BREEDING FOR RESISTANCE TO MOSAIC VIRUSES IN PUMPKIN (*Cucurbita moschata* Poir)

By

JESSY M. KURIAKOSE

THESIS

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Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF OLERICULTURE
COLLEGE OF HORTICULTURE
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KERALA, INDIA

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DECLARATION

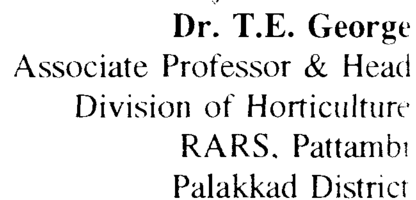
I hereby declare that the thesis entitled "**Breeding for resistance to mosaic viruses in pumpkin (*Cucurbita moschata* Poir)**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara,
01.09.1998.

JESSY M. KURIAKOSE

CERTIFICATE

Certified that the thesis entitled "**Breeding for resistance to mosaic viruses in pumpkin (*Cucurbita moschata* Poir)**" is a record of research work done independently by **Smt. Jessy M. Kuriakose**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.



Pattambi,
01.09.1998.

Dr. T.E. George
Associate Professor & Head
Division of Horticulture
RARS, Pattambi
Palakkad District

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Smt. Jessy M. Kuriakose**, a candidate for the degree of **Doctor of Philosophy in Horticulture**, agree that the thesis entitled "**Breeding for resistance to mosaic viruses in pumpkin (*Cucurbita moschata* Poir)**" may be submitted by Smt. Jessy M. Kuriakose, in partial fulfilment of the requirement for the degree.

Dr. T.E. George
Major Advisor, Advisory Committee
Associate Professor and Head
Division of Horticulture
RARS, Pattambi

Dr. S. Rajan
Professor and Head i/c
Department of Olericulture
College of Horticulture
Vellanikkara
(Member)

Dr. S. Balakrishnan
Professor
Department of Plant Pathology
College of Agriculture
Vellayani
(Member)

Dr. A. Augustin
Associate Professor
College of Horticulture
Vellanikkara
(Member)

Smt. S. Santhakumari
Professor and Head (Retired.)
Sugarcane Research Station
Thiruvalla
(Member)

EXTERNAL EXAMINER

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Introduction

INTRODUCTION

Introduced to our country from South American centre of crop origin by foreign navigators and emissaries, pumpkin, (*Cucurbita moschata* Poir) is now grown throughout the length and breadth of India. Pumpkin, an important cucurbitaceous vegetable of tropics cultivated for its mature fruit, is one of the most popular vegetables grown in Kerala and is known for its high vitamin A content and suitability for prolonged storage.

Among the various biotic and abiotic factors which limit the production of pumpkin, vulnerability to mosaic viruses is the most serious. Tripathi and Joshi (1985) reported the occurrence of several mosaic viruses in pumpkin namely, Yellow Vein Mosaic Virus, Pumpkin Mosaic Virus, Bottle Gourd Mosaic Virus, Cucumber Mosaic Virus, Cucumber Mottle Mosaic Virus, Cucumber Necrosis Virus, Squash Mosaic Virus and Watermelon Mosaic Virus. The cultivation of pumpkin suffered a set back during the last few years due to severe outbreak of mosaic diseases, particularly Pumpkin Mosaic and Yellow Vein Mosaic (Balakrishnan, 1988). Latha (1992) observed that Yellow Vein Mosaic, Pumpkin Mosaic, Bottle Gourd Mosaic, Water Melon Mosaic and Cucumber Mosaic are the major mosaic diseases causing crop loss in pumpkin in Kerala.

Incidence of mosaic diseases in pumpkin is very severe in Kerala during summer season, making the farmers reluctant to take up its cultivation during this otherwise most congenial growing season. Widespread occurrence of these mosaic diseases poses a big threat to pumpkin cultivation in Kerala. Balakrishnan (1988) found that the reduction in yield was even 100 percent in pumpkin when the plants were infected by mosaic viruses in the seedling stage. The virus menace has thus caused a drastic reduction in the yield and production of the crop in the state. This dismal scenari865o calls for an effective strategy for controlling the mosaic viruses in pumpkin.

The conventional plant protection measures for the control of vectors are inefficient and undesirable from the point of view of environmental pollution. The chemotherapic and physicotherapic procedures used *in vivo* against viruses are not so effective in practice. Therefore the only resource left to the grower to combat viral diseases is the use of virus resistant varieties (Horvath, 1984). Unfortunately all the existing released varieties

of pumpkin like Pusa Viswas, Arka Chandan, Co1, and Ambili are susceptible to pumpkin mosaic virus and yellow vein mosaic virus (Jayasree, 1984 and Suresh Babu, 1989).

The development of elite genotypes and the genetic upgrading of crops generally follow two pathways namely, production breeding and resistance breeding. To improve any crop, both these approaches should go complementary to each other.

Resistance breeding is the only feasible way for effective control of virus diseases. Evolution of a virus resistant variety in any crop requires long periods of screening in multi-environmental situations. Also, stability of resistance to viruses in crop varieties is another concern. Eventhough the process of evolving virus resistant varieties is cumbersome, the benefit of such research would be far-reaching and outstanding.

Work on genetic improvement of pumpkin, especially with focus on mosaic virus resistance has been conducted only on a very limited scale in India. It is in this context that the present research programme on Breeding for resistance to mosaic viruses in pumpkin (*Cucurbita moschata* Poir) becomes relevant and significant.

The study attempts to isolate sources of combined resistance to PMV and YVMV and to throw light on the genetic and biochemical mechanism of resistance to mosaic viruses. It will also lead to the evolution of mosaic virus resistant pumpkin genotypes which will have tremendous practical significance in boosting pumpkin production in the state Hence the present investigation was undertaken with the following broad objectives.

i) To identify sources of resistance to pumpkin mosaic virus and yellow vein mosaic virus
ii) To study the genetics of mosaic virus resistance
iii) To ascertain the biochemical mechanism of mosaic virus resistance and
iv) To select mosaic resistant plants with desirable horticultural attributes

Review of Literature

REVIEW OF LITERATURE

Pumpkin (*Cucurbita moschata*) is widely grown throughout India. Because of its high carotene content and good keeping quality, it is considered as a vegetable of immense value and it is a popular vegetable throughout Kerala. The crop often suffers from viral diseases which inflict significant losses in yield. According to Tripathi and Joshi (1985) pumpkin is infected by Bottle gourd Mosaic Virus (BMV), Cucumber Mosaic Virus (CMV), Cucumber Mottle Mosaic Virus (CMMV), Cucumber Necrosis Virus (CNV), Yellow Vein Mosaic Virus (YVMV), Squash Mosaic Virus (SqMV), Watermelon Mosaic Virus (WMV) and several other viruses. Yellow Vein Mosaic (YVM), Pumpkin Mosaic (PM), Bottlegourd Mosaic (BM), Watermelon Mosaic (WM) and Cucumber Mosaic (CM) are the major mosaic diseases causing crop loss in pumpkin in Kerala (Latha,1992).

Jayasree (1984) and Umamaheswaran (1985) reported that YVM and PM are very common and devastating mosaic diseases of pumpkin in Kerala. Widespread occurrence of these mosaic diseases poses a big threat to pumpkin cultivation in Kerala. Balakrishnan (1988) found that the reduction in yield was to the tune of 100 per cent in pumpkin when the plants were infected by mosaic viruses in the seedling stage.

Very limited work has been conducted so far for the genetic improvement of the crop especially with respect to mosaic virus resistance even though these viral diseases had been reported very early (Varma, 1955 and Shankar *et al.*, 1972). A review of literature pertaining to the common viral diseases of cucurbits - their sources of resistance, genetics, resistance mechanisms and breeding strategy is presented in this chapter.

2.1 Sources of resistance to mosaic virus

2.1.1. Yellow Vein Mosaic Virus (YVMV) and Pumpkin Mosaic Virus (PMV)

Suresh Babu (1989) and Latha (1992) reported that the introduced breeding line of pumpkin, Nigerian Local (CM 214) was extremely resistant to both YVMV and PMV. The seed germination, fruit setting and fruit development were quite erratic in this line and the fruits were warty and knobbed. Moreno *et al.* (1993) found that the agrestis type melon

(*Cucumis melo* var. *agrestis* genotype Garterslaber, UM 190/1982) exhibited low susceptibility to *Bemicia tabaci*, the vector of YVMV. Field evaluation of thirty different genotypes of bittergourd against YVMV showed that BG-14-4, BL-240, BG-14, HK-12 and Palwal Sel.-1 had no incidence of YVMV (Thakur, 1996).

2.1.2. Cucumber mosaic virus (CMV)

Cucurbita ecuadorensis has been reported to be resistant to CMV by Provvidenti et al. (1978). Lecoq and Pitrat (1980) reported that *C. melo* lines PI161375, Green Makuwa, Kanro Makuwa, Shiroubi Okayama, PI 164320 and PI 414723 were resistant to CMV and he attributed this resistance to non preference of vector *Aphis gossypii*. Rosemeyer and Bemis (1981) observed resistance to CMV in *C. foetidissima*. Munger *et al.* (1983) developed CMV resistant Summer squash (*Cucurbita pepo*) genotypes W 255 and No.469.

Resistance to CMV was noted in wild species of *Cucurbita* namely, *C. ecuadorenis* and this could be successfully hybridized with *C. pepo* for transferring CMV resistance (Washek, 1983). Walkey and Pink (1984) reported that *C. pepo* cultivar (cv.) Cindrella was resistant to two British strains of CMV and the resistance was heritable. According to Pink and Walkey (1988) this resistance in Cindrella had increased by selection and appeared to be fixed in the S4 generation. This resistance was effective in the glass house against 8 strains of the virus. Provvidenti (1985) reported that TMG-1, a single plant selection of a cucumber cultivar from Taiwan was resistant to CMV.

Velich and Tobias (1985) reported that muskmelon variety Shiroubi Okayama showed resistance to the virulent local strain of CMV-S4 at Hungary. Peterson *et al.*(1986) observed resistance to CMV in the cucumber variety WI 5207 G at ARS, USDA, USA. Resistance to CMV was noted in *C. colocynthis* PI 494528 and PI 494532 (Provvidenti, 1986). Field resistance to CMV was noted in a slicing cucumber variety, Milo, derived from a cross between the University of Hawaii inbred line 79-25 and 75 AI (Sekioka *et al.*, 1988). Ahemiya *et al.* (1991) found that most of the CMV resistant cultivars of Muskmelon were derived from Makuwauri (*C. melo* var. Makuwa).

Among 25 accessions of pumpkin (*C. moschata*) screened against different viruses, Horvath (1992) observed 16 as immune to CMV. According to Fiedorow (1993) pumpkin cultivar 390 and Zucchini cultivar Nimba had shown field resistance against CMV.

Studies at the Laboratory of Virolog, Ariana, Tunisia revealed that foreign melon lines PI 161375, Ginsen Makuwa and Freemans cucumber were resistant to the Tunisian common (TC) pathotype of Cucumber mosaic cucumovirus and Kanro Makuwa was resistant to the common strain TL from France (M, Nari *et al.*, 1993).

Yoshioka *et al.* (1993) developed transgenic melon plants expressing the CMV coat protein gene that were resistant to infection with CMV under green house conditions. After inoculation with 1 μg of purified CMV per ml of phosphate buffer transgenic plants did not develop symptoms during a 46-day observation period. After self pollination also, the seedlings of progenies of transgenic plants were resistant to CMV. Landraces belonging to *C. sativus* cultivar Soh-khia collected from Meghalaya and Assam have been found to possess high level of resistance to CMV (Sharma and Hore, 1996).

2.1.3. Cucumber Green Mottle Mosaic Virus (CGMMV)

Experiments at Indian Agricultural Research Institute, New Delhi revealed that two non-dessert types of phoot *C. melo var. momordica* and Kachri grown mostly in North India and FM-1 and FM-5 (Cornell breeding lines 83-273-6R and 83-275-6L respectively) were resistant (symptomless carrier type) to CGMMV (Rajamony *et al.*, 1987). Rajamony *et al.* (1990) screened 187 collections including wild Cucumis species against CGMMV under artificial inoculation and they found that *C. figarei*, *C. myriocarpus*, *C. myriocarpus* 3, *C. africanus*, *C. africanus* 2, *C. meeurii*, *C. zeyheri* 2 and *C. ficifolius* were resistant to the virus. Iroquois variety of muskmelon was reported to be medium resistant to CGMMV by Pan and More (1996).

2.1.4. Watermelon Mosaic Virus (WMV)

Provvidenti and Robinson (1977) reported resistance to WMV-1 in *Cucumis metuliferus*. Subsequently *Cucurbita foetidissima* and *C. ecuadorensis* had been identified to be highly resistant to WMV and WMV-1 (Papaya ring spot virus, PRSV-W) by Provvidenti *et al.* (1978) and Rosemeyer and Bemis (1981). An accession of *C. maxima* (ZR-1, a selection from Zapallito Redondo from Uruguay) was later recognized as resistant to PRSV-W (Provvidenti, 1982).

In cucumber, cultivar Surinam was found to be resistant to WMV-1 (Wang *et al.*, 1984). A melon breeding line 91213 was observed to be resistant to WMV-2 by Moyer *et al.* (1985) and the resistance was due to low rate of multiplication of the virus within the resistant plants. In *C. maxima*, variety Varzea Alegre had been reported as resistant to WMV-1 (PRSV-W) (Maluf *et al.*, 1985). Provvidenti (1985) reported that TMG-1 and Surinam in cucumber were resistant to WMV-1 and WMV-2. Vecchia and Avila (1985) could isolate a melon line W6 as resistant to WMV-1. Involving this resistant line and susceptible high yielding cultivar Amarelo, Pessoa *et al.* (1988) developed a promising WMV-1 resistant melon variety Eldorado-300 through resistance breeding.

Studies on virus transmission by *Aphis gossypii* revealed that three melon cultivars from Spain namely, Ariso, Invernizo and Escrito showed resistance to WMV-2 transmission (Pitrat *et al.*, 1988). Sekioka et al. (1988) observed resistance to WMV-2 in cucumber variety Milo. Kishaba *et al.* (1992) reported that the aphid resistant muskmelon breeding line AR Top Mark was resistant to WM 2 potyvirus. In melon, the breeding line PI 414723, derived from the Indian introduction PI 371795 by selfing and sib-pollination, was found to be resistant to melon aphid and hence resistant to WM2 potyvirus (Mc Creight *et al.*, 1992). The cultivar Menina from Portugal was found resistant to WMV-2 (Gilbert *et al.*, 1993).

Coat protein transgenic resistance to WMV in three yellow crookneck squash (*C. pepo var. melo pepo*)and five cantaloupe (*C. melo reticulatus* group) was reported by Clough and Hamm (1995). Useful resistance to WMV-2 strains from Florida, Arizona, California and New York was found in three lines of *Citrullus lanatus* i.e., PI189316, PI248178 and Egun (Gillaspie and Wright, 1995).

2.1.5. Zucchini Yellow Mosaic Virus (ZYMV)

Lecoq et al. (1981) and Provvidenti et al. (1984) observed resistance to ZYMV in *C. ecuadorensis*. Resistance to ZYMV had been found in the Muskmelon line PI 414723 from India. This resistance was effective against the ZYMV strains E 15 and 1318 belonging to the NF and F pathotypes respectively (Lecoq and Pirat, 1984 and Pitrat and Lecoq, 1984). Provvidenti *et al.* (1984) identified sources of resistance to ZYMV-CT and ZYMV-F_2 in accessions of *Citrullus colocynthis, Cucurbita sp., C. sativus, C. melo and Lagenaria Siceraria.*

The resistance of Nigerian Local to ZYMV and its possible role in the development of resistant variety had been reported by Provvidenti (1984). Provvidenti (1985) reported that TMG-1, a single plant selection of a cucumber cultivar from Taiwan was resistant to ZYMV. Provvidenti (1986) observed resistance to ZYMV in *C. colocynthis* PI 494528 and PI 494532. The reaction of these lines to ZYMV appeared to be influenced by temperature and light intensity as the plants were completely resistant during summer months while pre-mature leaf death occurred during winter. Paris *et al.* (1988) found resistance to ZYMV in an inbred line of *C. moschata* cultivar Menina.

Plants of *C. ecuadorensis* reacted to ZYMV with a few scattered chlorotic spots on the inoculated leaves, but the virus failed to move systemically (Robinson *et al.*, 1988). In a Dutch green house cucumber cultivar Dina, resistance reaction to ZYM potyvirus was noted, characterised by complete absence of symptoms both in seedlings and in mature plants when inoculated at 3-4 leaf stage (Hayja and Shahwan, 1991 and Shahwan *et al.*, 1995).

A high level of resistance to ZYM potyvirus was found in four land races of *C. lunatus* ie., PI 482322, PI 482299, PI 482261 and PI 482308 (Provvidenti, 1991). Out of 153 introduced watermelon and related breeding lines and commercial cultivars tested by mechanical inoculation technique in the green house, Boyhan *et al.* (1992) identified resistance reaction to Florida strain of ZYM poty virus in 482261-6 and Egun (*C. lanatus*) and in PI494528, PI 386025 and PI 386026 (*C. colocynthis*). The cultivar Menina from Portugal was found resistant to ZYMV (Gilbert *et al.*, 1993). According to Boyhan (1994), cultivar Egun had high level of resistance to the Egyptian strain of ZYMV.

2.1.6. Squash Mosaic Virus (SqMV) and Squash Leaf Curl Virus (SqLCV)

Salama and Still (1968) reported a moderate degree of resistance to SqMV in accession of *C. moschata*, *C. pepo* and *C. maxima*. Provvidenti and Robinson (1977) reported resistance to SqMV in *Cucumis metuliferus* while resistance to SqMV was also noted in *C. ecuadorensis* (Lecoq *et al.*, 1981 and Provvidenti *et al.*, 1984). *C. ecuadorensis*, *C. lundelliana* and *C. martinezii* were virtually immune to squash leaf curl virus (SqLCV) transmitted by the sweet potato white fly *Bemisia tabaci*. *Benincasa hispida*, *Cucurbita ficifolia*, *Lagenaria siceraria*, *Luffa acutangula*, *Cucurbita aegyptiaca* and *Lagenaria graveolens* were resistant to SqLCV in green house and field tests (Mc Creight and Kishaba, 1991).

2.1.7. Cucurbit Aphid Born Yellow Virus (CABYV)

Resistance to a yellowing disease caused by the virus CABYV transmitted by *Trialeurodes vaporariorum* was observed in *Cucumis anguria* var. longipes and *C. zeyheri* and in one accession of C. myriocarpus (Esteva *et al.*, 1988). Also tolerance to this virus was noted in plant introduction lines PI 157084, PI 161375 and Nagagate Kim Makuwa melons. Resistance to the vector of this virus was observed in *C. ficifolius* (Laska and Lebeda, 1989). One accession of *C. melo* var. *agrestis* displayed high level of resistance to CABYV (Nuez *et al.*, 1991). Resistance to this virus was observed in melons Faizabad Phoot, accession Nos. PI 90625, PI 124112, PI124440, PI 255478, PI 282448 and PI 414723 (Dogimont *et al.*, 1996).

2.1.8. Other viruses

A resistant source to Melon Mosaic Virus (MMV) was identified in the cucumber cultivar Kyoto-3-feet by Cohen *et al.* (1971). Resistance to Tomato ring spot virus was observed in *C. foetidissima* by Rosemeyer and Bemis (1981). Horvath (1992) screened 25 accessions of pumpkin against different viruses and observed that nine were immune to Cucumber fruit streak virus and three to Cucumber spot carmo virus. The various reports on sources of resistance to mosaic viruses are presented in Table 1.

2.2 Genetics of resistance

2.2.1. Whitefly transmitted viruses and PMV

Banerjee and Kalloo (1987a) found that the resistance to white fly transmitted Tomato leaf curl virus (TLCV) in the wild tomato *Lycopersicon hirsutum f. glabratum* B 6013 was controlled by two epistatic genes. A single dominant gene conferred resistance to the white fly transmitted yellowing disease in *Cucumis anguria* var. *longipes* (Esteva *et al.*, 1988). The resistance to yellow mosaic virus (YMV) in black gram was conferred by a single recessive gene while in *Vigna subulata* the resistance was digenic recessive (Pal et al., 1991). Latha (1992) reported that the resistance to YVMV and PMV in pumpkin accession CM 214 was governed by single dominant and recessive gene respectively. Bal *et al.* (1995) found that the resistance against leaf curl virus (LCV) in chilli was monogenic recessive.

2.2.2. CMV

Shifriss et al. (1942) investigated the genetic control of virus symptoms in cucumber and reported that three complementary genes controlled the ability or the failure to produce chlorosis at the cotyledon stage whereas in the true leaf stage they observed a constant changing of the ratio suggesting the role of several gene modifications in the genetic control of viral symptoms.

From the analysis of the F_1, F_2 and back cross generations involving the resistant and susceptible varieties of cucumber, Wasuwat and Walker (1961) observed that resistance to CMV was governed by a single dominant gene. A high degree of resistance to cucumis virus-1 in cucumber was characterised by intermediate inheritance and seemed to be based on three genes, each with partial resistance (Kooistra, 1969).

Table 1. Sources of mosaic virus resistance

No	Crop/Source of resistance	Type of mosaic virus	Authority
I	**Pumpkin**		
1	Nigerian Local	ZYMV	Provvidenti, 1984
		YVMV, PMV	Suresh Babu, 1989
		YVMV, PMV	Latha, 1992
2	Menina	ZYMV	Paris *et al.*, 1988
3	Cultivar 390	CMV	Fiedorow, 1993
4	Zucchini Cultivar Nimba	CMV	Fiedorow, 1993
II	**Musk melon**		
1	Green Makuwa	CMV	Lecoq and Pitrat, 1980
2	PI 164320	CMV	Lecoq and Pitrat, 1980
3	PI 414723	CMV	Lecoq and Pitrat, 1980
4	Shiroubi Okayama	CMV	Lecoq and Pitrat, 1980
5	PI 414723	ZYMV	Pitrat and Lecoq, 1984
6	Line 91213	WMV-2	Moyer et. al., 1985
7	Shiroubi Okayama	CMV-S_4 strain	Velich and Tobias, 1985
8	Line W6	WMV-1	Vecchia and Aviala, 1985
9	Phoot (*C. melo* var. *momordica*)	CGMMV	Rajamony *et. al.*, 1987
10	Kachri	CGMMV	Rajamony *et. al.*, 1987
11	FM-1	CGMMV	Rajamony *et. al.*, 1987
12	FM-5	CGMMV	Rajamony *et. al.*, 1987
13	Eldorado 300	WMV-1	Pessoa *et. al.*, 1988
14	Ariso	WMV-2, ZYMV	Pitrat *et. al.*, 1988
15	Invernizo	WMV-2, ZYMV	Pitrat *et. al.*, 1988
16	Escrito	WMV-2, ZYMV	Pitrat *et. al.*, 1988
17	AR Top Mark	WMV-2, ZYMV	Kishaba *et. al.*, 1992
18	PI 414723	WM-2 Poty virus	Mc Creight *et. al.*, 1992
19	Line 161375	CMV-TC	M, Nari et al.,1993
20	Ginsen Makuwa	CMV-TC	M, Nari et al.,1993
21	Freemans cucumber	CMV-TC	M, Nari et al.,1993
22	Kanro Makuwa	CMV-TL	M, Nari et al.,1993
23	Green slaber	YVMV	Moreno *et al.*, 1993
24	Transgenic Melon	CMV	Yoshioka *et al.*, 1993
25	Iroquois	CGMMV	Pan and More, 1996
III	**Bitter gourd**		
1	BG14-4	YVMV (Field resistance)	Thakur, 1996
2	BL-240	YVMV (Field resistance)	Thakur, 1996
3	BG-14	YVMV (Field resistance)	Thakur, 1996
4	HK-12	YVMV (Field resistance)	Thakur, 1996
5	Palwal Sel.1	YVMV (Field resistance)	Thakur, 1996

(Continued........)

Table 1. **Sources of mosaic virus resistance (Continued.....)**

No	Crop/Source of resistance	Type of mosaic virus	Authority
IV	***Cucurbita pepo***		
1	Cindrella	CMV	Walkey and Pink, 1984
V	***Cucurbita maxima***		
1	ACC. No.ZR-1	PRSV-W	Provvidenti, 1982
2	Varzea Alegre	WMV-1	Maluf *et al.*, 1985
VI	**Cucumber**		
1	Kyoto-3-feet	MMV	Cohen *et. al.*, 1971
2	Cultivar Surinam	WMV-1 WMV-1, WMV-2	Wang *et. al.*, 1984 Provvidenti, 1985
3	TMG-1	CMV, WMV-1, WMV-2, ZYMV	Provvidenti, 1985
4	WI 5207 G	CMV	Peterson *et. al.*, 1986
5	Milo	CMV, WMV-2	Sekioka *et. al.*, 1988
6	Cultivar Dina	ZYM poty virus	Hayja and Shahwan, 1991 Shahwan *et. al.*, 1995
7	Cultivar Soh-Khia	CMV	Sharma and Hore, 1996
VII	**Wild *cucurbita* sp.**		
1	*C. ecuadorensis*	CMV, WMV, WMV-1 ZYMV, Sq MV	Provvidenti *et al.*, 1978 Lecoq *et al.*, 1981 Provvidenti *et al.*, 1984
		CMV	Washek, 1983
		ZYMV SqLCV	Robinson *et al.*, 1988 Mc Creight and Kishaba, 1991
2	*C. foetidissima*	WMV-1 CMV, WMV, TRSV	Provvidenti *et al.*, 1978 Rosemeyer and Bemis, 1981
3	*C. martinezii*	CMV SqLCV	Washek, 1983 Mc Creight and Kishaba, 1991
4	*C. lundelliana*	SqLCV	Mc Creight and Kishaba, 1991

(Continued...

Table 1. Sources of mosaic virus resistance (Continued........)

No	Crop/Source of resistance	Type of mosaic virus	Authority
VIII	**Wild *cucumis* sp**		
1	*C. metuliferus*	WMV-1, Sq MV	Provvidenti and Robinson, 1977
2	C. anguria var. longipes	CABYV	Esteva et al., 1988
3	*C. zeyheri*	CABYV	Esteva *et al.*, 1988
		CGMMV	Rajamony *et al.*, 1990
4	*C. myriocarpus*	CABYV	Esteva *et al.*, 1988
	C. myriocarpus 1	CGMMV	Rajamony *et al.*, 1990
	C. myriocarpus 3	CGMMV	Rajamony *et al.*, 1990
5	*C. ficifolius*	CABYV	Laska and lebeda, 1989
	C. ficifolius	CGMMV	Rajanmony *et al.*, 1990
	C. ficifolius var. agrestis	CABYV	Nuez et al., 1991
6	C. figarei	CGMMV	Rajamony *et al.*, 1990
7	C. africanus	CGMMV	Rajamony *et al.*, 1990
8	C. meeurii	CGMMV	Rajamony *et al.*, 1990
IX	***Citrullus lanatus***		
1	PI 482322	ZYMV	Provvidenti, 1991
2	PI 482299	ZYMV	Provvidenti, 1991
3	PI 482261	ZYMV	Provvidenti, 1991
4	PI 482308	ZYMV	Provvidenti, 1991
5	PI 494528	ZYM Potyvirus	Boyhan et al., 1992
6	PI 386025	ZYM Potyvirus	Boyhan et al., 1992
7	Egun	ZYM-Potyvirus	Boyhan et al., 1992
		ZYMV- egyptian strain	Boyhen, 1994
		WMV- 2	Gillaspie and Wright, 1995
8	PI 189316	NMV-2	Gillaspie and Wright, 1995
9	PI 248178	NMV-2	Gillaspie and Wright, 1995
X	***Citrullus colocynthis***		
1	PI 494528	YVMV, CMV	Provvidenti, 1986
2	PI 494532	YVMV, CMV	Provvidenti, 1986
3	PI 386026	ZYM Potyvirus	Boyhan *et al.*, 1992

YVMV - Yellow Vein Mosaic Virus
ZYMV - Zucchini Yellow Mosaic Virus
CGMMV-Cucumber Green Mottle Mosaic Virus
PRSV-Papaya Ring Spot Virus
Sq MV-Squash Mosaic Virus
TRSV - Tomato Ring Spot Virus
CGMMV - Cucumber Green Mottle Mosaic Virus
PMV - Pumpkin Mosaic Virus
CMV - Cucumber Mosaic Virus
WMV-Watermelon Mosaic Virus
MMV-Melon Mosaic Virus
SqLCV-Squash Leaf Curl Virus
CABYV- Cucurbit Aphid Born Yellow Virus

According to Karchi *et al.* (1975) the resistance to CMV in melons was controlled by three recessive factors, while Lecoq and Pitrat (1980) reported that the resistance to one CMV strain in a resistant line of *C. melo* was governed by a single dominant gene. The resistance was due to non preference of the vector *Aphis gossypii*.

According to Takada (1982) the resistance to CMV in muskmelon Mitang was governed by 2-3 genes. Pitrat and Lecoq (1984) noted that the resistance to CMV in melon (due to resistance to aphid vectors) was controlled by 3-4 recessive genes. In cultivar Cindrella (*C. pepo*) the resistance to CMV appeared to be quantitative and had been increased by selection and it appeared to be fixed in the S_4 (Pink and Walkey, 1985). This resistance to CMV was effective against a range of strains suggesting that it might be durable. According to Velich and Tobias (1985), the resistance to CMV (S_4 strain) in muskmelon variety Shiroubi Okayama was digenic recessive. Meyer *et al.* (1987) observed quantitative resistance to CMV in cucumber which was found to be influenced by genotype, effective inoculum dose, plant age and virulence of the virus isolate used. Studies on lines derived from crosses between CMV resistant cultivar Cindrella and susceptible cultivar suggested that the resistance was under complex genetic control and that its expression was affected by environment (Pink and Walkey, 1988). In summer squash the resistance to CMV was controlled by single dominant gene as observed by Arora *et al.* (1992).

2.2.3. CGMMV

The studies by Rajamony *et al.* (1990) on inheritance of resistance to CGMMV in melon showed that the resistance was governed by polygenes with recessive nature. Out of 15 crosses studied 10 were found to be interacting. All the interacting crosses (except Phoot x Harela) showed duplicate type of epistasis.

2.2.4. WMV

In *Cucumis metuliferus*, the resistance to WMV-1 was governed by a single, completely dominant gene in a cross between the resistant PI 292190 and susceptible ACC 2459 (Provvidenti and Robinson, 1977). Similar results were obtained in case of papaya ring spot virus-water melon strain (PRSV-W Syn. WMV-1) (Provvidenti and Gonsalves, 1982).

In crosses between the resistant cucumber cultivar Surinam and the susceptible line Wisconsin 2757, the resistance to WMV-1 was monogenic and recessive. The symbol WMV-1 was assigned to the gene concerned (Wang *et al.*, 1984 and Provvidenti, 1985).

The inheritance of resistance to PRSV-W from C. maxima Varzea Alegre was studied by Maluf et al. (1985). Resistance was found to be controlled by polygenes with predominantly additive gene action. According to Vecchia and Avila (1985), the resistance to WMV-1 in the resistant melon W6 was conferred by a single dominant gene. Herrington *et al.* (1989) studied the inheritance of PRSV-W resistance from *C. ecuadorensis* to *C. maxima* cultivar Queenland Blue and suggested a polygenic control of resistance with predominance of additive gene effects.

Investigations on the inheritance of resistance to PRSV-W carried out by Maluf *et al.* (1997) in two resistant *C. maxima* accessions-ABL-010 and Redland Trailblazer (RT) revealed that resistance imparted by ABL in cross with susceptible Butternut could be explained by the action of three genes with partial dominance while that by RT in cross with Butternut was found to be under control of at least two genes with additive effects.

2.2.5. ZYMV

Lecoq and Pitrat (1984) and Pitrat and Lecoq (1984) noticed that the resistance to Zucchini yellow mosaic virus (ZYMV) in an acid melon from India PI 414723 was governed by a single dominant gene ZYM. Similar genetic mechanism was observed in cucumber by Yang et al. (1986) while Provvidenti (1985) noted that the cultivar Surinam (*C. sativus*) carried a single recessive gene for resistance to ZYMV.

Provvidenti (1986) reported that the resistance to ZYMV in a Nigerian squash (*C. moschata*) was partially dominant. According to Munger and Provvidenti (1987) the single gene conferring resistance to ZYMV when homozygous in *C. moschata* conferred a high level of resistance to ZYMV. The inheritance studies of resistance to ZYMV in cucumber revealed that the resistance to Connecticut strain of ZYMV was governed by a single recessive gene (Provvidenti, 1987). Investigations on the inheritance of resistance to

ZYMV in the cross *C. maxima* cultivar Queensland Blue x *C. ecuadorensis* showed that the major gene models did not fit all the data well, but estimates of heritability were high implying that selection of *C. maxima* genotypes with resistance to ZYMV should be effective (Herrington *et al.*, 1988). Paris et al. (1988) found that the resistance to ZYMV was controlled by a single dominant gene in the resistant inbred line of *C. moschata* cultivar Menina in crosses involving susceptible Walthan Butternut and Menina.

The findings of Robinson *et al.* (1988) showed that the resistance to ZYMV in *C. ecuadorensis* was controlled by a single major gene. They also observed that in heterozygous plants the degree of symptom expression varied due to the presence of modifying genes influencing the major gene. Quantitative inheritance of ZYMV resistance was reported in *C. ecuadorensis* by Paran *et al.* (1989). Several genes with major effects along with genes of minor effects seemed to control ZYMV resistance. They observed significance in additive and dominant gene action as well as in interaction of genes governing resistance. Hayja and Al-Shahwan (1991)reported that the resistance to ZYMV in cultivar Dina was governed by a single recessive gene. Provvidenti (1991) noticed that the resistance to florida strain of ZYMV in *Citrullus lunatus* PI 482261 was governed by a single recessive gene.

According to Gilbert-Albertini *et al.* (1993), the cultivar Menina (*C. moschata*) resistant to ZYM poty virus and WM poty virus was known to carry the same single dominant gene ZYM for resistance to both the viruses or perhaps two closely linked dominant genes. The resistance to ZYMV carried by the melon genotype PI 414723 was oligogenic with the number of genes observed to segregate in crossing depending on the genotype of the susceptible parent (Danin-Poleg *et al.*, 1997).

Table 2. **Inheritance of resistance to mosaic viruses**

No	Crop	Virus	Inheritance/gene action	Authority
1	Pumpkin	ZYMV	Partially dominant	Provvidenti, 1986
		ZYMV	Single dominant	Paris *et al.*, 1988
		WMPV	Single dominant	Gilbert-Albertini *et al.*, 1993
		YVMV	Single Dominant	Latha, 1992
		PMV	Single recessive	Latha, 1992
2	Blackgram	YMV	Single recessive	Pal *et al.*, 1991
3	*Lycopersicon hirsutum*	TLCV	Two epistatic genes	Banerjee and Kalloo, 1987 a
4	Chilli	LCV	Monogenic recessive	Bal *et al.*, 1995
5	Melons	CMV	Three recessive factors	Karchi et al., 1975
		CMV	Single dominant	Lecoq and Pitrat, 1980
		MNV	Single recessive	Coudriet *et al.*, 1981
		CMV	Oligogenic	Takada, 1982
		ZYMV	Single dominant	Lecoq and Pitrat, 1984
		CMV	Three/four recessive genes	Pitrat and Lecoq, 1984
		ZYMV	Single dominant	Pitrat and Lecoq, 1984
		CMV	Digenic recessive	Velich and Tobias, 1985
		WMV-1	Single dominant	Vecchia and Avila, 1985
		CGMMV	Polygenic recessive	Rajamony *et al.*, 1990
		ZYMV	Oligogenic	Danin-Poleg *et al.*, 1997
6	Cucumber	CMV	Three complementary genes with modifiers	Shifriss *et al.*, 1942
			Single dominant	Wasuvat and Walker, 1961
			Quantitative	Meyer *et al.*, 1987
		MMV	Single dominant	Cohen *et al.*, 1971
		ZYMV	Single dominant	Yang et al., 1980
				Provvidenti, 1985
			Single recessive	Provvidenti, 1987
			Single recessive	Hayja and Al-Shahwas, 1991
		WMV-1	Monogenic recessive	Wang et al., 1984
			Monogenic recessive	Provvidenti, 1985
		Cv-1	Three genes with partial resistance	Velich and Tobias, 1985
7	*Cucurbita pepo*	CMV	Quantitative	Pink and Walkey, 1985
			Complex genetic control	Pink and Walkey, 1988
			Single dominant gene	Arora *et al.*, 1992
8	*Cucurbita maxima*	PRSV-W	Polygenic	Maluf *et al.*, 1985
			Three genes with partial dominance 2-3 genes with additive effects	Maluf *et al.*, 1997
9	*Cucumis metuliferus*	WMV-1	Single dominant	Provvidenti and Robinson, 1977
		PRSV-N	Single dominant	Provvidenti and Gonsalves, 1982
10	*Cucumis anguria* var. longipes	WTYD	Single dominant	Esteva et al., 1988
11	*Cucurbita ecuadorensis*	ZYMV	Single dominant	Robinson et al., 1988
			Quantitative	Paran *et al.*, 1989
		PRSV-W	Polygenic	Herrington *et al.*, 1989

YVMV-Yellow Vein Mosaic Virus, PMV- Pumpkin Mosaic Virus, ZYMV- Zucchini Yellow Mosaic Virus, LCV-Leaf Curl virus
WMPV-Water Melon Poty Virus, YMV-Yellow Mosaic Virus, TLCV-Tomato Leaf Curl Virus, CV-Cucumis Virus
CMV-Cucumber Mosaic Virus, WMV-Water Melon Mosaic Virus, MNV-Melon Necrotic Virus, PRSV-Papaya Ring Spot Virus
CGMMV-Cucumber Green Mottle Mosaic Virus, MMV-Melon Mosaic Virus, WTYD- Whitefly Transmitted Yellowing Disease

2.2.6. MMV

In *C. sativus* cultivar Kyoto-3-feet, Cohen *et al.* (1971) identified a single dominant factor for the control of resistance to melon mosaic. In resistant plants there was low concentration of the virus. The various reports on genetics of resistance to mosaic viruses are presented in Table 2.

2.3 Biochemical mechanism of resistance

Plants have their own built-in defence mechanism against all microorganisms. Most plants are immune to most viruses and susceptibility is therefore the exceptional conditions. In a few cases the pathogen overcomes this defence barrier with offensive chemicals and cause diseases.

The most common resistance mechanism affecting the establishment and localisation of spread of virus is hypersensitivity where infected cells die pre-maturely. Muller (1959) and Cruickshank (1963) stated that the host might have two kinds of defence factors namely prohibitors and phytoalexins.

2.3.1 Defence mechanisms of the host

A wide range of chemicals especially phytoalexins, phenolics, proteins and host enzymes predominantly peroxidase (PRX), polyphenol oxidase (PPO) and esterase (EST) show antifungal and antiviral activities in plant disease resistance.

2.3.1.1 Phytoalexins and Phenolics

Jejelowo (1995) observed that three different phytoalexins-camalexin and two new phytoalexins accumulated in leaves of *Arabidopsis thaliana* following inoculation with the fungus *Cochliobolus carbonum*.

Toxic quinones are commonly formed from dihydroxic phenolic compounds (Hampton and Fulton, 1959) and quinones are both virus inhibitor and phytotoxic to host cells. Protective role of phenolics against bacterial wilt disease was reported by many scientists (Rajan, 1985 and Sadhankumar, 1995 in tomato, Gangappa, 1986 and Gopinath and Madalageri, 1986 in brinjal). Rajan (1985) observed a negative correlation between resistance and total phenol content in tomato, due to increased rate of oxidation of phenolics. In many plant pathogen interactions, the synthesis of phenolics is activated after infection and high amount of phenolics synthesised rapidly suppress the pathogen development (Vidhyasekharan, 1990). Sadhankumar (1995) indicated a high significant correlation of phenol content with resistance to bacterial wilt in tomato.

2.3.1.2 Protein

Enhanced protein synthesis appears to be a universal phenomenon in compatible host pathogen interactions. De novo synthesis of new proteins was also reported (De Wit and Bakkar, 1980). Russel (1981) suggested that antiviral factors (AVF) are involved in localised and systemic induced resistance to virus diseases and the AVF concerned with limiting systemic virus spread are proteins or polypeptides.

