STUDIES ON PAPAYA RINGSPOT VIRUS INFECTING *Carica* species

BY

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CANDIDATE'S DECLARATION

I hereby declare that the dissertation or part thereof has not been previously submitted by me to any other University or Institute for a degree or diploma

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Shri P. Ramakrishna Rao has satisfactorily prosecuted his course of research for a period of not less than four semesters and that the dissertation entitled " STUDIES ON PAPAYA RINGSPOT VIRUS INFECTING Carica species" submitted by him is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the dissertation or part thereof has not been previously submitted by him for a degree of any University.

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CERTIFICATE- II

This is to certify that the dissertation entitled " STUDIES ON PAPAYA RINGSPOT VIRUS INFECTING Carica species" submitted by Shri P. Ramakrishna Rao to the Marathwada Agricultural University, Parbhani, in partial fulfilment of the requirements for the degree of " Doctor of Philosophy in the subject of Plant Pathology", has been approved by the student's Advisory Committee after oral examination in collaboration with the external examiner.

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INTRODUCTION

1. INTRODUCTION

The papaya (*Carica papaya* L.) is an unusually interesting plant of tropical and subtropical regions of the world producing fruits of many uses. It is a native of tropical America and was introduced into India in the 16th century. The papaya is now grown in all the tropical and subtropical countries including Australia, Bangladesh, Brazil, Burma, Caribbean Islands, Central and South Africa, Hawaii, India, Mexico, Pakistan, Peru, Phillippines, Puerto Rico, Sri Lanka, Taiwan; the USA (Texas, Florida and California) and Venezuela.

Botanically, *Carica papaya* L., is the type species of the family Caricaceae within the order Passiflorales. According to Harms (1925), the Caricaceae are divided into four genera viz., *Carica*, *Monoica*, *Jacaratia* and *Cylicomorpha*. The first three belong to tropical America, particularly Mexico, and *Cylicomorpha* had its original habitat in Africa. The genus *Carica* contains about 40 species, but only three are of horticultural importance. These are *C. papaya*, the common papaya; *C. candamarcensis*, known as mountain papaya, and *C. monoica* found in Amazon basin.

Papaya not only a source of food but has many uses in industry and medicine. The fruits can be used in the preparation of salads, pies, jams, ice-cream flavouring, crystallised fruits and are canned in syrup. The seeds are also used for their medicinal value and unriped fruits are commonly used as vegetable in cooking. Papain, prepared from dried latex of immature fruits, is a proteolytic enzyme similar in action to pepsin, is in great demand in the international market, particularly in the UK and the USA. Papain has several uses in the industry as an essential ingredient in pharmaceutical, textile, tanning and brewing industries (Purseglove, 1968; Foyet, 1972). Papain is used in tenderising meat; cleaning of beer; in the manufacture of cosmetics; in the degumming of silk and rayon; in the pre-shrinking of wool and in the tanning of leather. It has also several uses in the medical field including treatment of necrotic tissues, dyspepsia, and other digestive ailments, ringworm and roundworm infections, skin lesions and ulcers, eczema and other skin diseases and in kidney disorders. Papain is used in detecting stomach and intestinal cancer and also in correcting diphtheria. According to Singh et al. (1983), an alkaloid carpaine produced by papaya has been utilized as a diuretic and a heart stimulant.

Additionally, papaya is also a wholesome, delicious and refreshing fruit occupying an important place in human nutrition as a rich source of vitamins and minerals. Aykroyd (1951) ranked it second only to mango as a source of the precursor of vitamin A. The ripe papaya fruit has the following composition and food value: moisture 89.6 per cent, protein 0.5 per cent, fat 0.1 per cent, carbohydrate 9.5 calcium 0.01 per cent, phosphorus 0.01 per cent, iron 0.4 per cent, vitamin A 2020 IU/100 g, B2 0.04 mg/100 g, vitamin C 40 mg/100 g, nicotinic acid 0.2 mg/100 g, riboflavin 250 mg/100 g, and calorific value 40 per 100 g (Muthukrishnan and Irulappan, 1985).

In India, papaya is grown in almost all the states. Though, it is successfully grown all over the country, the important papaya growing states are Bihar, Assam, Madhya Pradesh, Maharashtra, Tamil Nadu, Gujrat, Uttar Pradesh and Andhra Pradesh. According to Singh (1979), it is grown nearly on 10,000 hectares area in the country. In Maharashtra, the area under papaya is about 2,000 hectares, mostly concentrated in the districts of Jalgaon (500 ha), Dhule (200 ha), Nasik (100 ha), Thane (100 ha), Ahmednagar (100 ha), Pune (100 ha), Solapur (100 ha), Aurangabad (100 ha), Parbhani (100 ha), Buldana (100 ha), Akola (100 ha), Amravati (100 ha), Yavatmal (100 ha), Wardha(100 ha) and Nagpur (100 ha) (Anonymous, 1979- 80).

A large number of so-called papaya varieties is found in cultivation. As a matter of fact, none of these is a real variety, since papaya is a highly cross- pollinated crop, the plants raised from seeds of these varieties have mixed inheritance which makes them highly variable in performance. These varieties include Bangalore, Barwani, Ceylon, Co-1, Co-2, Co-3, Co-4, Co-5, Co-6, Gujrati, Honey Dew, Coorg Honey Dew, Kurghani, Madhu Bindu, Philippines, Poona Long, Poona Round, Pant Papaya-1, Pant Papaya-2, Pant Papaya-3, Pusa Delicious, Pusa Dwarf, Pusa Giant, Pusa Majesty, Ranchi, Red Fleshed, Selection No. 7, Singapore, Solo, Solo Hawaii, Sun Rise Solo and Washington. In Maharashtra, the variety Washington is popular.

The evergreen and fragile papaya bearing edible fruits of many uses, is being subjected to attack by a large number of diseases. Several serious diseases of papaya have been reported. These include diseases caused by bacteria, fungi, mycoplasma- like organisms (MLOs), nematodes and viruses (Simmonds, 1965; Cook, 1972; 1975; Pathak, 1980; Verma and Prasad, 1985/86). The bacterial, fungal, MLO, nematode and viral pathogens infecting papaya are listed as below.

I. Bacterial diseases :

1.	Bacterial leaf spot	:	Pseudomonas carica-papayae Robbs.
2.	Bacterial necrosis	:	Bacillus papayae Rant.

II. Fungal diseases :

1.	Anthracnose	:	Colletotrichum gloeosporioides (Penz.) Sacc.
2.	Stem, foot or root rot and dampingoff	:	Pythium aphanidermatum (Eds.)Fitz. Phytophthora parasitica Dast. Corticium solani (Prill. & Del.) Bourd. & Galz.
3.	Curvularia leaf spot	:	Curvularia carica- papayae Srivastava and Bilgrami.
4.	Phyllosticta leaf spot.	:	Phyllosticta sulata Chowdhury.
5.	Ascochyta leaf spot	:	Ascochyta caricae- papayae Tar.
6.	Leaf blight	:	Helminthosporium rostratum Drechl.
7.	Cercospora leaf spot	:	Cercospora papayae Hansford.
8.	St.Croix decline	:	Corynespora cassicola(Berk. & Curt.) Wei.
9.	Powdery mildews	:	Ovulariopsis papayae Vander Bilz. Oidium caricae Noack. Sphaerotheca fuliginea(Schlecht. ex.Fr.)Poll.

10. Ripe fruit rots : Alternaria tenuis Ness.ex. Pers.

: Phomopsis caricae- papayae Petrk. & Cif.

: Ascochyta caricae Pat.

: Colletotrichum gloeosporioides (Penz.) Sacc.

: C. acutatum Simmonds.

: C. dematium (Pers.ex. Fr.) Grove.

11. Watery fruit rot : Rhizopus stolonifer(Ehrenb.ex.Fr.) Lind.

III. MLO diseases :

1. Bunchy top : Vectors: i) Empoasca papayae Oman. (Adsuar, 1946; Story and Halliwell, 1969).

ii) E. stevensi Young. (Haque and Parasram, 1973)

IV. Nematodes :

1. Root-knot nematode : Meloidogyne incognita(Kofold & White) Chitwood.

2. Reniform nematode : Rotylenchulus reniformis (Linford & Oliveria).

V. Virus diseases:

Quite a few virus diseases have been recorded on papaya, some of which have been well studied. The viruses naturally infecting papayas and their properties are summarised and presented in a tabular form.

Table showing characteristics of viruses infectuous to papaya (*Carica papaya* L.)

Sr. No.	Name of the disease	Transmission			Physical properties			Particle morphology		Virus group	Distribution	Key references
		Seed	Sap	Vector/Graft	TIP	DEP	LIV	Shape	Size (nm)			
1.	Apical necrosis	Not known	-	Leafhopper (*Empoasca papayae*)	Not known	Not known	Not known	Bullet shaped	180.254 x 80.98	Rhabdo-virus	USA., Venezuela	Lastra and Quintero, 1981; Wan and Conover, 1981
2.	Isabella mosaic	-	+	Nematode(*Trichodorus christiei*)	60°C	10^{-2}	72	Not known	Not Known	Not known	Puerto Rico	Adsuar, 1972
3.	Leaf curl	-	-	Whitefly(*Bemisia tabaci*)	Not known	Not known	Not known	Isometric,	18 x 30	Gemini-virus	India, Phillioines.	Sen *et al.*,1946; Nariani, 1956; Reyes *et al.*,1959; Goodman,1981
4.	Lethal yellowing	Not known	+	Not known	Not known	Not known	Not known	Isometric	29-32	Not known	Pernambuco	Loreto *et al.*,1983.
5.	Mosaic	-	+	Not known	73-76°C	10^{-4}	180 days	Flexuous filaments.	530	Potex-virus	USA,Venezulea, India, Africa	de Bokx,1965; Zettler *et al.*,1968; Purcifull and Hiebert,1971; Sureka *et al.*,1977.
6.	Ringspot	-	+	Aphics(*Aphis craccivora*, *A.gossypii*, *A.spiraecola*, *Myzus persicae* etc.)	54-65°C	10^{-3}	8-96 hrs.	Flexuous filaments.	700-800	Poty-virus	USA,Caribbeean Islands, South America, Africa, Asia,Far East.	Conover,1964a; de Bokx,1965; Zettler *et al.*,1968; Story and Halliwell,1969; Purcifull,1972; Sureka *et al.*,1977; Yemewar and Mali,1980; Purcifull *et al.*,1984.
7.	Spotted wilt	-	+	Thrips(*Frankliniella fusca*, *F. occidentalis*, *F.schultzei*, *Thrips tabaci*).	46°C	10^{-3}	12 hrs or less	Isometric	70-90	Tomato spotted wilt virus	Hawaii (USA)	Trujillo and Gonsalves, 1967; Gonsalves,1968; Best,1969; Ie, 1970.
8.	Tobacco ringspot	Not reported in papaya	+	Nematode(*Xiphinema americanum*)	70°C	10^{-4}	6-10 days	Isometric	28	Nepo-virus	Texas (USA)	McLean and Olson,1962; Lambe,1963; Stace-Smith,1970.

(+) = positive transmission, (-) = negative transmission.

Of the various diseases infecting papaya, "papaya ringspot virus" (PRSV), "papaya mosaic virus" (PMV) and stem rot or damping off are important. Furthermore, virus diseases of papaya, especially PRSV is known to reduce fruit yield, latex and sugar content of fruits (Holtzmann and Ishii, 1963; Ishii and Holtzmann, 1963; Khurana, 1970; Sanchez de Luque et al., 1980; Barbosa and Paguio, 1982b).

Papaya virus diseases are generally grouped as "ringspots" or " mosaics" and are usually associated with "poty" or "potex" virus groups (Cook, 1972). It has been suggested by de Bokx (1965) that the name " papaya ringspot virus" (PRSV) be used for viruses which are aphid transmissible and whose particles are approximately 780 nm long with "potyvirus group" characteristics (Harrison et al., 1971; Purcifull, 1972) and "papaya mosaic virus" (PMV) be used for viruses which are not aphid transmissible and whose particles are approximately 533 nm long with "potex virus group" characteristics (Purcifull and Hiebert, 1971).

Earlier, an aphid transmissible virus disease of papaya characterised by mosaic, mottling, leaf distortion and reduction of leaf-lamina into filiform structures; characteristically elongated dark green streaks on petioles and stem; spots and rings on fruits and stunting of whole plant has been reported from Marathwada region during

1977-78 (Yemewar and Mali, 1980). Based on limited studies like transmission, host range, physical properties and serological tests, an aphid transmissible virus disease of papaya prevalent in Marathwada region was identified as " papaya ringspot virus" (PRSV) (Yemewar and Mali, 1980).

The occurrence of PRSV at an alarmingly high proportions in Washington, Solo and other newly released varieties like Co-1, Co-2, Co-3, Co-4 and Co-5 in recent years has become a cause of concern to papaya cultivation and papain production in Maharashtra State. Since, earlier identification of PRSV (Yemewar and Mali, 1980) was based on limited studies, it was felt necessary to identify the virus unequivocally using biological, serological and physical tests, which may aid in devising suitable control strategies of the disease. Further, it was also felt necessary to ascertain the presence of PRSV in other regions of Maharashtra to know its distribution and to verify the natural reservoirs and vectors of the virus so as to elucidate its ecological aspects. The present investigation being therefore, planned towards the isolation, identification and partial characterisation of the virus inciting "ringspot" disease of papaya prevalent in Maharashtra and also to identify the sources of resistance in Carica species and varieties to PRSV. Therefore, following studies were

undertaken during the period of August 1985 to July 1987 and the results of which are embodied in this thesis.

1. Collection and establishment of PRSV isolates from different regions of Maharashtra State, to study and confirm the identity of the casual virus and also strains if any.

2. Transmission studies viz.,

   i) mechanical
   ii) insect (aphid)
   iii) seed
   iv) graft
   v) dodder

3. Physical properties viz.,

   i) thermal inactivation point (TIP)
   ii) dilution end point (DEP)
   iii) longevity <u>in vitro</u> (LIV)

4. Host range

5. Electron microscopy viz.,

   i) leaf-dip preparation
   ii) ultrathin sectioning

6. Serology

7. Screening of species and varieties of papaya against PRSV to identify the sources of resistance.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Virus diseases of papaya (*Carica papaya* L.) have been reported nearly from every continent where papaya is grown, and, in many areas, severely restrict profitable production (Cook, 1972). The first report of a papaya disease being attributed to a virus was made by Smith (1932) in Jamaica in 1929. Since then and until recently quite a few virus diseases have been recorded on papaya. These include "leaf curl" (Sen *et al.*, 1946; Nariani, 1956; Reyes *et al.*, 1959), " mosaic" (Conover, 1962; Purcifull and Hiebert, 1971), " ringspot" (Jensen, 1949a,b; Purcifull, 1972), "tomato spotted wilt" (Gonsalves, 1968), "tobacco ringspot" (McLean and Olson, 1962)," apical or droopy necrosis" (Lastra and Quintero, 1981; Wan and Conover, 1981), "lethal yellowing" (Loreto *et al.*, 1983) and "isabella mosaic" (Adsuar, 1972). Of these, "papaya mosaic" (PMV) and "papaya ringspot virus" (PRSV) are wide spread and extensively studied.

The world literature on papaya virus diseases, especially on "mosaic" and "ringspot" is very confusing. In many cases, only the symptoms of the disease have been described. This is probably the reason that the different names are used for apparently identical viruses or strains of the same virus. Further, the slight ambiguity shrouding arthropod involvement in "papaya mosaic virus (PMV)

transmission may perhaps be attributed to these nomenclature discrepancies. A virus causing "mosaic" of papaya would be distinct from the "papaya mosaic virus" proper and the properties assigned to the former may be confused with the latter and vice-versa leading to obvious disarrays. These nomenclatural discrepancies have been cleared through aphid transmission and electron microscopic studies made by de Bokx (1965) and Zettler et al. (1968), who suggested that the name "papaya mosaic virus" (PMV) be used for viruses that are non-aphid transmissible and whose particles are 533 nm long with "potexvirus group" characteristics (Purcifull and Hiebert, 1971; Smith, 1972; Purcifull and Edwardson, 1981); and "papaya ringspot virus" (PRSV) for viruses which are aphid transmissible and whose particles are 780 nm long with " potyvirus group" characteristics (Harrison et al., 1971; Purcifull, 1972; Edwardson, 1974; Edwardson et al., 1984; Purcifull et al., 1984).

Virus diseases of papaya reported since 1929 from Africa (Kulkarni, 1970), Australia (Simmonds, 1937, 1965), the Caribbean area (Acuna and Zayas, 1940, 1946; Baker, 1939; Gabrovska et al., 1967); the USA (Florida) (Conover, 1962, 1964a,b) and (Hawaii) (Parris, 1938; Holtzmann and Ishii, 1963; Ishii and Holtzmann, 1963), India (Capoor and Varma

1948, 1958; Garga, 1963; Singh, 1969a) and South America (Malaguti et al., 1957; Torres and Giacometti, 1966), have been given a variety of common names. On the basis of gross characterisation (severe reduction in laminae, no reduction in latex flow from wounds and transmission by mechanical means and by aphids), Cook (1972) considered several of the syndromes described as the manifestations of the "papaya ringspot virus" (PRSV).

Since, the present investigation confirmed the identity of the virus causing severe leaf distortion and rings on fruits of papaya in Maharashtra State as " papaya ringspot virus", a member of "potyvirus group"; the world literature pertaining to PRSV and other similar or; identical aphid transmissible virus diseases of papaya is only reviewed and presented below.

2.1 Geographical distribution :

"Papaya ringspot virus" (PRSV) causes one of the most destructive diseases of papaya (Cook, 1972; Purcifull, 1972) and has been reported to be a major limiting factor throughout the tropical and subtropical regions of the world wherever papaya is grown. Though its existence had been reported by earlier workers under different names (Simmonds, 1937; Parris, 1938; Acuna and Zayas, 1940, 1946), the "papaya ringspot virus" (PRSV), was first described by Linder et al.

(1945) from the USA (Hawaii) and experiments reported in abstract form by Jensen (1946, 1947) demonstrated the viral nature of the disease, its incubation period in the plant and Myzus persicae and three unnamed Aphis species as being shown capable of transmitting the virus. Since then, the occurrence of PRSV had been reported from several countries which include Africa (Jensen, 1949a; Kulkarni, 1970; Lana, 1980), Australia (Simmonds, 1937, 1965; Shanmuganathan, 1980), the Caribbean countries (Adsuar, 1947a; Story and Halliwell, 1969), China (Wu et al., 1983), Far East (Wang et al., 1978), the USA (Florida) (Conover, 1962, 1964a,b) and (Hawaii) (Parris, 1938; Linder et al., 1945; Holmes et al., 1948; Jensen, 1949a; Holtzmann and Ishii, 1963; Ishii and Holtzmann, 1963); India (Khurana, 1974; Sureka et al., 1977; Yemewar and Mali, 1980), Pakistan (Basit, 1974); South America (Jensen, 1949a; Herold and Weibel, 1962; Lima and Gomes, 1975), Sri Lanka (Rajapakse and Herath, 1981, 1982) and Vietnam (Brcak and Pozdena, 1976).

The Indian records of papaya viruses resembling PRSV in symptomatology and aphid transmissibility and some other properties like physical properties in crude sap and host range in some instances reported from different states include: "papaya mosaic" from Bombay Province (Capoor and Varma, 1948, 1958), and " a sap transmissible virus of papaya

in Marathwada" (Yemewar and Mali, 1980) from Maharashtra State; "mosaic of papaya" (Mishra and Jha, 1955; Varma, 1971) from Bihar; " a serious disease of papaya" (Garga, 1963) from Madhya Pradesh; "papaya leaf reduction virus" (Singh, 1969a), "papaya mosaic" (Singh, 1969b), "distortion ringspot" and "ringspot" (Khurana, 1974), and "papaya mosaic" (Bhaskar, 1983) from Uttar Pradesh; "mosaic disease of papaya" (Varma, 1971) from Haryana; "papaya ringspot virus" (Sureka et al., 1977) from Rajasthan; "papaya mosaic virus" (Cheema and Reddy, 1985a,b) from Punjab; and "papaya mosaic" (Susan John, 1985) from Andhra Pradesh.

2.2 Extent of losses

Although, losses attributed to PRSV have not been assessed, it has been considered a major disease in many papaya growing countries (Cook, 1972). Several epidemics of PRSV limiting the establishment of commercial plantations and profitable papaya production have been reported from various countries including Africa (Kulkarni and Sheffield, 1968), the Caribbean countries (Story and Halliwell, 1969), Colombia (Sanchez de Luque et al., 1980), the USA (Florida) (Conover, 1962; Cook and Zettler, 1970) and (Hawaii) (Ishii and Holtzmann, 1963); India (Yemewar and Mali, 1980; Mali, 1986); South America (Herold and Weibel, 1962) and Taiwan (Wang et al., 1978). The reports on yield and quality losses attributed

to PRSV are very few and the literature available on this aspect is reviewed below.

