EVALUATION AND MOLECULAR CHARACTERIZATION OF ADVANCED GENERATION DISTANT HYBRIDIZATION SELECTIONS OF OKRA [*Abelmoschus esculentus* (L.) Moench]

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THESIS

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DECLARATION

I hereby declare that the thesis entitled "**Evaluation and molecular characterization of advanced generation distant hybridization selections of okra [*Abelmoschus esculentus* (L.) Moench]"** is a bonafide record of research done by me during the course of study and the thesis has not previously formed the basis for the award 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 **"Evaluation and molecular characterization of advanced generation distant hybridisation selections of okra [*Abelmoschus esculentus* (L.) Moench]"** is a record of research work done independently by Arunkumar B. (2013 - 12 - 118) 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 him.

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Introduction

1. INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) is an important vegetable crop throughout tropical and subtropical low altitude regions in Asia, Africa and America. In India, it is one of the most important vegetable crops cultivated during summer and rainy seasons. It is valued very highly for its tender green fruits and is generally marketed in the fresh state, but sometimes in canned or dehydrated form.

Okra with considerable area under cultivation in both African and Asian countries is considered as important component of diet for nutritional balanced food mainly on account of being rich in vitamin A (86.67 µg/100 g), riboflavin (0.10µg / 100g), vitamin C (18µg / 100g) and minerals like calcium (66mg/100g), phosphorus, iodine, iron and potassium (Gopalan *et al.*, 2007). It is crucial vegetable crop to contribute towards nutritional security for food and even so far income diversification in the subsistence farming system which is predominant in the developed as well as underdeveloped countries.

Eight *Abelmoschus* species occur in India. Out of these, *A. esculentus* is the only known cultivated species. *A. moschatus* occurs as semi wild species and is also cultivated for its aromatic seed cot oil, while the rest six species are truly wild types. Species resistant to *Bhendi yellow vein mosaic viru*s (BYVMV) are *A. caillei, A. manihot, A. tetraphyllus* and *A. crinitus.* Wild species have not been fully utilized in breeding programmes due to crossing barriers. Resistance to BYVMV is not stable in the cultivated species and frequent breakdown of resistance has been observed in developed varieties. There is an urgent need to adopt appropriate method of breeding programmes for the development of lines resistant to BYVMV.

Cultivation of okra in India is challenged due to severe incidence of *Bhendi yellow vein mosaic viru*s (BYVMV). It infects all stages of the crop and severely

reduces plant growth and yield. The virus produces typical vein yellowing and thickening of leaves forming a network of mottled veins and veinlets in the infected leaves. Initially, the leaves exhibit only mild yellow coloured veins but under the severe infection, the leaves become completely chlorotic and turn yellow. There is reduction of leaf chlorophyll and the infected plants give a stunted look and produce small-sized pale yellow fruits (Gupta and Paul, 2001). The virus is neither sap nor seed transmissible. In nature the virus transmission occurs through the insect vector white fly (*Bemisia tabaci*). The production losses due to BYVMV have been reported to range from 50 to 94 per cent (Sastry and Singh, 1974).

As the causal organism is virus the only control measure is the control of vectors using pesticides. But the disease cannot be controlled properly by chemical means. The escalating cost of pesticides and the chemical residues which cause the health hazards warrant alternative methods. Uprooting of infected plants is not practical and economical because of heavy infection rate in the field. So the only practical solution for this problem is to develop resistant varieties (Horvath, 1984). Unfortunately many of the existing released varieties of okra are showing the signs of susceptibility to BYVMV. Several varieties exhibited tolerance / resistance to this virus at the time of release, but this tolerance / resistance have broken down with time. Since, there is no source of resistance to BYVMV in *A.esculentus,* interspecific hybridization for BYVMV 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 BYVMV. However resistant varieties developed by various research organizations by interspecific hybridization have also started showing signs of susceptibility probably due to the eruption of new virus strains. Hence it is imperative to find diverse sources of resistance to BYVMV and evolve BYVMV resistant varieties in a continuous manner by suitable gene introgression programmes.

In this context a semi cultivated okra species *Abelmoschus caillei* (A. Cher.) Steveis deserves importance. It adorns many remarkable traits such as resistance to BYVMV, adaptability and long fruiting period (Charrier, 1984; Chacko, 1996). This species is a complex polyploid considered to be originated by contributing genomes of *A.esculentus* and *A. manihot* (Siemonsuma, 1982).

A variety 'Susthira' belonging to species *A. caillei*, 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 (Sureshbabu *et al*., 2004). 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 BYVMV. Hence it cannot be grown during summer when the disease is more prevalent. In this regard it would be a viable proposal to transfer BYVMV resistance from 'Susthira' (*A. caillei* cv. Susthira) to 'Salkeerthi' (*A. esculentus* cv. Salkeerthi). Accordingly interspecific hybridization between *A. caillei* variety Susthira and *A. esculentus* variety Salkeerthi was done and the generations were advanced up to F_{12} to get stabilized selections. Thus in order to conclude the breeding programme, the F_{12} generation selections have to be further assessed with proper selection for BYVMV resistance and other desirable traits.

In the distant hybridization programmes genetically diverse parents are involved and hence in the segregating generations there is more scope for the selection of desirable recombinants. Assessing the genetic variability among the advanced generation selections in comparison with parents will show their extent of possession of desirable genes. Molecular characterization will further ascertain the diversity and aid in fingerprinting of selected genotypes for registration of varieties

and identification of trait specific markers for BYVMV resistance will further helps in marker assisted selection and isolation of genes responsible for resistance. Thus the proposed study will pave a way for the final selection of lines having resistance to BYVMV combined with other desirable traits.

The major objectives of the present study are:

- To evaluate eight okra selections of the F_{12} generation of the cross *Abelmoschus caillei* cv. Susthira x *Abelmoschus esculentus* cv. Salkeerthi to study the extent of variability.

- To identify the most promising selections having high level of resistance to BYVMV combined with other desirable traits.

- Molecular characterization of the promising advanced generation selections using RAPD and ISSR marker systems.

Review of literature

2. REVIEW OF LITERATURE

Okra (*Abelmoschus esculentus* L. Moench) is one of the important tropical vegetable crops grown for its tender green pods. It is rich in important nutritive and mineral elements. But the susceptibility of most of the okra varieties to BYVMV is a major problem limiting the production of this crop. There is no source of resistance in *Abelmoschus esculentus*. Hence, there is a need to develop new okra varieties resistant to BYVMV by transferring resistance genes from diverse wild species by interspecific hybridization followed by selection of promising lines. The pertinent literature on the present study is reviewed under the following heads citing the research work on okra and other vegetable crops.

2.1 ORIGIN AND CYTOGENETICS

Okra belongs to the genus *Abelmoschus* which was established by Medikus (1787). The genus is believed to be of Asiatic origin whereas centre of origin of the major cultivated species *A. esculentus* is controversial- the species is believed to have originated in India (Masters, 1875); Ethiopia (Candolle, 1883); West Africa (Vavilov, 1951) and Tropical Asia (Grubben, 1977). Index kewensis lists over 30 species of *Abelmoschus* in the old world, four in the new world and four in Australia. Waalkes (1966) has a more conservative point of view retaining only six species. These are *A. moschatus* Medikus*, A. manihot* (L.) Medikus*, A. esculentus* (L.) Moench*, A. ficulneus* (L.) Wt and Art. ex Wt, *A. crinitus* Wall. and *A. angulosus* Wall ex Wt and Art. The former three species consisted of wild and cultivated forms and the latter three species consisted of wild forms only. Bates (1968) suggested some additional modifications like inclusion of *A. tuberculatus* and the grouping of all subspecies and varieties of *A. manihot.* The genus became more complex by discovery of an African cultivated species by Siemonsuma (1982) and described it as *A. caillei* (A. Cher.) Stevies. Based on the available cytogenetical evidence, the International Okra Workshop (1990) adopted a classification in which nine species were included in the

genus *Abelmoschus.* This classification also included the new cultivated species, *Abelmoschus caillei* which was wrongly identified earlier as *A. manihot* ssp. *manihot.*

Joshi and Hardas (1956) proposed a polyphyletic origin for the species. They reported an allopolyploid genome for cultivated okra. The chromosome polymorphism has been reported in okra that is chromosome count within a species exhibit a wide range of variation. The somatic chromosome number reported for *A. esculentus* varied greatly from 2n=72 to 144. However, the most frequently observed chromosome number was 2n=130 (Siemonsuma, 1982). Dutta and Nang (1968) proposed that the 2n numbers, 2n=72,108,120, 132 and 144 were an indication of a regular polyploid series with x=12.

2.2 BHENDI YELLOW VEIN MOSAIC VIRUS (BYVMV)

Yellow Vein Mosaic is the most serious disease of okra and is transmitted by white fly (*Bemisia tabaci* Gen.) (Ali *et al*., 2012). In India, the occurrence of this disease was first reported by Kulkarni (1924) in Bombay province. Later it was studied by Uppal *et al.* (1940) and Kapoor and Varma (1950). Infection of 100 per cent plants in a field is very usual and yield losses ranges from 50 to 94 per cent depending on the stage of crop growth at which infection occurs (Sastry & Singh, 1974). This disease adversely affects the quantity and quality of the fruits.

2.2.1 Incidence of BYVMV

The average incidence of disease varied from 0.5 to 73.02 per cent (Sangar, 1997). Sharma and Sharma (1984) reported the range of incidence from 3.2 to 97.8 %. The disease not only reduces yield substantially (10 per cent to 90%) but also affects marketability of the fruits (Jambhale and Nerkar, 1986).

Mehra *et al.* (2008) revealed that Arka Abay and Arka Anamika were resistant, whereas Pusa Sawni was susceptible to BYVMV during rainy season.

Fajinmi and Fajinmi (2010) studied degree of okra mosaic virus at different growth stages of plants. Virus infection was severe at growth stages earlier than four weeks. Late infection of BYVMV had little or no effect on performance of okra, but early infection had a significant effect on growth and yield.

Reddy and Sridevi (2014) had observed 0 % to 85.09 % of BYVMV incidence in the advance breeding lines of okra. Arka Anamika and Arka Abhay were observed to be highly susceptible (85.09%) and (56.21%) respectively.

2.2.2 Screening for BYVMV

Screening of 941 indigenous and exotic germplasm lines of okra for BYVMV field resistance under natural epiphytotic condition revealed that none of the accessions was immune or highly resistant, 43 were moderately resistant and three accessions, IC-218887, IC-69286 and EC-305619, were resistant (Abdul *et al.,* 2004).

Tripathy *et al.* (2007) evaluated the hybrids of okra against BYVMV and observed that the incidence of BYVMV was low in Makhamalli (10.1%), while it was high in Indo7215(47.9%).

Biswas *et al.* (2008) studied the reaction of 14 cultivars /lines of bhendi to BYVMV. Found that none of the cultivar /line was immune to the disease and the disease incidence varied from 18.25 % (ZOH-3002) to 64.96% (VB-9801).

Prasanth *et al.* (2008) revealed that out of fifty five screened genotypes, five were highly resistant, thirteen were resistant, seventeen were moderately resistant, thirteen were moderately susceptible, five were susceptible and two were highly susceptible based on coefficient of infection.

Tripathy *et al.* (2008) conducted screening experiment during summer and kharif season under reduced level of chemical fertilizers supplemented with organic

manures. BYVMV disease incidence was ranged from 22.48 per cent (Arka Anamika) to 43.96 per cent (Sansar selection).

Phadvibulya *et al*. (2009) in his experiment, two okra varieties (Annie and Okura) were subjected to irradiation. Screening for BYVMV resistance was conducted for M_3 and M_4 plants. One M_4 plant of Okura (B-21) was found highly resistant, but none of Annie. Ten resistant lines obtained by screening for BYVMV resistance up to the M_7 generation were selected for yield trial observations. Only a small portion of the plants of the mutant lines appeared to be resistant throughout the whole growth duration.

Aparna *et al.* (2012) crossed ten homozygous okra genotypes in all possible combinations, in diallele fashion and evaluated a total of 55 treatments (ten parents and 45crosses). They have identified that the six out of top ten high yielding cross genotypes marked to exert highly resistant (Arka Abhay **x** Hissar Unnat), resistant (Hissar Unnat **x** Lam-1), moderately resistant (VRO-6 **x** Pusa Makhmali, Parbhani Kranti **x** VRO-6, and Parbhani Kranti **x** Arka Abhay), and highly sensitive (Parbhani Kranti **x** Hissar Unnat) reactions in both rainy and late rainy seasons.

In a field screening experiment of five okra varieties, High degree of resistance was obtained in variety VRO-6 with 13.5 per cent disease intensity, besides that two varieties i.e., VRO-3 and HRB-9.2 were found to be moderately resistant. The rest two vatieties i.e., Pusa Makhamali and Pusa Savani showed moderately susceptible and highly susceptible respectively.(Tiwari *et al.,* 2012)

A field experiment carried out to screen for BYVMV resistance in okra, it was observed that in both the years, the entry VRO-6 has recorded mild incidence of BYVMV(2.6%) and the entry Akola Bahar has shown severe intensity of disease (63.9%)with respect to fruit yield , JOL-2KN-19 and VRO-6 recorded maximum yield.(Vijay and Joshi, 2013)

Nataraja *et al*. (2013) evaluated twenty one genotypes/ cultivars. They concluded that the genotypes viz., IC 331217, IC 332453 and IC 342075 and cultivars viz., Monisha 211 and Arka Anamika showed tolerant reaction to BYVMV.

Sergius and Esther (2014) carried out field screening of twenty three (23) *Abelmoschus esculentus* and *A. caillei* cultivars for the resistance against Okra Mosaic Viral Diseases (OMVD) and Okra Leaf Curl Disease (OLCD). They found that all the *A. esculentus* cultivars are susceptible to the two diseases, their findings also indicate that contrary to the general belief, not all *A. caillei* cultivars are resistance to these viral diseases.

Sixty five entries consisting of fifty seven inbred lines and seven checks were srceened under unprotected condition during summer and the results exhibited that four lines were highly resistant, ten lines were moderately resistant, twenty six lines tolerant, ten moderately suscetible, six were susceptible and one was highly susceptible (Reddy and Sridevi, 2014).

Screening for identificaion of genotypes resistant to BYVMV disease during 2011-2013 has brought one accession IC 117222, resistant to BYVMVD consistently for three years (Gangopadyay *et al*., 2014).

2.2.3 Inheritance of BYVMV resistance

In inter-specific crosses involving *A. manihot* ssp. *manihot,* the susceptibility to BYVMV was controlled by two dominant genes (Thakur, 1976), while Jambhale and Nerker (1981) observed single dominant gene to control BYVMV resistance in *A. manihot* and *A. tetraphyllus*.

The inheritance of resistance to BYVMV in okra was studied in crosses involving three resistant parents (Arka Anamika, Punjab Padmini and Arka Abhay) and three susceptible parents (Pusa Sawani, Local and Pusa Makhmali). The inheritance pattern suggested that resistance to BYVMV was controlled by two

complementary dominant genes in susceptible × susceptible (S × S) and susceptible × resistant (S × R) crosses but in resistant £ resistant (R × R) crosses by two duplicate dominant genes (Pullaiah et al. 1998).

It was revealed from the results of grafting tests that tolerance/resistance in okra is genetic and not due to escape (Ali *et al*. 2000). They selected a okra variety, Okra 1, tolerant to BYVMV was crossed to three susceptible genotypes, Parbhani Kranti, SL-44 and SL-46, to determine the nature of inheritance of tolerance. From the segregation pattern for disease reaction in F2 and BC1 generations of the three crosses, it could be hypothesized that the tolerance to BYVMV in IPSA Okra 1 is quantitative, with possibly two major factors and is dependent on gene dosage with incompletely dominant gene action (Ali *et al.,* 2000). But Vashisht *et al*. (2001), based on nine generations derived from crosses involving resistant (Parbhani Kranti) and susceptible cultivars (Punjab 8, Punjab Padmini, Pusa Makhmali and Pusa Sawani), reported that additive gene effects were more significant than dominance gene effects.

2.2.4 Breeding for resistance to BYVMV

2.2.4.1 *Selection*

Joshi *et al.* (1960) used treatment line IC 1542 from West Bengal for developing the resistant varieties.

AE 286 (*A. caillei*)), an BYVMV resistant edible perennial okra line developed through single plant selection (Sureshbabu *et al.,* 2002).

2.2.4.2 *Hybridization and Selection*

Singh *et al.* (1962) developed "Pusa Sawani" from the cross between IC-1542 and Pusa Makhmali. Dhankar *et al*. (1996) bred BYVMV resistant okra "Varsha Uphar" out of the cross between Lam Selection 1 x Parbhani Kranti.

Deo *et al.* (2000) reported that Parbhani Kranti and its hybrid Parbhani Kranti x HRB-9-2 were highly resistant to BYVMV. Rattan and Bindal (2000) in their experiment to develop okra hybrids resistant to BYVMV found that lines 407, 409, 417, 430 were completely resistant. The F_1 hybrids between the resistant lines were resistant, and that of susceptible parents susceptible. The studies indicated that resistance to disease is monogenic and dominant. Maximum number of fruits and yield per plant was recorded by hybrid 410 x 407 followed by 409 x 421 and 409 x 408 involving resistant x resistant and resistant x susceptible crosses, respectively.

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 BYVMV.

2.2.4.3 ***Inter specific hybridization in okra***

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

Arumugham and Muthukrishnan (1978) developed four BYVMV resistant F_1s by crossing two resistant forms of *A. manihot* with susceptible okra cultivars, Pusa Sawani and CO 1.

Mamidwar *et al.* (1980) 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 while studying crosses between cultivars of *A. esculentus* and *A. manihot*.

Jambhale and Nerkar (1981) in his experiment, two *Abelmoschus* species, viz., *A. manihot* (L.) Medik and *A. manihot* (L.) Medik ssp. *manihot*, resistant to yellow vein mosaic (YVM) were crossed with *A.esculentus* cv. 'Pusa Sawani', a susceptible culture. The hybrids were resistant and partially fertile. Segregation pattern for disease reaction in F_2, BC_1 and subsequent generations of the two crosses

revealed that resistance to YVM is controlled by a single dominant gene in each species.

Nirmaladevi (1982) revealed that *A. manihot* was crossable with *A. esculentus*. The interspecific F_1 hybrid exhibited resistance to BYVMV. She observed significant genetic distance between *A. esculentus* and *A. manihot.*

Martin (1982) crossed unnamed West African species of *Abelmoschus* and *A. esculentus.* The F_1 hybrids were comparatively sterile but a few produced germinable seeds. Back crosses were mere fertile with almost complete fertility in BC_2.

Jambhale and Nerkar (1983) observed resistance to BYVMV in plants which were obtained from backcrosses of *A. esculentus* and *A. manihot* to *A. esculentus* cv. Pusa Sawani. Seed fertility in the plants was 58 to 88 per cent.

Pillai (1984) developed interspecific hybrids from *A. manihot* and BYVMV susceptible *A. esculentus* cultivars. The hybrids were found to be resistant to the BYVMV 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.

Sharma and Sharma (1984) used *A. manihot* as a male parent in hybridization with Pusa Sawani for developing resistant 'Punjab Padmini'.

Nerkar and Jambhale (1985) used the BYVMV resistant Ghana line *A.manihot* ssp. *manihot* in their hybridization programme which resulted in the variety 'Parbhani Kranti'.

Mathews (1986) observed preponderance of low yielding YVM resistant plants similar to semi-wild parent among the F_2 population of the interspecific hybrids between the YVM susceptible cultivars of *A. esculentus* and YVM resistant semi wild species of *A. manihot* . Varying degrees of sterility were observed in the F_2

progenies. He also reported high phenotypic and genotypic coefficients of variation for weight of fruits per plant, number of leaves per plant and height of plants.

Prabha (1986) made cross between the BYVMV disease susceptible varieties of *A. esculentus* and resistant 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 genus.

Sureshbabu (1987) produced vigorous F_1 hybrid between *A. esculentus* and *A. manihot* ssp. *tetraphyllus* var. *tetraphyllus.* Sterility in the hybrid was attributed to the failure of development of female gamete.

Reciprocal crosses between *A. manihot* ssp. *manihot* and *A. tetraphyllus* and also between *A. manihot* ssp. *manihot* and induced amphidiploid of (1) *A. esculentus* x *A. tetraphyllus* and (2) *A. esculentus* x *A. manihot* revealed that *A. manihot* ssp. *manihot* (hexaploid) contained two genomes from *A. tetraphyllus* and a third from *A. manihot* (Kondaiah *et al*., 1990).

Sureshbabu and Dutta (1990) produced 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 colchicines treatment, resembling the F_1 plants with BYVMV 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 BYVMV resistance of the wild species and the desirable fruit characters of the cultivated species with high level of pollen fertility.

Dutta (1991) developed the okra lines having high yield, quality and resistance to BYVMV by interspecific hybridization between *A. esculentus* and BYVMV resistant wild species *A. manihot ssp. tetraphyllus.*

Nerkar (1991) noticed the importance of wild okra with respect to resistance to okra BYVMV, powdery mildew (*Erysiphae cichoracearum*), Jassids (*Empoasca* spp.) in breeding programmes to develop pest and disease resistant varieties.

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 cross between *A. esculentus* x *A. moschatus*. This suggested the potential of tissue culture methods to overcome the post zygotic incompatibility barriers in interspecific crosses.

Interspecific hybridization between *A. esculentus* and *A. manihot* was successful when *A. manihot* was used as the female parent (Chacko, 1996).

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.

Dhankar *et al*. (2005) reported the nature of inheritance for resistance to BYVMV in inter specific cross of 'Hissar Unnat' *A. esculentus* × *A. manihot subsp.*

Manihot. The resistance showed Mendelian segregation as per the condition governed by two complimentary dominant genes.

Crossability studies between *A.esculentus* and *A.caillei* revealed that crosses were more successful when *A.caillei* was used as female parent (Kousalya, 2005). The F_1 hybrid was also secured in the cross *A.esculentus* x *A.caillei* but 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.

Pitchaimuthu *et al.* (2009) reported that interspecific crosses involving *A. tetraphyllus, A. tuberculatus*, IIHR-223 (Red Bhendi) and Arka Anamika were found to be promising with least Per cent Disease Incidence (PDI) of BYVMV.

Jaseena (2008) found that six promising lines selected from F_5 generation of the corss *A. caillei* x *A. esculentus* were highly resistant to BYVMV.

Sureshbabu *et al*. (2009) reported high degree of variability in the F_6 generation of the cross *A. caillei* x *A. esculentus* and selected six promising lines expressing high levels of resistance to BYVMV.

Yamuna (2012) reported high degree of variability in the F9 generation of the cross cross *A. caillei* x *A. esculentus* and selected six promising lines expressing high levels of resistance to BYVMV.

Dankar (2012) in his studies crossed Hissar Unnat with the wild sub species *manihot,* partial fertile F1thus obtained were further back crossed and even involved for three way crosses for improving the fruit shape and colour characters.

Kishore *et al*. (2012) screened 36 accessions of okra (*A. esculentus*) and identified five as resistance to BYVMV. They also reported hybrid combinations such as Holavanalli Local x Mallapalli Local, Thirumala Local x Punjab Palguni,

Thirumala Local x Kattakada Local and Kunnapuzha Local x Punjab Phalguni as BYVMV resistant.

2.2.4.3.1 ***Interspecific Hybrid Sterility***

Teshima (1933) observed that *A. esculentus* and *A. manihot* crossed only when the former was used as 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). Genetic sterility is typically due to the genetic constitution of the organism and so is diplontic (Dobzhansky, 1951).

Pal *et al.* (1952) made 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.

Arumugham *et al.* (1975) reported about 90 per cent sterility in interspecific hybrid between *A. esculentus* x *A. manihot.* In interspecific hybridization between different *Abelmoschus* spp. viable seeds could be obtained only in cross between *A. ficulneus* (2n=72) and *A. tuberculatus* (2n=58), resulting plants were sterile (Siemonsuma, 1982). Partial seed fertility of 5.9 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).

Chacko (1996) reported that in the interspecific hybrid of *A. esculentus* x *A. manihot* pollen stainability was only 18.26 per cent. The mean diameter of sterile pollen grains was 0.03 µm where as fertile pollen grain was 0.062 µm.

Kousalya (2005) observed that in the cross *A.caillei* x *A.esculentus* the F_1 hybrid was partially sterile. This can be attributed to the cytological irregularities

including the presence of lagging chromosomes, occurrence of micronuclei and multipolar spindle formation.

From the studies conducted since 2000 at CCSHAU, Hissar revealed that Varsha Uphar is poor cross compatible with wild relative such as *Abelmoschus manihot* ssp *manihot* and *Abelmoschus tetraphyllus* in comparison to cultivar Hisar Unnat (Dankar, 2012).

In a study on inter specific crosses in *Abelmoschus esculentus* and its wild relatives, Alien pollen tubes showed significantly high growth inhibition in okra (*A. esculentus*) pistils. Pollen tube growth was normal in *A. esculentus*×*A. caillei* and its reciprocal cross. The results indicated that the crosses *A. manihot* subsp. *tetraphyllus* var. *pungens*×*A. esculentus* had high incompatibility, while *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*×*A. esculentus* were partially compatible and *A. esculentus*×*A. caillei* were fully compatible (Patil *et al*., 2013).

The results of cross compatibility studies conducted by Prabu and Warade (2013) revealed that cultivated *A.esculentus* cultivars were found compatible both directly and reciprocally with *A. tuberculatus*, *A.tetraphyllus*, *A.caillei*, *A.manihot* (L.) Medikus, *A.manihot spp. manihot and A.manihot spp. tetraphyllus* while, *A. moschatus* was found incompatible on both ways with *A. esculentus.*

2.2.4.3.2 *Segregating progenies*

Mathews (1986) evaluated the F_2 population 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 recombination.

Gill *et al.* (1983) successfully transferred Yellow Mosaic Virus resistance from black gram (*Phaseolus mungo*) to mung bean (*Phaseolus aureus*) by advancing the segregating generations and subsequent selections. BYVMV resistant mung bean lines were recovered in advanced generations of interspecific cross involving the mosaic susceptible mung bean line SML32 and the resistant black gram variety Saradhu, without backcrossing (Pal *et al.*, 1991).

Ali *et al*. (2000) crossed an okra variety, IPSA Okra 1, tolerant to BYVMV with three susceptible genotypes viz., Parbhani Kranti, SL-44 and SL-46 to determine the nature of inheritance of tolerance of IPSA Okra 1. Grafting test was also done to know the nature of tolerance. It was revealed from the results of grafting test that the tolerance in IPSA Okra 1 is genetic, not due to escape. The F_1 hybrids were tolerant to BYVMV. From the segregation pattern for disease reaction in F_2 and BC_1 generations of the three crosses, it could be hypothesized that the tolerance to BYVMV in IPSA Okra 1 is quantitative, with possibly two major factors, and dependent on gene dosage with incompletely dominant gene action.

Singh *et al*. (2000) noticed that when ten okra genotypes and five F_1s derived from them were screened for resistance to yellow vein mosaic virus, HRB-55 x Arka Anamika, Prabhani Kranti x HRB-9-2 and BO-1 x P-7 were highly resistant to the virus, while BO-1 x Pusa Sawani was susceptible.

The interspecific hybridization between *A. esculentus* x *A. caillei* showed that F_1 and F_2 generations were partially fertile, Kousalya (2005). She also observed that the F_2 plants were free of BYVMV infection.

Jaseena *et al*. (2008) evaluated F_4 and F_5 generations of the cross *A. caillei* x *A. esculentus* and reported high levels of variability and resistance to BYVMV in the segregating population. F_5 generation plants exhibited high degree of pollen fertility.

2.3 ACHIEVEMENTS IN BREEDING FOR RESISTANCE TO BYVMV

Since some of the tolerant varieties as well as inter varietal hybrid derivatives of okra lost their resistance to BYVMV disease in due course, attempts have been made to incorporate the resistant genes from wild species to susceptible commercial types of okra through interspecific breeding programmes.

Pusa Sawani, once most widely cultivated variety of okra developed from a cross between 1C 1542, an indigenous stock with symptom less carrier type of resistance and Pusa Makhmali, a high yielding, adapted but susceptible variety of *A. esculentus* (Singh *et al.*, 1962). However the initial resistance was given way to high susceptibility due to genetic and environmental factors.

Sharma (1982) made interspecific hybridization between *A. esculentus and A. manihot* ssp. *manihot* and the segregating generations were advanced upto F_8 followed by selection to develop Punjab Padmini, an BYVMV resistant variety.

The Maharashtra state seed Committee in 1985, released an BYVMV resistant variety Parbhani Kranti developed from cross of *A. esculentus* cv Pusa Sawani x *A. manihot* (Jambhale and Nerkar, 1986).

P-7, an BYVMV resistant variety was evolved from the cross between *Abelmoschus esculentus* cv Pusa Sawani and *A. manihot* ssp. *manihot*. The F_1 was backcrossed to the cultivated parent for four generations and selection was followed in the selfing generations up to F_8 (Thakur and Arora, 1988).

Selections from IIHR, Bangalore, Viz., Selection-4, Selection-7, Selection-9, Selection-10 and Selection-12 processed YVM diseases resistance and was derived from the cross of *A. esculentus* x *A. manihot* var. *tetraphyllus* (Markose and Peter, 1990).

Arka Anamika, high yielding BYVMV resistant variety obtained by interspecific hybridization between *A. esculentus and A. manihot* ssp *tetraphyllus* was released by IIHR Bangalore for National level cultivation. Arka Abhay, another high yielding and resistant line derived from the same cross was released for state level cultivation (Dutta, 1991).

Tamilnadu Agricultural University, Coimbatore developed one okra variety, MDU-1 in 1978. It is an induced mutant isolated from Pusa Sawani and had been notified by the Central Seed Committee in 1985 (Ram, 1998).

Varsha Uphar (HRB 9-2), an BYVMV resistant variety had been developed by Haryana Agricultural University, Hisar from the cross, Lam Selection-1 x Parbhani Kranti following pedigree selection method. It was released in 1992 and notified in 1995 by the Central Sub- Committee on Crop Standards (Ram, 1998).

Pusa A4 has been released by IARI in 1994 as a substitute for Pusa Sawani. It is also resistant to BYVMV (Ram, 1998).