Ming and Xian (1988) studied the resistance to fusarium wilt fungus in 79 watermelon varieties using seedling root dip inoculation technique. He observed that the resistant varieties had a higher content of glycine, serine alanine, threonine, proline and arginine than the susceptible varieties. Ahmed *et al.* (1994) observed that in okra cultivars resistant to YVMV the total protein and soluble protein were high.

2.3.1.3 Host enzymes

Oh (1988) found that in soybean the peroxidase activity markedly increased by the infection with soyabean necrotic virus (SMV-N) and was higher in the susceptible variety than in the resistant line. Reuveni et al. (1990) observed a high correlation ($P<0.05$) between peroxidase activity in the leaves of melon and resistance to *Pseudomonas cubensis* suggesting that this rapid assay was possible in preliminary selection of melons resistant to the fungus.

Ahmed *et al.* (1994) observed high polyphenol oxidase activity in okra varieties resistant to YVMV than in the susceptible varieties. In groundnut, Duan *et al.* (1994) found that there was no significant difference in pre-inoculation polyphenol oxidase activity between the bacterial wilt resistant and susceptible varieties, but the differences were significant after inoculation.

Shan and Tan (1994)observed increased esterase activity only in the susceptible groundnut cultivars in the case of bacterial wilt disease while Xia *et al.* (1994) reported higher esterase activity in verticillium wilt resistant cotton varieties than in the suscepts. In the former case esterase activity was inhibited in the resistant cultivars.

2.3.2. Protein and enzyme pattern Vs disease resistance

Several workers have reported the possibilities offered by the protein and enzyme pattern in plant tissues to locate resistant genes against diseases thereby enabling early screening against diseases. Gel electrophoresis of soluble proteins and enzyme create a powerful, well defined and effective method to detect the genetic differences among individuals. Isozyme variations are used as powerful tools to complement the conventional biochemical and genetic studies. The utility of electrophoretic isozyme method in studying the biochemical mechanism of disease resistance was reported by Kato *et al.* (1978) in *C. melo.*

2.3.2.1 Protein Pattern

Gabriel and Ellingboe (1982) conducted protein gel electrophoresis in isogenic lines of wheat for resistance to *Erysiphae graminis f. sp. tritici* and reported that existing proteins may not be involved in disease resistance. The pattern of protein in non-infected leaves of six barley cultivars showing various levels of resistance to powdery mildew was similar. Babu (1987) reported that Mungbean plants infected with yellow mosaic virus exhibited 11 protein bands in their leaves while three bands seen in the diseased were absent in the healthy ones.

Biles *et al.* (1989) observed variant protein banding pattern between watermelon cultivars differentially susceptible to races 0, 1 and 2 of *Fusarium oxysporum f sp. niveum*. The susceptible cultivar Black Diamond lacked protein bands that were present in other cultivars.

Sarkar *et al.* (1990) conducted PAGE of acid soluble basic proteins of seeds of disease resistant and susceptible linseed cultivars and observed that the protein bandings were 12 and 7 respectively for the resistant and susceptible genotypes respectively.

Accumulation of pathogenesis related proteins is thought to play a role in pathogen induced plant defence responses. Lawrence *et al.* (1996) studied the accumulation patterns of specific isozymes of pathogenesis related proteins in three resistant and one susceptible varieties upon challenge with the fungus *Alternaria solani*. Wastern blot analysis demonstrated that four isozymes of chitinase (26,27,30 and 32 KDa) were induced in all genotypes, but only resistant lines had significantly higher constitutive levels of the 30 KDa as well as total chitinase activity. Two isozymes of β-1,3-glucanase (33 and 35 KDa) were detected in all genotypes but a slightly higher constitutive level was detectable in all resistant lines compared to the suscepts. Only basic isozymes of chitinase and β-1,3-glucanase were inhibitory to the pathogen. This study suggested that a higher constitutive level of chitinase and beta-1,3-glucanase and the induction pattern of a 30 KDa chitinase isozyme in early blight resistant breeding lines related to genetically inherited resistance of tomato to *A. solani*.

Markose (1996) reported that chilli variety Ujwala (resistant to bacterial wilt) and Pusa Jwala (susceptible) possessed similar protein bands with identical electrophoretic mobility. On inoculation the susceptible variety exhibited an additional band in the leaves.

2.3.2.2 Enzyme Pattern

Farkas and Stahmann (1966) found that the peroxidase zymogram pattern of Southern bean mosaic virus infected leaves of *Phaseolus vulgaris* exhibited two new peroxidase isozymes II and III whereas the uninfected leaves showed peroxidase isozymes I and IV. Gabriel and Ellingboe (1982) noted genotypic differences in the banding pattern of

esterase in barley cultivars showing various levels of resistance to powdery mildew. Out of eight bands differences were exhibited in EST 4, EST 5 and EST 6 (Hwang *et al.*, 1982). Based on these differences they classified the cultivars into highly resistant, moderately resistant and highly susceptible. Hulupi *et al.* (1988) studied the role of isozymes against resistance to leaf rust in coffee. Based on PPO isozyme bands they classified the genotypes into three groups, same as to their resistance to leaf rust.

Ming and Xian (1988) conducted isozyme analysis of PRX and EST through PAGE in different organs of 11 watermelon varieties differing in resistance to fusarium wilt disease. There were some differences in PRX and EST isozyme spectrum in stems and leaves and the varieties differing in FWR. Therefore isozyme method could be used to screen watermelon varieties for FWR. Oh (1988) conducted studies on the electrophoretic pattern of peroxidase in resistant as well as susceptible soybean varieties, in early period of infection by soybean necrotic virus (SMV-N). He found that the electrophoretic isozyme pattern was changed by the infection in the susceptible variety while no new isozyme bands were observed in the incompatible host parasite combination.

Kato and Jodo (1989) observed that the acid phosphatase banding pattern in muskmelon genotypes varied with levels of resistance to powdery mildew. The banding pattern in the F2 of the cross between resistant muskmelon cultivar MSII and susceptible cultivar Earls' Favourite were of three kinds : 22 resembling MS II, 24 that of Earls Favourite and 14 that of the F1 hybrid.

Konishi *et al.* (1989) noted that one of the barley mosaic virus resistant genes in the barley landrace Mokusekko 3 was tightly linked to the esterase isozyme loci Est-1-Est-2-Est-4. Le Gouis *et al.* (1995) reported that Ym4-a barley mild mosaic virus resistant gene was tightly linked to these enzyme loci.

Yu and Wang (1990) reported that the seedling of susceptible watermelon cultivars to fusarium wilt disease had one or two isozyme bands more than those of the resistant cultivars and hence this factor could be used for early screening against diseases.

Studies by Wagih (1992) revealed that when infected with tobacco necrosis necro virus, the affected cucumber seedlings showed an additional major isozyme band of amylase (Rf 0.7), with respect to the healthy plants.

By electrophoretic analysis, only one band of β-1,3-glucan hydrolase was certified in the enzyme extract from healthy as well as TMV infected leaves of tobacco cultivar white Burley. Two isozyme bands were seen in tobacco leaves with induced resistance to TMV-T (tomato strain), after inoculation (Sanada *et al.*, 1994). Guldur and Yilmaz (1995) attempted for the rapid detection of tomato mosaic virus by agarose gel electrophoresis and reported that two main bands were produced from infected plants and no bands were detected in healthy plants.

Markose (1996) observed that the number of bands of peroxidase in roots was two in Ujwala (resistant to bacterial wilt) while it was three in Pusa Jwala (susceptible). In leaves, Ujwala had only one band while it was two in Pusa Jwala. In case of esterase enzyme, there were three medium thick bands in Ujwala whereas Pusa Jwala had only one feeble band. Inoculation had no effect in the banding pattern for both the enzymes.

2.4 Improving the suscepts through transfer of mosaic virus resistance

Hybridization involving crossing between the disease susceptible, but otherwise desirable plants with less desirable but resistant sources had been relatively successful in producing promising hybrids resistant to many pathogens. Planting resistant varieties is by far the most desirable method of plant disease control (Juan, 1988).

2.4.1. Resistance to CMV

Through resistance breeding, Takada (1982) developed three melon varieties Ano-1, Ano-2 and Ano-3 with resistance to CMV, from the F_3 of (Hiratsuka 3 x Mitangting) F6 x Maruike 3 of which Mitangting was resistant to CMV and the others susceptible, but

otherwise superior varieties. Munger *et al.* (1983) developed CMV resistant summer squash (*C. pepo*) genotypes W255 and No.469.

2.4.2. Resistance to whitefly transmitted viruses

Gill *et al.* (1983) successfully transferred YMV resistance from blackgram to mungbean. Kalloo and Banerjee (1990) developed six tomato line H-2, H-11, H-17, H-23, H-24 and H-36 resistant to TLCV transmitted by the white fly *B. tabaci* with controlled introgression of *L. hirsutum* f. *glabratum* into *L. esculentum* through pedigree selection in the BC-6 population. The fruit size and days to maturity in resistant lines were close to those of the cultivated susceptible varieties included. YVMV resistant mungbean lines were recovered in advanced generations of interspecific cross involving the mosaic susceptible mungbean line SML 32 and the resistant blackgram variety Saradhu, without back crossing (Pal *et al.*, 1991). Latha (1992) observed pumpkin seedlings with resistance to YVMV in the segregating population of Ambili(S) x CM 214(R).

2.4.3. Resistance to ZYMV

Among 300 muskmelon plants inoculated with ZYMV in the BC progenies [PI 414723 (R) X Doublon (S) x Doublon], Pitrat and Lecoq (1984) found that 143 were resistant to ZYMV, enabling selection for plants with desirable horticultural attributes from among these resistant populations. Munger and Provvidenti (1987) attempted to transfer the resistance to ZYMV from the resistant source Nigerian local to Butternut Squash (BN). In the BC_1F_2 generation they could select resistant plants with similarity to BN in shape, size, colour and quality of cooked flesh. Robinson *et al.* (1988) reported that it had been possible through selection and selfing to develop breeding lines from the back cross (*Cucurbita ma ima* cultivar Butternut(S) x (*C. ecudorensis* (R) x *C. ma ima* cultivar butternut), that have good fertility and a high degree of resistance to ZYMV.

2.4.4. Resistance to WMV

Pessoa *et al.* (1988) could develop a WMV-1 resistant promising melon variety namely, Eldorado-300 from the cross between W6(R) and cultivar Amarelo (S) through resistance breeding. The average yield of the variety was 7.71 t/ha, with an average fruit weight of 1.50 kg. The variety Redlands Trail blazer in C. maxima was produced by backcross transferring of resistance to WMV-2, PRSV-W and ZYMV from *C. ecuadorensis* into *C. ma ima* (Duch) (Herrington *et al.*, 1991). Munger (1993) reported that the hybrids of PRSV resistant cornell line PM 339 with MR 324, Jam Uvalde and Gulfcoat had superior resistance to its parental genotypes PM 339. Gilbert *et al.* (1994) could transfer the resistance to WM 2 poty virus from *C. melo* PI 414723 to three melon varieties by successive backcrossing with the resistant source.

2.4.5. Resistance to CGMMV

In view of the polygenic nature of inheritance of resistance to CGMMV in melon lines Phoot and FM I, More *et al.* (1993) carried out sibbing between selected resistant plants in the crosses Phoot (R) x Monoecious 4 (S) and FMI (R) x Pusa Madhuras (S) in the F_2 and F_3 generations. From the F_4 to F_8, selected resistant plants were selfed and advanced. Then selection was carried out for desirable horticultural characteristics. Thus they developed six highly resistant melon lines with desirable horticultural characters from the F_3-F_{10} namely VRM 5-10, VRM 29-2, VRM 31-1, VRM 31-2, VRM 42-4 and VRM 43-6.

Materials and Methods

MATERIALS AND METHODS

The present investigations envisaged to locate sources of resistance in pumpkin to PMV and YVMV, study the genetics of resistance to mosaic viruses, ascertain the biochemical mechanism of resistance to mosaic virus and select mosaic virus resistant genotypes with desirable horticultural attributes was carried out in the research plots attached to the Sugarcane Research Station, Thiruvalla during the period from 1994 to 1998. The experimental field was located at an altitude of 2.5m above MSL and 10^0 N latitude and 76^0 50′ E longitude. The area enjoys a warm humid tropical climate. The weekly weather parameters during the period of experimentation were recorded (Appendix I). The experimental site is flood prone with transported acidic alluvial soil having a pH of 5.5. The agenda of the research programme included the following main experiments.

1. Identification of source(s) of resistance to PMV and YVMV in pumpkin and related species
2. Studying the genetics of resistance to mosaic viruses
3. Ascertaining the biochemical mechanism of resistance to mosaic viruses
4. Selection of mosaic virus resistant plants possessing desirable horticultural attributes, from segregants of cross between virus resistant genotypes and important released varieties of pumpkin

3.1 Identification of Source(s) of resistance to PMV and YVMV

The experiment material comprised of 103 genotypes belonging to six species of *Cucurbita* which included both exotic and indigenous collections. The selfed seeds of these lines were sown in seed pans/polythene bags filled with potting mixture kept in an insect proof polyhouse during 1994-95. Seed pans were drenched with 0.2% Indofil-M-45 before sowing to protect the seedling from soil born pathogens. The number of seedlings screened per genotype against each virus was thirty. The seedlings were artificially inoculated by mechanical inoculation technique with standard extract of the virus in case of PMV and by white fly transmission in case of YVMV, when they were 10-14 days old. One set of thirty seedlings were inoculated with PMV and the other set with YVMV.

3.1.1 Mechanical inoculation of PMV

3.1.1.1 Preparation of inoculum

Collection of PMV isolates was done from field grown pumpkin in the research station. Leaves showing typical symptoms of PMV (light green and dark green patches with dark green blisters on the lamina) were collected and brought to the laboratory for preparation of the inoculum. The leaves were washed thoroughly in running tap water and dried between the folds of blotting paper. They were then macerated using sterilized mortar and pestle, using 1 ml of 0.067 M phosphate buffer(pH 7.2). The resultant pulp was squeezed through double layers of muslin cloth. The extract thus obtained was used as standard inoculum.

3.1.1.2 Inoculation technique

Carborundum powder (600 mesh size) was added to the inoculum at 0.025 g/ml as an abrasive. A small piece of sterilized absorbent cotton wool soaked in the extract was gently rubbed on the upper surface of the young leaves of 10-14 days old seedlings of the genotypes under study. The inoculated leaves were washed immediately to remove the excess of inoculum with a fine jet of distilled water from a squeeze bottle. The seedlings were then kept for symptom expression. Inoculation and establishment of the viruses were done under insect-proof conditions in the glass house.

3.1.2 Whitefly transmission

Whitefly (*B. tabaci*), the vector of YVMV was used for inoculation in case of YVMV. Plastic transmission cages were used for the transmission. After subjecting the adult flies for half an hour fasting, they were transferred to the cages and allowed to feed on YVM affected leaves of pumpkin (characterised by yellow net work of veins and veinlets and chlorotic interveinal areas on the lamina) for one hour to acquire the virus. Then they were transferred to healthy 10-14 days old pumpkin seedlings of 1-2 leaf stage of the genotypes under study and allowed to feed for 24 hours. After the inoculation feeding period, the plants were sprayed with 0.05% Rogor to kill the flies. Seedlings were then kept for symptom expression.

3.1.3 Scoring procedure

The plants were scored for leaf symptom expression as per the methodology outlined by George *et al.* (1992) for twenty one days in case of YVMV and for 28 days in case of PMV, at weekly interval using 1-3 scale (plates 1 and 2) as follows.

1 No symptom (R)

2 Mild mosaic symptoms / local lesions on inoculated leaves (MR)

3 Severe mosaic symptoms (S)

The plants which did not show symptoms were again inoculated with the respective virus and scored. The mean disease score, infection per centage and coefficient of infection (PDVR, 1997) were worked out in the case of each genotype. For calculating the coefficient of infection the scores 1, 2 and 3 were arbitrarily taken as 0, 1 and 2 respectively.

3.1.4 Confirmation of virus resistance

3.1.4.1 Back inoculation

The genotype expressing resistance to PMV was indexed back on ten healthy seedlings of the susceptible variety Ambili at 10-14 days old stage. The inoculated seedlings were observed for symptom expression.

3.1.4.2 Grafting

The method of grafting followed was wedge grafting. The root stocks comprised of 25 day old seedlings of susceptible variety Ambili inoculated with YVMV and showing symptoms of mosaic. The scion used was the top of 25 days old seedlings of both the resistant genotype NL and susceptible variety Ambili. The grafted plants were kept in an insect proof glass house and noted for disease reaction.

Scoring of leaf symptom expression of Pumpkin mosaic

Plate 1

Scoring of leaf symptom expression of Yellow vein mosaic

Plate 2

Resistant parent Nigerian Local

Plate 3

Female flowers of Nigerian Local

Plate 4

Fruits of Nigerian Local

Plate 5

1
2
3

1
2
3

NL

NIGERIAN LOCAL

3.1.4.3 Multi-environment testing

The resistant accession NL along with Ambili was grown for three seasons i.e., September-December 95, December-March 96 and April-July 96, to assess its resistance expression to PMV and YVMV in different environments. Scoring was carried out during the three seasons for the symptom expression of both the viruses as done in initial screening.

3.2 Genetics of resistance to mosaic viruses

3.2.1 Crossing programme

Based on the information obtained on resistance to PMV and YVMV, Nigerian Local (NL) (plates 3 to 5) identified as resistant to both the viruses, was selected as the resistant parent in the crossing programme. The four susceptible parents selected were Ambili (Amb), Pusa Viswas (PV), Arka Chandan (AC) and Co 1 (plates 6 to 15).

The crossing programme was started in August 1996. Crossing was carried out to get sufficient F_1 seeds of all cross combinations including the reciprocals. The top of corolla of the young unopened flowers expected to open the next day was tied with rubber band. The pollen grains collected from the pre-marked male flowers of the male parent were dusted on the stigma of the covered female flowers of the female parent before 7.30 am, next day.

The selected F_1 hybrids (plates 16 to 19) and their parents were planted in the field during February, 1997. Back crosses were made using both the parents in each cross combination to obtain first back cross generation seeds (B_1 and B_2). In the same season, F_1 plants were selfed to get the F_2 generation seeds.

3.2.2 Raising seedlings and planting

Seedlings were raised in green house in polythene bags filled with potting mixture. In the case of NL, the seeds were pre-treated and germinated in the laboratory and then transferred to the polythene bags. Ten day old seedling were then transferred to the prepared field and planted in pits of 60 cm diameter and 30 cm depth at the rate of 2-3 plants per pit. The spacing between pits was 3m x 3m. FYM @ 20-25 t/ha was applied as basal dose along

Susceptible parent Ambili

Plate 6

Ambili infected with Pumpkin mosaic virus

Plate 7

Ambili infected with Yellow vein mosaic virus

Plate 8

Susceptible parent Pusa Viswas

Plate 9

Pusa Viswas infected with Pumpkin mosaic virus

Plate 10

AMB

AMB

YELLOW VEIN MOSAIC
AMBILI

PV

PV

Susceptible parent Arka Chandan

Plate 11

Arka Chandan infected with Pumpkin mosaic virus

Plate 12

Arka Chandan infected with Yellow vein mosaic virus

Plate 13

Susceptible parent Co1

Plate 14

Co1 infected with Pumpkin mosaic virus

Plate 15

AC

AC

Arka Chandan

Co1

with half dose of N (35 kg) and full dose of P (25 kg) and K (25 kg) per hectare at the time of preparation of pits. The remaining dose of N (35 kg/ha) was applied in two equal splits at the time of vining and at the time of full blooming. All crop management as well as crop protection operations were carried out as per the Package of Practices Recommendations of Kerala Agricultural University (1993).

3.2.3 Evaluation for resistance to PMV

The evaluation of the parents along with the F_1, F_2, B_1 and B_2 generations of the following crosses for resistance to PMV was carried out in protected condition during July - October, 1997 following the procedure adopted in initial screening. The number of seedlings screened were 40 in the parents and F_1s, 300 in the F_2 and 100 each in the B_1 and B_2 generations.

1. Amb x NL
2. PV x NL
3. AC x NL
3. Co 1 X NL

3.2.4 Evaluation for resistance to YVMV

Scoring of the parents along with the F_1, F_2, B_1 and B_2 generations of the above crosses for resistance to YVMV was carried out under similar conditions adopting the technique and procedure followed in initial screening. The number of seedlings screened were the same as in case of PMV.

3.2.5 Determination of reciprocal effects on resistance to PMV and YVMV

The following F_1s and their reciprocals were evaluated for resistance to PMV and YVMV.

Amb x NL — NL x Amb
PV x NL — NL x PV
AC x NL — NL x AC
Co 1 x NL — NL x Co 1

F_1 - Ambili x Nigerian Local

Plate 16

F_1 - Pusa Viswas x Nigerian Local

Plate 17

F_1 - Arka Chandan x Nigerian Local

Plate 18

F_1 - Co1 x Nigerian Local

Plate 19

AMB x NL

PV x NL

AC x NL

Co1 x NL

3.2.6 Performance of parents

The performance of the susceptible parents, Amb, PV, AC, and Co 1 and the resistant parent NL were observed for their seed germination per cent (before and after seed coat removal), number of female flowers produced per plant, number of developed fruits per plant and fruit development per centage.

3.2.7 Chi-square test

The plants were classified into 2 categories namely, resistant to virus and susceptible to virus. Plants with numerical rating of disease score with value 1 and 2 were classified as resistant and those with score 3 were classified as susceptible. The gene action of virus resistance was determined by subjecting the F_2 and back cross ratios to chi-square test (Fischer, 1950). Penetrance of the gene for disease resistance/susceptibility was estimated as suggested by Avdeyev (1979).

3.3 Biochemical mechanism of mosaic virus resistance

The protein pattern and isozyme (peroxidase and esterase) profiles in the seedlings (three weeks old) of the resistant line NL, the susceptible varieties Amb, PV, AC and Co 1 and their F_1 hybrids namely, Amb x NL, PV x NL, AC x NL and Co 1 x NL were analysed using PAGE before and after inoculation of both the viruses. The viruses were inoculated seven days before sampling. The parents and hybrids were given code numbers as given below.

Amb	: 1	Co 1	: 4	PV x NL	: 7
PV	: 2	NL	: 5	AC x NL	: 8
AC	: 3	Amb x NL	: 6	Co 1 x NL	: 9

3.3.1 Protein Profile

3.3.1.1 Extraction

The extraction was carried out as per Sadasivam and Manickam (1992). Leaf samples were used for the study. The extraction buffer had the following composition.

Tris HCl	0.05M (adjusted to pH 7.40)
Cysteine	0.1%
Ascorbic acid	0.1%
Sucrose	17%

Plant material weighing three grams was crushed in 1.50ml of Tris HCl extraction buffer in a chilled mortar and pestle. The homogenates were filtered through a cheese cloth into centrifugation tubes and centrifuged at 20,000 rpm for 20 minutes at 4^0C. The clear supernatant liquid was collected in eppendorf tubes and one drop of bromophenol blue was added to each sample. An aliquot (0.5ml) of the extract was mixed with 0.5ml of Sodium Dodecyl Sulphate (SDS) sample buffer in eppendorf tube, heated exactly for five minutes in boiling water bath and freezed until use. Composition of the SDS sample buffer is given below:

<u>Composition of the SDS sample buffer</u>

0.1M Tris-HCl buffer, pH 6.8

Tris	-	0.606g
SDS	-	2.5 g
Sucrose	-	25 g
Mercapto ethanol	-	1.25 ml
Bromophenol blue	-	5 ml (0.5/w/v)

The volume was made up to 50ml and the required quantity was diluted five times to get the sample buffer.

3.3.1.2 Electrophoretic run

The procedure of Sadasivam and Manickam (1992) was followed for the electrophoretic run of the samples.

3.3.1.2.1 Running buffer-stock solution

A stock solution was prepared by mixing 6.00g Tris, 15.02g Glycine and 2g SDS. The pH of the solution was adjusted to 8.90 with 1M Tris. Out of this solution, 500ml was diluted to 1000ml.

3.3.1.2.2 Composition of the gel

Composition of the different reagents and their respective concentrations in the spacer and separation gels are presented below:

Sl. No.	Reagents	Composition	Quantity (g)	pH
1	Stock A (100 ml)	1.50 M Tris	18.171	8.9
2	Stock B (100 ml)	1.00 M Tris	12.14	6.7
3	Stock C (100 ml)	4.20 M Acrylamide 0.065M bisacrylamide	1.00	
4	Stock D*	10% APS	0.20	
5	Stock E	10% SDS	10g	
6	TEMED			

** Prepared at the time of mi ing*

Composition of different solutions for the preparation of one gel slab

Stock solution	Spacer gel (4%) (ml)	Separation gel (10%) (ml)
C	2.50	13.30
A	-	10.00
B	5.00	--
Distilled water	5.60	14.30
D	0.20	0.40
Stock E	0.10	0.30
TEMED	0.01	0.02

3.3.1.2.3 Preparation of the gel

The polyacrylamide gel used for resolving the protein and isozymes consisted of a four per cent spacer gel poured over a ten per cent separation gel. Two gel Plates were adhered together with a grease coated teflon spacer of 1mm thickness. The separation gel was prepared by pouring the mixture in the gel casting set, leaving 2.5cm from the top. After the polymerisation, the mixture for spacer gel was poured over this and immediately inserted a comb into the spacer gel for making wells. Care was taken to avoid bubbles in the gel while pouring the gel mixtures. After polymerisation the comb was removed for electrophoretic run.

3.3.1.2.4 Electrophoresis

The polymerised gel was fixed in an electrophoretic tank. 50 μl each of the sample was loaded in the wells. The upper and lower tanks were filled with running buffers. The gel was run at 20mA. About 6-7 hours were taken for completing the electrophoretic run at low (5^0C) temperature. After completion of running, the gel was transferred to the staining and substrate solutions respectively.

3.3.1.2.5 Staining of the gel

Staining of the gel was carried out by silver staining technique of Hames (1994) (Appendix II).

3.3.2. Isozymes

Two isozyme systems were analysed namely, peroxidase and esterase. Extraction, gel preparation and running procedures are the same as in case of proteins, but no SDS is added to the extract, gel or running buffer.

3.3.2.1. Staining of gel

The staining procedure of Vallejos (1983) as given below was followed.

Peroxidase

Reagents

0.2 M Acetate buffer pH 5.6	- 200 ml
Benzidine	- 0.2 g
H_2O_2 3%	- 0.80 ml
Water	- 200 ml

Fresh staining solution was prepared each time. Acetate buffer and benzidine were mixed, heated to boil, cooled, filtered and then hydrogen peroxide was added. The gels were immersed in staining solution till the brown bands of peroxidase appeared and destained in seven per cent acetic acid. As the bands faded on standing for long time, photographs were taken on the same day of staining. The zymogram was sketched and the Rm values were calculated.

Esterase

Reagents

Sodium dihydrogen phosphate	- 2.80 g
Disodium hydrogen phosphate	- 1.10 g
Fast Blue RR salt	- 0.12 g
1-naphthyl acetate	- 0.08 g
EDTA	- 0.06 g
Distilled water	- 200 ml

Sodium dihydrogen phosphate and disodium hydrogen phosphate were dissolved in 200 ml water to which EDTA, Fast blue and 1-naphthyl acetate (dissolved in 4 ml acetone) were added and filtered. The gel was then incubated in the staining solution at 37°C till light brown bands appeared. Then the solution was drained off and seven per cent acetic acid was added. The electrophorogram was photographed, sketched and the Rm values calculated.

3.4 Selection of resistant plants with desirable horticultural attributes

The F_1, F_2 and back cross populations of all cross combinations (25 seedlings each) were raised in polythene bags under insect proof conditions of net house and were subjected to artificial inoculation with PMV when the seedlings were 5 days old. The seedlings were then scored for symptom expression for twenty one days at weekly interval. The plants with scores 1 and 2 were again inoculated with YVMV and waited for symptom expression for ten days. The plants showing resistance (scores 1 and 2) to both the viruses were then transferred to field for continuing their further growth and development. The cultural practices adopted were same as those of previous seasons. The plants were then observed for important horticultural attributes. The plants of these populations displaying resistance to PMV and YVMV and at the same time free from incidence of other mosaic viruses and possessing desirable horticultural attributes were ultimately selected. A viable breeding strategy for developing high yielding pumpkin varieties with combined resistance to PMV and YVMV and possessing desirable horticultural attributes was formulated.

3.5 Observations recorded

Observations were recorded on the following quantitative and qualitative characters using standard procedures.

<u>Quantitative characters</u>

- i Days to anthesis of first female flower
- ii Node at which the first fruit was developed
- iii Length of Vine (m)
- iv Female flowers per plant
- v Days to maturity of first fruit
- vi Number of fruits per plant
- vii Fruit length (cm)
- viii Fruit diameter (cm)
- ix Average fruit weight (kg)

x Fruit yield per plant (kg)

xi Flesh thickness (cm)

xii Number of seeds per fruit

xiii Total soluble solids (%)

xiv Carotene content (IU/100g) of fruits

xv Incidence of mosaic viruses

Qualitative characters

i Fruit colour at maturity

ii Flesh colour

iii Fruit shape

iv Rind character : Smooth/warty

The total soluble solids (TSS %) was measured using hand refractometer and the carotene content expressed as Vitamin A (IU/100g) was estimated as per the procedure outlined by Srivastava and Kumar (1988).

3.6 Statistical Analysis

The data obtained from different experiments were subjected to statistical analysis. Analysis of variance of different characters under study was carried out as per Panse and Sukhatme (1978) (Appendix III).

Results

RESULTS

4.1 Isolation of sources of resistance to PMV and YVMV

One hundred and three genotypes of pumpkin and related species were screened against PMV and YVMV. They included 95 genotypes in *C. moschata*, three in *C. maxima*, two in *C. pepo* and one line each in *C. foetidissima, C. martinezii* and *C. ecuadorensis*. The results are presented in Table3.

Other than the introduced breeding line Nigerian Local, all the genotypes evaluated for resistance to PMV and YVMV including the feral species showed varying degrees of susceptibility to both the viruses, irrespective of their species status (Table 3). The mean disease score in NL against both the viruses was one while in others it ranged from 2.07 to 3.00 in case of PMV and 2.00 to 3.00 in case of YVMV. The coefficient of infection in these genotypes ranged from 20.82 (SRS-53) to 100 for PMV and 13.34 (SRS-19) to 100 for YVMV. In NL all the plants inoculated were free from both PMV and YVMV infection with zero coefficient of infection for both the viruses. In others the infection percentage ranged from 35 to 100 for PMV and from 26.67 to 100 in case of YVMV. The released varieties of pumpkin namely, Ambili, Pusa Viswas, Arka Chandan and Co1, were highly susceptible to both the viruses with severe systemic symptoms (Plates 20 to 22). The mean disease score four weeks after inoculation was 3 and the infection percentage and coefficient of infection were 100 each. The weekly data on infection percentage and mean disease score are presented in Table 4.

Within one week of inoculation of YVMV, 0.63 per cent plants showed susceptibility with a mean disease score of 1.35. On an average 33.75 per cent plants expressed susceptibility reaction after two weeks of inoculation, the mean disease score being 2.08. In case of PMV even moderate symptoms of disease expression was not noticed in any of the varieties within one week of inoculation of the virus. During the second week also only 0.008 per cent showed susceptibility reaction. During third week of inoculation, 22.50 per cent plants were infected with PMV whereas hundred per cent infection occurred during the fourth week.

Table 3. Disease reaction of *Cucurbita* species to PMV and YVMV

Sl. No.	Variety/ Cultivar/ Line	Accession No.	Source	Reaction to PMV				Reaction to YVMV			
				Mean disease score	Infection %	Coefficient of infection	Classification	Mean disease score	Infection %	Coefficient of infection	Classification
A. *Cucurbita moschata* genotypes											
1	Ambili	SRS-102	College of Hort., Vellanikkara	3.00	100.00	100.00	HS	3.00	100.00	100.00	HS
2	Pusa Viswas	SRS-103	IARI, New Delhi	3.00	100.00	100.00	HS	3.00	100.00	100.00	HS
3	Arka Chandan	SRS-104	IIHR, Bangalore	3.00	100 00	100.00	HS	3.00	100.00	100.00	HS
4	Co 1	SRS-105	TNAU, Coimbatore	3.00	100.00	100.00	HS	3.00	100.00	100.00	HS
5	Nigerian Local	SRS-7	Cornell University.,USA	1.00	0.00	0.00	HR	1.00	0.00	0.00	HR
6	Butternut	SRS-10	Cornell University.,USA	3.00	100.00	100.00	HS	2.93	93.33	90.06	HS
7	NIC10170	SRS-13	NBPGR, New Delhi	2.37	50.00	34.25	MS	2.20	48.28	28.97	MS
8	NIC10171	SRS-14	NBPGR, New Delhi	2.60	66.67	53.34	S	2.93	90.00	86.85	HS
9	NIC10173	SRS-15	NBPGR, New Delhi	2.34	55.17	36.96	MS	2.45	62.07	45.00	S
10	NIC10178	SRS-16	NBPGR, New Delhi	2.70	80.00	68.00	S	2.33	63.33	42.11	S
11	NIC10181	SRS-17	NBPGR, New Delhi	3.00	100.00	100.00	HS	2.82	83.33	75.83	HS
12	NIC10184	SRS-18	NBPGR, New Delhi	2.80	86.20	77.58	HS	2.70	80.00	68.00	S
13	NIC10185	SRS-19	NBPGR, New Delhi	2.45	68.97	50.00	S	2.00	26.67	13.34	MR
14	NIC10186	SRS-20	NBPGR, New Delhi	2.63	70.00	57.05	S	2.37	66.67	45.69	S
15	NIC10188	SRS-21	NBPGR, New Delhi	2.63	70.00	57.05	S	2.20	45.20	27.12	MS
16	NIC10190	SRS-22	NBPGR, New Delhi	2.79	82.76	74.07	HS	2.41	62.07	74.79	HS
17	NIC10193	SRS-23	NBPGR, New Delhi	2.43	56.67	40.52	S	2.53	60.00	45 90	S
18	NIC10194	SRS-24	NBPGR, New Delhi	3.00	100.00	100.00	HS	2.92	96.15	92.30	HS
19	NIC10195	SRS-25	NBPGR, New Delhi	2.43	53.30	38.11	MS	2.23	53.30	32.78	MS
20	NIC10197	SRS-26	NBPGR, New Delhi	2.57	79.30	62.25	S	2.44	81.48	58.67	S

HR - Highly resistant (0-4), R - Resistant (5-9), MR - Moderately resistant (10-19),
MS - Moderately susceptible (20-39), S - Susceptible (40-69), HS - Highly susceptible (70-100)

(Continued......)

Table 3 Continued......

Sl. No.	Variety/ Cultivar/ Line	Accession No.	Source	Reaction to PMV				Reaction to YVMV			
				Mean disease score	Infection %	Coeffi-cient of infection	Classifi-cation	Mean disease score	Infection %	Coeffi-cient of infection	Classifi-cation
21	IHR-15	SRS-29	IIHR, Bangalore	2.51	67.86	46.55	S	3.00	100.00	32.00	MS
22	IHR-20	SRS-27	IIHR, Bangalore	2.47	63.33	83.70	HS	2.20	53.33	72.69	HS
23	IHR-21	SRS-32	IIHR, Bangalore	2.37	55.17	51.20	S	2.26	55.55	100.00	HS
24	IHR-22	SRS-28	IIHR, Bangalore	2.80	93.00	58.57	S	2.10	69.23	23.18	MS
25	IHR-23	SRS-30	IIHR, Bangalore	2.64	71.43	67.85	S	2.07	43.33	30.25	MS
26	IHR-24	SRS-33	IIHR, Bangalore	2.36	46.43	37.79	MS	2.50	75.00	35.00	MS
27	IHR-26	SRS-31	IIHR, Bangalore	2.77	76.67	37.50	MS	2.21	50.00	38.89	MS
28	IHR-31	SRS-34	IIHR, Bangalore	2.36	46.43	31.57	MS	2.50	75.00	56.25	S
29	IHR-33	SRS-42	IIHR, Bangalore	2.34	55.17	45.53	S	2.17	43.33	36.65	MS
30	IHR-41	SRS-35	IIHR, Bangalore	2.50	60.71	50.76	S	2.19	61.59	40.07	S
31	IHR-42	SRS-44	IIHR, Bangalore	2.45	52.00	57.40	S	2.32	56.00	35.46	MS
32	IHR-43	SRS-36	IIHR, Bangalore	2.55	65.50	31.62	MS	2.32	60.71	40.13	S
33	IHR-44	SRS-37	IIHR, Bangalore	2.61	71.43	33.07	MS	2.33	53.33	100.00	HS
34	IHR-46	SRS-38	IIHR, Bangalore	2.31	48.28	29.93	MS	2.07	75.00	45.33	S
35	IHR-47	SRS-39	IIHR, Bangalore	2.37	48.28	45.83	S	3.00	100.00	30.89	MS
36	IHR-51	SRS-40	IIHR, Bangalore	2.24	48.28	36.96	MS	2.44	62.96	25.35	MS
37	IHR-52	SRS-43	IIHR, Bangalore	2.37	55.56	38.06	MS	2.37	50.00	34.25	MS
38	IHR-54	SRS-41	IIHR, Bangalore	2.50	61.11	37.70	MS	2.39	44.44	36.96	MS
39	S-501	SRS-45	Pazhayarikandam, Idukki	2.71	64.71	55.33	S	2.10	44.00	24.20	MS
40	S-502	SRS-46	Kattappana	2.41	54.55	38.46	MS	2.27	77.27	49.07	S
41	S-503	SRS-47	Kattappana	2.56	66.67	52.00	S	2.05	35.00	18.38	MR
42	S-504	SRS-48	Kunjikuzhi, Idukki	2.30	48.15	31.30	MS	2.21	46.43	28.09	MS

(Continued.....)

Table 3 Continued......

Sl. No.	Variety/ Cultivar/ Line	Accession No.	Source	Reaction to PMV				Reaction to YVMV			
				Mean disease score	Infection %	Coefficient of infection	Classification	Mean disease score	Infection %	Coefficient of infection	Classification
43	S-505	SRS49	Mavelikkara	2.52	66.67	50.67	S	2.21	54.17	32.77	MS
44	S-506	SRS-50	Pampady	2.38	53.85	37.16	MS	2.13	43.10	24.35	MS
45	S-507	SRS51	Ettumanoor	2.30	68.18	44.32	S	2.59	68.18	54.20	S
46	S-508	SRS-52	Kuravilangad	2.28	59.10	37.83	MS	2.44	68.00	48.96	S
47	S-509	SRS-53	Kaduthuruthy	2.18	35.29	20.82	MS	2.39	55.56	38.61	MS
48	S-510	SRS-54	Mallappally	2.16	45.00	26.10	MS	2.40	65.00	45.50	S
49	S-511	SRS-55	Kadakkad	2.29	50.00	32.25	MS	2.33	60.00	39.90	S
50	S-512	SRS-56	Bangalore	2.16	44.44	25.78	MS	2.19	47.62	28.33	MS
51	S-513	SRS-57	Vellanikkara	2.56	66.67	52.00	S	2.26	47.37	29.84	MS
52	S-514	SRS-58	Sultanbattery	2.44	55.56	40.00	S	2.01	27.78	13.89	MR
53	S-515	SRS-59	Thrikkodithanam	2.27	53.33	60.53	S	2.47	60.00	44.10	S
54	S-516	SRS-60	Chandanappally	2.27	45.45	28.86	MS	2.05	31.82	16.71	MR
55	S-517	SRS-61	Eraviperur	2.20	44.00	26.40	MS	2.20	54.17	32.50	MS
56	S-518	SRS-62	Bangalore	2.17	47.06	51.06	S	2.45	70.00	50.75	S
57	S-519	SRS-63	Mavelikkara	3.00	100.00	100.00	HS	2.73	77.27	66.84	S
58	S-520	SRS-64	Angamali	2.25	62.50	39.06	MS	2.05	35.38	18.57	MR
59	S-521	SRS-65	Angamali	2.17	41.67	24.38	MS	2.54	61.54	47.39	S
60	S-522	SRS-66	Angamali	2.32	52.00	34.32	MS	2.43	62.96	45.02	S
61	S-523	SRS-67	Angamali	2.11	42.00	23.31	MS	2.45	65.00	47.13	S
62	S-524	SRS-68	Angamali	2.18	40.91	24.14	MS	2.39	52.17	36.26	MS
63	S-525	SRS-69	Anakkayam	2.50	66.67	50.00	S	2.25	66.67	41.67	S
64	S-526	SRS-70	Anakkayam	2.52	70.40	53.50	S	2.14	32.14	18.32	MR
65	S-527	SRS-71	Sreekaryam	2.40	60.00	42.00	S	2.05	35.00	18.38	MR

(Continued......)