Parris (1938) attributed a yield loss ranging from 6 to over 30 per cent to a new mosaic disease, now called as "papaya ringspot virus", which was prevalent in Waialua and Lualualei on the island of Oahu in Hawaii. A mosaic disease of papaya occurring in Puerto Rico, which according to Cook and Asenjo (1941) markedly reduced the latex flow and papain and protein contents of fruits. Vasudeva (1958) recorded yield losses as high as 66 to 85 per cent in papaya plants infected with mosaic disease. Rosenberg (1962) from Hawaii noted that "papaya mosaic virus" reduced soluble salts in fruits from diseased trees by 29 per cent. The Hawaiian isolate of PRSV reported by Ishii and Holtzmann (1963) caused severe reduction in fruit set and noticeable differences in total soluble solids, flavour and aroma in fruits that develop on plants subsequent to infection (Holtzmann and Ishii, 1963) and also caused yield losses ranging between 5 and 20 per cent, but in some instances the losses reported were upto 75 per cent (Ishii and Holtzmann, 1963). According to Khurana (1970), the "distortion ringspot virus" (DRV) and " ringspot virus" (RSV) (considered to be PRSV strains now) infecting papaya in Gorakhpur area, of Uttar Pradesh, caused marked reduction in yield and quality

of fruit. According to him DRV was found to reduce latex and sugar contents of fruits by 55 per cent and fruit yield, whereas RSV was found to reduce sugar content only by 42 per cent and a deterioration in the latex quality. Sánchez de Luque et al. (1980) from Colombia observed that in PRSV susceptible varieties of papaya, the number of fruits, total weight of fruits per tree and individual fruit weight were less by 69 per cent, 78 per cent and 29 per cent respectively, as compared to those in a resistant variety. Furthermore, observable reductions in pulp content, aroma and values for brix in the fruits of 14 susceptible cultivars were also noted by him. Barbosa and Paguio (1982b) estimated yield losses in papaya infected with PRSV. When individual PRSV infected plants were compared by them with apparently healthy ones, the estimated losses in fruit yield and number of fruits per plant and individual fruit weight were 68.0 to 72.1, 59.1 to 61.5; and 19.4 to 25.0 per cent respectively.

2.3 Symptomatology

Parris (1938) described a new virus disease of papaya discovered at Waialua and Lualualei, Oahu in 1937. According to him, diseased plants were stunted with chlorotic leaves. The petioles of the affected plants were bent downward at the point of attachement. Linear, dark green, raised hydrotic streaks measuring 1/8 to 1" in length by 1/32" to 3/8" in width were evident on stem and petioles.

Diseased leaves were found to fall in 4 to 6 weeks after the appearance of first symptoms. As such the stem was left bare except for a few stunted and distorted leaves at the apex and frequently a fringe of lower leaves which were developed prior to symptom expression. Fruits on diseased trees were small and bled profusely.

Baker (1939) reported the common occurrence of "papaya mosaic" in Trinidad. It was characterised by water-soaked areas labelled as " oily-spots" on petioles and marked mosaic on the newly formed leaves, while older leaves being unaffected.

The first record of a probable virus disease of papaya in Florida (USA) was that of Townsend and Andrews (1940) who reported mottled foliage, rings on fruits, necrotic streaks on stems and stunting or death of apical bud without, however, demonstrating the viral nature of the disease.

Acuna and Zayas (1940, 1946) reported a "Cotorro mosaic" from Cuba (resembling PRSV in many properties) which was recognised by circular, greenish spots on the base of young petioles and sometimes on stems, expanding with the growth of the plant to several centimeters in length and 1 cm

in width and becoming "hook" or "U-shaped". In heavily infected areas, fruits show spotting, but lesions fail to extend as in case of petioles and stem. Another distinctive feature of the disease being the abundant flow of latex resulting from puncture in fruits, consequently failing to mature and are of insipid flavour. The basal leaves instead of developing at right angles to the stem, have an obtuse angle or form clusters, often persisting after the defoliation of the upper part of the tree; in some cases, a new tuft of small leaves are produced higher up the trunk. The flowers drop simultaneously, with the leaves interrupting the fruit setting. In affected seedlings and sometimes even in older plants, only fibrous tissues of leaves develop and the veins instead of being arrange in pinnate form, run in parallel lines separated by the conjuctive tissue devoid of chlorophyll. Severely infected young leaves may show necrotic areas, suggestive of a chemical injury.

Chatterji (1943) described a virus disease of papaya from Dacca, Bangaladesh, with following characteristic symptoms. The earliest symptoms of the disease include chlorosis and downward curling of young leaves. The leaves showed malformation and blistering with the advancement of time. In mild cases, malformation causes reduction in width and more pronounced dissection of leaf blade, while in

severe cases, some leaves showing malformation get reduced to midrib only. The older leaves are found to turn brown and fall off and the entire plant show a gradual decline in growth bearing a small central cluster of pale green, reduced and malformed leaves.

Jensen (1946, 1947, 1949a) from island of Oahu, Hawaii demonstrated the viral nature of papaya ringspot, its incubation period in the host plant and its aphid transmissibility and described the symptomatology in detail. Symptoms were produced in the foliage, fruit and stem of the plant. Rugosity and mottle without marked distortion, characterised the leaf symptoms. Reduced light enhanced the expression of leaf mottle. Yellow spots or yellow rings with green centres were produced on diseased fruits at the mature green stage. The fruits infected when young, developed yellow rings at maturity, while fruits 2/3 grown when infected, produced only solid yellow spots. Small, inconspicuous, light green rings sometimes also occurred on very young fruits on diseased plants. Young plants showed slow growth when infected and sometimes displayed water-soaked spots and streaks on stem.

Adsuar (1947a,b,c) described a sap, graft and aphid transmissible virus disease of papaya under the name

"papaya mosaic" from Puerto Rico which has now been considered as PRSV (Cook, 1972). Progressive symptoms of the disease included chlorosis, mottling, wrinkling and puckering of the top leaves. As the disease progressed, the plants became stunted and the leaves were severely deformed and reduced to filiform structures. The stem showed dark green, elongated streaks and fruits bore green and dark brown rings.

Holmes et al. (1948) published the results of a ringspot disease survey made on main islands of the Hawaiian group. They noted that the most distinctive and reliable symptom of the papaya ringspot was the appearance of yellow rings with green centres, measuring 1/8" to 3/4" in diameter on the surface of fruits while still they were mostly green. The foliage symptoms included puckering of young leaves and irregular mosaic in expanded leaves. The mosaic symptoms were more pronounced in the field trees during winter season, became masked in varying degrees during summer. Diseased plants showed retarded growth and were weak.

Capoor and Varma (1948, 1958) studied and described a mechanical, graft and aphid transmissible "mosaic" disease of papaya from erstwhile Bombay Province

of India. The natural infection of papaya by mosaic which was carried mostly through aphid vectors was characterised by profuse mottling and puckering of young foliage. Within 30-40 days after the appearance of first symptoms, plants showed degeneration and marked reduction in growth. The younger leaves were markedly reduced in size, chlorotic and malformed and older ones gradually fallen off leaving trees almost denuded except for a tuft of small leaves at the top. Conspicuous dark green spots, elongated streaks of "water-soaked" areas or " oil-spots" were evident on petioles and stem. The fruits produced on affected trees were found to develop innumerable circular or concentric "water-soaked" lesions with a central solid spot. In experimentally inoculated plants, the first symptoms were faint chlorotic spots followed by premature drooping and wilting of leaves. Subsequent leaves showed mild chlorotic spots followed by mosaic mottling, puckering and blistering. As the disease advanced, the young developing leaves showed much reduction in size and distorted. Water-soaked areas or oil-spots and elongated streaks were evident on stem and petioles of sap-inoculated plants.

A serious " mosaic" disease of papaya infecting 90 per cent plants and making papaya cultivation unprofitable was described by Mishra and Jha (1955) from Bihar State of

India. In less severely infected plants mottling was frequently observed, but constrictions and elongated water-soaked areas on stems and petioles were rarely seen and no flow of latex from the fruit or dark green or brown rings observed.

Reyes et al. (1959) reported a virus disease of papaya designated as " leaf curl", found in an orchard in Diliman, Rizal, Philippines. The disease was characterised by the reduction of the size of leaves and petioles, upward curling of leaves and tapering of the upper portion of the stem. The leaves were markedly distorted. Although they retained the peltate shape, they became deeply lobed, with lobes pointing upwards. Vein-clearing and thickening was visible when the leaves were viewed through transmitted light and these symptoms were more easily detectable on the under surface of the leaf.

Herold and Weibel (1962) reported that PRSV prevalent in Venezuela could be transmitted readily by mechanical sap inoculation and also through aphids. In green house, papaya plants inoculated with PRSV, the first symptoms shown were the appearance of dark green spots on the upper third of the stem almost 3 to 4 weeks after inoculation. Some days later, fine vein-clearing of young

leaves was observed. Simultaneously, the older leaves became yellow and fall off. Later, leaves with vein-clearing became mosaic spotted. New leaves became mosaic spotted and deformed as soon as they appeared. Mosaic and deformation were much stronger in green house grown plants than in field.

Conover (1962, 1964a,b) described and reported three virus diseases viz., " distortion ringspot" (DRV), "faint mottle ringspot" (FMRV) and " mild mosaic" (MMV) affecting papaya in Florida (USA) and causing diseases singly and in certain combination. The symptoms produced by "distortion ringspot" (DRV) were narrowing and distortion of one or more leaf lobes and greasy-appearing streaks on petioles, stems and rings on fruits which turned grey with age on fruits. "Faint mottle ringspot (FMRV) had similar markings on petioles, stems and fruits which remained brown, but leaves showed only a faint mottle. While mild mosaic (MMV) was characterised by green mottle devoid of leaf distortion or symptoms on petioles, stems and fruits. Conover (1964b) considered FMRV and DRV as closely related, but distinct strains of the same virus.

Garga (1963) reported a serious graft and aphid transmissible virus disease of papaya from the State of Madhya Pradesh in India. The leaves of affected plants

developed transluscent areas adjoining the veins, followed by puckering of the lamina, dark green raised areas and slight curling of leaf margins. Subsequently developed leaves were reduced in size and showed pronounced distortion. In some cases, the leaf lamina was represented only by main veins. The older leaves turned brown, fell down and the entire stem became denuded with only small cluster of reduced and much distorted leaves at the top. Plants infected in the early stages of their growth were severely stunted and failed to bore fruits. Circular rings were evident on fruits and flow of latex was not affected by the disease. The infected plants were not killed out right but survived for several years. Masking of disease symptoms during summer months was observed. Those plants which showed severe disease symptoms during winter, displayed almost normal foliage in summer.

Ishii and Holtzmann (1963) described a papaya mosaic virus disease from Hawaii. In the field, young and old trees affected with mosaic exhibited mild to severe chlorosis in the crown region; in some, a stunting of crown leaves and petioles occurred. In severe cases, cessation of growth and loss of leaves resulted in death of trees. Initially, the affected leaves showed rugose appearance. Young leaves of crown were generally stunted and severely chlorotic with

transparent oily-areas either scattered over the lamina or as vein-banding. Mature leaves were found to exhibit chlorotic pattern in the form of extensive vein-clearing and numerous small rings ranging from transparent yellow to tan colour. The leaves became stunted with the progress of the disease. On the stems of infected plants, water-soaked spots developing into lineal, concentric ring patterns or into diffused areas were produced. Considerable stunting of internodes was evident in severe infections. Lineal or elliptical hydrotic-spots of irregular distribution, being lighter in colour than those produced on stems, were of common occurrence on petioles. Severely infected petioles were stunted and bent downwards.

Distinctive fruit symptoms were manifested at all stages of maturity. Small dark green ringspots, 1 to 2 mm in diameter were evident on fruits of about two weeks old. Initially the rings may be incompletely closed and irregular, but as the fruit develops target like spots, increase in diameter from 1mm consisting of only one ring, to about 15 mm with as many as eight or more distinct, slightly raised, concentric brownish rings within a green outside ring. Frequently this entire spot may be slightly sunken. Although, rings average 4 to 8 mm in diameter, two or more rings may coalesce to form larger and varied shaped patterns.

In trees severely affected and denuded of leaves, the surface of younger fruits became deformed and secreted droplets of latex.

Simmonds (1965) reviewed the papaw diseases occurring in Queensland. In this review, Simmonds described a mosaic disease of papaw often reaching epidemic proportions in some districts of Queensland. The " Queensland papaya mosaic" was characterised by the initial chlorosis and stunting of younger leaves associated with the development oftypical narrow water-soaked streaks on petioles and upper portion of the stem and characteristic light green areas delimited by dark green areas on younger fruits. Latex flow became reduced or was often absent. As the disease progressed, the terminal leaves withered resulting in death of the top, that assumed an appearance difficult to distinguish from dieback. Subsequently, the died top apparently recovered, the only symptoms remained were certain stunting and crinkling of older leaves with petioles characteristically shortened. Mosaic infected plants showed a tendency to throw multiple side shoots, which remained stunted and often subsequently developed mosaic symptoms.

de Bokx (1965) from Florida (USA) studied the host range and electron microscopy of two virus diseases of papaya viz., "distortion ringspot" (DRV) and " mild mosaic"(MMV)

in order to establish the relationship between them. Based on the results, de Bokx attributed distortion ringspot and mild mosaic diseases to two different viruses, the former belonging to " potyvirus group" and the latter to "potexvirus group" and each producing distinct symptoms. The papaya plants inoculated with DRV- isolate produced vein-clearing and severe shoestringing on the foliage, the symptoms being more severe during winter, while the plants inoculated with MMV- isolate produced only severe mosaic, but no distortion on foliage.

Torres and Giacometti (1966) reported two virus diseases naturally infecting papaya in Cauca valley of Colombia. These viruses were provisionally named as "type A" and " type B", as their symptomatology resembled respectively " distortion ringspot virus" (DRV) in Florida (USA) and Cotorro mosaic virus" in Cuba. In " type A virus" infection, young leaves became pale, the leaf edges curved upwards and shoot turned light green. Oily-green spots, circles and lines generally appeared near the stem and base of petioles of young leaves. Green fruits showed small rings.

The presence of " distortion ringspot virus"(DRV) of papaya reported from Dominican Republic was initially indicated by the development of oil-like streaking and

spotting on main stem, followed by vein-clearing and mosaic of young foliage. The chronic stages of the disease resulted in narrowing and distortion of one or several lobes of occasional leaves resulting in filiform structures. In older plants, early symptoms were streaking on stems and petioles and/ or green ring spotting on the fruits. With the progress of disease mosaic mottle of younger leaves occurred followed by distortion (Story and Halliwell, 1969).

Singh (1969a,b) described two sap and aphid transmissible virus diseases of papaya viz., "papaya leaf reduction virus" (PLRV) and " papaya mosaic virus" (PMV) prevalent in Gorakhpur area of Uttar Pradesh. In PLRV-infected plants, leaves displayed mosaic and in advanced stages deformation and reduction, assuming thread-like appearance. Further, PLRV- infected plants showed severe stunting and latex flow from them was greatly reduced. While in PMV- infection the visible symptoms were vein-clearing, mosaic mottling and downward and inward curling of leaves and the appearance of circular rings and streaks on the petioles, stems and fruits.

From papaya plants showing field decline symptoms in Tanzania and Kenya, East Africa, Kulkarni (1970) isolated three viruses. Each virus caused distinct type of local lesion in Chenopodium quinoa and a fourth type of local

lesion was induced when transmission was from a source containing two of the three viruses. The viruses causing local lesions were designated as " chlorotic lesion virus" (CLV), "chlorotic ringspot virus" (CRV), " necrotic lesion virus" (NLV) and " yellow necrotic lesion virus" (YNLV). When, these isolates were reinoculated to papaya alone or in combination, they caused stunting and decline symptoms similar to those observed in the field. Affected plants in the field were stunted with a reduction in leaf number and size; young leaves occasionally showed vein- clearing. In severe cases, youngest leaves were distorted, crinkled, back-rolled and more rarely blistered. Infrequently, a bright yellow mottle or small discrete yellow spotting was evident. Fruits often withered, abscissed when 3 to 5 cm long or were few in number. The tips of main shoots showed either " small head" or " pencil point" and apical death.

During a survey of papaya virus diseases in Gorakhpur (1965-68), Khurana and Bhargava (1970) observed fruits on some papaya plants that had varying degree of deformations and abnormalities. These plants were found to be infected with a strain of " distortion ringspot virus" (DR) which has been shown to be related to " distortion ringspot virus" reported from Florida (USA) (Conover, 1962; de Bokx, 1965) and " ringspot" from Venezuela (Herold and

Weibel, 1962). The DR strain was found to be sap and aphid transmissible. Fruits on infected trees showed a tendency to fall early, but some retained to maturity. Forty to fifty day old fruits which remained on such trees exhibited dark green " oily" or water-soaked, concentric coalescent rings turning brown at maturity. Abnormal fruit showing induced "apocarpy" and "double papaya" were noted in papaya cultivars like African Giant, Honey Dew, Ranchi, Simulata Giant and Washington.

Varma (1971) reported high incidence (about 90 per cent) of a sap and aphid transmitted virus disease of papaya under the name," mosaic disease of papaya" which prevailed in several orchards of Haryana and Rajasthan. Some of the diseased trees bore few small and deformed fruits. Such fruits produced dark green or yellow necrotic spots, blisters or rings on their skin. The symptoms in the field were vein-clearing or faint vein-banding and mosaic mottling on newly infected young leaves. Older infected leaves showed marginal curling, downward bending, chlorosis, necrosis ultimately resulting in premature leaf fall. New leaves developed subsequent to infection, showed intense yellow mosaic with dark green patches or blisters interspersed over yellow background of leaf lamina. The lobes of leaf lamina were reduced or deformed and the leaf looked like a palm

with straightened fingers. The lobes of the leaves were overlapped due to abnormal growth. In some leaves half of the lamina was deformed while the other half remained normal. The infected leaves were also variously malformed, crinkled, modified into tendril- like structures, greatly reduced in size and stood upright on the top of the plants. Such symptoms were evident in both young as well as old plants. Severely infected plants lingered on for several months with a tuft of few reduced and deformed leaves and ultimately denuded and wilted.

Farqui et al. (1972) described a new virus disease of papaya from Pakistan under the name," shredded leaf". Plants infected with shredded leaf virus showed gradual deterioration of leaves as a result each rib carried a reduced and distorted lamina. The initial symptoms of the disease were upward curling and blistering of leaves. Subsequently, with the increase in the intensity of blistering, leaves became paler and reduced. Fruits on diseased trees were also malformed.

Khurana (1974) described three virus diseases on papaya, from Uttar Pradesh, India. These diseases were designated as " mild mosaic" (MM), " distortion ringspot" (DR) and " ringspot" (RS) and could be diagnosed by the

symptoms viz., MM inciting only mild mosaic; DR by distortion of leaves associated with marked reduction in leaf size, oily spots on stem, petioles and concentric, coalescent rings on fruits and RS by interveinal puckering of foliage, oily spots on stem and non-coalescent rings usually encircling a spot on fruits.

Brcak and Pozdena (1976) while surveying virus and virus- like diseases of some crop plants in Vietnam and Combodia, observed two symptomatically different serious diseases of papaya near Hanoi: (1) heavy leaf deformation in which attempts to detect virus in leaf tissues were unsuccessful and (2)" mosaic and ringspot". The symptoms in mosaic and ring spot were almost identical with those described for PRSV by Jensen (1949a). The causal virus was identified to be a member of potyvirus groups of Harrison et al. (1971).

Sanchez de Luque and Martinez Lopez (1976a,b) based on aphid transmission, symptoms, host range and physical properties confirmed the identity of a virus as "papaya ringspot virus" (PRSV), which limited commercial cultivation of papaya in Colombia. The Colombian isolate of PRSV caused chlorosis and mosaic of young leaves followed by distortion and reduction of leaf laminae into filiform structures. In

the infected trees, the stem displayed dark green, water-soaked patches and water-soaked linear streaks on petioles. The petioles also showed a tendency to bend downwards. Typical concentric rings were produced on all over the surface of fruits.

During the course of investigations on virus diseases of papaya occurring in and around Udaipur, Rajasthan, Sureka et al. (1977) encountered with two sap transmissible virus diseases viz., "papaya mosaic virus" (PMV) and "papaya ringspot virus" (PRSV) in single and mixed infections. PMV and PRSV induced distinguishable natural symptoms in single infection but in mixed infections symptoms of PMV were supressed by PRSV. In PMV- infected trees, the leaves displayed mosaic, vein banding and in few cases vein-clearing symptoms. Trees severely infected by PMV had fewer and smaller fruits. PRSV- induced foliar symptoms included distinct chlorotic spots, chlorotic rings, various types of line patterns, blisters and distortion. Premature leaf fall was also common and in severe cases, only a bunch of leaves was seen on the top with all the lower leaves defoliated. Petioles of affected trees possessed round to elliptical and elongated oily rings and streaks distributed over the whole surface. Infected trees were very much stunted with hardened stems bearing oily spots, streaks

and rings. Fruits on some of the infected trees displayed dark green oily or water-soaked circular, concentric rings. In some cases fruits were deeply lobed with various degrees of induced apocarpy resulted in the formation of bi-to-hexalobate fruits. Symptoms of the disease were severe only in winter months and heavily masked in summer.

