits per plant

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

16) Number of harvests

The total number of harvests was recorded from first to final harvest.

17) Crop duration

Time taken for last harvest from sowing was recorded separately.

18) Yield per plant

Weight of fruits harvested from the observation plants in each genotype was taken and their average was taken to get yield per plant

19) Incidence of other pests and diseases

Incidence of other pests and diseases such as shoot and fruit borer, *Cercospora* leaf spot, jassids *etc.*, recorded from the observation plants.

3.2.3 Screening for resistance to YVMV

The parental species, F_1 hybrids and F_2 generation plants were subjected to different screening techniques to assess their reaction to YVMV.

3.2.3.1 Field Screening

Genotypes were screened for resistance to YVMV by providing sufficient amount of field inoculation of virus by planting highly susceptible check (AE-202) around the field. Observations on disease incidence and disease severity were recorded.

Observations on disease incidence and disease severity were recorded as per the approved methods. Disease severity was scored using 0-5 scale suggested by Deo *et al.* (2000)

Grade	Per cent leaves infected
0	Symptom absent
1	< 25% leaves (Mild)
2	25-50% leaves
3	51-75% leaves
4	76-90% leaves
5	>90% leaves
6	

Per cent Disease severity (PDS) was calculated using the formula given below.

$$PDS = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves observed}} \times \frac{100}{\text{maximum disease grade}}$$

Per cent Disease Incidence (PDI) was calculated by using the formula given below:

$$PDI = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

Based on the per cent disease incidence and severity, coefficient of infection (C.I.) was calculated as suggested by Datar and Mayee (1981)

$$CI = \frac{\text{Per cent Disease incidence} \times \text{Per cent Disease severity.}}{100}$$

Based on the CI the genotypes were categorized into five categories as shown below.

CI	:	Category
0-4	:	Highly Resistant (HR)
4.1-9	:	Resistant (R)
9.1-19	:	Moderately Resistant (MR)
19.1-39	:	Moderately Susceptible(MS)
39.1-69	:	Susceptible (S)
69.1-100	:	Highly Susceptible (HS)

3.2.3.2 Artificial inoculation by grafting technique

Artificial inoculation of YVMV through grafting techniques suggested by

Capoor and Varma (1950), Salehuzzaman (1985) and Fugro and Rajput (1999) were followed to confirm the level of resistance to YVMV in the selected treatment genotypes.

The species and F1 hybrids which were found resistant in the field screening were subjected to artificial inoculation by grafting. In this method healthy resistant genotypes were grafted with diseased susceptible genotype plants, (Salkeerthi) by approach grafting. For this screening technique both the healthy genotypes and susceptible diseased genotype plants were raised in poly bags / tube pots. Nearly one month old seedlings were selected for approach grafting.

The grafted portions were tied with thread and covered with moistened cotton. Grafted seedlings were covered with insect proof nets. The complete graft union occurred within a month. The daily observations were taken for about a month for symptom expression in resistant genotypes.

3.3 CYTOLOGICAL AND CYTOGENETICAL STUDIES.

Cytological studies on the species *A.esculentus* and *A.caillei* 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. An elaborate pollen fertility study was also made on the parental species, interspecific hybrid and selected F_2 plants.

3.3.1 Fixation of flower buds

Flower buds of appropriate maturity were collected from *A.esculentus* variety Salkeerthi, *A. caillei* variety Susthira and their interspecific F_1 hybrids between 5.15 a.m. to 6.30 a.m when the maximum number of Pollen Mother cells (PMC_S) were in the stages of division. The collected flower 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 per cent alcohol.

3.3.2 Preparation of slides

For the preparation of slides two or three anthers from a flower bud were taken at a time on a clean slide. Acetocarmine stain of one per cent was added and a cover slip was placed over it. Then it was subjected to heat near boiling. For obtaining the desirable spread of chromosomes pressure was applied on the cover slip 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.

3.3.3 Pollen fertility studies

For studying the pollen fertility, pollen grains were collected from flowers within half to one hour after anthesis. Pollen grains were dusted on a clean slide using a camel hair brush and a drop of one per cent acetocarmine stain was added to it and kept for five minutes for staining after that covered with cover slip. The slides were observed under microscope at different fields. In each field the number of stained and unstained pollen grains were noted. The pollen fertility per cent was assessed by calculating the mean stained and unstained pollen grains.

3.4 BIOCHEMICAL ANALYSIS

Under this study activity of peroxidase, polyphenol oxidase and protein estimation, protein banding pattern of the parental species, interspecific F_1 and F_2 population were studied by standard procedures.

3.4.1 Activity of Peroxidase (PO)

The procedure given by Sadasivam and Manickam (1991) was followed. One gram seed sample were extracted in 3 ml of the phosphate buffer of 0.1 < of pH 7.0. Ground well in the ice tray at 4°C, centrifuged at 1000 rpm in the Remi cool centrifuge for 15 minutes. The supernatant was taken for the assay.

The analysis was done with the following :

1. Phosphate buffer of pH 7.0 of 0.1*M* – 3 ml.
2. Guaiacol solution 20m*M* (Disolve 240 mg Guaiacol in water – 0.05 ml and make up to 100ml.)
3. Plant tissue supernatent – 0.1 ml
4. Hydrogen peroxidase solution (0.042%) - 0.03 ml (Dilute 0.14 ml of 30% H_2O_2 to 100ml.
5. Distilled water – 0.2 ml.

Blanks :

1. 5ml PO_4 buffer (PH 7.0) – (absorbance had been set at 'O' with this)
2. 3.0 ml PO_4 buffer + 0.05 ml Guaiacol + 0.03 ml of 0.042 % H_2O_2 + 0.2 ml distilled water (reading recorded)
3. Exclude only H_2O_2 from the above mixture 2 and added with 0.1 ml of the plant sample (reading recorded).

The readings were taken at 436 nanometer for every thirty seconds up to 3 minutes.

3.4.2 Activity of Polyphenol Oxidase (PPO)

The procedure given by Sadasivam and Manickam (1991) was followed. Seed sample of 200 mg was homogenized in one ml 0.1 *M* Sodium Phosphate buffer (pH 6.5), Centrifuged at 1500 rpm for 15 min and the supernatant was used. To a clean cuvette, added 3 ml buffered catechol solution (0.01 *M* catechol freshly prepared in 0.1 *M* PO4 buffer at pH 6.0) and the absorbance was set as zero at 495 nm. To this 1ml of enzyme

extract was added, mixed gently and placed in the spectrophotometer. Recorded the absorbance for every 30 seconds up to 5 min.

Preparation of catechol 0.01 *M*

For 1*M* Catechol - 110.11 g/lit.

0.01*M* – 1.1g/lit or 0.11g/100ml.

Blanks

1. Phosphate buffer of pH 6.5 – 4 ml.
2. Buffered Catechol 3 ml + 1 ml distilled water.
3. Buffered Catechol 3 ml + Enzyme extract 1 ml.

3.4.3 Protein Estimation

The protein content of the genotypes were estimated using the method described by Lowry *et al.* (1951). The procedure is described below:

Preparation of the sample

1 gram of seed sample was extracted in 3 ml of 0.1 *M* Tris – HCL buffer of pH 7.6. Ground well in the ice tray at 4°C centrifuged at 15000 rpm for 15 minutes in Remi cool centrifuge. The supernatant was used for the estimation. The following reagents were prepared.

Reagent A. 100 ml of 0.1 *N* NaOH + 2g Na_2 CO_3

Reagent B. 1 g Sodium Potassium tartrate in 100 ml DW + 0.5 g CuSO4

Reagent C. 50 ml of Reagent A + 1 ml Reagent B (freshly prepared)

Reagent D. Folincio Calteau's phenol reagent at 1:1 dilution.

The reagents were prepared and mixed well with the sample supernatent as follows:

In a clean test tube, 0.2 ml of the supernatant was taken and made into 1 ml with distilled water. Add 5 ml of reagent C to this mix and kept for 10 minutes. After 10

minutes, 0.5 ml Reagent D was added to this mixture. Then it was incubated for half an hour in the dark for the formation of the colour. After the incubation was over, the absorbance of the solution was measured at 660 nm. The protein content of the genotypes were estimated by plotting these values in the standard graph.

The standard graph was prepared using the values obtained from the above same analysis done with the standard chemical Bovine Serum Albumin powder (v). The stock solution of the standard was prepared by dissolving 50 mg BSA dissolved and make up to 50 ml.

3.4.4 Protein banding pattern

Polyacrylamide gel electrophoresis (PAGE) using Hoefor Mighty Small TM II System was used for the purpose. Acrylamide monomeis were polymerized with N-N methylene bis acrylamide. [CH_2 (NH CONH = CH_2)2 Bis] to obtain the gel. N,N,N',N' – tetramethyl ethylene diamine (TEMED) was acted as formative and preparative agent and freshly prepared ammonium persulphate acted as catalyst. Polyacrylamide gel was preferred because of its chemical inertness, high resolution, easiness in handling, transparency of the gel and easiness in preparation.

Preparation of the sample

The seed samples of all genotypes were soaked in water over night and seed coat was removed, 1 gm of kernel of seeds weighed in electronic balance and ground in a pre cooled mortar along with extraction buffer (0.1 *M* Tris – HCl, PH 7.6). The extraction buffer was added to the samples in suitable proportion around 5-10°C by keeping in an ice tray. From the different proportions tried, it was found that a sample buffer ratio of 1:1 to 1:3 was ideal to get the required concentration of sample for the study. During rainy season, the quantity of buffer was reduced due to high moisture content of the leaves. The homogenized sample was centrifuged at 15000 rpm for 15 minutes in a Remi Cool Centrifuge at 5°C. The supernatant was taken into vials and stored at subzero temperature.