Hisar Unnat (HRB 55), an BYVMV resistant variety developed by Haryana Agricultural University, Hisar from the cross, Sel-2 x Parbhani Kranti had been released by the Central Variety Release Committee and notified in 1996 (Ram, 1998).

EMS-8 (Punjab-8) had been developed by PAU, Ludhiana in 1989. It is an induced mutant derived from Pusa Sawani treated with 1% EMS. The final selection was made in the M_8 generation. It has field resistance to BYVMV (Ram, 1998).

A. caillei variety Susthira had been developed in the Department of Olericulture, College of Horticulture, Kerala Agricultural University, Vellanikkara by selection (Sureshbabu *et al.,* 2004).

KAU had released two yellow vein mosaic resistant varieties, 'Manjima' of the cross Goreesapattom local x NBPGR/ TCR- 874 and 'Anjitha' by interspecific hybridization followed by mutation breeding and selection (KAU, 2007).

Several BYVMV resistant varieties like Kashi Vibhuti, Kashi pragati, Kashi Satdhari, Kashi Bhirav, Kashi Mahima had been released by IIVR, Varanasi (Pradeepkumar *et al.,* 2008).

Okra variety Kashi Kranthi has been developed through hybridization between VRO-6 and 67-10-02, foll0wed by pedigree selection at Indian Institute of Vegetable Research, Varanasi. It is an early, high yielding and possess resistance to yellow vein mosaic disease and okra enation leaf curl virus (Sanwal *et al.,* 2014).

Screening for identification of genotypes resistant to BYVMV disease during 2011-2013 has brought one accession IC 117222, resistant to BYVMV consistently for three years (Gangopadyay *et al*., 2014)

2.4 GENETIC VARIABILITY IN OKRA

Variability may be defined as the amount of variation present among the members of a population or species for one or more characters at genotypic or phenotypic levels. Presence of variability among genotypes is a prerequisite for any crop improvement programme.

Gandhi *et al*. (2001) evaluated 44 okra genotypes for genetic diversity and results shown significant variability for all the thirteen traits under investigation including plant height, inter nodal length, fruits and branches per plant, length and Fruit girths and yield per plant.

Lal *et al*. (2001) assessed the response of three okra varieties to varying sowing dates under Tarai foot hills of Himalayas and obtained the highest green pod yield (85.9 q/ha) for Parbhani Kranti, followed by Pusa Sawani (8.4 q/ha) and P-7

(72.5 q/ha), P-7 exhibited the lowest YVM virus infection (0.3 %) while Pusa Sawani showed the highest (41.4%).

Dhankar and Dhankar (2002) observed broad range of variation and high mean values for fruit yield and plant height in, rainy and spring- summer seasons. Singh *et al.* (2006) observed the significant differences among genotypes for all the characters under study.

Sharma and Mishra (2007) evaluated the induced genetic variation in okra cv. Ankur-40 under gamma radiation treatment. Nine characters were evaluated in the M_2 population. Variation was observed for days to flower bud initiation, fruit length, fruit yield, seed number and seed yield. Maximum range of statistical and genetic variation was observed for fruit yield/plant.

Prakash and Peechimuthu (2010) evaluated the forty four okra genotypes for genetic variability of yield contributing characters, and the genetic diversity. Analysis of variance indicated significant differences among the genotypes for different morphological characters.

Akotkar *et al.* (2010) evaluated the genetic variability and genetic diversity of fifty okra genotypes. Analysis of variance indicated significant difference among the genotypes for different morphological characters.

Genetic variability on 100 genotypes of okra revealed high magnitude of genetic variability and high degree of transmission of majority of the growth, earliness and yield associated traits under study (Reddy *et al.,* 2012).

Morey *et al.* (2012) studied the genetic variability for growth and yield contributing characters in twenty two genotypes of okra collected from NBPGR, New Delhi. Results indicated significant differences among the genotypes.

Ahiakpa *et al*. (2013) observed variations among the different accessions based on their vegetative traits, inflorescence, fruit and seed characteristics. They also noticed a wide variation in plant height among the accessions flowering and fruiting periods varied considerably among all accessions based on the output of the Principal Components, Correlation and Cluster analyses.

Kumar and Kumar (2014) analyzed the genetic variability of fifty five diverse genotypes including ten parents and forty five crosses of okra for fruit yield and its component traits. From the analysis of variance, they observed that the mean squares due to genotypes were significant for all the traits, indicating the presence of genetic variability in the experimental material.

2.5 COEFFICIENT OF VARIATION

Bindu *et al.* (1997) observed high genotypic coefficient of variation for plant height, fruit weight, number of effective nodes, number of branches per plant, fruit yield per plant and high phenotypic coefficient of variation observed for plant height ,number of effective nodes and number of branches per plant in okra.

Among okra genotypes high PCV and GCV were observed for branches per plant, fruits per plant and plant height in both rainy and spring-summer seasons (Dhankar and Dhankar, 2002). For fruit yield and plant height, values of PCV and GCV were almost equal indicating the environment had little effect but days to first flower and fruits and branches per plant had some influence by environment. Moreover, PCV and GCV were higher for all the traits during rainy season than spring summer.

Bendale *et al.* (2003) examined thirty okra genotypes for first flowering node, pod length, pod weight, plant height, nodes per plant, internodal length, number of branches per plant, seeds per pod, 100 seed weight, number of pods per plant and yield per plant. The phenotypic coefficient of variation for all the characters was

higher than genotypic coefficient of variation. Number of branches per plant, yield per plant and number of pods per plant showed high genotypic coefficient of variation and high phenotypic coefficient of variation.

Sindhumole (2003) observed high PCV and GCV for most of the traits including yield and its major components. However GCV was moderate for fruit girth, ridges and seeds per fruit and leaf axil bearing first flower but low for plant duration and YVM incidence at 30 days after sowing.

Dakahe *et al.* (2007) found that the magnitudinal differences between GCV and PCV estimate was maximum for fruit length, number of fruits per plant and fruit girth suggesting the influence of environment on these traits.

Singh *et al*. (2009) reported high GCV for plant height and fruit yield per plant and this finding had been supported by Gandhi *et al.* (2001).

Jindal *et al*. (2010) reported high genotypic and phenotypic coefficients of variation for number of primary branches per plant indicating maximum variability among the different genotypes. Akotkar, *et al*. (2010) observed higher GCV for number of fruiting nodes, number of ridges per fruit, plant height and number of fruiting nodes indicated these characters might be controlled by additive genes.

Prakash *and* Pichaimuthu (2010) revealed that high values of GCV and PCV was observed for number of fruiting nodes, number of ridges per fruit, plant height and number of fruiting nodes indicating these characters might be controlled by additive genes.

Adiger *et al*. (2011) reported that GCV values were higher for plant height, fruit yield per plant, fruit weight and days to 50 per cent flowering. The Fruit yield has significantly positive correlation with plant height, number of branches per plant,

inter nodal length, fruit length, fruit weight and number of fruits per plant at both genotypic and phenotypic level, indicating mutual association of these traits.

Chaukhande *et al.* (2011) revealed that highest genotypic coefficient of variation (GCV) as well as phenotypic coefficient of variation (PCV) was observed for incidence of yellow vein mosaic virus. The maximum difference between GCV and PCV was noted for inter nodal length.

Adeoluva and Kehinde, (2011) observed higher GCV and PCV for pod yield per plant and peduncle length respectively. They also reported that phenotypic variances were generally higher than their respective genotypic variances thus revealing the role of environmental factors.

Vani*, et al.* (2012) revealed that the characters like number of branches, fruit width and node at which first flower appeared showed low GCV and high PCV highlighting the usefulness of selection based on the phenotypic performance of genotypes.

Soyab *et al*. (2013) revealed that plant height (cm), number of fruits per plant and number of seeds per fruit observed high GCV and PCV. Katagi, *et al*. (2013) observed the higher GCV values for plant height, fruit yield per plant, fruit weight and days to 50 per cent flowering in the double cross F_2 population.

Yamuna *et al*. (2013) revealed that incidence of BYVMV has exhibited higher GCV and PCV values, whereas duration of the crop and Fruit girth exhibited low phenotypic and genotypic coefficient of variation. Hence, suggested that low variability noticed for these characters indicate the difficulty in improving these characters by selection.

Kumar *et al*. (2014) observed higher genotypic variances for first fruiting node, days to first picking, number of branches per plant, plant height, number of fruits per plant and yield per plant.

Reddy and Dhaduk, (2014) carried out an investigation to study the genetic variability induced through physical and chemical mutagenes, they observed higher GCV and PCV with high heritability for BYVMV incidence at 0.25% EMS treatment in GO-2 and 40kR treatment in GJO-3.

2.6 HERITABILITY AND GENETIC ADVANCE

The relative amount of heritable portion of total variation was found out with the help of heritability estimates and genetic advance. Lush (1940) defined the broad sense heritability as the ratio of genetic variance to the total variance. Robinson *et al.* (1949) defined the narrow sense heritability as the ratio of additive genetic variance to phenotypic variance.

Jaiprakashnarayan *et al.* (2006) observed high heritability coupled with high genetic advance for plant height 100 days after sowing, internodal length, number of nodes on main stem, number of nodes at first flowering and number of leaves at 45 days after sowing. High heritability with low genetic advance observed for days to first flower and days to 50% flowering in okra.

Singh and Singh (2006) noted high heritability for days to first flowering, first fruiting node length and high heritability with high genetic advance was observed for first fruiting node length, number of branches per plant, tapering length and fruit yield per plant.

Sunil *et al.* (2007) observed high heritability coupled with moderate genetic advance for days to flowering, number of node per plant, internodal length, fruit number per plant and yield per plant. High heritability coupled with low genetic advance was observed for plant height. Low heritability coupled with high genetic

advance for fruit width, tapering Fruit length and low heritability with low genetic advance for fruit length in okra.

Singh *et al.* (2007) estimated high values of heritability for plant height, number of fruits per plant, fruit yield, fruit length, fruit girth and number of branches per plant. High heritability coupled with moderate genetic advance for all the characters except for nodes at which first flower appear, indicating that additive gene affects were more important for these characters.

Akotkar *et al.* (2010) noticed high heritability and genetic advance for number of fruiting nodes, number of ridges per fruit, plant height and number of fruiting nodes. AdeOluwa and Kehinde (2011) observed very low heritability estimate for number of ridges per fruit (7.1%). Moderately high heritability (78.99%) for peduncle length; moderately high PCV and GCV 35.71% and 31.74%, respectively but low genetic advance of 1.99%.

Nwangburuka *et al.* (2012) observed high heritability and genetic advance in traits such as plant height (90.7, 51.5), fresh pod length (98.5, 48.8), fresh pod width (98.5, 48.8), mature pod length (98.5, 52.3), branching per plant (82.3, 54.8) and pod weight per plant (90.0, 63.3).

Soyab *et al.* (2013) studied the heritability and genetic advance as percent of mean among the 25 germplasm lines of okra. Significant differences among genotypes were observed for all the characters under study. Plant height (cm), number of seeds per fruit, and number of fruits per plant recorded high heritability coupled with high GCV and high genetic advance as per cent of mean indicates selection could be effective for improvement in these characters.

Seeds of two popular okra varieties *viz.* GO-2 and GJO-3 were treated with 20 kR, 30 kR and 40 kR of gamma rays. In M2 population of GO-2, high heritability and genetic advance was observed for some important yield contributing characters like

fruit length at 40 kR and 0.25% EMS; fruit weight at 20 kR and 40 kR; and fruit yield per plant at 20, 30, 40 kR and 0.15% EMS. Similarly in GJO-3, high heritability coupled with high genetic advance was observed for fruit length at 30 kR and fruit yield per plant at 20 kR (Reddy and Dhaduk, 2014).

2.7 CORRELATION STUDIES

Yield is the complex character hence it is necessary to know the importance and association of various yield contributing components with yield and within themselves. This is possible by determining the correlation coefficients (r) between the combining traits and yield.

Gandhi *et al.* (2001) reported that the dry fruit yield was highly and significantly dependent on number of nodes per plant, internodal length, number of fruits per plant and seed yield per plant. The interdependency of other characters on each other's was also recorded.

Dhankar and Dhankar (2002) observed that fruit yield was significantly and positively correlated with the number of fruits and branches per plant and plant height but was negatively correlated with days to 50% flowering. The number of fruits per plant was positively associated with number of branches per plant and plant height was negatively correlated with days to 50% flowering. Fruit yield can be improved through selection for higher number of fruits and branches and medium height

Jaiprakashnarayan and Mulge (2004) noticed that total yield per plant was positively and significantly correlated with number of fruits per plant, average fruit weight, number of nodes on main stem, fruit length, plant height at 60 and 100 days after sowing and number of leaves at 45 and 100 days, but negatively and significantly correlated with number of locules per fruit, number of nodes at first flowering and first fruiting.

Mohapatra *et al.* (2007) evaluated 23 genotype of okra for different yield traits as well as yellow vein mosaic virus and estimated that total fresh yield per plant had a positive and significant phenotypic and genotypic correlation with number of fruits per plant, fruit girth, fruit diameter, internodal distance and fruit weight.

Dhake *et al*. (2007) revealed that days to 50% flowering and days to maturity are significantly associated, which are most important traits for exploiting earliness and also suggested that for increasing green pod yield due emphasis should be given to number of fruits, number of internodes, plant height and fruit length as all these characters possess highly significant positive correlation with fruit yield.

Singh *et al.* (2007) observed that fruit yield had significant positive genotypic and phenotypic correlation with number of fruit, fruit length and plant height. Number of fruit showed significant positive genotypic and phenotypic associations with plant height and fruit length.

Ali *et al.* (2008) estimated the correlation coefficients among parents, F1 hybrids and F2 population separately. They observed that correlation coefficients were consistently significant and positive in all the three population between fruit yield per plant and number of fruits per plant. The consistency was also observed in F1 and F2 generation between fruit yield per plant and plant height.

Significantly positive correlations were recorded between yields per plant with plant height, intermodal length, fruits per plant, branches per plant, fruit weight, fruit girth, number of nodes to first flower and fruit length (Sengupta and Verma, 2009).

Solankey and Singh (2010) revealed that single fruit weight, number of fruits per plant and number of seeds per fruit were identified as important fruit yield component in kharif season. However, number of fruits per plant, plant height and stem diameter were most crucial yield components for summer season.

Balakrishnan and Sreenivasan (2010) in their study revealed that fruit yield was positively associated with number of fruits, number of internodes, fruit weight and fruit length. Shoot and fruit borer infestation recorded negative association with plant height, flowering period, fruit number, fruit yield and internodal length.

Studies on correlation revealed that fruit yield per plant exhibited significant and strong positive association with plant height, fruit length, number of fruits per plant and days from flowering to maturity and also the higher magnitude of genotypic correlations than the corresponding phenotypic ones, indicates the inherent association among the various traits (Kumar *et al*,. 2011).

Correlation and path analysis studies in 75 diverse Okra genotypes revealed that yield per plant exhibited positive and significant correlation with plant height, number of flowering nodes on main stem, number of fruits per plant, average weight of fruit (Chaukhande *et al*,. 2011).

Amoatey *et al*. (2015) studied the correlation coefficients in twenty nine (29) local and exotic lines (accessions), of okra (*Abelmoschus* spp L.) and they found that Seven pairs of quantitative traits were positive and significantly correlated ($P \leq 0.05$) while three were highly significantly associated ($P \leq 0.01$). The highest correlation ($r = 0.95$) was between number of days to 50% flowering (NDFl) and number of days to 50% fruiting (NDFr).

2.8 MUCILAGE EXTRACTION STUDIES

Wolfe *et al.* (1977) reported that a typical Ghanian okra soup was containing approximately 0.2-0.3 per cent by weight of mucilage.

Thampi and Indira (2000) evaluated 20 genotypes of thamara venda along with Pusa Sawani for nutritive value and organoleptic qualities. The mucilage content of Thamara venda genotypes was higher than the control variety Pusa Sawani.

Girase *et al*. (2003) observed a marked genetic variation for cortex mucilage contents (1.02 to 1.51%) in 15 okra cultivars including four wild species. The fresh cortex tissue was contained more mucilage (1.49%) than green mature fruits (0.57%) and green leaves (0.05%).

Kadlag *et al*. (2005) studied the influence of integrated plant nutrient supply on yield, quality and nutrient uptake of okra. He reported that application of inorganic fertilizers increased the mucilage content of fruits.

Chavan *et al*. (2007) obtained mucilage powder from fresh stems of okra plants. The mucilage content was about four per cent.

Girase *et al*. (2008) studied the cortex mucilage content at various growth stages of okra. Mucilage content was maximum (0.94%) at 90 days growth stage compared to 45, 60, 75 days growth stage.

2.9 MOLECULAR MARKERS

Genetic markers are the biological features that are determined by allelic forms of genes are genetic loci and can be transmitted from one generation to another, and thus can be used as an experimental probe or tags to keep track of an individual, tissue, a cell, a nucleus, a chromosome or a gene. Genetic markers used in genetics and plant breeding can be classified in to two categories: classical markers and DNA markers (Xu, 2010).

Classical markers include morphological markers, cytological markers and biochemical markers. DNA marker have developed into many systems based on different polymorphism-detecting techniques or methods which includes southern blotting – nuclear acid hybridization, PCR based methods such as RFLP, AFLP, RAPD, SSR, ISSR, SNP etc. (Collard *et al.,* 2005).

2.9.1 DNA makers

DNA marker is defined as a fragment of DNA that reveal molecular variations, which can be used to detect polymorphism between different genotypes or alleles of a gene for a particular sequence of DNA in a population or gene pool. Such fragments are associated with a certain location within the genome and may be detected by means of certain molecular technology. In short, DNA marker is a small region of DNA sequence showing polymorphism (base, deletion, insertion and substitution) between individuals. There are two basic methods to detect the polymorphism: Sothern blotting which is a nuclear acid hybridization (Southern, 1975), and PCR, a polymerase chain reaction (Mullis, 1990).

2.9.1.1 *Randomly Amplified polymorphic DNA (RAPD)*

Randomly Amplified polymorphic DNA (RAPD) is one such method (Welsh and McClelland, 1990) of identifying polymorphism that can be used to elicit information on molecular differences among individuals of a population between lines or accessions or any breeding material. RAPD markers are generated by the use of short (10-mer)synthetic oligonucleotides in a single strand primer (Williums *et al.,* 1990). In this technique, a decamer primer of arbitrary sequence is allowed to anneal at a relatively low temperature priming the amplification of DNA fragments distributed at random in the genome. Amplification products are visualized by separation on agarose and stained with ethidium bromide. They usually result in DNA fragments patterns that are polymorphic between genotypes, by their detecting diversity within them (Tommercup *et al.,* 1998). However, a key requirement for reliable and reproducible RAPD results is a consistent approach to sample preparation and DNA isolation.

There are several advantages of RAPD compared to other DNA based techniques. It is simple, fast, low cost and it can be done with small amount of DNA also it does not involve radioactivity (Varghese *et al.,* 1997). Another advantage of the RAPD method is that a universal set of random primers can be used for genomic

analysis of any organism (Welsch and McClelland, 1990). Short random primers have been used to reproducibly amplify segments of genomic

Genetic diversity in *A. esculentus* and the four related sp. (*A. ficulneus, A. manihot, A. moschatus.* and *A. tuberculatus*) was studied using isozymes and RAPD techniques by Bhat *et al.* (1995). This study covered allelic variations at 13 isozyme loci and 189 amplification products obtained by random amplification of genomic DNA using 22 random primers of ten nucleotide lengths. They reported moderate genetic diversity within *A, ficulneus, A. moschatus* and *A. esculentus. A. moschatus* genome was quite distinct from that of the other species. Gene duplication was common in all the species studied.

Martinello *et al*. (2001) investigated the genetic diversity of 39 *Abelmoschus* spp accessions at the DNA level with the random amplified polymorphic DNA (RAPD) procedure and at the phenotypic level with stable and highly heritable morphological characters. Dendrograms were generated for genetic distance based on molecular data (RAPD) and the morphological data and obtained similar results for grouping of all genotypes based on the two methods with a correlation of 0.62 between molecular and morphological data.

Aladele *et al*. (2008) collected 93 accessions of okra which consisted of 50 West African genotypes (*Abelmoschus caillei*) and 43 Asian genotypes (*A. esculentus*) and assessed for genetic distinctiveness and relationships using random amplified polymorphic DNA (RAPD). The molecular analysis showed that all the thirteen primers used revealed clear distinction between the two genotypes. There were more diversity among the Asian genotypes; this might be due to the fact that they were originally collected from six different countries in the region.

Saifullah *et al.* (2010) investigated the genetic diversity and molecular phylogeny of hundred and twenty genotypes of okra. In their study, out of 39 primers

screened, only five gave 38 clear and bright fragments. There were 32 polymorphic bands (6.40 diversity/primer) out of 38 reproducible products (7.6 fragments/primer) which were amplified from the selected five primers, corresponding to 86.70% diversity of the amplification bands.

Twenty-two genotypes of *Abelmoschus esculentus L. Moench,* including resistant varieties, hybrid varieties and susceptible varieties for BYVMV infection, were collected and analyzed for genetic diversity. 20 random primers were used of which 15 resulted in polymorphic, scorable and reproducible. The UPGMA based dendogram grouped 22 genotypes into three major clusters with one genotype placed independently at one end of the dendogram (Vaishali *et al*., 2010).

Prakash *et al.* (2011) used RAPD markers to assess genetic distinctiveness and relatedness of forty nine accessions of okra. The molecular analysis showed that all the fourteen primers used revealed clear distinction between the genotypes and they generated a total of 104 RAPD bands most of which were polymorphic across accessions (74.03%).

Nwangburuka *et al.* (2011) analyzed the genetic variability in twenty-nine okra accessions by using the random amplified polymorphic DNA (RAPD) technique. Eighty-four amplified products and 53 RAPD bands were scored with an average of 61.4% of them revealing polymorphism across accessions. Primer OPX 17 yielded the highest polymorphic band (8), with 67% polymorphism, while OPY 02 yielded 6 polymorphic bands with the highest percentage polymorphism (75%). The least number of polymorphic bands (3) as well as least percentage polymorphism (50%) was in OPX 18.

Morphological and molecular diversity among okra Germplasm was assessed by Kaur *et al.* (2013) using 40 RAPD primers. They obtained a total of 170 RAPD scorable fragments, of which 92.94% bands were observed to be polymorphic. PIC

values ranged from 0 (OPC-5) to 0.89 (OPC-2) with an average value of 0.75 indicating a wide genetic diversity among the germplasm.

Thirty nine okra genotypes were assessed by Ikram *et al*. (2013) for genetic variability using Random Amplified Polymorphic DNA (RAPD) markers. Twenty polymorphic RAPD primers amplified 111 DNA fragments, with an average of 5.5 fragments per primer. Among 39 okra genotypes, 107 fragments (96%) were found to be polymorphic.

2.9.1.2 *Inter Simple Sequence Repeats (ISSR)*

Inter Simple Sequence Repeats (ISSR) technique is a PCR based method, which involves amplification of DNA segments present at an amplifiable distance in between two identical microsatellite repeat regions, oriented in opposite direction. The technique uses microsatellites, usually 16-25 bp long, as primers in a single primer PCR reaction, targeting multiple genomic loci to amplify mainly the inter-SSR sequence of different sizes.

The microsatellite repeats used as primers can be di nucleotide, tri-nucleotide, tetra nucleotide or penta-nucleotide. The primers used can be either unanchored (Gupta *et al.,* 1994) or more usually anchored (Zietkiewicz *et al.,* 1994).

ISSRs have high reproducibility, possibly due to the use of longer primers (16-25 mers) as compared to RAPD primers (10-mer) which permits the subsequent use of high annealing temperature (45-60 ^{0}C) leading to higher stringency. ISSRs segregate mostly as dominant markers, following the simple Mendalian inheritance (Gupta *et al.,* 1994).

Inter-simple sequence repeat (ISSR) markers were employed to investigate the genetic diversity and differentiation of 24 okra genotypes. In this study, The 22 ISSR primers produced 289 amplified DNA fragments, and 145 (50%) fragments

were polymorphic. The unweighted pair-group method with arithmetic average (UPGMA) dendrogram analysis indicated that 24 okras were clustered into 4 geographically distinct groups. The average polymorphism information content (PIC) was 0.531929, which showed that the majority of primers were informative. The high values of allele frequency, genetic diversity, and heterozygosity showed that primer-sample combinations produced measurable fragments. The mean distances ranged from 0.045455 to 0.454545. The dendrogram indicated that the ISSR markers succeeded in distinguishing most of the 24 varieties in relation to their genetic backgrounds and geographical origins (Yuan *et al*., 2014).

Materials and methods

3. MATERIALS AND METHODS

The present investigation entitled 'Evaluation and molecular characterization of advanced generation distant hybridisation selections of okra [*Abelmoschus esculentus* (L.) Moench]' was carried out in the Department of Olericulture, college of Horticulture, KAU, Vellanikkara, Thrissur. During 2014–15. The materials used and the methods followed to conduct the experiment is explained bellow.

3.1 SITE, SOIL AND CLIMATE

The experimental site is located at an altitude of 22.5m above MSL. The experimental site has a sandy loam soil, which is acidic in reaction (pH 5.3). The area lies in tropical monsoon climatic region, with more than 80 per cent of the rainfall getting distributed through southwest and northeast monsoon showers. Data on temperature, rainfall, relative humidity, number of rainy days and sunshine hours during the entire cropping period were collected from meteorological observatory of College of Horticulture, Vellanikkara (Appendix 1).

3.2 SEASON OF EXPERIMENT

The experiment was conducted during June - October 2014 and consisted of the following aspects:

- Evaluation of the F_{12} generation plants for the selection of desirable lines.
- Screening for resistance to YVMV.
- Molecular characterization using RAPD and ISSR marker systems.

3.3 EXPERIMENTAL MATERIAL

The present study was carried out in the Department of Olericulture, College of Horticulture, Kerala Agricultural University, Vellanikkara, which accomplished an

interspecific hybridization between *Abelmoschus caillei* cv. Susthira (a semi wild yellow vein mosaic resistant species) and *Abelmoschus esculentus* cv. Salkeerthi (a high yielding, widely adapted, but YVMV susceptible variety). The generations out of this cross had been advanced up to F_{12} generation. The seeds of selected stabilizing superior F_{11} population generated the F_{12} population needed for the present study. Superior selections from the F_{11} generation were selected based on the plant morphology, fruit characters, fruit yield, pollen fertility and field resistance to yellow vein mosaic virus disease. These plants were genetically evaluated along with the parents and a standard variety Punjab 8. The source of materials used in the study is given in the Table 1.

3.4 EXPERIMENTAL METHOD

3.4.1 Design and layout

Design - Randomised Block Design

Replication - 3

Plot size - 4.5 × 1.8 m

Spacing - 60 × 45cm

Treatments - 11

Table 1. Source of materials used for the study

Sl. No	Variety/selection used	Species/others	Mode of evolution /parents	Reaction to YVMV	Evolved from
1	F_{12} -1 (T1)	Interspecific hybrid derivative	Susthira x Salkeerthi	To be tested	Department of Olericulture, COH, Vellanikkara
2	F_{12} -2 (T2)	Interspecific hybrid derivative	Susthira x Salkeerthi	To be tested	,,
3	F_{12} -3 (T3)	Interspecific hybrid derivative	Susthira x Salkeerthi	To be tested	,,
4	F_{12}-4 (T4)	Interspecific hybrid derivative	Susthira x Salkeerthi	To be tested	,,
5	F_{12} -5 (T5)	Interspecific hybrid derivative	Susthira x Salkeerthi	To be tested	,,
6	F_{12} -6 (T6)	Interspecific hybrid derivative	Susthira x Salkeerthi	To be tested	,,
7	F_{12} -7 (T7)	Interspecific hybrid derivative	Susthira x Salkeerthi	To be tested	,,
8	F_{12} -8 (T8)	Interspecific hybrid derivative	Susthira x Salkeerthi	To be tested	,,
9	Susthira (P1)* (T9)	*A. caillei*	Pure line Selection	Resistant	,,
10	Salkeerthi(21)* (T10)	*A. esculentus*	Pure line Selection	Susceptible	,,
11	Punjab 8 (T11)	*A. esculentus*	Mutant (EMS 8%)	Resistant	,,

P1* - Female parent

P2* - Male parent

3.4.2 Evaluation of the genotypes

Eight F_{12} generation selections along with their parents and Punjab 8 were evaluated during June to November (2014) in a Randomized Block Design (RBD) with three replications (Plate 1). Thirty plants were raised in each treatment in each replication. Highly susceptible okra line *A. esculentus* variety Salkeerthi was planted all around the field. The treatments received timely management and care as per the package of practice recommendation of KAU 2013. Ten plants selected at random from each treatment were used to take observations. No plant protection measures were taken up as it would reduce the vector population and thereby hinder the natural epiphytotic condition for Yellow Vein Mosaic Disease.

3.5 BIOMETRICAL OBSERVATIONS RECORDED

All the observation plants were tagged individually in each replication and their morphological characters were noted. The following observations were recorded and analyzed statistically

3.5.1 Qualitative characters:

1. Plant characters
    a. Plant habit : Branched or unbranched
2. Leaf characters
    a. Leaf lobing : Deeply lobed/narrowly lobed/serrated
    b. Colour of leaf base : Green / green with red tinge/red with green tinge
    c. Colour of leaf vein : Green/ whitish green
3. Flower characters
    a. Flower colour : Yellow/ golden yellow
    b. Flower size : Small/ medium/large

Plate 1: Field view of the experimental plot

c. Nature of corolla : Red throat/ purple throat

4. Fruit characters

a. Colour of fruit : Green/ dark green/ yellowish green/ red/ deep red / others

b. Pod pubescence : Smooth/less pubescent/ highly pubescent

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

3.5.2 Quantitative characters

1. Plant height (cm)

The height of the plant was measured from the base of the plant to tip at 100 days after sowing.

2. Internodal length (cm)

The length of the internode between sixth and seventh node of the plant was measured at 100 days after sowing.

3. Number of primary branches

The number of primary branches was counted at 60 days after sowing.

4. Length of epicalyx segment

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

5. Width of epicalyx segment

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

6. Petiole length (cm)

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

7. Days to flower

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.

8. Days to first harvest

The number of days taken to harvest the first fruit was noted and expressed in numbers.