Two isolates of PRSV, I and II, causing wilt and mosaic respectively have been described from Taiwan and squash being the best differential host for the two (Chang, 1979).

During a crop disease loss assessment survey of vegetables and fruits in Nigeria, Lana (1980) observed papaya plants being infected with "papaya mosaic virus"(PMV),"papaya ringspot virus"(PRSV) and " tobacco ringspot virus" (TRSV). Lana reported that PRSV recorded in Nigeria was sap and aphid transmissible. The field symptoms induced by Nigerian isolate of PRSV included vein-banding, spots, line pattern, mosaic, curling and distortion of leaves. In plants showing distortion symptoms, greasy streaks and grey spotting were usually found on stem. Ringspot symptoms some times appeared on the fruits, but virus could not be recovered from such fruits. Seedlings of Solo and some local cultivars sap-inoculated with PRSV exhibited vein-clearing, curling and ringspot symptoms on newly developed leaves.

Shanmuganathan (1980) during his survey on virus and virus- like diseases of plants, noted the occurrence of PRSV on papaya plants grown at Tarawa localities of Gilbert Islands. He observed PRSV to cause vein-clearing, mottling on fully expanded young leaves, leaf distortion and reduction of laminae. The expanded leaves were mildly mottled and rugose. In PRSV- infected plants however, he did not observe characteristic dark streaks on stems and petioles which usually associate with the disease. The trees were stunted and devoid of fruits.

A sap and aphid transmissible virus disease of papaya from Marathwada region characterised by the symptoms of mosaic mottling, leaf distortion, reduction of leaf lamina into filiform structures; streaks on the stem and petioles; rings and spots on fruits and stunting of plants has been reported by Yemewar and Mali (1980). The incidence of the disease was found to be 79 per cent during 1977-78. On the basis of transmission, host range, physical properties and serological tests, Yemewar and Mali (1980) confirmed the identity of sap and aphid transmissible virus disease of papaya occurring in Marathwada region as "papaya ringspot virus" (PRSV), a member of potyvirus group.

During a survey of papaya growing areas in Sri Lanka, Rajapakse and Herath (1982) noted several papaya plants grown in Kandy and Gampola showing abnormal symptoms like chlorosis, curling, stunting, vein-clearing and prominent mottling on the leaves. According to observed symptoms, they were categorised as "mosaic","ringspot" and "leaf curl" groups. On the basis of symptomatology, transmission and host range, mosaic, ringspot and leaf curl groups were identified as "papaya mosaic virus" (PMV), "papaya ringspot virus"(PRSV) and " papaya leaf curl virus" (PLCV) respectively. In sap inoculation on seedlings of two papaya varieties, Solo Hawaii and Washington, PMV produced systemic mosaic and vein-clearing, but PRSV showed systemic chlorosis, vein-clearing and ringspot, while PLCV displayed vein-clearing and leaf-roll symptoms. Insect transmission studies have indicated that both PMV and PRSV, but not PLCV were aphid transmissible. Papaya leaf curl was transmitted by whitefly vector, Bemisia tabaci.

A virus disease of papaya inducing mosaic, rugosity, reduction of leaf laminae, greasy appearing streaks on petioles and greasy-appearing ringspots on fruits observed in Pernambuco more than 10 years ago has been recently identified and designated as an isolate of "papaya ringspot virus" (PRSV-PE) based on aphid

transmission, host range, physical properties and particle and inclusion morphology (Barbosa and Paguio, 1982a).

The Venezuelan isolate of PRSV has been described with symptoms like vein-clearing, mosaic, phyllody, oily spots on petioles and stem and dark green concentric rings on the skin of fruits. Occasionally fruits on infected plants showed dark green areas and deformations (de la Rosa and Lastra, 1983).

Wan and Conover (1983) have reported the host symptomatology in respect of various papaya virus diseases, aiding in an easy diagnosis. "Papaya ringspot virus"(PRSV) was diagnosed on the basis of leaf distortion and greasy appearing streaks on stems and rings on fruits, whereas, "droppy necrosis virus" (DNV) was identified by the presence of recurvature, yellowing and stiffness of petioles. "Papaya mosaic virus" (PMV) could be diagnosed by the presence of green mottle on leaves without distortion and absence of symptoms on fruits and stems. "Papaya bunchy top" caused by a mycoplasma-like organisms could be diagnosed on the basis of the absence of latex flow from wounds made on field papaya plants.

Bhaskar (1983) reported a serious aphid-transmitted mosaic disease of papaya from Kanpur area of Uttar Pradesh. Papaya seedlings inoculated with mosaic through aphid vectors

developed dark green spots and elongated streaks which looked like water-soaked areas on the leaf laminae. When the disease was severe, the plants showed profuse mottling and puckering and became stunted. The petioles and stems were also infected.

In an interestingly titled paper, Rezende (1984) wondered whether "papaw mosaic" or "papaw ringspot" depicted the disease better in Brazil and choose "papaw mosaic" since the disease symptoms were generally mosaic on plants of all ages, the " ringspot" developing later on the fruits.

Yeh et al. (1984) described the symptoms produced by different isolates of PRSV on a papaya cultivar, Kapohosolo. The Taiwan isolates Su-mm, Su-sm and Su-smn manifested mild mottling, severe mottling and severe mottling associated with systemic necrosis and wilting respectively, while T-Chen and T-Wang respectively induced mottling and mosaic symptoms. The Hawaiian isolates of PRSV viz., HA and HB exhibited mosaic and leaf distortion symptoms. While the Florida isolate F-340 and Ecuador isolate EC were reported to produce mosaic and shoestringing symptoms. Further all these isolates were observed to cause water-soaked streaks on the stem and extreme retardation of plant growth. In winter months, all isolates except Su-mm caused severe distortion and shoestringing symptoms.

Susan John (1985) made detailed investigations on a sap and aphid transmissible mosaic disease of papaya occurring in Andhra Pradesh. Based on symptomatology, transmission, host range, physical properties and serology, the virus causing mosaic disease of papaya in Andhra Pradesh was identified as "papaya ringspot virus" (PRSV). PRSV incidence varied from 70 to 90 per cent. The disease was characterised by well marked mosaic, followed by reduction in size and distortion of foliage. Symptoms like vein-clearing, chlorotic spots and chlorotic rings were also evident on infected leaves. The stem possessed dark green oily-spots and streaks. On the fruits of infected plants, circular, concentric, water-soaked rings were present.

2.4 Effect on physiology and biochemistry of infected host :

The incitant of papaya ringspot disease reported under different names has been claimed to bring about a wide range of deviations in the physiology and biochemistry of infected host.

Singh (1971b) studied the oxygen uptake of papaya leaves infected with "papaya leaf reduction virus" (PLRV) and observed an increase in respiration eight days after virus inoculation. Respiration increased after tenth and

thirteenth days in infected and healthy leaves respectively, after which oxygen consumption being decreased in both, but more rapidly in healthy leaves. The virus was detected in inoculated plants on seventh day and reaching maximum concentration by twelfth day. He attributed initially higher rate of respiration to the entry of virus particles into cells and resultant senescence. In his further studies (Singh, 1972a, 1973) on the effects of PLRV in the peroxidase and catalase and on the total nitrogen and carbohydrate content of infected leaves; he noted that PLRV-infected leaves to show increased activity of peroxidase and catalase and their maximum activity coinciding with maximum virus titre (Singh, 1972a); and total nitrogen content being increased throughout disease development, while reducing sugars, non-reducing sugars and starch contents declining as compared to healthy leaves (Singh, 1973). "Papaya distortion mosaic virus" (PDMV) has been reported to increase peroxidase activity of infected leaves by 39.5 per cent in 9 days after virus inoculation, decreasing to the normal level in 15 days (Banarjee et al., 1976). In their analyses of PRSV- infected leaves (showing line pattern), Mathur andShukla (1977) observed an increase in the concentrations of amino acids like alanine, argenine, glycine, proline and serine, on the other hand leucine and valine were decreased. Glycine and serine increased by two folds and alanine by about three folds. Singh et al. (1977) reported destruction of

chlorophyll a and b in papaya leaves infected by an aphid - borne mosaic virus. They recorded significant reduction in the amounts of total chlorophyll, chlorophyll a and chlorophyll b by 56.71, 41.33 and 85.3 per cent respectively in infected leaves as compared to healthy ones. Singh et al. (1979) reported that " papaya leaf reduction virus" (PLRV) reduced the rate of hill reaction, gross dry matter production, chlorophyll and carbohydrate contents in papaya leaves, but increased the respiratory activity. The reductions were more pronounced in chlorotic than dark areas of the leaves showing mosaic symptoms. "Papaya ringspot mosaic virus" (PRMV) prevalent on papaya in South China has been found to cause reduction in chlorophyll and protein contents by 20.30 and 41.7 respectively, associated with a decrease in the activities of RUBP-ase (65 per cent), PEP-ase (46 per cent), malic enzyme and malate dehydrogenase in the infected leaves (Sun Guchou, 1985).

2.5 Histopathology

Chatterji (1943) observed several histological abnormalities in the root, stem, petiole and leaf of a papaya plant infected with " leaf deformation disease". Various histological abnormalities induced by the disease included degeneration of chloroplasts, latiferous cells and degeneration associated with necrosis of phloem and

hypertrophy and hyperplasia of latex, phloem and mesophyll cells. Thickening and distortion of leaves, production of many secondary longer veins were attributed to hypertrophy and hyperplasia of the infected cells. Hypoplasia was shown mainly in the dwarfed and stunted plants. Herold and Weibel (1962) in their electron-microscopic demonstration using papaya leaves infected with PRSV, have observed chloroplasts of infected tissues to contain only few starch grains or none and in heavily infected cells chloroplasts being often totally destroyed. Singh (1971a) investigated anatomical changes of leaves infected with "papaya leaf reduction virus" (PLRV) and reported that in PLRV-infected leaf, the palisade layers and spongy cells were smaller, contained fewer and smaller chloroplasts, had broad intercellular spaces and were deformed. Mathur and Shukla (1979) observed a variety of deviations in the histology of papaya leaves infected by PRSV. Alterations were quite distinct and varied with symptom expressions like ringspot and line pattern, blistering and distortion. In the ringspot and line pattern, the cells of upper and lower epidermis were deformed; in blisters the cells of upper epidermis were enlarged twice the normal size, but those of lower epidermis appeared shrunken and distorted, while in the leaves showing distortion, the cells of upper epidermis exhibited pronounced shrinkage, contrary to this, cells of lower epidermis were greatly enlarged and lost their

individuality. Palisade cells were reduced in size and contained more chloroplasts in sections cut through ringspot and line pattern symptom, but they were increased in size and contained more chloroplasts which stained heavily, while in sections through distorted leaf, palisade cells were enlarged and distorted heavily beyond recognition. Cells of spongy parenchyma were enlarged and deformed in all the types of symptoms with smaller intercullular spaces in the ringspot and line pattern, while in distortion symptom, exhibited enlargement and contained more chloroplasts. Vascular bundles were found badly damaged and distorted. The Taiwan isolate of "papaya ringspot virus" (PRSV) was reported to be responsible for degneration of chloroplasts in young infected leaves. The abnormal rounding and development of numerous vesicles were evident in mature mesophyll cells. Nucleus though apparently normal, was not centrally located in the cell (Chen, 1984).

2.6 Transmission :

2.6.1 Mechanical and other modes of transmission

Parris (1938) while working with a new virus disease of papaya in Hawaii observed more than 75 per cent increase in the mechanical transmission of the disease when carborundum was used as an abrasive. Symptoms were evident on test papaya seedlings within 16 to 21 days after virus inoculation.

The Puerto Rican mosaic disease of papaya caused by a sap and aphid transmissible virus was reported to be readily transmitted from papaya to papaya by "pin-puncture", "rubbing of infective sap with carborundum" and "grafting". The success of transmission was 37.93 per cent in case of "pin-puncture" and 82.14 per cent in case of "rubbing of infective sap with carborundum". Plants inoculated either by "pin-puncture" or "rubbing of infective sap with carborundum" developed symptoms within 8 to 15 days after inoculation, while grafted plants took 2 to 3 weeks (Adsuar, 1947a).

Capoor and Varma (1948, 1958) tested different inoculation techniques to transmit an aphid transmissible mosaic disease of papaya. Sap inoculation using carborundum as an abrasive gave highest percentage of transmission and the disease symptoms also appeared little earlier than in those plants which were inoculated without an abrasive. They also reported disease to be transmitted by "wedge-grafting" and "budding". The percentage transmission recorded for wedge grafting and budding was 72.5 and 100.0 respectively. In mechanical juice inoculation, the symptoms of the disease were visualised in 15 to 26 days after inoculation, whereas in wedge-grafted and budded plants, symptoms appeared 35 to 70 days after inoculation.

Conover (1962, 1964a,b) described three virus diseases of papaya viz., "mild mosaic virus"(MMV), "distortion ringspot virus"(DRV) and "faint mottle ringspot virus" (FMRV) from Florida (USA). DRV and FMRV has been considered to be closely related,but distinct strains of the same virus being aphid-transmitted, while MMV being non-aphid transmissible. Conover (1964a,b) reported that all the three viruses were readily transmitted by rubbing juice expressed from diseased leaves onto leaves of healthy plants. The results of transmission tests indicated that, though the mature plants were readily infected by leaf-rubbing method with juice expressed from DRV- infected plants, the highest percentage of transmission by DRV occurred when cotyledons of papaya seedling were inoculated before the true leaf was expanded. Mature plants showed symptoms 3 to 4 weeks after inoculation with DRV. Plants inoculated in their cotyledon stage developed symptoms within 5 to 8 days during summer months. Those intervals however, were, considerably longer in cool weather.

The Venezuelan isolate of PRSV has been reported to be readily transmitted by mechanical sap-inoculation and through aphid vector from papaya to papaya and to other test hosts. The first typical symptoms of the PRSV were evident 3 to 4 weeks after inoculation(Herold and Weibel, 1962).

Garga (1963) described a graft and aphid transmissible virus disease of papaya from Madhya Pradesh. The virus causing extreme reduction and marked distortion of papaya leaves in Madhya Pradesh have not been found sap-transmissible; the disease being successfully transmissible to healthy plants by wedge-grafting. When scions from diseased plants were wedge-grafted onto healthy stocks, typical symptoms of the disease appeared on the stocks in about 6 to 8 weeks.

The Hawaiian papaya mosaic disease described by Ishii and Holtzmann (1963) have been reported to be readily transmitted by both mechanical sap inoculation and aphid vectors. Experimental inoculations were made on papaya plants (cv. Solo) varied in age from 3 weeks to 4 months. Mechanical inoculations were made on lower 3 to 4 fully expanded leaves previously dusted with carborundum, by using cotton-tipped swabs dipped in clarified expressed sap from diseased leaves. In mechanically inoculated plants, symptoms normally appeared in 18 to 24 days after inoculation with an usual transmission ranging between 70 and 80 per cent.

In his host range studies on two papaya viruses viz., "mild mosaic" (MMV) and " distortion ringspot" (DRV), de Bokx (1965) made mechanical inoculations on papaya and other host species by dusting-over with carborundum and then rubbed

with crude, undiluted sap obtained from infected papaya leaves. Six weeks old papaya plants inoculated with sap from DRV-infected plants showed symptoms 4 weeks later, while those inoculated with MMV showed symptoms 15 to 20 days after inoculation.

In their studies on ultramicroscopic differences in inclusions of PMV and PRSV, Zettler et al. (1968) carried out all mechanical inoculations by dusting test plants with 600 mesh carborundum and rubbing them with a sterile cheese cloth pad dipped into water-diluted sap from infected papaya leaves.

According to Story and Halliwell (1969), the Dominican Republic isolate of DRV was mechanically transmitted to healthy papaya plants by sap inoculation. Symptoms appeared after15 to 17 days in plants in the 7 to 10 leaf stage. Plants in 3 to 5 leaf stage and plants older than 4 months required 20 to 25 days for symptoms to appear. The virus was transmitted from infected roots, fruit and leaves, but not from the latex.

Papaya leaf reduction virus (PLRV) was readily transmitted by mechanical sap inoculation and by aphid vector, but not by root inoculations(Singh, 1969a). Further, Singh (1972b) tested different mechanical root inoculation

methods i.e. (1) Pin-prick, (2) submerging injured roots in inoculum for 2 hours and (3) submerging intact roots in inoculum for 2 hours, to transmit " papaya mosaic virus" (PMV) earlier reported by him (Singh, 1969b) from Gorakhpur area. It was observed that papaya plants inoculated using expressed sap by "pin-prick" method showed higher rate of transmission (40 per cent) with earlier symptoms (8 to 12 days after inoculation), than those inoculated by second method (25 per cent transmission) with delayed symptom development (10 to 15 days), while those inoculated by third method displayed symptoms 20 days after inoculation with a rate of transmission as low as 6.6 per cent.

Three viruses designated as "chlorotic lesion virus" (CLV), "chlorotic ringspot virus"(CRV) and "necrotic lesion virus" (NLV) responsible for decline of papaya trees in East Africa were found to be readily transmitted by grafting and by mechanical sap inoculation. Inoculations carried out using infective sap diluted in 0.01 M phosphate buffer (pH 8.5) had resulted in the production of maximum number of lesions on Chenopodium quinoa and subsequently the same was used routinely for virus extraction and purification (Kulkarni, 1970).

An aphid transmissible mosaic disease of papaya was easily transmitted by mechanical inoculation using sap extracted and diluted in distilled water. In experimental transmissions, symptoms on 4 months old seedlings appeared after about 2 weeks of virus inoculation(Varma, 1971).

Cook and Milbrath (1971) effected the transmission of PRSV and PMV from papaya to papaya and to other hosts, using infected sap expressed either in water or 0.1 M phosphate buffer (pH 7.0) with 0.1 per cent sodium diethyldithiocarbamate and rubbed with carborundum on the leaves of various test plants.

In experiments of mechanical transmission to papaya plants, Sanchez de Luque and Martinez Lopez (1976a,b,1977) observed that the biological activity of PRSV was shown to be conserved within a broad range of concentrations and pH of phosphate buffer. In their successive trials of inoculum suspension, a concentration of 0.1 M phosphate buffer (pH 7.0) was choosen, because there was less fluctuation when one gram of tissue was suspended in 2 ml of buffer.

The " papaya ringspot virus "(PRSV) and "papaya mosaic virus" (PMV) from Udaipur, Rajasthan were reported to be easily transmitted to healthy papaya and other host plants by mechanical sap inoculation, using carborundum as an

abrasive. Inoculum was prepared by macerating infected papaya leaves with sterile mortar and pestle with 0.1 M neutral phosphate buffer (Sureka et al., 1977).

"Papaya ringspot virus" reported from Marathwada region of Maharashtra State by Yemewar and Mali (1980) was readily transmitted by sap inoculation and also be aphid vector. Sap was extracted by macerating infected leaves in a cold 0.1 M phosphate buffer (pH 7.0) containing 0.1 per cent sodium diethyldithiocarbamate. All sap inoculations were made by conventional leaf-rub method using carborundum 800 mesh as an abrasive.

The identification of viruses isolated from papaya in Nigeria was accomplished by insect transmission, grafting and by mechanical sap inoculations. Diseased leaves of papaya, ground in 1:1 mixture of 0.01 M phosphate buffer (pH 7.2) and 0.1 per cent sodium diethyldithiocarbamate served as inocula. The resulting inocula were then rubbed on leaves of test plants using celite or carborundum as an abrasive (Lana, 1980).

In Sri Lanka, Rajapakse and Herath (1981, 1982) identified three virus diseases of papaya viz., "papaya ringspot virus" (PRSV), "papaya mosaic virus" (PMV) and "papaya leaf curl virus" (PLCV) by mechanical sap inoculation, vector transmission and host range studies. In all mechanical

transmissions, infected sap extracted in 0.01 M phosphate buffer, pH 7.2 (1:1 W/V) was rubbed on the leaves of host plants using charcoal powder or carborundum as an abrasive.

Barbosa and Paguio (1982a,b) has reported that the Brazilian isolate of PRSV being readily transmitted by mechanical sap inoculation and by aphid vectors. Mechanical inoculations on test plants were effected by conventional leaf-rub method with sap extracted and diluted (1:9) in either 0.1 M(pH 7.0) or 0.7 M (pH 7.9) phosphate buffer containing 1.0 per cent celite.