Preparation of the gel.

Reagents

The protein banding pattern was carried out in anionic system. The following stock solutions were prepared.

Solution A

Tris----------36.6 g
TEMED--------0.23 ml

Adjusted the pH to 8.9 with 1 *N* HCl
Volume made up to 100 ml with distilled water

Solution B

Acrylamide ---------- 28.0 g
N' N' Methylene bisacrylamide – 0.735 g
Volume made up to 100 ml with distilled water

Solution C (freshly prepared)
Ammonium presulphate –0.14 g
Volume made up to 100 ml with distilled water

Preparation of the gel column

The Hoefer Mighty Small ™ II system of Pharmacia Biotech Inc, California was used. The size of the gel was 8.0 cm x 9.4 cm. The gel preparation was carried out as follows:

	Acrylamide concentration		
	7.5%	8.5%	10.5%
Solution A (ml)	2	2	2.5
Solution B (ml)	4.285	4.860	7.497
Solution C (ml)	9.715	9.150	10.003

Mixed the stock solution A, B and C in the above quantities to get the required gel recipes Solution A and B were stored in amber coloured bottles. Of the above, 7.5% gel was observed as the best for the samples. The quantities of various stocks given for 7.5% gel strength were mixed serially. They were stirred and injected after removing the air into the gel caster with the help of guard bulb pipette. The combs were pushed in between the caster plate for making wells and allowed to polymerize for about 45 – 90 min. Stacking gel to a height of 1-1.5 cm was also used for better resolution of band for all enzymes. Care was taken to remove air bubbles.

Stacking gel solution

Stacking gel solution was prepared by using the following solutions:

1. Monomer stock solution (30% acry., 2.7% bis.)
   Acrylamide ----------30.0 g
   Bis acrylamide-------0.8 g
   Volume made up to 100 ml with distilled water
   Stored at 4^0C away from light
2. 4X stacking gel buffer (0.5 *M* Tris-HCl, pH 6.8)
   Tris base – 0.6 g
   Adjust the pH to 6.8 with 1 *N* HCl
   Volume made up to 100 ml with distilled water

3. Initiator (10% APS, Prepared freshly)
   Ammonium presulphate –0.1g

Volume made up to 1 ml with distilled water

4. N, N, N', N' – tetrmethyl ethylene diamine (TEMED)

Staking gel was prepared by using the above solutions and the quantity of the solutions used were,

30% monomer solution --------- 0.7 ml

Stacking gel buffer ----------1.25 ml

TEMED---------- 10 µl

APS---------- 50µl

Distilled water----------3.0 ml

Electrophorentic run

The following two solutions were prepared

Electrode buffer

Stock solution

Tris 6g,Glycine 28.8g

Volume made up to one litre with distilled water keeping the pH at 8.3. The stock buffer was diluted 1:9 before use.

Tracking dye

Bromophenol blue – 25 mg

Volume made up to 10 ml with Tris – HCl buffer , pH 6.7

Stored at 5^0C in the refrigerator up to two – three weeks for use.

Preparation of Tris- HCl buffer solution of pH 6.7 was done by using

Tris ----------5.98 g

TEMED---------- 0.46 ml

pH adjusted to 6.7 with l*N* HCl

Volume made up to 100 ml with distilled water

After polymerization, the gels were transferred to electrophoretic apparatus. The upper and lower tanks were filled with the pre-chilled electrode buffer. The ratio of the sample extract and the tracking dye used was 9:1. Fifteen µl of the sample: dye mixture was added to the wells after removing the combs by guard bulb pipette. Upper tank was connected to cathode and the lower one to anode. The enzyme extracts were subjected to electrophoresis under alkaline system of Davis (1964). The running was carried out at 5^0 C. A current of 6 mA was maintained per plate and it took 4 h. for completion of the run.

4. RESULTS

The present investigation was primarily aimed at the introgression of YVMV resistance from *A. caillei* into a popular but YVMV susceptible *A.esculentus*. The species were crossed in both the directions. The crossability between these species were analyzed. The F_1 plants obtained were further advanced to raise the F_2 generations. An elaborate morphological study on the parental species, F_1 hybrids and F_2 generations were made . The parental species and their derivatives were screened for their reaction to YVMV under field condition and by graft inoculation methods. A detailed cytological studies on *A.esculentus*, *A.caillei* and their interspecific F_1 hybrids were made to elucidate the genome affinity between the species. Biochemical analysis regarding the activity of peroxidase, polyphenol oxidase and protein banding pattern of the parental species and their derivatives in relation to YVMV resistance were studied. The results pertaining to the above aspects have been explained under appropriate headings in this chapter.

4.1. INTERSPECIFIC HYBRIDIZATION AND CROSSABILITY STUDIES.

The per cent of fruit set in cross between *A. esculentus* and *A. caillei* differ widely in direct and reciprocal crosses (Table 2). There was also much differences in seeds per pod depending upon the maternal parent. The fruit set per cent in direct cross when *A.esculentus* as female parent recorded 21.23 per cent. In the reciprocal cross when *A. caillei* was used as female parent, the recorded fruit set was 51.57 per cent. Seeds set was higher in crosses where *A.caillei* used as a female parent. The crossability index in the cross *A.esculentus* x *A.caillei* (Plate 3) was 15.3 per cent whereas that in the cross *A.caillei* x *A.esculentus* (Plate 4) was 42.6 per cent. The F_1 seeds obtained in both ways were used to raise the interspecific F_2 generation (Plate 5).

The fertility of F_1 hybrids were evaluated by observing the per cent of fruit set on selfing and number of seeds per F_1 fruit (Table 3). The efficiency of fruit set and seeds set was low in F_1 hybrids. The number of seeds per F_1 fruit ranged from 0 to 12, when *A.esculentus* as female parent and it was 0 to17 when *A. caillei* was used as female parent. The F_1 hybrids recorded a maximum seed germination of 42.6 per cent.

Plate 1. *A. esculentus* variety Salkeerthi

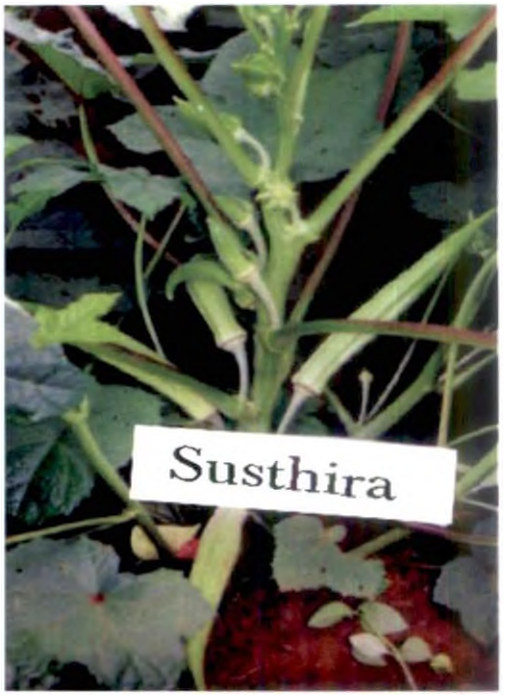

Plate 2. *A. caillei* variety Susthira

Plate 3. Interspecific F_1 hybrid of *A. esculentus x A. caillei*

Plate 4. Interspecific F_1 hybrid of *A. caillei x A. esculentus*

Table 2. Crossability between *A.esculentus and A. caillei*

Cross combination	Fruit set (%)	Number of seeds / fruit	Seed germi-nation (%)	Crossability Index (C.I.%)
A.esculentus x A.caillei	21.2	0-12	5.5	15.3
A.caillei x *A.esculentus*	51.5	0-41	49.4	42.6

Table 3. Fruit set, seed set and seed germination in parental species and its derivatives.

Sl.No.	Genotypes	No. of selfing	No. of fruit set	Fruit set (%)	No. of seeds/fruit	Seed germination (%)
1.	*A. esculentus*	50	48	96	42-72	81.2
2.	*A.caillei*	50	46	92	35-45	65.7
3.	F_1(*A.esculentus* x *A.caillei*)	15	9	60	0-12	5.5
4.	F_1 (*A.caillei* x *A.esculentus*)	50	37	74	0-17	42.6
5.	F_2 (*A.caillei* x *A.esculentus*)	50	42	82	13-34	52.7

4.2 COMPARISON OF QUALITATIVE CHARACTERS OF PARENTAL SPECIES AND THEIR DERIVATIVES

The parental species and F_2 generation plants were less branched , but F_1 hybrids were highly branched. The F_1 plants obtained by direct and reciprocal crosses were similar in all its qualitative characters. Leaf margin was deeply fid in *A.esculentus* and F_1 hybrids, but the other genotypes were narrowly fid . Flower colour was yellow in all the genotypes. Flower size was medium in both parental species and F_1 hybrids, whereas the F_2 plants had larger sized flowers. All the genotypes observed for purple throat at base of corolla on the inside only. Colour of leaf vein was green with purple tinge in all genotypes. Colour of fruit was light green in *A.esculentus* whereas that in *A.caillei,* F_1 hybrids and F_2 plants was green. Pod pubescence was absent in both the parents but the F_1 and F_2 pods had pubescence (Table 4).