9. First fruiting node

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

10. Fruit length (cm)

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

11. Fruit girth (cm)

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

12. Locules per pod

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

13. Number of ridges per pod

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

14. Number of fruits per plant

Total number of fruits borne on the observation plants was recorded

15. Number of harvests

Total number of harvests was recorded from first to final harvest.

16. Crop duration

Time taken for last harvest from sowing was done separately.

17. Yield per plant

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

18. Incidence of other pests and diseases

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

3.6 STATISTICAL ANALYSIS

The observations taken from the experimental plants were tabulated and subjected to statistical analysis.

3.6.1 Analysis of variance

Analysis of variance for RBD was carried out for comparison among different treatments and replications and to estimate variance components.

3.6.2 Estimation of variability components

The phenotypic and genotypic components of variance for each character were estimated by equating the expected value of mean squares (MS) to the respective variance components (Jain, 1982).

3.6.2.1 Phenotypic variance ($V_{(P)}$)

$$V_{(P)} = V_{(G)} + V_{(E)}$$

Where $V_{(G)}$ = Genotypic variance

$V_{(E)}$ = Environmental variance estimated as mean square due to error

3.6.2.2 Genotypic variance ($V_{(G)}$)

$$V_{(G)} = \frac{\text{Mean square (treatment) - Mean square (error)}}{\text{Number of replications}}$$

The phenotypic and genotypic coefficients of variation were worked out for each character by making use of the estimates of $V_{(P)}$ and $V_{(G)}$ and were expressed in percentage.

3.6.2.3 Phenotypic coefficient of variance (PCV)

$$PCV = \frac{\sqrt{V_{(P)}}}{\text{Mean}} \times 100$$

3.6.2.4 Genotypic coefficient of variance (GCV)

$$GCV = \frac{\sqrt{V_{(G)}}}{Mean} \times 100$$

In all cases, the mean of a character was calculated over all the treatments.

3.6.3 Estimation of heritability

The heritability (in broad sense) for each character was worked out as the ratio of genotypic variance to the phenotypic variance and was expressed as percentage (Jain, 1982).

$$\text{Heritability } (h^2) = \frac{V_{(G)}}{V_{(P)}} \times 100$$

3.6.4 Estimation of genetic advance

The expected genetic improvement by selection was proportional to the product of heritability and phenotypic standard deviation (Allard, 1960).

$$\text{Genetic advance (GA)} = k.h^2 \sqrt{V_{(P)}}$$

Where, ‘k’ is the standardized selection differential, usually taken as 2.06 (at 5 per cent selection) in large samples.

3.6.5 Correlation

The analysis of covariance was done between each pair of observations and the correlations were computed. The phenotypic correlation coefficient between two characters x and y was estimated as γp (x,y)

$$\gamma p\,(x,y) = \frac{Cov(p)\,(x,y)}{\sqrt{V_{(P)}\,x \;X\; V_{(P)}\,y}}$$

Where $Cov_{(P)}$ (x,y) denotes the phenotypic covariance between the characters x and y estimated by taking the respective expected values of mean sum of products.

$V_{(P)}$ x and $V_{(P)}$ y indicate the estimated phenotypic variances for x and y respectively.

The genotypic correlation coefficient between the characters x and y was estimated in the similar manner, replacing the phenotypic covariance by the genotypic covariance between the two characters and the phenotypic variances by the genotypic variances.

$$\gamma_G\,(x,y) = \frac{Cov_{(G)}\,(x,y)}{\sqrt{V_{(G)}\,x \;X\; V_{(G)}\,y}}$$

3.7 SCREENING FOR RESISTANCE TO YVMV

The parental species, F_{12} generation selections and Punjab 8 were subjected to standard screening techniques to assess their reaction to YVMV.

3.7.1 Field Screening

The treatment plants were selected for testing resistance to YVMV by providing sufficient amount of virus inoculum by planting highly susceptible variety 'Salkeerthi' in boarder rows.

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

Grade	Per cent leaves infected
0	No symptom
1	< 25
2	25-50
3	51-75
4	76-90
5	> 90

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

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

Per cent Disease Severity (PDS) was calculated using the formula given below,

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

Based on the per cent disease incidence and severity, coefficient of infection (CI) was calculated as suggested by Ravishankar (2012).

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

Based on the CI the genotypes were categorized into six categories as shown below (Ravishankar, 2012).

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)

3.7.2 Artificial inoculation of virus

3.7.2a Whitefly transmission

The promising F_{12} selections which were found to be resistant in the field screening were selected for this study along with the susceptible variety Salkeerthi. Whitefly (*Bemisia tabaci*), the vector of YVMV was used for artificial inoculation. Whiteflies were subjected to pre-acquisition fasting for half an hour and then for acquisition access for 24 h on YVMV infected plants followed by 24 h inoculation access period. Inoculated seedlings were kept under net house conditions for symptom expression.

3.2.2b Graft transmission technique

Artificial inoculation of YVMV was done through grafting technique. The method suggested by Kapoor and Varma (1950) to confirm the level of resistance to YVMV in the selected plants.

The existing healthy F_{12} generation plants in the field were pruned and retained for the development of new shoots. One month old YVMV infected Salkeerthi seedlings raised in polybags were grafted with newly emerged shoots of healthy plants.The grafted portions were tied with polythene tape and covered with moistened cotton. The daily observations were taken for about a month for symptom expression in resistant genotypes.

3.8 POLLEN FERTILITY STUDIES

For studying the pollen fertility in the treatment plants pollen grains were collected from flowers within 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. It was kept for five minutes for staining and then covered with a cover slip and observed under a microscope at different fields. In each field, the numbers of stained and unstained pollen grains were noted. The pollen fertility per cent was assessed by calculating the mean stained and unstained pollen grains.

3.9 EXTRACTION OF MUCILAGE

The mucilage content of the edible stage fruits was estimated by extracting the mucilage with ethyl alcohol (Thampi, 1998).

Twenty five grams of fresh fruit sample was taken, with that 100 ml of distilled water was added and kept for 24 h. Thus it was filtered through a muslin cloth into a flask. Fifty ml of alcohol was added to the flask and then it was filtered through a pre weighed filter paper. The filtrate along with the filter paper was dried

and weighed. The percentage of the mucilage content was calculated by the formula given below.

$$\text{Percentage of mucilage} = \frac{B - A}{\text{Weight of sample taken}} \times 100$$

B - Weight of the filter paper with mucilage

A - Weight of the filter paper alone

3.10 Molecular characterization

Molecular marker analysis of six okra genotypes (four YVMV resistant advanced generation selections along with their parents) were carried out with two marker systems viz. Random Amplified Polymorphic DNA (RAPD) and ISSR Inter simple sequence repeats (ISSR).

3.10.1 Genomic DNA isolation

Young tender, green leaves (first to third from the tip) were collected on ice from individual plants. The surface was cleaned by washing with sterile water and wiping with 70 per cent alcohol. The fresh leaves were ground to a fine powder with β -mercaptoethanol and PVP using ice-cold mortar and pestle in order to prevent browning due to phenol oxidase activity.

Among the most commonly used protocols, CTAB method developed by Singh and Kumar (2012) was used for the extraction of genomic DNA.

Procedure:

- 0.5 gram of clean leaf tissue was ground in pre-chilled mortar and pestle in the presence of β -mercaptoethanol and PVP.
- The homogenized sample was transferred into an autoclaved 2ml centrifuge tube and 1ml of pre-warmed extraction buffer was added.
- The contents were mixed well and incubated at 65ºC for 30 minutes with occasional mixing by gentle inversion.
- Equal volume (1ml) of chloroform: isoamyl alcohol (24:1) was added and mixed by inversion to emulsify.
- Spun at 12,000 rpm for 15 minutes at 4ºC.
- After centrifugation the contents got separated into three distinct phases.

Aqueous topmost layer	-	DNA and RNA
Interphase	-	fine particles and proteins
Lower layer	-	Chloroform, pigments and cell debris

- Transferred the top aqueous layer to a clean centrifuge tube and added equal volume of chloroform: isoamyl alcohol (24:1) and mixed by inversion.
- Centrifuged at 12,000 rpm for 15 minutes at 4ºC.
- Transferred the aqueous phase into a clean centrifuge tube and added 0.6 volume of chilled isopropanol and mixed by quick gentle inversion till DNA was precipitated. Kept at -20ºC for half an hour for complete precipitation.

- Centrifuged at 10,000 rpm for 15 minutes at 4ºC. Gently poured off the supernatant.
- Washed the DNA pellet with 70 per cent ethanol followed by 100 per cent ethanol.
- Spun for 5 min at 10,000 rpm and decanted the ethanol.
- Air dried the pellet, dissolved in 50µl of sterilized water and stored at -20ºC.

3.10.2 Purification of DNA

The DNA which had RNA as contaminant was purified by RNase treatment and further precipitation.

Procedure

- To 100 µl DNA sample, RNase solution (2 µl) was added and incubated at 37^0C in dry bath for 1 hour.
- The volume was made up to 250 µl with distilled water.
- Added equal volume of chloroform: isoamyl alcohol (24: 1) mixture and mixed gently.
- Centrifuged at 12,000 rpm for 15 minutes at 4^0C.
- Transferred the aqueous phase into a fresh micro centrifuge tube and added equal volume of chloroform: isoamyl alcohol (24: 1).
- Centrifuged at 12,000 rpm for 15 minutes at 4^0C.

- Transferred the aqueous phase into a clean centrifuge tube and added 0.6 volume of chilled isopropanol and mixed by quick gentle inversion till the DNA precipitated. Kept at -20ºC for half an hour for complete precipitation.

- Incubated the mixture at -20^{0}C for 30 minutes and centrifuged at 10,000 rpm for 15 minutes at 4^{0}C.

- Washed the DNA pellet with 70 per cent ethanol

- Air dried the pellet, dissolved in 50 to 100 µl sterilized water.

3.10.3 Quantification of DNA

3.10.3.1 *Assessment of quality and quantity of DNA by electrophoresis*

The quality of isolated DNA was evaluated through agarose gel electrophoresis.

Procedure

- The gel tray was prepared by sealing the ends with tape. Comb was placed in gel tray about 1 inch from one end of the tray and positioned the comb vertically such that the teeth are about 1 to 2 mm above the surface of the tray.

- Prepared 0.8 per cent agarose (0.8 g in 100ml) in a glass beaker or conical flask with 100 ml 1X TAE buffer. Micro waved for 45 to 60 seconds until agarose was dissolved and solution was clear.

- Solution was allowed to cool to about 42 to 45 ^{0}C before pouring. (4µl Ethidium bromide was added at this point to a concentration of 0.5 µg/ml) and mixed well.

- Poured this warm gel solution into the tray to a depth of about 5 mm. allowed the gel to solidify for about 30 to 45 minutes at room temperature.

- To run, gently removed the comb and the tape used for sealing, placed the tray in electrophoresis chamber, and covered (just until wells are submerged) with electrophoresis buffer (the same buffer used to prepare the agarose).

- To prepare samples for electrophoresis, added 1 µl of 6x gel loading dye for every 5µl of DNA solution. Mixed well and loaded 6µl DNA sample per well. Loaded suitable molecular weight marker (λDNA *Eco*RI/ *Hind*III double digest) in one lane.

- Electrophoresed at 70 volts until dye has migrated two third the length of the gel.

- Intact DNA appears as orange fluorescent bands. If degraded, it appears as a smear because of the presence of a large number of bands, which differ in base length. The gel profile was examined for intactness, clarity of DNA band, presence of RNA and protein.

3.10.3.2 *Gel Documentation*

Gel documentation was done with BioRad Gel Documentation System using PDQuest™ software. PDQuest is a software package for imaging, analysing, and databasing 2-D electrophoresis gels. PDQuest can acquire images of gels using any of several Bio-Rad imaging systems. An image of a gel is captured using the controls in the imaging device window and displayed on computer screen.

3.9.4 Assessing the quality and quantity of DNA by Nanodrop spectrophotometer

The purity of DNA was further checked by using NanoDrop ND-1000 spectrophometer. Nucleic acid shows absorption maxima at 260nm whereas proteins show peak absorbance at 280nm. Absorbance is recorded at both wavelength and

purity is indicated by the ratio OD_{260}/OD_{280}. The values between 1.8 and 2.0 indicate that the DNA is pure and free from proteins. The quantity of DNA in the pure sample was calculated using the relation 1 OD_{260} equivalent to 50µg double stranded DNA/ml sample.

1 OD at 260 nm = 50 µg DNA/ µl

Therefore $OD_{260} \times 50$ gives the quantity of DNA in µg/ µl.

Procedure for quantity detection using Nanodrop spectrophotometer

- Connected the Nanodrop spectrophotometer to the System and open the operating software ND-100.
- Selected the option Nucleic acid.
- With the sampling arm open, pipetted 1µl distilled water onto the lower measurement pedestal.
- Closed the sampling arm and initiated a spectral measurement using the operating software on the PC. The sample column is automatically drawn between the upper and lower measurement pedestals and the spectral measurement is made.
- Set the reading to zero with sample blank.
- 1µl sample was pipetted onto measurement pedestal and select measure.
- When the measurement was complete, opened the sampling arm and wiped the sample from both the upper and lower pedestals using a soft laboratory wipe. Simple wiping prevents sample carryover in successive measurements for samples varying by more than 1000 fold in concentration.

3.10.5 Molecular Markers used for the study

Two different marker systems were used for the study which included RAPD and ISSR assays. Under each marker analysis system, six genotypes were amplified separately with the selected primers.

3.10.5.1 ***RAPD (Random Amplified Polymorphic DNA)***

Good quality genomic DNA (30-40 µg/ µl) isolated from six okra genotypes by CTAB method were subjected to RAPD analysis as per the procedure reported by Williams *et al*. (1990). Okra specific random decamer primers with good resolving power were used for amplification of DNA (Table 2). Four primers reported by Kaur *et al*. (2013) viz. OPC-2, OPC-9, OPC-18 and OPB-2., three primers reported by Aladele *et al.* (2008) viz. OPX-18, UBC-465 and UBC-210 and three primers reported by Prakash *et al.* (2011) OPD-05, OPA-02 and OPT-02 were used for RAPD assay. The amplification was carried out in a thermal cycler (Agilent thermocycler^{R}).

Composition of the reaction mixture for PCR (20µl)

Materials	Quantity (µl)
a. Genomic DNA (40ng/µl)	2.0
b. 10X Taq assay buffer B	2.0
c. $Mgcl_2$	1.8
d. dNTPs (10mM)	1.5
e. Taq DNA polymerase (3U/µl)	0.4
f. Decamer primer	2.0
g. Autoclaved distilled water	10.3
Total volume	20

A master mixture with all reagents for the required number of reactions was prepared first and aliquots were dispensed into PCR tubes followed by addition of template DNA in each tube.

The PCR tubes were kept in the thermal cycler and the following programme was run.

Step 1.	93^0 c for 5 m	Initial denaturation.	
Step 2.	94^0 c for 1 m	Denaturation.	35 cycles
Step 3.	37^0 for 1 m	Primer annealing.	
Step 4.	72^0 for 1 m	Primer extension.	
Step 5.	72^0 for 10 m	Final extension.	
Step 6.	4^0 for infinity to hold the sample.		

The amplified products were run on 1 per cent agarose gel using 50X TAE buffer stained with ethidium bromide along with ladder (3kb). Electrophorosis was performed at 70 v. for 2 hours. The profile was documented using gel documentation system.

3.10.5.2 ***ISSR (Inter Simple Sequence Repeats)***

Good quality genomic DNA (30-40 ng/µl) isolated from six okra genotypes by CTAB method was subjected to ISSR analysis. ISSR primers with good resolving power were used for amplification of DNA. Ten ISSR primers reported by Yuan *et al*. (2014) in okra viz.UBC 811, UBC 817, UBC 818, UBC 823, UBC 830, UBC 834, UBC 842, UBC 846, UBC 848 and UBC 857 were used for ISSR assay. The amplification was carried out in a thermal cycler (Agilent thermocycler^R).

Composition of the reaction mixture for PCR (20µl)

Materials	Quantity (µl)
a. Genomic DNA (40 ng/µl)	2.0
b. 10X Taq assay buffer B	2.0
c. $Mgcl_2$	1.5

d.	dNTPs (10mM)	1.5
e.	Taq DNA polymerase (3U/µl)	0.4
f.	Primer	2.0
g.	Autoclaved distilled water	10.3
	Total volume	20

A master mixture with all reagents for the required number of reactions was prepared first and aliquots were dispensed into PCR tubes followed by addition of template DNA in each tube.

The PCR tubes were kept in the thermal cycler and the following programme was run.

Step 1.	93^0 c for 5 m	Initial denaturation.	
Step 2.	94^0 c for 1 m	Denaturation.	40 cycles
Step 3.	47^0 for 1 m	Primer annealing.	
Step 4.	72^0 for 1 m	Primer extension.	
Step 5.	72^0 for 10 m	Final extension.	
Step 6.	4^0 for infinity to hold the sample.		

The amplified products were run on 1.5 per cent agarose gel using 50X TAE buffer stained with ethidium bromide along with ladder (3kb). Electrophorosis was performed at 70 v. for 2 hours. The ISSR profile was documented using gel documentation system.

Table 2. RAPD primers used for characterizing okra genotypes

Sl. No.	Primer name	Nucleotide sequence (5'-3')	Annealing temp. (^{0}C)
1	OPC2	GTGAGGCGTC	37
2	OPC9	CTCACCGTCC	37
3	OPC18	TGAGTGGGTG	37
4	OPB2	TGATCCCTGG	37
5	OPX 18	GACTAGGTGG	37
6	UBC 465	GGTCAGGGCT	37
7	UBC 210	GCACCGAGAG	37
8	OPD 05	TGAGCGGACA	37
9	OPA 02	TGCCGAGCTG	37
10	OPT 02	GGAGAGACTC	37

Table 3. ISSR primers used for characterizing okra genotypes

Sl. No.	Primers name	Nucleotide sequence (5'-3')	Annealing temp. (^{0}C)
1	UBC 811	GAGAGAGAGAGAGAGAC	47
2	UBC 817	CACACACACACACACAA	47
3	UBC 818	CACACACACACACACAG	47
4	UBC 823	TCTCTCTCTCTCTCTCC	47
5	UBC 830	TGTGTGTGTGTGTGTGG	47
6	UBC 834	AGAGAGAGAGAGAGAGCT	47
7	UBC 842	GAGAGAGAGAGAGAGATG	47
8	UBC 846	CACACACACACACACAAT	47
9	UBC 848	CACACACACACACACAAG	47
10	UBC 857	ACACACACACACACACCG	47

Results

4. RESULTS

The data collected on the evaluation of eight F_{12} generation selections along with the parental varieties and Punjab 8 was tabulated and subjected to statistical analysis. The results obtained from the experiments are presented under following heads.

4.1 EVALUATION OF GENOTYPES

4.1.1 Qualitative characters

Leaf margin was deeply fid in *A. esculentus* and Punjab 8, but in *A. caillei* it was narrowly fid. In F_{12} lines it was varied from narrowly fid to deeply fid. Flower colour was yellow in the parents, F_{12} lines and Punjab 8. Flower size was medium in both the parental species and Punjab 8 where as advanced generation selections had large and medium sized flowers. Parents, Punjab 8 and F_{12} selections had purple throat at base of corolla in the inside and colour of leaf vein was green with purple tinge. Color of leaf base was red with green tinge in the parents, selections and Punjab 8. Colour of fruit was light green in *A. esculentus* whereas that in *A. caillei* and Punjab 8 was green. The F_{12} selections produced green, light green and dark green fruits. Pod pubescence was absent in both the parents and Punjab 8 but the F_{12} lines were slightly pubescent (Table 4). The variability expressed by the genotypes in epicalyx segment, fruit and leaf is shown in the Plate 2.

4.1.2 Quantitative characters

Mean values for the 20 characters relating to different treatments are given in Table 5. Analysis of variance for different quantitative characters is given in Table 6.

Height of the plant varied significantly among the treatments. The mean values ranged from 86.623 cm in T10 to 188.733 cm in T7.

2a. Variability in leaf shape and size

2b. Variability in epicalyx segment

2c.Variability in fruit shape and size

Plate 2. Variability in leaf, epicalyx segment and fruit

Significant differences were observed for internodal length. The mean values for the character ranged from 6.467 cm in T10 to 13.63 cm in T7.

Significant differences were observed for number of primary branches among the treatments. The mean values for the character ranged from 2.29 (T8) to 3.30 (T7).

Length of epicalyx segement revealed significant differences among the treatments. The maximum and minimum values for the character were recorded in T1 (1.913) and T14 (1.347) respectively.

Width of epicalyx segement varied significantly among the genotypes. The means ranged from 0.357 in T11 to 1.07 in T9.

Petiole length revealed high significant differences among the treatments. The mean values for the character ranged from 13.28 cm in T11 to 43.707 cm in T7.

High significant differences were present among the treatments for days to first flowering. The mean values for the character ranged from 40.487 in T11 to 57.94 in T4.

Days to first harvest revealed significant differences among the treatments. The maximum and minimum mean values for the character were recorded in T4 (64.38) and T11 (40.48) respectively.

Number of harvests revealed significant differences among the genotypes. The means ranged from 6.35 (T10) to 13.37 (T7).

Significant differences were noticed for first fruiting node among the genotypes. The mean values ranged from 4.24 (T10) to 6.543 (T5).

Table 4. Comparison of qualitative characters of parental species, Punjab 8 and F_{12} selections

Treatments	Leaf margin	Flower colour	Flower size	Purple throat at base of corolla	Colour of leaf vein	Colour of leaf base	Colour of fruit	Pod pubescence
T1	Narrowly fid	Yellow	Large	Present inside	Green with purple tinge	Red with Green tinge	Dark green	Slightly pubescent
T2	Narrowly fid	Yellow	Large	Present inside	Green with purple tinge	Red with Green tinge	Dark green	Slightly pubescent
T3	Narrowly fid	Yellow	Large	Present inside	Green with purple tinge	Red with Green tinge	light green	Slightly pubescent
T4	Narrowly fid	Yellow	Large	Present inside	Green with purple tinge	Red with Green tinge	Dark green	Slightly pubescent
T5	Deeply	Yellow	Medium	Present inside	Green with purple tinge	Red with Green tinge	Light green	Slightly pubescent
T6	Narrowly fid	Yellow	Large	Present inside	Green with purple tinge	Red with Green tinge	Light green	Slightly pubescent
T7	Narrowly fid	Yellow	Large	Present inside	Green with purple tinge	Red with Green tinge	Light green	Slightly pubescent
T8	Narrowly fid	Yellow	Medium	Present inside	Green with purple tinge	Red with Green tinge	Light green	Slightly pubescent
T9(P1)*	Narrowly fid	Yellow	Large	Present inside	Green with purple tinge	Red with Green tinge	Light green	Slightly pubescent
T10 (P2)*	Deeply	Yellow	Medium	Present inside	Green with purple tinge	Red with Green tinge	Light green	Absent
T11	Deeply	Yellow	Medium	Present inside	Green with purple tinge	Red with Green tinge	Light green	Absent

Fruit length varied significantly among the treatments. The means ranged from 9.733 cm in T11 to 20.127 cm in T1.

Fruit girth revealed significant differences among the treatments. The maximum and minimum values for the character were recorded in T7 (7.8) and T11 (5.910).

Significant differences were observed for number of locules per pod. The mean values ranged from 4.10 in T10 to 6.64 in T2.

Number of fruits per plant varied significantly among the genotypes. The maximum and minimum values for the character were recorded as 15.94 in T7 and 6.36 in T11 respectively.

Number of ridges per pod varied significantly among the treatments. The means ranged from 4.18 (T10) to 6.75 (T7).

Significant differences were observed for duration of the crop among the treatments. The mean values ranged from 96.803 days in T10 to 180.127 days in T7.

Yield per plant exhibited high significant differences among the treatments. Yield was maximum for T7 (381.770 g) and minimum for T10 (112.513 g).

The differences in pollen sterility (%) among the treatments were highly significant with both the parents. The mean values for pollen sterility ranged from 0.31 in T9 to 2.31 in T5. Parent P1 (T9) was on par with parent P2 (T10) in pollen sterility**.**

Significant differences were observed among the treatments for mucilage content (g/100g). The mean values were ranged from 0.353 (T1) to 0.613 (T15).

Table5. Quantitative characters of F_{12} selections, parental species and Punjab 8

Treatments	Plant Height (cm)	Internodal length (cm)	No. of primary branches	Length of epicalyx segment (cm)	Width of epicalyx segment (cm)	Petiole length (cm)	Days to flower	Days to first harvest	Number of harvests	First fruiting node
	1	2	3	4	5	6	7	8	9	10
T1	182.200	12.233	2.490	1.913	0.931	39.240	55.100	61.050	13.133	5.303
T2	167.007	12.333	2.350	1.953	0.833	40.320	49.450	55.917	10.790	5.700
T3	163.343	11.433	2.553	1.877	0.899	38.583	54.883	60.333	9.083	5.487
T4	159.003	11.167	2.467	1.860	0.950	42.470	57.940	64.380	9.973	6.393
T5	126.987	8.417	2.333	1.707	0.821	34.300	57.717	64.303	6.497	6.543
T6	176.617	10.817	2.500	1.917	0.966	36.000	54.617	60.700	13.037	5.150
T7	188.733	13.633	3.300	1.833	0.940	43.707	49.333	55.400	13.37	4.533
T8	131.843	8.603	2.290	1.637	0.630	35.150	50.883	56.737	6.907	6.303
T9(P1)*	174.260	12.170	3.080	1.793	1.073	40.880	57.800	65.287	11.500	5.603
T10 (P2)*	86.623	6.467	2.833	1.563	0.357	14.820	40.703	46.403	6.350	4.240
T11	99.627	6.717	3.017	1.347	0.383	13.283	40.487	46.417	6.397	4.683
CD	8.26	1.93	0.453	0.12	0.07	8.87	1.32	1.54	0.63	0.513

*P1: Parent 1 (*A. caillei*)

*P2: Parent 2 (*A. esculentus*)

T1-T8: F_{12}-1 to F_{12}-8 selections

T11: Punjab 8

Table 5. Quantitative characters of F_{12} selections, parental species and Punjab 8 continued..

Treatments	Fruit length (cm) 11	Fruit girth (cm) 12	Locules per pod 13	No. of fruits per plant 14	No. of ridges per pod 15	Crop duration (days) 16	Yield per plant (g) 17	Pollen sterility (%) 18	Mucilage content (g/100g) 19	Coefficient of infection 20
T1	20.127	7.010	5.900	14.883	6.267	176.353	376.223	1.280	0.353	0.000
T2	17.450	6.440	6.647	13.660	6.647	166.853	329.890	1.337	0.460	0.000
T3	17.783	6.233	5.340	11.860	5.473	168.200	280.277	1.467	0.443	6.797
T4	17.410	6.203	5.367	12.320	5.397	162.187	258.340	1.350	0.387	6.143
T5	17.017	6.253	5.467	7.850	5.483	136.790	135.367	2.313	0.430	40.573
T6	18.537	6.667	5.433	13.897	5.433	164.557	355.283	1.377	0.613	0.000
T7	15.600	7.800	6.43	15.940	6.755	180.127	381.770	1.293	0.453	0.000
T8	15.243	6.600	5.433	7.650	5.433	148.403	166.413	1.913	0.430	31.963
T9(P1)*	16.160	6.940	5.797	13.437	5.797	172.820	339.423	0.313	0.637	2.283
T10 (P2)*	10.042	5.032	4.100	7.267	4.180	96.803	112.513	0.377	0.450	99.847
T11	9.733	5.910	4.300	6.367	4.300	127.070	124.490	1.377	0.493	42.963
CD	2.43	0.89	0.42	4.91	0.51	8.81	25.88	0.05	0.093	2.49

*P1: Parent 1 (*A. caillei*)

*P2: Parent 2 (*A. esculentus*)

T1-T8: F_{12}-1 to F_{12}-8 selections

T11: Punjab 8

Table 6: ANOVA (mean squares) for different quantitative character

SI.No	Characters	Treatments	Replications	Error
1	Plant height (cm)	35337.87	0.10	2.34
2	Internodal length (cm)	17.61**	1.02	1.03
3	Number of primary branches	0.35**	0.02	0.0
4	Length of epicalyx segment (cm)	0.17**	0.00	0.00
5	Width of epicalyx segment (cm)	0.16**	0.00	0.00
6	Petiole length (cm)	349.67**	2.98	2.58
7	Days to flower	120.16**	0.51	0.60
8	Days to first harvest	131.05**	2.07	0.82
9	Number of harvests	24.29**	0.39	0.31
10	First fruiting node	01.77**	0.01	0.0
11	Fruit length (cm)	32.16**	0.1	2.05
12	Fruit girth (cm)	1.35**	0.61	0.27
13	Locules per pod	1.74**	0.32	0.06
14	Number of fruits per plant	74.15**	4.63	2.65
15	Number of ridges per pod	2.01**	0.44	0.10
16	Crop duration (days)	1920.75	4.05	2.34
17	Yield per plant (g)	3199.68**	0.64	0.58
18	Pollen sterility (%)	0.98**	0.046	0.02
19	Mucilage content (g/100g)	0.2**	0.02	0.00
20	Coefficient of infection	304.77**	0.35	2.03

** Significant at 5% ; * Significant at 1%

4.2 GENETIC PARAMETERS

The genetic parameters, viz., the phenotypic and genotypic coefficients of variation, heritability and genetic advance for each character under study were estimated and presented in Table 7.

4.2.1 Phenotypic and genotypic coefficients of variation

The maximum value for phenotypic and genotypic coefficient of variation was recorded for coefficient of infection of YVMV (149.00 and 148.85 respectively) followed by Yield per plant (39.91 and 39.51 respectively) (Fig 1).

The phenotypic coefficient of variation was minimum length of epicalyx segment (10.98) followed by days to first harvest (11.49), days to flower (12.30) and Fruit girth (12.37).

The genotypic coefficient of variation was minimum for Fruit girth (9.29) followed by length of epicalyx segment (10.22), days to first harvest (11.38) and days to flower (12.21).

4.2.2 Heritability and genetic advance

Majority of the characters exhibited very high heritability, the minimum being 59.9 for number of primary branches. The heritability value was maximum (99.8) for coefficient of infection and days to flower (98.6) closely followed by days to first harvest (99.7) (Fig 2).