Bhaskar (1983) in his confirmatory studies on the etiology of papaya mosaic occurring in Kanpur area of Uttar Pradesh, reported that the virus could be transmitted through sap and aphid vectors. He found that the infectivity of the virus to be increased when sap was extracted in 0.1 M phosphate buffer.

de la Rosa and Lastra (1983) were successful in transmitting Venezuelan isolate of PRSV from papaya to papaya and other indicator hosts by conventional leaf-rub method with sap extracted in 0.1 M potassium phosphate buffer (pH 7.5) containing 1.0 per cent potassium trisilicate.

Host range studies of 9 isolates of papaya ringspot virus and 3 isolates of watermelon mosaic virus-1 were reported to be accomplished by conventional leaf-rub method using sap of individual isolates extracted in 0.01 M potassium phosphate buffer (pH 7.0). 600 mesh carborundum was used as an abrasive (Yeh et al., 1984).

2.6.2 Aphid transmission and virus-vector relationship and ecology :

A number of studies have been conducted on the aphid species responsible for the transmission of papaya virus diseases.

Adsuar (1947b) reported the transmission of "papaya mosaic virus" in Puerto Rico by the green citrus aphid, Aphis spiraecola. Positive transmissions were obtained to the extent of 45.45 per cent with A. spiraecola when fed on diseased papaya leaves for periods of time varying from 8 minutes to 1 hour. Both nymphs and winged adults transmitted the disease with equal ease. Later, Adsuar (1947c) demonstrated that A. spiraecola to retain the virus the first 3 hours but failed to infect a second lot of plants after that time.

Capoor and Varma (1948, 1958) reported that a papaya mosaic disease was readily transmitted by six species of aphids viz., Myzus persicae (80.0 per cent), Aphis malvae (66.66 per cent), A. gossypii (47.05 per cent), A. medicaginis (35.71 per cent); Aphis sp., from Euphorbia hirta (26.66 per cent); and Macrosiphum sonchi (21.42 per cent). Negative transmissions were obtained with Aphis nerii, Pentalonia nigronervosa and Toxoptera citricidus. Of various aphid species transmitting the disease, M. persicae was proved to be the most efficient vector of the virus. In serial transfer tests involving viruleferous M. persicae and A. gossypii to 3 to 5 healthy papaya plants, it was shown that M. persicae was able to infect 3 out of 10 plants of the second series, but A. gossypii did not infect any plant after it had fed on the first set of healthy plants, indicating the non-persistent type of transmission.

In Hawaii, Jensen (1949a,b) carried out a series of aphid transmission tests to establish the virus and vector relationship for "ringspot" disease of papaya. Positive non-persistent transmission of the virus was obtained with six aphid species viz., Myzus persicae (51 per cent), Aphis medicaginis (45 per cent), A. rumicis (37.66 per cent) A. gossypii (33.33 per cent), Macrosiphum solanifolli (25 per cent) and Micromyzus formosanus (14 per cent). Additional

species of aphids which failed to transmit the disease included Aulacorthum sonchi, Rhopalosiphum pseudobrassicae and Brachycaudus helichrysi. M. persicae was found to acquire the virus in an acquisition feeding time of 2 minutes and infected healthy plants in an inoculation feeding time of 5 minutes. There was no demonstratable latent period of the virus in the aphid vector and the virus was not retained by the vector for inoculation of a second test plant series. Groups of 150 to 200 M. persicae induced infection of 100 per cent of the test plants after transfer from trees showing disease symptoms for only 10 days, while similar number of aphids transmitted the disease to 53 per cent of the test plants after transfer from trees which had been diseased for 3 months, indicating a decline in virus titre in old infected leaves available for aphid transmission. M. persicae failed to transmit ringspot virus from infected fruits (Jensen, 1949b). The first evidence of the disease in the plants inoculated with ringspot virus by means of Myzus persicae was puckering or bulging of leaves between the secondary veins and veinlets on the upper surface of young leaves. A tendency to roll downward and inward was also evident on the margins and distal point of leaves. Primary foliage symptoms usually appeared in from 9 to 21 days after infection. Plants making rapid and vigorous growth typically expressed symptoms in 9 to 14 days when inoculated by M. persicae. On the other hand, plants showing retarded and lanky growth at the time of

Gabrovska et al.(1967) in Cuba, reported the transmission of " papaya mosaic virus" with Aphis gossypii, A. craccivora, A. illinoisensis, A. nerii, A. spiraecola, Myzus persicae, Rhodobium porosum, Rhopalosiphum maidis and Acyrthosiphon pisum. They concluded that A. gossypii was major summer vector and M. persicae was the major winter vector.

Zettler et al. (1968) conducted transmission studies on "mild mosaic virus" (MMV) and "distortion ringspot virus" (DRV) of papaya with two species of aphids viz., Aphis craccivora and Myzus persicae using papaya and snapdragon as the source and test plant for MMV and papaya and pumpkin as the source and test plants for DRV. Non-viruleferous aphids were starved for 6 hours and the transmission was effected following an acquisition and inoculation feeding periods of 1 minute and 12 to 24 hours respectively. The results of this study indicated that only M. persicae was able to transmit DRV in a non-persistent manner from papaya to pumpkin, pumpkin to papaya and pumpkin to pumpkin, but not from papaya to papaya. MMV was found to be non-aphid-borne. A. craccivora failed to transmit DRV.

Transmission of two apparently related virus diseases of papaya viz., "distortion mosaic virus" (DMV) and "papaya mosaic virus" (PMV) occurring in Puerto Rico was

investigated by Schaefers (1969). In these studies 13 species of aphids viz., Aphis craccivora, A. nerii, A. gossypii, A. illinoisensis, A. spiraecola, Carolinaia cyperi, Dactynotus ambrosiae, Hyperomyzus lactucae, Myzus persicae, Rhopalosiphum maidis, R. nymphaeae, Sipha flava and Toxopotera aurantiae were investigated as potential vectors of one or both viruses. The acquisition feeding period in different tests was 1 minute or less, 5,30 or 60 minutes. Where 1 or 5 minutes access feeds were utilized, the aphids were subjected to 1 to 2 hour pre-acquisition fasting. The minimum inoculation feed period allowed was 1 hour. The number of aphids per test plant, among various tests ranging from 5 to 50, 10 being the most commonly used number. The results of this study confirmed the capability of aphid species, viz., Aphis nerii, A. gossypii, A. spiraecola and Myzus persicae as vectors of both DMV and PMV. In terms of efficiency of transmission, M. persicae appeared to be most efficient. In addition, the species, Carolinaia cyperi, and Dactynotus ambrosiae were found to be efficient vectors of DMV. No transmission occurred with A. craccivora, A. illinoisensis, Hyperomyzus lactucae, Rhopalosiphum maidis, R. nymphaeae, Toxoptera aurantiae and Sipha flava. The time from aphid inoculation to early symptom development was between 10 and 14 days.

Marin (1969) from Venezuela reported, Aphis nerii as a new vector of "distortion ringspot virus" (DRV) of papaya. Adult aphids of A. nerii were observed to be most numerous during May to June and December to January and their flight was shown to be less frequent during the hottest period of the day.

Papaya leaf reduction virus (PLRV) was reported to be readily transmitted by the aphid vector, Myzus persicae but not by Aphis gossypii, A. malvae, Rhopalosiphum maidis and Bemisia tabaci (Singh, 1969a). In an other investigation, he (Singh, 1971c) studied the vector relationship of PLRV using Myzus persicae. The results of transmission tests have indicated that the percentage infection increased with increase in the number of aphids (M. persicae) and maximum infection (100 per cent) was achieved with 10 aphids per plant. Pre-acquisition fasting was found to increase the efficiency of M. persicae and maximum infection (100 per cent) was recorded with 4 hours fasting and as it increased beyond 4 hours the efficiency showed decrease. Transmission efficiency of M. persicae was also found to increase with an increase in the acquisition feeding time and it was highest (90 per cent) at an acquisition feeding period of 15 minutes. Similarly, an increase inoculation feeding time was also observed to increase the infectivity by M. persicae and it

was maximum (100 per cent) with inoculation feeding periods of 15, 20, 25 and 30 minutes (Singh, 1971c). A "mosaic disease" of papaya from Gorakhpur region of Uttar Pradesh has also been reported to be readily transmitted by three species of aphids, Myzus persicae, Aphis gossypii and A. malvae in a non-persistent manner (Singh, 1969b). Subsequently, detailed investigations carried out by him (Singh, 1972c) on the relationship of "papaya mosaic virus" (PMV) and A. gossypii, showed that A. gossypii lost PMV more rapidly as it failed to produce infection on more than one plant in a series indicating a non-persistent manner of transmission. Pre-acquisition starvation increased the efficiency of the vector as the maximum infection (75 per cent) was obtained after 1 hour pre-acquisition starvation and as it increased beyond 1 hour the efficiency of A. gossypii showed a progressive decline. Transmission efficiency of A. gossypii was found to enhance with a progressive increase in the acquisition feeding time and it was maximum (75 per cent) at an acquisition feeding time of 15 minutes and showed a progressive decline with acquisition feeding times that were beyond 15 minutes. Similarly, increase in inoculation feeding time on test plants, was also found to increase the efficiency of A. gossypii and it was highest (75 per cent) at an inoculation

feeding time of 15 minutes. Infection increased with the increase in number of aphids per test plant, as the maximum infection (75 per cent) was obtained with 10 apterous adults per plant. Minimum inoculation and acquisition threshold periods were found to be ½ and 1 minutes respectively. Apterous adults of A. gossypii were efficient vectors of PMV than nymphs and alate adults (Singh, 1972c).

Khurana and Bhargava (1971) tested seven aphid species for their ability to transmit three virus diseases of papaya i.e. "mild mosaic virus" (MMV)," distortion ringspot virus" (DRV) and " ringspot virus" (RSV). Mild mosaic virus (MMV) was transmitted by Aphis craccivora (30 per cent). A. gossypii (90 per cent) and Myzus persicae (90 per cent). Distortion ringspot virus (DRV) was transmitted by A. nerii (30 per cent), Lipahis pseudobrassicae (40 per cent) and M. persicae (80 per cent), while ringspot virus (RSV) was transmitted only by M. persicae (90 per cent). Negative transmission was obtained with Longiunguis sacchari, Rhopalosiphum maidis and Aphis craccivora. A. nerii and Lipaphis pseudobrassicae were recorded as new vectors.

Higa and Namba (1971) in Hawaii, studied the vectors of "papaya mosaic" (PMV). The results of these transmission tests showed that PMV was readily transmitted

to test papaya plants by four species of aphids viz., Rhopalosiphum maidis (53 per cent), Macrosiphum euphorbiae (20 per cent), Aphis gossypii (81.25 per cent) and A. craccivora (6.66 per cent). No transmission occurred with Hyperomyzus lactucae.

Namba and Higa (1972, 1975, 1977) extensively worked out PMV transmission by Myzus persicae. The results of host susceptibility tests have indicated that cultivars differed in their susceptibility to PMV when transmission was carried through viruleferous aphids of M. persicae. Cucumber cultivars, Lehua 64A1 and 70-A-68 were found to be more susceptible than Colorado Long. Similarly, papaya cultivar, Line-8 was more susceptible than Waimanalo Solo and Kapoho Solo cultivars of papaya (Namba and Higa, 1972). With subsequent pre-acquisition fasting periods (that were grouped into 10 minutes increments ranging from 31-40 to 121-150 minutes), M. persicae were allowed one probe lasting anything between 10 to 30 seconds on infected plants before being transferred to healthy papaya or cucumber and left for 30 minutes, the aphid displayed a peak transmission efficiency of 45.5 per cent after fasting for 31 to 40 minutes which stepped down to an average value of 39.2 per cent in case where fasting lasted for 41 to 100 minutes and touched its lowest value of 28.7 per cent when the aphids were fasted for 121 to 130 minutes. The maximum number of probes (78.1 per cent) were recorded after 121 to 130 minutes

fasting. The least number of acquisition probes, on the other hand (53.1 per cent) were displayed when the aphids were subjected to a preliminary fasting of 31 to 40 minutes (Namba and Higa, 1975). Late instar apterae of M. persicae, under controlled laboratory conditions, transmitted PMV upto 7 hours after its acquisition. However, the rate of transmission dropped considerably after the first 30 minutes. Further, there was no difference in the retention of inoculativity of PMV between aphids held at 25°C and 5°C (Namba and Higa, 1977).

A serious mosaic disease infecting several papaya orchards in Haryana and Rajasthan was proved to be readily transmitted by five aphid species, viz., Myzus persicae, Aphis gossypii, A. nerii, Macrosiphum sp., and Rhopalosiphum maidis in a non-persistent manner. Of these, M. persicae, R. maidis and Macrosiphum sp., were found to be more efficient vectors of papaya mosaic virus (Varma, 1971).

"Papaya ringspot virus" (PRSV) described by Sanchez de Luque and Martinez Lopez (1976a,b) was reported to be transmitted more efficiently by Myzus persicae than by means of mechanical inoculation (Sanchez de Luque and Martinez Lopez, 1976a,b, 1977). It was also reported that the cotton aphid, Aphis gossypii being more prevalent in papaya growing areas than M. persicae, was also proved to be a

vector of the PRSV. Both the aphids transmitted PRSV in the non-persistent manner (Sanchez de Luque and Martinez Lopez, 1976a,b).

" Papaya ringspot virus" (PRSV) described in Marathwada region by Yemewar and Mali (1980) have been reported to be readily transmitted by the cotton aphid, Aphis gossypii from papaya to papaya and to cucumber, ashgourd, muskmelon and pumpkin with brief probes of 50 to 60 seconds, thereby indicating a non- persistent manner of transmission (Yemewar and Mali, 1980). The "papaya ringspot virus" (PRSV) reported from Marathwada region of Maharashtra (Yemewar and Mali, 1980) have successfully been demonstrated to be transmitted from papaya to papaya with five aphid species viz., Aphis craccivora (32 per cent), A. gossypii (64 per cent), A. nerii (32 per cent), Dactynotus sonchi (68 per cent) and Myzus persicae (68 per per cent). Two aphid species viz., Longiunguis sacchari and Rhopalosiphum maidis have been proved to be non-vectors of the PRSV (Dake, 1986).

In Nigeria, a study was made of the aphid transmission of four virus isolates which were isolated from papaya viz., "papaya mosaic virus" (PMV),"papaya ringspot virus (PRSV), "tobacco ringspot virus" (TRSV) and a "graft transmissible virus-like isolate", employing five

species of aphids viz., Aphis craccivora, A. gossypii, A. maidis, Myzus persicae and Toxoptera aurantiae. The aphid transmissibility tests revealed that the papaya ringspot virus (PRSV), but not PMV, TRSV and a graft transmissible virus, was readily transmitted by Myzus persicae (36.58 per cent), Aphis gossypii (72.55 per cent), A. craccivora (2.44 per cent), A. maidis (22.10 per cent) and Toxoptera aurantiae (32.55 per cent). Aphis gossypii was found to be the most efficient vector of PRSV although, M. persicae was more prevalent in papaya plantations (Lana, 1980).

Rajapakse and Herath (1981, 1982) tested different species of aphids found in the vicinity of papaya gardens in Sri Lanka as vectors of papaya virus diseases viz.,"papaya mosaic virus"(PMV), "papaya ringspot virus" (PRSV) and "papaya leaf curl virus "(PLCV). PMV and PRSV, but not PLCV, have been found to be aphid transmissible. PMV was proved to be readily transmitted by five species of aphids viz., Aphis craccivora (50 per cent), A. gossypii (70 per cent), A. nerii (40 per cent), A. spiraecola (33 per cent) and Myzus persicae (100 per cent) but A. malvae, Lipaphis pseudobrassicae and Toxoptera aurantiae were non-vectors of PMV (Rajapakse and Herath, 1981). Moreover, M. persicae, A. gossypii, A. craccivora and A. spiraecola were found to transmit both PMV and PRSV, nevertheless, A. nerii specifically transmitted only

PMV. Cent per cent disease transmission was recorded in case of PMV with M. persicae and A. craccivora and with A. spiraecola in case of PRSV. Aphis craccivora transmitted both PMV (100 per cent) and PRSV (91.66 per cent) efficiently. Three aphid species viz., Aphis malvae, Lipaphis pseudobrassicae and Toxoptera aurantiae were non-vectors of PMV and PRSV (Rajapakse and Herath, 1982).

In Taiwan, "papaya ringspot virus"(PRSV) was tested for its aphid transmissibility. Six species of aphids viz., Aphis gossypii, A. medicaginis, A. nerii, Myzus persicae, Rhopalosiphum maidis and Sinomegoura citricola readily transmitted PRSV in a non-persistent fashion. Optimum transmission by previously fasted M. persicae occurred with an acquisition feeding time of 2 to 5 minutes. Infectivity of M. persicae was lost after 2 hours of post-acquisition fasting or after 20 minutes fasting on broccoli. Although, a single aphid could transmit PRSV to papaya seedlings, a high infective rate was achieved when 5 to 10 aphids were used (Wang, 1981).

Barbosa and Paguio (1982a) in Brazil, identified the causal virus involved in "papaya ringspot disease" as PRSV-PE, a member of potyvirus group, based on symptomatology, aphid transmissibility, host range and inclusion morphology. They reported that PRSV-PE prevalent in Pernambuco, Brazil, was readily transmitted by Toxoptera citricidus and

Dactynotus sp.

Aphid transmissibility of "papaya mosaic virus" (PMV) prevalent in Kanpur area of Uttar Pradesh, was investigated by Bhaskar (1983); his results indicated that PMV was readily transmitted by Aphis gossypii (66.66 per cent), A. malvae (16.66 per cent), A. craccivora (50.00 per cent) and Myzus persicae (66.66 per cent). Apterous aphids (A. gossypii) were proved to be more efficient vectors of PMV than winged ones.

Transmission experiments conducted in net house, in Taichung, Taiwan, indicated that "papaya ringspot virus" (PRSV) could be transmitted by green peach aphid, Myzus persicae and corn leaf aphid, Rhopalosiphum maidis from papaya to papaya in a non-persistent manner. PRSV was transmitted in the laboratory by M. persicae for 6 hours after acquisition feeding and its infectivity was evident 2 weeks after inoculation (Hwang and Hsieh, 1984).

At Punjab Agricultural University, Ludhiana, detailed investigations were undertaken by Cheema and Reddy (1985a,b) to establish the relationship of a virus causing "papaya mosaic disease" (PMV) and corn leaf aphid, Rhopalsiphum maidis, which was earlier reported by them as a new and efficient vector of PMV (Cheema and Reddy, 1985a). The results of these investigations indicated that: the

unstarved R. maidis transmitted the virus to the extent of 20 per cent; pre-acquisition starvation, however, increased the efficiency of vector and maximum infection (60 per cent) was obtained with 30 minutes pre-qcquisition starvation; efficiency of the vector increased with the increased acquisition access and it was highest (90 per cent) at an acquisition feeding period of 15 minutes; infectivity by R. maidis was found to increase with an increase in the inoculation feeding time and it was highest (70 per cent) with an inoculation feeding time of 15 minutes; the infectivity by R. maidis showed a progressive decrease after the optimum pre-acquisition fasting (30 minutes), acquition and inoculation feeding (15 minutes)times; the infection being increased with the increase in number of aphids per test plant and maximum infection was obtained with 5 apterous adults per plant; apterous and alate forms transmitted the virus with the same efficiency; adults were more efficient vectors than nymphs and R. maidis lost the virus more rapidly after inoculating the first series of test plants indicating a non-persistent manner of transmission (Cheema and Reddy, 1985b).

Aphid transmission tests conducted on "mosaic disease of papaya" at Andhra Pradesh Agricultural University, Hyderabad, revealed that virus could be readily transmitted by two species of aphids, Aphis gossypii and Brevecoryne brassicae in a non-persistent manner to an extent of 50.0

and 16.6 per cent respectively. Two aphid species viz., Aphis craccivora and Dactynotus carthami failed to transmit the virus (Susan John, 1985).

Hsieh and Hwang (1986) investigated some ecological aspects of green peach aphid (GPA) transmitting "papaya ringspot virus" in Taiwan. Their investigationson host plants of green peach aphid (Myzus persicae) showed that it could survive and multiply on weeds such as Solanum nigrum, Amaranthus mangostanns, Alternanthera sessilis, Ilysanthes antipoda but not on Carica papaya. The flight velocity of M. persicae was 1.4 ± 0.4 KM/hr. Observation on the dispersal behaviour of M. persicae indicated that there was no regular pattern, the aphid landed on the papaya plant one hour after release and took off 1 hour later or stayed over night before departure. The preference of aphid to colour was as follow: Yellow > orange > green. Seasonal monitoring of alate aphids in papaya orchard showed that M. persicae was the vector that occurred most consistently. The population of M. persicae initially appeared in September to October of warm and dry season, reached the peak in November or December, and declined in January. Two density peaks in February and during April to May were probably due to warm weather and spring migration. From June to August were hot and rainy summer, the population density of alate M. persicae declined to the lowest level.