4.3 COMPARISON OF QUANTITATIVE CHARACTERS OF PARENTAL SPECIES AND F_1 HYBRIDS (TABLE 5)

1. Plant height

   A.esculentus and A.caillei had plant height of (82.2 cm) and (88.8 cm) respectively. Plant height in both direct and reciprocal interspecific F_1 hybrids recorded (106.6 cm 111.1 cm).

2. Internodal length.

   Internodal length was highest in *A.esculentus* (5.2 cm) followed by the F_1 hybrid (4.9 cm) and *A. caillei* recorded an internodal length of (4.41 cm).

3. Number of primary branches

   Number of primary branches produced by *A. esculentus* was (3.1). F_1 hybrids recorded (2.98) followed by *A. caillei* (2.84).

Plate 5. An F_2 selection out of the cross *A. caillei* x *A. esculentus*

Plate 6. L.S. of fruits of parantal species and F_1 hybrid

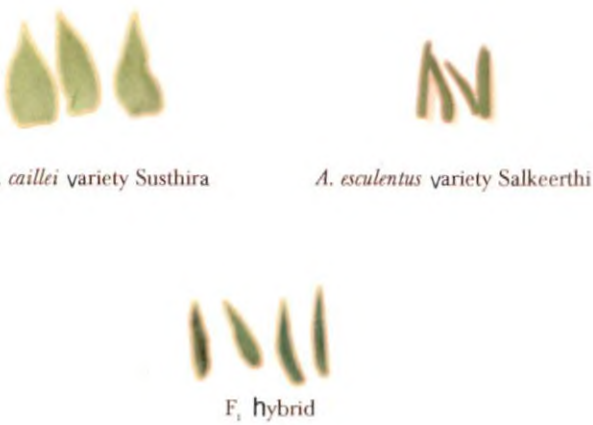

Plate 7. Variability in width of epicalyx segments in *A. esculents*, *A. caillei* and F_1 hybrid

Table 4. Comparison of qualitative characters of parental species and derivatives.

Sl. No.	Characters	*A.esculentus*	*A.caillei*	F_1 *(A.esculentus x A.caillei)*	F_1 *(A.caillei x A.esculentus)*	F_2 of *(A.caillei x A.esculentus)*
1	Plant habit	Less branched	Less branched	Highly branched	Highly branched	Less branched
2	Pubescence Stem Lamina Petiole	 smooth smooth smooth	 smooth smooth smooth	 smooth smooth smooth	 smooth smooth smooth	 smooth smooth smooth
3	Pigmentation Stem Petiole colour	 green green with purple tinge	 green green with purple tinge	 green green with purple tinge	 green green with purple tinge	 green green with purple tinge
4	Leaf margin	Deeply fid	Narrowly fid	Deeply fid	Deeply fid	Narrowly fid
5	Leaf tip	Pointed	Pointed	Pointed	Pointed	Pointed
6	Flower colour	Yellow	Yellow	Yellow	Yellow	Yellow
7	Flower size	Medium	Medium	Medium	Medium	Large
8	Purple throat at base of corolla	Present inside	Present inside	Present inside	Present inside	Present inside
9	Colour of leaf vein	green with purple tinge	green with purple tinge	green with purple tinge	green with purple tinge	green with purple tinge
10	Colour of fruit	Light green	green	green	green	green
11	Pod pubescence	Absent pubescence	Absent pubescence	Less pubescence	Less pubescence	Less pubescence

Table 5. Comparison of quantitative characters of parental species and F_1 hybrids.

Sl.No	Genotypes / Charaacters	*A.esculentus*		*A.caillei*		F_1 (*A.esculentus* x *A.caillei*)		F_1 (*A.caillei* x *A.esculentus*)	
		Mean	cv	Mean	cv	Mean	cv	Mean	cv
1	Plant height (cm)	82.2	0.76	88.8	0.83	106.6	1.43	111.1	1.58
2	Internodal length (cm)	5.2	0.74	4.41	0.47	4.72	0.707	4.9	0.84
3	No. of primary branches	3.1	0.71	2.84	0.74	2.97	0.47	2.98	0.50
4	Length of epicalyx segment (cm)	2.0	0.47	1.16	0.40	1.56	0.62	1.51	0.56
5	Width of epicalyx segment (cm)	0.5	0.29	1.12	0.59	1.16	0.71	1.54	0.66
6	Petiole length (cm)	26.6	0.70	28.1	0.74	31.1	1.83	33.3	2.03
7	Days to flower	35.6	1.14	45.2	3.11	54.4	1.58	53.4	1.14
8	Days to first harvest	41.6	1.14	52	0.70	59.2	1.48	60.6	1.14
9	First fruiting node	4.0	0	6.0	0	6	0	6	0
10	Length of fruit (cm)	28.1	0.74	17.94	0.75	20.32	1.17	19.9	17.8
11	Girth of fruit (cm)	7.48	0.31	8.32	0.13	8.4	0.15	8.46	0.11
12	Locules per pod	5	0	6	0	6	0	6	0
13	No.of ridges per pod	5	0	6	0	6	0	6	0
14	No. of fruits per plant	12.6	1.14	14.4	1.34	9.2	0.44	11.6	1.14
15	*Yield per plant(gm)	268.6	14.57	224	18.01	173.6	7.40	176.6	8.08

* Estimated yield

4. Length of epicalyx segment

   Length of epicalyx segment was maximum in *A.esculentus* (2.0 cm) and *A. caillei*, F_1 hybrid recorded 1.16 cm and 1.5 cm respectively (Plate 7).

5. Width of epicalyx segment

   Width of epicalyx segment was maximum in interspecific F_1 hybrid (1.12 cm). *A.esculentus* and *A. caillei* had 0.5 cm and 1.1 cm respectively.

6. Petiole length

   Petiole length was highest in F_1 hybrid (33.3 cm) followed by *A. caillei* (28.1 cm) and *A. esculentus* (26.6 cm).

7. Days to flower

   Days to first flower was minimum in *A.esculentus* (35.6 days). *A.caillei* was late in flowering (45.2 days). Both direct and reciprocal crosses took more days to flower (54.4 and 53.4 days) and later than both the parents.

8. Days to first harvest

   A.esculentus took 41.6 days for the first fruit harvest whereas in *A.caillei* and F_1 recorded 52 days and 59.2 days respectively.

9. First fruiting node

   A. esculentus fruited at 4^{th} node of main stem, while in F_1 hybrid and *A.caillei* fruiting was at 6^{th} node.

10. Length of fruit

    Among the parents *A.esculentus* had longer fruits (28.1 cm) compared to *A.caillei* (17.94 cm). The direct and reciprocal crosses had intermediate values 20.32 cm and 19.9 cm respectively.

11. Girth of fruit

    The F_1 hybrid *A.caillei x A.esculentus* recorded the maximum girth of fruit

(8.46 cm) compared to the parents *A.caillei* 8.3 cm, *A. esculentus* 7.48 cm.

12. Locules per pod

All the fruits of *A.esculentus* had five locules. The direct and reciprocal crosses of *A.esculentus* and *A.caillei* behaved like the semi wild parent *A.caillei* and recorded six locules per fruit.

13. Number of ridges per pod

The lowest number of ridges observed in *A.esculentus* (5 ridges/pod), *A.caillei* and F_1 hybrid recorded (6 ridges/pod).

14. Number of fruits per plant

A.esculentus produced more number of fruits (14.4) compared to *A.caillei* (12.6). Both the direct and reciprocal crosses had less number of fruits (9.2 and 11 respectively.

15.Yield per plant

The estimated mean yield per plant was highest in *A.esculentus* (268.6 g/plant) followed by *A. caillei* (224 g/plant), F_1 hybrid recorded it as (176.6 g/plant.)

4.4 COMPARISON OF QUANTITATIVE CHARACTERS OF F_2 POPULATION.

A comparison of quantitative characters in the 25 selected F_2 plants has been presented in (Table 6). The F_2 plants studied were more similar to the semi wild okra species *A.caillei* with respect to their morphological characters. Prominent *A. caillei* traits such as less lobbed leaf, winged epicalyx segments and medium sized green fruits were expressed by most of the F_2 population. None of the F_2 population showed the typical fruit and leaf characters of *A.esculentus* variety Salkeerthi. That did not express extreme recombinants but a prominent shift towards *A.caillei* in almost all characters was

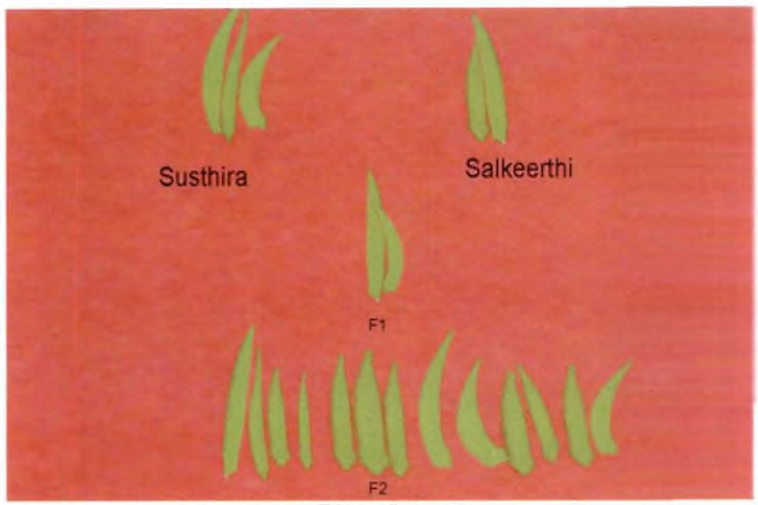

Plate 8a.
Variability in fruit shape and size in parents, F_1 and F_2.