Table 3 Continued......

Sl. No.	Variety/ Cultivar/ Line	Accession No.	Source	Reaction to PMV				Reaction to YVMV			
				Mean disease score	Infection %	Coeffi-cient of infection	Classifi-cation	Mean disease score	Infection %	Coeffi-cient of infection	Classifi-cation
66	P1	SRS-72	College of Hort.,Vellanikkara	2.35	56.50	38.14	MS	2.17	56.52	33.06	MS
67	P2	SRS-73	College of Hort.,Vellanikkara	2.29	50.00	32.25	MS	2.26	45.44	28.63	MS
68	P3	SRS-74	College of Hort.,Vellanikkara	2.14	45.45	25.91	MS	2.26	47.83	30.13	MS
69	P4	SRS-75	College of Hort.,Vellanikkara	2.20	50.00	30.00	MS	2.32	53.57	35.36	MS
70	P5	SRS-76	College of Hort.,Vellanikkara	2.29	52.94	34.15	MS	2.11	42.11	23.38	MS
71	P6	SRS-77	College of Hort.,Vellanikkara	2.50	66.67	50.00	S	2.77	83.33	73.74	HS
72	P7	SRS-78	College of Hort.,Vellanikkara	2.42	50.00	35.50	MS	2.58	56.67	44.77	S
73	P8	SRS-79	College of Hort.,Vellanikkara	3.00	100.00	100.00	HS	2.14	42.86	24.43	MS
74	P9	SRS-80	College of Hort.,Vellanikkara	2.41	58.52	41.26	S	2.39	61.11	42.47	S
75	P11	SRS-81	College of Hort.,Vellanikkara	2.42	57.89	41.10	S	2.20	45.00	27.00	MS
76	P12	SRS-82	College of Hort.,Vellanikkara	2.48	60.00	44.40	S	2.31	50.00	32.75	MS
77	P13	SRS-83	College of Hort.,Vellanikkara	2.30	55.00	36.75	MS	2.35	55.00	37.13	MS
78	P14	SRS-84	College of Hort.,Vellanikkara	2.39	52.17	36.26	MS	2.29	50.00	32.25	MS
79	P15	SRS-85	College of Hort.,Vellanikkara	2.42	58.33	41.41	S	2.17	37.50	21.94	MS
80	P16	SRS-86	College of Hort.,Vellanikkara	2.39	61.11	42.47	S	2.33	55.56	36.95	MS
81	P17	SRS-87	College of Hort.,Vellanikkara	2.30	50.00	32.50	MS	2.40	40.00	28.00	MS
82	P18	SRS-88	College of Hort.,Vellanikkara	2.57	69.56	54.60	S	3.00	100.00	100.00	HS
83	P19	SRS-89	College of Hort.,Vellanikkara	2.50	62.50	46.88	S	2.04	40.00	20.80	MS
84	P20	SRS-90	College of Hort.,Vellanikkara	2.20	35.00	21.00	MS	2.27	58.00	36.83	MS
85	P21	SRS-91	College of Hort.,Vellanikkara	2.07	40.74	21.80	MS	2.44	59.26	42.67	S
86	P22	SSRS-92	College of Hort.,Vellanikkara	2.40	46.67	32.67	MS	2.32	57.14	37.79	MS
87	P23	SRS-93	College of Hort.,Vellanikkara	2.55	68.18	52.85	S	2.05	36.36	19.09	MR

(Continued......)

Table 3 Continued......

Sl. No.	Variety/ Cultivar/ Line	Accession No.	Source	Reaction to PMV				Reaction to YVMV			
				Mean disease score	Infection %	Coeffi- cient of infection	Classifi- cation	Mean disease score	Infection %	Coeffi- cient of infection	Classifi- cation
88	P24	SRS-94	College of Hort.,Vellanikkara	2.14	45.45	24.32	MS	2.56	78.26	61.04	S
89	P27	SRS-95	College of Hort.,Vellanikkara	2.39	41.18	28.62	MS	2.31	48.28	31.62	MS
90	P28	SRS-96	College of Hort.,Vellanikkara	2.41	62.50	44.06	S	2.46	47.17	34.43	MS
91	P29	SRS-97	College of Hort.,Vellanikkara	3.00	100.00	100.00	HS	2.44	60.00	43.20	S
92	P30	SRS-98	College of Hort.,Vellanikkara	2.47	60.00	44.10	S	2.66	80.00	66.40	S
93	P31	SRS-99	College of Hort.,Vellanikkara	2.52	59.26	45.04	S	2.42	61.54	43.69	S
94	528	SRS-100	Thiruvalla	2.24	44.83	27.80	MS	2.58	67.74	53.51	S
95	529	SRS-101	Vadakkanchery	2.30	46.63	30.31	MS	2.34	51.72	34.65	MS
B. ***Cucurbita maxima* genotypes**											
1	E.C.367659	SRS-2	Cornell University, USA	2.43	56.67	40.52	S	2.20	89.29	53.57	S
2	E.C.367660 (Queenland)	SRS-5	Cornell University, USA	2.63	85.33	69.54	HS	2.36	55.17	37.52	MS
3	E.C.367664 *Zapallito rotunda*	SRS-4	Cornell University, USA	2.61	75.00	60.38	S	2.60	63.00	50.40	S
C. ***Cucurbita pepo* genotypes**											
1	E.C.373225 (Cindrella)	SRS-1	NVRS, U.K.	2.53	61.30	46.89	S	2.6	73.33	58.67	S
2	Zucchini Hybrid	SRS-8	Cornell University, USA	2.39	50.00	34.75	MS	2.34	58.67	39.31	MS
D. **Feral and related species of pumpkins**											
1	*C. foetidissima* (EC 367661)	SRS-9	Cornell University, USA	2.20	46.67	28.00	MS	2.30	46.67	30.34	MS
2	*C. martinezii*	SRS-6	Cornell University, USA	2.76	82.78	72.85	HS	2.50	72.42	54.32	S
3	*C. ecuadorensis*	SRS-3	Cornell University, USA	2.56	70.00	54.60	S	2.37	86.21	59.05	S

Table 4. Symptom expression of inoculated susceptible varieties

Variety	Disease evaluation	YVMV			PMV			
		1st week	2nd week	3rd week	1st week	2nd week	3rd week	4th week
Amb	Infection per cent	0.00	40.00	100	0	0	30.00	100
	Mean disease score	1.35	2.23	3	1	1.38	2.15	3
PV	Infection per cent	0.00	37.50	100	0	0	15.00	100
	Mean disease score	1.40	2.25	3	1	1.15	2.05	3
AC	Infection per cent	2.50	35.00	100	0	0	22.50	100
	Mean disease score	1.43	2.08	3	1	1.08	2.18	3
Col	Infection per cent	0.00	22.50	100	0	0.03	22.50	100
	Mean disease score	1.23	1.75	3	1	1.15	2.18	3
Mean	Infection per cent	0.63	33.75	100	0	0.008	22.50	100
	Mean disease score	1.35	2.08	3	1	1.19	2.14	3

4.1.1 Confirmation of resistance

The resistant line NL was subjected to resistance confirmation techniques of back inoculation, graft transmission and multi-environment tests.

4.1.1.1 Back inoculation

Back inoculation was carried out to determine whether the resistance to PMV in NL is true or due to tolerance of symptomless carrier type. The results confirmed that the resistance in NL is true since the susceptible plants did not show any disease symptoms upon back inoculation with the sap of NL, pre-inoculated with PMV. The sap from the symptomatic plants of Ambili produced symptom of PMV when inoculated on the susceptible seedlings of Ambili.

Susceptibility of Co1 and Pusa Viswas to Pumpkin mosaic virus inoculation

Plate 20

Susceptibility of Arka Chandan and Ambili to Yellow vein mosaic virus inoculation

Plate 21

Susceptibility of Pusa Viswas to Pumpkin mosaic virus inoculation

Plate 22

Resistance confirmation test through grafting in case of Yellow vein mosaic virus (1)
R - Nigerian Local, S - Ambili

Plate 23

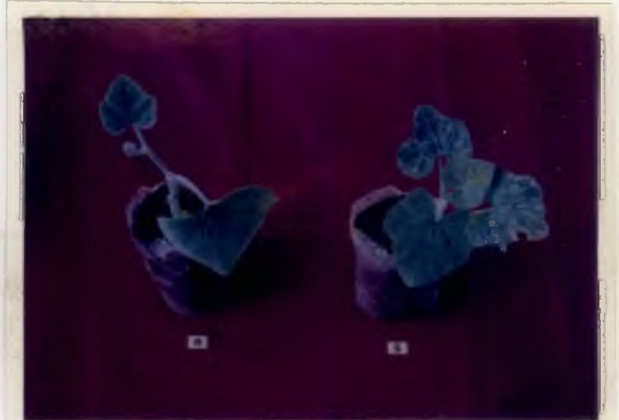
Resistance confirmation test through grafting in case of Yellow vein mosaic virus (2)
R - Nigerian Local, S - Ambili

Plate 24

CO1
3
PUMPKIN MOSAIC

Arka Chandan
3
Ambili
YELLOW VEIN MOSAIC

PV



4.1.1.2 Grafting

Grafting of resistant genotype on the YVMV inoculated susceptible symptomatic root stock showed no disease symptoms of YVMV on the resistant line, while the susceptible scions of Ambili showed complete susceptibility (Plates 23 and 24).

4.1.1.3 Multi-environment test

The resistant line along with the susceptible variety Ambili was grown in three seasons in the field to locate the environmental effect on the expression of disease resistance. The three seasons were September-December 1995, December-March 1996, and April-July 1996. In all the seasons, NL was found to be free from virus infection (score : one each) while Ambili showed susceptible reaction to PMV and YVMV with a mean disease score of three each. The data are presented in Table 5.

Table 5. Effect of environment on PMV/YVMV disease reaction

Environment	Mean disease score			
	Ambili		NL	
	PMV	YVMV	PMV	YVMV
E-1	3	3	1	1
E-2	3	3	1	1
E-3	3	3	1	1

E-1 - September - December 95, E-2 - December 95 - March 96, E-3 - April - July 96

4.2 Genetics of resistance to PMV and YVMV

Inheritance of resistance to PMV and YVMV was studied using the parents, F_1, F_2, B_1 and B_2 populations of four crosses involving resistant and susceptible genotypes. The four cross combinations were Ambili x NL, PV x NL, AC x NL and Col x NL. The respective viruses were inoculated into seedlings of the six generations of each cross combination, and then scored for symptom expression at weekly interval upto 28 days in case of PMV and upto 21 days in case of YVMV.

4.2.1 Reaction to PMV

The number of PMV resistant and affected seedlings in the six generations of the four cross combinations after 28 days of inoculation are presented in Tables 6 to 9.

4.2.1.1 Cross Amb x NL

The parent Ambili was found to be highly susceptible while NL was found to be resistant (Table 6). In the F_1 generation, all seedlings were affected by PMV i.e., all the ten seedlings in the four replications showed high susceptibility to PMV. In the F_2 generation, out of the total 300 plants, 64 were resistant (40R and 24 MR) while 236 showed susceptibility. This fitted very well into the monogenic Mendelian ratio of 3:1 ($\chi2$ = 2.15, P = 0.20-0.10). In the B_1 generation, out of 100 plants 84 were susceptible and 16 resistant while in the B_2, 52 survived and 48 succumbed to the virus. This fitted well into the ratio of 1:1 ($\chi2$ = 0.16, P = 0.70-0.50) (Fig. 1).

Table 6. Reaction of the parents, F_1, F_2 and backcross generations of the cross Amb x NL to PMV

Generations	Number of plants					Expected ratio	χ^2	Probability
	Total	Resistant			Susceptible			
		Score 1	Score 2	Total				
Amb	40			0	40			
NL	40	32	8	40	0			
F_1	40			0	40			
F_2	300	40	24	64	236	1R:3S	2.15	0.20-0.10
B_1	100	12	4	16	84			
B_2	100	36	16	52	48	1R:1S	0.16	0.70-0.50

R - Resistant, S - Susceptible, B_1 - (Amb X NL) x Amb, B_2 - (Amb X NL) x NL

4.2.1.2 Cross PV x NL

In the susceptible parent PV all the forty plants were severely affected by PMV inoculation (Table 7 and Fig. 1). In the resistant parent all seedlings were free from infection. All F_1 plants except two were highly susceptible with 95 per cent penetrance for susceptibility. In the F_2, assuming penetrance, the expected ratio of susceptible plants to resistant plants should be 218:82. The actual ratio of susceptible and resistant population was 228 and 72 respectively, fitting well into the expected ratio of 3:1 ($\chi2$ = 1.60, P = 0.30-0.20). In the B_1 generation, all but sixteen plants were susceptible. In the B_2 generation, the susceptibility-resistance ratio was 1:1 with 56 susceptible and 44 resistant plants (expected ratio 48:52) showing a good fit to the test cross ratio of 1:1 ($\chi2$ = 2.56, P = 0.20-0.10)

Table 7. Reaction of the parents, F_1, F_2 and backcross generations of the cross PV x NL to PMV

Generations	Number of plants					Expected ratio, assuming partial penetrance	χ^2	Probability
	Total	Resistant			Susceptible			
		Score 1	Score 2	Total				
PV	40			0	40			
NL	40	40		40	0			
F_1	40		2	2	38			
F_2	300	68	4	72 (82)	228 (218)	1.09R:2.91S	1.60	0.30-0.20
B_1	100	8	8	16	84			
B_2	100	40	4	44 (52)	56 (48)	1.04R:0.96S	2.56	0.20-0.10

R - Resistant, S - Susceptible, B_1 - (PV X NL) x PV, B_2 - (PV X NL) x NL
Figures in parentheses are the expected numbers assuming partial penetrance to susceptibility

4.2.1.3 Cross AC x NL

In AC all the seedlings showed susceptibility reaction to PMV while in the resistant parents all the plants showed resistance reaction (Table 8 and Fig. 1). The F_1 was susceptible (90%) with 36 susceptible out of 40. In the F_2 there were 240 susceptible and 60

resistant seedlings. This fitted into the genetic ratio of 3:1 ($\chi2$ = 3.48, P = 0.1-0.05). In the B_1, out of 100, 92 showed susceptibility. In B_2, 44 plants expressed resistance (28R and 16MR) while 56 showed susceptibility ($\chi2$ = 1.44, P = 0.30-0.20). The penetrance value in F_1 did not fit well with the frequencies of the resistant and susceptible plants in the F_2 and B_2 generations.

Table 8. Reaction of the parents, F_1, F_2 and backcross generations of the cross AC x NL to PMV

Generations	Number of plants					Expected ratio	χ^2	Probability
	Total	Resistant			Susceptible			
		Score 1	Score 2	Total				
AC	40			0	40			
NL	40	40		40	0			
F_1	40	4		4	36			
F_2	300	40	20	60	240	1R:3S	3.48	0.10-0.05
B_1	100	14	4	8	92			
B_2	100	28	10	44	56	1R:1S	1.44	0.30-0.20

R - Resistant, S - Susceptible, B_1 - (AC X NL) x AC, B_2 - (AC X NL) x NL

4.2.1.4 Cross Co1 x NL

In the susceptible parent Co1, all the seedlings screened against the virus showed susceptibility reaction while in the resistant NL, all plants were completely free from the disease (Table 9 and Fig. 1). In the F_1, all the forty seedlings were susceptible indicating the dominance of susceptibility. Out of the total number of 300 F_2 plants 236 were susceptible and 64 resistant fitting in the monogenic ratio of 3:1 ($\chi2$ = 2.15, P = 0.20-0.10). In B_1 out of the 100 seedlings, 84 were susceptible. The B_2 generation segregated into 44 resistant and 56 susceptible ($\chi2$ = 1.44, P = 0.30-0.20).

Table 9. Reaction of the parents, F_1, F_2 and backcross generations of the cross Col x NL to PMV

Generations	Number of plants					Expected ratio	χ^2	Probability
	Total	Resistant			Susceptible			
		Score 1	Score 2	Total				
Col	40				40			
NL	40	40		40				
F_1	40			0	40			
F_2	300	48	16	64	236	1R:3S	2.15	0.20-0.10
B_1	100	16		16	84			
B_2	100	32	12	44	56	1R:1S	1.44	0.30-0.20

R - Resistant, S - Susceptible, B_1 - (Col X NL) x Col, B_2 - (Col X NL) x NL

4.2.2 Reaction to YVMV

The number of survived and susceptible plants in the six generations after inoculation with YVMV, among the four cross combinations are given in Tables 10 to 13 and Fig. 2.

4.2.2.1 Cross Amb x NL

All the plants of the susceptible variety Ambili exhibited susceptibility reaction while NL showed complete resistance. In the F_1, out of 40 plants, 32 were resistant (Table 10, Fig. 2). In F_2, a total number of 300 plants segregated into 216 resistant and 84 susceptible ($\chi 2$ = 1.44, P = 0.30-0.50). In the B_1 generation 52 had resistance and 48 had susceptibility ($\chi 2$ = 0.16, P = 0.70-0.50). In B_2, 92 were resistant with only eight susceptible. The frequencies of segregation of the resistant and susceptible plants in the F_2 and B_1 generations did not fit with the penetrance value for resistance in the F_1 (80%).

Table 10. Reaction of the parents, F_1, F_2 and backcross generations of the cross Amb x NL to YVMV

Generations	Number of plants					Expected ratio	χ^2	Probability
	Total	Resistant			Susceptible			
		Score 1	Score 2	Total				
Amb	40			0	40			
NL	40	40		40	0			
F_1	40	20	12	32	8			
F_2	300	156	60	216	84	3R:1S	1.44	0.30-0.20
B_1	100	32	20	52	48	1R:1S	0.16	0.70-0.50
B_2	100	68	24	92	8			

R - Resistant, S - Susceptible, B_1 - (Amb x NL) x NL, B_2 - (Amb X NL)X Amb

4.2.2.2 Cross PV x NL

The parent PV was found to be highly susceptible to YVMV whereas NL was found to be highly resistant (Table 11 and Fig. 2). Except four, all the F_1 plants were resistant (32R and 4MR) with 90 per cent penetrance for resistance. The F_2 data revealed 220 plants as resistant (196R and 24MR) and 80 as susceptible, against the expected frequencies of 210 and 90 assuming the penetrance. The probability value indicated the non-significance of difference of observed and expected frequencies ($\chi2$ = 1.60, P = 0.30-0.20). The B_1 generation segregated into 52 resistant and 48 susceptible. The expected frequencies assuming penetrance were 45 resistant : 55 susceptible. The observed frequencies showed a good fit to the test cross ratio of 1:1 ($\chi2$ = 1.98, P = 0.20-0.10). In the B_2 generation, out of the 100 seedlings 92 were resistant and 8 susceptible.

Table 11. Reaction of the parents, F_1, F_2 and backcross generations of the cross PV x NL to YVMV

Generations	Number of plants					Expected ratio assuming partial penetrance	χ^2	Probability
	Total	Resistant			Susceptible			
		Score 1	Score 2	Total				
PV	40				40			
NL	40	40		40				
F_1	40	32	4	36	4			
F_2	300	196	24	220 (210)	80 (90)	2.80R:1.20S	1.60	0.30-0.20
B_1	100	44	8	52 (45)	48 (55)	0.90R:1.10S	1.98	0.20-0.10
B_2	100	72	20	92	8			

R - Resistant, S - Susceptible, B_1 - (PV X NL) x PV, B_2 - (PV X NL) x NL

The figures in parentheses are expected numbers assuming partial penetrance to susceptibility

4.2.2.3 Cross AC x NL

All the seedlings in variety AC were susceptible to YVMV whereas NL was completely free from the disease (Table 12 and Fig. 2). The F_1 gave 28 resistant plants and 12 suscepts, out of the 40 plants screened. The 300 plants in the F_2 segregated into 220 resistant (180R and 40MR) and 80 susceptible, fitting in an expected ratio of 3:1 ($\chi 2$ = 0.44, P = 0.70-0.50). In B_1, 48 plants possessed resistance and 52 had susceptibility showing a good fit to the ratio of 1:1 ($\chi 2$ = 0.16, P = 0.70-0.50). In the back cross with the resistant parent 88 were resistant (72R and 16MR) and 12 susceptible out of the 100 plants screened. The penetrance value of the gene for resistance to YVMV (70%) did not agree with the frequencies of the resistant and susceptible plants in the F_2 and B_1 generations.

Table 12. Reaction of the parents, F_1, F_2 and backcross generations of the cross AC x NL to YVMV

Generations	Number of plants					Expected ratio	χ^2	Probability
	Total	Resistant			Susceptible			
		Score 1	Score 2	Total				
AC	40				40			
NL	40	36	4	40				
F_1	40	28		28	12			
F_2	300	180	40	220	80	3R:1S	0.44	0.70-0.50
B_1	100	36	12	48	52	1R:1S	0.16	0.70-0.50
B_2	100	72	16	88	12			

R - Resistant, S - Susceptible, B_1 - (AC x NL) x AC, B_2 - (AC x NL) x NL

4.2.2.4 Cross Col x NL

In Col, all the forty plants were susceptible. In F_1, 32 showed resistant reaction (24R and 8MR) while eight were susceptible. The F_2 generation segregated into 224 resistant (164R and 60MR) and 76 susceptible plants. This segregation is in agreement with the Mendelian ratio of 3:1 ($\chi2$ = 0.02, P = 0.90-0.80). In back cross with the susceptible parent, out of the 100 seedlings screened, 44 (32R and 12MR) possessed resistance to the disease and 56 were severely affected. This ratio very well fitted into the expected genetic ratio of 3:1 ($\chi2$ = 1.44, P = 0.30-0.20). In back cross with NL, out of the 100 plants, 84 (68R and 16MR) had resistance (Table 13 and Fig. 2). In this case also the penetrance for resistance expressed by the gene in the F_1 (80%) did not agree with the number of resistant and susceptible plants segregated in the F_2 and B_1 generations.

4.2.3 Determination of reciprocal effects in resistance to PMV and YVMV

The four F_1 hybrids and their respective reciprocal F_1s were tested for resistance to PMV and YVMV (Table 14). In the F_1s and in the reciprocal F_1s, the disease reaction against both the viruses was the same. The F_1 hybrids Amb x NL, PV x NL, AC x NL and Col x NL and their reciprocals NL X Amb, NL x PV, NL x AC, and NL x Col were

found to be susceptible to PMV while resistant reaction was observed in case of YVMV. This behaviour clearly showed that there is no cytoplasmic effect with regard to PMV and YVMV disease reactions.

Table 13. Reaction of the parents, F_1, F_2 and backcross generations of the cross Col x NL to YVMV

Generations	Number of plants					Expected ratio	χ^2	Probability
	Total	Resistant			Susceptible			
		Score 1	Score 2	Total				
Col	40							
NL	40	40		40				
F_1	40	24	8	32	8			
F_2	300	164	60	224	76	3R:1S	0.02	0.90-0.80
B_1	100	32	12	44	56	1R:1S	1.44	0.30-0.20
B_2	100	68	16	84	16			

R - Resistant, S - Susceptible, B_1 - (Col X NL) x Col, B_2- (Col X NL) x NL

Table 14. Reaction of F_1s and reciprocals against PMV and YVMV

Cross	Reaction to PMV			Reaction to YVMV		
	Infection %	Disease score	Class	Infection %	Disease score	Class
Amb x NL	100.00	3.00	S	20.00	1.68	R
NL X Amb	97.50	2.98	S	17.50	1.53	R
PV x NL	95.00	2.95	S	12.50	1.45	R
NL x PV	92.50	2.93	S	12.50	1.43	R
AC x NL	92.50	2.93	S	32.50	1.80	R
NL x AC	95.00	2.95	S	25.00	1.70	R
Col x NL	100.00	3.00	S	15.00	1.50	R
NL x Col	97.50	2.98	S	17.50	1.53	R
C.D (0.05)	NS	NS	--	NS	NS	--

Although having resistance to both the viruses, the plants of NL are shy bearing with limited fruit set. The seed germination percentage, fruit set and fruit development are quite erratic. Further, the fruits are non-appealing because of the warty surface.

The seed germination percentage (normal and after seed coat removal), the number of female flowers per plant, number of developed fruits per plant and fruit development percentage in the parents were compared and presented in Table 15 and Fig 3.

Table 15. Seed germination and fruit development in the parents

Parents	Seed germination %		No. of female flowers/plant	No. of fruits /plant	Fruit developme nt %
	Normal	after seed coat removal			
NL	19.00	62.00	14.80	1.20	8.06
Ambili	91.00	94.00	6.90	1.40	20.39
PV	87.00	91.00	13.80	1.80	12.23
AC	93.50	97.00	11.60	1.40	12.08
Col	80.00	92.00	10.40	1.60	15.55
C.D. (0.05)	5.51*	6.34*	3.32*	0.39*	2.09*

Under normal conditions, the seed germination in the resistant parent NL was only 19 per cent while in the susceptible parents AC, Amb and PV the germination percentage were 93.50, 91.00 and 87 respectively. Compared to Ambili, PV and AC, the seed germination percentage was significantly low in Col i.e., 80 per cent germination. After seed coat removal, the seed germination in Amb, PV, AC and Col were on par. Through seed coat removal, 43 per cent increase in seed germination per cent (62%) was noted in NL. In Ambili and Pusa Viswas the increase in seed germination percentage through seed coat removal were 3 and 4 respectively. In AC, the increase was not at all significant (1%) whereas in Col, 12 per cent increase in seed germination percentage could be achieved.

The number of female flowers produced per plant varied from 6.90 in Ambili to 14.80 in NL. In PV, AC and Co1 the per plant production of female flowers were 13.80, 11.60 and 10.40 respectively. The variety PV produced the highest number of fruits per plant (1.80) followed by Co1 (1.60), Ambili and AC (1.40 each). the lowest per plant fruit production was noticed in NL i.e., 1.2. The fruit development percent was the highest for Ambili (20.39) and minimum for NL (8.06). In Co1, PV and AC, the fruit development percentage were 15.55, 12.23 and 12.08 respectively.

4.3 Biochemical mechanism of mosaic virus resistance

4.3.1. Protein pattern

Protein pattern during electrophoresis is a genetic expression. The protein pattern of the susceptible and resistant parents and their F_1s was analysed using PAGE before and after inoculation of the viruses using the leaf extract. The resolution of bands is presented in Figure 4 and plate 25.

All the genotypes including the hybrids possessed similar bands of identical electrophoretic mobility before inoculation of virus. The resistant genotype, susceptible varieties and their F_1 hybrids did not show any difference in the protein pattern with respect to the number of bands and their Rm values. There were seven bands with Rm values 0.17, 0.22, 0.27, 0.42, 0.58, 0.62 and 0.73.

After inoculation of both the viruses, the protein zymogram exhibited identical protein pattern, the number and Rm values being the same for all the genotypes.

4.3.2. Isoenzyme analysis

4.3.2.1. Peroxidase

Leaf samples of three week old seedlings of the four susceptible parents (Amb, PV, AC and Co1), resistant parent NL and their F_1s were taken for this study before

inoculation and after inoculation of PMV and YVMV and the peroxidase profiles are depicted in plates 26 to 28 and Figures 5a, 5b and 5c.

Before inoculation of the viruses the resistant parent expressed only one peroxidase band (PRX1) with Rm value 0.083, while the susceptible parents had three bands (PRX1, PRX3 and PRX6) (plate 26 and Fig. 5a.) with Rm values 0.083, 0.342 and 0.583. Dense bands of PRX3 and PRX6 were obtained in Amb and AC while feeble bands of the same were seen in PV and CO1. The electrophorogram of the hybrids Amb x NL and CO1 x NL showed two common bands of PRX1 and PRX6 as in Ambili and CO1, but PRX-3 band was absent as in the resistant parent. The hybrid PV x NL had additional bands of PRX2, PRX4 and PRX7 with Rm values 0.275, 0.392 and 0.642 respectively. The banding patterns of AC and AC x NL were similar.

The peroxidase zymogram of the parents and their hybrids after PMV inoculation is presented in plate 27 and Fig. 5b. In this zymogram, PRX1 band was not expressed in any of the genotypes. The susceptible plants had two bands PRX3, PRX6 and an additional band, PRX7. The resistant plants had two bands of PRX3 and PRX6 similar to the susceptibles and an additional band, PRX5 with Rm value 0.463. So consequent to inoculation, an additional band (PRX5) with Rm value 0.463 was observed in the resistant plant. The band PRX7 with Rm value 0.642 recorded in susceptible plants was absent in resistant ones. The isozyme profile of the hybrids was similar to its respective female parents.

The peroxidase zymogram after seven days of inoculation of YVMV is presented in plate 28 and Fig. 5c. The figure clearly showed that after inoculation of YVMV, the isozyme PRX1 had disappeared while the band PRX7 (Rm value 0.642) is seen additionally in both resistant and susceptible parents. Before inoculation, the resistant parent NL had only PRX1 band. The electrophorogram after inoculation of YVMV showed different isozyme profile in resistant and susceptible parents.

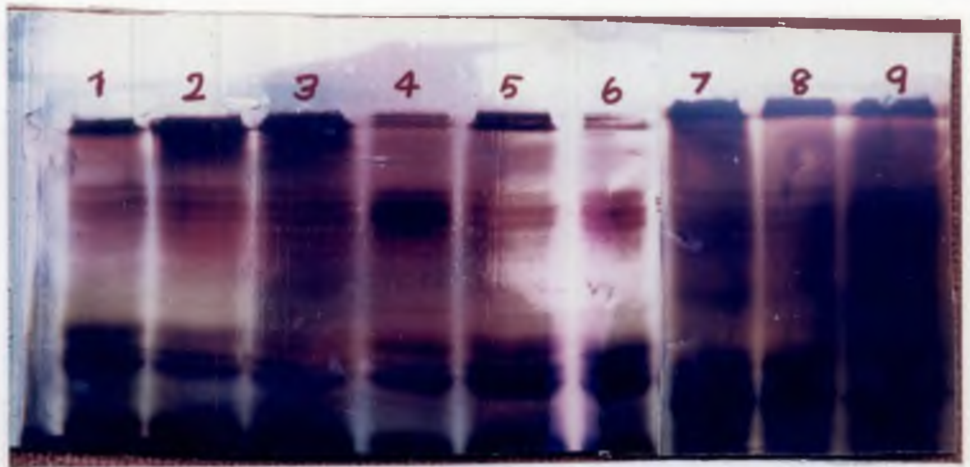
The esterase isozyme profile of the parents - both resistant and the susceptible and their F1 hybrids before virus inoculation is presented in plates 29a and 29b and in Fig. 6a and 7a. A total of six bands were observed in the zymogram of susceptible parents. But

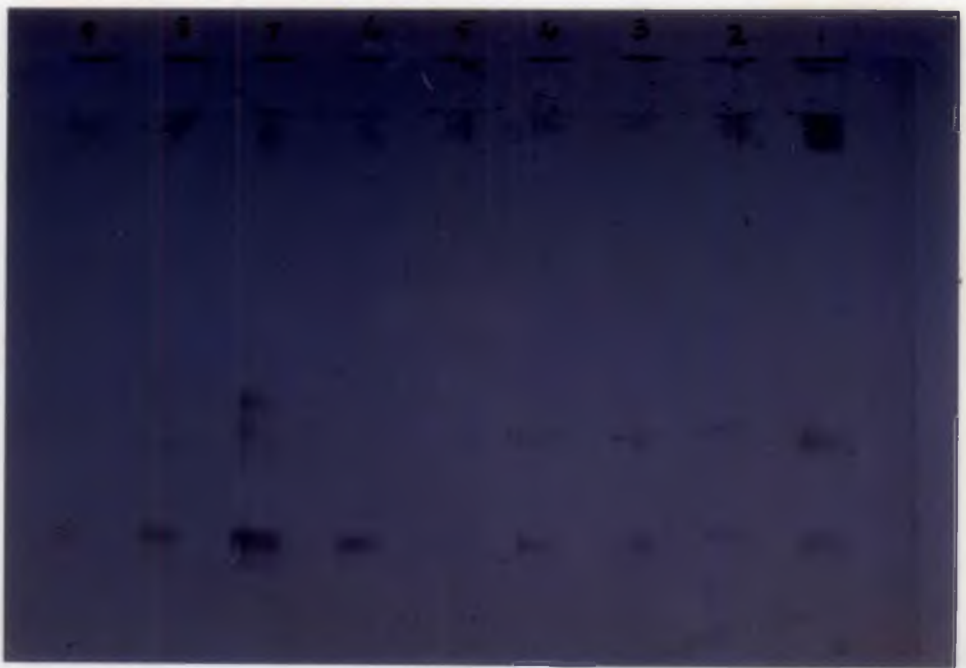
Protein eloctrophorogram before inoculation of virus

Plate 25

Peroxidase eloctrophorogram before inoculation of virus

Plate 26


1 2 3 4 5 6 7 8 9



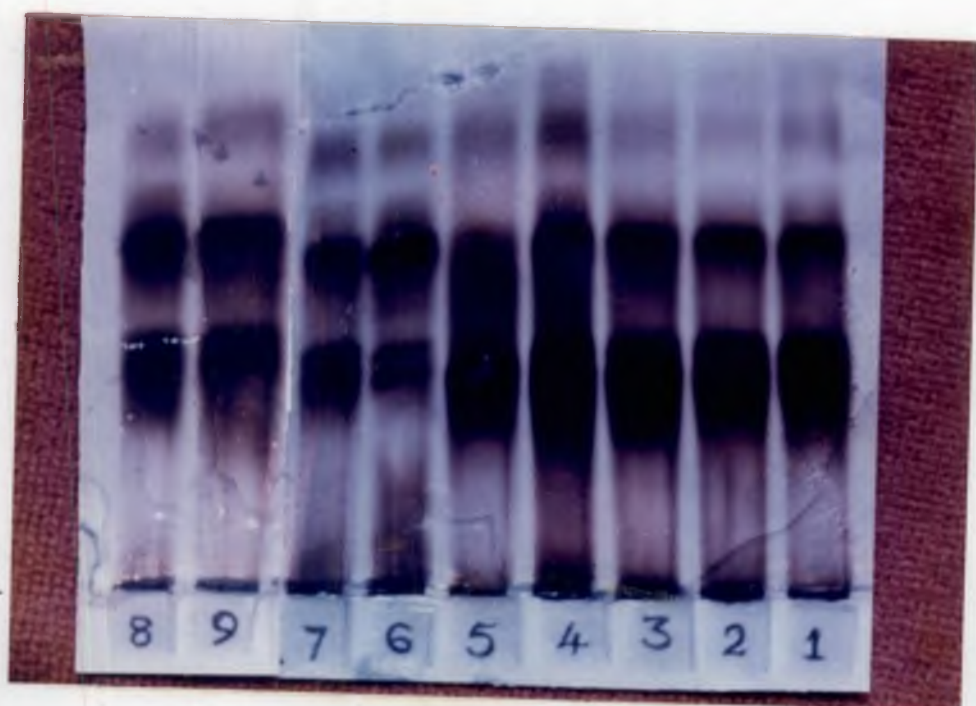
Peroxidase electrophorogram after inoculation of pumpkin mosaic virus

Plate 27

Peroxidase electrophorogram after inoculation of Yellow vein mosaic virus

Plate 28

1 2 3 4 5 6 7 8 9

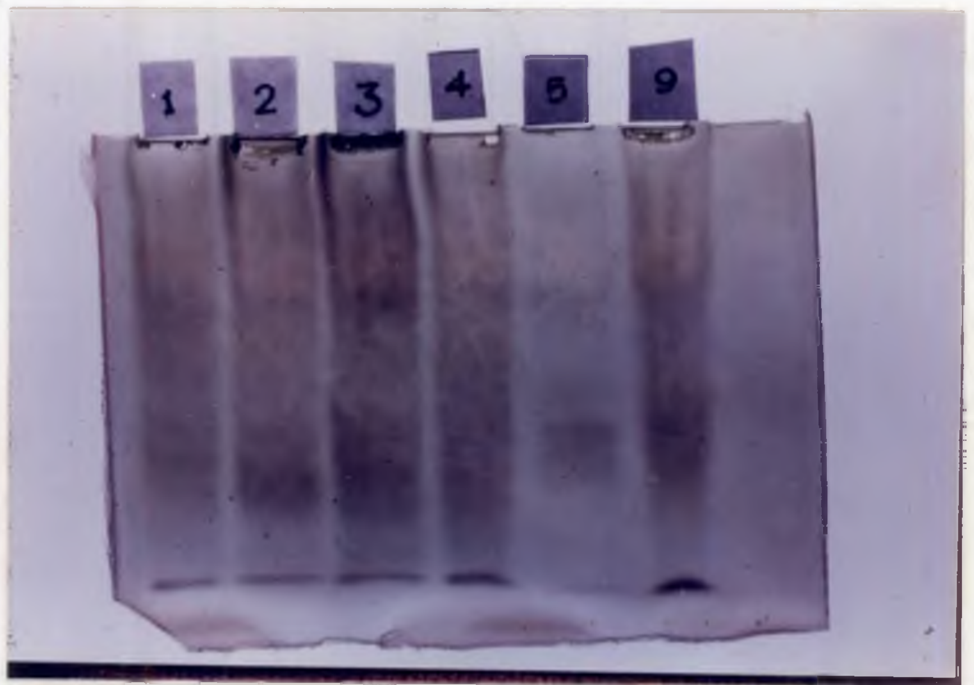

8 9 7 6 5 4 3 2 1

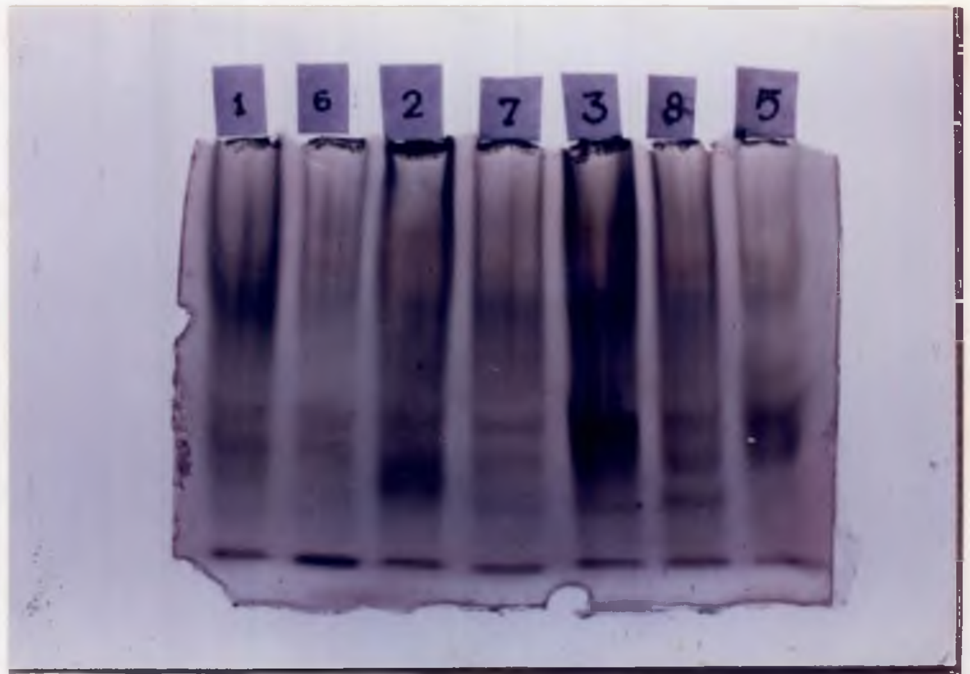
Estarase electrophorogram before virus inoculation