The initial infection of papaya orchard perhaps was transmitted by the viruleferous alate aphids, then the papaya infected with virus disease served as the virus source, during aphids migrating period, the papaya ringspot virus disease might account for rapid spread in the papaya orchard.

Reports on the occurrence of aphids on papaya and on the crops grown in and around papaya plantations would appear to be of pertinent interest, particularly with regards to the feasibility of disease management through vector control. Capoor and Varma (1958) noted the absence of aphids on papaya in Bombay Province, India. Papaya has been reported to be a poor host for aphids in Cuba, although mature alate of several species have frequently been observed on the host including A. gnaccivora, A. gossypii, A. middletonii, A. spiraecola, Aulacarthum circumflex, Dactynotus ambrosiae, Myzus persicae, M. euphorbiae, Protaphis sp., Toxoptera aurantiae and Rhopalosiphum maidis. Myzus persicae often reached damaging proportions on papaya in Hawaii (Holdaway and Look, 1941; Look and McAfee, 1944; Jensen, 1949b; Sherman and Tamashiro, 1959). Aphis spiraecola has been reported to occur on papaya in Puerto Rico (Adsuar, 1947b; Wolcott, 1948, 1955) and in abundance on this host in Florida (Martorell and Adsuar, 1952).

Aphis gossypii was of common occurrence on papaya in Hawaii (Holdaway and Look, 1941; Look and McAfee, 1944; Jensen, 1949b; Sherman and Tamashiro, 1959), Florida (Martorell and Adsuar, 1952) and also on papaya fruits in Venezuela (Kralovic, 1967), but has not been observed to colonise this host in Puerto Rico (Schaefers, 1969). Rhopalosiphum fitchii has been observed to occur in abudance on papaya in Florida (Martorell and Adsuar, 1952). Look and McAfee (1944) in Hawaii, noted the occurrence of two additional aphid species viz., Aulacarthum circumflex and Aphis middletonii on papaya. Schaefers (1969) reported that he has collected mature alate on papaya with some frequency. The aphid species collected by him include A. spiraecola, A. gossypii, A. nerii and Aphis coreopsidis, with A. spiraecola being by far the most commonly collected species. But he failed to observe successful colonisation of papaya by any of these aphids in Puerto Rico. Recently, Wan and Conover (1983) while surveying virus diseases of papaya in Florida, noted the colonisation of papaya by whiteflies and mites (Tetranychyus sp.), but not at all by aphids. Subsequently, investigations made on host plants of green peach aphid, Myzus persicae, a major vector of PRSV in Taiwan, showed that M. persicae could survive and multiply on certain weed hosts, but not at all on Carica papaya and alate aphids were proved to be responsible in spreading PRSV during their migration (Hsieh and Hwang, 1986).

Aphid trap collection studies carried out in Florida by Wolfenbarger (1966) indicated that most of the aphids were in flight in the months of January, February, March and April, but fewest in the months of July, August and September and that sufficient aphids appeared to be present, however, to transmit papaya viruses at all times. Adult aphids of A. nerii, a potential vector of "distortion ringspot virus" (DRV) of papaya were found to be most numerous during May, June, December and January and their flight was shown to be less frequent during hottest period of the day.

During the survey of "papaya mosaic disease" (PMV) in Haryana and Rajasthan, Varma (1971) recorded 100 per cent incidence of PMV, where, wheat and brassica crops were grown in the vicinity of papaya orchards, but in one orchard where berseem was grown as intercrop, the plants happened to be free from PMV. He attributed high incidence of PMV in certain orchards to the aphids which bred on rose, wheat and brassica being grown in the vicinity.

2.6.3 Seed transmission :

Many aphid-borne virus diseases of papaya displaying symptoms similar to "papaya ringspot virus" (PRSV), but described under different names and the "papaya ringspot virus" in particular have not been reported to be seed-borne in papaya. These include "mosaic" diseases reported from

erstwhile Bombay Province, India (Capoor and Varma, 1948, 1958), Hawaii (Ishii and Holtzmann, 1963), Gorakhpur, India (Singh, 1969b); "distortion ringspot virus" (Conover, 1964a) and "faint mottle ringspot virus" (Conover, 1964b) from Florida; "papaya decline viruses" from East Africa (Kulkarni ,1970) and " papaya ringspot virus" reported from Florida (de Bokx, 1965) and from India (Yemewar and Mali, 1980; Susan John, 1985; Deke, 1986).

2.6.4 Pollen transmission :

No information is available on the pollen transmission of papaya ringspot virus. However, three viruses, namely "chlorotic lesion virus" (CLV), chlorotic ringspot virus" (CRV) and " necrotic lesion virus" (NLV) isolated from papaya showing field decline symptoms in East Africa having similarities in foliar symptoms and particle morphology (750 x 12 nm rods) to PRSV, were proved to be pollen transmissible from papaya to papaya (Kulkarni, 1970).

2.6.5 Graft transmission :

Information in respect to graft transmission of PRSV is lacking in the literature surveyed. However, three aphid transmissible mosaic diseases of papaya described and reported from Puerto Rico (Adsuar, 1947a), erstwhile Bombay Province of India (Capoor and Varma, 1948, 1958) and Madhya

Pradesh of India (Garga, 1963) were reported to be graft transmissible.

Adsuar (1947a) reported that Puerto Rican "papaya mosaic virus" (PMV) was successfully transmitted from PMV-infected to healthy seedlings of papaya, when pieces of stem tissues from infected plants were grafted onto healthy plants. Test plants inoculated by grafting showed symptoms typical of PMV within 2 to 3 weeks after graft inoculation.

An aphid transmissible mosaic disease infecting papaya in erstwhile Bombay Province, India, was reported to be successfully transmitted to healthy seedlings of papaya by "wedge-grafting", with a rate of transmission as high as 72.5 per cent. Typical symptoms of PMV were evident on grafted stocks within about 6 to 8 weeks after graft inoculation (Capoor and Varma, 1948, 1958).

Garga (1963) reported that a serious aphid transmissible "papaya mosaic virus" (PMV), infecting papayas in Madhya Pradesh, was successfully transmitted from infected to healthy seedlings of papaya by " wedge-grafting", when scions were wedge-grafted onto healthy stocks. Typical symptoms of PMV appeared on the stocks in about 6 to 8 weeks after graft inoculation.

2.6.6 Dodder transmission :

Information in respect to dodder transmission of PRSV is lacking in the literature surveyed. A single available report by Garga (1963) from Madhya Pradesh indicated that an aphid-borne virus disease of papaya displaying symptoms similar to PRSV, could not be transmitted from papaya to papaya through the agency of dodder inspite of dodder (Cuscuta sp.) had been well established on both diseased and healthy plants of papaya.

2.7 Host range :

Jensen (1949a) using aphid (M. persicae) inoculations reported that all the 16 plant species representing 12 families viz., Commelina diffusa, Bryophyllum claycinum, Beta vulgaris, Lactuca sativa, Cucumis sativus, Brassica chinensis, Malvastrum coromandelianum, Mirabilis jalapa, Crotalaria incana, Passiflora foetida, P. pfordti, Portulaca oleracea, Capsicum frutescens, Lycopersicon esculentum, Solanum tubersoum and Nicotiana tabacum were non-hosts of the PRSV.

Capoor and Varma (1948, 1958) using mechanical sap inoculation tested 56 plant species in 15 families and reported 10 plants species belonging to Cucurbitaceae as hosts of a virus causing aphid transmitted papaya mosaic in Bombay Province, India. The plant species in Cucurbitaceae

included bottlegourd, cucumber, roundgourd, ridgegourd, snakegourd, muskmelon, watermelon, pumpkin, squash and vegetable marrow. However, bittergourd, ashgourd and spongegourd were immune to the virus.

Of the 49 test plants in 21 species, only one test plant has been reported to be the host of "papaya mosaic disease" in Puerto Rico. Melothria quadaleupensis was only a susceptible host and proved to be symptomless carrier of the virus (Adsuar, 1950).

Herold and Weibel (1962) observed mild mosaic mottling and scattered pale green spots on the leaves of squash (Cucurbita pepo var. Zuchini-Italian Vegetable Marrow) upon mechanical inoculation of cotyledons with Venezuelan isolate of papaya ringspot virus.

A graft and aphid transmissible virus disease of papaya from Madhya Pradesh had a restricted host range to Caricaceae. Plant species viz., Lycopersicon esculentum, Nicotiana tabacum cv. White Burley, Datura innoxia, Solanum melongena, Zinnia elegans, Luffa acutangula and Citrullus lanatus were non-hosts of the virus (Garga, 1963).

Ishii and Holtzmann (1963), in Hawaii, have indicated that host plants of papaya mosaic caused by PRSV were species limited to the family Cucurbitaceae.

These included: Cucumis melo var. reticulata, Cucumis sativus, Cucurbita maxima, Cucurbita pepo and Citrullus lanatus. Cucumis sativus showed only light to mild mosaic; Cucurbita pepo, Cucurbita maxima, Cucumis melo var. reticulata and Citrullus lanatus showed mild to severe mosaic with scattered green blisters in addition to leaf distortion. The average incubation period on Cucurbits was 12 days as compared to 21 days on papaya. Plant species that failed to show symptoms were: Gomphrena globosa, Vinca rosea, Impatiens balsamina, Beta vulgaris, Spinacia oleracea, Zinnia elegans, Raphanus sativus, Brassica rapa, Momordica balsamina, Phaseolus vulgaris var. humilis, Vigna sinensis, Nicotiana glutinosa, N. rustica, N. tabacum, Petunia hybrida and Apium graveolens var. dulce.

Conover (1964a,b) reported that the host range of all the three viruses (DRV, FMRV, MMV) was restricted only to plant species belonging to Caricaceae and Cucurbitaceae. Hosts in Caricaceae, susceptible to MMV, DRV and FMRV included: Carica cauliflora, C. goudotiana, C. monoica. Carica candamarcensis was susceptible only to MMV, while C. quercifolia was immune to all the three viruses. Non-cucurbitaceous hosts which were susceptible to MMV, DRV and FMRV included Cyclanthera pedata and Melothria pendula, whereas, cucurbitaceous hosts viz., cucumber,

muskmelon, watermelon and summersquash were readily infected by DRV and FMRV, but not by MMV. Plants not susceptible to MMV, DRV and FMRV were: Luffa cylindrica, Momordica charantia, Nicotiana tabacum, N. tabacum x glutinosa, Phaseolus vulgaris, Passiflora edulis f. flavacarpa, Sicana oderifera and Vigna sinensis.

de Bokx (1965) while studying MMV and DRV described by Conover (1962, 1964a,b) reported that MMV was detected by means of local lesion hosts such as Gomphrena globosa, Chenopodium amaranticolor, and Cassia occidentalis. Antirrhinum majus and Sesamum indicum were systemic hosts, while Vinca rosea and Zinnia elegans were symptomless hosts of MMV. The systemic hosts of DRV were pumpkin (Cucurbita pepo) cv. small sugar and squash (C. pepo var. melopepo), which exhibited mosaic symptoms 12 to 15 days after virus inoculation. The non-hosts of DRV isolate were: cucumber cv. Chicago pickling; watermelon cv. Stone Mountain; Gomphrena globosa, Chenopodium amaranticolor, and Cassia occidentalis. Contrary to Conover's findings, squash (Cucurbita pepo var. melopepo) and pumpkin (C. pepo) were the only cucurbitaceous hosts of the DRV isolate observed in this study by de Bokx.

With "papaya mosaic disease" (PMV) Namba and Kawanishi (1966) found that papaya, cucumber and watermelon, which were important hosts of the virus in Hawaii, were

equally susceptible to PMV when transmitted by the green peach aphid, Myzus persicae.

The susceptibility of different hosts to MMV and DRV as confirmed by Conover (1962, 1964a) and de Bokx (1965) was re-evaluated by Zettler et al., (1968) through differential aphid transmission and mechanical inoculation using de Bokx's isolates. These studies have indicated that in addition to papaya, cucumber (Cucumis sativus) cvs. Chicago Pickling, National Pickling, A & C, Marketer and Ohio MR 17; and watermelon (Citrullus lanatus) cv. Stone Mountain were susceptible to both MMV and DRV showing systemic symptoms. The susceptibility of other cucurbitaceous test hosts varied with MMV and DRV isolates. Thus pumpkin (Cucurbita pepo) cvs. Small Sugar and Jack-O-Lantren; and summersquash (C. pepo var. melopepo)cvs. Early Prolific Straightneck and Yellow Prolific Straightneck were infected systemically only by DRV, but not by MMV. Similarly, muskmelon (Cucumis melo) was found to be systemically infected by MMV only. The non-cucurbitaceous hosts, Antirrhinum majus and Gomphrena globosa were susceptible only to MMV isolate and showed systemic and local symptoms respectively. Their results were in agreement with the findings of Conover (1962, 1964a,b) and de Bokx (1965) regarding the susceptibility of pumpkin and summersquash to MMV and DRV, while results

regarding the susceptibility of cucumber, watermelon and muskmelon, however, failed to confirm the results of either Conover (1962, 1964a,b) or de Bokx (1965), even though same isolates were employed by all the investigators. In Conover's host range studies, cucumber, watermelon and muskmelon were found to be non-hosts of MMV, while in de Bokx's studies, Chicago Pickling cultivar of cucumber and Stone Mountain cultivar of watermelon were not infected by DRV.

Hosts of DRV isolate from Dominican Republic determined by mechanical inoculation were found only in the Cucurbitaceae and Caricaceae (Story and Halliwell, 1969). Cucurbit hosts of the virus included pumpkin (cv. Small Sugar) and squash (cv. Early Prolific Straightneck). Hosts within the Caricaceae was the Solo variety of papaya. Plants not infected by DRV were: muskmelon, watermelon, cucumber, bushbean (Phaseolus vulgaris), tobacco, Gomphrena globosa, Antirrhinum majus, Vinca rosea, Zinnia elegans and Chenopodium amaranticolor. The inability to infect cucumber, muskmelon and watermelon with DRV was in disagreement with the reports by Conover (1964a), de Bokx (1965) and Zettler et al. (1968). Disagreement of researchers in the reported DRV cucurbit host range was partially attributed to the differential susceptibility of these hosts when inoculated and it was opined that the susceptibility of cucurbits was limited to seedling stage, particularly

just before the emergence of the primary leaves. And it was supported by the fact that the DRV often failed to infect pumpkin or squash when inoculated in their primary leaf stages (Story and Halliwell, 1969).

Singh (1969a,b) reported that the host range of PLRV and PMV (possibly isolates of PRSV) was restricted only to the family Caricaceae. Fifty six species of plants belonging to 16 families (Apocynaceae, Balsaminaceae, Caparidaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Cucurbitaceae, Geraniaceae, Leguminosae, Malvaceae, Plantaginaceae, Schrophulariaceae, Solanaceae, and Tropeolaceae), when separately inoculated mechanically with these viruses, PLRV and PMV could not infect any other plants than Carica papaya.

Cook and Zettler (1970) while screening 90 accessions of Carica papaya received from different sources, using two isolates each of PRSV and of PMV originated from Florida and Venezuela, have not found any differential cultivar. For instance, PRSV and PMV isolates from Florida have been found indistinguishable from their respective counterpart, Venezuelan isolates in symptomatology in C. papaya. However, they have been able to identify diagnostic plant species for PRSV and PMV. For instance both the PRSV isolates systemically infected Cucurbita peop cv. Small Sugar, but neither of them infected C. globosa. Both the PMV isolates failed to infect C. pepo cv. Small Sugar, but readily

infected G. globosa and produced identical local lesions on the inoculated leaves. Plants of Jacaratia mexicana related to Carica species were apparently immune to PRSV and were hypersensitive to PMV.

Host range studies made on three viruses viz., CLV, CRV, and NLV isolated from papaya showing " field decline symptoms" in East Africa showed that CLV, CRV when maintained in papaya were found to infect Chenopodium amaranticolor, C. quinoa, Carica papaya and soybean. The host plants susceptible only to NLV were: Antirrhinum majus and groundnut. All the three viruses failed to infect cucumber, muskmelon, watermelon, wintersquash and pumpkin apart from other species of plants (Kulkarni, 1970).

Cook and Milbrath (1971) attempted to verify the identity of viruses infectuous to papaya grown in the island of Oahu, Hawaii, and to determine possible sources of resistance and other additional diagnostic hosts of these viruses. Their identification of "papaya ringspot virus" (PRSV) was based on systemic infection in Cucurbita pepo cv. Small Sugar and failure to infect G. globosa, while that of PMV was based on the production of necrotic local lesions on G. globosa and Cassia occidentalis and failure to produce symptoms on C. pepo cv. Small Sugar. Nicotiana glutinosa and Datura stramonium were insusceptible to both PRSV and PMV, while these hosts were

used as diagnostic hosts for the identification of "tomato spotted wilt" (TSWV) infecting papaya. Plants of Vicia faba, Celosia plumosa were systemically infected, while that of Ocimum basilicum developed chlorotic rings on inoculated leaves following infection with PMV, but not at all by PRSV. Chenopodium amaranticolor was found to be infected by both the viruses. Leaves of C. amaranticolor inoculated with PRSV developed red-bordered chlorotic local lesions easily distinguishable from those caused by PMV. Plants of Carica cauliflora, C. goudotiana, C. monoica, C. papaya cv. Solo; C. parviflora and C. pubescens were found susceptible, while those of related Jacaratia spinosa were immune to both PRSV and PMV.

A Venezuelan isolate of PRSV described by Lopez Pinto (1972) have been reported to infect Cucurbita pepo and a weed host Melothria guadalupensis in addition to papaya.

In their extensive host range studies oriented towards the identification of indicator hosts of Colombian isolate of PRSV, Sanchez de Luque and Martinez Lopez (1976b, 1977) failed to locate not even a single local lesion host from among the 33 plant species belonging to 13 families (Amaranthaceae, Caricaceae, Chenopodiaceae, Convolvulaceae, Compositae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Hypercaceae, Leguminosae, Mimosaceae, Portulaceae

and Solanaceae) tested as possible hosts. The systemic hosts of PRSV were : Carica goudotiana, Citrullus lanatus, Cucumis melo and Cucurbita pepo cvs. Senator and Small Sugar. C. pepo cv. Small Sugar was found to be the most promising plant to be used as a indicator host for PRSV diagnosis. For systemic hosts not being worthy indicator plants of the virus, they indicated that these hosts might play an important role in the epidemiology of the disease, as they preserve the virus in the absence of papaya (Sanchez de Luque and Martinez Lopez, 1977). None of the weeds studied was a host of the virus. Cucumis sativus, C. dipsaceus, Cucurbita moschata, Momordica charantia, C. amaranticolor and Cassia occidentalis were non-hosts of PRSV in addition to several leguminous, solanaceous and other test hosts.

The host range of "papaya ringspot virus" (PRSV) described and reported from Udaipur, India, include plant species belonging to Caricaceae, Chenopodiaceae and Cucurbitaceae. The virus induced chlorotic local lesions surrounded by red-borders in C. amaranticolor; systemic mosaic mottling in pumpkin (C. maxima), squash (C. pepo) and vegetable marrow (C. pepo var. medullosa). The virus failed to produce symptoms on cucurbitaceous hosts like Cucumis melo, Citrullus lanatus, C. fistulosus, Lagenaria siceraria, Luffa acutangula and Tricosanthes anguina (Sureka et al., 1977).

In contrast to the report of Sanchez de Luque and Martinez Lopez (1977), Wang et al.(1978) in Taiwan, observed Chenopodium amaranticolor and C. quinoa to react with local lesions following inoculation with Taiwan isolate of PRSV. The virus also caused systemic necrosis on melon and mosaic on other cucurbits (Wang et al., 1978).

Chenopodium amaranticolor and C. quinoa have been found to be hypersensitive to PRSV in Taiwan, inspite of the fact that the virus was recoverable only from C. quinoa. Two isolates of PRSV, virus-I causing wilting and virus-II producing conspicuous mosaic have been recognised and squash being indicated as the best differential host for the two (Chang, 1979).

Marathwada isolate of PRSV has been reported to infect plant species in Chenopodiaceae and Cucurbitaceae families. The systemic hosts of the virus reported include ashgourd, spongegourd, longmelon, cucumber, pumpkin and squash. C. amaranticolor and C. quinoa have been found to be local lesion hosts. Watermelon, bottlegourd, ridgegourd, bittergourd and two species of Chenopodium viz., C. album and C. murale, apart from plant species belonging to Amaranthaceae, Compositae, Leguminosae and Solanaceae have been recorded as non-hosts of the virus (Yemewar and Mali, 1980).

Lana (1980) reported that the Nigerian isolate of PRSV had a narrow host range restricted to Chenopodiaceae, Caricaeae and Cucurbitaceae. The local lesion hosts reported include C. amaranticolor and C. quinoa; and systemic ones inducing mosaic include Cucumis sativus cv. Money Maker; faint mottle in Cucurbita pepo cv. Zuchini; and vein-clearing, leaf curl, and ringspot in papaya cvs. Local and Solo. Non-hosts of the virus included Citrullus lanatus, G. globosa, Vigna unguiculata and Nicotiana tabacum.