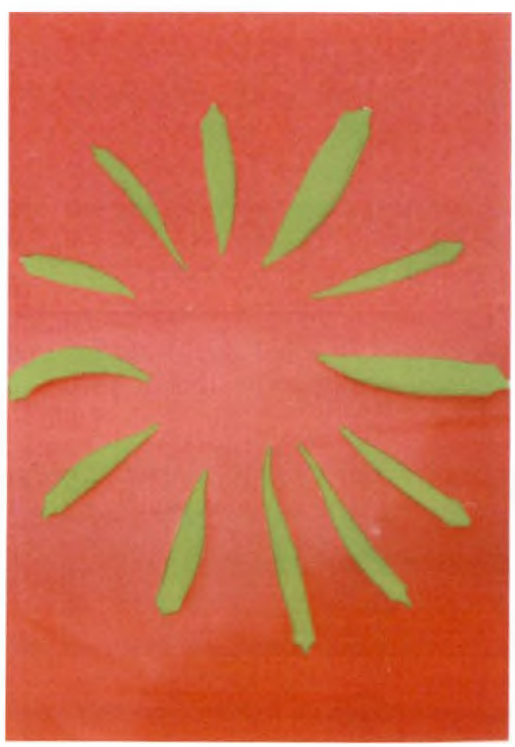

Plate 8b.
Variability in fruit shape and size in the F_2 segregants.

Table 6. Comparison of quantitative characters of selected F_2 plants

F_2 Selection	Plant height (cm)	Internodal length (cm)	No.of primary branches	Length of epi-calyx (cm)	Width of epi calyx (cm)	Petiole length (cm)	Days to flower	Days to first harvest	First fruiting node	Length of fruit (cm)	Girth of fruit (cm)	Locules per pod	No. of fruits per plant	No. of ridges per pod	Crop duration	*Yield per plant (g)
F2–1	82	3.9	2	2.2	0.2	30	53	60	6	15.5	7.2	6	10	6	119	175
F2-2	80	3.8	2	2.4	0.1	34	54	62	5	17.9	8.6	6	11	5	115	183
F2-3	84	3.9	3	1.5	1	37	54	63	4	16.2	8.2	5	9	5	123	176
F2-4	86	4.1	3	1.4	1	36	55	63	6	18.5	7.4	5	9	5	124	170
F2-5	89	4.2	4	1.9	1.1	36.5	53	60	5	17.5	6.5	5	10	5	122	169
F2-6	90	4.2	2	2	0.5	37	52	59	6	20	8.3	5	12	5	124	182
F2-7	94	4.4	2	2.4	0.4	39	53	58	6	12	8.5	6	18	6	123	190
F2-8	96	4.4	2	2.5	0.5	38	55	57	6	16	7.5	6	17	6	110	173
F2-9	82	4.5	3	2.7	0.4	37.5	54	59	6	16.5	7.7	6	16	6	110	189
F2-10	83	5	3	1.9	0.6	36	54	59	6	15	5.5	6	12	6	99	187
F2-11	87	5.5	4	1.3	0.5	36.5	53	63	6	12	5.9	6	15	5	96	172
F2-12	79	5.4	4	1	0.4	34	54	60	5	16	6.9	6	12	6	99	167
F2-13	89	4.4	4	1.2	1	30	56	62	4	16	7	5	14	5	99	178
F2-14	93	4.5	2	2.6	1.2	34.5	56	61	6	18	7.3	6	12	6	89	179
F2-15	92	4.6	3	2.5	1.1	35	55	56	6	16.5	7.5	6	13	6	112	183
F2-16	97	4.8	2	2.3	1.2	34.6	56	56	6	18	7.6	6	12	5	123	182
F2-17	101	4.9	3	2.3	0.6	36.9	62	62	6	19	8.2	6	11	6	122	195
F2-18	102	6	3	2.6	0.5	36.5	53	57	6	17	8.1	6	10	6	122	169
F2-19	106	6.2	3	2.3	0.4	36.8	54	58	6	15.5	8.5	6	12	6	124	179
F2-20	85	6.9	3	2.2	0.5	32	52	60	6	16.9	8.3	6	15	6	125	178
F2-21	102	5.3	2	1.9	0.3	35	50	57	6	17	8.6	5	12	6	123	184
F2-22	106	6.4	3	1.9	0.4	34	51	56	6	15.2	8.5	6	11	5	99	189
F2-23	102	6	2	2.3	0.5	36	52	61	6	12	8.4	6	12	6	98	179
F2-24	95	4.9	3	2.5	0.4	35	53	62	5	15	8.5	6	9	6	98	185
F2-25	82	4.7	4	2	0.5	39	54	62	5	17	7.5	6	11	6	102	185
Mean	91.36	4.91	2.84	2.8	0.61	35.47	54.04	61.16	5.64	17.68	7.68	6.12	12.2	6.04	113.04	179.92
CV	8.43	0.84	0.74	0.74	0.32	2.31	2.33	5.20	0.71	3.85	0.84	0.66	2.43	0.61	11.77	7.37

* Estimated yield

observed. However some segregants such as F_2-2, F_2-4, F_2-6 were selected based on its promising fruit character tending towards *A. esculentus*. Their fruit length ranged from

17-20 cm, expressed some degree of fruit length variability (Plate 8b) compared to other F_2 segregants and they were also having ideal number of ridges on the fruit.

4.5 CYTOLOGICAL AND CYTOGENETICAL STUDIES

A detailed meiotic observations were carried out on parental species and the interspecific F_1 hybrid *(A.caillei x A.esculentus)* to ascertain their chromosome number and cytogenetical relationship.

4.5.1 Meiosis in *A.esculentus*

Cytological studies confirmed the chromosome number of *A.esculentus* as 2n = 130. The chromosome configuration at metaphase I was regular forming 65 bivalents (Plate 9). A few Pollen Mother Cells (PMCs) having up to four univalents were also observed due to the precautious separation of some bivalents during anaphase I (Table 7). At anaphase I disjunction of chromosome was regular without any abnormalities. Distribution of chromosomes to the poles was equal at anaphase I and II. Pollen grains formed were normal and highly fertile.

4.5.2 Meiosis in *A. caillei*

Meiotic studies in *A.caillei* established its chromosome number as 2n = 184. At metaphase I the chromosomal association consisted of 92 bivalents (Plate 11). Due to the early separation of some bivalents a few cells showing up to four univalents were also observed (Table 8). At anaphase I and II normal disjunction of chromosomes to the poles were observed in all the PMCs studied. At telophase I and II two and four groups were formed respectively. At tetrad stage four microspores were observed. Further microspores developed into highly fertile pollen grains.

Plate 9. Metaphase I in *A. esculentus* showing 65 bivalents (x 1000)

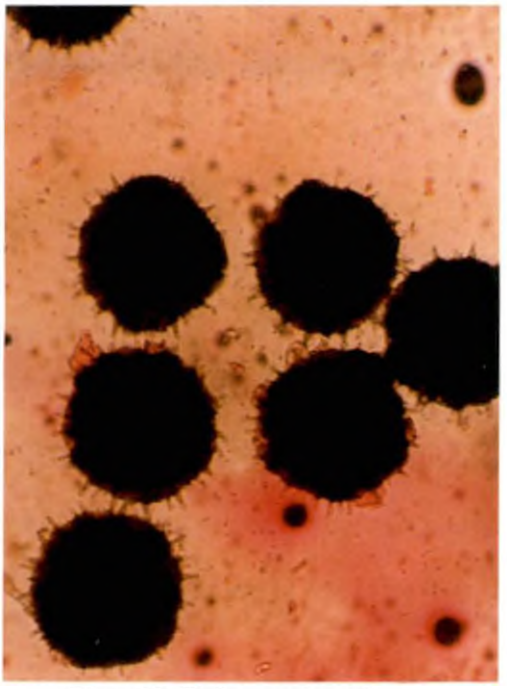

Plate 10. Pollen grains of *A. esculentus* (x 400)

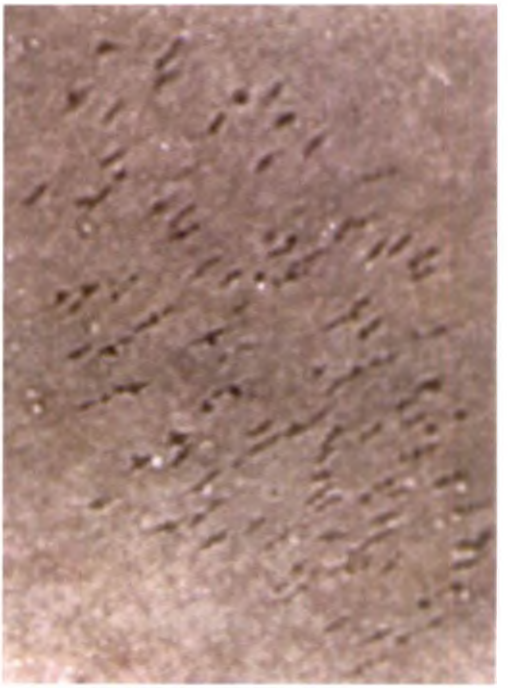

Plate 11. Metaphase I in *A. caillei* showing 92 bivalents (x 1000)

4.5.3 Meiosis in the interspecific F_1 hybrid (*A.caillei* x *A. esculentus*)

Meiosis in the interspecific F_1 hybrid (Plate 13) was critically observed and its chromosome number ascertained as 2n = 157. Chromosomal association in metaphase I consisted of bivalents and univalents (Table 9) in the PMCs examined. The number of bivalents ranged from 63 to 65 and the maximum number of bivalents of 65 and 64 per cent were observed in 80 per cent of the PMCs. Bivalents were oriented in the equatorial plane 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 resultant pollen grains were partially fertile.