Plate 29 a

Estarase electrophorogram before virus inoculation

Plate 29 b


1
2
3
4
5
9


1
6
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3
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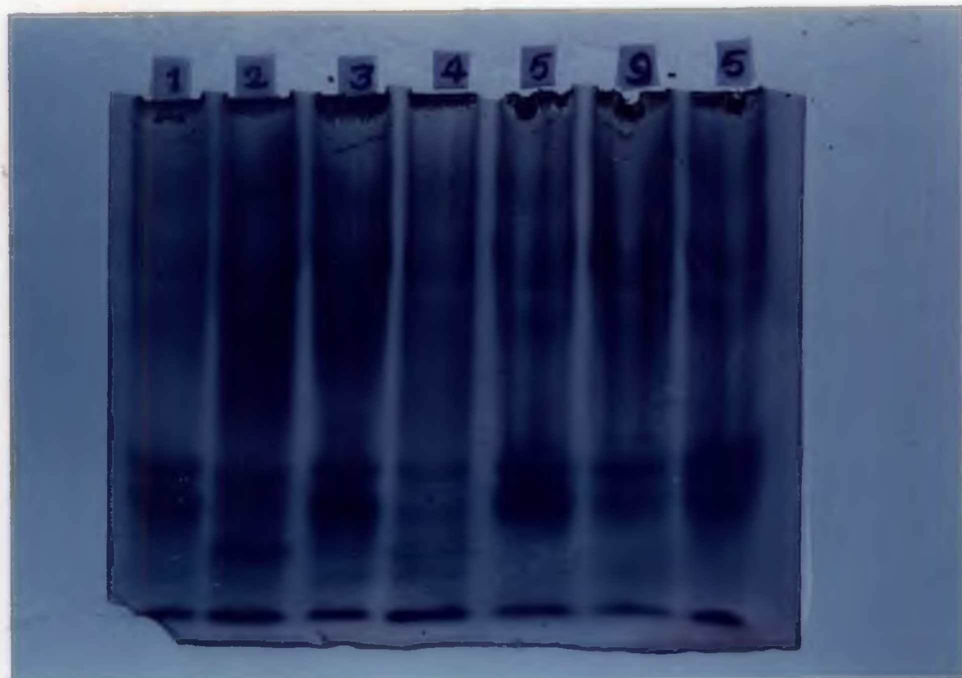
5

Estrerase electrophorogram after Pumpkin mosaic virus inoculation

Plate 30 a

Estrerase electrophorogram after Pumpkin mosaic virus inoculation

Plate 30 b


1
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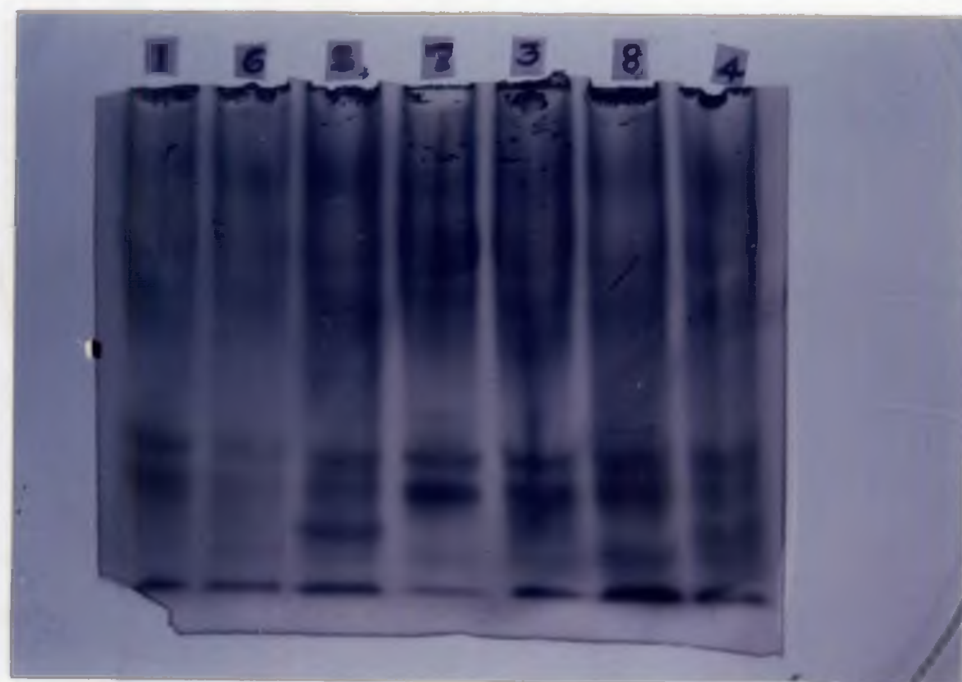
1
6
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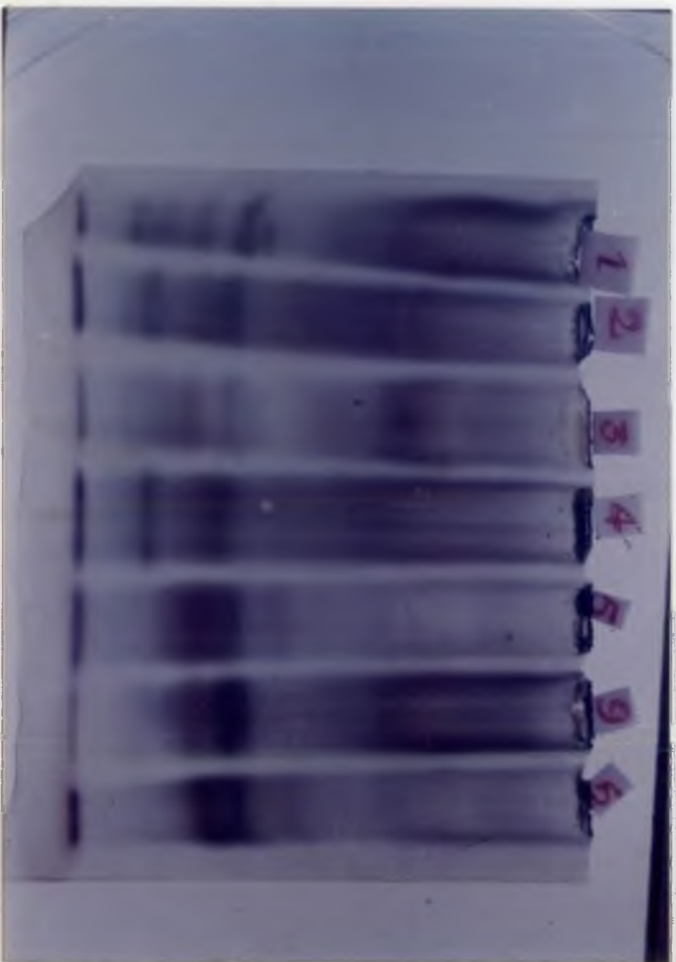
4

Estrerase electrophorogram after Yellow vein mosaic virus inoculation

Plate 31 a

Estrerase electrophorogram after Yellow vein mosaic virus inoculation

Plate 31 b



the isozymes EST 1 (0.368), EST 4 (0.632) and EST 6 (0.809) were absent in the resistant parent. The hybrid Co1 x NL had an isozyme profile similar to its female parent CO1. In the F1s, Amb x NL, PV x NL and AC x NL the isozyme band EST 4 with Rm value 0.632 was not seen while all the other five bands expressed in the susceptible parents were present.

After inoculation of PMV there were only five bands in the susceptible parents the band EST 7 with Rm value 0.868 being absent (Fig 6b and 7b; Plates 30a and 30b). Before inoculation, the resistant parent had only three bands, while it was five after inoculation, the additional bands being EST 1 (0.368) and EST 5 (0.721). In Co1 x NL, EST 6 (0.809) was not expressed, as in the case of the resistant parent. Other than the resistant parent and CO1 all others expressed the EST 6 isoform.

Consequent to inoculation of YVMV there were seven bands for the esterase isozyme in the susceptible parent and four bands in the resistant parent (Fig. 6c and 7c; Plates 31a and 31b). EST 2 (0.412) and EST 5 (0.721) were additional bands in the susceptible parents, while in the resistant parent EST 1 (0.368) and EST 4 (0.632) were additional. The isozyme EST 4 was very active with very thick bands. The hybrids did not express EST 2 (0.412)and EST 5 (0.721) present in the female parents except Co1 x NL. These two bands were absent in NL also. In NL only EST 1, EST 3, EST 4 and EST 8 (1.00) were present. Consequent to inoculation, the additional bands seen in NL were EST 1 (0.368) and EST 4 (0.632). EST 4 was very thick, showing high activity.

4.4 Selection of mosaic resistant plants

4.4.1 Isolation of plants with combined resistance to PMV and YVMV

Twenty five plants each in the F_1, F_2 and back cross generations of Amb x NL, PV x NL, AC x NL and Co1 x NL were evaluated for their resistance to PMV and YVMV sequentially so as to identify plants with resistance to both the viruses. Initial screening was done against PMV in five day old seedlings. The plants with scores 1 and 2 were sequentially inoculated with YVMV. The results are presented in Table 16 and Fig. 8.

4.4.1.1 Amb x NL

In the cross Amb x NL, out of 25 F_1s only one plant showed resistance to PMV. This was further inoculated with YVMV and the plant showed moderate resistance (MR) reaction. In the F_2 after sequential inoculation with PMV and YVMV, four plants were observed as resistant to both the viruses. After PMV inoculation six plants showed resistant expression with a mean score of 1.50. In B_1 three plants expressed resistance to PMV of which two were found resistant to YVMV with a mean disease score of one. Fifteen plants in the B_2 generation exhibited resistance reaction to PMV with a mean score of one and among these ten were found resistant to YVMV. All the plants expressing resistance to both the viruses in the F_1, F_2, B_1 and B_2 generations were planted in the main field for further growth and development.

4.4.1.2 PV x NL

From among 25 plants evaluated for resistance to PMV in the F_1 generation of PV x NL, nine showed resistant reaction. The mean disease score was 1.22. These were sequentially inoculated with YVMV and only two expressed resistance with a mean score of one. The F_2 segregated into six resistant and 19 susceptible plants against PMV, the mean resistance score being 1.33. Out of these six, only four had resistance to YVMV and the mean score was 1.50. In the B_1 generation only four plants expressed resistance to PMV and the mean score was one. Further screening of these plants against YVMV resulted in the identification of two resistant (R) plants. Inoculation with PMV in 25 seedlings in the B_2 generation identified 12 plants as resistant, 10R and 2MR. These genotypes were inoculated with YVMV and nine plants were found to possess resistance to the virus, the mean disease score being 1.10. All the above 17 plants identified as resistant to both the viruses in the four generations were planted in the main field for further selection.

Table 16. Disease reaction of F_1, F_2 and backcross generation against PMV and YVMV

Generation	No. of plants screened against PMV	No. of plants survived& screened against YVMV	Mean disease score	No. of plants survived& transferred to the field	Mean disease score
Amb x NL					
F_1	25	1	2.00	1	2.00
F_2	25	6	1.50	4	1.00
B_1	25	3	1.00	2	1.00
B_2	25	15	1.00	10	1.00
PV x NL					
F_1	25	9	1.22	2	1.00
F_2	25	6	1.33	4	1.50
B_1	25	4	1.00	2	1.00
B_2	25	12	1.70	9	1.10
AC x NL1.33					
F_1	25	5	1.40	3	1.33
F_2	25	8	1.00	6	1.00
B_1	25	5	1.20	3	1.67
B_2	25	15	1.13	11	1.36
Col x NL					
F_1	25	4	1.75	2	1.50
F_2	25	5	1.20	3	1.33
B_1	25	7	1.30	2	1.50
B_2	25	10	1.40	7	1.14

4.4.1.3 AC x NL

Out of the total number of 25 F_1 seedlings screened against PMV, disease symptoms were expressed only in 20 plants, five being resistant. There were three resistant and two moderately resistant plants. These resistant ones were subjected to subsequent inoculation with YVMV and three plants having resistance to YVMV were identified. In the F_2 there were eight plants resistant to PMV out of 25 seedlings screened. Further screening of these resistant segregants against YVMV brought out six plants with R reaction. Consequent to inoculation with PMV in 25 B_1 seedlings, five plants were found possessing resistance to PMV with a mean disease score of 1.2.

These resistant seedlings were sequentially inoculated with YVMV yielding three resistant plants, two being MR. The mean disease score was 1.67. Sequential screening against PMV and YVMV in 25 seedlings in the B_2 generation resulted in the isolation of 11 plants with combined resistance to both the viruses. All the 23 plants found as resistant to both PMV and YVMV were further evaluated in the main field for their subsequent performance.

4.4.1.4 Col x NL

The results of sequential screening against PMV in the six generations of the cross Col x NL are presented in Table 16. Evaluation of 25 seedlings in the F_1 generation against PMV gave one R and three MR plants. Subsequent inoculation of YVMV in these resistant seedlings brought out two plants with resistant reaction, the mean score being 1.5. In the F_2, out of 25, five showed resistant reaction (4R and 1MR) to PMV, the mean score being 1.20. These were inoculated with YVMV on the 21st day of inoculation of PMV and the results revealed that three seedlings had resistance to YVMV, two with R and one with MR reaction. In the B_1 generation scoring against PMV isolated seven resistant genotypes and the mean disease score was 1.30. Subsequent inoculation with YVMV in these plants identified two resistant plants, one R and the other MR. In the B_2 generation, 40 per cent plants showed resistant reaction to PMV and the sequential inoculation with YVMV could isolate seven plants possessing combined resistance to both the viruses.

All the plants with combined resistance to PMV and YVMV in the four generations were then transferred to main field for further evaluation.

4.4.2 Selection of plants with desirable horticultural attributes

The field performance of the mosaic resistant F_1, F_2 and back cross plants was evaluated in terms of their quantitative and qualitative characters including the incidence of other mosaic diseases. These observations are presented in Tables 17 to 24.

The plants found free from incidence of mosaic viruses were renumbered generation-wise as given in Appendix IV.

Amb x NL

The data in Table 17 showed that the days taken to anthesis of the first female flower varied from 45 days in the F_1-1 to 63 in B_2-5. The node of first fruit development in F -1 was 27 while in B_2-5 it was 39. In plant number F_2-2 also, the fruit development was observed in the twenty seventh node and the days required for anthesis of the first female flower was 46 days after sowing (DAS). The mean performance of the limited available plants in the F_2, B_1 and B_2 generations revealed that these took 50,75, 48 and 55.40 days respectively for anthesis of the first female flower. The node number of development of the first fruit was on an average 29.50 in the F_2 and B_1 plants while it was 31.10 in the B_2s.

The length of main vine was the highest for F_2-1 (10.30m) followed by 8.90m in F_1-1. The lowest vine length (4.50) was observed in B_2-8. The number of Female flower produced per plant varied from five in F_2-2 to 16 in B_2-6. The F_2 plants on an average produced 10 flowers per plant, while the B_1 and B_2 plants had 12 and 9.40 numbers of female flowers respectively per plant. Maximum number of fruits per plant was noticed in B_2-6 namely, four against the lowest number of one in F_2-2, B_2-1, B_2-5, B_2-7 and B_2-10. The F_1 plant F_1-1 gave three fruits out of 12 female flowers, the fruit development being 25 per cent. In B_2-3 and B_2-6 also the fruit production was 25 per cent. The lowest fruit recovery was seen in B_2-2 (15.38%). On an average, the fruit yield in number with respect to the number of

female flowers produced was 22.50 per cent in F_2 plants, 16.67 per cent in B_1s and 19.15 per cent in B_2s.

The number of days taken for maturity of the first fruit was minimum in F_1-1 i.e., 95 days from the date of sowing while B_2-5 took the maximum number of 127 days. On an average the F_2 plants required 107.75 days for the first fruit development while the B_1 and B_2 plants needed 115 and 111.80 days respectively. Plant number B_2-9 produced fruits with an average maximum length of 19.80m while the fruit diameter was the highest in the fruits of B_2-3 (23.05cm) (Table 18). Minimum fruit length was noted in F_2-4 (10.90cm). The fruit diameter was the lowest (12.50cm) for fruits of B_2-4 and B_2-9. The fruit yield was the highest in B_1-1 (9.85kg) with an average fruit weight of 4.93kg, closely followed by F_1-1 with 9.60kg fruit yield and 3.20kg average fruit weight. The genotype B_2-6 gave 9.40kg fruits consisting of four fruits of average fruit weight 2.35kg. On an average, the F_2 plants produced 4.14kg fruits with an average weight of 1.87kg against 9.60kg in F_1-1. The per plant mean fruit yield in the B_1 and B_2 population was 7.43 and 3.49kg respectively with an average fruit weight of 3.72kg for the former and 3.49kg for the latter.

The flesh thickness varied from 1.90cm in F_2-3 and B_2-2 to 3.26cm in fruits of B_2-3. The average flesh thickness of the fruits of F_1 plants was 2.83cm while the mean values for the B_1 and B_2 plants were 2.57cm and 2.39cm respectively. The number of seeds per fruit was the highest in F_1-1 (256). The F_2 plants on an average had 166.25 numbers of seeds per fruit while the B_1 and B_2 plants produced 246.50 and 134 numbers of seeds per fruit respectively.

The T.S.S. values ranged from ten per cent in the F_1-1 to 3 per cent in B_2-4 and B_2-8. The mean values for T.S.S. in fruits of F_2, B_1 and B_2 generations were 5.15, 5.35 and 4.69 per cent respectively.

Maximum carotene content namely, 33.22 IU/100g was observed in the fruits of F_1-1, followed by F_2-2 (32.16 IU), F_2-4 (32 IU) and F_2-1 (31.85 IU). Fruits of B_2-6 had the lowest carotene content (14.02 IU). The mean carotene content in the fruits of F_2, B_1 and B_2 plants were 30.98 IU, 29.41 IU and 21.44 IU respectively.

Table 17. Biometric characters of plants resistant to PMV and YVMV in the cross Amb x NL

Genotype		No. of days to first FF anthesis	Node of first fruit development	Length of main vine (m)	No. of FF	No. of days to first fruit maturity	No. of fruits	Fruit yield (kg)	Mosaic incidence
F_1	1	45	27	8.90	12	95	3	9.60	Nil
F_2	1	56	31	10.30	13	104	3	5.80	Nil
	2	46	27	7.20	5	102	1	2.35	Nil
	3	50	28	6.10	13	104	3	6.10	Nil
	4	57	32	6.60	9	121	2	2.30	Nil
Mean		50.75	29.50	7.80	10	107.75	2.25	4.14	
B_1	1	46	28	6.10	12	114	2	9.85	Nil
	2	50	31	5.90	12	116	2	5.00	Nil
Mean		48.00	29.50	6.00	12	115	2	7.43	
B_2	1	58	35	5.80	6	120	1	1.20	CMV
	2	53	36	6.80	13	109	2	3.90	CMV
	3	53	29	7.90	8	103	2	7.50	Nil
	4	57	28	7.20	11	115	2	2.31	CMV
	5	63	39	6.50	6	127	1	2.15	WMV
	6	52	30	8.10	16	110	4	9.40	Nil
	7	59	28	6.70	6	117	1	1.20	Nil
	8	50	28	4.50	11	101	2	3.20	CMV
	9	59	35	6.60	12	110	2	2.65	CMV
	10	50	29	8.10	5	106	1	1.40	Nil
Mean		55.40	31.10	6.82	9.40	111.80	1.80	3.49	

FF - Female flowers

Table 18. Fruit characters of plants resistant to PMV and YVMV in the cross Amb x NL

Genotype		Av. length (cm)	Av. diameter (cm)	Av. fruit weight (kg)	Av. flesh thickness (cm)	Av. no. of seeds/ fruit	T.S.S. %	Carotene content IU/100g	Fruit smoothness	Fruit colour	Flesh colour	Fruit shape
F_1	1	15.00	18.50	3.20	2.83	256	10.00	33.20	3	Greyish dark green with white patches and powdery coat	Yellow	Round
F_2	1	15.40	18.00	1.93	2.43	155	6.60	31.85	1	Cane with light powder coat and faded cream patches	Orange	Round
	2	15.60	20.40	2.35	2.70	130	6.60	32.16	3	Light greyish green	Yellow	Round
	3	14.10	18.00	2.03	1.90	200	4.20	27.89	2	greyish dark green	Yellow	Round
	4	10.90	16.50	1.15	2.23	180	3.20	32.00	1	Cane with faded white patches	Deep yellow	Round
Mean		14.00	18.23	1.87	2.31	166.25	5.15	30.98				
B_1	1	18.80	22.80	4.93	2.73	268	6.80	29.79	1	Cane with faded cream patches and waxy coat	Orange	Round
	2	13.85	20.75	2.50	2.41	225	3.90	29.03	1.50	Green with greyish waxy coat	Yellow	Round
Mean		16.33	21.78	3.72	2.57	246.50	5.35	29.41				
B_2	1	11.50	15.00	1.20	2.30	225	5.80	19.13	3	Green with cane projections	Yellow	Round
	2	11.00	14.00	1.95	1.90	102	6.00	21.49	3	Cane	Yellow	Round
	3	16.30	23.05	3.75	3.26	93	5.40	28.15	1.25	Dark green with thick greyish wax coat	Yellow	Round
	4	14.30	12.50	1.16	1.92	178	3.00	27.81	2	Green with white patches	Yellow	Oblong round
	5	15.60	18.30	2.15	2.48	121	8.80	25.15	2	Green	Yellow	Round
	6	15.20	16.50	2.35	2.53	130	4.05	14.02	3	Greyish green	Yellow	Oblong
	7	11.50	15.00	1.20	2.25	125	6.00	18.44	2	Greyish green	Yellow	Round
	8	13.20	18.00	1.60	2.95	31	3.00	28.22	2	Cane with light white patches	Yellow	Round
	9	19.80	12.50	1.33	2.05	120	4.40	14.71	3	Dark green	Creamy yellow	Round
	10	12.00	14.30	1.40	2.30	215	4.00	17.30	2	Cane with faded white patches and green shade	Yellow	Oblong
Mean		14.00	15.92	3.49	2.39	134	4.69	21.44				

Fruit smoothness : 1 = Smooth, 2 = Moderately warty, 3 = Warty

The fruits from all the plants were graded for their external colour, flesh colour, fruit shape and smoothness (Table 18). The external fruit colour ranged from greyish dark green colour of NL to cane colour of Ambili and a complexion of these colours was noticed in some fruits. The flesh colour noted were orange/deep yellow colour of Ambili, creamy yellow colour of NL and a blend of these colours i.e., yellow, produced by 76.47 per cent of the plants. Regarding the fruit shape, 82.35 per cent plants produced round fruits. Four plants i.e., F_2-1, F_2-4, B_1-1 and B_2-3, produced smooth fruits while the plant B_1-2 produced almost smooth fruits. These plants were free from incidence of other mosaic viruses also. The per plant fruit yield of the said genotypes were 5.80kg, 2.30kg, 9.85kg, 7.50kg and 5.00 kg respectively. The plants F_2-3 and B_2-6 (reassigned number B_2-2) produced 6.10kg and 9.40kg fruits respectively, but the warty nature of fruits (score 2 and 3) is an undesirable character.

PV X NL

The data on the performance of the PMV and YVMV resistant genotypes comprising the F_1, F_2, B_1 and B_2 generations of the cross PV x NL is presented in Tables 19 and 20.

The number of days required for the anthesis of the first female flower ranged from 36 in the B_1 plants to 51 in the plant B_2-7. The plant B_2-8 (38) clearly followed the B_1s in earliness. The F_2-2 and B_2-6 plants took 39 days for anthesis of the first female flower. The days taken for anthesis of the first female flower were 41.50, 43.75, 36.00 and 45.33 respectively in the F_1, F_2, B_1 and B_2 plants. The node number of first fruit development ranged from 19 in the B_1 plants to 36 in B_2-9 (Table 19). On an average, the node number of first fruit development was the highest for the B_2 plants namely, 26.67. The genotype F_1-2 produced the shortest main vine (3.90m) and B_2-8 had the longest (8.25m). The main vine length showed an increasing trend from the F_1 genotype (4.80m) upto the B_2 genotypes namely, 4.63m, 5.53m, and 6.50m respectively for the B_1, F_2, and B_2 generations. The genotypes F_2-4 and B_2-1 gave the highest number of female flowers per plant (21.00) and fruit development was 9.53 and 14.29 per cent respectively. This was followed by F_2-1, F_2-3 and B_2-2 with 19 female flowers each, the fruit development being 10.53, 10.53 and 15.79 per cent respectively.

Table 19. Biometric characters of plants resistant to PMV and YVMV in the cross PV x NL

Genotype		No. of days to first FF anthesis	Node of first fruit develop ment	Length of main vine (m)	No. of FF	No. of days to first fruit maturity	No. of fruits	Fruit yield (kg)	Mosaic incidence
F_1	1	42	23	5.70	18	91	3	3.96	Nil
	2	41	21	3.90	15	89	2	2.15	Nil
Mean		41.50	22.00	4.80	16.50	90.00	2.50	3.06	
F_2	1	49	22	5.40	19	94	2	2.44	Nil
	2	39	20	4.90	8	89	1	1.18	CMV
	3	42	26	5.60	19	90	2	2.10	Nil
	4	45	21	6.20	21	95	2	2.40	Nil
Mean		43.75	22.50	5.53	16.75	91.50	1.75	2.03	
B_1	1	36	19	4.70	11	86	2	3.60	Nil
	2	36	19	4.55	9	86	1	2.00	Nil
Mean		36.00	19.00	4.63	10	86.00	1.5	2.80	
B_2	1	47	25	7.90	21	95	3	5.70	Nil
	2	49	23	5.90	19	103	3	2.90	BMV
	3	48	28	6.30	16	100	2	2.80	CMV
	4	45	22	6.00	10	93	1	1.10	WMV
	5	48	31	7.50	7	115	1	2.20	CMV
	6	39	23	5.00	18	90	3	3.50	CMV
	7	51	28	4.90	16	109	5	5.80	CMV
	8	38	24	8.25	11	86	2	6.60	Nil
	9	46	36	6.80	17	101	2	2.70	CMV
Mean		45.33	26.67	6.50	15.00	99.11	2.44	3.70	

FF - Female flowers

Table 20. Fruit characters of plants resistant to PMV and YVMV in the cross PV x NL

Genotype		Average length (cm)	Average diameter (cm)	Av. fruit weight (kg)	Av. flesh thickness (cm)	Av. No. of seeds/ fruit	T.S.S. %	Carotene content IU/100g	Fruit smooth ness	Fruit colour	Flesh colour	Fruit shape
F_1	1	13.70	13.50	1.32	1.78	236	3.00	20.65	3	Deep green with white patches	Yellow	Conical
	2	12.45	10.75	1.08	1.71	269	4.20	20.80	3	Deep green with white patches	Yellow	Conical
Mean		13.08	12.13	1.20	1.75	252.50	3.60	20.73				
F_2	1	9.95	10.85	1.22	1.73	53	2.40	31.09	1	Cane	Yellow	Oblong
	2	10.10	9.30	1.18	1.63	121	3.80	13.49	3	Cane with green shades	Yellow	Conical
	3	14.25	11.55	1.05	1.65	185	3.60	30.83	1	Cane	Orange	Round oblong
	4	11.20	13.30	1.20	2.38	210	4.60	22.86	2	Canish dark green	yellow	Oblong
Mean		11.40	11.25	1.16	1.85	142.25	3.60	24.57				
B_1	1	19.40	14.30	1.80	2.46	203	5.50	33.91	1	Greyish light cane	Orange	Oblong
	2	18.80	18.00	2.00	3.08	121	6.00	22.48	2	Cane	Yellow	Round with either side conical
Mean		19.10	16.15	1.90	2.77	212	5.75	28.20				
B_2	1	14.80	16.50	1.90	2.28	150	3.00	15.55	3	Green	Yellow	Oblong
	2	14.17	12.30	0.97	1.50	60	3.00	14.10	2	Dark green	Creamy yellow	Bottle
	3	17.00	18.50	1.40	2.90	110	4.20	17.68	2	Cane	Yellow	Round
	4	15.00	10.50	1.10	1.83	94	5.00	15.70	3	Dark Greyish green	Yellow	Round
	5	16.70	19.00	2.20	2.40	150	3.40	22.02	1	Cane	Yellow	Oblong
	6	16.40	15.30	1.17	1.93	139	4.20	21.18	3	Greyish green	Yellow	Oblong
	7	9.60	15.30	1.16	1.80	49	2.20	23.38	3	Greyish canish green	Yellow	Round and oblong
	8	18.40	25.20	3.30	2.03	175	4.40	30.94	3	Light canish cream	Creamy yellow	Round
	9	10.50	15.40	1.35	1.21	108	4.00	14.93	3	Dark green	yellow	Round conical
Mean		14.73	16.44	1.62	2.10	115	3.71	19.50				

Fruit smoothness : 1 = Smooth, 2 = Moderately warty, 3 = Warty

The female flower production was the lowest in B_2-5 (7), but the fruit development percentage was 14.29. The fruit production in terms of number of fruits and development percentage was maximum in B_2-7 i.e., out of 15 female flowers produced, five developed in to mature fruits, the development percentage being 31.25. On an average, the F_2 plants produced the highest number of female flowers per plant (16.75) closely followed by the F_1 genotypes (16.50) whereas the fruit yield per plant was maximum in the F_1s (2.50kg) closely followed by the B_2 plants (2.44kg). In general the per cent fruit development was the highest for the B_2 plants (16.27), followed by the F_1 (15.15), B_1 (15.00) and F_2 (12.45) plants.

The number of days required for maturity of the first fruit showed the same trend as that of the days to first female flower anthesis. The B_1 plants took the shortest period of 86 days followed by the F_1, F_2 and B_2 plants (90.00, 91.50 and 99.11 days respectively).

The highest fruit yield per plant was recorded in B_2-8 (6.60kg) comprising two fruits with an average weight of 3.3kg. The average fruit length was 18.40cm, fruit diameter 25.20cm and flesh thickness 2.03cm. The second highest yield was obtained from B_2-1 i.e., 5.70kg with an average fruit weight of 1.90kg, fruit length 14.80cm, fruit diameter 16.50cm and flesh thickness 2.28cm. The yield was minimum in B_2-4 (1.10kg). In general, the B_2 plants produced an average yield of 3.70kg from 2.44 fruits of average length 14.73cm, average diameter 14.73cm, average weight 1.62kg and flesh thickness 2.10cm. This was followed by the F_1s (3.06kg), B_1s (2.80kg) and F_2s (2.03kg) respectively. The flesh thickness on an average was the highest for B_1 plants (2.77cm), B_1-4 recording the maximum flesh thickness of 3.08cm.

The number of seeds per fruit ranged from 269 in F_1-2 to 49 in B_2-7. The average number of seeds per fruit was maximum in the F_1 plants (252.50), followed by the B_1 (212.00), F_2 (142.25) and B_2 (115) plants.

The T.S.S. content of the fruits ranged from 2.2 per cent in B_2-7 to 6 per cent in B_1-2. The second highest T.S.S. value of 5.50 per cent was recorded by B_1-1. So the average value for T.S.S. was maximum in the fruits of B_1 plants i.e., 5.75 per cent. The B_2 plants recorded an average value of 3.71 per cent T.S.S. while the F_1 and F_2 plants produced fruits with average T.S.S. value of 3.60 per cent.

The carotene content in fruits ranged from 13.49 IU/100g in F_2-2 to 33.91 in B_1-1. The second and third highest values in carotene content were noted in fruits of B_2-8 (30.94) and F_2-3 (30.83). The mean carotene content in the fruits of F_1, F_2, B_1 and B_2 plants were 20.73, 24.57, 28.20 and 19.50 respectively.

The data on fruit characters and mosaic disease incidence is given in Table 20. A range of external fruit colour was observed in the different genotypes under evaluation. There were fruits expressing the external colour of PV (cane) and greyish green colour of NL. A blend of these colours was noted in others. The flesh colour also varied in the different genotypes ranging from orange colour of PV to creamy yellow colour of NL.

Regarding the fruit shape, round, conical, oblong , round oblong and bottle shaped fruits were produced by the different genotypes. Out of the seventeen genotypes screened only four produced smooth fruits i.e., F_2-1, F_2-3, B_1-1 and B_2-5. Four plants had moderately warty fruits while warty fruits were seen in other nine plants. Incidence of CMV/WMV/BMV was noted in eight genotypes under study.

AC x NL

The performance of the different genotypes comprising the F_1, F_2, B_1 and B_2 population of the cross AC x NL evaluated in the field for selection of genotypes with desirable horticultural characteristics is presented in Tables 21 and 22.

The minimum duration for anthesis of the first female flower was noted in F_1-2 with 46 days. B_2-7 took the longest period of 60 days for the first female flower anthesis. The F_1 plants on an average took 47 days for anthesis of the first female flower while the genotypes in F_2, B_1 and B_2 generations required 50.67, 50.33 and 52.36 days respectively. The node of first fruit development was the lowest in F_2-3, the node number being 26. Plant number B_2-8 developed the first fruit only in 41st node. The node of first fruit development was the lowest in the F_1 plants, the node number being 30. The fruit development in the F_2 plants on an average occurred in node number 32.50 while in the B_1 and B_2 plants the node numbers were 32 and 35.50 respectively. The main vine length was the highest for B_2-8 i.e., 11.90 followed by B_2-3 (11.40m). The shortest main vine was produced by F_2-3 (4.25m). The average length of main vine in the F_1, F_2, B_1 and B_2 generations was 7.95m, 8.10m, 7.80m and 9.17m respectively (Table 21).

The female flower production in the plants ranged from 6 in B_2-5 to 17 in F_2-6. On an average, the F_1 plants produced the highest numbers of female flowers per plant i.e., 15.67, while the B_2s produced 10.64 female flowers per plant. The fruit development per cent was the highest in plant number F_2-6 i.e., 23.52, closely followed by B_1-1 (21.43). The average fruit production per cent in the F_1s, F_2s B_1s and B_2s were 14.87, 14.95, 18.18 and 14.57 respectively. There were four fruits each in F_1-3 and F_1-6. The per plant fruit production was the highest in the F_1 generation i.e., 2.33, followed by the F_1 (2.1), F_2 (1.67) and B_2 (1.55) generations.

Table 21. Biometric characters of plants resistant to PMV and YVMV in the cross AC x NL

Genotype		No. of days to first FF anthesis	Node of first fruit development	Length of main vine (m)	No. of FF	No. of days to first fruit maturity	No. of fruits	Fruit yield (kg)	Mosaic incidence
F_1	1	47	31	7.00	15	101	2	2.80	WMV
	2	46	29	7.85	9	97	1	3.90	WMV
	3	48	30	8.90	23	101	4	5.00	CMV
Mean		47	30	7.95	15.67	99.60	2.33	3.90	
F_2	1	56	34	6.40	7	118	1	1.20	Nil
	2	51	33	4.75	10	102	1	2.30	Nil
	3	48	26	4.25	8	107	1	2.00	BMV
	4	49	34	5.15	14	109	2	1.70	Nil
	5	51	36	4.75	11	103	1	2.50	Nil
	6	49	32	8.10	17	98	4	7.10	Nil
Mean		50.67	32.50	5.57	11.17	106.17	1.67	2.43	
B_1	1	51	33	5.20	14	100	3	3.80	Nil
	2	48	28	9.70	9	103	2	6.25	Nil
	3	52	35	8.50	10	109	1	1.50	CMV
Mean		50.33	32	7.80	11	104	2	3.85	
B_2	1	48	32	9.40	13	102	2	3.90	CMV
	2	51	34	7.95	9	106	1	1.91	CMV
	3	53	37	11.40	7	113	1	1.60	CMV
	4	56	36	8.50	11	108	2	3.10	CMV
	5	47	34	8.10	6	103	1	1.30	CMV
	6	51	38	10.20	9	104	1	1.80	Nil
	7	60	39	8.30	15	115	3	2.50	WMV
	8	49	41	11.90	14	110	2	1.86	CMV
	9	58	36	9.30	8	101	1	1.10	Nil
	10	59	28	6.60	13	104	2	6.40	Nil
	11	52	36	9.20	12	105	1	1.10	CMV
Mean		52.36	31.55	9.17	10.64	106.45	1.55	2.42	

FF Female flowers

Table 22. Fruit characters of plants resistant to PMV and YVMV in the cross AC x NL

Genotype		Av. length (cm)	Av. diameter (cm)	Av. fruit weight (kg)	Av. flesh thickness (cm)	Av. no. of seeds/ fruit	TSS %	Carotene content IU/100g	Fruit smoothness	Fruit colour at maturity	Flesh colour	Fruit shape
F_1	1	15.20	16.10	1.40	2.18	349	5.40	23.44	2.00	Green	Yellow	Bottle
	2	16.50	18.00	3.90	2.68	267	5.00	22.17	2.00	Greyish green	Yellow	Bottle
	3	14.20	12.50	1.25	2.70	350	5.00	30.02	2.00	Light greyish	Yellowish Orange	Oblong
Mean		15.30	15.58	2.18	2.52	322	5.13	25.21				
F_2	1	10.20	16.40	1.20	2.43	205	5.80	41.10	1.00	Light candy	Yellow	Flat round
	2	11.00	17.00	2.30	2.69	115	4.20	31.78	3.00	Light green with light canish projections	Orange	Round
	3	11.70	18.00	2.00	2.88	177	7.20	26.11	1.00	Greyish candy	Orange	Bottle, round
	4	9.50	13.60	0.85	2.50	80	6.00	22.76	2.00	Greyish dark green	Orange	Round
	5	12.20	20.10	2.50	2.98	240	5.00	21.69	3.00	Light greyish green	Yellow	Flat round
	6	15.40	17.20	1.78	1.58	210	3.60	29.79	2.00	Greyish green	Yellow	Oblong
Mean		11.67	17.05	1.77	2.50	171.17	5.30	28.87				
B_1	1	10.00	18.00	1.27	2.25	293	4.00	23.10	3.00	Green with candy projections	Yellow	Round
	2	18.10	24.35	3.13	3.05	310	6.90	27.89	1.25	Greyish candy	Cane Yellow	Flat round
	3	12.00	16.00	1.50	2.70	175	7.20	21.87	1.50	Candy	Cane Yellow	Flat round
Mean		13.37	19.45	1.97	2.67	259.33	6.03	24.28				

(Continued....)

Table 22. Fruit characters of plants resistant to PMV and YVMV in the cross AC x NL (Continued........)

Genotype		Av. length (cm)	Av. diameter (cm)	Av. Fruit weight (kg)	Av. Flesh thickness (cm)	Av. No. of seeds/ fruit	TSS %	Carotene content IU/100g	Fruit smoothness	Fruit colour at maturity	Flesh colour	Fruit shape
	1	17.00	18.00	1.95	2.85	208	4.80	24.23	1.50	Green	Yellowish orange	Round
	2	16.00	17.80	1.91	2.18	169	3.60	17.15	2.00	Green	Light yellow	Round
	3	16.00	18.00	1.60	2.50	131	4.20	31.40	3.00	Light green with candy projections	Yellow	Round
	4	15.20	17.90	1.75	2.40	162	4.80	24.16	1.00	Light candy	Yellow	Flat round
	5	19.20	18.30	1.30	2.20	159	2.80	26.06	2.00	Green	Yellow	Bottle
B_2	6	10.40	18.50	1.80	1.90	160	4.80	22.02	3.00	Light green with ivory projections and waxy powder coating	Yellow	Flat round
	7	14.20	14.50	2.50	2.18	120	5.20	21.41	3.00	Greyish	Light orange	Round
	8	15.00	15.50	1.86	2.13	108	4.00	25.22	3.00	Candy	Yellowish orange	Flat round
	9	10.00	15.00	1.10	2.08	128	2.80	17.60	3.00	Greyish candy	Yellow	Flat round
	10	15.50	18.50	6.40	2.50	130	5.00	17.76	2.00	Light Candy	Light yellow	Round
	11	13.00	14.00	1.10	2.90	160	3.40	30.79	1.00	Dark green	Light orange	Round
Mean		14.71	16.91	2.42	2.35	148.64	4.13	23.44				

Fruit smoothness : 1 = Smooth, 2 = Moderately warty, 3 = Warty

The fruit yield per plant ranged from 7.10kg in F_2-6 (comprising four fruits of average length 15.40cm, average diameter 17.20cm, average fruit weight 1.78kg and flesh thickness 1.58cm) to 1.10kg in B_2-9 (consisting of only one fruit with length 10cm, diameter 15cm and flesh thickness 2.08cm).B_2-10 and B_1-2 were ranked as the second and third high yielders with 6.40kg and 6.25kg fruit yield respectively. The average values for fruit length, fruit diameter, fruit weight and flesh thickness in B_2-10 were in the order of 15.50cm, 18.50cm, 3.20kg and 2.90cm (Table 22). The fruits of B_1-2 were 18.10cm long and 24.35cm in diameter with average fruit weight of 3.13kg and flesh thickness 3.05cm. The fruit yield per plant on an average was maximum in the F_1 plants (3.90kg), closely followed by the B_1 generation with 3.85kg fruits. The F_2s and B_1s gave an average yield of 2.43kg and 2.42kg fruits per plant. The F_1 plants on an average comprised of 2.33 fruits with average fruit length 15.30cm, fruit diameter 15.58cm, fruit weight 2.18kg and flesh thickness 2.52cm. The highest flesh thickness was noted in fruits of B_1-2 (3.05cm) followed by that in F_2-5 (2.98cm).