Maximum genetic advance was exhibited by yield per plant (210.08) followed by plant height (96.84) and coefficient of infection (64.19). Most of the characters exhibited low genetic advance values and the minimum value expressed for the character, length of epicalyx (0.34) followed by width of epicalyx segment (0.48) and number of primary branches (0.50).

4.3 CORRELATION STUDIES

The data relating to the characters studied in the F_{12} generation were subjected to correlation analysis and the results are presented in Table 8.

4.3.1 Phenotypic correlation

Days to first flower exhibited high positive phenotypic correlation with first fruiting node, locules per pod, number of ridges per pod, plant height, and crop duration and significant negative correlation with Fruit length, number of fruits per plant, number of harvests per plant and coefficient of infection.

First fruiting node had significant positive phenotypic correlation with days to flower, days to first harvest, number of ridges and petiole length and significant negative correlation with Fruit length, number of fruits per plant, number of harvests per plant yield per plant and coefficient of infection.

Days to first harvest was having high positive phenotypic correlation with first fruiting node, Fruit length, locules per pod, number of ridges per pod and crop duration significant negative correlation with yield per plant.

Number of primary branches had high positive phenotypic correlation with plant height, number of harvest and crop duration and negatively correlated with days to first harvest, Fruit girth and locules per pod.

Fruit length had significant positive phenotypic correlation with days to first harvest, plant height, number of harvest and yield per plant and highly significant negative correlation with Fruit girth and petiole length.

Table 7. Genetic parameters for different quantitative characters

Sl. No	Characters	PCV	GCV	Heritability	Genetic advance
1	Days to first flowering	12.30	12.21	98.6	12.91
2	First flowering node	14.68	13.83	88.6	1.46
3	Days to first harvest	11.48	11.38	98.1	13.44
4	Number of primary branches	15.15	11.73	59.9	0.50
5	Fruit length (cm)	21.83	19.91	83.1	5.95
6	Fruit girth (cm)	12.37	9.29	56.4	0.93
7	Locules per pod	14.44	13.69	89.9	1.46
8	Number of ridges	15.49	14.35	85.7	1.52
9	Number of fruits per plant	31.6	29.71	84.4	6.56
10	Plant height (cm)	22.95	22.73	98.1	96.84
11	Petiole length (cm)	33.63	28.78	73.3	17.43
12	Internodal length (cm)	25.03	22.51	80.9	4.3
13	Length of epicalyx segment (cm)	10.98	10.22	86.6	0.34
14	Width of epicalyx segment (cm)	30.22	29.81	97.3	0.48
15	Number of harvests	29.66	29.06	96.0	5.71
16	Crop Duration	16.60	16.26	95.9	50.70
17	Yield per plant (g)	39.91	39.53	98.1	10.08
18	Coefficient of Infection (%)	149.01	148.85	99.8	64.19

Significant positive correlation was observed for Fruit girth with locules per pod, number of ridges and plant height and negatively correlated with coefficient of infection.

Significant positive correlation was noticed for locules per pod with days to flower, days to first harvest, Fruit girth, number of ridges per pod and petiole length and intermodal distance and highly significant negative correlation with number of primary branches, number of harvests per plant and yield per plant.

Number of ridges per pod recorded highly significant positive phenotypic correlation with days to flower, first fruiting node, days to harvest, Fruit girth, locules per pod, petiole length and highly significant negative correlation with yield per plant and coefficient of infection.

Significant positive phenotypic correlation was observed for Number of fruits per plant with plant height, internodal distance, number of harvests, crop duration and yield per plant while the correlation was negative and significant with days to flower, first fruiting node and coefficient of infection.

Plant height had significant positive correlation with number of primary braches, Fruit length, Fruit girth, fruits per plant, internodal distance, crop duration and yield per plant and significant negative correlation with coefficient of infection.

Petiole length showed high positive phenotypic correlation with first fruiting node, locules per pod, number of ridges per pod, and significant negative correlation with Fruit length, number of harvests per plant and coefficient of infection.

Positive phenotypic correlation was observed for internodal length with locules per plant, number of fruits per plant and plant height and significant negative correlation with width of epicalyx and coefficient of infection.

Length of epicalyx segment showed significant positive phenotypic correlation with plant height, internodal length, width of epicalyx and significant negative phenotypic correlation was presented for duration of crop, yield per plant and coefficient of infection.

Significant negative correlation was observed for width of epicalyx segment with number of primary branches, internodal length, length of epicalyx segment and number of harvest and significant negative correlation with coefficient of infection.

Number of harvests per plant recorded highly significant positive phenotypic correlation with number of primary branches, Fruit length, number of fruits per plant, intermodal length, width of epicalyx, duration of crop and yield and significant negative correlation with days to flower, days to first harvest, fruiting node, locules per pod, number of ridges per pod, petiole length and coefficient of infection.

Crop duration showed high positive phenotypic correlation with days to flower, days to first harvest, and number of fruits per plant, plant height internodal length, width of epicalyx crop duration and yield per plant and highly significant negative correlation with length of epicalyx and coefficient of infection.

Yield per plant showed high positive phenotypic correlation with number of primary branches Fruit length, number of fruits per plant, plant height, crop duration and number of harvests per plant and highly significant negative correlation with first flowering node, days to first harvest, locules per pod and number of ridges per pod, length of epicalyx and coefficient of infection.

Coefficient of infection exhibited significantly negative correlation with all the characters observed.

4.3.2 Genotypic correlation

Days to flower had high positive genotypic correlation with first fruiting node, Fruit girth, locules per pod, number of ridges per pod, pollen sterility, petiole length and days to first harvest and significant negative correlation with number of fruits per plant and number of harvests per plant and coefficient of infection.

First fruiting node had significant positive genotypic correlation with days to flower, days to first harvest, number of ridges per pod, petiole length, days to first flowering, days to first harvest, and significant negative correlation with number of fruits per plant and duration of crop and number of harvests per plant and coefficient of infection.

Days to first harvest showed significant positive genotypic correlation with first flowering node, Fruit girth, locules per pod, number of ridges per pod, plant height and significant negative correlation with number of fruits per plant and coefficient of infection.

Number of primary branches had significant positive correlation with plant height and significant negative genotypic correlation with number of ridges per pod, crop duration and coefficient of infection.

Fruit length had significant positive genotypic correlation with first fruiting node, Fruit girth, number of harvests per plant, yield per plant and highly significant negative correlation with locules per pod, number of primary branches, petiole length, inter nodal length and coefficient of infection.

Fruit girth showed high positive genotypic correlation with days to flowering, days to harvest, Fruit length, number of harvests and significant negative correlation with locules per pod, petiole length, internodal length and coefficient of infection.

Significant positive correlation was noticed for locules per pod with days to flowering, days to harvest, Fruit girth, number of ridges per pod, petiole length, and highly significant negative correlation with Fruit length, number of fruits per plant, internodal length and coefficient of infection.

Number of ridges per pod recorded highly significant positive genotypic correlation with days to flowering, days to harvest, Fruit girth, locules per pod, and highly significant negative correlation with number of harvests per plant, duration of crop, yield per plant and coefficient of infection.

Number of fruits per plant showed significant positive genotypic correlation with plant height, intermodal length, crop duration, number of harvests and yield per plant, while the correlation was negative and significant with days to flowering, first fruiting node and days to harvest, Fruit girth, locules per pod, petiole length and coefficient of infection.

Plant height had high positive genotypic correlation with days to flowering, days to harvest, number of primary branches, number of fruits per plant, number of harvests per plant and significant negative correlation with coefficient of infection.

Petiole length had high positive genotypic correlation with days to flowering, first fruiting node days to harvest, locules per pod, intermodal distance, length of epicalyx, width of epicalyx, number of harvest, and significant negative correlation with Fruit length, number of fruits per plant and coefficient of infection.

Internodal length had high positive genotypic correlation with first fruiting node, days to harvest, number of fruits per plant, length of epicalyx segment, width of epicalyx segment, number of harvest and crop duration and significant negative correlation with Fruit length Fruit girth, locules per pod and coefficient of infection.

Length of epicalyx segment showed significant positive correlation with days to harvest, width of epicalyx segment, number of harvest and significant negative genotypic correlation with Fruit girth and coefficient of infection.

Width of epicalyx segment had significant positive correlation with days to flowering, days to harvest, number of harvest and significant negative genotypic correlation with Fruit girth and coefficient of infection.

Number of harvests per plant had high positive genotypic correlation with Fruit length, number of fruits per plant, plant height, crop duration, yield per plant and significant negative correlation with days to flower, locules per pod, number of ridges per pod and coefficient of infection.

Duration of crop showed high positive genotypic correlation with number of fruits per plant yield per plant and highly significant negative correlation with number of primary branches and coefficient of infection.

Yield per plant showed high positive genotypic correlation with days to harvest, Fruit length, Fruit girth, number of fruits per plant and highly significant negative correlation with first fruiting node, number of ridges and coefficient of infection. Coefficient of infection exhibited significantly negative correlation with all the characters observed.

Table 8. Genotypic and phenotypic correlation coefficients for 18 characters in the treatment genotypes

	1	2	3	4	5	6	7	8	9	10
1. Days to first flowering		0.686*	0.396	-0.344	-0.620*	0.410	0.732*	0.471*	-0.471*	0.356
2. First flowering node	0.736**		0.486*	-0.369	0.481	0.290	0.284	0.634*	-0.995*	0.164
3. Days to first harvest	0.597	0.737**		-0.324*	0.808*	0.409	0.523*	0.673*	-0.468*	0.349
4. Number of primary branches	-0.416	-0.102	-0.381		-0.448	-0.535*	-0.995**	-0.070	0.228	0.652*
5. Fruit length	0.473	0553*	0.550	-0.521**		-0.471*	0.381	-0.255	0.129	0.773*
6. Fruit girth	0.548*	0.138	0.556*	0.190	0.696**		0.653*	0.708*	0.250	0.739*
7. Locules per pod	0.741*	0.334	0.548*	-0.143	-0.656**	0.914**		0.936**	0.238	0.107
8. Number of ridges	0.613*	0.220	0.578*	-0.429	0.367	0.928**	0.915**		0.255	0.311
9. Number of fruits per plant	-0.508*	-0.735**	-0.512*	0.131	0.350	-0.826**	-0.403*	0.151		0.928**
10. Plant hight	0.668**	0.165	0.664**	0.835**	0.136	0.128	0.359	0.285	0.962**	
11. Petiol length	0.850**	0.571*	0.744**	-0.259	-0.859**	0.217	0.890**	0.405	-0.616*	0.108
12. Internodal distance	0.203	0.614*	0.608**	0.037	-0.821**	-0.653*	-0.525**	0.456	0.779**	0.489
13. Length of epicalyx	0.346	0.385	0.724**	-0.425	0.168	-0.499*	0.257	0.551	-0.628	0.239
14. Width of epicalyx	0.880**	0.412	0.885**	-0.090	0.389	-0.399*	0.171	0.467	0.347	0.327
15. Number of harvests	-0.438*	-0.160	0.440	0.209	0.691**	0.465	-0.610*	-0.759**	0.987**	0.925**
16. Crop Duration	0.467	0.363	0.464	-0.550**	0.534	0.288	0.356	-0.874**	0.887**	0.279
17. Yield per plant	0.489	-0.753**	0.488*	0.116	0.749**	0.876**	0.493	-0.837**	0.989**	0.464
18. Coefficient of Infection	-0.655**	-0.323	-0.654**	-0.757	-0.8155**	-0.899**	-0.807**	-0.801**	-0.831**	-0.931**

Table 8. Genotypic and phenotypic correlation coefficients for 18 characters in the treatment genotypes continued...

	11	1 2	1 3	14	15	16	17	18
1. Days to first flowering	0.469	0.529	0.393	0.265	-0.482*	0.648*	-0.419	-0.651*
2. First flowering node	0.662*	0.100	0.294	0.370	-0.136	-0.448	-0.921**	-0.298
3. Days to first harvest	0.368	0.524	0.415	0.167	0.422	0.624*	-0.476*	-0.648*
4. Number of primary branches	-0.155	0.207	-0.410	0.679*	0.483*	0.492	0.647*	-0.064*
5. Fruit length	-0.693*	0.320	0.206	0.126	0.616*	0.442	0.674*	-0.745*
6. Fruit girth	0.312	0.246	0.364	0.344	0.431	0.513	0.679	-0.686*
7. Locules per pod	0.664*	0.790*	0.345	0.332	-0.680*	0.213	-0.657*	-0.773*
8. Number of ridges	0.706**	0.200	0.319	0.208	0.409	0.306	-0.760*	-0.747**
9. Number of fruits per plant	-0.299	0.923**	0.213	0.609	0.941**	0.845**	0.959**	-0.783*
10. Plant hight	0.326	0.933**	0.769**	0.412	0.403	0.652*	0.959**	-0.920**
11. Petiol length		0.302	0.231	0.851	-0.620*	0.302	0.207	-0.777**
12. Internodal distance	0.922**		0.701**	0.824**	0.824**	0.470	0.401	-0.824**
13. Length of epicalyx	0.869**	0.839**		0.795*	0.466	-0.597*	-0.637*	-0.680**
14. Width of epicalyx	0.953**	0.904**	0.844**		0.759**	0.171	0.214	-0.851**
15. Number of harvests	0.9707**	0.918**	0.735**	0.785**		0.831**	0.960**	-0.778**
16. Crop Duration	0.324	0.583*	0.448	0.496	0.831**		0.896**	-0.961**
17. Yield per plant	0.476	0.466	0.392	0.422	0.978**	0.911**		-0.849**
18. Coefficient of Infection	-0.864**	-0.910**	-0.726**	-0.864**	-0.792**	-0.979**	-0.859**	

** Significant at 5% ; * Significant at 1%

4.4 POLLEN FERTILITY STUDIES

The pollen fertility of parental lines, Punjab 8 and F_{12} selections was studied by staining with one per cent acetocarmine. Pollen fertility in the parental species *A. esculentus* variety Salkeerthi was as high as 99.68 per cent (Plate 3a) and *A. caillei* variety Susthira recorded 99.62 per cent pollen stainability (Plate 3b). Variety Punjab 8 recorded 98.68 per cent pollen stainability (Plate 3c). The highly YVMV resistant and high yielding plants selected out of F_{12} selections viz., F_{12}-1 F_{12}-2, F_{12}-6 and F_{12}-7 expressed high levels of pollen fertility in the range of 98.63 to 98.72 (Plate 3d).

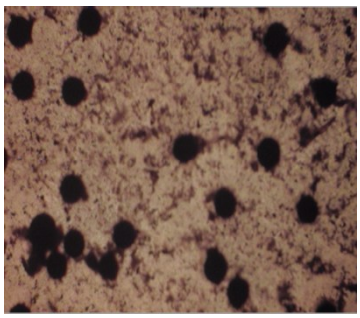
4.5 REACTION TO YVMV

4.5.1 Field screening

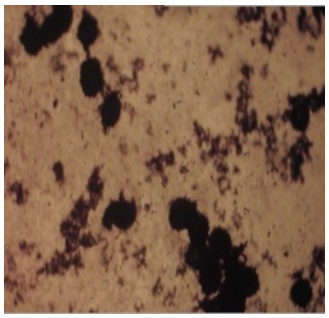
Field screening trial for resistance to YVMV showed that the parent *A. esculentus* variety Salkeerhti as highly susceptible (CI=69.21) whereas, the other parental species *A. caillei* as resistant (CI=99.847) and four F_{12} selections (F_{12}-1, F_{12}-2, F_{12}-6 and F_{12}-7) were completely free from YVMV. Variety Punjab 8 recorded coefficient of infection of 42.96 (Susceptible). The other F_{12} lines showed coefficient of infection ranging from 6.14 to 40.57 (Moderately resistant to susceptible). Reaction of the parental species, Punjab 8 and F_{12} selections to YVMV in the field screening studies is given in Table 9.

4.5.2 Screening by whitefly transmission

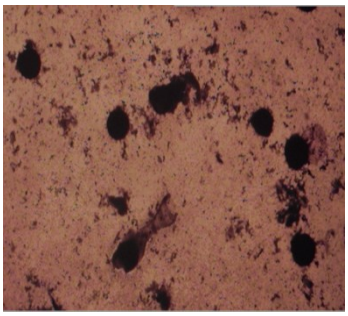
For the confirmation of disease resistance whitefly transmission studies were carried out. Four genotypes (F_{12}-1, F_{12}-2, F_{12}-6 and F_{12}-7) which found highly resistant in the field screening were artificially inoculated with viruliferous whiteflies and their reaction to YVMV is given in Table 9. All four genotypes were highly resistant to YVMV as they did not show any disease symptoms even after 30 days of inoculation whereas *A. esculentus* var. Salkeerthi showed typical symptoms of YVMV.



3a. *A. caillei* (10x)

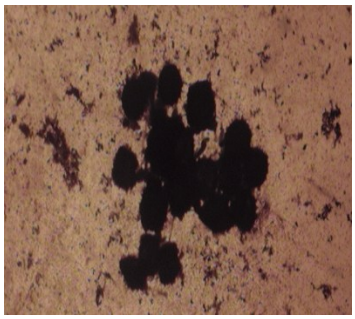


3b. *A. esculentus* (10x)

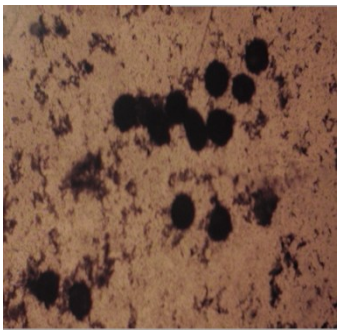


3c. Punjab 8 (10x)

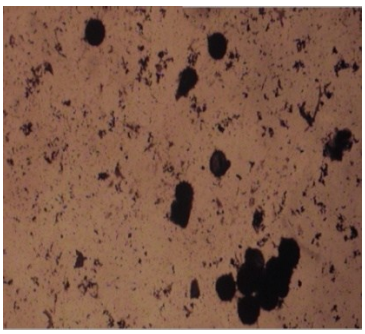
3d. Pollen grains of promising F_{12} selections



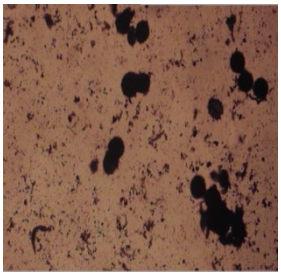
3d (1). F_{12}-1 (10X)



3d (2). F_{12}-2(10X)



3d (3). F_{12}-6 (10X)



3d (4). F_{12}-7(10X)

Plate 3. Pollen grains of parents, Punjab 8 and promising F_{12} selections showing fertile and sterile pollen grains

4a. F_{12}-1 grafted with *A. esculentus esculentus*

4b. F_{12}-2 grafted with *A.*

4c. F_{12}-6 grafted with *A. esculentus esculentus*

4d. F_{12}-7 grafted with *A.*

Plate 4. Absence of YVMV symptoms in F_{12} selections in the graft combination with diseased *A. esculentus*

5a. Absence of YVMV symptoms in F_{12} selections by whitefly transmission

5b. *A. esculentus* showing symptoms of YVMV by whitefly transmission

Plate 5. Artificial inoculation of YVMV by whitefly transmission

F_{12}-1

F_{12}- 2

F_{12}- 6

F_{12}- 7

Plate 6. Promising selections from F_{12} generation

4.5.3 Screening by graft transmission

Four genotypes (F_{12}-1-12, F_{12}-2-8, F_{12}-6-11 and F_{12}-7-14) identified highly resistant to YVMV in the field screening as well as to white fly transmission studies were artificially inoculated by approach grafting. All four genotypes were found to be resistant and did not show any symptoms even after 30 days and their reaction to YVMV is given in Table 9. The symptoms of YVMV on susceptible *A. esculentus* var. Salkeerthi is shown in Plate 4.

4.6 Selection of desirable promising lines from F_{12} generation

The F_{12} generation plants showed considerably good amount of variability with respect to plant, leaf, flower and fruit characters. The F_{12} generation lines were morphologically more similar to parent, *A. esculentus*. Four selections (F_{12}-1, F_{12}-2, F_{12}-6 and F_{12}-7) remained highly resistant to YVMV in screening trials. The pods of these lines also have less mucilage content. These selections have attractive fruit colour, desirable number of ridges per fruit, high yield, less mucilage content considerably good amount of pollen fertility and high level of resistance to YVMV.

Table 9. Reaction of genotypes to YVMV in the field screening, graft transmission and whitefly transmission studies

Sl. No	Field screening			Whitefly transmission		Graft transmission	
	Genotypes	CI	Disease reaction	Genotypes	Disease reaction	Genotypes	Disease reaction
1	F12-1	0.000	HR	F_{12}-1	HR	F_{12}-1	HR
2	F12-2	0.000	HR	F12-2	HR	F12-2	HR
3	F12-3	6.797	R	F12-6	HR	F12-6	HR
4	F12-4	6.143	R	F12-7	HR	F12-7	HR
5	F12-5	40.573	S				
6	F12-6	0.000	HR				
7	F12-7	0.000	HR				
8	F12-8	31.96	MS				
9	*A. caillei* cv Susthira	2.283	HR				
10	*A. esculentus* cv. Salkeerthi	99.84	HS				
11	Punjab 8	42.96	S				

4.7 GENOMIC DNA ISOLATION FROM DIFFERENT OKRA GENOTYPES

4.7.1 Source of DNA

For isolation of genomic DNA, leaf samples were collected from plants grown at Dept. of Olericulture, College of Horticulture, Vellanikkara. As reported in many other crops, young, tender green coloured leaves were found best for the recovery of good quality DNA in sufficient quantity from okra.

4.7.2 Isolation and purification of DNA

Genomic DNA isolated through CTAB method reported by Singh and Kumar (2012) was not pure and had RNA contamination (Plate 7a). However RNAse treatment after the DNA isolation resulted in good quality DNA (Plate 7b).

4.7.3 Quantification of DNA

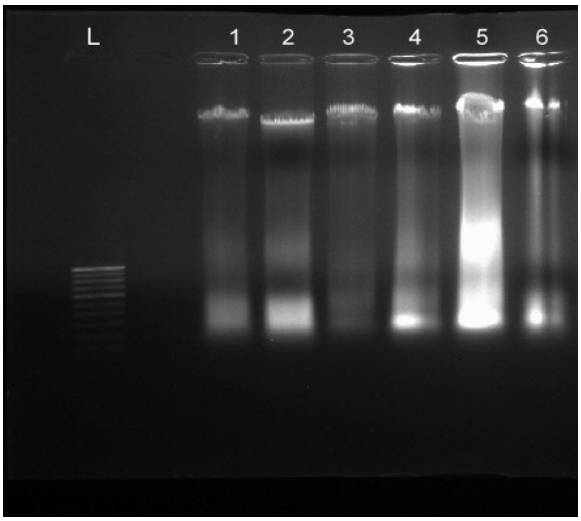
The quality and quantity of isolated DNA was analysed using both electrophoresis and NanoDrop^{R} ND-1000spectrophotometer. Intact clear bands indicated that DNA extracted was non-degraded and was of good quality. The ratio of absorbance for the DNA isolated ranged from 1.9 to 2.1, which indicated that the quality of DNA was good (Table 10). The DNA after appropriate dilutions was used as template for RAPD and ISSR analyses.

4.8 MOLECULAR CHARACTERIZATION

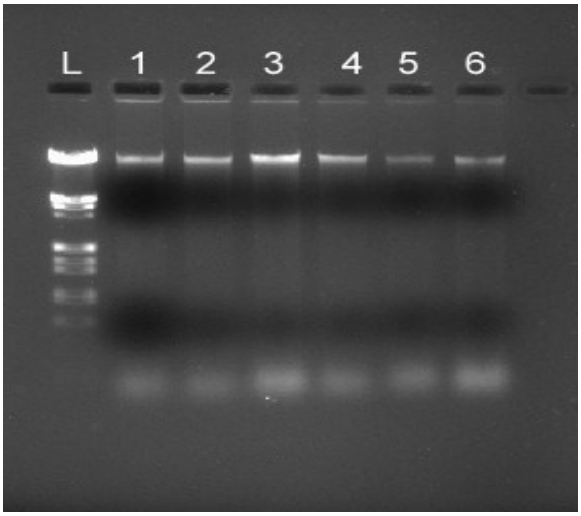
Two maker systems viz., RAPD and ISSR were used for molecular characterization. RAPD and ISSR analysis were done with already reported okra specific primers.

4.8.1 Random Amplified polymorphic DNA (RAPD) analysis

The RAPD analysis with thermal settings described in the section 3.3.1 gave good amplification. List of RAPD primers used for study is given in Table 2. Based



a. Isolated DNA



b. Isolated DNA after RNase treatment

L: Lamda DNA (*EcoR1/ Hind III* digest 1000 bp), 1- Susthira, 2- Salkeerthi, 3- Selection 1, 4- Selection 2, 5- Selection 6, 6- Selection 7

Plate 7. Isolated DNA from different okra genotypes.

on the previous reports ten okra specific primers were selected for RAPD assay which were OPC02, OPC9, OPC18, OPB2, OPX 18, UBC 465, UBC 210, OPD 05, OPA 02 and OPT 02. Finally the analysis of RAPD data was carried using NTSYS.

4.8.1.1 *Molecular characterization in different okra genotypes using RAPD primers*

Amplification of DNA from different okra genotypes was done using already reported okra specific primers for RAPD marker system. The details of amplification with the 10 RAPD primers are provided in Table 11. Observations as follows:

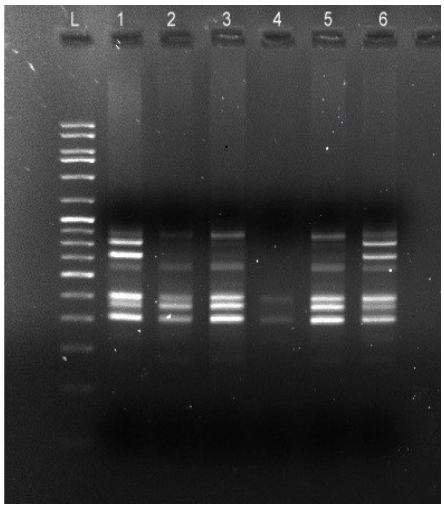
OPC 2

A total of seven amplicons ranged in size 400-900 bp were produced by the primer OPC 2. They were clear, distinct and reproducible. It could generate five polymorphic bands out of seven amplicons (plate 8a) and the percentage of polymorphism was 71.42.

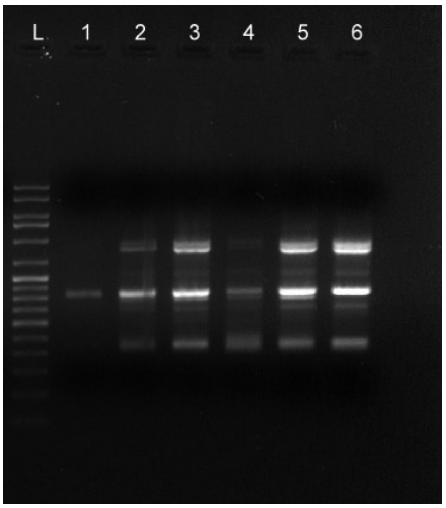
One loci of size 800 bp were found in Susthira and Selection 7. One loci of size 450 bp was found in all the genotypes except Selection 2. Three loci of size 400 bp, 500 and 600 bp was found in all the genotypes.

OPC 9

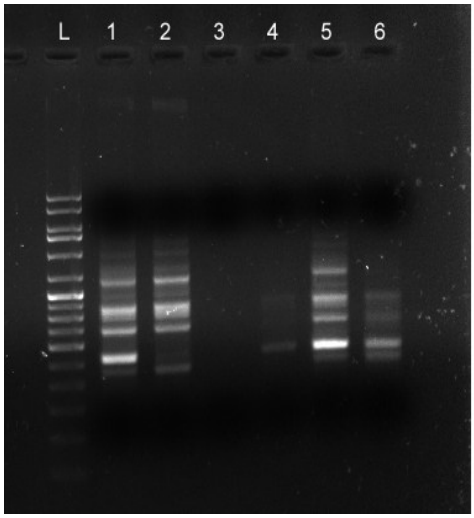
OPC 9 generated six clear, distinct and reproducible amplicons ranged in size from 400-1500 bp and five of them were polymorphic (Plate 8b) and the percentage of polymorphism was 83.33.



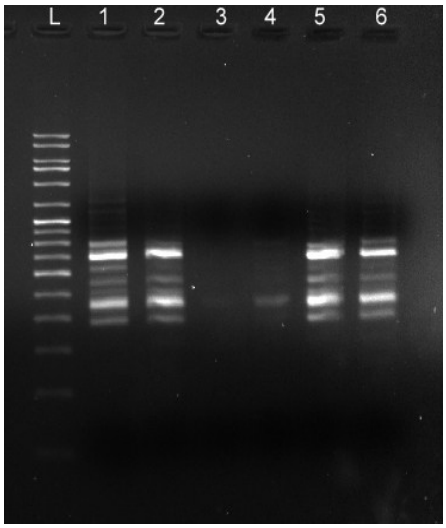
a. Primer OPC 2



b. Primer OPC 9



c. Primer OPC 18



d. Primer OPB 2

L- Ladder (3 kb), 1- Susthira, 2- Salkeerthi, 3- Selection 1, 4- Selection 2, 5- Selection 6, 6- Selection 7

Plate 8. Amplification patterns in different okra genotypes with RAPD primers

Table 10. Assessment of quality and quantity of DNA isolated from okra genotypes by NanoDrop spectrophotometer

Genotypes	A260/280	Qantity (ng/µl)
Abelmoschus caillei var. Susthira	2.03	2405.9
A. esculentus var. Salkeerthi	1.9	1322.5
Selection-1	1.89	1610.4
Selection-2	1.88	2255.6
Selection-6	1.97	2063.7
Selection-7	1.96	1438.6

One loci of size 1500 bp was found in Salkeerthi, Sel. 1, Sel. 6 and Sel.7. One loci of size 800 bp was preset in all the genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7. A locus of size 700 bp is found only in Sel. 1, Sel.3 and Sel. 7.

OPC 18

OPC 18 generated eight clear, distinct and reproducible amplicons ranged in size from 300-900 bp (plate 8c) and seven of them were polymorphic and the percentage of polymorphism was 87.5.