4.6 POLLEN FERTILITY STUDIES

The pollen fertility of parental lines, interspecific F_1 hybrids and the selected F_2 plants were studied by staining with one per cent acetocarmine (Table 10). Pollen fertility in the parental species *A. esculentus* variety Salkeerthi was as high as 99.5 per cent (Plate 10). *A. caillei* variety Susthira recorded 98.7 per cent pollen stainability (Plate 12). The interspecific F_1 hybrid (*A.caillei* x *A. esculentus*) and F_2 plants expressed 19.2 and 62.5 per cent pollen fertility respectively (Plate 14 and Plate 15).

4.7 SCREENING FOR YVMV RESISTANCE

4.7.1 Field Screening

The parental species, interspecific F_1 hybrids and F_2 plants were screened for YVMV resistance (Table 11). *A. esculentus* variety Salkeerthi expressed 70.7 per cent coefficient of infection to YVMV. *A.caillei* variety Susthira showed a very mild infection of 0.1 per cent. It expressed a mild vein clearing which later reverted back to healthy leaves. Interspecific hybrids both direct and reciprocal crosses showed coefficient of infection of 18.9 per cent (Moderately Resistant) and 16.8 per cent (Moderately Resistant) respectively The F_2 plants were free of YVMV infection. The direct and reciprocal F_1 hybrids showed YVMV symptoms at early stage and disappeared on maturity of 60-90 days (Plate 16).

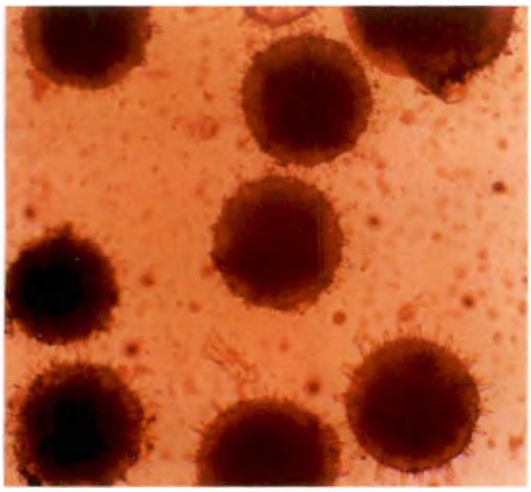

Plate 12. Pollen grains of *A. caillei* (x 400)

Plate 13. Metaphase I in *A. caillei* x *A. esculentus* showing 65 bivalents and 27 univalents (x 1000)

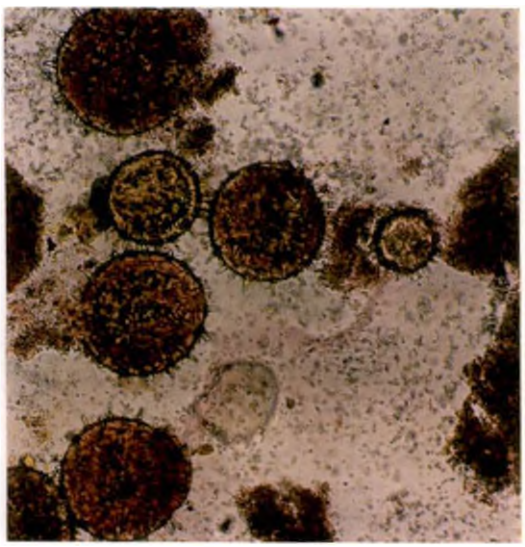

Plate 14. Pollen grains of interspecific F_1 hybrid (x 400)

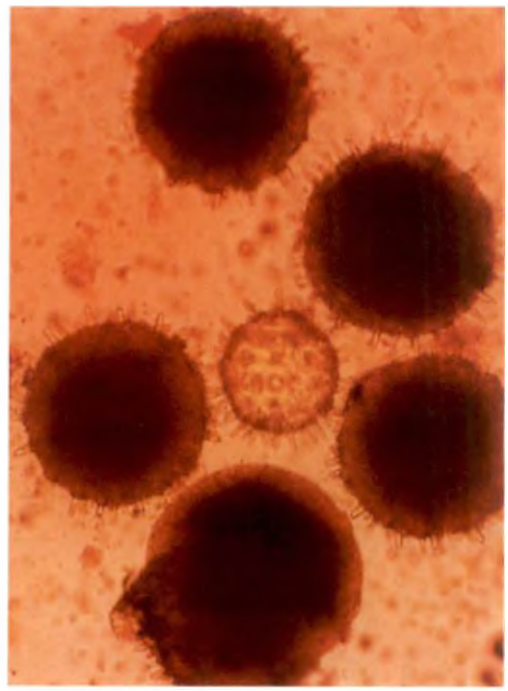

Plate 15. Pollen grains of F_2 generation (x 400)

Table 7. Chromosome association in metaphase I in PMCs of *A.esculentus* variety Salkeerthi

IV	III	II	I	Frequency No.	Frequency (%)
-	-	65	-	4	40
-	-	64	2	4	40
-	-	63	4	2	20
Range		63-65	2-4	Total No.10	
Mean/cell		64.2	1.6	of cells	

I - Univalents

II - Bivalents

III - Trivalents

IV - Tetravalents

Table 8. Chromosome association in metaphase I in PMCs of *A.caillei* variety Susthira

IV	III	II	I	Frequency No.	Frequency (%)
-	-	92	-	8	80
-	-	91	2	1	10
-	-	90	4	1	10
Range		90-92	2-4	Total No.10	
Mean/cell		691.7	0.6	of cells	

I - Univalents

II - Bivalents

III - Trivalents

IV - Tetravalents

Table 9. Chromosome association in metaphase I in PMCs of Interspecific F_1 hybrid of *A.caillei* x *A.esculentus*

IV	III	II	I	Frequency No.	Frequency (%)
-	-	65	27	7	70
-	-	64	29	2	20
-	-	63	37	1	10
Range		63-65	27-29	Total No.10	
Mean/cell		64.4	27.8	of cells	

I - Univalents

II - Bivalents

III - Trivalents

IV - Tetravalents

Table 10. Estimation of pollen fertility in parents and its derivatives by staining with acetocarmine.

Sl. No.	Genotypes	Pollen stained (%)	Unstained (%)	Estimation of fertile pollen per cent
1.	*A. esculentus*	99.5	0.5	99.5
2.	*A.caillei*	98.7	1.3	98.7
3.	F_1 (*A.esculentus* x *A.caillei*)	21.2	78.8	21.2
4.	F_1 (*A.caillei* x *A.esculentus*)	19.2	80.8	19.2
5	F2 of *A.caillei* x *A.esculentus*	62.5	37.5	62.5

Table 11. Field Screening for YVMV resistance

Sl. No.	Genotypes	Coefficient of infection (C.I)	Disease Reaction
1.	*A. esculentus* variety Salkeerthi	70.7	HS
2.	*A.caillei* variety Susthira	0.1	HR
3	F_1 (*A.esculentus* x *A.caillei*)	18.9	MR
4.	F_1 (*A.caillei* x *A.esculentus*)	16.8	MR
5.	F2 of (*A.caillei x A.esculentus*)	0	HR

HS - Highly Susceptible

HR – Highly Resistant

MR – Moderately Resistant

Plate 16. YVMV symptoms recovering F_1 hybrid plant

Plate17. YVMV symptoms not expressed in F_1 hybrid plant in the graft combination of F_1 hybrid + diseased *A. esculentus*

4.7.2 Artificial inoculation of YVMV by approach grafting

Artificial inoculation of YVMV by approach grafting diseased *A.esculentus* with *A. caillei* did not showed disease symptom on *A.caillei* but symptom expressed in scion. Grafting diseased *A.esculentus* with F_1 hybrids at 30 days old seedling stage did not showed symptoms of YVMV in F_1 hybrids (Plate 17) but the newly emerging leaves of *A.esculentus* showed symptom of YVMV. The healthy *A.esculentus* was grafted with F_1 plants which showed symptoms of YVMV in early stages but the symptoms disappeared in later stage. The resulted grafts did not show any symptoms of YVMV in *A.esculentus.*

4.8 ACTIVITY OF PEROXIDASE

There was much variation in the activity of peroxidase in the parental species and its derivatives. The Optical Density (OD) values were recorded for different time intervals as (30 to 180 sec.) The genotypes showed a sequential increase in activity trend was obtained only up to 60 sec. thereafter the reading was steady. The resistant parent *A.caillei* showed highest activity (81.75 units/g/min.), followed by F_2 of (*A.caillei x A.esculentus*), (68.25 units/g/min.). The activity of *A.esculentus* recorded (58.94 units/g/min) and F_1(*A.caillei x A.esculentus*), (31.41 units/g/min.) The results revealed the peroxidase activity is not independent of phenotypic expression (Table 12).