Plant number F_1-3 produced four fruits containing an average number of 350 seeds, very closely followed by F_1-1 with two fruits having 349 seeds per fruit on an average. The fruits of B_1-2 had a mean number of 310 seeds per fruit. In general, the F_1 fruits contained the highest number of 322 seeds per fruit, followed by the B_1 -5 with 259.33 seeds.

The T.S.S. value of fruits ranged from 2.80 per cent (B_2-5 and B_2-9) to 7.20 per cent in the F_2-3 plant. The B_1 lines recorded the highest average T.S.S. of 6.03 per cent while the F_2s had 5.30 per cent, F_1s had 5.13 per cent and B_2s had 4.13 per cent T.S.S.

Among the genotypes evaluated, F_2-1 produced fruits with the highest content of carotene i.e.,41.10 IU/100g, followed by F_2-2 (31.78 IU), B_2-3 (31.40 IU), B_2-11 (30.79 IU) and F_1-3 (30.02 IU). Fruits of F_2-6 had a carotene content of 29.79 IU. The lowest quantity of carotene was recorded in fruits of B_2-2 (17.15 IU). The mean carotene content in the fruits of F_1, F_2, B_1 and B_2 generations were 25.21, 28.87, 24.28 and 23.44 IU respectively.

The fruit characters along with the incidence of mosaic diseases noted in the genotypes belonging to the different generations of the cross AC x NL is given in Table 22. The plants produced fruits with colour ranges greyish green, green, light grey, greyish candy,

candy and light candy. The fruits had flesh colours ranging from light yellow, yellow, yellowish orange, light orange, orange and cane yellow. The fruit shape varied as bottle, oblong, flat round, round and round bottle. Only four plants produced smooth fruits, while eight had moderately warty fruits and others gave highly warty fruits. Attack by either of the viruses CMV, WMV and BMV was noted in 13 genotypes under study. Only ten plants were found free from these diseases.

Col x NL

The field performance of the genotypes from the cross Col x NL comprising the F_1, F_2 B_1 and B_2 generation are presented in Tables 23 and 24.

The number of days required for the anthesis of the first female flowers varied from the minimum 53.00 (F_1-1) to maximum 63 (B_1-7). The F_1s took a mean duration of 53.50 days for anthesis of the first female flower, while the F_2s, B_1s and B_2s took 62.33, 59.00 and 59.00 days respectively. The node number of first fruit development ranged from 29 in F_1-1 to 45 in B_1-2 (Table 23).

The main vine length was the longest for F_1-2 with 9.50m, B_2-4 ranking second with 9.40m. The overall performance of the lines in the different generations revealed that the F_1 plants had the maximum vine length with 9.25m, followed by the B_1s (8.68m), B_2s (8.03m) and F_2s (6.80m) respectively.

The female flower production was the highest in B_2-1 (17.00), with a fruit yield of three numbers. So the fruit development percentage was 17.65. The lines F_2-3, B_1-2 and B_2-6 produced 15 female flowers each and their fruit development was in the order of 26.67, 13.33 and 13.33 per cent respectively. The highest fruit development occurred in F_1-2 (30 per cent) followed by F_2-3 (26.67 per cent). Considering the average, the female flower production per plant was the highest for the B_2s (12.71) closely followed by the F_2s (12.67). The F_1s on an average produced three fruits per plant while the F_2s, B_1s and B_2s had a per plant yield of 2.67, 1.50 and 1.71 fruits. Considering the mean values, the F_1s had 28.57 per cent fruit development, B_1s 17.28, F_2s 14.28 and B_2s 13.41 per cent.

Table 23. Biometric characters of plants resistant to PMV and YVMV in the cross Co1 x NL

Genotype		No. of days to first FF anthesis	Node of first fruit development	Length of main vine (m)	No. of FF	No. of days to first fruit maturity	No. of fruits	Fruit yield (kg)	Mosaic incidence
F_1	1	53	29	9.00	11	114	3	5.35	WMV
	2	54	32	9.50	10	111	3	7.50	CMV
Mean		53.50	30.50	9.25	10.50	112.50	3	6.43	
F_2	1	59	30	5.20	10	117	2	6.80	Nil
	2	62	32	7.70	13	118	2	1.50	CMV
	3	66	34	7.50	15	124	4	4.56	BMV
Mean		62.33	32	6.80	12.67	119.67	2.67	4.29	
B_1	1	55	35	8.15	7	113	1	1.95	WMV
	2	63	45	9.20	15	131	2	7.30	Nil
Mean		59	40	8.68	11	122	1.50	4.62	
B_2	1	59	42	8.10	17	123	3	9.75	WMV
	2	56	43	7.30	12	117	1	3.10	CMV
	3	59	38	5.00	9	130	1	1.52	CMV
	4	62	41	9.40	14	125	2	2.60	CMV
	5	59	42	8.10	9	123	1	3.25	CMV
	6	56	39	8.90	15	115	2	3.70	CMV
	7	62	32	8.70	13	110	2	8.00	Nil
Mean		59	37.72	8.03	12.71	120.43	1.70	4.56	

FF - Female flowers

Table 24. Fruit characters of plants resistant to PMV and YVMV in the cross Co1 x NL

Genotype		Av. length (cm)	Av. diameter (cm)	Av. weight (kg)	Av. flesh thickness (cm)	Av. no. of seeds/ fruit	T.S.S. %	Carotene content IU/100g	Fruit smooth ness	Fruit colour at maturity	Flesh colour	Fruit shape
F_1	1	14.70	18.00	1.78	2.35	240	5.20	16.54	2	Greyish green	Light yellow	Round
	2	18.40	21.60	2.50	2.70	292	5.40	15.85	3	Greyish green	Yellow	Round
Mean		16.55	19.80	2.14	2.53	266	5.30	16.20	-	-	-	-
F_2	1	16.10	15.27	3.40	2.93	100	5.80	23.55	1	Cane	Yellow	Round
	2	11.95	12.65	0.75	1.75	182	3.80	15.62	1	Cane	Yellow	Round
	3	16.30	15.10	1.14	1.83	90	3.60	22.56	1.50	Canish grey	Yellow	Round
Mean		14.45	14.01	1.76	2.17	124	4.40	20.58	-	-	-	-
B_1	1	11.00	14.00	1.95	1.90	109	3.80	11.35	2	Greenish cane	Light yellow	Round
	2	15.20	19.30	3.65	3.00	190	5.60	22.71	1	Greyish cane	Yellow	Round
Mean		13.10	16.65	2.80	2.45	149.5	4.70	17.03	-	-	-	-
B_2	1	13.20	19.80	3.25	2.90	190	3.80	19.58	3	Light cane	Yellow	Round
	2	14.30	19.10	3.10	2.76	123	3.20	13.11	3	Green	Ivory	Round
	3	11.70	14.20	1.52	2.40	163	4.60	10.74	1	light greenish candy with white patches	Light yellow	Round
	4	12.50	13.80	1.30	2.35	134	4.20	17.22	2	Light cane	Ivory	Round
	5	15.40	17.00	3.25	2.50	109	3.80	18.06	3	Light green with canish projections	Ivory	Round
	6	11.00	17.80	1.85	2.20	155	5.00	21.03	3	Light green	Ivory	Flat round
	7	19.10	21.40	4.00	3.30	182	3.80	22.17	3	Light green	Yellow	Round
Mean		13.89	17.50	2.61	2.63	150.86	4.06	17.42	-	-	-	-

The genotype B_2-1 gave the highest yield of 9.75kg comprising three fruits of average length 13.20cm, average diameter 19.80cm, average weight 3.25kg, average flesh thickness 2.90cm and T.S.S. 3.80 per cent. This was followed by B_2-10 producing two fruits weighing a total of 8kg and the average values for fruit length, fruit diameter, fruit weight, flesh thickness and T.S.S. were 19.10cm, 21.40cm. 4.00kg, 3.30cm and 3.80 per cent respectively. On an average, the F_1 plants gave the highest yield of 6.43 kg per plant from three fruits with average fruit length 16.55cm, fruit diameter 19.80cm, average weight 2.14kg, flesh thickness 2.53cm and average T.S.S. 5.30 per cent. The flesh thickness was maximum for the fruits in the B_2 generation i.e., 2.63cm, followed by those in the F_1 generation i.e., 2.53cm. The T.S.S. percentage ranged from 3.20 (B_2-2) to 5.80 (F_2-1). The carotene content of fruits ranged from 10.74 IU in B_2-3 to 23.55 IU/100g in F_2-1. the fruits of B_1-2 (22.71), F_2-3 (22.56IU) and B_2-7 (22.17IU) were also comparatively high in carotene content.

The fruit characteristics of the genotypes evaluated are presented in Table 24. The fruits varied in colour ranges namely, greyish green, green, light green, greyish cane, canish grey, light cane and cane. F_2-1, F_2-2, B_2-1 and B_2-2 had smooth fruits while F_2-3 had almost smooth fruits (score 1.5). The flesh colour of fruits ranged as light yellow, yellow and ivory. Fifty per cent plants produced fruits with light yellow flesh and 28.57 per cent had ivory fleshed fruits. Except B_2-6, all others produced round fruits. Out of the 14 genotypes only two lines, F_2-2 and B_1-7 produced smooth fruits.

Most of the plants were seen affected by either of the viruses WMV, CMV and BMV. Only three were free from incidence of these viruses.

An overall review of the performance of the progenies in the cross combination of Amb x NL, PV X NL, AC X NL and Col x NL is presented in Table 25. The data revealed that the progenies belonging to the F_1, F_2 B_1 and B_2 generations of the cross PV X NL on an average took the minimum number of days (41.65) for the first Female flower anthesis, followed by Amb x NL, AC X NL, and Col x NL taking a duration in the order of 49.79, 50.10 and 58.46 days for the first female flower anthesis. The node of first fruit development ranged from 22.48 in PV x NL to 35.06 in Col x NL. The progenies in the cross PV X NL had the shortest main vine with a length of 5.37m. the main vine length was maximum for Col x NL (8.19).

Table 25. Mean performance of the population in the four cross combinations , comprising the F_1, F_2, B_1 and B_2 generations

Cross combinations	No. of days to first FF anthesis	Node of first fruit developm ent	Length of main vine (m)	No. of FF	No. of days to first fruit maturity	No. of fruits /plant	Fruit length (cm)	Fruit diamet er(cm)	Av. fruit weigh (kg)t	Fruit yield /plant (kg)	Flesh thickens (cm)	No. of seeds /fruit	T.S.S %	carotene content (IU/100g)
Amb x NL	49.79	29.28	7.38	10.85	107.39	2.26 (20.83)	14.83	18.67	2.65	6.17	2.53	200.69	6.23	28.76
PV x NL	41.65	22.48	5.37	14.56	91.65	2.05 (14.08)	14.27	13.99	1.48	2.90	2.12	180.44	4.17	22.11
AC x NL	50.10	32.51	7.60	12.12	104.07	1.89 (15.59)	13.76	17.23	1.88	3.15	2.51	225.29	5.15	25.45
Col x NL	58.46	35.06	8.19	8.98	118.65	2.22 (24.72)	14.50	17.01	2.33	4.98	2.45	172.59	4.59	19.72

Figures given in parenthesis are the fruit development percentage FF - Female flowers

The first fruit in the progenies of PV x NL matured with an average duration of 91.65 days while those in AC x NL took 104.07 days. The plants of Amb x NL required an average duration of 107.39 days for maturity of its first fruit while Col x NL took the maximum duration of 118.65 days. Among the four crosses, progenies of PV x NL recorded the highest number of female flower per plant i.e. 14.56. The minimum number of female flower per plant were observed in Col x NL (8.98). The number of fruits produced per plant ranged from 1.89 in AC x NL to 2.26 in Amb x NL. The fruit development percentage was the highest (24.72) for Col x NL lines followed by Amb x NL lines (20.83). The fruit development was the least in PV x NL (14.08%). The progenies of Amb x NL gave the highest per plant yield of 6.17kg. The mean values for fruit length, fruit diameter, average fruit weight, flesh thickness and T.S.S. for these lines were 14.83cm, 8.61cm, 2.65kg, 2.53cm and 6.23 per cent respectively. The second highest yield per plant (4.98kg) was recorded by the progenies of Col x NL with 2.26 fruits having average length 14.50cm, average diameter 17.01cm and average fruit weight 2.33kg. The flesh thickness and T.S.S. percentage were 2.45cm and 4.59cm respectively.

The mean number of seeds per fruit was the highest for AC x NL lines (225.29), followed by Ambili x NL (200.69), PV x NL (180.44) and Col x NL (172.59) respectively. The mean T.S.S. percentage and carotene content of fruits were on top for Amb x NL plants (6.23% and 28.76 IU respectively), followed by AC x NL lines (5.15% and 25.45 IU respectively).

Based on the non incidence of mosaic diseases, fruit smoothness and per plant yield, eight most promising superior genotypes from among the four cross combinations were selected and the data on their performance is presented in Tables 26 and 27 and Figures 9 and 10. They included four lines from the segregating population of Amb x NL namely, F_2-1, B_1-1 (Plate 45), B_1-2 (Plate 46) and B_2-3 (reassigned number B_2-1), one each from PV x NL (B_1-1 with reassigned number B_1-3, Plate 47) and AC x NL (B_1-2 with reassigned number B_1-6, Plate 48) and two from Col x NL i.e., F_2-1 and B_1-2 (reassigned numbers F_2-13 and B_1-7 respectively; Plates 49 and 50). Among the seven, B_1-1 had the highest yield (9.85kg). There were two fruits per plant with average fruit weight 4.93kg. It took only 46 days for anthesis of the first female flower and the node of first fruit development was 28. It produced 12

Table 26. Biometric characters of selected promising plants resistant to PMV and YVMV

Genotype	Reassigned Numbers	No. of days to first FF anthesis	Node of first fruit development	Length of main vine (m)	No. of FF	No. of days to first fruit maturity	No. of fruits	Fruit yield (kg)	Mosaic incidence
Amb x NL									
F_2-1	F_2-1	56	31	10.30	13	104	3	5.80	Nil
B_1-1	B_1-1	46	28	6.10	12	114	2	9.85	Nil
B_1-2	B_1-2	50	31	5.90	12	116	2	5.00	Nil
B_2-3	B_2-1	53	29	7.90	8	103	2	7.50	Nil
PV x NL									
B_1-1	B_1-3	36	19	4.70	11	86	2	3.60	Nil
AC x NL									
B_1-2	B_1-6	48	28	9.70	9	103	2	6.25	Nil
Col x NL									
F_2-1	F_2-13	59	30	5.20	10	117	2	6.80	Nil
B_1-2	B_1-7	63	45	9.20	15	131	2	7.30	Nil

FF - Female flowers

Table 27. Fruit characters of selected promising plants resistant to PMV and YVMV

Geno-type	Reassigned numbers	Av. length (cm)	Av. diameter (cm)	Av. fruit weight (kg)	Av. flesh thickness (cm)	Av. no. of seeds/ fruit	T.S.S. %	Carotene content IU/100g	Fruit smoo-thness	Fruit colour at maturity	Flesh colour	Fruit shape
Amb x NL												
F_2-1	F_2-1	15.40	18.00	1.93	2.43	155	6.60	31.85	1	Cane with light powdered coat and faded cream patches	Orange	Round
B_1-1	B_1-1	18.80	22.80	4.93	2.73	268	6.80	29.79	1	Cane with faded cream patches and waxy coat	Orange	Round
B_1-2	B_1-2	13.85	20.75	2.50	2.41	225	3.90	29.03	1.5	Green with greyish waxy coat	Yellow	Round
B_2-3	B_2-1	16.30	23.05	3.75	3.26	93	5.40	28.15	1.25	Dark green with thick greyish waxy coat	Yellow	Round
PV x NL												
B_1-1	B_1-3	19.40	14.30	1.80	2.46	203	5.50	33.91	1	Greyish light cane	Orange	Oblong
AC x NL												
B_1-2	B_1-6	18.10	24.35	3.13	3.05	310	6.90	27.89	1.25	Greyish candy	Cane yellow	Flat round
Col x NL												
F_2-1	F_2-13	16.10	15.27	3.40	2.93	100	5.80	23.55	1	Cane	Yellow	Round
B_1-2	B_1-7	15.20	19.30	3.65	3.00	190	5.60	22.71	1	Greyish cane	Yellow	Round

female flowers and the fruit development percentage was 16.67. The mean flesh thickness was 2.73cm. The fruits contained a mean number of 268 seeds. The T.S.S. percentage and carotene content were in the order of 6.80 and 29.79 IU. The flesh colour was either that of the parents (Plates 32 to 34) or a blend of these two colours.

Plant numbers B_2-1 and B_1-7 produced fruits to the tune of 7.50kg and 7.30 kg respectively, followed by F_2-13 (6.80kg), B_1-6 (6.25kg), F_2-1 (5.8kg) and B_1-2 (5.00kg). The flesh thickness was maximum for fruits of B_2-1 (3.26cm). The highest T.S.S. value was recorded in fruits of B_1-6 (6.90%), closely followed by B_1-1 (6.80%) and F_2-1 (6.60%). The carotene content was the highest in B_1-3 (33.91 IU) and lowest in B_1-7 (22.71 IU).

But for the warty fruits, there were some mosaic resistant high yielding plants identified from these studies. They included F_1-1, F_2-3 and B_2-6 (reassigned number B_2-2) belonging to the cross Amb x NL, B_2-1 and B_2-8 (reassigned numbers B_2-5 and B_2-6 respectively) from the cross PV x NL, F_2-6 and B_2-10 (reassigned numbers F_2-12 and B_2-9 respectively) from AC x NL and B_2-7 (reassigned number B_2-10) from Co1 x NL (Plates 35 to 37).

The variability in fruit characters in the F_2, B_1 and B_2 generations of the cross Amb x NL, PV x NL, AC x NL and Co1 x NL is presented in Plates 38 to 44.

Flesh colour of Nigerian Local

Plate 32

Flesh colour of Ambili, Co1 and Pusa Viswas

Plate 33

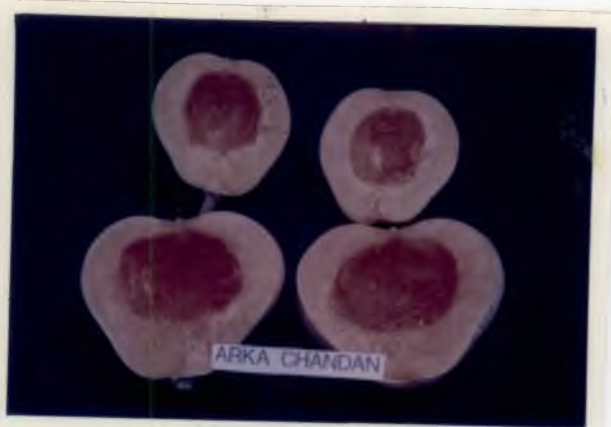
Flesh colour of Arka Chandran

Plate 34

B_2-2 High yielding plant with warty fruits

Plate 35

NIGERIAN LOCAL


ARKA CHANDAN

B2 -2

B_2-6 High yielding plant with warty fruits

Plate 36

B_2-10 High yielding plant with warty fruits

Plate 37

Variability in fruit characters - Ambili x Nigerian Local - F_2

Plate 38

Variability in fruit characters - Ambili x Nigerian Local - B_1 and B_2

Plate 39

B2-6

B2-10

Variability in fruit characters - Pusa Viswas x Nigerian Local - F_2

Plate 40

Variability in fruit characters - Pusa Viswas x Nigerian Local - B_1 and B_2

Plate 41

Variability in fruit characters - Arka Chandan x Nigerian Local - F_2

Plate 42

Variability in fruit characters - Arka Chandan x Nigerian Local - B_1 and B_2

Plate 43

Variability in fruit characters - Co1 x Nigerian Local - F_2

Plate 44

Discussion

DISCUSSION

Pumpkin mosaic and Yellow vein mosaic are two serious viral diseases of pumpkin in Kerala. Because of the devastating nature of these diseases the area under pumpkin in Kerala is getting reduced year after year (Balakrishnan, 1988). Attempts on management of these viral diseases could not so far result in the formulation of any effective and efficient control strategy. Therefore the viable technology left to combat the viral diseases is use of resistant varieties (Horvath, 1984). None of the pumpkin varieties released so far possesses resistance to either of the virus under study. In this backdrop, investigations were carried out in pumpkin with the objectives of (i) identification of source(s) of resistance to PMV and YVMV, (ii) studying the genetics of mosaic virus resistance, (iii) ascertaining the biochemical mechanism of mosaic virus resistance and (iv) identifying desirable plants which possess combined resistance to PMV and YVMV.

5.1 Identification of sources of resistance to PMV and YVMV

Among 95 genotypes of *C. moschata* screened against PMV and YVMV, only one accession Nigerian Local (SRS 7), introduced from Cornell University possessed high level of resistance to both the viruses with a mean disease score of one, zero infection percentage and zero coefficient of infection. This resistance in NL was earlier reported by Suresh Babu (1989) and Latha (1992). The resistance in NL against ZYMV had been observed by Provvidenti (1984).

Other than NL, all the genotypes screened showed varying degrees of systemic symptoms against both the viruses. The commercially superior released pumpkin varieties Ambili, PV, AC and Co1 were highly susceptible. In all these varieties, the mean disease score for both the viruses was three, the infection percentage and coefficient of infection being 100. The accessions belonging to *C. maxima* and *C. pepo* were also susceptible to PMV and YVMV. Cooper and Jones (1983) had reported that most accessions of cultivated *Cucurbita* species lacked resistance or tolerance to viruses. Observations in similar lines were also recorded by Suresh Babu (1989)

Among the feral species namely, *C. foetidissima, C. martinezii* and *C. ecuadorensis*, none expressed resistance reaction to either viruses. These lines had been reported as multi-virus resistant sources by several scientists. In *C. ecuadorensis* resistance to CMV, WMV, WMV-1, ZYMV and SqMV was observed by Provvidenti *et al.* (1978) and Lecoq *et al.* (1981). Resistance in *C. foetidissima* against CMV, WMV, and TRSV (Rosemeyer and Bemis, 1981) and resistance to CMV and Sq LCV in *C. martinezii* (Washek, 1983 and Mc Creight and Kishaba, 1991) had been identified. However, results of the present study indicate that these lines do not possess resistance to either PMV or YVMV strains prevalent in this part of the globe.

After inoculation of YVMV, the symptom expression started even within one week. After two weeks of inoculation, 33.75 per cent plants became susceptible. According to Jayasree (1984), pumpkin plants inoculated with YVMV started showing symptoms of the mosaic disease by tenth day onwards. In case of PMV, even during second week of inoculation only 0.008 per cent showed susceptibility reaction. During third week the infection per cent was only 22.50 and hundred per cent infection was noted during the fourth week.

Confirmation studies were carried out in the line NL, identified as resistant to both the viruses in order to ascertain the nature of resistance, since the resistant reaction expressed consequent to virus inoculation can be due to escape, symptomless carrier type of tolerance or due to true resistance. Lack of symptoms or tolerance is considered as a form of resistance only from the practical point of view as no symptoms develop in this case and hence do not cause any yield reduction (Cooper and Jones, 1983). However, symptomless plants can accumulate as much virus as plants showing symptoms (Beekman, 1987 and Valkonen and Makarainen, 1993). This type of tolerance is useful, if and only if, no sources of other types of resistance are available (Kurpa, 1989).

Neither the inoculated leaves nor the uninoculated leaves of the susceptible variety Ambili showed symptoms when back inoculated with sap from the resistant source in case of PMV. Through back inoculation viruses could not be isolated from this genotype thereby proving that the resistance is not symptomless carrier type of tolerance. The reaction of plants in which neither the inoculated nor the non-inoculated leaves showed symptoms of

virus and back inoculation failed to re-isolate the virus from them to indicator / known susceptible plants is called extreme resistance (Horvath, 1986). Hence the line NL is having extreme resistance to both PMV and YVMV.

Graft transmission was done in case of YVMV. NL did not develop any symptoms, even after grafting on the infected symptomatic root stock thereby confirming that the line is possessing extreme resistance to YVMV.

The effect of environment on disease resistance of genotypes was reported in case of Tobacco Mosaic Virus (TMV) in pepper (Zatyko, 1981). According to Latha (1992) the incidence of PM and YVM in pumpkin varied with environment. In this study the environmental fluctuations did not influence the resistance expression of NL against either PMV or YVMV indicating the stability of virus resistance in this genotype.

The mosaic resistance confirmation studies namely, back inoculation, grafting and multi-environment study unambiguously established that the reaction in NL can be considered as extremely resistant and stable. This is in consonance with the report of Suresh Babu (1989).

5.2 Genetics of virus resistance

To formulate breeding strategies for evolving disease resistant varieties, knowledge of inheritance pattern of disease resistance is considered as a pre-requisite.

5.2.1 Resistance to PMV

Resistance to PMV was evaluated in five parents and their F_1, F_2 and backcross generations of four cross combinations namely, Amb x NL, PV x NL, AC x NL and Col X NL (Fig.1).

In the F_1, 96.25 per cent plants showed susceptibility. This points to the dominance of susceptibility over resistance. The F_1s in Amb x NL and Col x NL showed cent

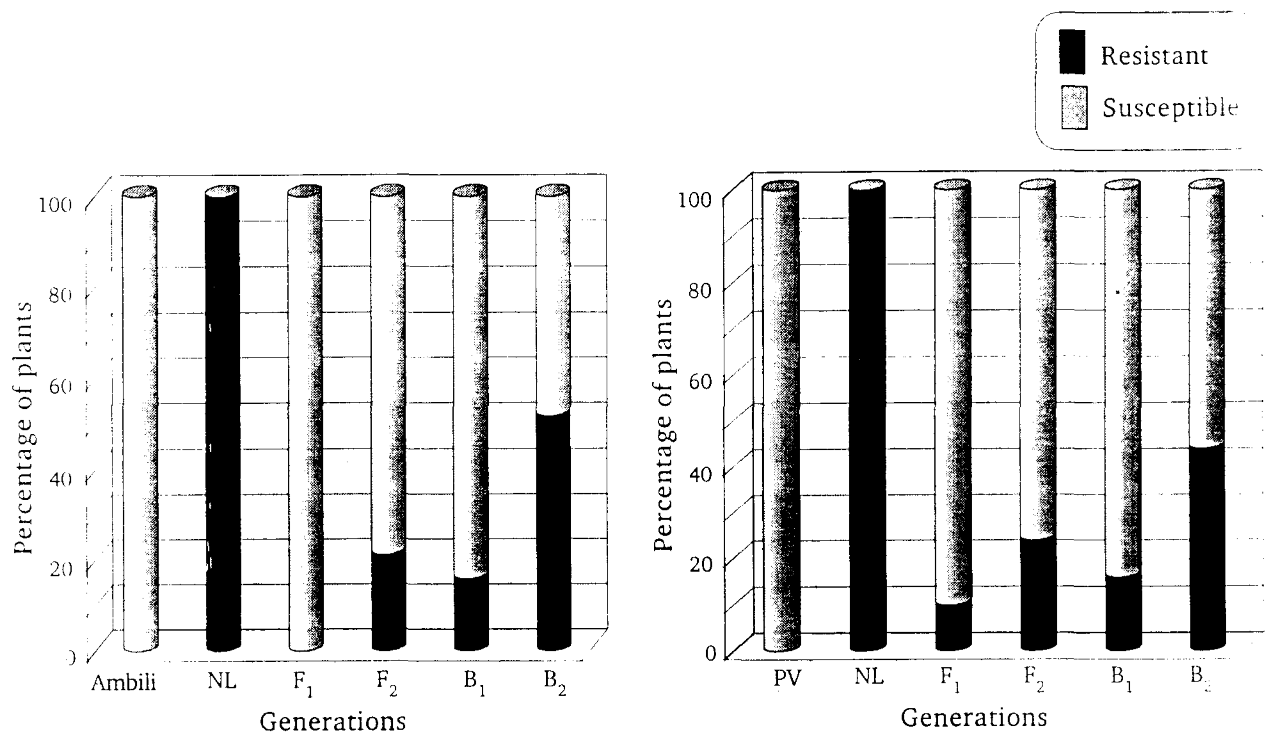
per cent susceptibility while the gene for susceptibility in PV x NL had only 95% penetrance under different genetic background. Markose (1996) observed 92 per cent penetrance for bacterial wilt resistance in chilli in the resistant parent Ujwala.

In AC x NL F_1s, only 90 per cent plants were susceptible to PMV but the frequency of resistant and susceptible plants did not fit well with the penetrance and expressivity values in F_2 and B_2 generations. This might be due to the presence of modifying genes. Robinson *et al.* (1988) reported the presence of modifying genes influencing the major gene for resistance to ZYMV in *C. ecuadorensis*. He noticed variation in the degree of symptom expression in the heterozygous plants under different genetic backgrounds. Here also the presence of similar action of modifying genes might have slightly influenced the disease symptom expression of the major gene in the F_1s of AC x NL.

Evaluation of reciprocal crosses ruled out the cytoplasmic effect with respect to PMV resistance. The F_2 segregation ratio in all the crosses was in agreement with the Mendelian genetic ratio of 3:1 (susceptible : resistant). The reactions of the test crosses (F_1 back crossed to NL) confirmed this with a genetic ratio of 1:1 (resistant : susceptible) in all the four crosses studied.

The inheritance studies in six generations of all the four cross combinations clearly revealed that the resistance to PMV in pumpkin is basically controlled by a single recessive gene. Latha (1992) observed that the gene action for resistance to PMV in pumpkin is monogenic and recessive. Wang *et al.* (1984) and Provvidenti (1985) noted monogenic recessive nature of inheritance to WMV-1 in cucumber. The resistance gene to ZYMV in cucumber is monogenic recessive (Provvidenti, 1987 and Hayja and Al-shahwan, 1991).

As the gene governing resistance to PMV in NL is monogenic recessive a series of backcrosses each followed by selfing, screening for resistance and evaluation for yield and desirable horticultural attributes is required to incorporate PMV resistance into commercially viable superior varieties.



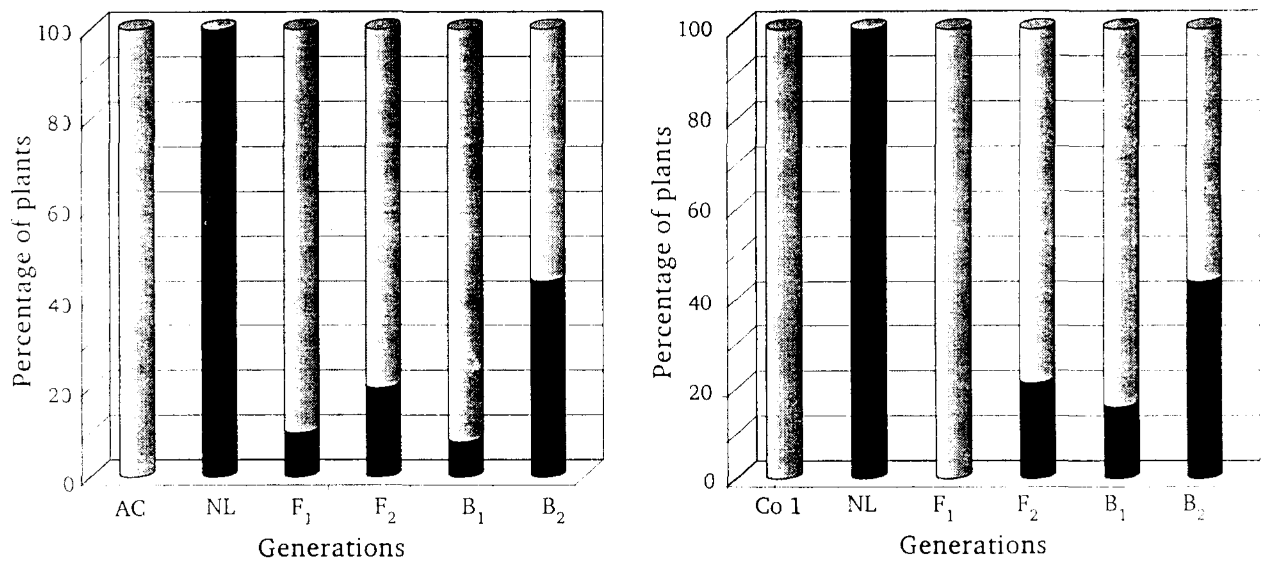


Fig. 1. Reaction of parents, F1, F2 and Backcross generations to PMV

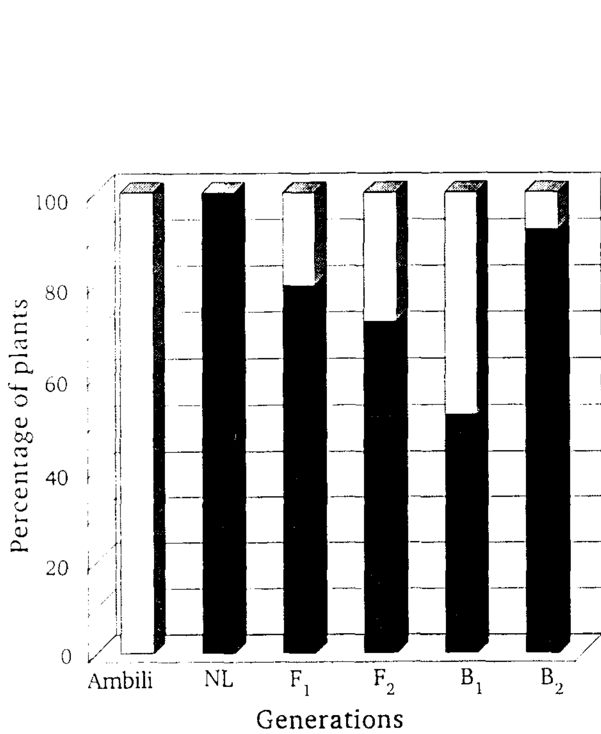
5.2.2 Resistance to YVMV

Reaction to YVMV in the six generations i.e. P_1, P_2, F_1, F_2, B_1 and B_2 of the above four cross combinations was studied. In the F_1 on an average out of 40 plants 32 were resistant. The reciprocal crosses also behaved in a similar fashion. This shows the dominance of resistance over susceptibility and rules out any cytoplasmic effect. The gene for resistance in PV x NL had a penetrance value of 90 per cent. In the other crosses, the frequencies of resistant and susceptible plants did not fit well with the penetrance and expressivity values in the F_2 and B_1 generations. Similar trend was observed by Munger and Provvidenti (1987) and Robinson *et al.* (1988). Accordingly, in the heterozygous plants, the presence of modifying genes might have slightly influenced the disease symptom expression of the major gene in the F_1s of Amb x NL, AC x NL and Co1 x NL. Further investigations seem to be necessary to ascertain the number of gene modifiers involved in the resistance expression of these hybrids.

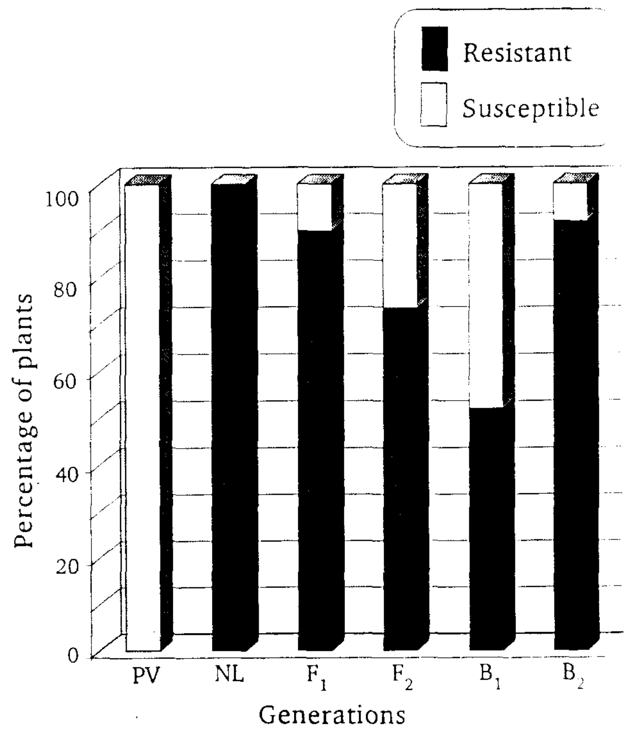
Out of 300 F_2 plants in each cross, a mean number of 220 plants were resistant and 80 susceptible, fitting in a 3:1 ratio indicating the monogenic dominance of resistance. When F_1 was back crossed to the susceptible parent, the segregation of resistance and susceptibility was in equal proportion i.e., out of a mean number of 100 B_1 plants inoculated 48 were resistant. This very well fitted into 1:1 ratio of resistance : susceptibility (Fig. 2). So, resistance to YVMV is governed by a single dominant gene which is slightly influenced by gene modifiers.

Monogenic dominant gene action for YVMV resistance in pumpkin was observed by Latha (1992). Resistance to ZYMV also in pumpkin is governed by singly dominant gene (Paris *et al.*, 1988 and Gilbert-Albertini *et al.*,1993).

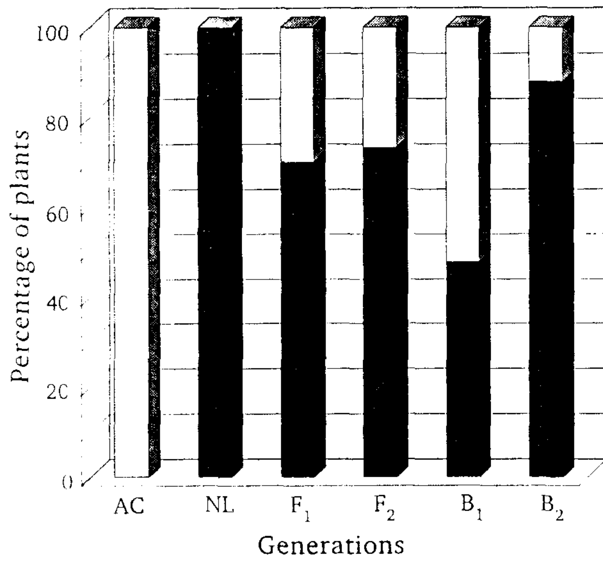
Although NL is a good source of resistance to both the viruses under study, they do not set fruit well. The seed germination, fruit setting and fruit development are quite erratic and the fruits are warty and knobbed (Munger and Provvidenti, 1987 and Suresh Babu, 1989). According to Latha (1992), the seed germination was as low as eight per cent and even germinated seeds needed much care for further development. Germination was appreciable (71%) consequent to seed coat removal.