Two loci of size 1150 bp and 1500 bp were found in Susthira, Salkeerthi, Sel. 6. Two loci of 800 bp and 700 bp were found in Susthira, Salkeerthi, Sel. 6 and Sel. 7. A locus of size 500 bp was present in Susthira, Sel. 2, Sel. 6 and Sel. 7.

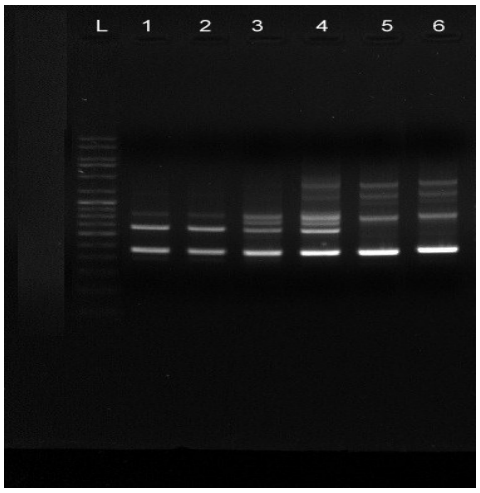
OPB 2

Six clear, distinct and reproducible amplicons were produced by primer OPB 2 (plate 8d) ranged from 300-900 bp It could detect five polymorphic amplicons out of six and the percentage of polymorphism was 83.33.

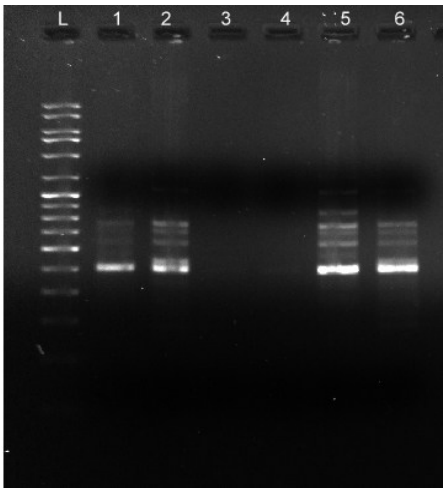
Three loci of size 500 bp, 600 bp and 800 bp were found in all the genotypes except Sel. 1, Sel. 2. Primer OPB 2 has not produced any amplicons in Selection 1.

OPX 18

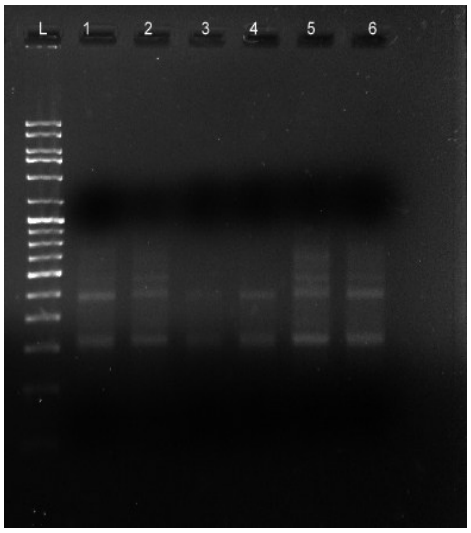
The primer OPX 18 could generate a total of six clear, distinct and reproducible bands (300-1200 bp). But out of which five were polymorphic and the percentage of polymorphism was 83.33. OPX 18 produced similar banding pattern in both Susthira and Salkeerthi. The amplification profile is given in Plate 9a.



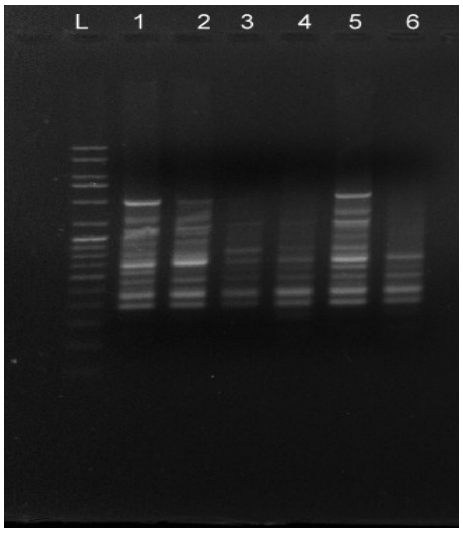
a. Primer OPX 18



b. Primer UBC 465



c. Primer UBC 210



d. Primer OPD 05

L- Ladder (3 kb), 1- Susthira, 2- Salkeerthi, 3- Selection 1, 4- Selection 2, 5- Selection 6, 6- Selection 7

Plate 9. Amplification patterns in different okra genotypes with RAPD primers

Table 11. Amplification pattern in okra genotypes with RAPD primers

Sl. No.	Primer	Total no. of amplicons	No. of polymorphic amplicons	No. of monomorphic amplicons	Polymorphism (%)	Size of amplicons (range-bp)
1	OPC2	7	5	2	71.42	400-900 bp
2	OPC9	6	5	1	83.33	400-1500 bp
3	OPC18	8	7	1	87.5	300-900 bp
4	OPB2	6	5	1	83.33	300-900 bp
5	OPX 18	6	5	1	83.33	300-1200 bp
6	UBC 465	8	7	1	87.5	500-1000 bp
7	UBC 210	4	2	2	50	300-800 bp
8	OPD 05	10	8	2	80	400-1500 bp
9	OPA 02	13	12	1	92.37	450-1100 bp
10	OPT 02	3	2	1	66.66	1000-1500 bp
Total		71	58	13		
Average		7.1	5.8	1.3	81.69	

Two loci of size 1300 bp and 1185 bp were found in Sel. 2, Sel. 6 and Sel. 7. A single locus of size 650 bp was found in Susthira, Salkeerthi and a locus of size 800 bp is present only in Sel. 1 and Sel. 2. Two loci of size 850 bp and 450 bp were present in all genotypes.

UBC 465

Amplification of okra genotypes with UBC 465 primer produced eight clear, distinct and reproducible bands ranged in size 500-1000 bp (Plate 9b). Seven of the eight amplicons were polymorphic and the percentage of polymorphism is 87.5.

Three loci of size 400 bp, 450 bp and 700 bp were found in Susthira Salkeerthi, Sel.6 and Sel. 7. One loci of size 600 bp was found only in Susthira.

UBC 210

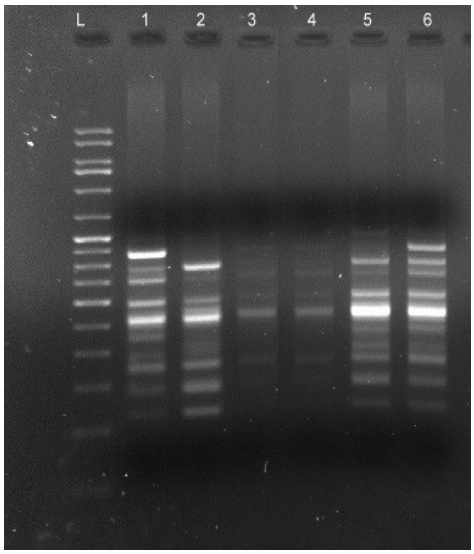
The decamer primer UBC 210 could generate four clear, distinct and reproducible amplicons (Plate 9c) ranged from 300-800 bp. Two of them were polymorphic across the genotypes and the polymorphism calculated is 50 per cent.

Two loci of size 800 bp and 550 bp were present in Salkeerthi, Sel. 6 and Sel. 7. Two loci of 500 bp and 300 bp were found in all the genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7.

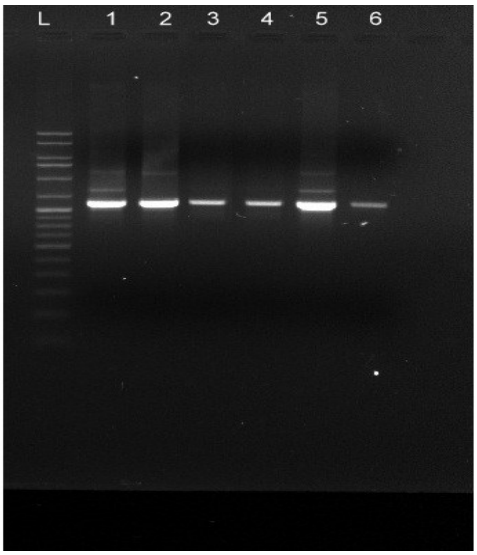
OPD 05

OPD 05 could generate a total of ten clear, distinct and reproducible amplicons (plate 9d) ranged from 400-1500 bp. Of the ten amplicons, eight were polymorphic across the genotypes and the polymorphism is found to be 80 per cent.

A single locus of size 1500 bp was found in Susthira, Salkeerthi and Sel. 6. A single locus of size 600 bp was found in Susthira and Sel.6. Five loci of size 1000 p,



a. Primer OPA 02



b. Primer OPT 02

L- Ladder (3 kb), 1- Susthira, 2- Salkeerthi, 3- Selection 1, 4- Selection 2, 5- Selection 6, 6- Selection 7

Plate 10. Amplification patterns in different okra genotypes with RAPD primers

700 bp, 550 bp, 450 bp and 400 bp were found in all genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7.

OPA 02

The primer OPA 02 could produce thirteen clear, distinct and reproducible amplicons (Plate 10a) ranged from 450-1100 bp. Out of thirteen bands, twelve were polymorphic and the polymorphism was found to be 92.7 per cent.

One loci of size 900 bp was found in Susthira and Sel. 7. One loci of size 450 bp was present in Susthira, Sel. 6 and Sel. 7. Five loci of size 350 bp, 500 bp, 600 bp, 700 bp and 800bp were found in all genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7.

OPT 02

Amplification of okra genotypes with the primer OPT 02 has resulted in three clear, distinct and reproducible amplicons (Plate 10b) ranged from 1000-1500 bp. Of the three amplicons, two were polymorphic and the polymorphism is found to be 66.66 per cent.

A locus of size 1800 bp was seen in Susthira, Salkeerthi and Sel. 6. A locus of size 1200 bp was observed in only Susthira and Sel. 6. A locus of size 1100 bp was present in all genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7.

4.8.1.2 RAPD data analysis

Reproducible, well resolved fragments were scored using Quantity one software (Biorad) and each scorable band was scored for presence (1) or absence (0). RAPD analysis using ten selected primers produced a total of 78 fragments in the six okra genotypes (Table 11) the numer of scorable bands produced by each primer ranged from thirteen (OPA 02) to three (OPT 02) with an average of 7.8 marker per primer.

The numerical scores for the presence or absence of bands were entered into a binary matrix and used for calculating the similarity coefficient using Jaccard's coefficient (Jaccard, 1908) the main similarity coefficient for each accession pair was calculated and used for cluster analysis using UPGMA method and a dendrogram generated using the software package NTSYS pc version 2.02i (Rohf, 1993). The dendrogram generated using NTSYS is given in Figure 3.

The dendrogram generated using NTSYS grouped the okra genotypes into two major clusters with a Jaccard's similarity coefficient of 0.50 to 0.79. The overall variability observed among the six genotypes studied was 50 percent. In the first cluster two selections (Sel. 6 and Sel. 7) and two source parent varieties (Susthia and Salkeerthi) were grouped which were sixty one percent similar. Second cluster included the remaining two selections (Sel. 1 and Sel. 2) which were sixty eighty percent similar. Highest similarity of seventy nine percent was observed between Salkeerhti and Selection 6.

Jacard's similarity coefficient with RAPD data of okra genotypes is presented in Table 12. The pairwise similarity coefficient values varied between 0.4366 and 0.7887 indicating variability of 22-57 percent in the okra genotypes.

4.8.2 Inter Simple Sequence Repeats (ISSR) analysis

The ISSR analysis with thermal settings described in the section 3.3.2 gave good amplification. Based on the previous reports ten okra specific primers were selected for ISSR assay UBC 811, UBC 817, UBC 818, UBC 823, UBC 830, UBC 834, UBC 842, UBC 846, UBC 848 and UBC 857. Finally the analysis of ISSR data was carried using NTSYS.

4.8.2.1 Molecular characterization in different okra genotypes using ISSR primers

Amplification of DNA from individual genotypes was carried out using already reported okra specific primers for ISSR marker system. The details of amplification with the 10 primers are provided in Table 13. Observations were as follows:

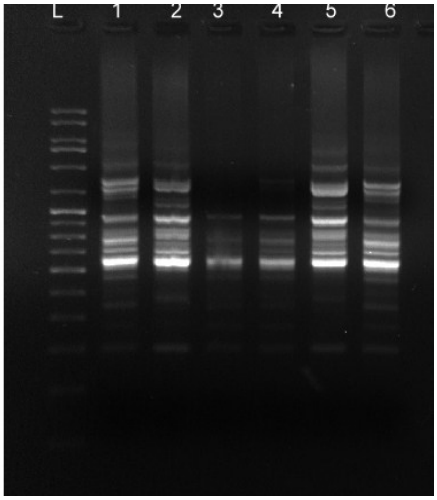
UBC 811

A total of twelve amplifications ranged from 200-1815 bp were produced by the primer UC 811. They were clear, distinct and reproducible (Plate11a). It could generate eleven polymorphic amplicons with a polymorphism percentage of 91.89.

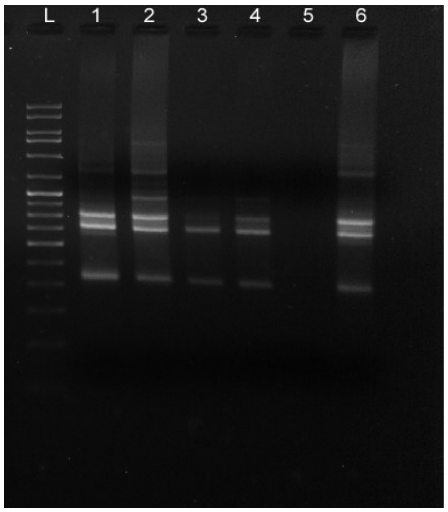
One loci of size 1000 bp was found in Salkeerthi and Sel. 3. Two loci of size 1815 bp and 1185 bp were found in Susthira, Salkeerthi, Sel. 6 and Sel. 7. Six loci of size 980 bp, 750 bp, 700 bp, 650 bp, 450 bp and 400 bp were found in all the genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7. A single locus of size 550 bp was found in only Susthira, Sel.1, Sel. 2 ad Sel. 7.

UBC 817

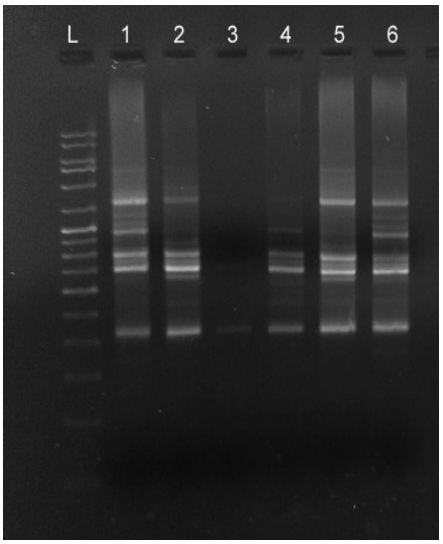
The primer UBC 817 could generate a total of eight clear, distinct and reproducible bands (400-1815 bp). But out of which four were polymorphic and the percentage of polymorphism was 50. The amplification profile is given in Plate 11b.



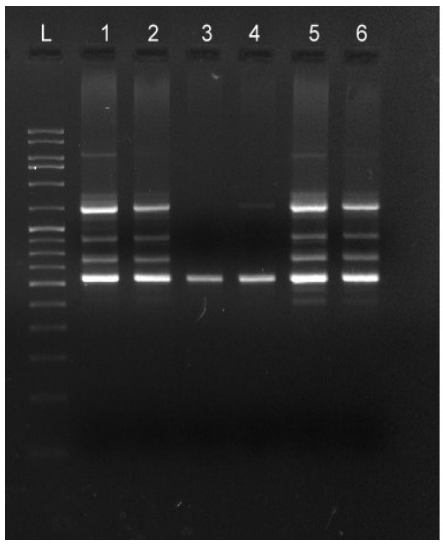
a. Primer UBC 811



b. Primer UBC 817



c. Primer UBC 818



d. Primer UBC 832

L- Ladder (3 kb), 1- Susthira, 2- Salkeerthi, 3- Selection 1, 4- Selection 2, 5- Selection 6, 6- Selection 7

Plate 11. Amplification patterns in different okra genotypes with ISSR primer

One loci of size 1000 bp was present in Salkeerthi and Sel.2. A single locus of size 1200 bp was found in Susthira, Selkeerthi and Sel. 7. Three loci of size 800 bp, 700 bp and 420 bp were found in all genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7.

UBC 818

The primer UBC 818 could produce nine clear, distinct and reproducible amplicons ranged from 400-1815 bp (Plate 11c). Out of nine bands, seven were polymorphic and the polymorphism is found to be 77.77 per cent.

Two loci of 1200 bp and 1100 bp were found in Susthira and Sel. 7. Three loci of size 1150 bp, 800 bp and 700 bp were found in Susthira, Salkeerthi, Sel.2, Sel. 6. and Sel. 7. A locus of size 680 bp was found in only Salkeerthi and Sel. 6. A locus of size 420 bp was seen in all genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7.

UBC 832

A total of nine amplicons ranged in size 500-2000 bp were produced by the primer UBC 832. They were clear, distinct and reproducible. It could generate eight polymorphic bands out of nine amplicons (plate 11d) and the percentage of polymorphism was 88.88.

A locus of size 2000 bp was found in Susthira and Sel. 6. Two loci of size 580 bp and 550 bp were seen in Sel. 6 and Sel. 7. Three loci of size 1150 bp, 900 bp and 800 bp were found in all the genotypes except Sel. 1 and Sel. 2. A locus of size 620 bp was found in all the genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7.

UBC 830

Amplification of okra genotypes with the primer UBC 830 has resulted in fourteen clear, distinct and reproducible amplicons ranged from 450-1700 bp (Plate 12a). Of the fourteen amplicons, twelve were polymorphic and the polymorphism is found to be 69.23 per cent.

Three loci of size 900 bp, 1500 bp and 1815 bp were found in Susthira and Sel. 7. A locus of size 1100 bp was found in only Salkeerthi and Sel. 6. Three loci of size 1050 bp, 1000 bp and 710 bp were present in Susthira, Salkeerthi, Sel. 6 and Sel. 7. A single locus of size 650 bp was seen exclusively in Salkeerthi and Sel. 6. A locus of size 580 bp was found only in Salkeerthi, Sel. 2, Sel. 6 and Sel. 7.

UBC 834

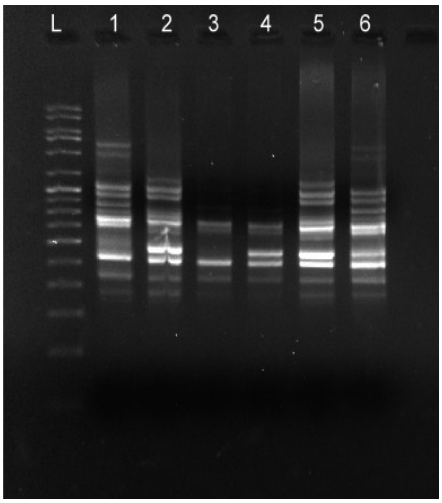
Six clear, distinct and reproducible amplicons were produced by primer UBC 834 (plate 12b) ranged from 400-1815 bp. It could detect three polymorphic amplicons out of six and the percentage of polymorphism was 50.

Three loci of size 800 bp, 750 bp and 700 bp were found in Susthira, Sel. 2, Sel. 6 and Sel. 7. Three loci of size 900 bp, 600 bp and 550 bp were found in all the genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7.

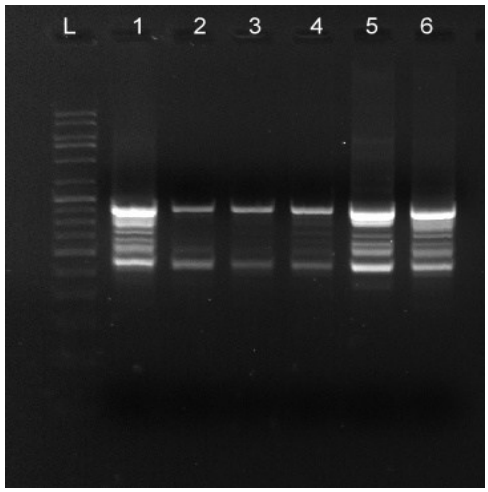
UBC 842

UBC 842 could generate a total of six clear, distinct and reproducible amplicons (plate 12c) ranged from 600-1500 bp. Of the six ands, five were polymorphic across the genotypes and the polymorphism is found to be 83.33 per cent.

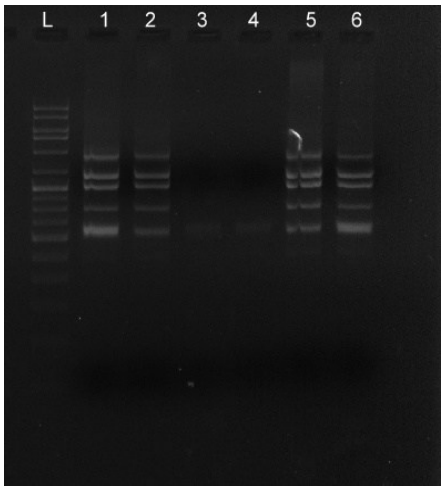
Four loci of size 1300 bp, 1100 bp 1000 bp and 800 bp were found Susthira, Salkerthi, Sel. 6 and Sel. 7. A locus of size 650 bp was present in all the genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7.



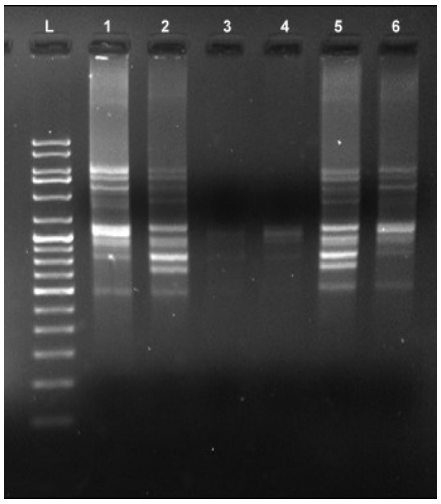
a. Primer UBC 830



b. Primer UBC 834



c. Primer UBC 832



d. Primer UBC 846

L- Ladder (3 kb), 1- Susthira, 2- Salkeerthi, 3- Selection 1, 4- Selection 2, 5- Selection 6, 6- Selection 7

Plate 12. Amplification patterns in different okra genotypes with ISSR primers

Table 13. Amplification pattern in okra genotypes with ISSR primers

Sl. No.	Primer	Total no. of amplicons	No. of polymorphic amplicons	No. of monomorphic amplicons	Polymorphism (%0	Size of amplicons (range-bp)
1	UBC 811	12	11	1	91.89	200-1815 bp
2	UBC 817	8	4	4	50	400-1815 bp
3	UBC 818	9	7	2	77.77	420-1600 bp
4	UBC 823	9	8	1	88.88	500-2000 bp
5	UBC 830	13	9	4	69.23	450-1815 bp
6	UBC 834	6	3	3	50	400-1815 bp
7	UBC 842	6	5	1	83.33	600-1500 bp
8	UBC 846	10	7	3	70	600-2000 bp
9	UBC 848	9	7	2	77.77	300-1400 bp
10	UBC 857	10	7	3	70	200-1500
Total		92	68	24		
Average		9.2	6.8	2.4	73.91	

UBC 846

A total of ten amplicons ranged in size 600-2000 bp were produced by the primer UBC 846. They were clear, distinct and reproducible. It could generate seven polymorphic bands out of ten amplicons (plate 12d) and the percentage of polymorphism was 70.

A locus of size 700 bp was found in Salkeerthi and Sel. 6. Four loci of size 2500 bp, 2000 bp 1900 bp and 1700 bp were found in Susthira, Salkeerthi, Sel. 6 and Sel. 7. Three loci of size 1000 bp, 850 bp and 600 bp were seen is all genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7.

UBC 848

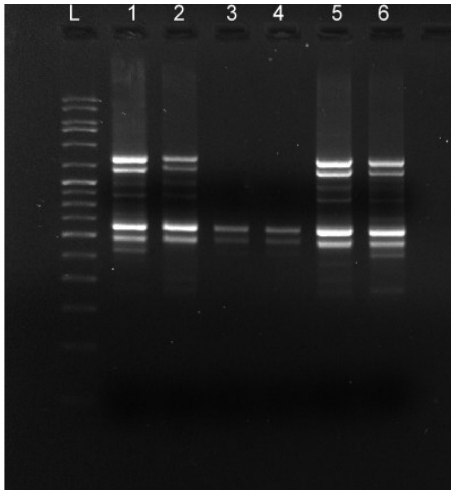
Nine clear, distinct and reproducible amplicons were produced by primer UBC 848 (plate 13a) ranged from 300-1400 bp. It could detect seven polymorphic amplicons out of nine and the percentage of polymorphism was 77.77.

A locus of size 550 bp was found in only Susthira and Sel. 7. Four loci of size 1300 bp, 1100 bp, 1000 bp and 900 bp were found in Susthira, Salkerthi, Sel. 6 and Sel. 7. Two loci of size 650 bp and 490 bp were seen is all genotypes viz., Susthira, Salkeerthi, Sel.1, Sel. 2, Sel. 6 and Sel. 7. Two loci size 400 bp and 350 bp were found I only Salkeerthi and Sel. 6.

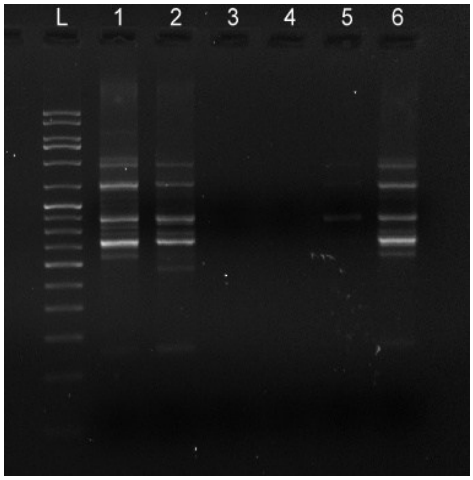
UBC 857

UBC 857 could generate a total of ten clear, distinct and reproducible amplicons (plate 13) ranged from 300-1500 bp. Of the ten bands, seven were polymorphic across the genotypes and the polymorphism is found to be 70 per cent.

A single and of size700 bp was observed in Susthira and Sel. 7. One loci of size 600 bp was seen in only Salkeerthi. Three loci of size 1500 p, 1150 bp and 900 bp were found in Susthira, Salkerthi, Sel. 6 and Sel. 7.



a. Primer UBC 848



b. Primer UBC 857

L- Ladder (3 kb), 1- Susthira, 2- Salkeerthi, 3- Selection 1, 4- Selection 2, 5- Selection 6, 6- Selection 7

Plate 13. Amplification patterns in different okra genotypes with ISSR primers

4.8.2.2 ISSR data analysis

Reproducible, well resolved fragments were scored using Quantity one software (Biorad) and each scorable band was scored for presence (1) or absence (0). ISSR analysis using ten selected primers produced a total of 92 fragments in the six okra genotypes (Table 12) the number of scorable bands produced by each primer ranged from six (UBC 834) to thirteen (UBC 830) with an average of 9.2 marker per primer.

The numerical scores for the presence or absence of bands was entered into a binary matrix and used for calculating the similarity coefficient using Jaccard's coefficient (Jaccard, 1908) the main similarity coefficient for each accession pair was calculated and used for cluster analysis using UPGMA method and a dendrogram generated using the software package NTSYS pc version 2.02i (Rohf, 1993). The dendrogram generated using NTSYS is given in Figure 4.

The dendrogram generated using NTSYS grouped the okra genotypes into two major clusters with a Jaccard's similarity coefficient of 0.43 to 0.91. The overall variability observed among the six genotypes studied was 57 percent. First cluster included two selections (Sel. 6 and Sel. 7) and two source parent cultivars (Susthia and Salkeerthi) which were sixty five percent similar. Second cluster included the remaining two selections (Sel. 1 and Sel. 2) which were seventy six percent similar. Highest similarity of ninety percent was observed between Susthira and Selection 7.

Jacard's similarity coefficient with ISSR data of okra genotypes is presented in Table 14. The pairwise similarity coefficient values varied between 0.415 to 0.906 indicating variability of 10-59 percent in the okra genotypes.

Table 12. Jacard's similarity matrix for okra genotypes with RAPD data

	Susthira	Salkeerthi	Selection 1	Selection 2	Selection 6	Selection 6
Susthira	1.0000					
Salkeerthi	0.7183	1.0000				
Selection 1	0.4478	0.5079	1.0000			
Selection 2	0.4853	0.5231	0.6818	1.0000		
Selection 6	0.7432	0.7887	0.4366	0.4930	1.0000	
Selection 7	0.6818	0.5352	0.5000	0.5714	0.6338	1.0000

Tale 14. Jacard's similarity matrix for okra genotypes with ISSR data

	Susthira	Salkeerthi	Selection 1	Selection 2	Selection 6	Selection 7
Susthira	1.0000					
Salkeerthi	0.6163	1.0000				
Selection 1	0.4167	0.3472	1.0000			
Selection 2	0.5067	0.4400	0.7317	1.0000		
Selection 6	0.6744	0.7375	0.3077	0.4125	1.0000	
Selection 7	0.9067	0.6429	0.4225	0.5556	0.6437	1.0000

Discussion

5. DISCUSSION

Nation has to manage through agriculture and agriculture has to manage through high yielding cultivars of different crops with desirable quality to achieve food and nutritional security. The targeted food production has to come from dearing resources without adversely affecting the environment.

Among the various options available to enhance production the development and use of high yielding varieties is widely acknowledged as the most potential tool. Therefore the development of high yielding varieties with resistant to major pest and diseases is considered to be the good option for enhancing yield in okra.

The present investigation was aimed at the evaluation of the genetic variability in the F_{12} generation selections rose out of the cross *A. caillei* var.Susthira x *A. esculentus* var.Salkeerthi and to identify YVMV resistant high yielding lines from these advanced generation selections and also to undertake the molecular assay of those identified YVMV resistant high yielding lines using RAPD and ISSR systems. In interspecific hybridization programmes, progenies developed from the crosses are expected to exhibit a broad spectrum of genetic variability, there by offering great scope for isolating desirable selections in the advanced generations. The salient results gathered in the light of the present study are discussed here under.