4.9 ACTIVITY OF POLYPHENOL OXIDASE

The activity of polyphenol oxidase also showed variation among the genotypes as expressed in peroxidase. The OD values were recorded and analysed. The results revealed high activity for *A.caillei* (26.97 units/g/min) followed by F_2 of (*A.caillei x A.esculentus*) (15.12 units/g/min). The susceptible parent had the lowest activity of 14.1 units/g/min. The F_1 (*A.caillei x A.esculentus*) recorded 14.37 units/g/min (Table 13) which can be comparable to F_2, lower than the resistant parent.

4.10 PROTEIN ESTIMATION

The soluble protein content of the seed samples of parental species and its derivatives were estimated by Lowry's method and expressed in mg/g of sample. Variation in soluble protein was observed among the genotypes. The low protein content recorded for *A.esculentus* (9.8 mg/g of seed). The protein content for *A. caillei* (13 mg/g of seed) and F_1 *(A.caillei x A.esculentus)* was 11.8 mg/g of seed. The protein content recorded for F_2 15 mg/g of seed. The susceptible genotypes had lower protein content, while the resistant genotypes recorded highest (Table 14).

4.11 PROTEIN BANDING PATTERN STUDIES

The protein banding pattern for parental species and its derivatives were studied by polyacralamide gel electrophoresis. There was a common protein band for all the genotypes studied, which may be the evidence of common origin (Rm value =0.788). The genotype *A.caillei*, F_2 of *(A.caillei x A.esculentus)* showed additional protein band of Rm value = 0.211 whereas the additional protein band of F_1 *(A.caillei x A.esculentus)* had Rm value = 0.322. The susceptible one expressed only one protein band of Rm value of 0.788 which was the common for all the genotypes. The expression of the protein band with Rm value 0.211 which may reveal a specificity of protein in resistant genotype.

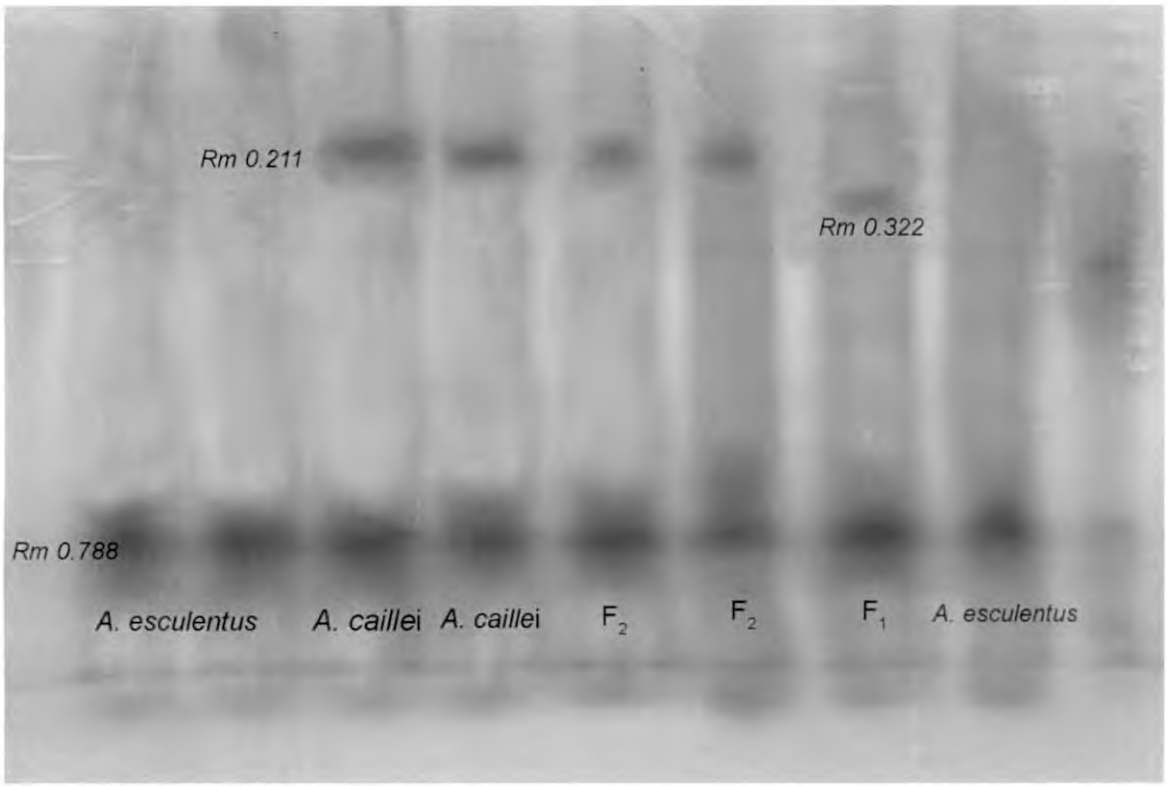

Plate 18. Protein banding pattern of *A. esculentus, A. caillei*, F_1 *(A. caillei x A. esculentus)* and F_2 generation

Table 12. Peroxidase activity in parental species and its derivatives at 30°C for 30sec.

Sl.No.	Species/species derivatives	Peroxidase activity (units/g/min)
1	*A.esculentus*	58.94
2	*A.caillei*	81.75
3	F_1 *(A.caillei x A.esculentus)*	31.41
4	F_2 of *(A.caillei x A.esculentus)*	68.25

Table 13. Polyphenoloxidase activity in parental species and its derivatives at 30°C for 30sec.

Sl.No.	Species/species derivatives	Polyphenol oxidase activity (units/g/min)
1	*A.esculentus*	14.04
2	*A.caillei*	26.97
3	F_1 *(A.caillei x A.esculentus)*	14.37
4	F_2 of *(A.caillei x A.esculentus)*	15.12

Table 14. Protein content of the parental species and its derivatives

Sl.No.	Species/species derivatives	Protein content (mg/g of seed)
1	*A.esculentus*	9.8
2	*A.caillei*	13
3	F_1 *(A.caillei x A.esculentus)*	11.8
4	F_2 of *(A.caillei x A.esculentus)*	15

5. DISCUSSION

The present study was aimed at making initial steps to introgress YVMV resistance from *A.caillei* into *A.esculentus*. Under this study, parental species were crossed to raise interspecific F_1 hybrids and F_2 generation plants. Then these generations were screened for resistance to YVMV. A detailed cytological study on *A.esculentus* and *A. caillei* was made to ascertain their chromosome number. Cytogenetical study by estimating the chromosome affinity between the parental species was also conducted by observing meiotic stages in the interspecific F_1 hybrid plants. Activity of peroxidase, polyphenol oxidase and protein banding pattern in relation with YVMV resistance in parental species and their derivatives were also studied. The results obtained in the study are discussed under the following headings.

5.1. INTERSPECIFIC HYBRIDIZATION AND CROSSABILITY STUDIES

The result of the crossability studies between the two species showed that crosses were more successful when *A.caillei* was used as female parent (Crossability Index = 42.64%). The F_1 hybrid was also secured in the cross *A.esculentus* x *A. caillei* but in this direction crossability index was less (15.3%). The cross *A. caillei* x *A.esculentus* was more successful, probably due to the higher ploidy level of female parent *A.caillei* which provided better embryo endosperm balance as per Kalloo (1988). Chacko *et al.* (1996) also reported higher degree of crossability on the cross of *A. caillei* x *A.esculentus*. The F_1 seeds obtained in the parent study could be used to raise F_2 generation plants.

5.2. SCREENING FOR YVMV RESISTANCE

In the field screening trials the *A.esculentus* line Salkeerthi was highly susceptible toYVMV (C.I = 70.7) where as *A. caillei* variety Susthira was highly resistant (C.I=0.1),(Fig.3.). This observation was in accordance with Sureshbabu *et al.* (2002). The interspecific F_{1s} were moderately resistant to YVMV (C.I = 18.9). This result is in agreement with Cheriyan (1986). The susceptibility of F_1 plants to YVMV shows that the genetic mechanism of resistance to this disease may be either recessive or polygenic. Most of the F_1 YVMV susceptible plants reverted back to healthy plants, probably due to

some inherent genetic mechanisms. All the F_2 generation plants were resistant to YVMV. This might be attributed to the phenomenon of one sided segregation expressed by F_2 plants of interspecific crosses as per Kalloo (1988).

In the YVMV screening trials by grafting technique, in the graft combination of *A.caillei* + *A.esculentus* the *A.caillei* did not express YVMV symptoms whereas *A. esculentus* scion expressed symptoms indicating inherent resistance to YVMV in *A. caillei*. But *A.caillei* must be a symptomless carrier to YVMV. In the graft combination of interspecific F_1 + *A.esculentus* the disease was expressed by *A.esculentus* which shows viral infection in the F_1 hybrids. This result is in accordance with Cheriyan (1986).

In the F_1 plants which expressed YVMV symptoms showed a gradual disappearance of the disease expression and later it recovered fully. *A.esculentus* was grafted on such YVMV recovered plants, interestingly the scion did not express YVMV symptoms. This might be attributed to disappearance of the virus from the F_1 plants due to some inborn mechanism present in these F_1 plants, which need further detailed study.