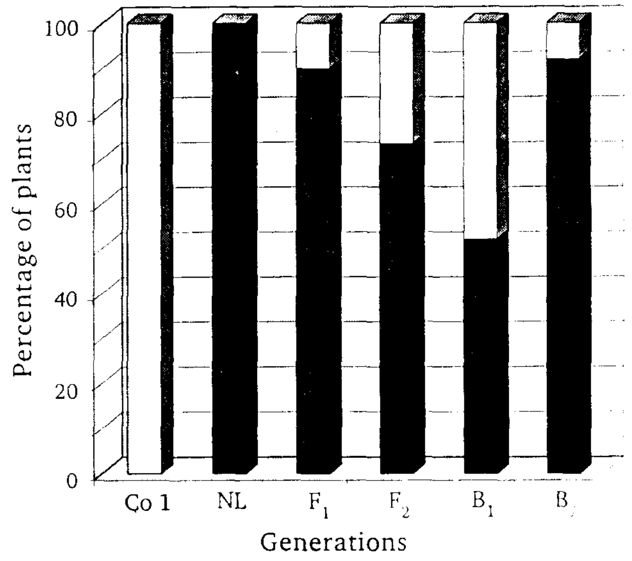
Cross : Ambili X Nigerian Local



Cross : Pusa Viswas X Nigerian Local

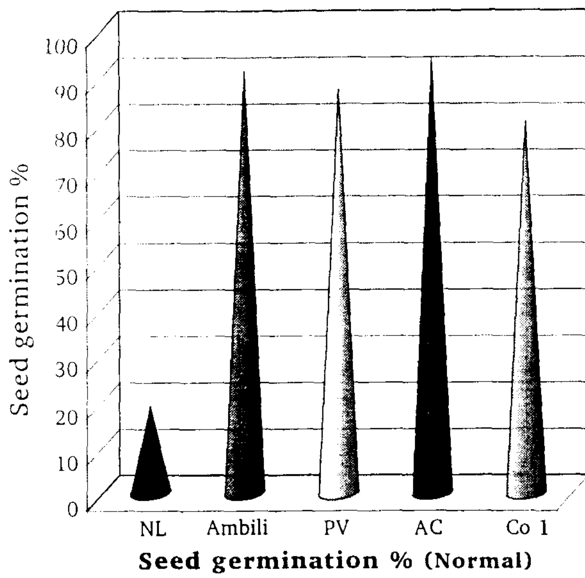


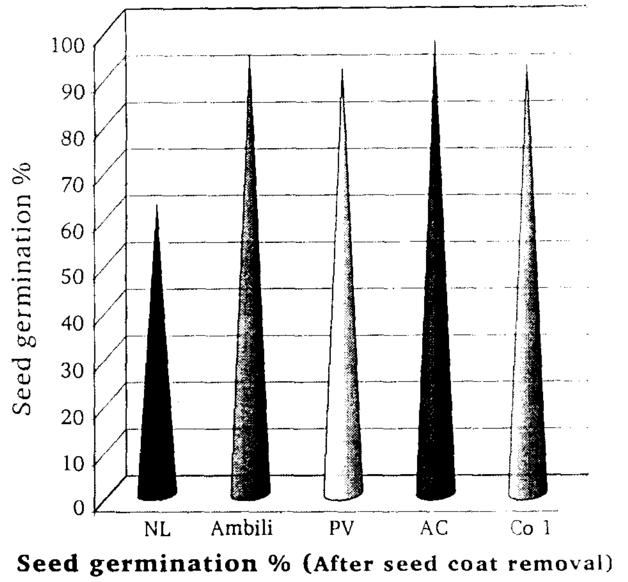
Cross : Arka Chandan X Nigerian Local

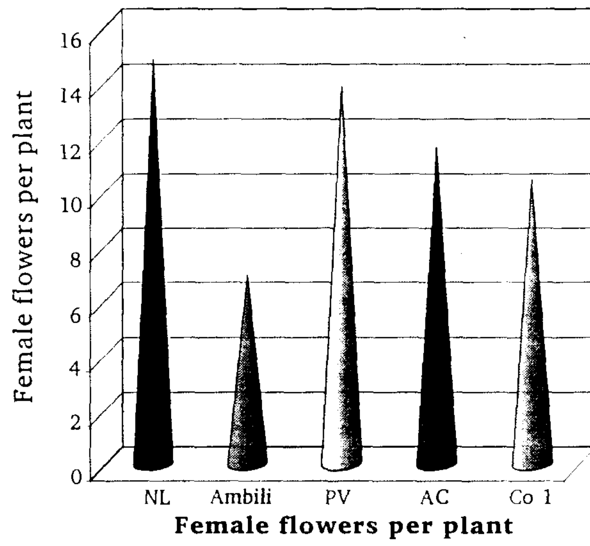


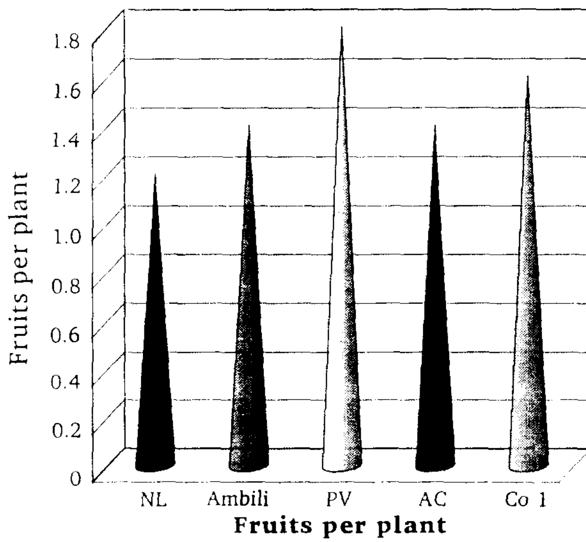
Cross : Co 1 X Nigerian Local

Fig. 2. Reaction of parents, F1, F2 and Backcross generations to YVMV









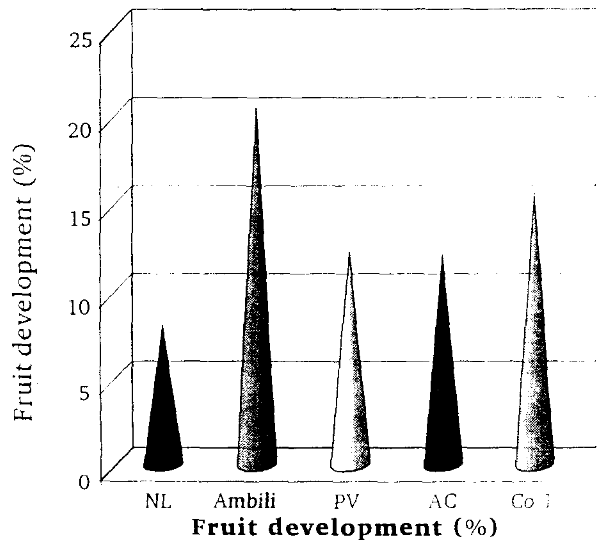


Fig. 3. Seed germinaton and fruit development in parents

Observations in the present study were also in similar lines. In case of NL the seed germination was as low as 19 per cent, while it was above 80 per cent in the parents. Through seed coat removal, 43 per cent increase in seed germination (62%) was noted in NL, while the increase was only 3,4 and 12 per cent respectively in Amb, PV and Col. In AC the increase in germination was not at all significant (Fig. 3). In NL, the presence of hard seed coat might have restricted the absorption of water and oxygen essential for germination of seeds. The removal of seed coat enabled easy penetration of water and oxygen resulting in emergence of the embryo. In musk melon seeds significant increase in oxygen uptake as a result of seed coat removal was reported by Pesis and Na (1986). An increase in germination rate and percentage by removal of seed coat was earlier reported by Kim and Jeong (1990) in *Cucumis melo* var. *microspermus*.

5.3 Biochemical mechanism of mosaic virus resistance

5.3.1 Protein pattern

Gel electrophoresis of proteins is a powerful, well defined and effective method to detect genetic differences among individuals. This method reveals electrophoretic variation of the different molecular forms of proteins in a slab of gel. The shape and size of pores of the electrophoretic matrix influence its migration. The fraction that determines the number of bands observed on a gel is the quaternary structure of the protein products (sub units).

SDS gel electrophoresis was carried out to find out the polymer status of infected and non-infected plants. The electrophorogram revealed that the banding patterns were same for both infected and non infected plants. All the genotypes including the resistant, susceptible and their F_1 hybrids showed no difference in the number of protein bands and their relative mobility, both before and after the inoculation of the viruses. Also, similar protein pattern was observed in all the genotypes before and after host pathogen interaction (Fig. 4). Markose (1994) also observed similar results in the bacterial wilt resistant and susceptible chilli varieties before inoculation of the bacteria.

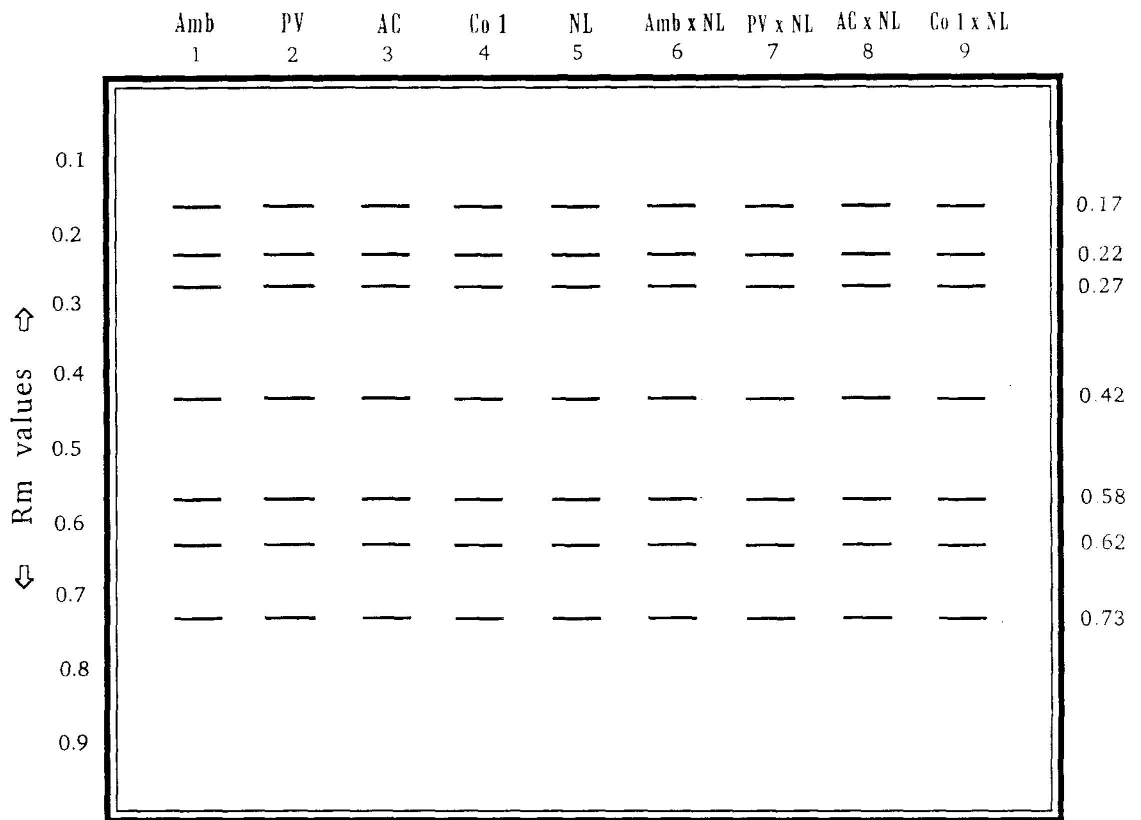


Fig. 4 . Protein electrophorogram before inoculation of virus

5.3.2 Isozymes analysis

Isozyme analysis by electrophoresis provides a well defined and effective method to detect genetic differences among individuals. Isozyme profiles might be used as a tool to compare healthy and pathological stages of plants for yielding valuable information on pathogenesis. Isozyme studies complement the conventional biochemical and genetic analysis. The utility of electrophoretic method of isozyme study in the biochemical mechanism of disease resistance was reported by Kato *et al* (1978) in *C. melo*.

Changes in enzymatic activities are associated with virus infection (Visedo *et al*, 1990). In the present study, morphological observations and the isozyme pattern of peroxidase and esterase enzymes in susceptible and resistant genotype of pumpkin as well as their F_1 hybrids were analysed, before inoculation and after inoculation of both viruses.

The resistant and susceptible genotypes showed marked differences in their peroxidase profile. The susceptible parents had three bands namely, PRX 1, PRX 3 and PRX 6, while the resistant parent NL expressed only one isoform - PRX 1 (0.083) before virus inoculation (Fig. 5a). This is in agreement with the findings of Linde and Rhodes (1988) in cucumber against anthracnose resistance, Ming and Xian (1988) in watermelon against fusarium wilt disease and Markose (1996) in chilli against bacterial wilt resistance. According to Markose (1996) the three band expression of peroxidase in susceptible genotypes showed low enzyme activity than the single band expressed resistant genotype. Due to absence of polymorphism in the resistant genotype, the activity was substantially high which implies that protein sub-units have an influence in activity and resistance.

The hybrids Amb x NL and Co1 x NL showed similarities of both parents. As in case of NL the band PRX 3 was not expressed. The hybrid PV x NL exhibited six bands with three additional bands (PRX2, PRX4 and PRX7) whereas the hybrid AC x NL was similar to AC in the peroxidase banding pattern. Ming and Xing-ping (1988) found that in watermelon the F_1 hybrids had the same zymogram as their two parents or maternal parents or paternal parents or had 'hybrid enzyme' or lacked some zymographic bands of their parents. Similar results were obtained in these cases also.

After inoculation of PMV, the banding pattern of peroxidase changed. The low Rm value PRX 1 band expressed before inoculation was not expressed in both the resistant and susceptible parents as well as in their F_1 hybrids after inoculation. The additional band PRX 7 (Rm value 0.642) shown by PV x NL before inoculation was noted in all the other genotypes except in the resistant one. NL (resistant parent) had an additional band of PRX5 (Rm value 0.463). This isoform might be responsible for resistance to PMV in NL (Fig. 5b). Siegel, (1993) reported that such isoforms provide an efficient mechanism for removing peroxide as well as for immobilising toxic phenolic lignin precursors. Alger *et al.* (1995) noted an increase in peroxidase activity in conjunction with the appearance of an additional isoperoxidase band specific for the resistant cultivar in ***Capsicum annum*** when infected with *Phytophthora capsici.* After inoculation of YVMV the susceptible parents had three isizyme bands i.e. PRX 3 (0.342), PRX 6 (0.583) and PRX 7 (0.642) while the resistant parent had a streaking band showing the expression of PRX3, PRX4, PRX5 and PRX6. These findings are in conformity with the report of Siegel (1993) who suggested that peroxidases often increased as a response to stress. The resistant hybrids had the additional band PRX4 with Rm value 0.392 over the susceptible parents (Fig. 5c).

Out of the six isozyme bands expressed in PV x NL before inoculation of the virus, PRX1, PRX3 and PRX6 were common for other genotypes, though PRX3 and PRX5 were for the resistant parent NL, Amb x NL and Col x NL and PRX6 absent in NL. PRX4 was expressed after inoculation of YVMV in the resistant parent and in the resistant hybrids and PRX7 was expressed after inoculation of both the viruses in all the genotypes except the resistant parent. So the only additional band, PRX2 for PV x NL can be attributed to the earliness in growth and development of this hybrid compared to the other hybrids and parents.

The resistant and susceptible genotypes showed different electrophoretic pattern of EST before and after inoculation of the viruses except EST8. There were six bands of esterase in the susceptible parent and only three in the resistant before virus inoculation. A perceptible difference in the esterase profile is thus evident in susceptible and resistant genotypes. Gabriel and Ellingboe (1982) also reported similar differences in the banding patten of esterase showing various levels of resistance to powdery mildew in wheat. In the

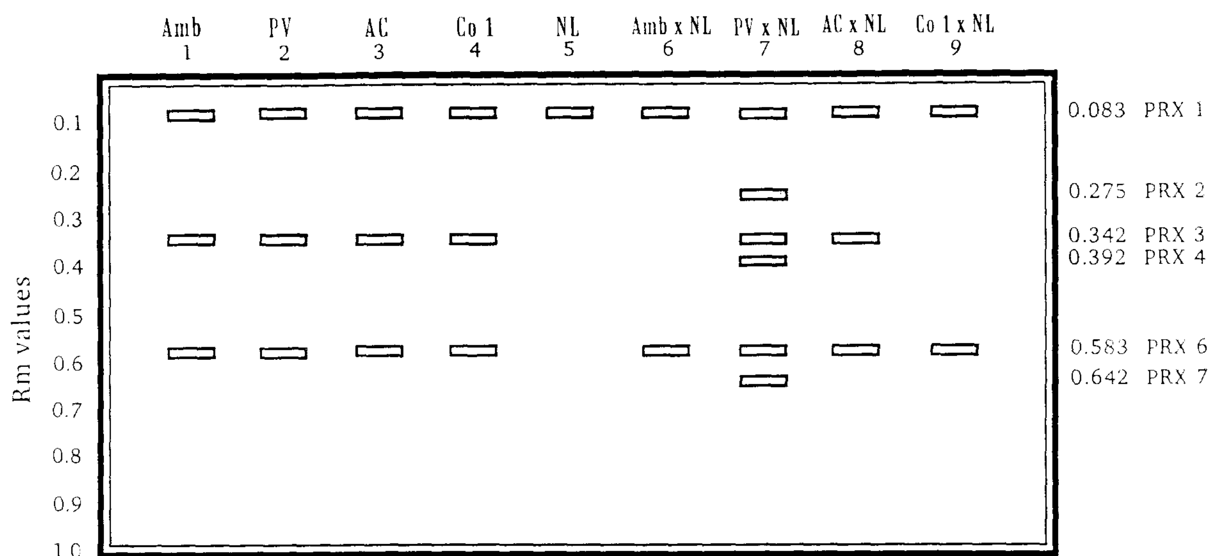


Fig. 5 a. Peroxidase electrophorogram before virus inoculation

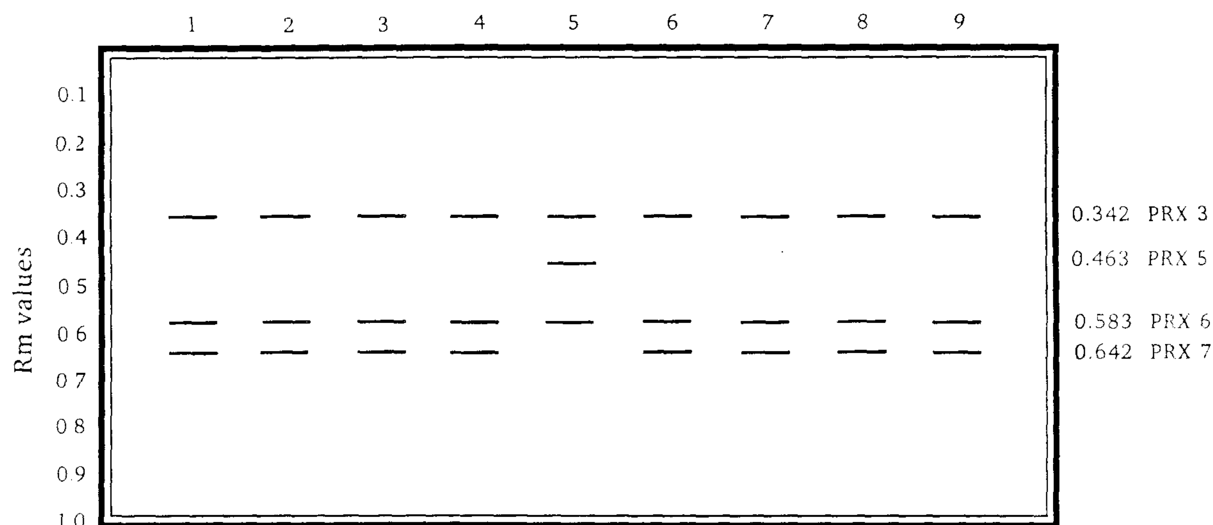


Fig. 5 b. Peroxidase electrophorogram after PVY inoculation

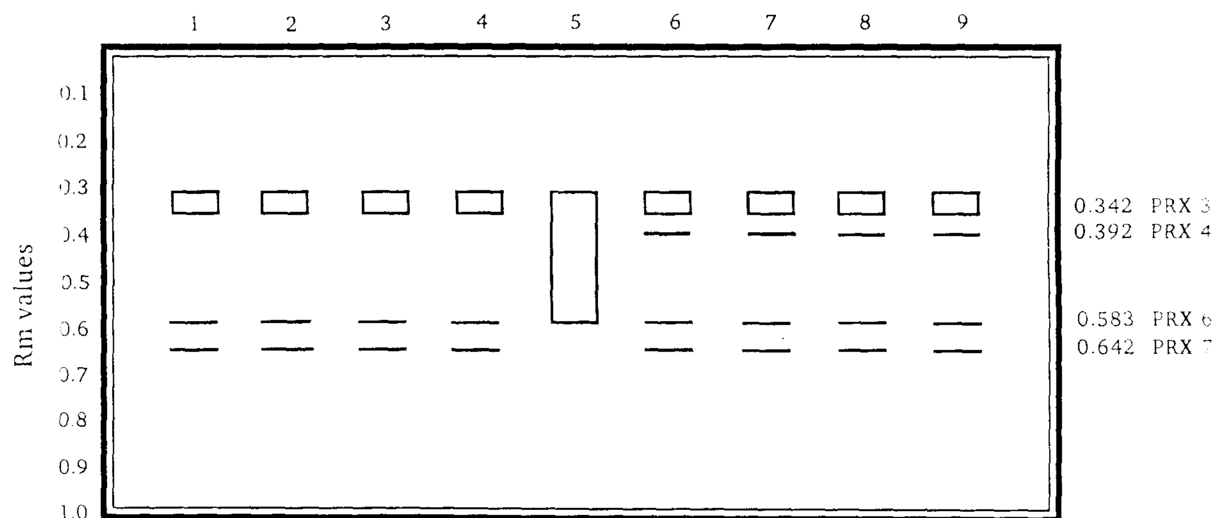


Fig. 5 c. Peroxidase electrophorogram after YVMV inoculation

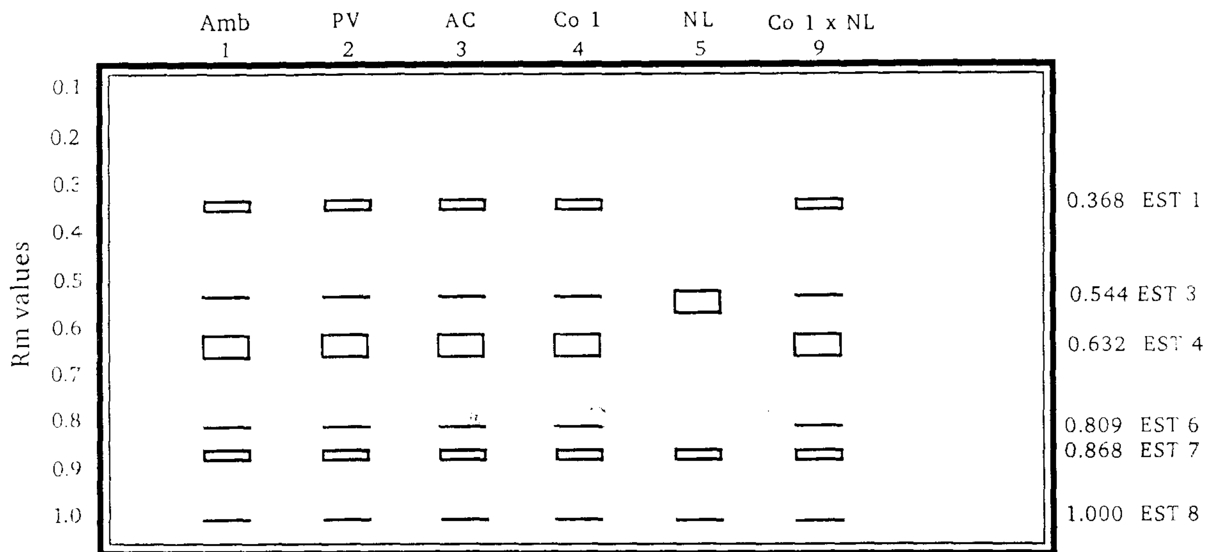


Fig. 6 a. Esterase electrophorogram before virus inoculation

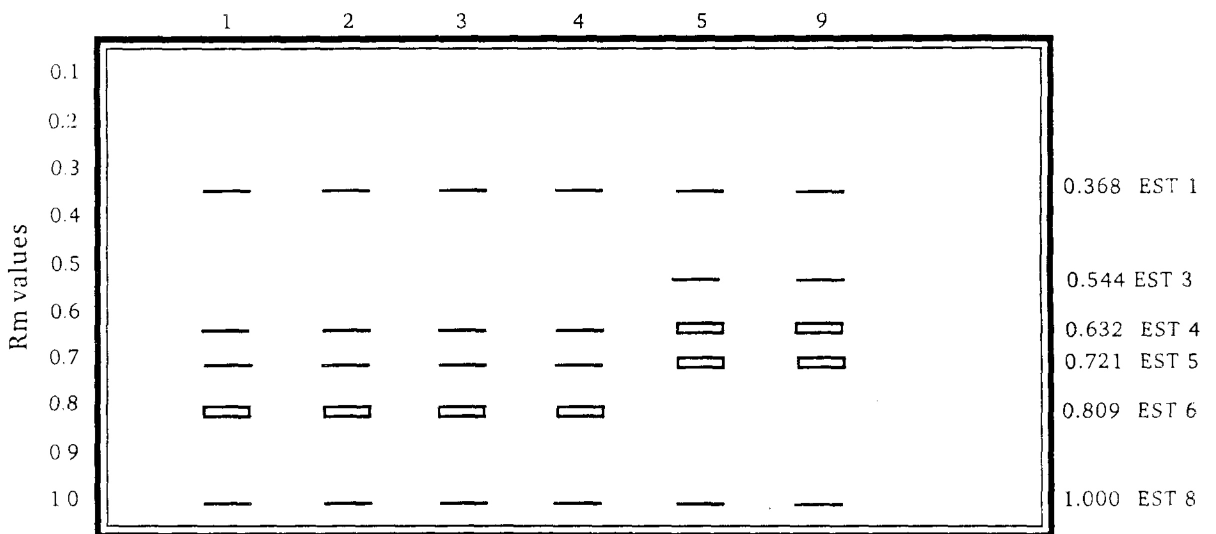


Fig. 6 b. Esterase electrophorogram after PMV inoculation

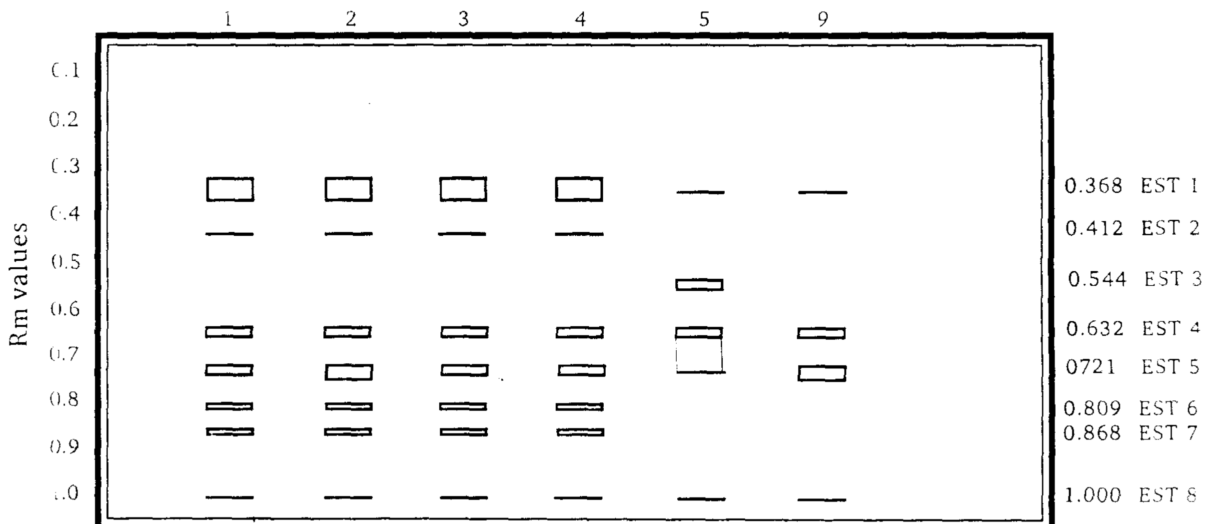


Fig. 6 c. Esterase electrophorogram after YVMV inoculation

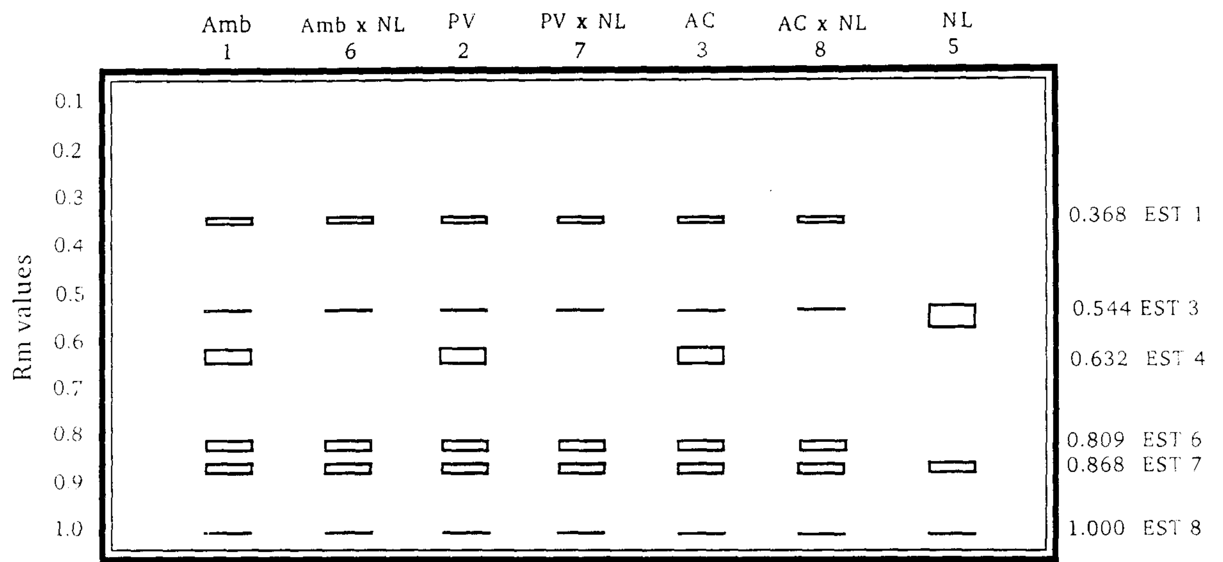


Fig. 7 a. Esterase electrophorogram before virus inoculation

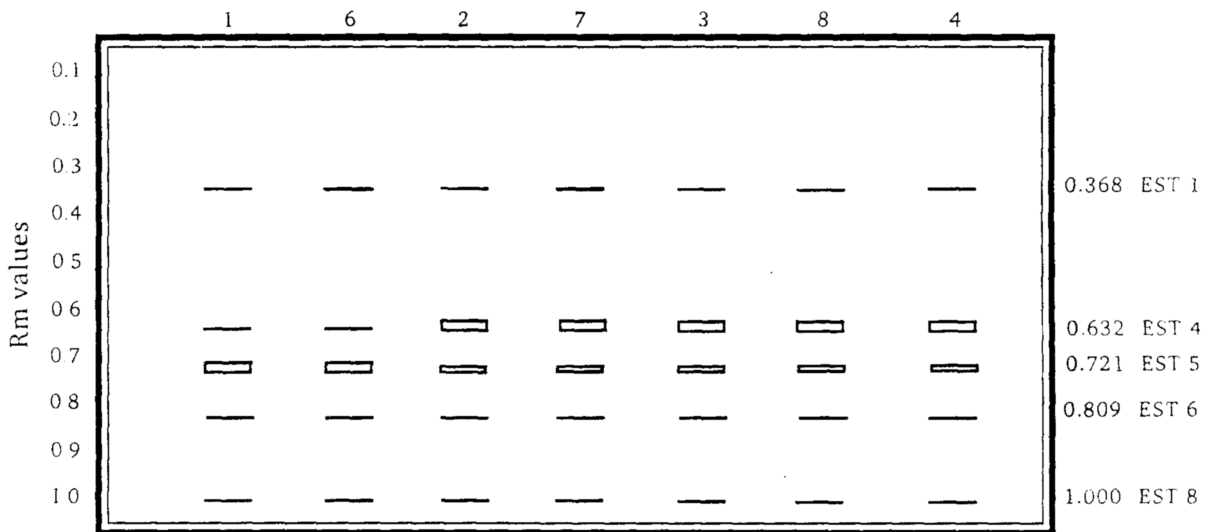


Fig. 7 b. Esterase electrophorogram after PMV inoculation

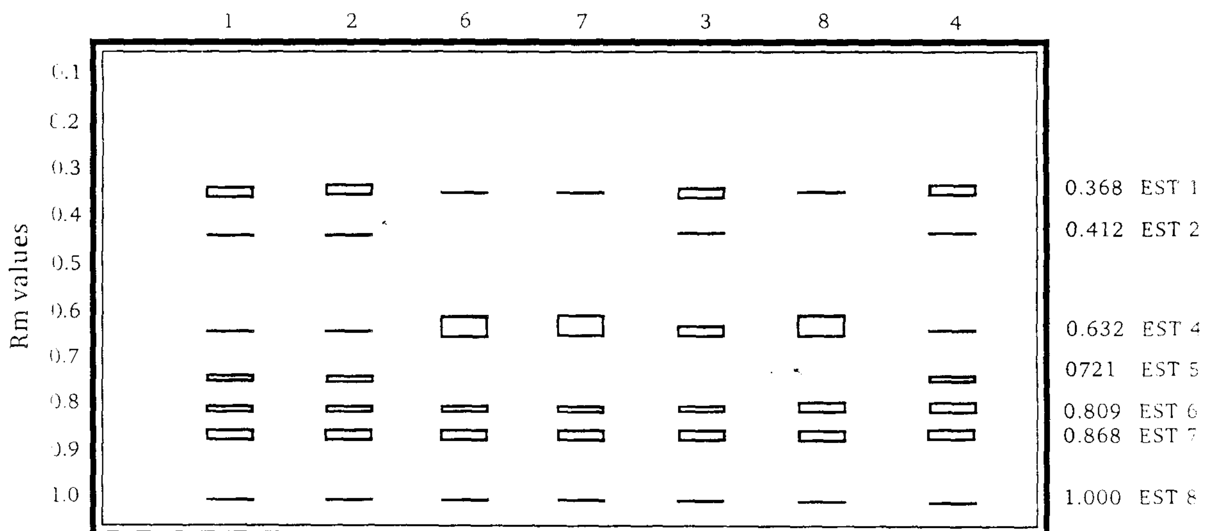


Fig. 7 c. Esterase electrophorogram after YVMV inoculation

F_1s (Amb x NL, PV x NL and AC x NL) the isozyme band EST4 with Rm value 0.632 present in the parents and in Col x NL was not expressed (Fig. 6a and 7a). Findings in similar lines were also furnished by Endo (1971) who observed the intensity ratio of isozyme bands deviated from the expected distribution pattern in the F_1 plants resulting from a cross between two strains of *Oryza perennis*. Ming and Xing-ping (1988) also observed such a trend in the isozyme profiles of hybrids in watermelon. Even though the EST4 band was absent in the three hybrids, the same was expressed in all the genotypes studied after inoculation of both the pathogens.

The susceptible parents expressed an esterase band of EST7 (0.868) before inoculation and disappeared the same after PMV inoculation and expressed a new esterase band, EST5 (0.721). This is also in agreement with the findings of Oh (1988). The resistant parent also after inoculation of PMV, did not express EST7 but exhibited three additional bands EST 1 (0.368), EST4 (0.632) and EST 5 (0.721) (Fig.6b and 7b). The hybrids expressed the varying isozyme profiles as observed by Ming and Xing-ping (1988). Consequent to YVMV infection, the additional band of EST 4 (0.632) was observed in NL and might be responsible for the resistance mechanism. This is seemingly the reason for the resistance expression of NL to YVMV (Fig. 6c and 7c).

The expression of additional band is a common feature in the resistant parent after the host-virus interaction in case of both PRX and EST isozymes. The total system of EST was affected by the virus infection while PRX was comparatively stable. The resistant expression of NL against PMV infection can be attributed to the expression of the additional band PRX5 (0.463) while the resistant mechanism against YVMV in NL as well as in the F_1 hybrids can be due to the activity of isoform PRX4 (0.392).

5.4 Selection of mosaic resistant desirable plants

Sequential inoculation with PMV followed by YVMV was carried out in the F_1, F_2, B_1 and B_2 generations of the four cross combinations namely, Amb x NL, PV x NL, AC x NL and Col x NL to identify plants with combined resistance to both the viruses (Fig. 8).

In the F_1s, eight plants were identified as resistant to both the viruses i.e., one in Amb x NL, two in PV x NL, three in AC x NL and two in Col x NL. Resistance in the F_1 generation had been observed in melon by Munger (1993) and in bhindi by Nerkar and Jambhale (1987). Munger (1993) found that the hybrids of Cornell line PM 339 (PRV resistant) with susceptible MR 324, Tam Uvalde and Gulfcoat possessed superior resistance to its resistant parent. In bhindi Nerkar and Jambhale (1987) identified YVMV resistant F_1 hybrids in the crosses *Abelmoschus manihot* (R) with *A. esculentus* (S)and *A. tetraphyllus* (R) with *A. esculentus* (S).

From among 100 plants each evaluated sequentially for PMV and YVMV resistance in the F_2 and B_1 generations belonging to the four cross combinations, 17 segregants in F_2 and nine plants in B_1 were isolated as resistant to both the viruses. In the B_2 population, out of 100 there were 37 seedlings resistant to both the viruses. They included ten from the cross Amb x NL, nine from PV x NL, eleven from AC x NL and seven from Col x NL. Velich and Tobias (1985) observed melon plants with resistance to S4 strain of CMV in the F_2 generation of the cross Shiroubi Okayama (R) and Korad S100 (S). Pitrat and Lecoq (1994) identified 143 plants as resistant to ZYMV in the B_2 progeny of the cross PI 414723 (R) x Doublon (S) and suggested that selection is possible from among the population for desirable resistant plants.

All the 71 seedlings possessing combined resistance to PMV and YVMV were further evaluated in the field for their morphological and horticultural characters.

In the cross Amb x NL, earliness as revealed by the shortest number of days taken for anthesis of the first female flower (45) was noted in F_1-1. The B_2 generation took the longest period (55.40) for opening of the first female flower. In the PV x NL, the F_1s took the shortest period (41.50) for anthesis of the first female flower, while the B_2s took the longest period in flowering. Same trend was observed in AC x NL and Col x NL for earliness. Except Col x NL, in all the other crosses, B_2 plants flowered comparatively late. Doijode and Sulladmath (1981) reported additive gene action, dominance and epistasis in the control of number of days taken for anthesis of the first female flower. In all the crosses except PV x NL the node of first fruit development was the shortest in the F_1s. This is also

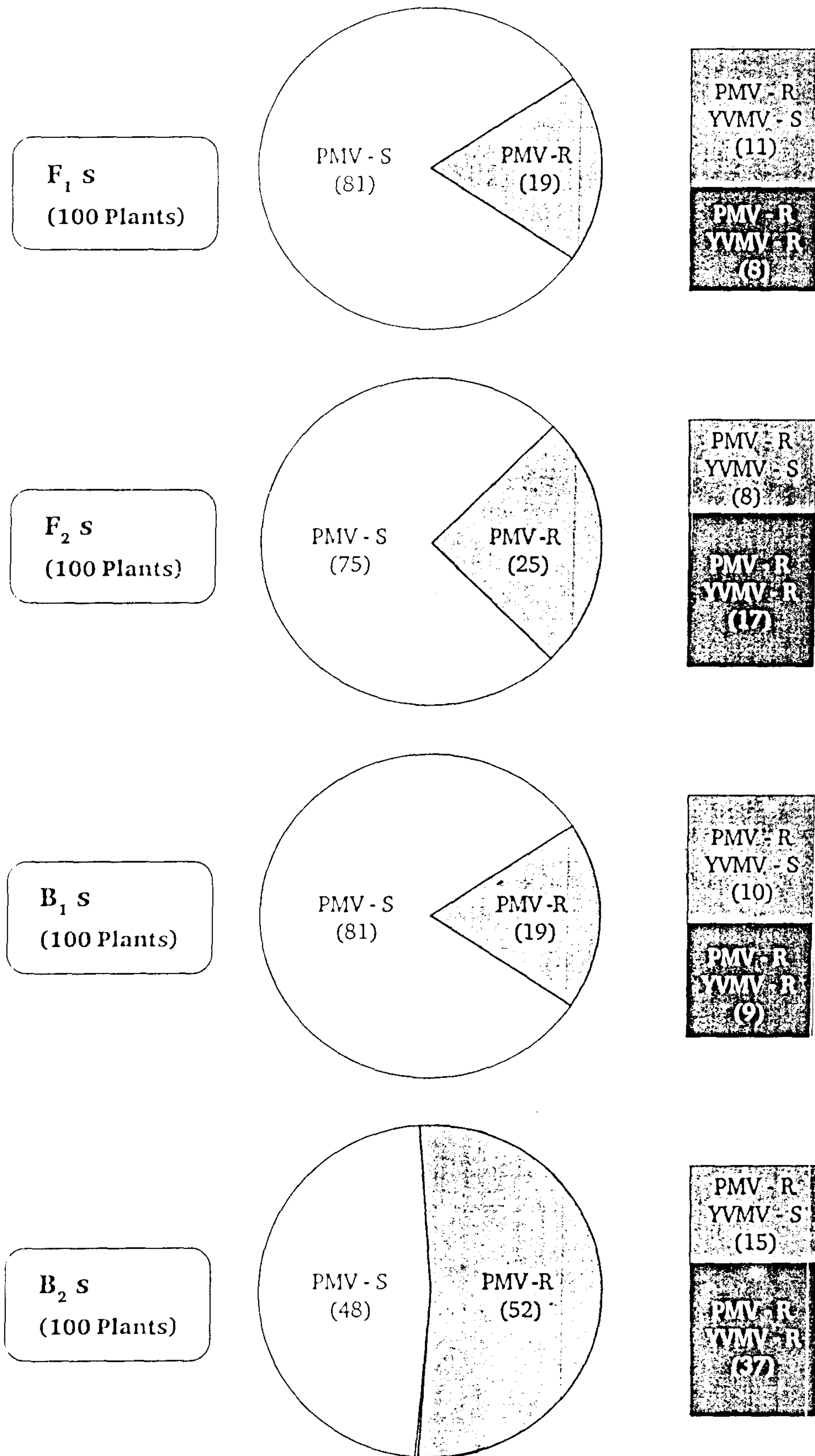


Fig. 8 . Reaction of F_1s, F_2s, B_1s and B_2s to sequential inoculation to PMV and YVMY

indication of earliness of F_1s compared to F_2s, B_1s and B_2s. According to Curtis (1941) the F_1 hybrids are earlier and more uniform in maturity.