5.1 EVALUATION OF GENOTYPES

Significant variations among the treatments were observed for most of the characters under study (Table 5). The *A. esculentus* parent had the lowest mean value for first fruiting node. Many of the F_{12} selections resembled the wild parent with respect to this character. This result is in accordance with the findings of John (1997) and Reddy *et al*. (2012).

The promising selections showed characters similar to the cultivated species *A. esculentus* line Salkeerthi such as longer fruit length, less number of ridges per

pod, reduced width of epicalyx segment etc (Table5). This shows the transfer of desirable genes from cultivated parent to F_{12} generation selections.

5.2 GENETIC PARAMETERS IN THE GENOTYPES EVALUATED

5.2.1. Variability

Analysis of variance for different characters under study revealed that the treatment effects were highly significant suggesting existence of high genetic variability in the population. The presence of such variability in the population under study is the ultimate result of variability in the genetic constitution of various individuals. Such variability is desirable and can be utilized for developing new genotypes in okra. The progress in breeding programme depending upon availability of genetic variability and understanding this variability provides many avenues for genetic improvement of crop without which neither the improvement in an existing lines nor is development of new lines feasible. More the variability higher is the chance of improvement of crop species. In okra great variability in qualitative and quantitative characters had been observed by many workers like Dhankar and Dhankar (2002); Prakash and Pechaimuthu (2010).

In the present study, first flowering node, days to flower, days to first harvest, Fruit length, number of fruits per plant, plant height, petiole length, crop duration, yield per plant and coefficient of infection exhibited considerable variability. Prakash and Pechaimuthu (2010) also reported considerable variability for plant height, length and number of fruits and yield per plant. Reddy *et al*. (2012) observed significant variability for plant height, number of marketable fruits per plant, yield per plant and incidence of YVMV. The variation in fruit weight was also reported by Bendale *et al.* (2003) and Singh *et al.* (2006). Varietal differences for fruit length have been reported by Bindu *et al.* (1997) and Mohapatra *et al.* (2007). Significant variations with regard to number of fruits per plant have been reported by

Dhankar and Dhankar (2002), Bendale *et al.* (2008), Singh *et al.* (2006) and Akotkar *et al.* (2010).

Twenty two okra genotypes exhibited wide variation for plant height, days to first flower, fruits per plant and yield (Soyab *et al*. (2013). Gandhi *et al.* (2001) observed significant variability for plant height, fruits per plant, Fruit lengths and yield per plant. Highest variability for average fruit length and number of fruits per plant was observed by Jindal *et al*. (2010).

Adiger *et al*. (2011) observed highest variability for fruit yield per plant, followed by plant height and number of fruits per plant. Chaukhande *et al.* (2011) reported wide range of variation for most of the traits including fruit length, days to first flower, and plant height and fruit weight per plant.

5.2.2 Coefficient of variation

Genetic variability is the tendency of individuals in population to vary from one another. Variability is different from a genetic variation, which is the actual amount of phenotypic variation seen in a particular population. The variability of a character describes how much that trait tends to vary in response to genetics of a population. Statistically, the total variability is expressed in terms of phenotypic coefficient of variation (PCV) and the genotypic variability is expressed in terms of genotypic coefficient of variation (GCV). These parameters of variability are particularly very informative when a breeder is interested in having a stock of the comparative account of variability present in different traits, which might have been measured in different units. Variability in population, especially in respect to the character for which improvement is sought, is a prerequisite for successful selection. In the present study GCV and PCV for all characters are presented in Fig 1.

The estimates of phenotypic coefficient of variation were comparable with genotypic coefficient of variation for all the characters studied. However the

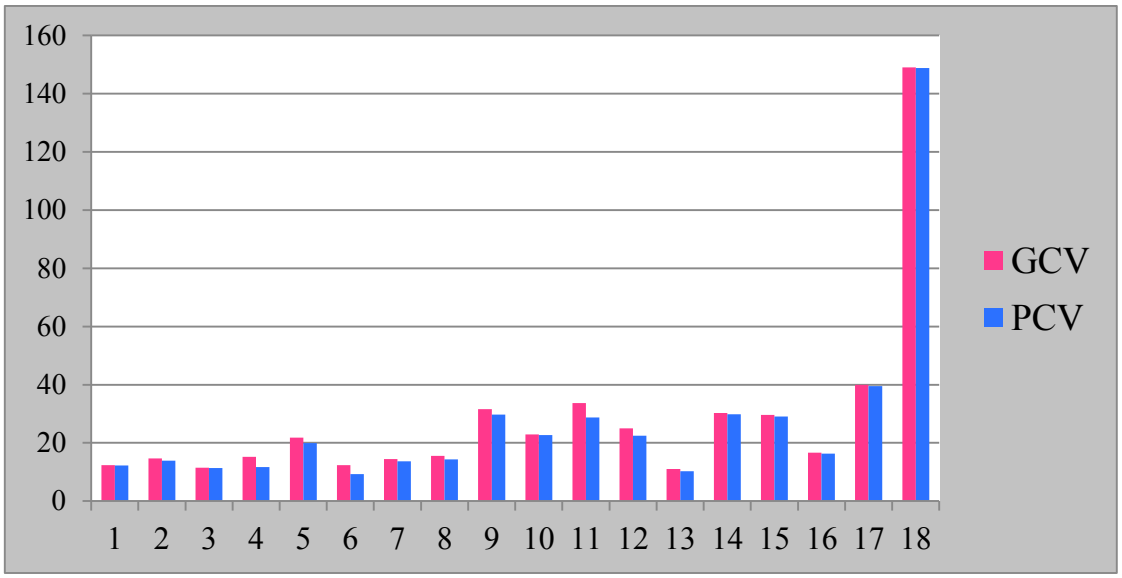


Fig.1. GCV and PCV for characters in the genotypes

1. Days to first harvest
2. First fruiting node
3. Days to first harvest
4. No. of primary branches
5. Length of fruit (cm)
6. Girth of fruit (cm)
7. Locules per pod
8. Number of ridges per pod
9. Number of fruits per plant
10. Plant height
11. Petiole length (cm)
12. Inter nodal length
13. Length of epicalyx segment (cm)
14. Width of epicalyx segment (cm)
15. Number of harvests
16. Crop duration (days)
17. Yield per plant (g)
20. Coefficient of infection

estimates of PCV were higher than estimates of GCV for all the treatments suggesting the role of environmental variance. Similar results were observed by Soyab *et al*. (2013) and Kumar and Kumar (2014).

High GCV and PCV values were exhibited by coefficient of infection. Same results were obtained by Chaukhande *et al*. (2011), but John (1977) reported low phenotypic and genotypic coefficient of variation for YVMV incidence.

Duration of the crop and Fruit girth exhibited low phenotypic and genotypic coefficient of variation. John (1997) also observed low genotypic and phenotypic coefficients of variation for these characters. The low variability noticed for these characters indicates the difficulty in improving these characters by selection.

5.2.3 Heritability and Genetic Advance

High PCV or GCV will not provide a clear picture of the extent of genetic gain to be achieved from selection for the phenotypic traits unless the heritable fraction was known (Burton, 1953).

Heritability which denotes the proportion of genetic controlled variability expressed by a programme for a particular character or a set of character is very important biometrical tool for guiding plant breeder's procedures. Thus wide range of variability and high heritability values are essential for improvement through selection. Low heritability of the character indicates that the character is largely influenced by environment. In such cases large population is required for selection of desirable genotypes. The magnitude of improvement through selection programme is detected by genetic advance. Heritability estimates alone are not useful in predicting the results about the selection, unless it is accompanied by genetic advance (Johnson *et al.* 1955).

Selection acts on genetic differences and gains from selection for a specific character depend largely on the heritability of the character (Allard, 1960). Heritable

variation may be efficiently used with greater degree of accuracy when heritability is studied on conjunction with genetic advance (Majumdar *et al.,* 1974).

High heritability was observed for all the 18 characters under study which indicates that the environment plays a little role on inheriting these traits to progenies (Fig. 2).

High heritability coupled with high genetic advance was shown by incidence of YVMV. This indicates the presence of additive genes and shows that these characters can be improved by selection. Chaukhande *et al*. (2011) reported high genetic advance for above character. Reddy and Sridevi (2014) also observed high estimates of heritability coupled with genetic advance.

High heritability along with high genetic advance was shown by plant height and yield per plant. It is in accordance with findings of Bindu *et al.* (1997), Singh *et al.* (2007) and Akotkar *et al.* (2010), who observed high heritability along with high genetic advance for above characters.

High heritability coupled with low genetic advance was observed for first fruiting node, locules per pod, number of primary branches. Same results were observed by Jaiprakashnarayan *et al.* (2006).

5.2.4 Correlation studies

Correlation coefficient measures the relationship between two or more variables. They are helpful in determining component characters of complex characters. Many of these characters are interrelated in desirable and undesirable direction. Correlation studies measure the mutual relationships between various characters and help in determining the component characters on which selection can be based. The efficiency of selection mainly depends upon the direction and magnitude of correlation between the different characters studied. Correlation analysis provides reliable information on nature, extent and direction of selection. Yield is a complex character resulting from the

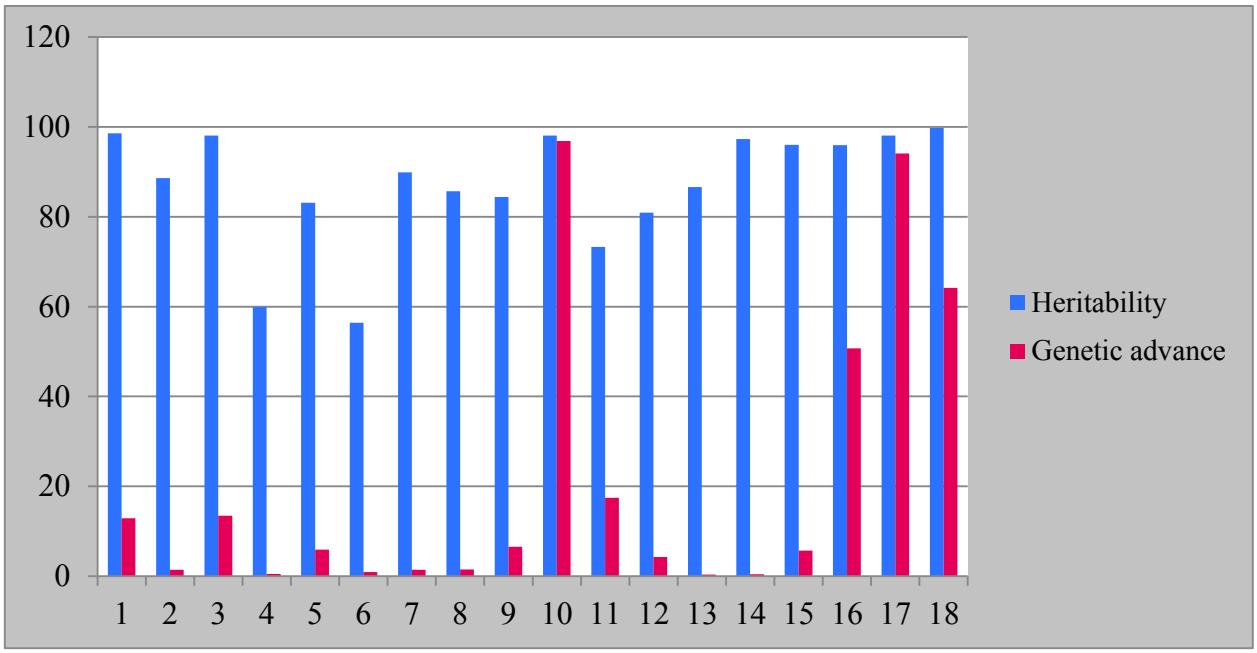


Fig.2. Heritability and Genetic advance for characters in the genotypes

1. Days to first harvest
2. First fruiting node
3. Days to first harvest
4. No. of primary branches
5. Length of fruit (cm)
6. Girth of fruit (cm)
7. Locules per pod
8. Number of ridges per pod
9. Number of fruits per plant
10. Plant height
11. Petiole length (cm)
12. Inter nodal length
13. Length of epicalyx segment (cm)
14. Width of epicalyx segment (cm)
15. Number of harvests
16. Crop duration (days)
17. Yield per plant (g)
20. Coefficient of infection

interaction of a number of factors and the environmental conditions. In order to develop a high yielding genotype, selection based on the performance of the yield is usefully not very efficient but when it is based on the component characters it may give more efficient results.

The magnitude and direction of association among the 18 characters studied in the genotypes were assessed by means of correlation analysis (Table 8).

In the present investigation, the characters exhibiting significant positive phenotypic correlation with yield are number of fruits per plant, weight of fruit, Fruit length, plant height, crop duration, number of branches per plant, fruit girth, and crop duration. Thus, it indicates the importance of these characters in selection.

Number of fruits per plant was positively correlated with yield. The same result was also obtained by Gandhi *et al.* (2002), Dhankar and Dhankar (2002), Ali *et al.* (2008). and Jaiprakashnarayan and Mulge (2004) suggested the importance of fruit number per fruit as a selection criterion for increasing yield. Singh *et al.* (2007) also observed strong positive association between number of fruits per plant and weight of fruits per plant.

Days to first flowering was negatively correlated with number of fruits per plant and yield per plant. This is in agreement with findings of Alex (1988) and Adiger *et al*. (2011).

The positive significant association of plant height with number of fruits per plant, Fruit lengths, yield per plant, yield per hectare, weight of fruits, number of seeds per fruit, number of branches per plant at maturity and crop duration. It indicates that yield could be increases due to increase in plant height. These findings are supported by Mohapatra *et al.* (2007), Singh *et al.* (2007), Kumar *et al*. (2011), Chaukhande *et al*. (2011) had reported the significant and positive association between plant height and Fruit lengths.

Significant positive phenotypic and genotypic correlation with yield was shown by fruit length and fruits per plant indicating that an improvement of these characters will produce a simultaneous improvement in yield. This had also been reported by Singh *et al.* (2007) and Sengupta and Verma (2009) and Balakrishnan and Sreenivasan (2010). Significant positive association of fruit yield per plant with number of fruits per plant has been reported by several workers (John, 1997, Dhankar and Dhankar, 2002, Ali *et al,*. 2008).

Total yield had negative and significant association with number of locules per fruit as observed by Sood *et al*. (1995) and days to flowering according to Majumdar *et al*. (1974) and Adiger *et al*. (2011) in okra.

Nodes at first flowering had positive and significant correlation with days to first flowering. This result is in accordance with the findings of Parthap *et al.* (1979) Singh *et al* (2006).

5.3 MUCILAGE CONTENT

Okra pods are especially characteristic for their mucilage content. The mucilage content of edible stage fruits was estimated by extracting the mucilage with ethyl alcohol. This method was followed by many researchers like Thampi and Indira (2000); Ravisankar (2002) and Kadlag *et al*. (2005). Presence of less mucilage content in okra fruits is a desirable character. Mucilage content in the parental species *A. esculentus* variety Salkeerthi was as low as 0.45 g/100g and *A. caillei* variety Susthira recorded 0.69 g/100g which is high among all the genotypes under study. Similar results were obtained by Thampi and Indira (2000) and Ravisankar (2002). In the F_{12} generation lines, it ranged from 0.35 - 0.61 g/100g. Variety Punjab 8 recorded 0.49 g/100g. The mucilage content of desirable promising F_{12} selections was in the range of 0.35-0.45 g/100g which was on par with Punjab 8.

5.4 POLLEN FERTILITY STUDIES

The pollen fertility in *A. esculentus* and *A. caillei* were 99.68 and 99.62 per cent respectively due to their regular chromosome behavior during meiosis. Similar results were reported by Jaseena *et al*. (2008) and Yamuna *et al*. (2013). Pollen stainability in the F_{12} lines varied from 97.68 to 98.72 per cent. In case of standard check variety Punjab 8 pollen fertility was 98.68 per cent. The promising lines selected from F_{12} generation viz., F_{12}-1, F_{12}-2, F_{12}-6 and F_{12}-7 showed pollen fertility in the range of 98.63 to 98.72 per cent.

Pollen fertility studies shows that high amount of pollen fertility exists in F_{12} generation lines on par with Punjab 8. Jaseena *et al.* (2008) reported 12.47 per cent pollen sterility in the F_5 generation of the cross *A. caillei* x *A. esculentus.* The present study indicated that the degree of pollen sterility decreased significantly in the succeeding generations and achieved securing of highly fertile advanced generation lines combined with desirable traits. Similar line of work has been reported by Jambhale and Nerkar (1983) and SureshBabu and Dutta (1990).

5.5 SCREENING FOR YVMV RESISTANCE

5.5.1 Field screening

In the field screening trials the *A. esculentus* variety Salkeerthi was highly susceptible to YVMV (CI=99.847) whereas *A. caillei* variety Susthira was resistant (CI=2.283). This observation is in accordance with the findings of Sureshbabu *et al*. (2002) and Yamuna *et al*. (2013).

Even though Punjab-8, a popular okra variety was reported to be highly resistant by Singh *et al.* (2007) and moderately resistant to YVMV by Kiran and Pathak (2012), it appeared to be susceptible (CI=42.96) in the present investigation. In F_{12} lines, four were highly resistant (F_{12}-1, F_{12}-2, F_{12}-6 and F_{12}-7) and remaining

were moderately resistant (CI=6.14 to 40.57) to YVMV. This result is in agreement with the findings of Philip *et al* 2000.

YVM disease resistance identified in the F_{12} selections wes further confirmed by conducting following methods:

5.5.2 Whitefly transmission

All four selections highly resistant to YVMV in field screening were found highly resistant with whitefly transmission also and only the susceptible check Salkeerthi showed symptoms of the disease. Similarly Ravisankar, 2002, Sindhumole *et al*. (2003) also recorded transmission YVMV through the whiteflies.

5.5.3 Grafting transmission method

Graft transmission studies were carried out in order to ascertain the nature of resistance since the resistant reaction expressed consequent to virus inoculation can be either due to escape or due to true resistance. Out of the four selections tested, all the selections (F_{12}-1, F_{12}-2, F_{12}-6, and F_{12}-7) were completely free of YVMV in graft transmission confirming the true resistance of these genotypes. Similar grafting trial for confirming the YVM resistance in okra was performed previously by Ali *et al*. (2000); Ravisankar, 2002; Sindhumole *et al*. (2003) and Jaseena (2008). The present study also showed that graft inoculation is more effective method for screening of YVMV in okra.

5.6 Stable YVMV resistant F_{12} selections

As evidenced in the above mentioned confirmation tests, the highly YVMV resistant selections (F_{12}-1, F_{12}-2, F_{12}-6, and F_{12}-7) possessed true and stable resistance which can be developed as new YVM resistant okra varieties. More over they possess desirable features such as dark green fruit colour, reduced number of ridges and optimum fruit length.

The occurrence of highly YVMV resistant advanced F_{12} generation lines clearly shows that the flow of desirable YVMV resistant genes from the semi-wild species *A. caillei* to the cultivated species *A. esculentus* had been successful. Jambhale and Nerkar (1983), SureshBabu and Dutta (1990) and Philip (2000) have reported similar type of gene introgression in okra.

5.7 ISOLATION, PURIFICATION AND QUANTIFICATION OF THE GENOMIC DNA

The plant part used for DNA isolation was young leaves from six okra genotypes. The method used for isolation of DNA was CTAB method as suggested by Singh and Kumar (2012).

Grinding in liquid nitrogen was found to degrade the DNA content, hence liquid nitrogen was not added while grinding. The addition of antioxidants like β-mercaptoethenol and sodium metabisulfite during grinding was found effective for removal of phenolic compounds. Similar results were reported by Nwangburuka *et al.* (2011) and Singh and Kumar (2012) in okra.

The detergent used in the extraction buffer for DNA isolation is CTAB (Cytyle Trimethyl Ammonium Bromide), a cationic detergent, which helps in the disruption of the cell membrane thus releasing nucleic acid into the extraction buffer and prevents co-precipitation of polysaccharides with nucleic acid by acting as a selective precipitant of nucleic acids.

In the present study, the CTAB was found superior. The useful effects of CTAB on the quality of DNA were also reported by Singh and Kumar (2012) and Prakash *et al.* (2011). CTAB effectively disrupts the cell membrane and together with NaCl separates the polysaccharides. Double treatment with chloroform: isoamylalcohol mixture and centrifugation effectively removes the pigmants and proteins. The addition of chilled isopropanol precipitates the DNA and washing the

pellet with 70 percent alcohol followed by absolute alcohol removes the traces of CTAB Singh and Kumar (2012).

The quality of DNA was tested by subjecting it to agarose gel electrophoresis as well as spectrophotometric method. In the former, the DNA was visualized on 0.8 percent agarose gel under UV light by ethidium bromide staining.

A DNA sample was reported as high quality if it had a band of high molecular weight with little smearing and low amount of RNA (Wettasingf and Peffley, 1998). The DNA extracted showed high amount of RNA as a smear bellow it. To remove RNA, RNase was used. Use of RNase was reported Nwangburuka *et al.* (2011) and Saifullah *et al.* (2010).

In the present study, the RNase treated DNA sample when electrophoresed showed a high molecular weight DNA, which formed a single band just below the well. This indicated that the DNA under test was of good quality (Plate7b)

In spectrophotometer method, the ratio of optical density at 260 and 280 nm was worked to test the quality. The absorbance ratio was calculated as a 260/280, for the various samples. Quantification of the DNA on the basis of UV absorbance at 260 indicated substantial yield of DNA from the tissue samples. Spectrophotometric determination of concentration of DNA was also reported by Saifullah *et al.* (2010). The samples with ratio between 1.5 and 2 were considered to be of high quality. All the samples recorded a ratio between 1.5 and 2 (Table 10).

5.8 MOLECULAR MARKER ANALYSIS

Molecular markers have been proved to be a fundamental and reliable tool for fingerprinting varieties, establishing the fidelity of progenies and germplasm characterization. Molecular markers provide an important technology for evolving levels of genetic variability and have been utilized in a variety of plant species. Molecular markers which detect variation at DNA level overcome most of the

limitations of morphological and biochemical markers. Molecular markers are independent of developmental stages of the crop and are not influenced by the varying environmental conditions. Hence molecular markers are preferred for variability analysis than traditional morphological markers (Agarwal *et al.,* 2008).

Most of the molecular markers are developed by PCR (Polymerase Chain Reaction) technology and amplifies unique regions in the genomic DNA based on the primers designed for amplification. In the present study, two such PCR based marker systems viz. RAPD and ISSR were used for molecular characterization of advanced generation selections of okra.

5.8.1 Molecular marker analysis in okra

Reports on molecular marker development in okra are very scanty and have been limited to characterization of cultivars. An agreement between clustering patterns obtained from morphological traits and molecular markers in *Abelmoschus spp*. has been demonstrated (Mortinello et al., 2001). Ninety-three accessions of common (*A. esculentus*) and West African (*A. caillei*) could be distinguished using random amplified polymorphic DNA (RAPD) markers (Aladele *et al*., 2008). Use of sequence related amplified polymorphism (SRAP) in marker aided selection (MAS) for various traits in Turkish germplasm of okra has been suggested (Gulsen *et al*., 2007). Recently, 20 okra accessions from Burkina Faso were analyzed using 16 primers designed to amplify SSR regions of *Medicago truncatula*. Two accessions were found distinct from the other 18, based on the presence of an unique 440 bp fragment generated with primer MT-27 and also based on presence of hairs on fruits and delayed maturity of these two accessions (Sawadogo *et al.*, 2009).

5.8.1.1 *RAPD assay of okra genotypes*

After the invention of Polymerase Chain Reaction (PCR) technology, a large number of approaches for generation of molecular markers based on PCR were

detailed, primarily due to its apparent simplicity and high probability of success. Williams *et al.* (1990) were the first to use RAPD markers. The basis of RAPD technique is differential PCR amplification of genomic DNA. It deduces DNA polymorphisms produced by rearrangements or deletions at or between oligonucleotide primer binding sites in the genome using short random oligonucleotide sequences. RAPD markers are dominant in nature because polymorphisms are detected as the presence or absence of bands. Use of high quality DNA is shown to be a key factor in obtaining reproducible RAPDs bands (Penner *et al.,* 1993). In the present study the use of high quality DNA helped in getting reproducible bands using the standardized conditions for the thermal cycler.

However, the advantages of RAPD include simplicity, rapidity, requirement of only a small quantity of DNA and ability to generate numerous polymorphisms Agarwal *et al.* (2008). Use of RAPD markers for estimating the genetic diversity in *Abelmoschus sp.* was attempted by Martinello *et al*. (2001), Saifullah *et al.* (2010), Prakash *et al*. (2011) and Kaur *et al.* (2013). Molecular characterization of okra accessions using RAPD was carried out by Vaishali *et al*. (2010) and Nwangburuka *et al.* (2011). RAPD markers were also used by Aladele *et al*. (2008) to assess genetic distinctiveness between *Abelmoschus esculentus* and *A. caillei*.

Random decamer primers with good resolving power were used for amplification of DNA. Four decamer primers reported by Kaur *et al*. (2013) viz. OPC-2, OPC-9, OPC-18 and OPB-2., three decamer primers reported by Aladele *et al.* (2008) viz. OPX-18, UBC-465 and UBC-210. And three primers reported by Prakash *et al.* (2011) OPD-05, OPA-02 and OPT-02 were used for RAPD assay.

The amplification conditions standardized in the present study were suited to mastercycler personal thermocycler from Agilent technologies. Also the annealing temperature was identified as the most critical with respect to number of amplified fragments and reproducibility of result. Kaur *et al.* (2013), Vaishali *et al*. (2010) and

Aladele *et al*. (2008) suggested 37^0C as the best annealing temperature and hence same temperature was used in the present investigation.

The amplification pattern produced by the different combinations of the ingredients of the reaction mixture indicated that the most important factor affecting the specificity and yield of amplification was concentration of $MgCl_2$ in the buffer as well as concentration of DNA polymerase enzyme. In present investigation, the enzyme used was *Taq* DNA polymerase supplied by Genei, Bengaluru. The concentrations of primers as well as dNTPs were also found to affect the intensity and number of amplifications. All reactions were carried out in 20µl final volume.

5.8.1.2 *Molecular characterization of okra genotypes using RAPD*

The RAPD markers generated using the selected primers were visualized by electrophoresis, in a one percent agarose gel stained with ethidium bromide.

The selected primers produced clear and distinct amplification pattern (Table 11) with the four selections of okra and their source parental varieties selected for the study. There were total of 71 amplicons of which 58 were polymorphic giving a polymorphism of 8.69 percent. The number of amplicons produced ranged from three to thirteen with an average of 7.1 amplicons per primer and a mean of 5.8 polymorphic bands per primer. The number of markers detected by each primer depends on primer sequence and the extent of variation is genotype specific (Upadhyaya *et al.,* 2004). This is understandable as product amplified depends upon the sequence of random primers and their compatibility within genomic DNA. Saifullah *et al.* (2010) reported in their study, five decamer primers gave 38 clear and bright fragments. There were 32 polymorphic bands (6.40 diversity/primer) out of 38 reproducible products (7.6 fragments/primer).

(Vaishali *et al*., 2010). Characterized Twenty-two genotypes of *Abelmoschus esculentus L. Moench,* including resistant varieties, hybrid varieties and susceptible varieties for YVMV infection using 20 random primers of which 15 resulted in

polymorphic, scorable and reproducible. Of the ten primers studied, all the primers had given polymorphism of more than 80 percent except OPC 2 (71.42 percent) while primer OPA 02 gave highest polymorphism of 92.37 percent.

RAPD primers such as OPC 9 and OPC 18 produced specific bands (700 bp and 550 bp respectively) which are present only in Selections and absent in parental varieties. Decamer primer OPX 18 produced distinct bands specifically in Selections (1300 bp and 1150 bp in Sel.2, 6 and 7; 800 bp in Sel. 1 and Sel. 2) which were absent in parent varieties. RAPD assay showed clear and distinct variations between YVMV resistant (Susthira and four Selections) and susceptible (Salkeerthi) lines. Among the selected RAPD primers, the unique bands produced by OPC 2 (400 bp and 450 bp), OPT 02 (1200 bp) and OPA 02 (450 bp) were specific to only resistant lines (Susthira and four Selections). Hence it can be assumed that any one of these bands may be associated with the genes responsible for YVMV resistance.

5.8.1.4 *RAPD data analysis*

A binary data matrix was used for calculating the similarity coefficient using Jaccard's coefficient (Jaccard, 1908). The mean similarity coefficient was calculated and used for cluster analysis using the UPGMA method and a dendrogram generated using the software package NTSYS pc 2.02i (Rohf, 1993).

The dendrogram generated using NTSYS grouped the okra genotypes into two major clusters. Cluster I includes two selections (Sel. 6 and Sel. 7) and two source parent varities (Susthira and Salkeerthi). Remaining two selections (Sel. 1 and Sel. 2) formed the second main cluster. Cluster I was divided into two main sub clusters. Two parental varieties (Susthira and Salkeerthi) and Selection 6 were grouped in first sub cluster and only Selection 7 appeared in second sub cluster (Figure3).