5.3 INTROGRESSION OF YVMV RESISTANCE

In the present study YVMV susceptible *A.esculentus* variety Salkeerthi was crossed with YVMV resistant species *A.caillei* and the resulting F_1 generation could be advanced to F_2 generation. Morphologically the F_1 plants were more similar to *A. caillei*, but the plant height recorded maximum of 111.1 cm (Fig.1.). All the F_2 generation plants were resistant to YVMV, and they also expressed semi-wild morphological features of *A.caillei*. The frequency of recombinants expressed by F_2 generation plants was very low. Three F_2 selections (F_2-2,F2-4, F_2-6) having YVMV resistance, long fruits (17 cm to 20cm) and five ridges on the fruits could be selected. In general the F_2 populations showed prominent *A.caillei* characters such as winged epicalyx segments, fruit length, more ridges per pod etc., rather than expressing extreme recombinants having prominence of *A.esculentus* characters(Fig.2). This might be due to the one sided segregation as explained by Kalloo (1998) in several segregating generation of interspecific crosses. The possible reasons for this phenomenon is due to reduced pairing of chromosome in the

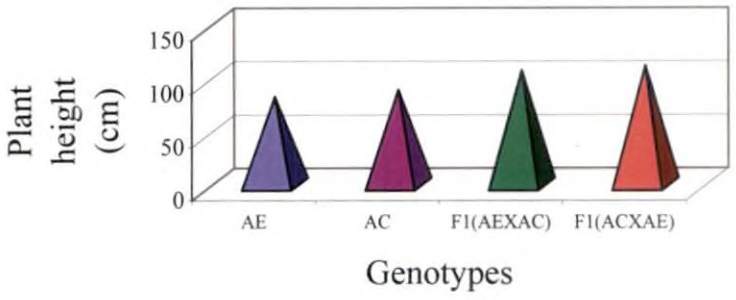

Fig. 1. Variability in plant height in the parental species and F_1 hybrids

AE - *A. esculentus*; AC - *A. caillei*

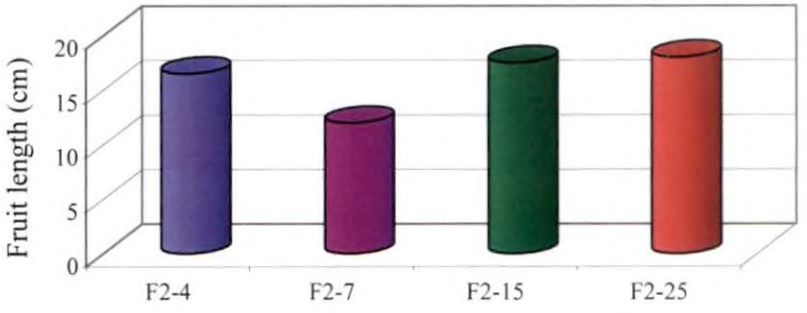

Fig. 2. Variability in fruit length in F_2 generation plants

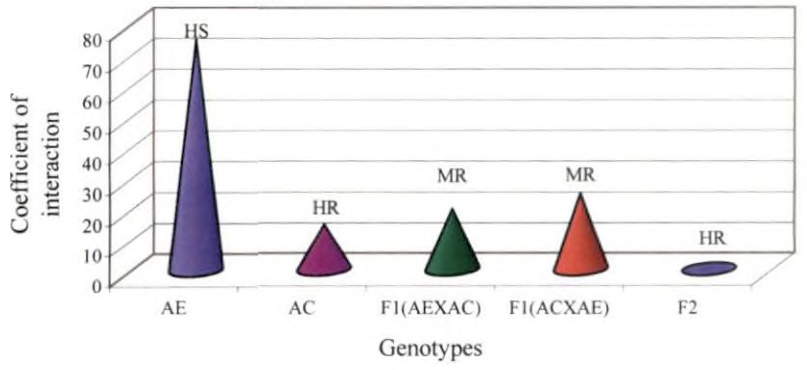

Fig. 3. Field screening for YVMV resistance

interspecific F_1, chromosome differentiations restricted recombination, linkage, gametic and zygotic elimination. Mathews (1986) also got similar pattern of F_2 segregation in the cross of *A. manihot* x *A.esculentus*. He observed a preponderance of YVMV resistant plants similar to semi-wild parents in the F_2 population, suggesting the presence of powerful genetic mechanism which restrict free recombination.

The three F_2 selections (F_2-2, F_2-4, F_2-6) which showed considerably good amount of pollen fertility (62.5 %) had higher level of seed germination (52.7 %) and resistance to YVMV. These selections can be further advanced to future generations so that stabilized lines having excellent agronomic traits and fruit quality combining resistance to YVMV can be secured.

5.4. CYTOGENETICAL STUDIES

In the present investigation, cytogenetical interrelationship between *A.esculentus* and *A. caillei* could be worked out based on its chromosome number and chromosome homology.

5.4.1. The chromosome number of *A.esculentus* and *A.caillei*

Meiotic association in *A.esculentus* during the metaphase –I was mostly of 65 bivalents, confirming its chromosome number 2n=130. This is in agreement with Joshi *et al.*(1974), Joshi and Hardas (1976), Sureshbabu and Dutta (1987) and Chacko (1996). Cytological studies on *A.caillei* evoked considerable interest. It was established in the present study that its chromosome number as 2n=184. Chacko (1996) had reported chromosome number of a semi-wild okra form of Kerala known as "Thamaravenda" considered as *A.caillei* as 2n=184. This observation is also in accordance with Sureshbabu *et al* (2002). The high level pollen fertility in *A.esculentus* and *A.caillei* is attributed to their regular chromosome behaviour during meiosis.

5.4.2. Cytology of interspecific hybrid

In the interspecific hybrid almost all the haplophase chromosomes (n=65) of *A. esculentus* were found to pair with the homologus counterparts of *A.caillei* forming mostly 65 bivalents and 27 univalents. This confirms the proposal of Siemonsuma (1982) that *A.esculentus* had contributed its genomes for the evolution of *A.caillei*. Having a high degree of chromosome affinity between *A.esculentus* and *A. caillei*, observed in the present study confirmed that the gene introgression between these species is quite possible and this information can be utilized in the future breeding programme. Chacko (1996) also observed similar degree of genome affinity between *A.esculentus* and *A.caillei*.

The interspecific F_1 hybrid was found to be partially sterile. This can be attributed to the cytological irregularities including the presence of lagging chromosomes, occurrence of micronuclei and multipolar spindle formation. Pioneer workers like Pal *et al.* (1952), Stebbins (1988), Arumugham *et al* (1975) and Siemonsuma (1982) reported similar reasons for the hybrid sterility in several interspecific crosses.

5.5 BIOCHEMICAL STUDIES

The present study on activity of peroxidase, polyphenol oxidase and protein estimation, protein banding pattern in relation to YVMV resistance in the parental species, F_1 hybrid and F_2 generation. The genotype *A.esculentus* showed maximum YVMV infection (C.I. 70.7) in the field screening studies, in which low peroxidase activity (58.94 units/g/min.) and polyphenol oxidase (14.1 units/g/min.) supporting with low protein content of 9.8 mg/g of seed. *A.esculentus* showed only one protein band of Rm = 0.788 which was common for all genotypes studied. It implies that the resistant factor acquired in *A.caillei* as well as F_2 has no place in the susceptible species *A.esculentus*.

A.caillei with high peroxidase, polyphenol oxidase and high protein content (15 mg/g of seed), additional protein band (Rm value = 0.211) influenced on resistance to YVMV. The disease incidence in F_1 plants showed symptom at 35 days after sowing the symptom of YVMV disappeared at the stage of maturity in F_1 which was clearly indicated by one additional protein band (Rm value = 0.322). This protein band (Rm value = 0.322) which may be performing the function of the resistance/ tolerance of the resistant

species and F_2 plants in which the protein banding was expressed (Rm value = 0.211). The disease infection in the early stage may be due to the cumulative action of low peroxidase, low polyphenol oxidase and low protein (11.8 mg/g of seed) which was lower than resistant parents.

F_2 generation plants were free from symptom of YVMV infection which may be influenced by high peroxidase, high polyphenol oxidase and high protein content, additional protein band (Rm value = 0.211). Similar to that of resistant parent *A. caillei* indicated that semi-wild parental resistant character was expressed in the F_2 generation.

6. SUMMARY

The present investigation on "Introgression of Yellow Vein Mosaic Virus resistance from *Abelmoschus caillei* (A.Cher.) Steveis into *A.esculentus* (L.) Moench" was carried out during 2003-2004 at the Department of Olericulture, College of Horticulture, Thrissur. The main objective of the study was to facilitate transfer of YVMV resistance from *A.caillei* into *A.esculentus* by interspecific hybridization. Cytological and cytogenetical studies on the parental species and the F_1 hybrid were made to confirm the chromosome affinity between the species. The F_1 plants obtained were further advanced to raise the F_2 generations. The morphologiocal traits of parents interspecific F_1 hybrids and F_2 plants were compared. The evaluation of YVMV resistance in the parents and their derivatives was made by field screening and artificial inoculation by grafting technique. The disease incidence and disease severity were assessed for all the genotypes. Biochemical analysis like activity of Peroxidase (PO), Polyphenol Oxidase (PPO), protein estimation and protein banding pattern for further characterization of genotypes based on their reaction to YVMV were also conducted.

Interspecific hybridization between *A.esculentus* and *A.caillei* showed that F_1 and F_2 generations were partialy fertile. The crossability index in the cross *A.caillei x A.esculentus* was 42.64 per cent and that in the cross *A. esculentus x A.caillei* was 15.3 per cent.