In Amb x NL, the longest vine was produced by the F_1 (8.90m) and shortest by B_1s (6.00m). In the cross PV x NL, the length was comparatively higher for the B_2 plants, followed by F_2, F_1 and B_1. A similar trend in performance of the different generations in pumpkin in vine length was observed by Latha (1992). In the cross Col x NL, the F_1 plants exhibited the same trend as that of Amb x NL.

The range in number of female flowers per plant in the different generations of Amb x NL was 12 in F_1 and B_1, 10 in F_2 and 9.40 in B_2. In AC x NL also, the highest number of female flowers was produced in the F_1 generation. The fruit yield in terms of number and weight of fruits in all the crosses was maximum for the F_1 plants. This is in agreement with the findings of Singh *et al.* (1976) in muskmelon, Brar and Nandpuri (1978) in watermelon for yield per plant and in pumpkin for fruits per plant, fruit yield and number of female flowers per plant by Latha (1992).

In all the crosses, the plants belonging to the F_1, F_2, B_1 and B_2 generations produced fruits with fruit colour of either the female parent or with dark greyish green colour of NL or with a blend of these two colours. In flesh colour also same trend was observed. Latha (1992) observed similar pattern in pumpkin.

Smooth fruits, freedom from mosaic disease incidence and fruit yield were taken as the criteria for selection of desirable plants. Gopalakrishnan (1979) suggested that selection of plants considering yield *per se* was more efficient than selection for component characters in pumpkin. In the cross Amb x NL, plant numbers F_2-1, B_1-1 (Plate 45), B_1-2 (Plate 46) and B2-1 (reassigned numbers), in PV x NL plant number B_1-3 (reassigned number) (Plate 47), in AC x NL plant number B_1-6 (reassigned number) (Plate 48) and in Col x NL plant numbers F_2-13 and B_1-7 (reassigned numbers) (Plate 49 and 50) were selected based on the above criteria. The fruit yield (kg) of the selected plants ranged from 9.85 kg (B_1-1) to 3.60 kg (B_1-3). According to Suresh Babu (1989) the fruit yield in pumpkin ranged from 0.90 kg to 13.40 kg per plant. The second highest yield was recorded by B_2-1 (7.50 kg).

B_1-1 Mosaic resistant promising selection from the cross Ambili x Nigerian Local

Plate 45

B_1-2 Mosaic resistant promising selection from the cross Ambili x Nigerian Local

Plate 46

B_1-3 Mosaic resistant promising selection from the cross Pusa Viswas x Nigerian Local

Plate 47

B_1-6 Mosaic resistant promising selection from the cross Arka Chandan x Nigerian Local

Plate 48

F_2-13 Mosaic resistant promising selection from the cross Co1 x Nigerian Local

Plate 49

B_1-7 Mosaic resistant promising selection from the cross Co1 x Nigerian Local

Plate 50

B1 - 1

B1 -2

B1-3

B1-6

F2 -13

B1 - 7

The selected plants had main vine length ranging from 10.30 in F_2-1 to 4.70 in B_1-3. The plant B_1-6 had 9.70m long main vine followed by B_1-7 (9.20m). Observations in similar lines were reported by Gopalakrishnan (1979). According to Suresh Babu (1989) the main vine length in pumpkin ranged from 6.69m to 13.99 m. Plant number B_1-7 produced the highest number of female flowers per plant (15), followed by F_2-1 (13). The number of female flowers was minimum in B_2-1 (8). In F_2-1, the number of fruits was three while in all others, it was two. The average fruit weight was the highest for B_1-1 (4.93 kg) followed by B_2-1 (3.75 kg). The average fruit weight was the least in B_1-3 (1.80 kg). The carotene content was the highest in fruits of B_1-3 (33.91 IU/100 g) followed by F_2-1 (31.85 IU/100g) and B_1-1 (29.79 IU/100 g). Gopalakrishnan (1979) and Suresh Babu (1989) observed wide variation in pumpkin in all the above characters. The T.S.S. content was the highest in B_1-6 (6.90%) followed by B_1-1 (6.80%) and F_2-1 (6.60%) (Fig. 9 and 10).

An overall perusal of the performance of the selected plants showed that plant number B_1-1 is the most promising with 9.85 kg fruit yield. It was comparatively early in flowering. It took only 46 days for anthesis of the first female flower and the node of first fruit development was 28. The main vine length was 6.10m and it took 114 days for maturity of the first fruit. There were twelve female flowers and two fruits, the fruit development per cent being 16.67. The fruits of B_1-1 were on an average 18.80 cm long and 22.80 cm in diameter with an average fruit weight of 4.93 kg. The flesh was 2.73 cm thick. The number of seeds (268) TSS content (6.80%) and carotene content (29.79 IU/100g) were comparatively high (Fig. 9 and 10). The fruits were round and canish in colour with faded cream patches and waxy powder coat. The flesh colour was orange. This plant in general, resembled mostly to its female parent Ambili.

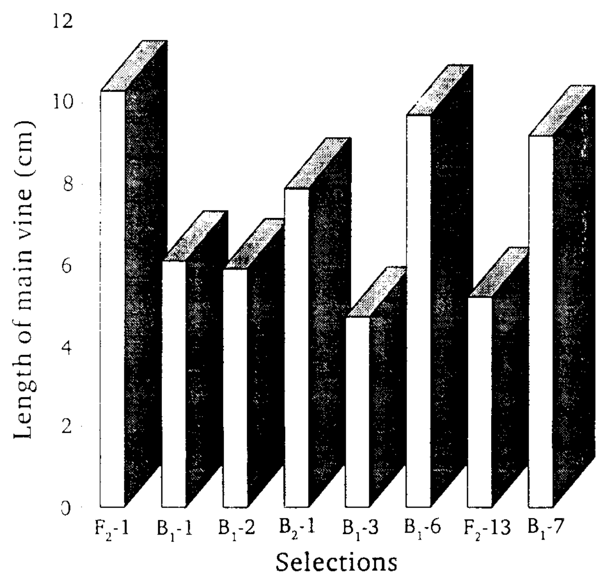
The second highest yield (7.5 kg) was recorded by plant number B_2-1. The fruit development percentage (25%) was the highest for this plant. The first fruit matures within a period of 103 days. The average fruit weight was 3.75 kg. The fruits had a carotene content of 28.15 IU/100g. Maximum flesh thickness (3.26 cm) was noted in this fruit. The fruit is dark green with thick greyish waxy coat. The fruits have got yellow flesh. This is a blend of colours of both parents i.e., Ambili and NL.

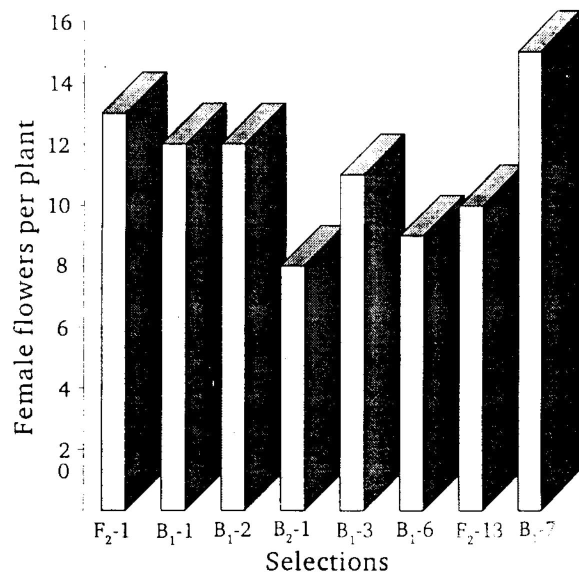
Plant numbers B_1-7 and F_2-13, produced 7.30 kg and 6.80 kg fruits respectively, the average fruit weight being 3.65 and 3.40 kg. Both the plants were comparatively late in flowering, the number of days to first female flower anthesis being 63 and 59 respectively. The average fruit weight in B_1-7 and F_2-13 were 3.65 kg and 3.40 kg respectively. Fruits of F_1-7 were greyish cane in colour. The greyish colour was inherited from NL while the cane colour was from the female parent CO1.

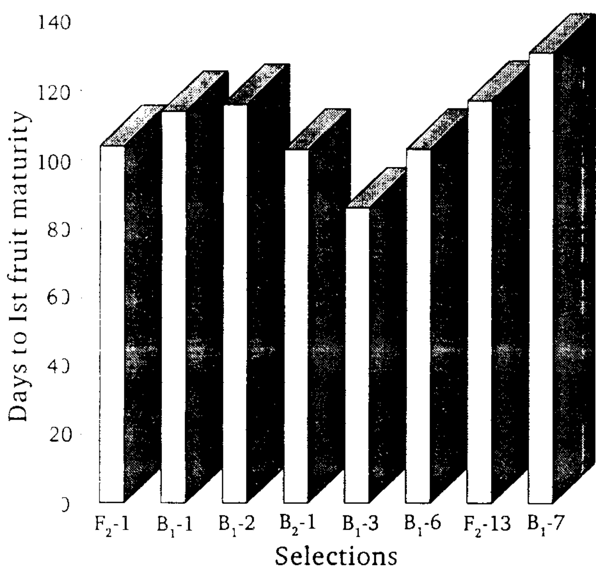
Plant number B_1-6 produced 6.25 kg fruits. The fruit diameter (24.35 cm), number of seeds per fruit (310) and T.S.S. content (6.90%) were maximum for B_1-6. Plant numbers F_2-1, B_1-2 and B_1-3 gave 5.80, 5.00 and 3.60 kg fruits respectively. B_1-3 took the shortest period for the first female flower anthesis (36) and for the first fruit maturity (86) indicating that it is the earliest among the selections. Fruits of B_1-3 had high carotene content (33.91 IU/100 g) followed by F_2-1 (31.85 IU/100 g). F_2-1 expressed the fruit colour of Ambili, while B_1-2 had green coloured fruits i.e., intermediate colour of Ambili and NL (Latha, 1992). The greyish light cane colour of B_1-3 fruits was inherited from both the parents i.e., cane colour from Pusa Viswas and greyish waxy coating from NL.

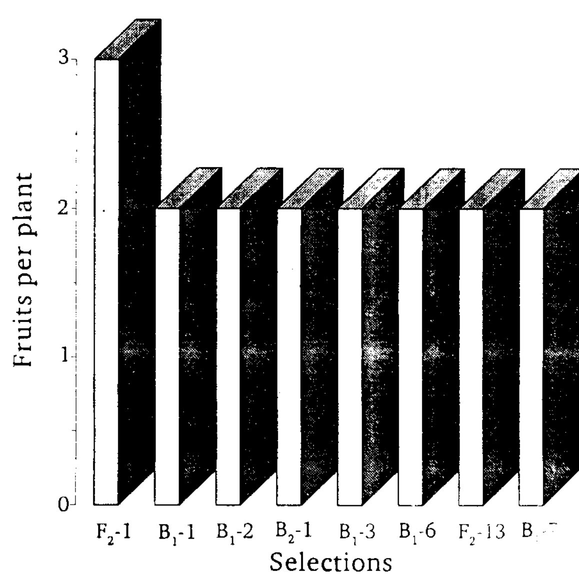
World wide attempts to transfer mosaic virus resistance from resistant source to otherwise commercially superior varieties had been carried out in cucurbits. Munger and Provvidenti (1987) attempted to transfer the resistance to ZYMV in NL to Butternut squash through hybridization and back crossing. In the BC_3 F_2 generation they could select resistant plants with similarity to BN in shape, size, colour and quality of cooked flesh. The high yielding, WMV-1 resistant melon variety Eldorado-300 was developed through resistance breeding from the cross between W6 (R) and CV. Amarelo (S). Herrington *et al.* (1991) developed a high yielding promising variety in *Cucurbita maxima* i.e., Redlands Trail blazer with resistance to WMV-2, PRV-W and ZYMV by backcross transferring of the resistance to these viruses from the source *Cucurbita ecuadorensis into Cucurbita maxima.*

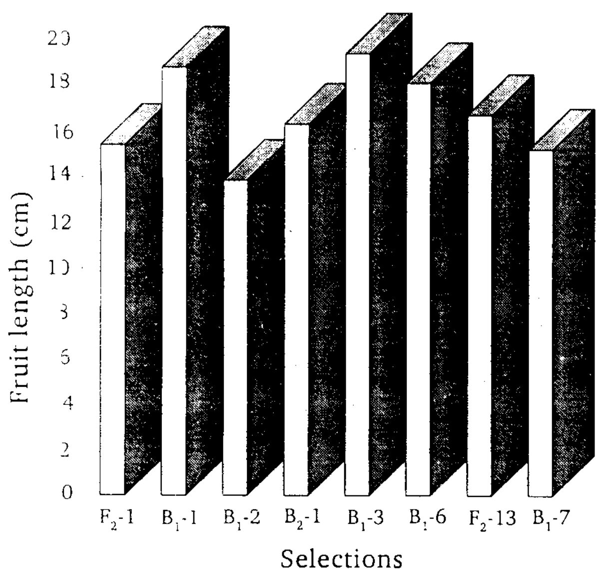
The mosaic resistant high yielding selected plants with desirable fruit characters need further selfing and screening for PMV and YVMV resistance followed by selection in the field. The plants with desirable fruit characters combined with high yield again need further selfing, screenig for mosaic resistance and then evaluation in the field. This cycle is











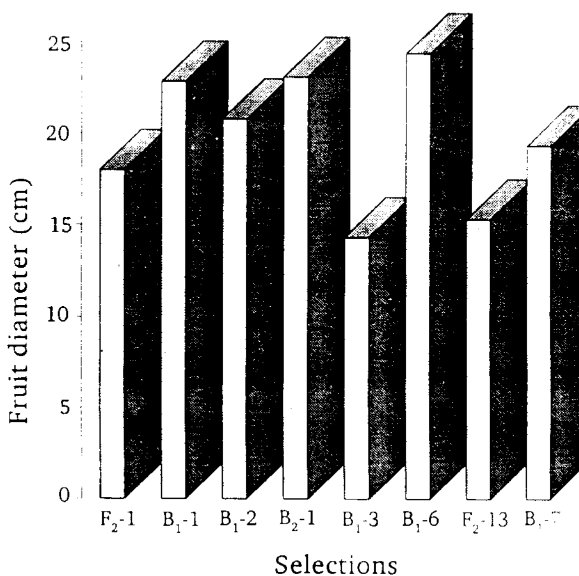


Fig. 9. Performance of selections resistant to PMV and YVMV

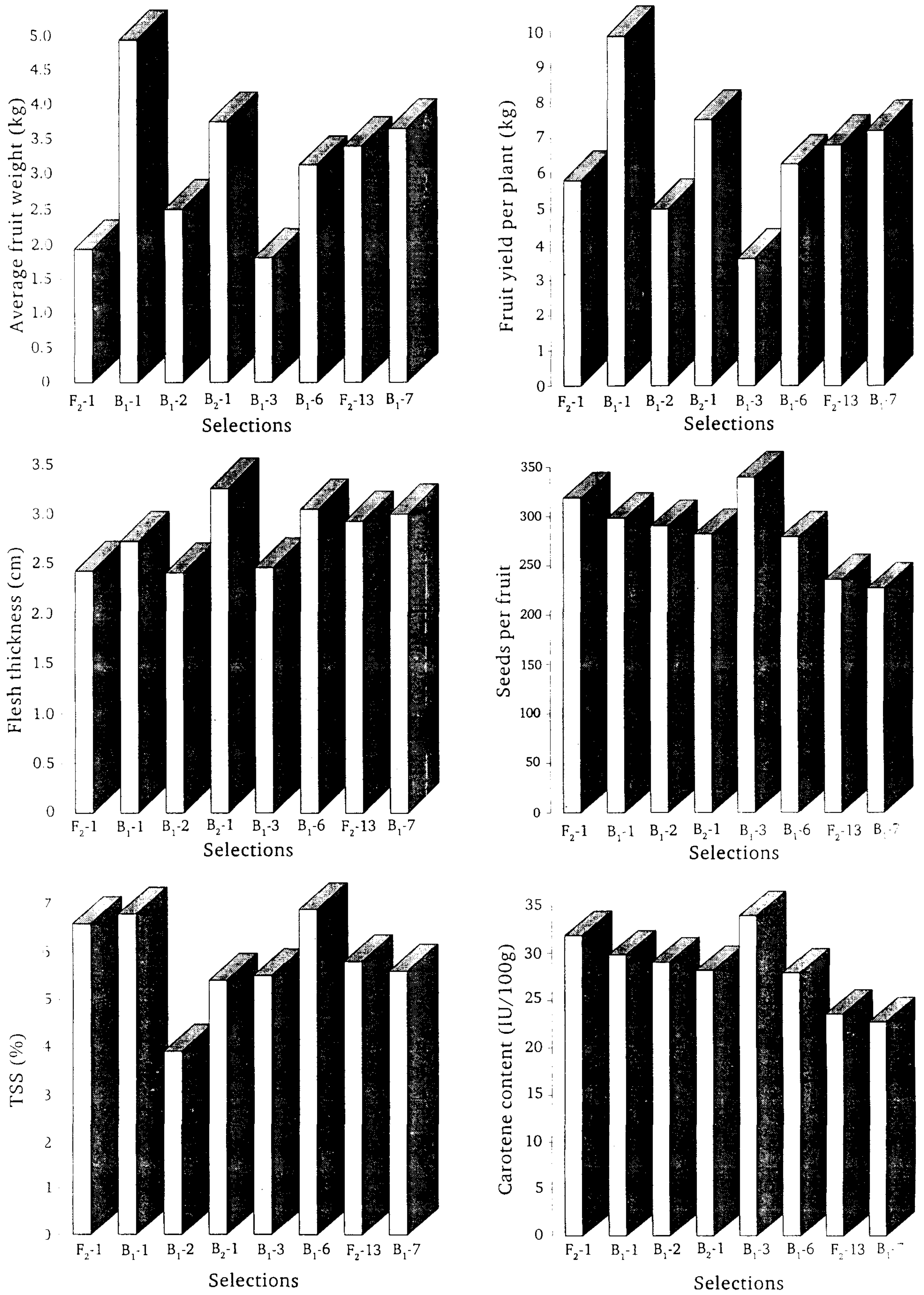


Fig. 10. Performance of selections resistant to PMV and YVMV

to be followed until the desired characters are stabilised. Takada (1982) could develop three melon varieties, Ano-1, Ano-2 and Ano-3 with resistance to CMV from the F_3 of (Hiratsuka 3 x Mitangting) F_6 x Maruike of which Mitangting was resistant to CMV than the other otherwise superior varieties.

In addition to the eight selections described above, there were some high yielding mosaic resistant plants evolved from the study which were but handicapped by warty fruits. They included F_1-1, F_2-3 and B_2-2 (reassigned number) belonging to the cross Amb x NL, B_2-5 and B_2 6 (reassigned number) from the cross PV x NL, F_2-12 and B_2-9 (reassigned number) from cross AC x NL and B_2-10 (reassigned number) from the cross Col x NL. These can be improved through back crossing with the respective female parent, selfing, screening for virus resistance and selection as suggested by Robinson *et al.* (1988) in case of ZYMV resistant genotypes developed from the back cross of *Cucurbita maxima* cv. Butter cup (S)x *C. ecuadoresis* (R) x *C. maxima* cv. Buttercup.

To sum up, studies on sources of resistance to PMV and YVMV in pumpkin had brought out that Nigerian Local has got combined resistance to PMV and YVMV. Crosses involving this resistant source and the four mosaic susceptible released pumpkin varieties under study namely, Amb, PV, AC and Col revealed that the resistance to PMV is controlled by a single recessive gene (with involvement of gene modifiers in PV x NL) while the resistance to YVMV is basically monogenic dominant, but slightly influenced by gene modifiers. The biochemical mechanism of mosaic virus resistance can be attributed to peroxidase and esterase isozymes than the protein pattern since there is clear cut difference in the isozyme profiles of the resistant and susceptible parents before and after inoculation of PMV and YVMV. Based on resistance to PMV and YVMV, non incidence of other mosaic viruses, fruit smoothness and fruit yield, eight segregants belonging to the different cross combinations were selected for further progressing.

Summary

SUMMARY

Pumpkin, (*Cucurbita moschata* Poir) is grown throughout the length and breadth of India. It is an important and popular cucurbitaceous vegetable grown in Kerala which is known for its high Vitamin A content and suitability for long storage. The cultivation of pumpkin suffered a set back during the last few years due to severe outbreak of mosaic diseases, particularly pumpkin mosaic and yellow vein mosaic. The reduction in yield was even 100 per cent in pumpkin when the plants were infected by mosaic viruses in the seedling stage. This dismal scenario warrants the formulation of an effective strategy for controlling the mosaic viruses in pumpkin.

The only effective method to check viral diseases is to use virus resistant varieties. Unfortunately all the existing released varieties of pumpkin are susceptible to pumpkin mosaic virus and yellow vein mosaic virus. It is in this context that the present research programme, breeding for resistance to mosaic viruses in pumpkin, was undertaken. Investigations were carried out in pumpkin with the objectives of (i) identification of source(s) of resistance to PMV and YVMV, (ii) studying the genetics of mosaic virus resistance, (iii) ascertaining the biochemical mechanism of mosaic virus resistance and (iv) identifying desirable plants possessing combined resistance to PMV and YVMV.

Identification of sources of resistance to PMV and YVMV

The experimental material comprised 103 genotypes belonging to six species of *Cucurbita* which included both exotic and indigenous collections. Among 95 genotypes of *C. moschata* screened against PMV and YVMV, only one accession Nigerian Local (SRS 7), introduced form Cornell University possessed high level of resistance to both the viruses. Other than NL, all the genotypes screened showed varying degrees of systemic symptoms against both the viruses. The commercially superior released pumpkin varieties Ambili, PV, AC and Co1 showed high susceptibility. The accessions belonging to *C. maxima* and *C. pepo* were also susceptible to PMV and YVMV. Among the feral species namely, *C. foetidissima*, *C. martinezii* and *C. ecuadorensis*, none expressed resistance reaction to either viruses. After inoculation of YVMV, the symptom expression started even within one week. After two weeks of inoculation, 33.75 per cent plants were susceptible.

Confirmation studies were carried out in the line NL, identified as resistant to both the viruses in order to ascertain the nature of resistance, since the resistance expressed consequent to virus inoculation may be due to escape, symptomless carrier type of tolerance or immunity. Neither the inoculated leaves nor the uninoculated leaves of the susceptible variety Ambili showed symptoms when back inoculated with sap from the resistant source in case of PMV. Through back inoculation, viruses could not be isolated from this genotype thereby proving that the resistance is not symptomless carrier type of tolerance. So the line NL is having extreme resistance to PMV. Graft transmission was done in case of YVMV. NL did not develop any symptom even after grafting on the infected symptomatic root stock showing that the line is extremely resistant to YVMV. The environmental fluctuations also did not influence the resistance expression of NL against both PMV and YVMV indicating the stability of virus expression in these genotypes.

Genetics of virus resistance

Resistance to PMV was evaluated in five parents and their F_1, F_2 and backcross generations of four cross combinations namely, Amb x NL, PV x NL, AC x NL and Col X NL.

On an average 96.25 per cent plants in the F_1 showed susceptibility. This points to the dominance of susceptibility. In PV x NL, the gene for susceptibility to PMV had only 95 per cent penetrance. Involvement of gene modifiers that influence the disease symptom expression of the major gene for susceptibility was observed in the cross AC x NL. Evaluation of reciprocal crosses ruled out the cytoplasmic effect in PMV resistance. The F_2 segregation ratio in all the crosses was in agreement with the Mendelian genetic ratio of 3:1 (Susceptible : Resistant) The test crosses (F_1 back crossed to NL) confirmed this with a genetic ratio of 1:1 (resistant : susceptible) in all the four crosses studied. The inheritance studies in six generations of all the four cross combinations clearly revealed that the resistance to PMV in pumpkin is controlled by a single recessive gene. As the gene governing resistance to PMV in NL is monogenic recessive, a series of backcrosses each followed by selfing and selection for resistance is required to incorporate PMV resistance.

Reaction to YVMV in the six generations i.e., P_1, P_2, F_1, F_2, B_1 and B_2 of the four cross combinations under study revealed that the resistance is controlled by a single dominant gene. In the F_1, on an average, out of 40 plants 32 were resistant. The reciprocal crosses also behaved in a similar manner. The gene for resistance in PV x NL had a penetrance value of 90 per cent. In the other crosses role of gene modifiers that might be involved in the resistant expression of the major gene can not be ruled out. Out of 300 F_2 plants in each cross, a mean number of 220 plants were resistant and 80 susceptible, fitting in a 3:1 ratio indicating the monogenic dominance of resistance. When F_1 was back crossed to the susceptible parent, the segregation of resistance and susceptibility was in equal proportion i.e., out of a mean number of 100 B_1 plants inoculated 48 were resistant. This very well fitted into 1:1 ratio of resistance : susceptibility. As the resistance to YVMV being governed by basically a dominant gene, it is easy to transfer this resistance by simple back crossing. This throws light on the scope of using this resistance in F_1 seed production.

Although NL is a good source of resistance to both the viruses under study, they do not set fruit well. The seed germination was as low as 19 per cent, while it was above 80 per cent in the other parents. Through seed coat removal, 43 per cent increase in seed germination (62%) was noted in NL, while the increases were only 3,4 and 12 per cent respectively in Amb, PV and Col. In AC the increase in germination was not at all significant. In NL, the presence of hard seed coat might have restricted the absorption of water and oxygen essential for germination of seeds. The removal of seed coat enabled easy penetration of water and oxygen resulting in emergence of the embryo.

Biochemical mechanism of mosaic virus resistance

Study of protein pattern and isozyme analysis by electrophoresis provides a well defined and effective method to detect genetic differences among individuals. In the present study, the protein pattern and the isozyme pattern of peroxidase and esterase enzymes in susceptible and resistant genotype of pumpkin as well as their F_1 hybrids were analysed, before inoculation and after inoculation of both viruses.

All the genotypes including the resistant, susceptible and their F_1 hybrids showed no difference in the number of protein bands and relative mobility, both before and after inoculation of the viruses.

The resistant and susceptible genotypes showed clear cut differences in their peroxidase profile. The susceptible parents had three bands namely, PRX1, PRX3 and PRX6, while the resistant parent NL expressed only one isoform - PRX1 (0.083) before virus inoculation. Due to absence of polymorphism in the resistant genotype, the activity was substantially high which implies that protein sub-units have an influence in activity and resistance. The hybrids Amb x NL and Col x NL showed similarities of both parents. As in case of NL the band PRX3 was not expressed. The hybrid PV x NL exhibited six bands with three additional bands, PRX2, PRX4 and PRX7 whereas the hybrid AC x NL was similar to AC in the peroxidase banding pattern. The additional band PRX2 for PV x NL can be attributed to the earliness in growth and development of this hybrid compared to the other hybrids and parents.

After inoculation of PMV, the banding pattern of peroxidase changed. The low Rm value PRX1 band expressed before inoculation was not expressed in both the resistant and susceptible parents as well as in their F_1 hybrids after inoculation. The additional band PRX7 (Rm value 0.642) shown by PV x NL before virus inoculation was noted in all the other genotypes except in the resistant. In the resistant parent NL an additional band, PRX5 (Rm value 0.463) was noted. This particular isoform might be responsible for resistance to PMV in NL.

After inoculation of YVMV the susceptible parents had three isozyme bands i.e., PRX3 (0.342), PRX6 (0.583) and PRX7 (0.642), while the resistant parent had a streaking band showing the expression of PRX3, PRX4, PRX5 and PRX6. The hybrids had an additional band PRX4 (0.392) over the susceptible parents. This is seemingly the reason for the resistant expression of NL and the F_1 hybrids to YVMV.

There were six bands of esterase in the susceptible parent and only three in the resistant before virus inoculation. Consequent to host-pathogen (PMV) interaction one esterase band, EST 7 (Rm value 0.868) expressed before inoculation did not express in the susceptible parents and expressed as EST5 (0.721). The resistant parent also, after inoculation of PMV, did not express EST7, but exhibited three additional bands EST 1 (0.368), EST4 (0.632) and EST 5 (0.721). Consequent to YVMV infection, out of two additional bands seen in NL, EST 4 (0.632) was thick and the specific activity of this particular isoform also might be responsible for the resistance mechanism in addition to that of PRX4.

Selection of mosaic resistant desirable plants

Sequential inoculation with PMV followed by YVMV was carried out in the F_1, F_2, B_1 and B_2 generations of the four cross combinations namely, Amb x NL, PV x NL, AC x NL and Col x NL to identify plants with combined resistance to both the viruses.

In the F_1s, eight plants were identified as resistant to both the viruses i.e., one in Amb x NL, two in PV x NL, three in AC x NL and two in Col x NL. From among 100 plants each evaluated sequentially for PMV and YVMV resistance in the F_2 and B_1 generations belonging to the four cross combinations, 17 segregants in F_2 and nine in B_1 were isolated as resistant to both the viruses. In the B_2 population, out of 100 there were 37 seedlings resistant to both the viruses. They included ten from the cross Amb x NL, nine from PV x NL, eleven from AC x NL and seven from Col x NL.

All the 71 seedlings possessing combined resistance to PMV and YVMV were further evaluated in the field for their morphological and horticultural characters. In the cross Amb x NL, earliness as revealed by the shortest number of days taken for anthesis of the first female flower (45) was noted in F_1-1. The B_2 generation took the longest period (55.40) for opening of the first female flower. In PV x NL, the F_1s took the shortest period (41.50) for anthesis of the first female flower, while the B_2s was the latest in flowering. Same trend was observed in AC x NL and Col x NL for earliness. Except Col x NL, in all the other crosses, B_2 plants flowered comparatively late. In all the crosses except PV x NL the node of first fruit development was the shortest in the F_1s. This is also indication of earliness of F_1s compared to F_2s, B_1s and B_2s.

In Amb x NL, the longest vine was produced by the F_1s (8.90m) and shortest by B_1s (6.00m). In the cross PV x NL, the vine length was comparatively higher for the B_2 plants, followed by F_2, F_1 and B_1. In the cross Col x NL, the F_1 plants exhibited the same trend as that of Amb x NL.

The range in number of female flowers per plant in the different generations of Amb x NL was 12 in F_1 and B_1, 10 in F_2 and 9.40 in B_2. In AC x NL also, the highest number of female flowers was produced in the F_1 generation. The fruit yield in terms of number and weight of fruits in all the crosses was maximum for the F_1 plants.

In all the crosses, the plants belonging to the F_1, F_2, B_1 and B_2 generations produced fruits with fruit colour of either the female parent or with dark greyish green colour of NL or with a blend of these two colours. In flesh colour also same trend was observed.

Based on fruit smoothness, non-incidence of mosaic virus and yield of fruits, eight most promising superior segregants from among the four cross combinations were selected. They included four in Amb x NL, two from Col x NL and one each from PV x NL and AC x NL.

References

REFERENCES

Ahemiya, Y., Hirai, S., Higashiura, M. and Hirano, K. 1991. Susceptibility of melon cvs. to Cucumber Mosaic Virus. *Tech. Bull. Faculty of Hort. Chiba Univ.* 43: 191-198

Ahmed, N., Thakur, M.R., Bajaj, K.L. and Cheema, S.S. 1994. Biochemical basis of resistance to yellow vein mosaic virus in okra. *Pl.. Dis. Res.* 9(1):20-25

Alger, M.D., Egea, C., Espin, A. and Candela, M.E. 1995. Peroxidase isoenzymes in the defense response of *Capsicum annum* to *Pytophthora capsici.Physiologia Plantarum* 94:736-742

Arora, S.K., Singh, B., Dhanju, K.C. and Ghai, T.R. 1992. Genetics of resistance in summer squash to powdery mildew and CMV. *Pl. Dis. Res.* 7 (2) : 215-220

Avdeyev, Y.I. 1979. Inheritance of resistance to concentric fruit cracking. *Tomato Genet. Co-operative Rep.* 29 : 20-21

Babu, R. (1987) Studies on mungbean yellow mosaic virus disease. Ph.D. thesis, Tamil Nadu Agricultural University, Coimbatore.

Bal, S.S., Singh, J. and Dhanju, K.C. 1995. Genetics of resistance to mosaic and leaf curl viruses in chilli (*Capsicum annum* L.) *Indian J. Virol.* 11 (1) : 77-79

Balakrishnan, S. 1988. Mosaic diseases of pumpkin in Kerala. *Research Highlights on Vegetables.* Kerala Agricultural University, Thrissur, Kerala p. 44-46

Banerjee, M.K. and Kalloo 1987a. Inheritance of tomato leaf curl virus resistance in *Lycopersicon hirsutum f. glabratum. Euphytica* 36 : 581-584

Beekman, A.G.B. 1987. Breeding for resistance. *Viruses of Potatoes and Sweet Potato Production.* (Ed. De Bokx, J.A. and Vain Der Want, J.P.H.)(5th ed).PUDOC, Wageningen, p. 162-170

Biles, C.L., Martyn, R.D. and Wilson, H.D. 1989. Isozymes and general protein for various water melon cultivars and tissue types. *HortScience.* 24 (5) : 810-812

Boyhan, G.E., Norton, J.D., Jacobsen, B.J. and Abrahams, B.R. 1992. Evaluation of watermelon and related germplasm for resistance to ZYMV. *Pl. Dis.* 76 (3): 250-252

Boyhan, G.E. 1994. Evaluation of watermelon and related germplasm for resistance to Egyptian strain of ZYMV. *Pl. Dis.* 78 (1):100

Brar, J.S. and Nandpuri, K.S. 1978. Genetic analysis of yield and fruit number in watermelon (*Citrullus lunatus Thumb.* Mam). *Indian J. Hort.* 35 (3): 225-228

Clough, G.H. and Hamm, P.B. 1995. Coat protein transgenic resistance to watermelon mosaic and Zucchini Yellow mosaic virus in squash and cantaloupe. *Pl. Dis.* 79(11): 1107-1109

Cohen, S., Gertman, E. and Kedar, N. 1971. Inheritance of resistance to melon mosaic virus in cucumbers. *Phytopathology* 61 (2) : 253-255

Cooper, J.I. and Jones, A.I. 1983. Response of plants to viruses-proposals for the use of terms. *Phytopathology* 73 : 127-128

Coudriet, D.L., Kishaba, A.N. and Bohn, G.W. 1981. Inheritance of resistance to muskmelon necrotic spot virus in a melon aphid resistant breeding line of muskmelon. *J. Am. Soc. hort. Sci.* 106 (6) : 789-91

Cruickshank, I.A.M. 1963. Phytoalexins. *A. Rev. Phytopath.* 1:351-374

Curtis, L.C. 1941. Comparative earliness of the first and second generation squash (*Cucurbita pepo*) and the possibility of using second generation seed for commerical planting. *Proc. Am. Soc. hort. Sci.* 38: 596-596

Danin - Poleg, Y., Paris, H.S., Cohen, S., Rabinowitch, H.D. and Karchi, Z. 1997. Oligogenic inheritance of resistance to Zucchini Yellow mosaic virus in melons. *Euphytica* 93 (3) : 331-337

De Wit, P.J.G.M. and Bakkar, J. 1980. Differential changes in soluble tomato leaf proteins after inoculation with virulent and avirulent races of *Cladosporium fulvum (Syn. Fulvia fulva).* *Physiol. Pl. Path.* 17:121

Dogimont, C., Salama, S., Martin, J., Lecoq, H. and Pitrat, M. 1996. Sources of resistance to cucurbit aphid-borne yellow luteo virus in a melon germplasm collection. *Pl. Dis.* 80 (12) : 1379-1382

Doijode, S.D. and Sulladmath, U.V. 1981. Inheritance of earliness in pumpkin. *Haryana J. hort. Sci.* 10(3/4): 259-264

Duan, N.X., Peng, Z., Tan, U.J. and Jiang, H.F. 1994. Preliminary studies on the biochemical mechanisms of resistance to *Pseudomonas solanacearum* in groundnut. *Groundnut Bacterial Wilt Asia.* ICRISAT. 3:101-102

Endo, T. 1971. Expression of allelic peroxidase isozymes in heterozygotes of *Oryza perennis*. *Jap. J. Genet.* 46 : 1-5

Esteva, J., Nuez, F. and Cuartero, J. 1988. Resistance to yellowing disease in wild relatives of muskmelon. *Rep. Cucurbit Genet. Co-operative* 11 : 52-53

Esteva, J. Nuez, F. and Cuartero, J. 1988. A yellowing disease on melons in the south east coast of Spain. Cucurbitaceae 88 *Proc. of the Eucarpia meeting on cucurbit genetics and breeding,* Avignon, Montfavet, France, May 31-June 2.p.163

Farkas, G.L. and Stahmann, M.A. 1966. On the nature of changes in peroxidase isoenzymes in bean leaves infected by southern bean mosaic virus. Phytopathology. 56:669-671

Fiedorow, Z. 1993. Susceptibility of cucurbitaceous plants to CMV. *Ochrona Roslin* 37 (9): 11-12

Fischer, R.A. 1950. *Statistical Methods for Research Workers.* (3rd ed.)Oliver and Boyd, London

Gabriel, D.W. and Ellingboe, A.H. 1982. High resolution two-dimensional electrophoresis of protein from congenic wheat lines differing by single resistance genes. *Physiol. Pl. Path.* 20:349

Gangappa, B. 1986. Studies on resistance to bacterial wilt (*Pseudomonas solanacearum* E.F. Smith) in brinjal. M.Sc. thesis, University of Agricultural Sciences, Bangalore

Gay, P.A., Dodson, K.M. and Tuzun, S. 1995. Differential accumulation of anionic peroxidase isozymes in resistant and susceptible cabbage varieties during pathogenesis with *Xanthomonas campestris pv. campestris*. *Phytopathology* 85 (10) : 1122

George, T.E., Anand, N., Deshpande, A.A. and Singh, S.J. 1992. Studies on sources of resistance to Potato Virus Y (PVY) and Cucumber Mosaic Virus (CMV) in *Capsicum* species. *SABRAO J.* 24 (1) : 7-13

Gianinazzi, S. and Kassanis, B. 1974. Virus resistance induced in plants by polyacrylic acid. *J. Genet. Virol.* 23 (1) : 1

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

Gilbert-Albertini, F., Lecoq, H., Pitrat, M. and Nicolet, J.L. 1993. Resistance of *Cucurbita moschata* to WMV type 2 and its genetic relation to resistance to ZYMV. *Euphytica* 69 (3) : 231-237

Gilbert, R.Z., Kyle, M.M., Munger, H.M. and Gray, S.M. 1994. Inheritance of resistance to WMV in *C. melo*. *HortScience* 29 (2) : 107-110

Gillaspie, A.G. Jr. and Wright, J. M. 1995. Resistance of *Citrullus lanatus* germplasm to Watermelon mosaic virus 2 strain. *Phytopathology.* 85 (4) : 510

Gopalakrishnan, T.R. 1979. Gnetic variability and correlation studies in pumpkin (*Cucurbita moschata* Poir). M.Sc.(Hort.) thesis, Kerala Agricultural University, Thrissur, Kerala

Gopinath, G. and Madalageri, B.B. 1986. Bacterial wilt (*Pseudomonas solanacearum* E.F.Smith) reistance in eggplant. *Veg. Sci.* 13:189-195

Guldur, M.E. and Yilmaz, M.A. 1995. Rapid detection of tomato mosaic virus by agarose gel electrophoresis *J. Turkish Phytopath.* 23(2):87-90

Hames, B.D. 1994. One dimensional polyacrylamide gel electrophoresis. *Gel Electrophoresis of proteins : A practical approach.*.(Ed. Hames, B.D. and Rickwood D.) 2nd ed. Oxford University Press, New York. p.60

Hampton, R.E. and Fulton, R.W. 1959. Factors responsible for the instability of some labile plant viruses. *Phytopathology* 49 :540

Hayja, A. and Al-Shahwan, I.M. 1991. Inheritance of resistance to Zucchini Yellow Mosaic Virus in cucumber. *Zeitschrift fiir Pflanzenkran Kheiten und pflanzenschutz* 98 (3) : 301-304

Herrington, M.E., Greber, R.S., Brown, P.J. and Persley, D.M. 1988. Inheritance of resistance to ZYMV in *C. maxima* Queensland Blue x *C. ecuadorensis*. *Queensland J. agric. and Animal Sci.*. 45 (2) : 145-149

Herrington, M.E., Byth, D.E., Teakle, D.S. and Brown, P.J. 1989. Inheritance of resistance to papaya ring spot virus type W in hybrids between *Cucurbita ecuadorensis* and *C. maxima*. *Aust. J. exp. Agric.* 29 (2) : 253-259

Herrington, M.E., Prytz, S., Peter, B., Persley, D.M. and Greber, R. 1991. Resistance to Papaya Ring Spot virus-W, ZYMV and WMV-2 in *Cucurbita maxima*. *Rep.Cucurbit Genet.Co-operative* 14 : 123

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

Horvath, J. 1986. Compatible and incompatible relations between *Capsicum* species and viruses III. New incompatible host-virus relations (Resistant and immune plants). *Acta Phytopath.. Entomol..* Hungarika. 21 : 59-62

Hcrvath, J. 1992. Screening *C. moschata* accessions for resistance to different viruses. *Recent advances in vegetable virus research* - 7th Conference, ISHS Veg. Virus Working Group, Athens, Greece, July 12-16

Hulupi, R., Djojodirdjo, S. and Hartiko, H. 1988. Identification of Coffee cultivars and species based on Malate dehydrogenase and polyphenol oxidase isozyme banding patterns. *Pelita perkebunan (Indonesia)* 4 (1) : 1-12

Hwang, B.K., Wolf, G. and Heitfuss, R. 1982. Soluble proteins and multiple forms of esterases in leaf tissue at first and flag leaf stages of spring barley plants in relation to their resistance to powdery mildew. *Physiol. Pl. Path.*. 21:367

Jayasree, P.K. 1984. Yellow Vein Mosaic Disease of Pumpkin in Kerala. M.Sc. thesis, Kerala Agricultural University, Vellanikkara, Thrissur

Jejelowo, O. 1995. Accumulation of three different phytoalexins in leaves of *Arabidopsis thaliana* following inoculation with *Cochliobolus carbonum*. *Phytopathology* 85 (10) : 1202

Jimenez, L.D., Ayer, W.A. and Tewari, J.P. 1995. Elicitation of phytoalexins in *Capsella bursa-pastoris* in response to *Alternaria brassicae*. *Phytopathology* 5 (10):1189

Juan, M.O.S. 1988. Advances in plant disease management in the Phlippines. *Philipp. Phytopath.*. 24 (1 & 2) : 1-4

Kalloo,G. and Banerjee, M.K. 1990. Transfer of Tomato Leaf curl resistance from *Lycopersicon hirsutum f. glabratum* to *L. esculentum*. *Pl.. Breed.* 105 (2) : 156-159

Karchi, Z., Cohen, S. and Govers, A. 1975. Inheritance of resistance to cucumber mosaic virus in melon. *Phytopathology* 65 (4) : 479-481

Kato, M., Jodo, S. and Tokumasu, S. 1978. Application of electrophoretic isozyme method to muskmelon breeding. *J. Jap. Soc. hort. Sci.* 47 : 57-62

Kato, M. and Jodo, S. 1989. Application of the elecrophoretic isozyme method to muskmelon breeding. II Selection effects in F_2 and B_1F_3 generations.. *Memoirs College Agric. Ehime Univ.* 34 (1) : 15-24

Karwasra, S.S. and Parashar, R.D. 1988. Peroxidase and Polyphenol oxidase activity in relation to resistance in potatoes agaist bacterial soft rot. *Indian J. Mycol. Pl. Path.* 18 (1) : 109

K.A.U. 1993. *Package of Practices Recommendations-Crops 93.* Directorate of Extension, Kerala Agricultural Universaity, Vellanikkara, Thrissur, Kerala. p. 174-175

Kim, S.J. and Jeong, H.J. 1990. Studies on seed germination characteristics of Songhwan Oriental melon. *Cucumis melo* var. *microspermus J. Korean Soc. hort. Sci.* 31 (2): 114-120

Kishaba, A.N., Castle, S.J., Coudriet, D.L., Mc Creight, J.D. and Bohn, G.W. 1992. Virus transmission by Aphis gossypii Glover to aphid resistant and susceptibe musk melon. *J. Am . Soc. hort. Sci.* 117 (2) : 248-254

Konishi, T.N., Yoshida, K.H. and Soutome, K. 1989. Linkage relationships between two loci for the barley yellow mosaic resistance of Mokusekko and esterase isozymes in barley (*Hordeum vulgare* L.) *Jap. J. Breed.* 39 : 423-430

Kooistra, E. 1969. The inheritance of resistance to cucumis virus in cucumber (*Cucumis sativus* L.) *Euphytica* 18 : 326-332

Kulshreshtha, B.M. and Chauhan, R.K.S. 1985. Production of phytoalexins in the roots of raddish (*Raphanus sativus*) after inoculation with Alternaria alternata. *Indian Phytopath.* 38 (2) : 286-96.