The host range upon mechanical sap inoculation of PRSV isolate from Sri Lanka included Carica papaya, Cucurbita pepo, Cucumis sativus and Chenopodium amaranticolor. Carica papaya cvs. Solo Hawaii and Coimbatore Dwarf-1, reacted with systemic chlorosis, vein-clearing and ringspot; C. pepo, C. sativus and Chenopodium amaranticolor with systemic faint mottle, mosaic and necrotic local lesions respectively. The plants of N. tabacum, G. globosa, Vigna sinensis, Phaseolus vulgaris and Lycopersicon esculentum were non-hosts of the virus (Rajapakse and Herath, 1982).

Resistance to PRSV in PI-292190- an accession of Cucumis metuliferus has been reported to be conferred by a single dominant gene. Clones of F_2 and test-cross plants inoculated with PRSV or "water melon mosaic virus-1 (WMV-1) reacted identically, suggesting that the factor for resistance

to PRSV is closely linked to Wmv or may be the same factor. PRSV and WMV-1 are known to be closely related serologically. ACC-2459 of C. metuliferus has been found to be an ideal and valuable host for the propagation of isolates of PRSV (Provvidenti and Gonsalves, 1982).

The Pernambuco isolate of PRSV studied by Barbosa and Paguio (1982a,b) had a host range limited to Caricaceae and Cucurbitaceae. The virus caused systemic infection in Carica papaya, C. pepo cvs. Caserta, Early Prolific Straightneck, Zuchini; and Cucumis melo cvs. Amarelo Redonodo and Edisto. Cucumis sativus, Citrullus lanatus, N. tabacum, G. globosa, Antirrhinum majus, Chenopodium amaranticolor, Phaseolus vulgaris and Vigna unguiculata were non-hosts of the PRSV.

Wan and Conover (1983) observed that the leaves of Small Sugar Pumpkin inoculated with PRSV developed chlorotic spots, vein-clearing, vein-banding and systemic mottling.

The Chinese strain of PRSV has been reported to invade members of the Cucurbitaceae, in addition to the Caricaceae (Wu et al., 1983).

An aphid transmitted mosaic disease of papaya from Kanpur, India, have been reported to infect hosts like Cucumis sativus, Trichosanthes anguina, Cucurbita maxima,

Citrulus lanatus, Lagenaria siceraria and Cucumis melo. The virus produced characteristic local lesions on the inoculated leaves of Chenopodium amaranticolor (Bhaskar, 1983).

Of the several plants species belonging to the families of Amaranthaceae, Chenopodiaceae, Caricaceae, Cucurbitaceae, Leguminosae and Solanaceae, PRSV isolate from Venezuela has been shown to infect only some plant species belonging to Caricaceae viz., Carica papaya and Cucurbitaceae viz., Cucurbita pepo cv. Early Prolific Straightneck. The non-hosts of the virus were N. tabacum, Cassia occidentalis, Chenopodium amaranticolor, C. quinoa, G. globosa and Vigna sinensis (de la Rosa and Lastra, 1983).

While conducting comparative host range studies of nine isolates of PRSV from Taiwan, USA (Hawaii, Florida), Eucador; and three isolates of watermelon mosaic virus-1 (WMV-1) from the USA (New York, Virginia, Florida), Yeh et al. (1984) have reported that all the nine isolates of PRSV infected members of Chenopodiaceae, Caricaceae and Cucurbitaceae, while watermelon mosaic virus-1 isolates such as WMV-1NY and WMV-1VG infected only Cucurbitaceae, but WMV-1F infected both Chenopodiaceae and Cucurbitaceae. All the nine PRSV isolates and one of WMV-1 isolates viz.,

WMV-1F induced local lesions in both Chenopodium amaranticolor and C. quinoa. The major difference between PRSV and WMV-1 was that the former infected Carica papaya and the latter did not. Three species of Cucurbitaceae viz., Cucumis metuliferus (ACC-2459), Cucumis anguaria var. anguaria and Cucumis anguaria var. longipes were found to be valuable hosts for the propagation of PRSV. Cucumis metuliferus (PI-292194), Cucumis melo line B 66-5 and Cucumis sativus "Surinam", which possessed genes resistant to WMV-1, reacted identically to all isolates of WMV-1 and PRSV. All the isolates of WMV-1 and PRSV produced systemic symptoms on Cucumis melo cv. Gold Star, Cucumis meeusii, Cucumis dipsaceous, Cucumis dinteri, Cucumis hardwickii, Citrullus lanatus, Cucurbita moschata and Luffa acutangula. None of the PRSV or WMV-1 isolates infected Nicotiana benthamiana, Brassica campestris, Pisum sativum or Phaseolus vulgaris (Yeh et al., 1984).

The PRSV isolate from Andhra Pradesh had a restricted host range. Besides papaya, it produced systemic symptoms on Cucumis sativus, C. melo and Citrullus lanatus belonging to the family Cucurbitaceae and local lesions on Chenopodium amaranticolor and C. quinoa belonging to the family Chenopodiaceae. The virus failed to infect the following

cucurbitaceous hosts viz., Lagenaria siceraria, Luffa acutangula, Momordica charantia, Trichosanthes anguina and Cucurbita maxima (Susan John, 1985).

2.8 Physical properties:

Physical properties of "PRSV" and other "aphid-transmitted mosaic viruses" of papaya, now considered as "strains of PRSV" with potyvirus group characteristics have been reported by several workers.

Adsuar (1947c) reported that virus causing mosaic of papaya in Puerto Rico, was inactivated at 60°C, after 48 hours storage at room temperature and after dilutions of 10^{-3}.

According to Capoor and Varma (1948, 1958) aphid transmitted "papaya mosaic" lost its infectivity after 26 to 28 hours storage at room temperature, after heating for 10 minutes between 53 to 55°C and dilutions between 10^{-3} to 10^{-4}.

Aphid transmitted "papaya mosaic" (PMV) from Hawaii, has been reported with the following physical properties, dilution end point (DEP) being between 10^{-4} to 10^{-5}, thermal inactivation point (TIP) being at 54°C and longevity in vitro (LIV) for 8 hours (Ishii and Holtzmann, 1963).

Strains of PRSV viz., DRV and FMRV from Florida have also been studied for physical properties. No differences, however, have been noted in DEP (10^{-3}) or TIP (between 54-56°C) or LIV (8 hours) between these strains (Conover, 1964a,b).

Story and Halliwell (1969) reported the physical properties of "DRV" of papaya in Dominican Republic. The thermal inactivation point (TIP), dilution end point (DEP) and the longevity in vitro (LIV) recorded for this virus were: 50 to 55°C, 10^{-3} and 8 hours respectively.

Singh (1969a) reported that the "papaya leaf reduction virus" (PLRV) had a DEP between 10^{-2} to 10^{-3}, TIP between 45 to 50°C and a LIV between 3 to 4 days at room temperature.

Singh (1969b) reported that the aphid-transmitted "papaya mosaic virus" (PMV) was found to be inactivated in papaya sap at 60°C and between the dilutions of 10^{-4} and 10^{-5}. The virus was viable upto 24 hours but not upto 30 hours at room temperature.

Kulkarni (1970) has reported the physical properties of three viruses (with potyvirus group particle morphology) viz., CLV, CRV, and NLV. These viruses differed in physical properties. CRV had lower TIP (55 to 60°C), CLV intermediate (60 to 65°C) and NLV higher values (75-80°C);

whereas, NLV recorded lower values of LIV (24 hours), CRV, intermediate (6 days) and CLV, higher (8 days). Regarding DEP, CLV differed in having slightly lower value (10^{-5}), however, the DEP values of CRV and NLV were at par (10^{-6}).

The "distortion ringspot virus" (DRV) prevalent in Venezuela, have been reported to be inactivated between a TIP of 50 and 55°C and a LIV of 96 hours. The DEP was being at 10^{-3} (Lopez Pinto, 1972).

Lima and Gomes (1975) have reported that the PRSV isolate from Fortaleza, Brazil, was inactivated between 54 to 56°C and after an aging *in vitro* for 12 hours.

The Colombian isolate of PRSV had a LIV of 8 hours at 24°C and 2 days at 4°C, and TIP at 60°C (Sanchez de Luque and Martinez Lopez, 1976b).

Sureka *et al.* (1977) have reported that the Rajasthan isolate of PRSV was inactivated between 45 to 50°C. The DEP was 1:500 and the LIV 24 hours.

According to Wang *et al.* (1978) the PRSV isolate of Taiwan, in papaya leaf sap lost its infectivity between 50 to 55°C, 10^{-3} to 10^{-4} dilution or storage at 20°C for 10 hours.

Chang (1979) from Taiwan has recorded differences in the physical properties of the two isolates (Virus-I, Virus-II) of PRSV. The isolate Virus-I had slightly lower values of TIP (45- 50°C), than the isolate Virus-II but had higher values for DEP (10^{-4} to 10^{-5}) and LIV (10 to 11 days) than the isolate virus II (DEP, 10^{-3} to 10^{-4}; LIV, 7 to 8 days).

The "papaya ringspot virus" reported from Marathwada region was found to be inactivated between 60 to 65°C and at a dilution of 10^{-3} in papaya sap. The virus was viable upto 10 hours at 27 to 30°C (Yemewar and Mali, 1980).

The TIP, the DEP and the LIV reported for the "Nigerian isolate of PRSV" were: 57°C, 10^{-3} and 10 hours respectively (Lana, 1980).

Barbosa and Paguio (1982a) from Brazil have reported that the physical properties of the PRSV isolate, designated as " PRSV-PE". In papaya sap, the virus lost its infectivity between 55 to 60°C and on storage between 12 to 24 hours at room temperature. The DEP of the virus was between 10^{-2} to 10^{-3} .

An aphid transmitted "papaya mosaic virus"(PMV) reported from Kanpur, India, was inactivated by heating the sap between 55 to 60°C. The virus lost its infectivity

after 24 hours storage at room temperature and at a dilution of 10^{-3} (Bhaskar, 1983).

de la Rosa and Lastra (1983) from Venezuela have reported that PRSV was inactivated between 52 to 54°C. The DEP was 10^{-4} and the LIV was 24 hours.

Wu et al. (1983) from South China have reported that the infectivity of the PRSV in papaya sap was lost between 50 and 55°C, between 10^{-2} and 10^{-3} dilutions and after a storage between 8 to 16 hours at room temperature.

The infectivity of the PRSV isolate reported from Andhra Pradesh, was lost between 24 to 48 hours storage at room temperature (30 to 33°C), after heating between 45 to 50°C for 10 minutes and between dilutions of 10^{-2} to 10^{-3} (Susan John, 1985).

2.9 Strains :

Two types of isolates of "papaya ringspot virus" are now being recognised viz., type P isolates and type W isolates (Purcifull et al., 1984). The main difference between these isolates is that the type P isolates infect papaya whereas type W isolates does not (Gonsalves and Ishii, 1980; Yeh et al., 1984). In papaya, the type P isolates cause mottling and distortion of leaves, rings and spots on fruit and streaks on stem and petioles.

Affected plants are stunted and fruit set is reduced (Jensen, 1949a; Conover, 1964a). Cool weather favours the development of severe leaf distortion symptoms (Jensen, 1949a; Conover, 1964a; Lima and Gomes, 1975). In squash, watermelon and other cucurbits, type W isolates cause mottling and distortion of leaves and fruit (Halliwell et al., 1979). A variant found in Guadeloupe is serologically related to, but distinct from, typical P and W isolates (Sensa stricto, Purcifull et al., 1984). Isolates within type P differ in the symptoms they induce. These are well documented in the literature and designated as: "faint mottle ringspot virus" (FMRV) which induces milder symptoms in Carica papaya than "distortion ringspot virus" (DRV) in Florida (Conover, 1964a,b); non-sap transmissible mild strain of DRV characterised by leaf distortion and upward curling of young papaya leaves in Dominican Republic (Story and Halliwell, 1969); two isolates of PRSV from Hawaii (USA) viz., "PRV-HA" and " PRV-HB", the former causing severe leaf distortion in papaya and intensive mosaic and leaf distortion in Cucurbita pepo and the latter causing less severe leaf distortion in papaya and only mild mottling in C. pepo (Gonsalves and Ishii, 1980); variation in foliar symptom expression has been observed on papaya, inoculated with nine isolates of PRSV originating

from different geographic regions. For instance, five Taiwan isolates of PRSV viz., Su-mm caused mild mottling; Su-sm caused severe mottling, Su-smn induced systemic necrosis, and wilting apart from severe mottling, while isolates viz., T-Chen and T-Wang developed mottling and mosaic; two Hawaiian isolates of PRSV viz., PRV-HA and PRV-HB induced mosaic and leaf distortion symptoms; and the Florida isolate PRVF-340 and a Ecuador isolate PRV-ED produced mosaic and shoestringing symptoms. In the winter months all the isolates except Su-mm showed severe symptoms of leaf-distortion and shoestringing (Yeh et al., 1984). Mild strains of PRSV such as " Aparecida" in Brazil and a " nitrous acid induced mutant" PRVHA 5-1 in Florida, that cross-protect against severe ones have been reported (Rezende et al., 1981; Yeh and Gonsalves, 1984a). A strain of PRSV (Virus-I) that induced wilt in papaya has been reported from Taiwan (Chang, 1979). Some type W isolates such as WMV-1F, but not WMV-1NY or WMV-1VG, produced local lesions on Chenopodium amaranticolor and C. quinoa. Further, based on host reactions, it has been shown that WMV-1NY and WMV-1VG are infectious only to Cucurbitaceae, but WMV-1F being infectious to Cucurbitaceae as well as Chenopodiaceae (Yeh et al., 1984). Further, Yeh et al. (1984) opined that inability to produce local lesions on Chenopodium spp., by WMV isolates should not be used as criteria to distinguish WMV-1 from WMV-2. However, they

felt that local lesion production on Chenopodium quinoa and C. amaranticolor might provide a useful criteria for distinguishing strains of WMV-1.

2.10 Serology :

The virus is a good immunogen. Liquid immunoprecipitin tests with clarified plant extracts (Webb and Scott, 1965) or with purified virus particles (Milne and Grogan, 1969) have been used for virus detection and for studying the relationships. The intact virus particles do not diffuse readily in agar gels but sodium dodecyl sulphate (SDS)(Purcifull and Hiebert, 1979) or pyrrolidine (Shepard et al., 1974) can be added to disrupt the particles into diffusable fragments. SDS immunodiffusion tests have been used to detect isolates of types P and W in cucurbit leaf extracts with antisera to untreated virus (Purcifull and Hiebert, 1979; Russo et al., 1979) or with antisera to SDS treated particle protein (Gonsalves and Ishii, 1980). Freeze-drying of leaf extracts is a convenient method for preparing reference antigens for use in SDS- immunodiffusion tests (Purcifull and Hiebert, 1979). Virus detection in papaya by SDS-immunodiffusion is possible but unreliable (Wan and Conover, 1983).

Enzyme-linked immunosorbent assay (ELISA) has been used to detect the virus; much higher A 405 readings were obtained when extracts from infected papaya or squash

leaves were prepared in 0.25 M potassium phosphate + 0.1 M EDTA, pH 7.5, than in extracts prepared in standard phosphate-buffered saline (Gonsalves and Ishii, 1980). Immunoelectron miscroscopic procedures have also been used to study the relationships (Makkouk and Lesemann, 1980).

Antisera have been prepared to the proteins of cylindrical (Pinwheel) inclusions(CI) and amorphous inclusions (AI). The CI proteins have been detected using SDS- immunodiffusion methods (Baum and Purcifull, 1981; Yeh and Gonsalves, 1984b). Antisera prepared to purified CI proteins of type P and type W isolates had titres of 1/8 to 1/6 in SDS- immunodiffusion tests. With indirect ELISA, CI proteins have been detected at concentrations as low as 1.6 ng/ml and at sap dilutions of 3.2×10^5 (Yeh and Gonsalves, 1984b).

2.10.1 Relationships :

Its particle morphology, aphid transmissibility, serological relationships and ability to induce pinwheel inclusions in host cells placed "papaya ringspot virus" in the "potyvirus group" (Hollings and Brunt, 1981; Matthews, 1982). Edwardson (1974) assigned both type P and W isolates to sub-group I because they typically induced pinwheels and scrolls, but not laminated aggregates, in host cells.

"Watermelon mosaic virus" (WMV) has been divided into two groups of strains by Webb and Scott (1965) on the basis of failure in cross-protection tests, on major differences in host range and serological differences, inspite of the fact that Milne and Grogan (1969) have found a close serological relationship among various WMV-1 and WMV-2 isolates and had shown that they were the strains of the same virus. They had also shown that "papaya ringspot virus" was serologically closely related to both WMV-1 and WMV-2 isolates, but "potato virus Y" (PVY) and "bean yellow mosaic virus" (BYMV) were distantly related. However, more recent work with isolates from the USA, Europe, Australia and Mediterranean regions has indicated that there exists a significant serological, host range and cytological distinction between WMV-1 and WMV-2 isolates, although distant serological relationships have been detected in some cases. Further, the coat proteins and CI proteins of type W (WMV-1) were reported to be serologically very closely related, if not identical with those of type P isolates (Papaya ringspot virus) (Purcifull and Hiebert, 1979; Russo et al., 1979; Gonsalves and Ishii, 1980; Makkouk and Lesemann, 1980; Baum and Purcifull, 1981; Barbosa and Paguio, 1982a; Dodds et al., 1984 and Yeh and Gonsalves, 1984b).

Purcifull and Hiebert (1979) reported that in SDS- immunodiffusion tests WMV-1FL and WMV-2FL isolates were distinguishable and also by differences in their cross-reactivities with certain potyviruses. The "papaya ringspot virus" have been found to be closely related to WMV-1FL, but not with WMV-2FL. "Soybean mosaic virus" (SMV) has been observed to be closely related to WMV-2FL, but not to WMV-1FL. Moreover, they noticed very faint reactions of WMV-1FL with WMV-2FL antiserum with bleedings collected later than 7 months after immunization.

ELISA and SDS- immunodiffusion tests have been used to check the relationship of papaya ringspot virus isolate of Hawaii (PRSV-HA causing severe leaf distortion) to several potyviruses and isolates of PRSV. Watermelon mosaic virus-1 (WMV-1), PRSV-HA and Florida isolate of PRSV (PRSV-340) gave reactions of identity in SDS- immunodiffusion tests in which antisera to PRSV-HA and WMV-1 were used, while WMV-2 did not react with either antiserum. Antisera of PRSV-HA and WMV-1 also gave strong reactions to PRSV-HB (isolate from Hawaii causing leaf distortion to lesser extent), PRSV-340 and WMV-1 in ELISA tests. Isolates of "bean yellow mosaic virus" (BYMV), "cowpea aphid-borne mosaic virus" (CAMV) and "watermelon mosaic virus-2" (WMV-2) have failed to react with the antiserum of PRSV-HA in ELISA or SDS-immunodiffusion tests (Gonsalves and Ishii, 1980).

Makkouk and Lesemann (1980) have reported that in SDS- immunodiffusion tests the Lebanon isolate of WMV-1 reacted with WMV-1, but not with WMV-2. Using Derrick technique of immunoelectron microscopy, they observed strong specific trapping when grids were coated with WMV-1 antiserum, but no trapping with WMV-2 or "bean yellow mosaic virus" (BYMV) antisera. In decoration technique of immunoelectron microscopy, a strong effect was observed with WMV-1 antiserum, and a weak and no reactions were observed with BYMV and WMV-1 antisera respectively.

Antisera specific for cylindrical inclusions of a Jordanian isolate of WMV-1, a Florida isolate of WMV-2 and a Mexican isolate of WMV (WMV-M) did not react with their respective purified viruses or with healthy antigens in SDS-double immunodifussion tests. In reciprocal tests with antisera collected from 2 to 4 months after initial immunization, only the WMV-M inclusion antiserum reacted with crude sap or purified inclusions of other WMV- isolates. Intragel absorption of WMV-M inclusion antiserum with purified inclusions of WMV-1 and WMV-2 or WMV-M confirmed that WMV-M inclusions were immunochemically distinct from, but related to WMV-1 and WMV-2 (Baum and Purcifull, 1981).

Barbosa and Paguio (1982a) reported that in SDS-immunodiffusion tests the Pernambucan isolate of PRSV showed a close serological relationship with " watermelon mosaic virus-1" (WMV-1), but not with WMV-2.

Dodds et al. (1984) from the University of California observed a cross-reaction between WMV-1 antigen and WMV-2 antiserum in SDS- immunodiffusion test. The visible precipitate was closer to the antiserum well than the precipitate formed by the homologous reaction between WMV-2 and WMV-2 antiserum. This was obtained when California antiserum, but not when the Florida antiserum to WMV-2 was used and its development could be prevented by cross absorption, when SDS- treated sap from plants infected with WMV-1 was placed in the centre well 4 hours before placing California WMV-2 antiserum in the centre well. No cross-reaction between WMV-2 antigen and WMV-1 antiserum was detected. A cross-reaction between WMV-2 and WMV-1 antiserum was detected by ELISA.