The fruit set and seed set per cent were high when *A.caillei* was used as female parent. The pollen fertility studies revealed F_1 *A.caillei* x *A.esculentus* having fertility per cent of 21.2 and F_1 *A. esculentus x A.caillei* recorded 19.2 per cent of pollen fertility. The pollen fertility of F_2 generation plants was 62.5 per cent. Meiotic association in *A.esculentus* during the metaphase – I was mostly of 65 bivalents, confirmed its chromosome number 2n = 130. Cytological studies on *A.caillei* evoked considerable interest. It was established in the present study that its chromosome number as 2n = 184. The high level pollen fertility in *A.esculentus* and *A. caillei* is attributed to their regular chromosome pairing during meiosis.

In the interspecific F_1 hybrid between *A.caillei x A.esculentus* all the haplophase chromosomes (n=65) of *A.esculentus* were found to pair with the homologous counter parts of *A.caillei* forming mostly 65 bivalents and 27 univalents. This is an indication of high degree of chromosome affinity between *A.esculentus* and *A.caillei* and this information can be utilized in the future breeding programme.

The interspecific hybrid was found to be partially sterile, this might 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.

The parental species, interspecific F_1 hybrids and F_2 plants were screened for YVMV resistance. *A.esculentus* variety Salkeerthi expressed 70.7 per cent coefficient of infection to YVMV, whereas *A.caillei* variety Susthira showed a very mild infection of 0.1 per cent. It expressed a mild vein clearing which later reverted back to healthy ones. Interspecific F_1 hybrids of both direct and reciprocal crosses showed coefficient of infection of 18.9 per cent and 16.8 per cent respectively. The F_2 plants were free of YVMV infection. The direct and reciprocal F_1 hybrids also showed YVMV symptom at early stage and on maturity, symptom disappeared.

Artificial inoculation of YVMV by approach grafting of diseased *A.esculentus* with *A.caillei* did not show disease symptom of YVMV on *A.caillei*. Grafting on 30 days old seedling also did not show symptom of YVMV on F_1 hybrids, but symptom appeared in newly emerging leaves of *A.esculentus*. The same method was adopted for grafting F_1 plants which showed symptom of YVMV in early stages but the symptom disappeared in later stage F_1 plants with healthy *A.esculentus*. The resulted grafts did not show any symptom of YVMV in scion of *A.esculentus*. *A.caillei* must be a symptomless carrier of YVMV.

The activity of peroxidase enzyme showed much variation in parental species and its derivatives. The genotypes were observed to be in a sequential manner of highest activity for resistant one and lowest for F_1 which had different phenotypic characters that of parents. The resistant parent *A.caillei* showed highest activity (81.75 units/g/min.), followed by F_2 of *(A.caillei x A.esculentus)*, (68.25 units/g/min.) The activity in *A.*

esculentus was 58-94 units/g/min. and in F_1 (*A.caillei x A.esculentus*) was 31.41 units/g/min. The results revealed that peroxidase activity is not independent of phenotypic expression. The activity of polyphenol oxidase also showed variation for the genotypes as expressed in peroxidase. The results revealed high activity for *A.caillei* (26.97 units/g/min.) followed by F_2 of *(A.caillei x A.esculentus)* (15.12 units/g/min.). The susceptible parent showed the lowest activity of 14.1 units/g/min. The F_1 *(A.caillei x A.esculentus)* recorded 14.37 units/g/min.

The soluble protein content of parental species and its derivatives recorded lowest protein content for *A.esculentus* (9.8 mg/g of seed). The value recorded for *A.caillei* was 13 mg/g of seed and for F_1 was 11.8 mg/g of seed. The maximum protein content recorded for F_2 was 15 mg/g of seed. The susceptible genotypes had lower content, while the resistant genotypes recorded higher concentration.

Protein banding pattern of parental species and its derivatives showed a common band for all the genotypes which may be an evidence of common origin (Rm value = 0.788). The genotype *A.caillei* and F_2 showed additional band of Rm value = 0.211 whereas F_1 expressed a band of Rm value = 0.322. The susceptible one expressed only one band of Rm value of 0.788 which is common for all the genotypes. The expression of the protein band with Rm value 0.211 revealed a specificity of the protein in resistant genotypes.

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* Originals not seen.

Appendices

Appendix.I . Meteorological data (mean monthly)
Source: Department of Agricultural Meteorology, KAU, Vellanikkara.

Months	Max. temperature		Min.temperature (^{0}C)		RH(%)		Rain fall (mm)		Rainy days		Sunshine (hr.)		Windspeed (km/hr.)	
	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
January	33.2	33.4	22.9	22.3	50	58	0.0	0.0	0	0	9.4	9.6	8.6	7.7
February	34.7	35.2	23.6	22.5	63	50	162.1	0.0	5	0	9.2	9.6	5.1	6.2
March	34.6	36.5	24.1	24.2	67	61	94.8	8.6	4	1	8.5	8.6	3.7	4.7
April	34.6	34.8	25.0	25.2	72	69	23.8	60.2	3	6	7.5	7.4	3.2	4.3
May	34.0	30.4	25.0	23.6	72	84	40.3	578.3	3	21	6.3	3.4	3.8	3.7
June	30.9	29.6	23.8	22.3	80	85	570.6	786.0	19	24	4.0	3.3	3.2	4.0
July	29.5	29.3	22.2	23.4	84	85	492.6	369.6	22	24	2.5	3.3	2.9	3.4
August	28.9	28.5	23.4	23.1	83	83	490.1	386.9	19	14	4.2	4.4	3.1	3.8
September	31.0	30.8	22.7	23.6	79	80	53.7	208.8	7	10	7.3	5.1	3.3	3.6
October	30.8		23.1		81		276.6		14		5.6		3.9	
November	31.5		23.9		66		18.2		1		7.1		8.2	
December	32.2		21.9		61		0.0		0		9.1		7.6	

INTROGRESSION OF YELLOW VEIN MOSAIC VIRUS RESISTANCE FROM *Abelmoschus caillei* (A. Cher.) Steveis INTO *A. esculentus* (L.) Moench.

By

V. KOUSALYA

ABSTRACT OF THE THESIS

submitted in partial fulfilment of the requirement for the degree of

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ABSTRACT

The present investigation on "Introgression of Yellow Vein Mosaic Virus resistance from *A.caillei* (A. Cher.) Steveis into *A.esculentus* (L.) Moench" was conducted at the Department of Olericulture, College of Horticulture, KAU, Vellanikkara, Thrissur during 2003 to 2004. The major objective of this study was to transfer YVMV resistance from *A.caillei* variety Susthira to popular but highly susceptible variety Salkeerthi (*A.esculentus*).

The study was aimed at finding the chromosome affinity between two species involved in interspecific hybridisation. Biochemical characterization based on activity of peroxidase, polyphenol oxidase and protein content, protein banding pattern in parental species and its derivatives. *A.caillei* variety Susthira and *A.esculentus* variety Salkeerthi were crossed reciprocally and crossability index between the species was worked out. It was found that crossing between the species was more successful when *A.caillei* was used as female parent. The interspecific F_1 seeds obtained were used to raise the F_2 generation. The parental species, interspecific F_1 hybrids and F_2 generation were subjected to detailed testing programmes for reaction to YVMV. *A.caillei* and F_2 generation were highly resistant to YVMV. Whereas *A.esculentus* and F_1 generation plants were susceptible to YVMV. The F_2 generation plants were morphologically more similar to semi wild parent *A.caillei*. However, three F_2 selections viz.,F_2-2, F_2-4, F_2-6 having more fruit length and desirable number of ridges per fruit could be selected. These selections showed considerably good amount of pollen fertility and seed set. So these selections can be further advanced and stabilized with desirable agronomic traits like fruit characters can be selected in future.

The cytological study showed that the chromosome number of *A.esculentus* and *A.caillei* as $2n = 130$ and $2n = 184$ respectively. In the interspecific F_1 hybrid (*A.caillei* x *A.essculentus*) mostly 65 bivalents could be observed suggesting a good amount of affinity between the genome of two species.

Biochemical analysis on activity of peroxidase, polyphenol oxidase and protein estimation, protein banding pattern in relation to YVMV resistance in the parental species, F_1 hybrids and F_2 generation was carried out. The genotype *A.esculentus* showed maximum YVMV infection of (C.I. 70.7) in the field screening studies, in which low peroxidase activity (58.94 units/g/min.) and polyphenol oxidase (14.1 units/g/min.) supporting with low protein content of 9.8 mg/g of seed. *A.esculentus* showed only one protein band of Rm = 0.788 which was common for all genotypes studied. It implies that the resistant factor acquired in *A.caillei* as well as F_2 has no place in the susceptible species *A.esculentus*.

A.caillei with high peroxidase, polyphenol oxidase and high protein content (15 mg/g of seed), additional protein band (Rm value = 0.211) influenced on resistance to YVMV. The disease incidence in F_1 plants showed symptom at 35 days after sowing the symptom of YVMV disappeared at the stage of maturity in F_1 which was clearly indicated by one additional protein band (Rm value = 0.322). This protein band (Rm value = 0.322) which may be performing the function of the resistance/ tolerance of the resistant species and F_2 plants in which the protein banding was expressed (Rm value = 0.211). The disease infection in the early stage may be due to the cumulative action of low peroxidase, low polyphenol oxidase and low protein (11.8 mg/g of seed) which was lower than resistant parents.

F_2 generation plants were free from symptom of YVMV infection which may be influenced by high peroxidase, high polyphenol oxidase and high protein content, additional protein band (Rm value = 0.211). Which was similar to that of resistant parent *A. caillei* and indicated that semi-wild parental resistant character was expressed in the F_2 generation.