Kurpa, A. 1989. Reaction of potato cultivars to primary and secondary infection by potato moptop furovirus and strategies for virus detection. *EPPO Bull.* 19: 593-598

Laska, P. and Lebeda, A. 1989. Resistance in wild *Cucumis sp.* to glass house white fly *(Trialurodes vaporariorum). Archi fiir Zuchtung sforschung* 19(1) : 89-93

Latha, P. 1992. Selection for mosaic resistance in pumpkin. M.Sc. thesis, Kerala Agricultural University, Thrissur, kerala.

Lawrence, C.B., Joosten, M.H.A.J. and Tuzun, S. 1996. Differential induction of pathogenesis related proteins in tomato by *Alternaria solani* and the association of a basic chitinase isozyme with resistance. *Physiol. mol. Pl. Path.* 48 (6) : 361-377

Lecoq, H and Pitrat, M . 1980. resistance to cucumber mosaic virus transmission by *Aphis gossypii* in melon. *Rep. Cucurbit Genet.Co-Operative* 3 : 30

Lecoq, H., Pitrat, M. and Clement, M. 1981. Identification et caracterisation dun potyvirus Provoquant la maladie du rabougrisement jaune du melon. *Agronomie* 75 : 827-834

Lecoq, H and Pitrat, M. 1984. Interaction of ZYMV strain musk melon lines. *Rep.Cucurbit Genet. Co-operative* 7 : 43-44

Lecoq, H.,Bourdin, D., Wippf-scheibel, C., Bon, M., Lot, H., Lemaire, O. and Herrbach, E. 1992. A new yellowing disease of cucurbits caused by a luteovirus, Cucurbit aphid born yellow virus. *Pl. Path.* 41 : 749-761

Le Gouis, J., Erdogan, M., Friedt, W. and Ordon, F. 1995. Potential and limitations of isozymes for chromosomal localisation of resistance genes against barley mosaic virus (BAMV). *Euphytica* 82 (1) : 25-30

Li, S. Li, K.Q. and Yuan, W.J. 1991. Variation in enzyme activities in cucumber leaves infected by *Pseudoperonospora cubensis* (Berk *et* Curt) Rostov. *Acta Phytopath. Sinica* 21(4) : 277-283

Linde, D.C. and Rhodes, B.B. 1988. Preinoculation peroxidase activity in cucumber leaves not associated with race 2 anthracnose resistance. *Rep. Cucurbit Genet. Co-operative* 11 : 20

Loebenstein, G. 1972. Localization and induced resistance in virus-infected plants. *A. Rev. Phytopath.*10 : 177

Maluf, W.R., Pereira, J.J. and Figueira, A.R. 1997. Inheritance of resistance to the papaya ringspot virus-watermelon strain from two different accessions of winter squash *Cucurbita maxima* Duch. *Euphytica* 94 (2) : 163-168

Maluf, W.R., Silva, I.S. and Moura ,W.M. 1985. Inheritance of (WMV-1) resistance in squash *Cucurbita maxima. Revista Brasileira de Genet. (Brazilian J. Genet.)* 8 (1) : 175-82

Markose, B.L. 1996. Genetic and biochemical bases of resistance to bacterial wilt in chilli. Ph.D. (Hort) thesis, Kerala Agricultural University, Thrissur, Kerala

Mc Creight, J.D., Bohn, G.W. and Kishaba, A.N. 1992. Pedigree PI 414723 melon. *Rep. Cucurbit Genet. Co-operative* 15 : 51-52

Mc Creight, J.D. and Kishaba, A.N. 1991. Reaction of cucurbit species to squash leaf curl virus and sweet potato white fly. *J. Am. Soc. hort. Sci.* 116 (1) : 137-41

Meyer, U., Weber, I. and kegler, H. 1987. Characterisation of quantitative resistance of cucumber to cucumber mosaic virus - a model experiment. *Archivfur Gartenbau* 35 (8) : 425-439

Ming, W. and Xian, Z. 1988. Studies on watermelon germplasm sources resistant to Fusarium wilt disease at the seedling stage. *Rep.Cucurbit Genet. Co-operative* 11 : 68

Ming, W. and Xing-Ping, Z. 1988. Isozyme analysis of hybrids and their parents of watermelon (*Citrullus lanatus*). *Rep. Cucurbit Genet. Co-operative* 11:57

M, Nari, M.,Jebari, H. and Cherif,D. 1993. Study of strains of CMV on Tunisian melon and identification of resistant genotypes. *Cahiers Agric.* 2 (1) : 60-62

Mohammadi, A.G., Dwivedi, T.S., Sand, N.K. and Gupta, K.C. 1991. Determination of phenolic acids in chickpea by reversed- pase high performance liquid chromatography and their relation to fungal resistance. *Indian J. Pl.Path.* 9 (12) : 42-46

More, T.A., Varma, A., Seshadri, V.S., Somkuwar, R.G. and Rajamony, L. 1993. Breeding and development of Cucumber Green mottle melon (*C. melo*). *Rep. Cucurbit Genet. Co-operative* 16 : 44-46

Moreno, V., Aguilera, G.J.L., Arellano, G.C. and Roig, L.A. 1993. Preliminary screening of cucurbit species for *Bemisia tabaci* Genn. Whitefly resistance. *Rep. Cucurbit Genet. Co-operative* 16 : 87-89

Moyer, J.W., Kennedy, G.G. and Romanow, L.R. 1985. Resistance to Watermelon mosaic virus II multiplication in *Cucumis melo*. *Phytopathology* 75 (2) : 201-205

Muller, K.O. 1959. Hypersensitivity. *Plant Pathology - An Advanced Treatise* (Ed. Horsfall. J.G. and Diamond, A.E.) Academic Press, New York. p.469-519

Munger, H.M., Hentry, M. and Washek, R.L. 1983. Progress and procedure in breeding CMV resistant *Cucurbita pepo L.Rep. Cucurbit Genet.Co-operative* 6 : 82-83

Munger, H.M. and Provvidenti, R. 1987. Inheritance of resistance to Zucchini Yellow mosaic virus in *C. moschata*. *Rep.Cucurbit Genet.Co-operative* 10 : 80-81

Munger, H.M. 1993. The role of viruses in sudden wilt of melons in New York. *Rep Cucurbit Genet.Co-operative* 16 : 47-48

Nerkar, Y.S. and Jambhale, N.D. 1987. Breeding of okra for resistance to Yellow vein mosaic virus. *Indian J. Virol.* 3 (12): 94-98

Nuez, F., Esteva, J., Soria, C. and Guillamon, M.L.G. 1991. Research for sources of resistance to a whitefly transmitted yellowing disease in melon. *Rep.Cucurbit Genet Co-operative* 14 : 59

Oh, J.H. 1988. Changes in activity and electrophoretic pattern of peroxidase in early period of infection by soybean necrotic virus (SMV-N) in soybean genotypes. *Korean J. Pl Path.*4 (4) : 257-263

Pal, S.S., Dhaliwal, H.S. and Brains, S.S. 1991. Inheritance of resistance to Yellow mosaic virus in some *Vigna species. Pl.Breed.*106 (2) : 168-171

Pan, R.S. and More, T.A. 1996. Screening of melon (*Cucumis melo L.*) germplasm for multiple disease resistance. *Euphytica* 88 (2) : 125-128

Panse, V.G. and Sukhatme, P.V. 1978. *Statistical methods for Agricultural Workers.*(3rd. ed.), I.C.A.R., New Delhi. p. 68-75

Paran, I., Shifriss, C. and Raccah, B. 1989. Inheritance of resistance to Zucchini yellow mosic virus in the interspeicific cross *Cucurbita maxima X C. ecuadorensis. Euphytica* 42(3): 227-232

Paris, H.S., Cohen, S., Burger, Y. and Yoseph, R. 1988. Single gene resistance to zucchini yellow mosaic virus in *Cucurbita moschata. Euphytica* 37 (1) : 27-29

PDVR. 1997. *Proc. of the VI group meeting on vegetable research* held at Tamil Nadu Agricultural University. May 28 - 31, Project Directorate of Vegetable Research, Varanasi. p. 111

Pesis, E. and Na, T.J. 1986. The effect of seed coat removal on germination and respiration of muskmelon seeds. *Seed Sci. Technol.* 14(1): 117-125

Pessoa, H.B.S.V., Avila, A.C; Dellavecchia, P.T., Araujo, J.P. and Oliverira, D.L.O.B. 1988. Eldorado-300, a melon resistant to WMV-I. *Horticulture Brasileira* 6(1):40-41

Peterson, C.E., Staub, J.E., Crubaugh, L. and Palmer, M. 1986. Resistance to CMV in cucumber variety WI 5207. *HortScience* 21(2) : 335-336

Pink, D.A.C. and Walkey, D.G.A. 1985. Breeding for resistance to CMV in Courgette and Vegetable marrow. *Rep. Cucurbit Genet. Co-operative* 8: 74-75

Pink, D.A.C and Walkey, D.G.A. 1988. Breeding CMV resistant Courgette at the Institute of Horticultural Research, Wellesbourne. *Cucurbitaceae Proc. Eucarpia meeting Cucurbit Genetics and Breeding*, Avignon, Montfavet, France, May 31-June 2

Pitrat, M. and Lecoq, H. 1984. A rapid method to test muskmelon for several virus resitances. *Proc. of the third Eucarpia meeting on breeding of cucumber and melons*, 2-5 July. Plovdiv; Bulgaria. p 104-107

Pitrat, M. and Lecoq, H. 1984. Using different forms of virus resistance in melon. *Selectionneur Francais* 34: 29-37

Pitrat, M. and Lecoq, H. 1984. Inheritance of Zucchini Yellow Mosaic virus resistance in C. melo L. *Euphytica* 33: 57-66

Pitrat, M., Maestro, C., Ferriere, C., Ricard, M. and Alvarez, J. 1988. Resistance to *Aphis gossypii* in Spanish melon (*Cucumis melo*). *Rep. Cucurbit Genet. Co-operative* 11:50

Provvidenti, R. and Robinson, R.W. 1977. Inheritance of resistance to water melon mosaic virus-1 in *Cucumis metuliferus. J. Hered.* 68(1): 56-57

Provvidenti, R., Robinson, R.W. and Munger, H.M. 1978. Resistance in feral species to six viruses infecting *Cucurbita Pl. Dis. Rep.* 62:326

Provvidenti, R; Robinson, R.W. and Munger, H.M. 1978. Resistance in feral species to six viruses infecting *Cucurbita Pl. Dis. Rep.* 62(4): 326-329

Provvidenti, R. 1982. Sources of resitance or tolerance to viruses in accessions of *Cucurbita maxima. Rep. Cucurbit Genet. Co-operative* 5:46-47

Provvidenti, R. and Gonsalves, D. 1982. Resistance to papaya ring spot virus in *Cucumis metuliferus* and its relationship to resistance to watermelon mosaic virus-1. *J. Hered.* 73(3): 239-240

Provvidenti, R. 1984. Epidemics of Zucchini yellow mosaic virus and other cucurbit viruses in Egypt in the spring of 1983. *Rep. Cucurbit Genet. Co-operative* 7: 78-79

Provvidenti, R., Gonsalves, D. and Humaydan, H.S. 1984. Occurrence of Zucchini yellow mosaic virus in cucurbits from Connecticut, Newyork, Florida and California. *Pl. Dis.* 68(5) : 443-446

Provvidenti, R. 1985. Souces of resistance to viruses in two accessions of *Cucumis sativus Rep. Cucurbit Genet. Co-operative* 8:12

Provvidenti, R. 1986. Reaction of accessions of *Citrullus colocynthis* from Nigeria to ZYMV and other cucurbit viruses. *Rep. Cucurbit Genet. Co-operative* 9: 82-83

Provvidenti, R. 1986. Viral diseases of cucurbits and sources of resistance. *Tech. Bull. Food and Fertilizer Technology Centre*, Taiwan (93):36

Provvidenti, R. 1987. Inheritance of resistance to a strain of zucchini yellow mosaic virus in cucumber. *HortScience* 22(1):102-103

Provvidenti, R. 1991. Inheritance of resistance to the Florida strain of ZYMV in watermelon. *HortScience* 26(4):407-498

Rajamony, L., More, T.A., Seshadri, V.S. and Varma, A. 1987. Resistance to cucumber green mottle mosaic virus (CGMMV) in musk melon . *Rep. Cucurbit Genet. Co-operative* 10:58-59

Rajamony, L., More, T.A., Seshadri, V.S. and Varma, A. 1990. Reaction of muskmelon collections to cucumber green mottle mosaic virus *J. Phytopath.* 129(3) : 237-244

Rajamony, L., More, T.A. and Seshadri, V.S. 1990. Inheritance of resistance to cucumber green mottle mosaic virus in muskmelon (*Cucurmis melo* L.). *Euphytica* 47 (2):93-97

Rajan, S. 1985. Selection efficiency and genetic and biochemical bases of resistance to bacterial wilt in tomato. Ph.D. thesis, Kerala Agricultural University, Vellanikkara, Thrissur

Reuveni, R. and Bothman, G.C. 1985. The relationship between peroxidase activity and resistance to *sphaerotheca fuliginea* in melon. *Phytopathologische Zeitschrift* 114(3):260-267

Reuveni, R., Shimoni, M. and Karchi, Z. 1990. A rapid assay for monitoring peroxidase activity in melon as a marker for resistance to *Pseudoperonospora cubensis. J. Phytopath.* 129(4): 333-338

Robinson, R.W., Weeden, N.F. and Provvidenti, R. 1988. Inheritance of resistance to Zucchini yellow mosaic virus in the inter-specific cross *C. maxima* x *C. ecuadorensis. Rep. Cucurbit Genet. Co-operative* 11:74-75

Rosemeyer, M.E. and Bemis, W.P. 1981. Virus studies with *C. foetidissima. Rep.Cucurbit Genet. Co-operative* 4: 41-42

Russel, G.E. 1981. Resistance to virus diseases. In *Plant Breeding for pest and disease resistance.* (Ed. Russel, G.E.) (2nd ed.) Butterworths and Co; London. p. 216-221

Sadasivam, S. and Manickam, A. 1992. *Biochemical Methods for Agricultural Sciences.* Wiley Eastern Limited. p. 58-64

Sadhankumar, P.G. 1995. Incorporation of resistance to fruit cracking in a bacterial wilt resistance genetic background in tomato. Ph.D. thesis, Kerala Agricultural University, Vellanikkara, Thrissur

Salama, E.A. and Still, W.H. 1968. Resistance to squash mosaic virus strains among *Cucurbita species. Gran Kans Acad. Sci.* 71:62

Sanada, M., Itoh, R.T., Shimokawa, H. and Matsushita, K. 1994. Increase of β-1,3-Glucan hydrolase and acquisition of resistance in White Burley leaves by Tobacco mosaic virus and Salicylate. *Ann. Phytopath. Soc. Jap.* 60:469-477

Sarkar, S.J., Das, L. and Biswas, A.K. 1990. Electrophoretic evaluation of basic seed proteins of control and hybrid muskmelons of *Linun usitatissimum L. Exp. Genet.* 6 (1 & 2) : 6-9

Sekioka, T.T., Takeda, K., Tana, J.S. and Grilbert, J.C. 1988. Milo Cucumber *HortScience* 23 (3):640

Shahwan, I.M.A.I., Abdalla, O.A. and Saleh, M.A.A.I. 1995. Response of green house cucumber cvs. to an isolate of Zucchini yellow mosaic virus. *Pl. Dis.* 79 (9): 898-901

Shan, Z.H. and Tan, Y.J. 1994. A preliminary study of groundnut wilt resistance mechanisms *Groundnut Bacterial wilt in Asia*, ICRISAT 3 : 99-100

Shankar, G., Nariani, T.K. and Prakash, N. 1972. Studies on pumpkin mosaic virus (PMV) with particular reference to purification, electron microscopy and Serology. *Indian J. Microbiol.* 12: 154-165

Sharma, A., Uddhya, P.P. and Tiwari, C.B. 1990. Phenolic content of bottlegourd as influenced by the infection of CGMMV. *National Acad. Sci. letter* 13 (4): 115-116

Sharma, B.D. and Hore, D.K. 1996. Indian cucumber germplasm and challenges ahead. *Genet. Res.and Crop Evolution* 43 (1) : 7-12

Shifriss, O., Myers, C.H. and Chupp, C. 1942. Resistance to mosaic virus in the cucumber *Phytopathology* 32 (6): 773-784

Siegel, B.Z. 1993. Plant peroxidases-an organismic perspective. *Pl. Growth Regulation* 12: 303-312

Singh, D., Nandpuri, K.S. and Sharma, B.R. 1976. Inheritance of some economic quantitative characters in an inter-varietal cross of muskmelon (*Cucumis melo* L). *J. Res* 13(2):172-176

Singh, D.K. and Abidi, A.B. 1988. Biochemical transformation in leaves of *Lycopersicon esculentum Mill* varieties affected by leaf curl and mosaic disease. *Prog. Hort.* 20 (3-4): 185-89

Srivastava, R.P. and Kumar, S. 1988. Estimation of beta-carotene. *Fruit and vegetable Preservation-Principles and Practices.* International Book Distributing Co. p.236

Sudha, N. 1991. Evaluation of interspecific hybrid derivatives of tomato (*Lycopersicon esculentum Mull.*) for resistance to TSWV and economic characters. Thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

Suresh Babu, U. 1989. Divergence studies in pumpkin (*Cucurbita moschata* Poir). M.Sc. Thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala.

Takada, K. 1982. Breeding methods for disease resistance of melon and development of new lines with combined resistance. *Jap. agric. Res. q.*16(2): 94-104

Tan, Y.J., Duan, N.X., Liao, B.S., Xu, Z.Y., He, L.Y. and Zheng, G.R. 1994. Status of groundnut bacterial wilt research in China. *Groundnut Bacterial Wilt in Asia.* ICRISAT 3:107-113

Thakur, T.C., Khattra, A.S. and Dhanju, K.C. 1996. Evaluation of bittergourd genotypes against diseases and their correlation with other quantitative characters. *Punjab Vegetable Grower* 31: 25-28

Tripathi, G. and Joshi, R.D. 1985. Watermelon mosaic virus in pumpkin. (*C. maxima*) *Indian Phytopath.* 38(2): 244-247

Umamaheswaran, K. 1985. Transmission, physical properties and host range of pumpkin mosaic virus. M.Sc. Thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala.

Valkonen, J.P.T. and Makarainen, E. 1993. Symptom expression and accumulation of Potato virus Y (PVY) and Potato leaf roll virus in thirteen potato cultivars. *Agric. Sci. Finl* 2: 33-40

Vallejos, C.E. 1983. Enzyme staining In. *Isozymes in Plant Genetics and breeding.* Part A (Eds. S.D. Tanskley and T.J. Orton)Elsevier, Amsterdam, p. 469-516

Varma, P.M. 1955. Ability of the whitefly to carry more than one virus simultaneously. *Curr Sci.* 24 : 217-318

Vecchia, P.T. and Avila, A.C. 1985. Inheritance of resistance to Watermelon mosaic virus-1 (WMV-1) in melon. *Fitopatologia Brasileira* 10(3):467-474

Velich, I. and Tobias, I. 1985. Breeding for resitance to CMV in muskmelon *Proc. of the third Eucarpia meeting on breeding of cucumbers and melons.* p.108-111

Vidhyasekaran, P. 1990. *Physiology of Disease Resistance in Field Crops.* Today and Tomorrow's Printers and Publishers, New Delhi. p.137

Visedo, G., Piqueras, J.F. and Garcia, J.A. 1990. Isozyme profiles associated with hypersensitive response of *Chenopodium foetidum* to plum pox virus infection. *Physiologia Plantarum* 78: 218-224

Wagih, E.E. 1992. Amylase activity may play a role in the pathosmosis thought to develop in the areas of virus localisation in plants. *J. phytopath* 134 (1): 22-26

Wai, T., Stommel, J.R., Tousignat, M.E. and Kaper, J.M. 1995. Field test of satellite-transgenic tomato resistance against CMV. *Phytophthology* 85 (4) : 513

Walkey, D.G.A. and Pink, P.A.C. 1984. Resistance in vegetable marrow and other *Cucurbita* spp. to two British strains of CMV. *J. agric. Sci.* 102 (1) : 197-205

Wang, Y.J., Provvidenti, R. and Robinson, R.W. 1984. Inheritance of resistance to watermelon mosaic virus-1 in cucumber *HortScience* 19 (4) : 587-588

Washek, R.L. 1983. Cucumber mosaic resistance in summer squash (*C. pepo* L.) *Diss. Abstr. int.* 44 (6) : 1662 B

Wasuwat, S.L. and Walker, J.C. 1961. Inheritrance of resistance in cucumber to cucumber mosaic virus. *Phytopathology* 51 (7) : 423-428

Xia, Z.J., Gu, B.K., Wu, A.M., Li, J.Y., Chen, Y.L. and Chen, X.B. 1994. Studies on the biochemical components in cotton related to the resistance to *Verticillium dahliae*. *Acta Phytophylacia Sinica* 21(4):305-310

Yang, W.Z., Hsiao, C.H. and Chang, W.N. 1986. Screening cucumber for resistance to viruses and genetic studies on resistance to Zucchini Yellow Mosaic Virus. *J. agric. Res.* China 35 (2) : 192-201

Yoshioka, K., Hanada, K., Harada, T., Minobe, Y. and Oosawa, K. 1993. Virus resistance in transgenic melon plants that express the CMV coat protein gene and in their progeny *Jap. J. Breed.* 43 (4) : 629-34

Yu S.Q. and Wang, S.Z. 1990. Study on appraisal methods for assessing resistance to fusarial wilt disease in watermelon. *Sci. Agric. Sinica* 23 (1) : 31-36

Yurina, O.V., Anikina, I.I. and Yurina, T.P. 1990. Search for resistant forms. *Kartofel.ovoshchi* 3 : 25-26

Zatyko, L. 1981. Responses of varieties of pepper resistant to TMV infection under different conditions. *Zoldsegtermesztesi Kutato Intezit Bulletinji* 14 : 5-11

Appendices

APPENDIX I

Weather parameters of experimental area (Sugarcane Research Station, Thiruvalla)

Year / month	Temperature (°C)		Total rainfall (mm)	No. of rainy days	R.H. (%)
	Maximum	Minimum			
January, 1994	32.60	19.90	42.80	2	59.50
February, 1994	32.73	20.66	53.60	7	57.63
March, 1994	35.46	22.31	6.20	7	58.75
April, 1994	32.98	22.88	161.60	2	59.50
May, 1994	31.22	23.37	388.10	15	59.50
June, 1994	29.85	23.00	580.40	13	63.00
July, 1994	29.20	22.10	711.40	23	65.30
August, 1994	34.40	22.00	229.60	26	59.80
September, 1994	30.30	23.00	233.40	12	56.50
October, 1994	30.80	22.10	427.10	21	53.50
November, 1994	31.10	22.60	77.00	5	57.00
December, 1994	31.60	22.80	-------	---	58.26
January, 1995	32.80	22.00	28.00	2	58.10
February, 1995	33.50	22.20	33.60	4	58.20
March, 1995	34.00	24.60	3.00	1	56.30
April, 1995	34.80	26.30	86.00	7	58.70
May, 1995	37.00	23.00	293.52	16	60.40
June, 1995	30.00	23.00	693.00	28	65.80
July, 1995	30.60	22.50	690.00	28	66.20
August, 1995	35.00	22.60	232.00	14	63.40
September, 1995	32.00	23.80	212.00	15	56.50
October, 1995	30.40	21.80	228.00	13	52.60
November, 1995	31.20	21.80	156.00	12	55.00
December, 1995	30.87	20.60	43.00	2	55.60

(Continued.........)

APPENDIX I (Continued.....)

Weather parameters of experimental area (Sugarcane Research Station, Thiruvalla)

Year / month	Temperature (^{0}C)		Total rainfall (mm)	No. of rainy days	R.H. (%)
	Maximum	Minimum			
January, 1996	31.20	20.00	28.00	2	61.40
February, 1996	34.00	22.40	22.00	3	59.30
March, 1996	34.80	24.60	1.60	1	58.00
April, 1996	36.40	23.00	78.30	7	63.00
May, 1996	34.00	24.20	194.00	16	68.00
June, 1996	32.00	21.60	793.00	13	72.00
July, 1996	30.60	22.30	718.00	31	74.00
August, 1996	35.80	23.40	791.00	26	78.00
September, 1996	33.00	22.40	199.00	10	72.00
October, 1996	30.60	21.80	313.00	19	33.00
November, 1996	31.00	20.60	79.00	6	69.00
December, 1996	30.20	20.60	5.00	1	62.00
January, 1997	32.80	22.00	16.00	1	64.00
February, 1997	33.00	23.60	12.00	6	62.03
March, 1997	34.00	23.20	3.00	1	63.00
April, 1997	34.60	25.20	90.00	5	64.00
May, 1997	33.20	26.00	302.00	14	68.00
June, 1997	30.60	24.60	699.00	20	72.00
July, 1997	30.60	23.80	710.00	22	74.00
August, 1997	33.60	22.20	415.00	12	80.20
September, 1997	31.00	24.20	324.00	11	73.20
October, 1997	33.10	19.80	186.00	22	53.60
November, 1997	31.50	21.60	196.60	13	62.00
December, 1997	31.03	19.20	214.40	11	59.00
January, 1998	33.00	21.50	--------	----	58.00
February, 1998	34.20	22.20	--------	----	59.00
March, 1998	35.50	23.00	1.40	1	56.50
April, 1998	35.00	22.50	7.50	3	63.50
May, 1998	34.00	23.50	84.80	9	64.00
June, 1998	32.50	22.00	634.20	27	65.00

APPENDIX II

Monochromatic silver staining (Hames, 1994).

Prepared the following stock solutions using deionised water.

a. Solution A. 0.8 g Ag No3 in 4 ml distilled water

b. Solution B. Mixed 20 ml of 0.36% NaOH with 1.4 ml freshly prepared 14.8 M NH_4OH. (0.9 ml of NH_4OH diluted to 1.4 ml for a total stain solution volume of 100ml).

All the following procedures were carried out in a glass dish.

i) Soaked the gel in 50% MeOH for one hour.

ii) Prepared the silver stain by adding solution A dropwise to solution B with constant stirrring and then making up to 100 ml with deionised H_2O. Prepared silver stain just before use. Stained the gel in this solution for 15 minutes with gentle agitation, on a platform shaker.

iii) Washed the gel in deionised H_2O for five minutes with gentle agitation.

iv) Soaked the gel in developing solution until bands appeared.

v) Washed the gel with deionised H_2O and placed in 50% MeOH to stop further development.

Developing solution

25 ml of 1% citric acid was mixed with 0.25 ml of 37% formaldehyde and made upto 500 ml with deionised H_2O. The solutin was prepared just before use.

APPENDIX III

General analysis of variance

Sources of variation		Parents	Error
df		4	12
Seed germination	Normal	3899.20*	12.80
	After seed coat removal	814.80*	16.933
No. of fruits developed		0.211*	0.064
Fruit development percentage		84.83*	1.845
No. of female flowers per plant		38.56*	4.652

* Significant at 5% level

APPENDIX IV

Original and reassigned numbers of test plants

Amb x NL		PV x NL		AC x NL		Col x NL	
Original	Reassigned	Original	Reassigned	Original	Reassigned	Original	Reassigned
F_1-1	F_1-1	F_1-1	F_1-2	F_2-1	F_2-8	F_2-1	F_2-13
F_2-1	F_2-1	F_1-2	F_1-3	F_2-2	F_2-9	B_1-2	B_1-7
F_2-2	F_2-2	F_2-1	F_2-5	F_2-4	F_2-10	B_2-7	B_2-10
F_2-3	F_2-3	F_2-3	F_2-6	F_2-5	F_2-11		
F_2-4	F_2-4	F_2-4	F_2-7	F_2-6	F_2-12		
B_1-1	B_1-1	B_1-1	B_1-3	B_1-1	B_1-5		
B_1-2	B_1-2	B_1-2	B_1-4	B_1-2	B_1-6		
B_2-3	B_2-1	B_2-1	B_2-5	B_2-6	B_2-7		
B_2-6	B_2-2	B_2-8	B_2-6	B_2-9	B_2-8		
B_2-7	B_2-3			B_2-10	B_2-9		
B_2-10	B_2-4						

BREEDING FOR RESISTANCE TO MOSAIC VIRUSES IN PUMPKIN (*Cucurbita moschata* Poir)

By

JESSY M. KURIAKOSE

ABSTRACT OF A THESIS

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Kerala Agricultural University

DEPARTMENT OF OLERICULTURE
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 654
KERALA, INDIA

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ABSTRACT

Pumpkin Mosaic Virus (PMV) and Yellow Vein Mosaic Virus (YVMV) are the most devastating diseases of pumpkin in Kerala and the only way to manage this is to use virus resistant varieties. Investigations were carried out at Sugarcane Research Station, Thiruvalla, Kerala Agricultural University to identify source(s) of resistance to PMV and YVMV, to study the genetics and biochemical mechanism of mosaic virus resistance and to identify desirable segregants possessing combined resistance to PMV and YVMV.

Six species of *Cucurbita* (103 genotypes) were used for the study. Only one accession Nigerian Local (NL), introduced form Cornell University possessed high level of resistance to both the viruses.

The mosaic resistance confirmation studies namely, back inoculation, grafting and multi-environment study clearly established that the reaction in NL can be considered as stable. Neither the inoculated leaves nor the uninoculated subsequent leaves of the susceptible variety Ambili showed symptoms when back inoculated with sap from NL in case of PMV. Through back inoculation, viruses could not be isolated from this genotype thereby proving that the resistance is not symptomless carrier type of tolerance. NL did not develop any disease symptom even after grafting on the YVMV infected symptomatic root stock. The environmental fluctuations also did not influence the resistance expression of NL against both PMV and YVMV.

Resistance to PMV was evaluated in five parents and their F_1, F_2 and backcross generations of four cross combinations namely, Amb x NL, PV x NL, AC x NL and Col X NL. On an average 96.25 per cent plants in the F_1 showed susceptibility. The gene for susceptibility to PMV had only 95 percent penetrance in PV x NL. Under different genetic background the role of gene modifiers in the disease susceptibility expression of the major gene in AC x NL can not be ruled out. The F_2 segregation ratio in all the crosses was in agreement with the Mendelian genetic ratio of 3 : 1 (susceptible : resistant). The test cross (F_1 back crossed to NL) confirmed this with a genetic ratio of 1:1 (resistant : susceptible) in all the four crosses studied. The inheritance studies in six generations of all the four cross combinations clearly revealed that the resistance to PMV in pumpkin is controlled basically by a single recessive gene. The role of gene modifiers affecting the expression of this major gene is to be investigated further.

Reaction to YVMV in the six generations i.e., P_1, P_2, F_1, F_2, B_1 and B_2 of the four cross combinations under study revealed that the resistance is controlled by a single dominant gene. In the F_1 on an average out of 40 plants only 32 were resistant. Hence the influence of gene modifiers in the resistant expression of this dominant gene for resistance to YVMV in heterozygous conditions can not be ruled out and is to be further analysed. The reciprocal crosses also behaved in a similar fashion. Out of 300 F_2 plants in each cross, a mean number of 220 plants were resistant and 80 susceptible, fitting in a 3:1 ratio indicating the monogenic dominance of resistance. When F_1 was back crossed to the susceptible parent, the segregation of resistance and susceptibility was in equal proportion i.e., out of a mean number of 100 B_1 plants inoculated 48 were resistant. This very well fitted into 1:1 ratio of resistance : susceptibility. So resistance to YVMV is governed basically by a dominant gene, slightly influenced by gene modifiers.

Although NL is a good source of resistance to both the viruses under study, the seed germination was as low as 19 per cent. Through seed coat removal, 62 per cent seed germination (43%increase) was noted in NL.

The isozyme pattern of peroxidase and esterase enzymes in susceptible and resistant genotype of pumpkin as well as their F_1 hybrids was analysed, before inoculation and after inoculation of both viruses. The resistant and susceptible genotypes showed clear cut differences in their peroxidase profile. The susceptible parents had three bands namely, PRX1, PRX3 and PRX6, while the resistant parent NL expressed only one isoform - PRX1 (0.083) before virus inoculation. The hybrid PV x NL exhibited six bands with three additional bands, PRX2, PRX3 and PRX5. The additional band PRX2 can be attributed to the earliness in growth and development of this hybrid.

After inoculation of PMV, the band PRX1 expressed before inoculation was not expressed in both the resistant and susceptible parents as well as in their F_1 hybrids. In the resistant parent NL an additional band, PRX5 (Rm value 0.463) was noted. This particular isoform might be responsible for resistance to PMV in NL. After infection of YVMV, the resistant parent as well as the resistant F_1 hybrids had the additional band PRX4 (Rm value 0.392).

There were six bands of esterase in the susceptible parents and only three in the resistant before virus inoculation. The resistant parent after inoculation of PMV, exhibited three additional bands EST1 (0.368), EST4 (0.632) and EST5 (0.721). Consequent to YVMV infection, the additional band EST 4 (Rm value 0.632) observed in NL was thick and the specific activity of this particular isoform might also be responsible for the resistance mechanism.

Consequent to virus infection, the total system of esterase was affected, while peroxidase was comparatively stable. The resistant expression of NL against PMV infection can be attributed to the expression of the additional band PRX5 (0.463) and the resistant mechanism against YVMV in NL as well as in the F_1 hybrids can be due to the activity of the isoform PRX4 (0.392).

Sequential inoculation with PMV followed by YVMV was carried out in 25 seedlings each in the F_1, F_2, B_1 and B_2 generations of the four cross combinations namely, Amb x NL, PV x NL, AC x NL and Col x NL to identify plants with combined resistance to both the viruses.

In the F_1s, eight plants were identified as resistant to both the viruses i.e., one in Amb x NL, two in PV x NL, three in AC x NL and two in Col x NL. In the F_2, B_1 and B_2 populations, there were 17, 9 and 37 seedlings respectively, resistant to both the viruses.

All the 71 seedlings possessing combined resistance to PMV and YVMV were further evaluated in the field for their biometric and horticultural characters. The studies in general indicated earliness of F_1s compared to F_2s, B_1s and B_2s. On an average, the fruit yield in terms of number and weight of fruits in all the crosses was maximum for the F_1 plants.

In all the crosses, the plants belonging to the F_1, F_2, B_1 and B_2 generations produced fruits with external fruit colour as well as flesh colour of either the female parent or the male parent or with a blend of these two colours.

Based on resistance to PMV and YVMV, fruit smoothness, non-incidence of other mosaic viruses and yield of fruits, eight most promising superior segregants from among the four cross combinations were selected for further improvement. They included four in Amb x NL, two from Col x NL and one each from PV x NL and AC x NL.