Yeh et al. (1984) reported that in SDS-immunodiffusion tests, nine isolates of PRSV obtained from different geographical regions gave a reliable and consistent reactions to three isolates of WMV-1, when infected Cucumis metuliferus (ACC-2459) was used as crude antigen for PRSV

isolates and infected Zucchini squash for WMV-1 isolates. The reactions were determined by using either antiserum to dissociated coat protein of PRSV or antiserum to intact particles of WMV-1. The results indicated that all the nine isolates of PRSV namely, Su-mm, Su-sm, Su-smn, T-Chen and T-Wang all from Taiwan; PRSV-HA and PRSV-HB of Hawaii; PRSV-F340 from Florida and PRSV-ED from Ecuador and three isolates of WMV-1 viz., WMV-1VG (Virginia), WMV-1NY (New York) and WMV-1F (Florida) were serologically indistinguishable. Moreover, their host range studies indicated that Cucumis metuliferus (PI 292190), Cucumis melo line B 66-5 and Cucumis sativus "Surinam" which possessed a single recessive gene for resistance to WMV-1, reacted identically to all nine isolates of PRSV and three isolates of WMV-1. The similarities in resistant and susceptible host reactions and serology encountered in these studies have shown that PRSV and WMV-1 were very closely related.

Yeh and Gonsalves (1984b) observed no serological differences when purified PRSV CIP and WMV-1 CIP were tested against either PRSV CIP or WMV-1 CIP antiserum. Moreover, when crude antigens from infected tissues were tested against both CIP antisera, all the nine isolates of PRSV and three isolates of WMV-1 were serologically indistinguishable.

PRSV CIP antiserum and WMV-1 CIP antiserum did not react with sap from plants infected with WMV-2 NY, WMV-2F and WMV-2C. No precipitin lines were observed when crude antigens from plant tissues infected with PVY, TEV, BYMV, CAMV, BlCMV, LMV, and TuMV were tested against both antisera. The results of indirect ELISA confirmed SDS- immunodiffusion tests that PRSV CIP and WMV-1 CIP were serologically identical, but not related to WMV-2 CIP.

Considering the properties of type P (PRSV) and type W (WMV-1) and their biological and cytological similarities, Lovisolo (1980) and Yeh et al., (1984) suggested reclassification of PRSV and WMV-1 as strains of the same virus rather than two different viruses. Accordingly PRSV and WMV-1 isolates have been regrouped under one name, "papaya ringspot virus" as type P and type W isolates respectively (Purcifull et al., 1984).

PRSV has affinities with some potyviruses. Pyrrolidine degradation products of its particles reacted to the D- protein of "tobacco etch virus" (TEV) (Shepard et al., 1974), and the particle proteins and CIP of potyvirus isolated from cucurbits in Morocco (Fisher and Lockhart, 1974) were serologically related to, but distinct from, those of PRSV (Baum and Purcifull, 1981).

Lima and Gomes (1975) reported that in immunodiffusion tests Brazilian isolate of PRSV gave no specific reaction against the antisera specific for "papaya mosaic virus" (PMV) and "tobacco ringspot virus"(TRSV).

PRSV isolate described from Marathwada region of Maharashtra State was serologically related to PRSV isolate from Florida (USA) but apparently shared no common antigens with the antisera of "bean common mosaic virus"(BCMV), "bean yellow mosaic virus" (BYMV), "potato virus X" (PVX), "potato virus-M" (PVM), "tobacco mosaic virus" (TMV), "cucumber mosaic virus" (CMV) and " tobacco ringspot virus" (TRSV) (Yemewar and Mali, 1980; Dake, 1986).

Lana (1980) found that when detergent-treated antigens used in agar diffusion tests and precipitin tests with partially purified antigens of PRSV, the designated PMV and PRSV isolates gave positive reaction to their type strain antisera. The papaya decline virus (PDV) also reacted to the PRSV antiserum, indicating that all these isolates were serotypes of PRSV and PMV. PRSV gave negative serological reactions with the antisera of "papaya mosaic virus" (PMV), "potato virus X" (PVX), "potato virus Y" (PVY), "tobacco ringspot virus" (TRSV), "tomato ringspot virus" (TmRSV), "tobacco mosaic virus (TMV) and "cucumber mosaic virus" (CMV).

Partially purified preparation of PRSV described from Andhra Pradesh, in agar-gel diffusion tests gave positive reaction only to the PRSV antiserum, but not to the antisera of "pumpkin mosaic virus", "cucumber mosaic virus-1" and "bottlegourd mosaic virus" (Susan John, 1985).

2.11 Electron microscopy :

2.11.1 Particle morphology :

Electron microscopic observations of sections of leaf, fruit and stem tissues of papaya infected with PRSV showed filamentous particles in the cytoplasm. In the cell they were in a somewhat parallel arrangement or completely randomly distributed. The average length of the particles worked out include 800 ± 10 nm and a diameter of 12 nm. They had a channel of 3 nm diameter in the centre (Herold and Weibel, 1962).

de Bokx (1965) have reported long flexuous particles with a modal length of 780 nm in the leaf-dip preparations of papaya leaves infected by "distortion ringspot virus" (DRV), a strain of PRSV.

Three viruses from East Africa viz., CLV, CRV and NLV, were found to be similar in particle morphology. Negatively stained purified preparations of the three viruses contained stiff rod-shaped particles having a diameter of 750 x 12 nm (Kulkarni, 1970).

Smith (1972) determined the length of the "distortion ringspot virus" (DRV) a strain of PRSV to be between 700 to 800 nm.

Khurana (1974) reported that in leaf-dip preparations, the average particle length for " mild mosaic virus" (MMV), "distortion ringspot virus" (DRV) and "ringspot virus" (RSV) of papaya was 551, 763 and 721 nm respectively. Of these, only DRV and RSV resemble isolates of PRSV in particle size and morphology.

The Vietnam isolate of PRSV described and reported by Brcak and Pozdena (1976), had elongated flexuous virus particles in the diseased leaf tissues. Average length of the particles was 730 nm based on the means of main maximum ranging from 600 to 800 nm.

In leaf-tissue preparations stained with 2 per cent phosphotungstic acid and ultrathin sections of papaya infected with "papaya ringspot mosaic virus", filamentous virus particles of 200 to 1200 nm long (generally 700 to 800 nm) and 10 to 15 nm diameter had been observed by Ko et al. (1979) and these particles were found to occur in bundles in the cytoplasm near cell wall.

While working with the properties of viruses isolated from papaya in Nigeria, Lana (1980) found that the average dimensions of the 213 particles of purified PRSV

were 791 x 12 nm. The particles of the "papaya decline virus" (PDV) isolate measured 748 x 13 nm in crude sap of infected tissues.

Barbosa and Paguio (1982a) reported that in leaf-dip preparation, the particle length of the Pernambucan isolate of PRSV designated as PRSV-PE was found to range from 750 to 800 nm.

de La Rosa and Lastra (1983) while working with the Venezuelan isolate of PRSV, observed leaf-dip preparation to contain flexuous, rod-shaped particles. They reported a modal length of 782 nm based on 167 virus particle counts.

Electron microscopy of papaya leaves infected with Taiwan isolate of PRSV revealed the presence of filamentous particles with lengths ranging from 700 to 750 nm in negatively stained leaf-dip preparations (Chen, 1984).

The leaf-dip preparations of Marathwada isolate of PRSV contained elongated, flexuous, rod-shaped particles. The average length of virus determined from means of main maximum was 776 ± 5 nm (Dake, 1986).

2.11.2 Inclusion morphology :

Ultrathin sections of PRSV infected leaf tissues displayed "pinwheel" and "circular" inclusions which were considered to result from cross-sectioning of three dimensional inclusions (Zettler et al., 1968). Electron microscopy of ultrathin sections of "distortion ringspot

virus" (DRV) infected papaya leaf tissues revealed the presence of "pinwheel" and " circular" inclusions (Story and Halliwell, 1969). "Pinwheels" and"scrolls" have also been observed in papaya leaf tissues infected by isolates of PRSV from Pernambuco (Barbosa and Paguio, 1982a) and South China (Wu et al., 1983). Chen (1984), in addition to "pinwheels" and "circular" or "tubular" (scrolls) inclusions, reported the presence of "short laminated aggregates" in the tissue sections of papaya leaves infected by Taiwan isolate of PRSV. Edwardson et al. (1984) studied the ultrastructure of Cucurbita pepo leaf tissue infected with PRSV. They observed PRSV to induce cytoplasmic cylindrical inclusions (CCI) consisting of "pinwheels" and "scrolls", which is a characteristic feature of subdivision-I of the potyvirus group.

2.12 Cross protection :

Several unsuccessful attempts have been made to develop effective control measures for PRSV. Conover (1964a, b), Cook and Zettler (1970) and Cook and Milbrath (1971) failed to find resistance to the virus among the Carica papaya lines. However, some species of Carica such as C.candamarcensis, C. candicans, C. cauliflora, C. pubescens, C. quersifolia and C. stipulata and two members of Caricaceae viz., Jacaratia mexicana and J. spinosa have been reported to possess resistance

to PRSV (de Zerpa, 1958, 1962; Jimenez and Horovitz, 1958; Vasudeva, 1959; Capoor and Varma, 1961; Conover, 1964a,b; Horovitz and Jimenez, 1967; Cook and Zettler, 1970; Adsuar, 1971; Cook and Milbrath, 1971). Unfortunately, the species of Carica and Jacaratia carrying genes for resistance to PRSV were found to be incompatible with Carica papaya and conventional interspecific hybridization has been met with little or no success (Mekako and Nakasone, 1975). Furthermore, attempts to control PRSV through application of insecticides for control of aphid vectors, roguing of infected plants, oil and milk sprays and placement of reflective white plastic cloth to avoid landing of alate aphids have not been successful.

The unavailability of resistant sources among C. papaya, ineffectiveness of chemical control on account of stylet-borne nature of the virus, roguing being not a permanent solution and restricted host range of the virus, made cross protection an attractive alternative method of management of PRSV.

The occurrence of a mild isolate of PRSV designated as "Aparecida D'oeste" with a cross protective ability has been reported from Sao paulo, Brazil (Rezende et al., 1981). Papaya plants preimmunized with this isolate, that super inoculated and then exposed in the field for four months did not show any increase in mosaic symptoms or foliar

distortion and made good growth (Rezende et al., 1982). Subsequently, four months after challenge inoculation, all the field exposed challenge inoculated plants started showing mild symptoms at first and tend to increase in severity after 5 to 8 months (Rezende et al., 1983, 1984). The failure of cross protection or pre-immunization of PRSV was attributed to mutation and selective competition of mild isolates of the virus, but not to break-in protection (Rezende et al., 1983, 1984). The results of cross protection tests obtained in Brazil indicated that for pre-immunization to work in the control of PRSV, it was necessary to obtain mild protective isolates that are more stable and competetive (Rezende et al., 1984). Recently, Yeh and Gonsalves(1984a) have succeeded in inducing mild strains of PRSV with protective ability designated as PRVHA-5-1 and PRVHA-6-1. Papaya seedlings inoculated with these mild mutants, remained symptomless or with diffuse mottling with no reduction in plant size under green house conditions. Protection was observed when PRVHA 5-1 and 6-1 were used to protect papaya against different mechanical challenge inoculations with severe strain (Yeh and Gonsalves, 1984a). Results of small plot and large scale field trials conducted in more recent years indicated that mutants PRVHA-5-1 and 6-1 have been found to be highly valuable mild strains for controlling PRSV by cross-protection (Yeh et al., 1986).

2.13 Disease resistance :

Several unsuccessful attempts have been made to develop effective control measures for PRSV. Although, tolerant selections of papaya (AREC-7, AREC-12 and AREC-19) and their cross- combinations (like 7-A x 19-G, 12-B x 7-E, 12-C x 7-E and 19-B x 19-G) have been identified (Conover, 1976; Conover and Litz, 1978), resistance to PRSV have not been found to occur within Carica papaya (Cook and Zettler, 1970; Conover, 1976; Conover and Litz, 1978; Wang et al., 1978). Some species of Carica such, as C. candamarcensis, C. candicans, C. cauliflora, C. pubescens, C. quersifolia and C. stipulata, however, have been reported to be resistant to PRSV (Capoor and Varma, 1948, 1958, 1961; de Zerpa, 1958, 1962, 1967; Jimenez and Horovitz, 1958; Vasudeva, 1959; Conover, 1964a,b; Horovitz and Jimenez, 1967; Cook and Zettler, 1970; Adsuar, 1971). Among other genera of Caricaceae, two species of Jacaratia viz., J. mexicana (Cook and Zettler, 1970) and J. spinosa (Cook and Milbrath, 1971) have also been found to be resistant to PRSV. Unfortunately, the species of Carica carrying genes for resistance to PRSV have been found incompatible with Carica papaya and conventional interspecific hybridisation has met with little or no success (Mekako and Nakasone, 1975). The review pertaining to PRSV screening trials is presented below:

The results of "papaya mosaic" (considered to be caused by PRSV) screening trials carried out by Capoor and Varma (1948, 1958, 1961) have indicated that Carica papaya varieties namely, Poona Long, Poona Round, Bombay, Ceylon, Ranchi, Honey Dew, Hawaiian, Washington, Goda Kawala, Madhu Bindu, Kaajimuindeen, American, Solo Hawaii, Hortus Gold, Mammoth, Giant, Peterson, Bettina, Philippine, Paradenia, Solo Line-8, Venezuela and Coorg Honey Dew were susceptible (Capoor and Varma, 1948, 1958). Among the Carica species tested, only C. cauliflora was immune, whereas, C. candamarcensis, C. goudotiana and C. microspora have been found susceptible to the virus (Capoor and Varma, 1961).

Mechanical sap inoculation tests carried out in Venezuela with papaya mosaic caused by PRSV showed that C. cauliflora, C. candamarcensis were resistant, but C. monoica and C. microspora were susceptible to the virus (Malaguti et al., 1957).

According to Jimenez and Horovitz (1958) C. candamarcensis can be crossed with C. papaya, although seed fails to mature. In most cases, however, the immature embryos can be cultivated successfully.

In Venezuela, resistance to " distortion ringspot" (DRV) an isolate of PRSV was found only among Caricaceae other than papaya accessions. It was also observed that in reciprocal graft tests between infected C. papaya and C. cauliflora, the latter inhibited infection in C. papaya which could then give rise to healthy leaves. Among the Carica material tested for resistance to DRV, C. candicans, C. pubescens and C. stipulata have been found to be resistant (de Zerpa, 1958, 1962, 1967).

Vasudeva (1959) reported that three species of Carica viz., C. cauliflora, C. candamarcensis and C. microspora when tested against an aphid-transmitted unidentified "papaya mosaic" by mechanical sap inoculation, only C. cauliflora was found to be resistant.

Of the various Carica spp., tested in Florida for their resistance to " distortion ringspot virus" (DRV) and "faint mottle ringspot virus" (FMRV), strains of PRSV, two species viz., C. candamarcensis and C. quercifolia were resistant, while C. cauliflora, C. goudotiana and C. monoica were susceptible to both the strains (Conover, 1964a,b).

Horovitz and Jimenez (1967) observed that C. candicans, C. cauliflora and C. stipulata to be resistant to DRV in Venezuela. They also reported that attempts to incorporate DRV resistance into C. papaya from other Carica species failed on account of cross incompatability.

Screening trials carried out at Florida to locate PRSV resistance in 90 C. papaya accessions originated from Australia, Brazil, Guadeloupe, Honduras, India, Kenya, Okinawa, Puerto Rico, Panama, Philippines and Venezuela and related plants have disclosed that no resistance was found in 90 accessions of C. papaya to PRSV. Some of the C. papaya varieties found susceptible to PRSV were: Alargada, Alargada Rosada, Banglore, Bettina, Brookfield, Cotaxtla, Hawaiian, Hoffman, Honey Dew, Kiru, Madhubindu, Ranchi, Redfleshed, Redonda, Simultana Giant, Solo and Sunny Bank. Plants related to Jacaratia mexicana, however, were immune to PRSV (Cook and Zettler, 1970).

Carica candamarcensis was found to be resistant to all the three virus isolates (CLV, CRV, and NLV) reported from East Africa (Kulkarni, 1970).

The papaya varieties, Banglore, Mammoth, Ceylon, Ceylon Dwarf, Cochin, Coorg, Honey Dew, Dehradun, Faizabadi, Giant, Gujarati, Hawaiian, Jaunpuri Large, Mammoth Large, Ranchi and Washington have been found to be susceptible to a sap and unidentified aphid transmissible papaya mosaic virus reported from Haryana and Rajasthan (Varma, 1971).

Cook and Milbrath (1971) from Hawaii reported that plants of Jacaratia spinosa belonging to the family Caricaceae were found to be immune to PRSV.

Adsuar (1971) found that C. candamarcensis was immune to an aphid transmissible mosaic disease of papaya present in Puerto Rico. Carica candamarcensis plants inoculated with sap from and cleft-grafted with mosaic infected C. papaya plants, did not develop symptoms of the disease.

Mekako and Nakasone (1975) from the University of Hawaii, in their interspecific hybridization programme among six Carica species have observed that C. cauliflora and its F_1 hybrids were resistant to " distortion ringspot"(DRV) a strain of PRSV.

Conover (1976) summarised the results of breeding programme and achievements of tissue culture initiated at Homestead, Florida, which were aimed at the developing papaya varieties tolerant to DRV. The results of this programme have disclosed that of the 20 papaya stocks screened for their resistance to DRV, only two stocks viz., AREC-7, and AREC-19 had no plants severely affected by DRV. It was observed that tolerance to DRV was an inheritable trait. Tissue culture technique was believed to be very useful in papaya especially to overcome the problem of cross-incompatability between C. papaya and other Carica species. It was believed that the tissue culture would be quite helpful in the following ways: propagation of individual

tolerant plants to enable specific genotype to be maintained, which otherwise might be lost if propagated by seed and subsequently such plants could be used for production of F_1 seeds, as parents in a standard breeding programme, or, if easily propagated, set in the field for fruit production; anther cultures might be used to produce haploid plants which could be treated so as to become homozygous diploids which would breed true and this would results in great saving in time, since the selfing required to produce homozygous lines by normal breeding procedures being 8 to 10 years; an embryo culture might be used to overcome the problem of incompatability between C. papaya and Carica sp. This might make it possible to transfer the resistance to DRV in these species to papaya.

While evaluating first generation progeny from crosses within and between lines of papaya derived from plants selected individually for tolerance to PRSV, Conover and Litz (1978) found that tolerance to PRSV was inherited in a quantitative manner. The effectiveness of a given staminate or pistillate parent in transmitting tolerance to its progeny depended on the other parent, suggesting that the highest level of tolerance resulted from pairings which combined the greatest number of different factors for tolerance. A useful level of tolerance has not been found in red or orange-fleshed varieties. Among the parental lines tested for their tolerance, AREC-19 showed maximum number of tolerant

plants followed by AREC-7, AREC-12 and AREC-9. Among the various sib matings, mating between 19-A and 19-G resulted in maximum tolerance, while among the crosses, the crosses between 7-A and 19-G; 12-B and 7-E; and 12-C and 7-E have produced maximum tolerant plants.

Litz and Conover (1979) reported successful somatic embryogenesis in cell suspension of Carica stipulata, a PRSV- resistant relative of C. papaya.

In Sri Lanka no resistance has been found among 40 accessions of Carica papaya to an aphid transmissible papaya mosaic virus but Carica candamarcensis was found to be resistant (Rajapakse, 1981).

Provvidenti and Gonsalves (1982) reported that resistance to PRSV was conferred by a single dominant gene. Clones of F_2 and test-cross plants of Cucumis metuliferus when inoculated with "PRSV" or "watermelon mosaic virus-1" (WMV-1) reacted identically, thereby indicated that the factor for resistance to PRSV was closely linked to Wmv or being the same factor.

Of the 53 lines or varieties of papaya screened for PRSV tolerance in Taiwan, two varieties viz., FL-77-5 and Costa Rica have been found tolerant to PRSV (Wang, 1982).

Susan John (1985) from Andhra Pradesh found that papaya varieties like Co-1, Co-2, Co-3, Co-4, Honey Dew and Washington to be susceptible to PRSV.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Survey :

Papaya plantations in different localities of Maharashtra State, were surveyed during the months of September, October and November, 1985 and January, 1986 for a "papaya ringspot disease" caused by " papaya ringspot virus" (PRSV). Identification of the disease under field condition wasbased on characteristic symptoms like leaf distortion, streaks on petioles and stem and rings on fruits produced by PRSV. Percentage incidence of PRSV was calculated by actually counting diseased plants in the total population. In addition to disease survey, information regarding symptoms on PRSV- infected plants, intercropping, cropping pattern around papaya plantations and aphid colonisation on papaya, intercrops, and neighbouring crops was also recorded.