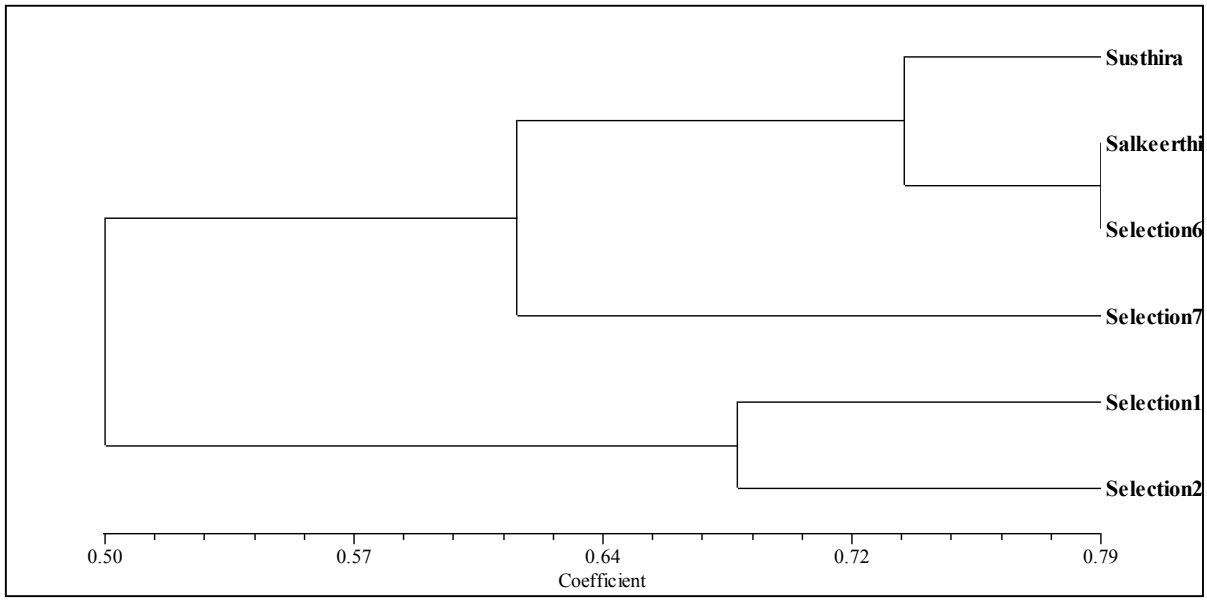


Figure 3. Dendrogram generated for selected okra genotypes using RAPD data

A genetic similarity matrix of different groups of okra genotypes based on proportion of shared ISSR fragments was also generated (Table 13). The pair wise similarity coefficient values varied from 0.4366 to 0.7887 in the present investigation. Prakash *et al*. (2011) reported genetic similarity coefficients (Jaccard's) in ISSR assay of okra in the rage of 0 to 81 units. The extent of variability in advanced generation selections from their parental varieties was found more in Selection 1 (66 % and 50% from Susthira and Salkeerthi respectively) and Selection 2 (52 % and 48 % from Susthira and Salkeerthi respectively).

5.8.2 ISSR analysis

The choice of a molecular marker technique depends on its reproducibility and simplicity. Since 1994, a molecular marker technique called Inter Simple Sequence Repeats (ISSR) has been available and is being exploited.

ISSRs are semi arbitrary markers amplified by PCR in the presence of one primer complimentary to a target microsatellite. Like RAPD, ISSR markers are quick and easy to handle, but they seem to have the reproducibility of SSR marker because of the longer length of their primer. ISSR targets the highly variable microsatellite regions of the nuclear genome providing a large number of polymorphic fragments (Gupta et al., 1994). Therefore this technique has been used extensively to evaluate genetic diversity both within and between populations in angiosperms and gymnosperms (Oshborn et al., 2005).

Primers with good resolving power were used for amplification of DNA. Ten ISSR primers reported by Yuan *et al*. (2014) were used for ISSR assay. Different sizes of amplicons were produced by different primers and the results are presented in Table 12.

The amplification conditions standardized in the present study were suited to mastercycler personal thermocycler from Agilent technologies. Also the annealing

temperature was identified as the most critical with respect to number of amplified fragments and reproducibility of result. Annealing temperature of 49^0 c as suggested by Yuan *et al*. (2014) gave good amplification.

The amplification pattern produced by the different combinations of the ingredients of the reaction mixture indicated that the most important factor affecting the specificity and yield of amplification were concentration of $MgCl_2$ in the buffer as well as concentration of DNA polymerase enzyme. In present investigation, the enzyme used was *Taq* DNA polymerase supplied by Genei, Bengaluru. The concentrations of primers as well as dNTPs were also found to affect the intensity and number of amplifications. All reactions were carried out in 20µl final volume.

5.8.2.1 ***Molecular characterization of okra genotypes using ISSR***

The selected ISSR primers produced a total of 92 amplicons of which 68 were polymorphic giving a polymorphism of 73.91 percent with an average of 9.2 markers per primer and a mean of 6.8 polymorphic bands per primer (Table 12). Rana *et al*. (2008) reported that seven primers produced a total 166 bands with average of 9.7 bands per primer. Out of 166 bands, 91 bands were found to be polymorphic. They showed 54.05 percent polymorphism and the average numbers of polymorphic bands per primer were observed was 5.3. Noormohammadi *et al*. (2013) studied the molecular genetic diversity in the Opal cotton (*Gossypium hirsutum*) cultivar and in F2 progenies using ISSR primers using ten ISSR primers and obtained a total of 206 reproducible bands, with 49.4% polymorphism. The 22 ISSR primers produced 289 amplified DNA fragments in okra of which 145 (50%) fragments were polymorphic Yuan *et al*. (2014).

Out of ten primers used, the primer UBC 811 gave the highest polymorphism of 91.89 percent and the lowest being the primer UBC 817 (50 percent). The amplicon size in the present investigation ranged from the molecular weight of 200 bp to 2000 bp.

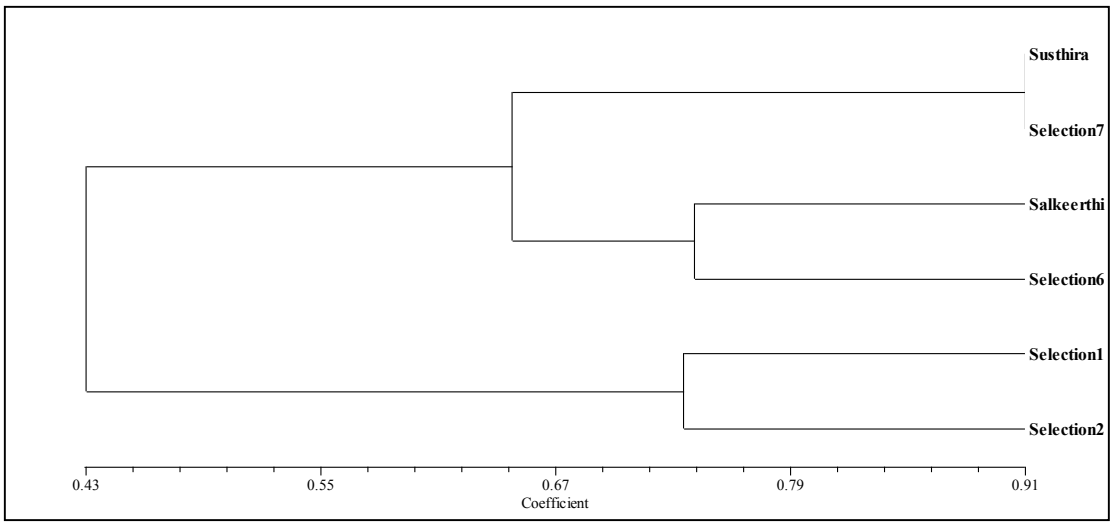


Figure 4. Dendrogram generated for selected okra genotypes using ISSR data.

ISSR primer UBC 832 produced two unique bands (580 bp and 550 bp) specific to Sel. 6. UBC 834 also produced unique band of size 500 bp specifically in Sel. 6. ISSR assay showed variation between the advanced generation selections and their parental species. Among the selected ISSR primers, three primers viz. UBC 834 (700 bp, 750 bp ad 800 bp), UBC 818 (1150 bp) and UBC 830(1815 bp and 1500 bp) produced unique amplicons which were present only in the resistant genotypes (Susthira and advanced generation selections). Hence it can be assumed that any one of these bands may be associated with the genes responsible for YVMV resistance. Primer UC 846 has produced a unique band of size 700 bp in only Salkeethi and Selection 6.

5.8.2.2 *ISSR data analysis*

A binary data matrix was used for calculating the similarity coefficient using Jaccard's coefficient (Jaccard, 1908). The mean similarity coefficient was calculated and used for cluster analysis using the UPGMA method and a dendrogram generated using the software package NTSYS pc 2.02i (Rohf, 1993). The dendrogram generated using NTSYS grouped the okra genotypes into two major clusters. Cluster I includes two selections (Sel. 6 and Sel. 7) and two source parent cultivars (Susthia and Salkeerthi). Remaining two selections (Sel. 1 and Sel. 2) formed the second main cluster. Cluster I was divided into two main sub clusters. Resistant parent Susthira and Selection 7 were grouped in first sub cluster and susceptible parent Salkeerthi and Selection 6 appeared in second sub cluster (Figure 4).

A genetic similarity matrix of different groups of okra genotypes based on proportion of shared ISSR fragments was also generated (Table 14). The pair wise similarity coefficient values varied from 0.415 to 0.906 in the present investigation. Yuan *et al*. (2014) observed genetic similarity coefficients (Jaccard's) in ISSR assay of okra in the rage of 0.04545 to 0.4545. The extent of variability in advanced generation selections from their parental varieties was found more in Selection 1 (59

% and 66% from Susthira and Salkeerthi respectively) and Selection 2 (50 % and 56% from Susthira and Salkeerthi respectively).

5.8.3 Comparison of RAPD and ISSR systems

RAPD produced a total of 71 amplicons of which 58 were polymorphic giving a polymorphism of 81.69 percent. The number of amplicons produced ranged from three to thirteen with an average of 7.1 amplicons per primer and a mean of 5.8 polymorphic bands per primer. The ISSR primers produced a total of 92 amplicons of which 68 were polymorphic giving a polymorphism of 73.91 percent. The number of amplicons were in the range of six to thirteen with an average of 9.2 markers per primer and a mean of 6.8 polymorphic bands per primer. In RAPD assay the highest percentage of polymorphism was given by OPA 02 (92.37%). In ISSR assay, the primer UBC 811 produced highest polymorphism percentage of 91.89 %. The ISSR marker systems produced more amplicons as compared to RAPD system with more number of markers per primer and more polymorphic amplicons per primer. The amplification patter observed in Selection 1 and Selection 2 was peculiar in both the marker systems. Molecular marker analyses could assess the variability among advanced generation selections and their parents evaluated in the present investigations. The study could locate some markers in the resistant genotypes which on further indepth study will aid in marker assisted selection for YVMV resistance. Further, molecular data generated in the present investigations will serve as a base for fingerprinting the elite genotypes for varietal registration.

Summary

6. SUMMARY

Okra has captured a prominent position among the vegetables due to its high nutritive value, year round cultivation and export potential. However many of the okra cultivars now in vogue are highly susceptible to YVMV disease which reduces the yield considerably. Hence it is essential to evolve varieties resistant to YVMV disease. The present investigation entitled 'Evaluation and molecular characterization of advanced generation distant hybridisation selections of okra [*Abelmoschus esculentus* (L.) Moench]' was carried out in the Department of Olericulture, College of Horticulture, KAU, Vellanikkara, Thrissur. During 2014–15. The main objective of the study was to study the variability in F_{12} generation of the cross *Abelmoschus caillei* var. Susthira x *Abelmoschus esculentus* var. Salkeerthi and to select the promising high yielding YVMV resistant lines from this population. The study also aims at molecular characterization of the selected promising advanced generation lines.

The F_{12} generation plants were raised in the field along with their parents and variety Punjab 8. The morphological traits of genotypes were compared. The evaluation of YVMV resistance in the genotypes was made by field screening and artificial inoculation by grafting, whitefly transmission techniques.

Evaluation of genotypes

The genotypes exhibited significant variation for all the characters studied. Less mucilage content and high level of pollen fertility was noted in the selected desirable lines. Incidence of YVM disease was much lower in the selected lines compared with the cultivated parent.

The PCV and GCV were maximum for incidence of YVMV disease whereas days to first flowering exhibited low variation. High heritability and genetic advance were noted for coefficient of infection of YVMV. This indicates the presence of

additive genes. High heritability coupled with low genetic advance was observed for first fruiting node, number of harvests.

Fruit yield per plant recorded significant positive correlation with number of fruits per plant and crop duration thereby indicating the plant and fruit characters that should be considered while selection for yield improvement.

Mucilage content

Presence of less mucilage content in okra fruits is a desirable character. Mucilage content in the parental species *A. esculentus* variety Salkeerthi was as low as 0.45 g/100g and *A. caillei* variety Susthira recorded 0.69 g/100g which is very high among all the genotypes under study. In the F_{12} generation lines, it ranged from 0.35-0.61 g/100g. Variety Punjab 8 recorded 0.49 g/100g. The desirable F_{12} lines (F_{12}-1, F_{12}-2, F_{12}-6 and F_{12}-7) were showing less amounts of mucilage content compared to wild parent.

Pollen fertility studies

The pollen fertility studies revealed that the parents *Abelmoschus caillei* var. Susthira and *Abelmoschus esculentus* var. Salkeerthi, standard check variety Punjab 8 and all advanced F_{12} generation lines had higher pollen fertility. The pollen fertility in the genotypes was ranged from 97.69 to 99.69 per cent. The F_{12} lines were on par with Punjab 8 in pollen fertility. The high level of pollen fertility in the parents must be attributed to their regular chromosome pairing during meiosis.

Screening for resistance to YVMV

All the genotypes under study were screened for YVMV resistance. Field screening trial for resistance to YVMV showed that the parent *A. esculentus* as susceptible (CI=99.84), while the other parental species *A. caillei* was resistant to YVMV. Standard check variety Punjab 8 was susceptible (CI=42.96). In F_{12} lines the

genotypes were ranged from highly resistant to moderately susceptible (CI=0 to 40.57).All six F_{12} selections highly resistant in field screening were found highly resistant to YVMV in both whitefly and graft transmission studies.

Selection of promising lines from the F_{12} population

Four advanced generation selections viz., (F_{12}-1, F_{12}-2, F_{12}-6 and F_{12}-7) showed characters such as less number of ridges per pod, longer fruit length, reduced width of epicalyx segment etc. morphologically similar to the cultivated species *A. esculentus* line Salkeerthi. Their fruit lengths ranged from 17-20 cm and were having green pods with five ribs and less mucilage content. They were also having more number of fruits per plant and high yield. These selections also showed high level of pollen fertility and resistance to YVMV.

The four F_{12} selections viz., (F_{12}-1, F_{12}-2, F_{12}-6 and F_{12}-7) made from this study can be recommended for multilocation testing and can be released as high yielding YVMV disease resistant varieties.

Molecular characterization

RAPD assay

The selected ten primers produced clear and distinct amplification pattern with the four Selections of okra and their source parent cultivars selected for the study. There were total of 78 amplicons of which 65 were polymorphic giving a polymorphism of 83.33 percent. The number of amplicons produced ranged from three to thirteen with an average of 7.8 amplicons per primer and a mean of 6.5 polymorphic ands per primer. Among the selected RAPD primers, the unique bands produced by OPC 2 (800 bp and 700 bp), OPC 18 (500 bp), OPD 05 (600 bp) and OPA 02 (900 bp) were found to be specific to only resistant lines (Susthira and four Selections). The dendrogram generated using NTSYS grouped the okra genotypes into two major clusters with a Jaccard's similarity coefficient of 0.50 to 0.79. The

overall variability observed among the six genotypes studied was 50 percent. Highest similarity of seventy nine percent was observed between Salkeerhti and Selection 6.

ISSR assay

The selected ISSR primers produced a total of 92 amplicons of which 68 were polymorphic giving a polymorphism of 73.91 percent with an average of 9.2 markers per primer and a mean of 6.8 polymorphic bands per primer. Out of ten primers used, the primer UBC 811 gave the highest polymorphism of 91.89 percent. Among the selected ISSR primers, three primers (UBC 834, UBC 818, and UBC 830) produced unique amplicons which were present only in the resistant genotypes. The dendrogram generated using NTSYS grouped the okra genotypes into two major clusters with a Jaccard's similarity coefficient of 0.43 to 0.91. The overall variability observed among the six genotypes studied was 57 percent.

References

7. REFERENCES

Adiger, S., Shanthakumar, G., Gangashetty, P. I. and Salimath, P. M. 2011. Association studies in okra (Abelmoschus esculentus (L.) Moench). *Elect. J. Plant Breed.* 2(4):568-573.

Abdul, N. M., Joseph, J. K. and Karuppaiyan, R. 2004. Evaluation of okra germplasm for fruit yield, quality and field resistance to yellow vein mosaic virus. *Indian J. Plant Genet. Resour*. 17: 241–244.

Adeoluva, O. O. and Kehinde, O. B. Genetic variability studies in West African okra (*Abelmoschus caillei). Agric. Biol. J. North Americ.* 10(2): 326-335.

Agarwal, M., Srivastava, N. and Padh, H. 2008. Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Rep.* 27: 617-631.

Akotkar, P. K. De, D. K. and Pal, A. K. 2010. Genetic Variability and Diversity in Okra (Abelmoschus esculentus L. Moench). *Elect. J. Plant Breed.* 1(4): 393-398.

Amoatey, H. M., Klu, G. Y., Quartey, E. K.,, Doku, H. A., Sossah, F. L. and MM Segbefia, M. M. Genetic Diversity Studies in 29 Accessions of Okra (*Abelmoschus* spp L.) Using 13 Quantitative Traits. *American J. Experi. Agric.* 5(3): 217-225.

Ahiakpa, J. K., Kaledzi, P. D., Adi, E. B., Peprah, S. and Dapaah, H. K. 2013. Genetic diversity, correlation and path analyses of okra (*Abelmoschus* spp. (L.) Moench) germplasm collected in Ghana. *Int. J. Dev. Sustain.* 2(2): 1396-1415.

Aladele, S. E., Ariyo, O. J. and Lapena, R. D. 2008. Genetic relationships among West African okra (*Abelmoschus caillei*) and Asian genotypes *(Abelmoschus esculentus)* using RAPD. *African J. Biotechnol.*7 (10):1426-1431.

Alex, R.1988. Progeny studies of interspecific crosses of *Abelmoschus* sp. M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 112p.

Ali, M., Hossain, M.Z., and Sarker, N.C. 2000. Inheritance of yellow vein mosaic virus (YVMV) tolerance cultivar of okra (*Abelmoschus esculentus* (L.) Moench). *Euphytica* 3 (3): 205-209.

Ali, M. I., Khan, M. A., Rashid, A., Ehetisham-ul-haq, M., Javed, M. T. and Sajid, M. 2012. Epidemiology of Okra Yellow Vein Mosaic Virus (OYVMV) and Its Management through Tracer, Mycotal and Imidacloprid. *American J. Plant Sci*. 3: 1741-45.

Ali, S. Singh, B., Dhaka, A. and Kumar, D. 2008. Study on correlation coefficients in okra (*Abelmoschus esculentus (L.) Moench*). *Plant Archives* 8(1): 405-407.

Aparna, J., Srivastava, K. and Singh, P. K. 2012. Screening of okra genotypes to disease reactions of yellow vein mosaic virus under natural conditions. *VEGETOS* 25(1): 326-328.

Allard, R.W. 1960. *Principles of Plant Breeding*. John Wiley and sons, New York, 432p.

Akotkar, P. K., De, D. K. and Pal, A. K. 2010. Genetic Variability and Diversity in Okra (*Abelmoschus esculentus (L. Moench*). *Electr. J. Pant Breed.* 1(4): 393-398.

Arumugham, R. and Muthukrishnan, C.R. 1978. Nitrogenous compounds in relation to resistance to yellow vein mosaic disease of okra. *Prog. Hort.* 10(2): 17-21.

Arumugham, R., Chelliah, S., and Muthukrishnan, C.R. 1975. *Abelmoschus manihot* - a source of resistance to bhindi yellow vein mosaic. *Madras Agric. J.* 62(5): 310-312.

Balakrishnan, D. and Sreenivasan, E. 2010. Correlation and Path Analysis Studies in Okra *Abelmoschus esculentus* (L.) Moench. *Madras Agric. J.* 97 (10-12): 326-328.

Bates, D.M. 1968. Notes on the cultivated malvaceae *Abelmoschus. Baileya* 16: 99-112.

Bhat, I. S., Mahajan, R. K. and Rana, R. S. 1995. Genetic diversity in South Asian okra (*Abelmoschus esculentus*) germplasm collection. *Ann. Appl. Biol.* 126:539-550.

Bendale, V.W., Kadam, S.R., Bhave, S.G., Sawant, S.S., and Desai, S.S. 2008. Evaluation of genetic divergence in okra. *J. Maharashtra Agric. Univ.* 33(1): 91-93.

Bendale, V. W., Kadam, S. R. Bhave, S. G. Mehta, U. B. Pethe, S. 2003. Genetic variability and correlation studies in okra. *Orissa J. Hort*. 31 (2): 1-4.

Bindu, K.K., Manju, P., and Sreekumar, S.G. 1997. Genetic variability in bhindi (*Abelmoschus esculentus* (L.) Moench). *S. Indian Hort*. 45: 286-288.

Biswas, N. K., Nath, P. S., Das, S., De, B. K. and Bhattacharyya, I. 2008. Field screening of different varieties/lines of bhendi [*Abelmoschus esculentus* (L.) Moench] against yellow vein mosaic virus disease in West Bengal. *Res. Crops* 9(2): 342-344.

Burton, G.W. 1953. Quantitative inheritance in grasse. *Proc. 6th Int. Grassland Congr*. 1 : 273 – 283.

Chacko, R.S. 1996. Genetic improvement and cytogenetical studies in thamaravenda (*Abelmoschus* manihot). M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 77p.

Candolle, A.P.De. 1883. *Origin Des Plantus Cultivees*. Paris, 150p.

Chandran, M. and Rajamony, L. 1997. Interspecific cross compatibility in *Abelmoschus. Proc. 9th Kerala Sci. Congr*; January 1997, Thiruvanandapuram, Kerala. p.186.

Chandran, M., Rajamony, L., Mohanakumaran, N., and Rajmohan, K. 1996. Embryos of interspecific crosses of *Abelmoschus* rescued invitro. *Proc. 2nd int. crop Sci. Congr.* November 17-24, 1996, New Delhi. pp. 165-166.

Charrier, A. 1984. Genetic resources of *Abelmoschus* (Okra) Report: *AGPC/ BPGR / 84/194* (Rome). pp. 10-20.

Chaukhande, P., Chaukhande, P.B., and Dod, V.N. 2011. Genetic variability in okra (*Abelmoschus esculentus* (L.) Moench). *The Asian J. Hort.* 6(1): 241-246.

Chaukhande, P., Chaukhande, P. B. and Dod, V. N. 2011. Correlation and path analysis studies in okra [*Abelmoschus esculentus (L.)Moench*]. *Asian J. Hortic.* 6(1): 203-205.

Chavan, J.K., Dalvi, U.S., Chavan, U.D. 2007. Isolation of lady's finger (okra) stem mucilage as clarificant in jaggery preparation. *J. Fd. Sci. Technol*. 44(1): 59-61.

Collard, b. C., Jahufer, M. Z., Brower, J. B. and Pang, E. C. 2005. An introduction to markers, quantitative loci (QTL) mapping and marker assisted selection for crop improvement: The basic concepts, Euphytica. 142: 169-196.

Dakahe, K., Patil, H.E., and Patil, S.D. 2007. Genetic variability and correlation studies in okra. *Asian J. Hort*. 2(1): 201-203.

Dakhe, K., Patil, H. E. and Patil, S. D. 2007. Genetic variability and correlation studies in okra (*Abelmoschus esculentus (L.) Moench*). *Asian J. Hortic.* 2(1): 201-203.

Deo, C., Singh, K. P., and Panda, P. K. 2000. Screening of okra parental lines and their F1s for resistance against yellow vein mosaic virus. *Veg. Sci*. 27(1): 78-79.

Dakahe, K., Patil, H. E. and Patil, S. D. 2007. Genetic variability and correlation studies in okra (*Abelmoschus esculentus L. Moench*.) *Asian J. Hort*. 2(1): 201-203.

Dhankar, B.S. and Dhankar, S.K. 2002. Genetic variability, correlation and path analysis in okra (*Abelmoschus esculentus* (L.) Moench). *Veg. Sci.* 29: 63-65.

Dankar, S. K. 2012. Genetic improvement of adopted okra cultivars for YVMV disease resistance involving wildrelatives in genus A*belmoschus.* Proceedins SEAVEG2012 Regional Symposium, 24-26 January 2012.

Dhankar, S. K., Dhankar, B.S., and Yadava, R.K. 2005. Inheritance of resistance to yellow vein mosaic virus in an interspecific cross of okra (*Abelmoschus esculentus*). *Indian J. Agric. Sci.* 75(2): 87-89.

Dobzhanansky, T. 1951. *Genetics and the Origin of Species*. Columbia University Press., New York, 364 p.

Dongre, A., Parkhi, V. and Gahukar, S. 2004. Characterization of cotton (*Gossypium hirsutum*) germplasm by ISSR, RAPD markers and agronomic values. *Indian J. Biotechnol*. 3: 388-393.

Dutta, O.P. 1991. Okra germplasm utilization at IIHR, Bangalore . *Int. workshop okra genet. resour*. NBPGR, New Delhi. pp. 114-116.

Dutta, P.C. and Nang, A. 1968. A few strains of (*Abelmoschus esculentus* (L.) Moench). their karyological study in relation to phylogeny and organ development. *Beitr. Biol. Pflanzen*. 45: 113-126.

Fajinmi, A. A. and Fajinmi, O. B. 2010. Epidemiology of okra mosaic virus on okra under tropical conditions. *Int. J. Vegetable Sci*. 16(3): 287-296.

Gandhi, H. T., Yadav, M. D., and Navale, P. A. 2001. Studies on variability in okra (*Abelmoschus esculentus* (L.) Moench). *J. Maharashtra Agric. Univ*. 26: 146-148.

Gangopadyay, K. K., ranjan, P., Bag, N. K., Srivastava, R., Roy, A., Bharadwaj, R., Prsad, T. V., Pandey, C. and Dutta, M. 2014. Vegetable genotypes for future. *Indian Hort.* 59(1): 3-5.

Gill, A.S., Verma, M.M., Dhaliwal, H.S. and Sandhu, T.S. 1983. Interspecific transfer of resistance to mungbean yellow mosaic virus from *Vigna mungo* to *Vigna radiata. Curr. Sci.* 52: 31-33.

Girase, S.M., Chavan, U.D., and Chavan, J.K. 2008. Accumulation of mucilage in cortex during growth of okra plants. *J. Maharashtra Agric. Univ*. 33(1): 90-91.

Girase, Y.P., Chavan, U.D., and Chavan, J.K. 2003. Mucilage from okra (*Abelmoschus esculentus*) cortex extraction and cultivar evaluation. *J. Fd. Sci. Technol*. 40(1): 118-119.

Gopalan, C., Sastri, B.V. R., and Balasubramanian, S. 2007. *Nutritive Value of Indian Foods.* National Institute of Nutrition, Hyderabad, 161p.

Grubben, G.J.H. 1977. Okra In: *Tropical Vegetables and Their Genetic Resources*. IBPGR, Rome, pp. 111-114.

Gulsen, O., Karagul. S. and Abak, K. 2007. Diversity and relationships among Turkish okra germplasm by SRAP and phenotypic marker polymorphism. *Biologia Bratislava* 62: 41-45.

Gupta, V.K. and Paul, Y.S. 2001. *Diseases of Vegetable Crops*. Kalyani Publishers, Ludhiana, 277p.

Gupta, M. Chyi, Y. S. and Romeo- Severson, J. L. 1994. Amplification of Random Amplified Polymorphic DNA in molecular ecology. *Mol. Ecol*.15(1): 1155-63.

Horvath, J. 1984. Virus resistance of species and varieties of peppers: incompatible host virus reaction. *Kertgazdasag* 16: 93-95.

Ikram, H. U., Khan, A. A. and Azmat, M. A.2013. Assessment of genetic diversity in okra (*Abelmoschus esculentus*(L.) Moench) using rapd markers. *Pak. J. Agri. Sci.* 50(4): 655-662.

Jaccard, P. 2008. Nouvelle research Sur la distribution florale Bull Soc Vaund. Sci. Nat. (French). 44: 223-270.

Jaiprakashnarayan, N.P and Mulge, R. 2004. Correlation and path analysis in okra (*Abelmoschus esculentus* (L.) Moench). *Indian J. Hort*. 61(3): 232-235.

Jaiprakashnarayan, R.P., R. Mulge, Y.K. Kotikal, M.P. Patil, M.B. Madalageri and B.R. Patil. 2006. Studies on genetic variability for growth and earliness character in okra. *Crop Res*. Hisar. 32 (3): 411 – 413.

Jambhale, N.D. and Nerkar, Y.S. 1981. Inheritance of resistance to okra yellow vein mosaic disease in interspecific crosses of *Abelmoschus. Theor. Appl. Genet*. 60: 313-316.

Jambhale, N.D. and Nerkar, Y.S. 1983. Interspecific transfer of resistance to yellow vein mosaic virus disease in okra. *J. Maharashtra. Agric. Univ.* 8: 197.

Jambhale, N.D. and Nerkar, Y.S. 1985. An unstable gene controlling developmental variation in okra. *Theor. Appl. Genet*. 71(1): 122-125.

Jambhale, N.D. and Nerkar, Y.S. 1986. Parbhani Kranti, an yellow vein mosaic resistant okra. *Hort. Sci*. 21: 1470-1471.

Jain, J. P. 1982. *Statistical Techniques in Quantitative Genetics*. Tata Mc Graw Hill Publishing Company, New Delhi, 103p.

Jaseena, P. 2008. Identification of promising segregants in F_4 and F_5 generations of the cross *Abelmoschus caillei* (A. Cher) Stevis x *A. esculentus* (L.) Moench. M Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 103p.

Jaseena, P., Sureshbabu, K.V., George, T.E., and Mathew, S.K. 2008. Identification of promising segregants in the F_4 generation of the cross *A. caillei* x *A. esculentus* . *Veg. Sci*. 35(2): 124-126.

Jindal, S. K., Arora, D. and Ghai, T. R. 2010. Variability studies for yield and its contributing traits in okra. *Electronic J. Pl. Breed.* 1(6): 1495-1499.

John, S. 1997. Genetic analysis of segregating generations of irradiated interspecific hybrids in okra (*Abelmoschus esculentus* (L.) Moench). MSc(Ag.) thesis, Kerala Agricultural University, Thrissur, 161p.

Johnson, H. W., HRobinson, H. F. and Comstock, R. E. 1955. Genotypic and phenotypic correlation in soybean and their implications in selection. *Agron. J.* 47 (10): 477 – 483.

Joshi, A.B., Singh, H.B. and Joshi, B.S. 1960. Why not grow Pusa Sawani *Indian Fmg*. 10: 6-7.

Joshi, A.B. and Hardas, M.W. 1956. Alloploid nature of okra, (*Abelmoschus esculentus* (L.) Moench). *Nature* 178: 1190-1191.

Kadlag, A.D., Kolekar, R.G., Kasture, M.C., and Sawale, D.D. 2005. Effect of integrated plant nutrient supply on yield, quality and nutrient uptake of okra. *Ann. Pl. Physiol.* 19(2): 191-195.

Kapoor, S.P. and Varma, P.M. 1950. Yellow vein mosaic of *Abelmoschus esculentus* L. *Indian J. Agric. Sci.* 20: 217-230.

KAU [Kerala Agricultural University]. 2007. KAU home page [online]. Available: http://www.kau.edu/varieties.htm. [14 June. 2012].

Kaur, K., Pathak, M., Kaur, S., Pathak, D. and Chawla, N. 2013. Assessment of morphological and molecular diversity among okra [*Abelmoschus esculentus* (L.) Moench.] germplasm. *African J. Biotechnol.*12(21): 3160-3170.

Katagi, A., Tirakannanavar, S., Jagadeesha, R. C., Jayappa, J. and Shankarappa. Genetic analysis of association studies in segregating population of okra [*Abelmoschus esculentus* (L.) Moench. *Intr. J. For. Crop Improv.* 4(1): 13-18.

Kishore. D. S. 2012. Variability for yield and resistance to yellow vein mosaic virus disease in okra [*Abelomoschus esculentus* (L) Moench] M.Sc.Thesis. Kerala Agricultural University. Thrissur, 144p.

Kondaiah, G.M., Kulkarni, U.G., and Nerkar, Y.S. 1990. The evolution of West African cultivated guinean okra. *J. Maharashtra Agric. Univ*. 15(1): 65-68.

Kousalya, V. 2005. Introgression of yellow vein mosaic virus resistance from *Abelmoschus caillei* (A.Cher.) Steveis into (*Abelmoschus esculentus* (L.) Moench. M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 67p.

Kulkarni, G.S. 1924. Mosaic and other related diseases of crops in Bombay presidency. *Poona Agric. Coll. Mag*. 16(1): 6-12.

Kumar, P. and Kumar, R. 2014. Variability, heritability and character association in okra [*Abelmoscus esculentus (L.) Moench*]. *Asian J. Bio Sci.* 9(1): 9-13.

Kumar, P., Singh, K. V., Singh, B., Kumar, S. and Singh, O. 2012. Correlation and path analysis studies in Okra (*Abelmoschus esculentus* (L.) Moench.). *Prog. Agric*. 12(2): 354-359.

Lush, J. l. 1940. Correlation and regression of offsprings ondam as method of estimating heritability characters. *Proc Amer. Soc. Anim. Prod.* 239-302.

*Masters, M.T. 1875. In: *Flora of British India* (Ed. Hooker, J.D.) Ashford Kent.1: 320-348.

Majumdar, M. K., Chatterjee, S. D. Bose, P. and Bhattacharya, G. 1974. Variability, inter-relationship and path coefficient analysis for some Quantitative characters in okra. *Indian Agriculturalist* . 18 (1) : 13–20.

Mamidwar, R.B., Nerkar, Y.S., and Jambhale, N.D. 1980. Cytogenetics of interspecific hybrids in the genus *Abelmoschus. Indian J. Hered.* 11(2): 35-40.

Markose, B.L. and Peter, K.V. 1990. *Review of Research on Vegetables and Tuber crops- Okra*. Directorate of Extension, Kerala Agricultural University, Thrissur, 109 p.

Martinello, G. E., Leal, N. R., Amaral, A. T., Pereira, M. G. and Daher, R. F. 2001. Comparison of morphological characteristics and rapd for estimating genetic diversity in *Abelmoschus* spp. *Acta Hort.* 546: 267-273.

Martin, F. W. 1982. A second edible okra species and its hybrids with common okra. *Ann. Bot*. 50: 277-283.

Mathews, H. 1986. Evaluation of the F2 generation of interspecific hybrids of *Abelmoschus* with reference to yellow vein mosaic resistance and yield. M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 112p.

Medikus, F. K. 1787. Ueber einige Kunstliche Gosch Lecture aus der Malven famile, den der klsee der Monadelphien. *Mannheion* 45-56.

Mehra, R., Dhawan, P. and Batra, V. 2008. Screening of okra germplasm against okra yellow vein mosaic virus disease for sustainable cultivation. *Veg. Sci.* 37(1): 121-122.

Morey, A. L., Nagre, P. K., Dod, V. N. and Kale, V. S. 2012. Genetic variability in okra. *Asian J. Hort*. 7(1): 1-4.

Mohapatra. M. R., Acharyya, P. and Sengupta, S. 2007. Variability and association analysis in okra. *Indian Agriculturist* 51 (1/2): 17 – 26.

Mullies, K. 1999. The unusual origin of polymerase chain reaction *Sci. Am*. 262(4):56-61.

Nataraja, M. V., Chalam, M. S. V., Madhumathi, T. and Rao, V. S. 2013. Screening of okra genotypes against sucking sests and yellow vein mosaic virus disease under field conditions. *Indian J. Pl. Protection* 41(3): 226-230.

Noormohammadi**1**, Z., Farahani**1**, Y. H. A., Sheidai, M., Ghasemzadeh-Baraki, S. and Alishah, O. 2013. Genetic diversity analysis in Opal cotton hybrids based on SSR, ISSR, and RAPD markers. *Genet. Mol. Res.* 12 (1): 256-269.

Nerkar, Y.S. and Jambhale, N.D. 1985. Transfer of resistance to yellow vein mosaic from related species into okra (*Abelmoschus esculentus* (L.) Moench). *Indian J. Genet.* 45(2): 268-270.

Nirmaladevi, S. 1982. Induction of variability in *Abelmoschus manihot* var. Ghana by irradiation. M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 85p.

Nerkar, Y. S. 1991. The use of related species in transferring disease and pest resistance genes to okra (*Abelmoschus esculentus* (L.) Moench). *Indian J. Agric. Res.* 31(3): 141-148.

Nwangburuka, C. C., Kehinde, O. B., Ojo, D. K., Popoola, A. R., Oduwaye, O., Denton, O. A. and Adekoya, M. 2011. Molecular characterization of

twenty-nine okra accessions using the random amplified polymorphic DNA (RAPD) molecular marker. *Acta SATECH* 4(1): 1 – 14.

Oshbon, B., Lightfrot, B. R., and Hill, A. 2005. Assessment of genetic polymorphism of *Pinus ponderosa* by Inter Simple Sequence Repeats (ISSR). *J. Sci*. 1:45-51.

Pal, B. P., Singh, H. B. and Swarup, V. 1952. Taxonomic relationships and breeding possibilities of species related to okra (*Abelmoschus esculentus* (L.) Moench). *Bot. Gaz.*113: 455-464.

Patil, P., Malik, S. K., Negi, K. S., John, J., Yadav, S., Chaudhari, G. and Bhat, K. V. 2013. Pollen germination characteristics, pollen–pistil interaction and reproductive behaviour in interspecific crosses among *Abelmoschus esculentus Moench* and its wild relatives. Grana 52(1): 1-14.

Patil, P., Malik, S. K., Negi, K. S., John, J., Yadav, S., Chaudhari, G., and Bhat, K. V. 2013. Pollen germination characteristics, pollen–pistil interaction and reproductive behaviour in interspecific crosses among *Abelmoschus esculentus* Moench and its wild relatives. *Grana* 52(1): 1-14.

Penner, G. A., Bush, A., Wise, R., Kim, W., Domier, L. and Kasha, K. 1993. Reproducibility of Random Amplified Polymorphic DNA (RAPD) analysis among laborotaries. *Genome. Res*. 12: 341-345.

Phadvibulya, V., Boonsirichai, K., Adthalungrong, A., and Srithongchai, W. 2009. Selection for resistance to yellow vein mosaic virus disease of okra by induced mutation. In: Shu, Q.Y. (ed.), *Induced Plant Mutations in the Genomics Era* (4th Ed.). Food and Agriculture Organization, Rome, pp. 349-351.

Philip, A.M.C., Manju, P., and Rajagopalan, B. 2000. Variability in F5 generation of irradiated interspecific hybrids in okra (*Abelmoschus esculentus* (L.) Moench). *J. Trop. Agric*. 38: 87-89.

Pitchaimuthu, M., Rao, E.S., Swamy, K.R.M., and Reddy, M. K. 2009. Developing pre breeding lines of okra (*Abelmoschus esculentus* (L.) Moench) resistant to yellow vein mosaic virus. [abstract]. In: *Abstracts, International*

Conference on Horticulture; 9-12, Nov, 2009, Premnath Foundation, Bangalore. pp. 329-351.

Pillai, U.P.R. 1984. Evaluation of Interspecific hybrids of bhindi with reference to yellow vein mosaic resistance and heterosis. M.Sc.(Ag.) thesis, Kerala Agricultural University, Thrissur, 120p.

Prabu, T. and Warade, S. D. 2013. Crossability studies in genus *Abelmoschus.* Veg. Sci. 40(1): 11-16.

Parthap, P.S., Dhankar, B.S., and Gautam, R.B. 1982. Genetics of earliness and quality in okra (*Abelmoschus esculentus* (L.) Moench). *Haryana Agric. Univ. J. Res*.12: 433-437.

Prabha, P. 1986. Cross compatibility between (*Abelmoschus esculentus* (L.) Moench) and *Abelmoschus manihot* and hybrid sterility. M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 105p.

Prakash,K., Pitchaimuthu. M. and Ravishankar. K. V. 2011. Assessment of genetic relatedness among Okra genotypes [*Abelmoschus esculentus* (L.) Moench] using RAPD markers. *Electr. J . Pl. Breed.* **2**(1): 80-86.

Prakash, K. and Pithaimuthu, M 2010. Nature and magnitude of genetic variability and diversity studies in okra (*Abelmoschus esculentus (L.) Moench*). *Electr. J. Pant Breed.* 1(6): 1426-1430.

Pradeepkumar, T., Suma, B., Jyothibhaskar, and Satheesan, K.N. 2008. *Management of Horticultural crops. Horticultural Science Series-11 Part-1.* (eds. Peter, K.V) New India Publishing Agency, New Delhi. pp. 141-142.

Prashanth, S.J., Jaiprakashnarayan, R.P., Mulge, R., and Madalageri, M.B. 2008. Screening for disease incidence of yellow vein mosaic virus in okra (*Abelmoschus esculentum* (L.) Moench*). Int. J. Pl. Prot*. 1(2): 78-80.

Pullaiah, N., Reddy, T. B., Moses, G. J., Reddy, B. M., Reddy, D. R. 1998. Inheritance of resistance to yellow vein mosaic virus in okra (Abelmoschus esculentus (L.) Moench). Ind J Genet Plant Breed. 58:349–352.

Ram, H.H. 1998. *Vegetable Breeding-Principles and Practices*. Lalyani publishers, New Delhi, 121p.

Rana, G., Tan, L. and Li, B. 2008. Optimization of ISSR- PCR reaction system for *Luffa cylindrical* (L.) Roem. *J. Shanxi. Agric. Sci*. 34:12-15.

Rattan, R.S. and Bindal, A. 2000. Development of okra hybrids resistant to yellow vein mosaic virus. *Veg. Sci*. 27(2): 121-125.

Ravisankar, J. 2002. Development of yellow vein mosaic virus infection on growth and yield of okra crop. M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 82p.

Reddy, A. M. And Sridevi, O. 2014. Evaluation of advanced breeding lines for yield and yield related components and resistance to okra yellow vein mosaic virus (OYVMV) disease in okra. *Intrl. J. Pl. Sci.* 9(1): 52-56.

Reddy, T. M., Babu, H. K., Ganesh, M., Reddy, C. K., Begum, H., Reddy, P. B., and Narashimulu, G. 2012. Genetic variability analysis for the selection of elite genotypes based on podyield and quality from the germplasm of okra (*Abelmoschus esculentus L. Moench*). *J. Agric. Technol.* 8(2): 639-655.

Reddy, P. S. and Dhaduk, L. K. 2014. Induction of genetic variability in okra [*Abelmoschus esculentus* (L.) *Moench*] by gamma and EMS. *Elect. J. Plant Breed.* 5(3): 588-593.

Robinson, H. F., R. E. Comstock and P.H. Harvey (1949). Genetic and Phenotypic correlations in corn and their correlation in corn and their implication in selection. Agron. J. 43 : 262 – 267.

Rohf, F. J. 2005. NTSYS, Numerical Taxonomy and Multivariate Analysis System. Version 2.02 Exter Softwares, New York.

Report of an International Workshop on Okra Genetic Resources, Held at the National Bureau for Plant Genetic Resources, New Delhi, India. 1990. Food and Agriculture Organization. 530 p.

Sangar, R.B.S. 1997. Field reaction of bhindi varieties to yellow vein mosaic virus. *Indian J. Virol*. 13: 131-134.

Sankar, A. A. and More, G. A. 2001. Evaluation of Inter Simple Sequence Repeat analysis for mapping in Cotrus and extension of genetic linkage map. *Theor. Appl. Genet*. 102:206-214.

Sastry, K.S. and Singh, S.J. 1974. Effect of yellow vein mosaic virus infection on growth and yield of okra crop. *Indian Phytopath*. 27: 295-297.

Sanwal, S. K. and Singh, B. 2014. Kashi Kranthi: new okra variety. Indian Hort. 59(2): 3-5.

Saifullah, M., Rabbani, M. G., Garvey, E. J. 2010. Estimation of genetic diversity of okra (*Abelmoschus esculentus* L. Moench) using RAPD markers. *SAARC J. Agric*.8(2): 19-28.

Sawadogo, M., Ouedraogo, J. T., Balma, D., Ouedraogo, M., Gowda, B. S., Botanga, C. and Timko, M. P. 2009. The use of cross species SSR primers to study genetic diversity of okra from Burkina Faso. *Afr. J. Biotechnol*. 8: 2476-2482.

Sengupta, S. K. and Verma,B. K. 2009. Genetic variability and correlation studies in okra (*Abelmoschus esculentus (L.) Moench*. *Haryana J. Hortic. Sci*. 38(3): 364-365.

Sergius, U. O. and Esther, D. U. 2014. Screening of *Abelmoschus esculentus* and *Abelmoschus caillei* cultivars for resistance against okra leaf curl and okra mosaic viral diseases, under field conditions in SouthEastern Nigeria. *African J. Biotechnol*. 13(48): 4419-4429.

Sharma, B. R. and Sharma, O. P. 1984. Breeding for resistance to yellow vein mosaic virus in okra. *Indian J. Agric. Sci.* 54(10): 917-920.

Sharma, B.R. 1982. Punjab Padmini- a new variety of okra. *Prog. Fmg.* 18: 15-16.

Sharma, B.K. and Mishra, M.N. 2007. Gamma-rays induced genetic variability in Ankur-40 variety of okra (*Abelmoschus esculentus* (L.) *Monech*). *Int. J. Pl. Sci*. 2(1): 57-59.

Siemonsuma, J.S. 1982. West African okra- morphological and cytological indications for the existence of natural amphidiploid of (*Abelmoschus*

esculentus (L.) Moench) and *Abelmoschus manihot* (L.) Medikar. *Euphytica.* 31(1): 241-252.

Sindhumole, P. 2003. Genetic analysis for yield and resistance of Yellow Vein Mosaic in okra (*Abelmoschus esculentus* (L.) Moench). Ph.D. thesis, Kerala Agricultural University, Thrissur, 167p.

Singh, K.P., Panda, P.K., and Deo, C. 2000. Screening of okra parental lines and their F_1s for resistance against YVMV. *Veg. Sci*. 27(1): 78-79.

Singh, A.K., Sharma, J.P., Kumar, S., and Sharma, N. 2007. Screening okra against yellow vein mosaic virus under sub tropical conditions. *Haryana J. Hort. Sci*. 36(3-4): 294-296.

Singh, A. K., Ahmed, N. Rajnarayan, H. and Chatoo, M. A. 2007. Genetic Variability, correlations and path analysis in okra under Kashmir conditions. *Indian J. Hort.* 64 (4) ; 472 – 474.

Singh, A.P., Bahadur, V., Toppo, R., and Singh, B. 2009. Genetic analysis in okra (*Abelmoschus esculentus* (L.) Moench). [abstract]. In: *Abstracts, International Conference on Horticulture;* 9-12, Nov, 2009, Premnath Foundation, Bangalore. pp. 332-333.

Singh, H. B., Joshi, B. S., Khanna, P. O., and Gupta, P. S.1962. Breeding for field resistance to YVMV in bhindi. *Indian J. Genet. Pl. Breed*. 22: 137-144.

Singh, V. and Kumar, V. 2012. An optimized method of DNA isolation from highly mucilaginous okra (Abelmoschus esculentus L. Moench) for PCR analysis. *Advancs Appl. Sci. Res*. 3(3): 1805-1810.

Singh, B., Pal, A. K. and Singh, S. 2006. Genetic variability and correlation analysis in okra [*Abelmoschus esculentus (L.) Moench*.]. *Indian J. Hort*. 63(3): 281-285.

Solankey, S. S. and Singh, A. K. 2010. Correlation studies in okra (Abelmoschus esculentus (L.) Moench). *Asian J. Hort*.5 (1): 70-75.

Sood, S., Arya, P.S., and Singh, Y. 1995. Genetic variability and correlation studies in okra (*Abelmoschus esculentus*). *Sci. Cult*. 35: 318-319.

Southern, E. M. 1995. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol*. 98:503-517.

Soyab, S. M., Shaikh, M. and Jadhav, R. S. 2013. Genetic variability, heritability and genetic advance in okra (*Abelmoschus Esculentus* L. Moench.). *Elect. J. Plant Breed*. 4(3): 1255-1257.

Sureshbabu, K.V. 1987. Cytological studies in okra (*Abelmoschus esculentus* (L.) Moench). Ph.D. thesis, University of Agricultural Sciences, Bangalore, 147p.

Sureshbabu, K.V. and Dutta, O.P. 1990. Cytogenetic studies of the F1 hybrid (*Abelmoschus esculentus* (L.) Moench) x *Abelmoschus tetraphyllus* and its amphiploid. *Agric. Res. J. Kerala* 28: 22-25.

Sureshbabu, K.V., Gopalakrishnan, T.R. and Mathew, S.K. 2004. Genetic variability, correlation studies, path analysis and reaction to YVMV. In: *Proc. 1st Indian Hort. Congr*. November 6-9, 2004, New Delhi. pp. 85-86.

Sureshbabu, K.V., Gopalakrishnan, T.R., Mathew, S.K. and Rajan, S. 2002. Crop improvement in yellow vein mosaic resistant underexploited okra type of Kerala. *Proc. Int. Conf. on vegetable.* November 11-14, Bangalore. pp. 382-383.

Sureshbabu, K. V., Jaseena, P., and George, T. E. 2009. Evaluation of YVMV resistant advanced generation lines of okra (*A. esculentus* (L.) Moench) rose out of interspecific hybridization. [abstract]. In: *Abstracts, International Conference on Horticulture;* 9-12, Nov, 2009, Premnath Foundation, Bangalore. pp. 52-53.

Sunil, K. Yadav, J. R. Mishra, G. Sanjeev Kumar, S. and B. Singh (2007). Estimation of heritability and genetic advance in okra. *Plant Archives* 7 (2): 923 – 924.

*Stebbins, G. L. 1950. *Variation and Evolution in Plants.* Columbia Univ. Press., New York, 643p.

Teshima, T.K. 1933. Genetic and Cytological studies in an interspecific hybrid of *Hibiscus esculentus* and *Hibiscus manihot. J. Fec. Agric. Hokkoido. Univ.* 34: 1-155.

Thakur, M.R. 1976. Inheritance of yellow vein mosaic in a cross of okra species (*Abelmoschus esculentus* (L.) Moench) x *Abelmoschus manihot. SABRAO J*. 8: 69-73.

Thakur, M.R. and Arora, S.K. 1988. Punjab-7- A virus resistant variety of okra. *Prog. Fmg.* 24: 13-14.

Tiwari, A., Singh, B. Sanval, S. K. and Pandey, S. D. 2012. Screening of okra varieties to yellow vein mosaic virus under field condition. *Hortflora Res. Spectrum* 1(1): 92-93.

Tripathy, P., Maity, T.K., and Patnaik, H.P. 2008. Field screening of open pollinated okra varieties against major pests in response to reduced level of chemical fertilizers and organic manures. *Pl. Prot. Environ*. 5(1): 38-44.

Tripathy, P., Maity, T. K. and Patnaik, H. P. 2007. Screening of okra hybrids against major pests under reduced level of chemical fertilizers supplemented with organic manures. *Indian J. Pl. protection* 35(2): 274-278.

Thampi, K.S. 1998. Nutritive value and organoleptic evaluation of thamara venda genotypes (*Abelmoschus caillei*). M.Sc. (Home Science) thesis, Kerala Agricultural University, Thrissur, 78p.

Thampi, K. S. and Indira, V. 2000. Nutritive value and organoleptic evaluation of thamara venda genotypes (*Abelmoschus caillei* (L)). *J. Tropic. Agrice.* 38(1-2): 38-40.

Tommercup, I. C., Barton, I. E., Sethuraj, M. R. and Ecke, J. 1997. Reliability of RAPD fingerprinting of three Basidiomycetes fungi Laccaria Hydnangium and Rhizoctonia. Mycol. Res. 99: 179-186.

Uppal, B. N., Varma, P. M., and Capoor, S. P. 1940. Yellow Vein Mosaic of Bhindi. *Curr. Sci.* 9: 27-28.

Upadhyay, A., Jayadev, K., Manimekalai, R. and Parthasarathy, V. A. 2004. Genetic relationship and diversity in Indian coconut accessions based on RAPD markers. *Scientia Hort*. 99: 353-362.

Varghese, V. A., Ranak, K., Sethuraj, M. R. and Ecke, J. 1997. Evaluation of Randomly Amplified

Vashista, R. N., Pandita, M. L., and Bhutani, R. D. 1982. Variability studies in okra (*Abelmoschus esculentus* (L.) Moench) under dry farming conditions. *Haryana. J. Hort. Sci*. 11: 117-121.

Vashisht, V. K., Sharma, B. R., Dhillon, G. S. 2001. Genetics of resistance to yellow vein mosaic virus in okra. *Crop Improv*. 28:18–25.

Vani, V. M., Singh, A. K., Raju, S. V. and Singh, S. P. 2012. Variability studies in Okra Abelmoschus esculentus L. Moench. *Envt. Ecol*. 30(3): 1203- 1206.

Vijay, M. and Joshi, V. 2013. Screening for yellow vein mosaic virus resistance under Hyderabad conditions. *Asian J. Hort*. 8(2): 763-766.

Vaishali, N., Agarwal, R., Singh, B. and Kumar, R. 2010. Molecular characterization and diversity analysis of *Abelmoschus esculentus* germplasm. *Veg. Sci.* 37(2): 146-148.

*Waalkes, Van, J.B. 1966. Malesian Malvaceae revised. *Blumea* 14: 1-251.

Wettasinghe, R. and Peffley, E. B. 1998. A rapid and efficient extraction method for onio DNA. *Plant Breed.* 117: 585-589.

Welsh, J. and McClelland, m. 1990. Fingerprinting of genomes usng PCR with arbitrary primers. *Nuclic Acids Res.* 18: 7213-7218.

Wolfe, M. L., Chaplin, M. F., and Otchere, G.1977. Studies on the mucilages extracted from okra fruits (*Hibiscus esculentus* (L.)) and baobab leaves (*Adansonia digitata). J. Sci. Fd. Agric.* 28(6): 519-529.

Williums, J. C. K., Kuelik, A. R. Livak, K. J. Rafalski, J. A. and Tengeeze, S. V. 1990. DNA Polumorphism Random Amplified polymorphic DNA markers. *Nucliec Acids Res.* 18:6531-6535.

Xu, Y. 2010. Molecular Plant Breeding. CAB International, Japan. 150p.

Yamuna, M., Sureshbabu, K. V., George, T. E., Prasanna, K. P., Mathew, S. K. and Krishnan, S. Evaluation of promising interspecific hybrid derivatives of okra (*Abelmoschus esculentus* (L.) Moench). *Veg. Sci.* 40(1): 99-101.

Yamuna, M. 2012. Evaluation of promising distant hybridization derivatives of okra [*Abelomoschus esculentus* (L) Moench] M.Sc.Thesis. Kerala Agricultural University. Thrissur, 84p.

Yuan, C.Y. , Zhan, C., Wang, P. S., Hu, S., Chang, H.P., Xiao, W.J., Lu, X.T., Jiang, S.B., Ye, J.Z., and Guo, X. H. 2014. Genetic diversity analysis of okra (*Abelmoschus esculentus* L.) by inter-simple sequence repeat (ISSR) markers. *Genet. Mol. Res.* 13 (2): 3165-3175.

Zietkiewie, E., Rafalski, A. and Labuda, D. 1994. Genome fingerprinting by Inter Simple Sequence Repeats (ISSR) anchored Polymerase Chain Reaction. *Genomics*. 20:176-183.

Appendices

Appendix I

A. Meteorological data during the crop growing period

Month	Mean max. Temp. (^{0} C	Mean min. Temp. (^{0} C	Mean RH (%)	Rainfall (mm)
June	28.5	22.7	90.00	1031.80
July	28.40	22.70	91.00	93230.0
August	29.90	22.0	84.00	478.23
September	30.00	22.2	88.00	344.7
October	30.80	23.0	85.00	355.22
November	32.6	22.00	73.00	332.45

B. Reagents for DNA isolation by modified CTAB method as per Singh and Kumar (2012)

1. CTAB buffer

- 2% CTAB (w/v)
- 100 mM Tris (pH 8.0)
- 0.5 M EDTA
- 1.4 M NaCl

C. Composition of buffer and dyes used for agarose gel electrophoresis

1. 50X TAE buffer (pH 8.0)

- 242 g Tris base
- 571.1 ml glacial acetic acid
- 100 ml 0.5 mM EDTA (pH 8.0)

2. Tracking/ Loading dye (6X)

- 0.25 % Bromophenol blue
- O.25% Xylene cynol
- 30% Glycerol in water

3. Ethidium bromide

- The dye was prepared as a stock solution of 10 mg/ ml in water and was stored at room temperature in dark bottle.

EVALUATION AND MOLECULAR CHARACTERIZATION OF ADVANCED GENERATION DISTANT HYBRIDIZATION SELECTIONS OF OKRA [*Abelmoschus esculentus* (L.) Moench]

by
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ABSTRACT OF THE THESIS

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ABSTRACT

Yellow Vein Mosaic Virus (YVMV) disease is a devastating disease infecting okra (*Abelmoschus esculentus* (L.) Moench), at all stages of crop growth, causing 50 to 94 per cent crop loss. The best way to tackle this disease is the use of resistant varieties developed by interspecific hybridization. There is no source of resistance to this disease in the species *Abelmoschus esculentus*. Hence a study was undertaken in the Department of Olericulture, College of Horticulture, Kerala Agricultural University, Vellanikkara during 2014-2015 for the evaluation of F_{12} selections of the cross between *Abelmoschus caillei* variety Susthira (YVMV resistant) and *Abelmoschus esculentus* variety Salkeerthi (high yielding, widely adapted but YVMV susceptible), with the objective of identifying promising lines with high level of resistance to YVMV.

Okra genotypes consisting of eight F_{12} selections along with their parents and Punjab 8 were evaluated for qualitative and quantitative characters, pollen fertility and reaction to YVMV in RBD with three replications. Variability and correlations were worked out for all the characters studied. Screening for YVMV resistance was done by creating artificial epiphytotic conditions in field, white fly transmission and graft transmission techniques. Four F_{12} selections (F_{12}-1, F_{12}-2, F_{12}-6 and F_{12}-7) exhibited high level of resistance to YVMV.

Evaluation of quantitative characters in the F_{12} selections showed significant variation among the genotypes for traits like, plant height, petiole length, days to first flowering, days to first harvest, length of fruit, number of fruits per plant, crop duration, yield per plant and coefficient of infection to YVMV.

The maximum values for both PCV and GCV were noticed for coefficient of infection of YVMV. Most of the traits possessed high heritability especially for the coefficient of infection of YVMV. High genetic advance could be noticed for plant height, yield per plant and coefficient of infection to YVMV. Correlation analysis indicated that fruit yield displayed positive genotypic association with length of fruit, number of fruits per plant and crop duration.

Pollen fertility studies indicated high level of pollen fertility in F_{12} selections. Mucilage extraction analysis revealed that only low amount of mucilage was present in F_{12} generation lines compared to the parent *A. caillei* variety Susthira.

Four F_{12} selections showed positive characters such as lower number of ridges per pod, longer fruit length, reduced width of epicalyx segment and less mucilage content similar to the parent Salkeerthi. Based on its promising fruit characters tending towards *A. esculentus,* selections such as F_{12}-1, F_{12}-2, F_{12}-6 and F_{12}-7 were identified. These selections expressed high resistance to YVMV and high yield.

Molecular characterization of promising selections (F_{12}-1, F_{12}-2, F_{12}-6 and F_{12}-7) and their parental varieties was carried out using RAPD and ISSR markers. RAPD produced a total of 71 amplicons of which 58 were polymorphic giving a polymorphism of 81.69 percent. The number of amplicons produced ranged from three to thirteen with an average of 7.1 amplicons per primer and a mean of 5.8 polymorphic bands per primer. The ISSR primers produced a total of 92 amplicons of which 68 were polymorphic giving a polymorphism of 73.91 percent. The number of amplicons were in the range of six to thirteen with an average of 9.2 markers per primer and a mean of 6.8 polymorphic bands per primer. In RAPD assay the highest percentage of polymorphism was given by OPA 02 (92.37%). In ISSR assay, the primer UBC 811 produced highest polymorphism percentage of 91.89 %. The ISSR marker systems produced more amplicons as compared to RAPD system with more number of markers per primer and more polymorphic amplicons per primer. The amplification patter observed in Selection 1 and Selection 2 was peculiar in both the marker systems. Molecular marker analyses could assess the variability among advanced generation selections and their parents evaluated in the present investigations. The study could locate some markers in the resistant genotypes which on further indepth study will aid in marker assisted selection for YVMV resistance. Further, molecular data generated in the present investigations will serve as a base for fingerprinting the elite genotypes for varietal registration.