3.2 Isolates of PRSV :

To confirm the identity, prevalence and possible existence of strains of PRSV on papaya in Maharashtra State, in all 10 samples of PRSV, each consisting of young symptomatic leaves were collected from different regions. PRSV samples were collected separately in polyethylene bags and were duly labelled. Each symptomatic leaf sample was designated as an individual isolate and named after a

locality from which it was collected. Thus 10 samples of PRSV collected from different localities were treated as 10 Isolates of PRSV. The particulars of these isolates are given as below :

Six isolates collected from C. papaya from different localities of Marathwada included Aurangabad (1), Latur (1), Nanded (1), Osmanabad (1) and Parbhani (2). Two isolates, one each from an interspecific hybrid between C. cauliflora x C. papaya and C. papaya from Pune. One isolate each was collected from C. papaya from Thane and Akola. Ten isolates were designated using abbreviations of localities from where these isolates were collected. These included AKL (Akola), AUR(Aurangabad), LTR (Latur), NAN (Nanded), OSB (Osmanabad); PBN (HG) and PBN (FF); PUN-HC-c.p.; PUN-C.p. and THA (Thane). Two Parbhani isolates viz., PBN (HG) and PBN (FF) were collected from Horticultural Garden, MAU, Parbhani and Farmer's field respectively. Similarly, Pune isolates viz., PUN-HC- c.p. and PUN-C.p. were collected from Ganesh Khind gardens from an interspecific hybrid between C. cauliflora x C. papaya and from C. papaya respectively.

3.3 Isolation and maintenance of isolates :

Isolates of PRSV, collected from different localities of Maharashtra State were established and maintained on two

hosts viz., papaya (cv. Washington) and summersquash (Cucurbita pepo var. melopepo cv. Patty Pan) by mechanical sap inoculation and by aphid transmission in an insect-free glass house. Isolates of PRSV maintained on these hosts served as sources of virus for biological and physico-chemical tests. Healthy test plants of papaya (cv. Washington) and summersquash (cv. Patty Pan) were raised from healthy seeds in earthen-pots (15 cm diameter) containing steam-sterilised soil, compost and sand (2:1:1) mixture. All the transmission tests were carried out, preferably on 50 days old seedlings in case of papaya and at cotyledon stage in case of summersquash. All the inoculated plants were maintained in an insect- free glass house and observations on the aspects like incubation period, percentage infectivity and symptoms were recorded for ten isolates separately.

3.4 Transmission studies :

3.4.1 Mechanical transmission :

Standard inoculum of different PRSV isolates was prepared in a cold 0.1 M phosphate buffer, pH 7.0 containing 0.1 per cent sodium diethyldithiocarbamate (DIECA), by titurating young symptomatic leaves in a chilled mortar and pestle. The resultant inocula were filtered separately through double-covered muslin cloth, before being used for

mechanical inoculation. Fifty days old plants of papaya (cv. Washington) were inoculated by conventional leaf-rub method using a cotton swab. Carborundum (800 mesh) was used as an abrasive. Immediately after inoculation, plants were washed thoroughly with water. Healthy seedlings of papaya (cv. Washington) inoculated similarly with distilled water were used as controls. For each inoculation, about 20 healthy seedlings of papaya were used. All the inoculated plants were maintained in an insect-free glass house and were regularly observed for development of symptoms. Observations on incubation period, sequence of symptom development and percentage transmission of the virus were also recorded.

3.4.2 Aphid transmission :

3.4.2.1 Maintenance of pure culture of different aphid species :

Eight aphid species viz., Acyrthosiphon pisum (Harris), Aphis craccivora Koch, Aphis gossypii Glover, Aphis nerii Boyer de Fonscolombe, Melanaphis (= Longiunguis van der Goot) sacchari Zehntner, Myzus persicae (Sulzer), Rhopalosiphum maidis (Fitch), and Uroleucon(= Dactynotus Rafinesque) compositae (Theobald) used in transmission tests, were collected from clusterbeans, Glyricidia maculata, cotton, Calotropis gigantea sorghum, cabbage, maize and safflower respectively. For all the aphid transmission tests, aphids raised from single aphid colonies were used. For raising

colonies of aphids of different species, healthy leaves of their respective feed-plants were placed on slightly moistened filter paper discs contained in Petri dishes. An apterous female aphid from each of the aphid species, was transferred separately onto these leaves in Petri dishes using a camel hair brush. Later, Petri dishes were incubated for 8 hours and the newly-born aphids (nymphs) were carefully picked up and transferred to their respective healthy feed-plants, maintained in muslin cloth cages, for their further multiplication. Different species of aphids used in this study, were identified as per the key and descriptions outlined by Blackman and Eastop (1984). These identifications were confirmed by Zoological Survey of India, Calcutta.

3.4.2.2 Aphid transmission of ten PRSV isolates :

The main objective of this study was to know the aphid transmissibility of different PRSV isolates and also to assess the efficiency of different aphid species in PRSV transmission. For aphid transmission of PRSV isolates, 8 aphid species viz., Acyrthosiphon pisum, Aphis craccivora, A. gossypii, A. nerii, Melanaphis sacchari, Myzus persicae, Rhopalosiphum maidis and Uroleucon compositae were used. In all the transmission tests, only adult apterous aphids were used. Adult apterous aphids from each species were given a pre-acquisition fasting period of 1 hour by keeping them in

Petri dishes containing moistened filter paper discs followed by an acquisition feeding time of 5 minutes on young symptomatic leaves of source plant (Papaya cv. Washington) of each of the 10 PRSV isolates. Then, they were, transferred onto a 50 days old healthy seedlings of papaya (cv. Washington) with the help of a camel hair brush and subsequently, followed by an inoculation feeding time of 4 hours. For each aphid-virus isolate combination, 20 healthy seedlings of papaya were used. For each test plant, 30 viruleferous aphids were used in transmission tests. Healthy seedlings of papaya (cv. Washington), similarly exposed to non-viruleferous aphids were used as controls. Later, aphids were killed by spraying 0.02 per cent dimethoate insecticide. All the test seedlings were maintained in an insect-free glass house and observations were recorded for incubation period, type of symptoms and percentage transmission in respect of each isolate.

3.4.3 Transmission of PRSV using different parts of infected papaya plant as a source of inoculum :

In these studies, Washington cultivar of papaya naturally infected by PRSV-PBN (FF) isolate and healthy 50 days old seedlings of papaya (cv. Washington) served as donor and test hosts respectively, to assess the virus inoculum potentiality of different infected tissues viz., leaf, petiole, stem and fruit, in PRSV transmission tests. For virus transmission studies, two methods of inoculation

viz., mechanical sap and aphid were employed. Symptomatic leaves showing distortion; petioles and stem showing streaks and fruits showing rings were utilised as source tissues in virus transmission tests. Young symptomatic leaves and thin peelings of infected tissues from petioles, stems and fruits, were separately washed in tap water to remove dirt and latex and dried between folds of blotter paper, before being used in transmission tests. For mechanical transmission, standard extracts of different virus source tissues were prepared in a cold neutral 0.1 M phosphate buffer containing 0.1 per cent DIECA, by triturating them, individually in a chilled mortar and pestle. The resultant inocula, were, then, filtered separately through a double-overed muslin cloth, before being used in transmission tests. Each of the standard extract was inoculated separately on about 20 healthy 50 days old seedlings of papaya by conventional leaf-rub method, using cotton swab and carborundum (800 mesh) as an abrasive. Immediately, after inoculation, plants were washed thoroughly with water. For aphid transmission, two species viz., Aphis gossypii and Myzus persicae were used. Adult apterate aphids of A. gossypii and M. persicae after a pre-acquisition fasting for 1 hour and acquisition feeding for 5 minutes on different virus source tissues, were transferred onto a separate sets of about 20 healthy seedlings of papaya in batches of 30 aphids per plant and were allowed for an inoculation feeding of 4 hours. Later, aphids were killed by spraying with 0.02

per cent dimethoate insecticide. Separate sets of healthy seedlings of papaya inoculated with distilled water and carborundum and exposed to aviruleferous aphids of A. gossypii and M. persicae, served as controls. All the inoculated plants were maintained in an insect-free glass house and observations were recorded with respect to incubation period, symptoms and percentage transmission for each virus source tissue-inoculation method combinations.

3.4.4 Virus-vector relationship :

Parbhani isolate of PRSV (PBN-FF) and two aphid species viz., A. gossypii and M. persicae, were used to determine the virus-vector relationship. Healthy seedlings of summersquash (C. pepo var. melopepo) cv. Patty Pan, preferably in cotyledon stage and just before the emergence of primary leaves, were used as test plants.

3.4.4.1 Efficiency of different morphological forms of the aphids in PRSV transmission :

In order to determine the efficiency of different morphological forms viz., nymphs, alate adults, apterate adults of A. gossypii and M. persicae, a pre-acquisition fasting period of 1 hour, followed by an acquisition feeding period of 5 minutes was given on PRSV- infected leaves of papaya. Later, these morphological forms were transferred

separately in a batches of 30 per plant onto separate sets of 20 healthy seedlings of summersquash cv. Patty Pan and were allowed for an inoculation feeding period of 4 hours. Later, aphids were killed by spraying 0.02 per cent dimethoate insecticide and the test plants were maintained in an insect-free glass house and observations in respect of symptom development and percentage transmission were recorded separately for each morphological form of an aphid species. Healthy seedlings of test plant inoculated with aviruleferous aphids of different morphological forms were used as controls.

3.4.4.2 Influence of the number of aphids in PRSV transmission :

A varying number of aphids viz., 1, 5, 10, 15, 20, 25, 30, 35, and 40 of A. gossypii and M. persicae were used in these tests. After a pre-acquisition starvation of 1 hour and acquisition feeding of 5 minutes on the virus source, aphids in different numbers were transferred separately to sets of 20 healthy seedlings of summersquash (cv. Patty Pan) and allowed for an inoculation feeding of 4 hours. Later aphids were killed by spraying 0.02 per cent dimethoate insecticide. The test plants were maintained in an insect-free glass house and observed for symptom development. The efficiency of each aphid treatment was assessed by computing per cent infectivity of PRSV based on 20 test plants.

3.4.4.3 Effect of pre-acquisition fasting periods given to aphid vector species on PRSV transmission :

Adult apterate aphids (A. gossypii and M. persicae) pre-starved for different periods i.e. 0, 0.5, 1, 2, 3, 4, 5 and 6 hours were given an acquisition feeding of 5 minutes on the virus source. Later, the aphids were transferred onto a separate sets of 20 healthy seedlings of test host in a batches of 30 aphids per plant. The inoculation feeding period allowed was 4 hours. At the end of inoculation feeding, aphids were killed by spraying dimethoate (0.02 per cent) insecticide. The test plants were maintained in an insect- free glass house and were regularly observed for symptom development. Transmission efficiency of aphids after having given pre-acquisition fasting for different periods was assessed by per cent infectivity based on 20 test plants. Healthy plants of summersquash exposed to aviruleferous aphids(that were subjected to different pre-acquisition fasting periods) served as controls.

3.4.4.4 Effect of different acquisition feeding periods given to aphid vector species on PRSV transmission :

To determine acquisition feeding period of PRSV, aphids (A. gossypii and M. persicae), after 1 hour pre-acquisition starvation, were allowed separately for different acquisition feeding periods of 1, 2, 5, 10, 15, 30, 60 and 120

minutes on virus source. Subsequently, they were transferred onto a separate sets of 20 healthy seedlings summersquash (cv. Patty Pan) in a batches of 30 per test plant and were allowed for an inoculation feeding for 4 hours. After killing the aphids by spraying dimethoate (0.02 per cent) insecticide all the test plants were maintained in an insect-free glass houseand regularly observed for symptom development. The efficiency of each feeding time was assessed by counting the number of plants infected.

3.4.4.5 Effect of different inoculation feeding periods given to aphid vector species on PRSV transmission :

To determine the inoculation access period of PRSV, aphids (A. gossypii and M. persicae), pre-starved for 1 hour and allowed for 5 minutes acquisition access on virus source, were transferred in a batches of 30 aphids per plant, onto a separate sets of 20 healthy seedlings of summersquash (cv. Patty Pan) and were allowed for different inoculation feeding periods of 1, 2, 5, 10, 15, 30, 60 and 120 minutes. At the end of each inoculation feeding period, aphids were killed by spraying with 0.02 per cent dimethoate insecticide and all the test plants were maintained in an insect-free glass house. Efficiency of each inoculation feeding period was judged by calculating percentage infectivity based on 20 test plants.

3.4.4.6 Persistence of PRSV in aphid vector species :

The retention of the virus by an aphid i.e. the number of plants an individual aphid can infect was determined by feeding an aphid on a series of 5 summersquash plants (cv. Patty Pan) for 1 and 2 minutes on each of the first four plants and left overnight on the last plant series. Prior to serial transmission, the aphids (A. gossypii and M. persicae) were given a pre-acquisition fasting of 1 hour followed by an acquisition feeding of 1 and 2 minutes on virus source. Later, the aphids were killed by spraying 0.02 per cent dimethoate insecticide and test plants were maintained in an insect- free glass house. Observations with respect to number of plants infected in each series were also recorded.

3.4.5 Seed transmission :

Seed transmission tests ("growing-on" and "indicator" inoculation tests) were conducted with seeds collected from papaya (cv. Washington) plants that were infected by PRSV. All the fruits selected for seed extraction from virus infected plants possessed ring spots typical of PRSV on the rind. Seeds collected from such fruits were washed in tap water and dried, before they were used in transmission tests. " Growing-on" and " indicator"

inoculation tests were usedin determining seed transmission. For these tests, about 2,200 seeds were sown in five lots in earthen-pots containing steam-sterilised soil, compost and sand (2:1:1) mixture. In each pot about 50 seeds were sown. About 2,200 seeds collected from healthy fruits (showing no symptoms of PRSV), and sown in five lots in earthen-pots similarly, served as controls. The pots were then maintained in insect-free glass house and watered regularly with a rose-can. The counts for germination and plants showing PRSV symptoms, if any, were recorded. Seedlings of papaya not showing symptoms of PRSV even after a period of two months, were indexed on test plants viz., papaya (cv. Washington) and summersquash (cv. Patty Pan) using pooled samples, each consisting of 100 plants, to detect symptomless infection if any by seeds. Test plants were inoculated with a sap from pooled samples, using conventional leaf-rub method. For this purpose 0.1 M phosphate buffer (pH 7.0) containing 0.1 per cent DIECA, was used as an extraction buffer and carborundum (800 mesh) as an abrasive.

3.4.6 Graft transmission :

Graft transmission test was conducted on 20 healthy and actively growing papaya (cv. Washington) plants ranging from 3½ to 4 months in age, grown in earthen-pots(15 cm diameter). The union between healthy and PRSV- PBN-FF-

infected papaya plants was established by "approach-grafting" as per the procedure outlined by Bos (1967). For this purpose, the cortical tissue (4 to 5 cm in length and 0.5 to 1 cm in width) of both healthy and PRSV-infected plants was carefully sliced with a sterile razor blade in order to expose the cambium in an equal pattern. The healthy and infected papaya plants were, then, tied together with polyethylene tape in order to secure the cut portions in a place and to ensure the maximum contant of cambium of infected plants with that of healthy ones and also to prevent a possible damage by desiccation. Later, the grafted plants were maintained in an insect-free glass house and regularly observed for symptom development. The observations with respect to incubation period, symptomatology and percentage infectivity were recorded.

3.4.7 Dodder transmission :

For dodder (Cuscuta species) transmission of PRSV isolate PBN-FF), virus free dodder culture was used. The virus free dodder culture was raised by sowing seeds collected from dodder and grown on healthy seedlings of papaya in earthen-pots. The stems of dodder seedlings after having attained the length of 2 to 4 cm, were placed on papaya seedlings with one end of the seedling in the soil and the other in contact with petiole or stem. Subsequently, when the dodder cultures were found well established and had

produced abundant number of new vines, they were used for transmission of PRSV from papaya to papaya, following the procedure outlined by Fulton (1964).

For effecting dodder transmission of PRSV, the virus free dodder was first established on PRSV-infected papaya plants. For this purpose, pieces of dodder collected from virus free culture were wrapped around petioles and stems of the infected papaya plants and were allowed to establish. After having ensured the establishment of dodder on infected plants, vigorously growing, uninjured tips of dodder vines were trailed and secured by wrapping onto healthy test plants of papaya (cv. Washington) of 2 to 3 months old. Later, all the dodder inoculated plants were maintained in an insect-free glass house. Observations with respect to incubation period, symptoms and percentage transmission of the virus on the dodder inoculated plants were recorded.

3.5 Host range :

For host range, plant species belonging to nine families viz., Amaranthaceae, Caricaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Labiateae, Leguminosae, Malvaceae and Solanaceae, were selected for comparing reactions of 10 PRSV isolates. All the test host plants were raised from healthy seeds sown in earthen-pots

containing steam-sterilised soil, sand and compost (2:1:1) mixture and were inoculated at their appropriate growth stage with ten PRSV isolates by mechanical sap and viruleferous aphid inoculations. All the leguminous plant species were inoculated, on primary leaves; cucurbitaceous plant species, invariably on cotyledons, just before the emergence of true leaves; while the other test plants were inoculated, when they were at 6 to 8 leaf stage or on fully expanded leaves. Plant species belonging to Chenopodiaceae were tested only by mechanical sap inoculation. For mechanical sap inoculation, inoculum was prepared separately for each of the PRSV isolate by triturating young symptomatic leaves of respective isolates with a cold mortar and pestle in a chilled, neutral 0.1 M phosphate buffer containing 0.1 per cent DIECA (1:1w/v) and the resultant sap extracts were filtered separately through double-overed muslin cloth, before being used for inoculations. A set of 10 healthy plants of each of the host species was used for inoculation of each of the PRSV isolate. Inoculations were made by conventional leaf-rub method, using a cotton swab and carborundum (800 mesh) as an abrasive. Immediately after inoculation, plants were washed throughly with water and maintained in an insect-free glass house. For aphid inoculations, viruleferous, apterous aphids of

Aphis gossypii were used. Adult apterous aphids of A. gossypii, after a pre-acquisition fasting of 1 hour and acquisition feeding time of 5 minutes on young symptomatic leaves of source plant (papaya cv. Washington) of each of the PRSV isolates, were transferred onto a separate sets of about 10 healthy seedlings of each of the host species, in batches of 30 aphids per plant and allowed for an inoculation feeding of 4 hours. At the end of inoculation feeding time, aphids were killed by spraying with 0.02 per cent dimethoate insecticide andmaintained in an insect-free glass house. Separate sets of 10 healthy seedlings of each of the test plant species, inoculated with distilled water and carborundum and also exposed to aviruleferous aphids (A. gossypii) served as controls. All the inoculated plants were watched regularly for 4 to 6 weeks to record observations with respect to incubation period, symptoms and percentage infectivity.

The host species that failed to express symptoms even after 4 to 6 weeks of virus inoculation, were back indexed on papaya (cv. Washington) and summersquash (cv. Patty Pan) test plants for detection of latent infection, if any.

The host range study was conducted during December 1986 to July 1987 and the weather data i.e., temperature and

humidity ranges prevailed during these months are presented as below :

Table showing mean monthly Meteorological observations :

Month/ Year	Temperature(°C)		Relative humidity(%)	
	Max.	Min.	A.M.	P.M.
December 1986	29.7	12.8	74.0	47.0
January 1987	30.4	14.1	79.0	35.0
February 1987	32.4	13.6	57.0	21.0
March 1987	36.1	16.3	43.0	15.0
April 1987	40.8	23.0	29.0	11.0
May 1987	39.8	24.3	44.0	18.0
June 1987	37.8	25.2	69.0	36.0
July 1987	33.1	24.3	81.0	55.0

The following plant species were used as test hosts:

I. Amaranthaceae :

1. Celosia argentea L.
2. Celosia plumosa L.
3. Gomphrena globosa L.

II. Caricaceae :

4. Carica cauliflora Jacq.
5. Carica papaya L. cv. Washington

III. Chenopodiaceae :

6. Beta vulgaris L.

7. Chenopodium album Red.

8. Chenopodium amaranticolor Coste. et. Reyn.

9. Chenopodium murale L.

10. Chenopodium quinoa Willd.

IV Compositae :

11. Zinnia elegans Jacq.

V. Cucurbitaceae :

12 Benincasa hispida Cogn. cv. Co-1.

13. Citrullus lanatus(Thumb)Matsum & Nakai.cv.Sugar Baby

14. Citrullus lanatus var. fistulosus Duth & Full cv. Arka Tinda.

15. Cucumis melo L. cv. Arka Jeet.

16. Cucumis sativus L. cvs. Banglore Dwarf, Banglore Special White and Local.

17. Cucurbita maxima Duchense. cv. Arka Suryamukhi

18. Cucurbita pepo L. cv. Arka Chandan.

19. Cucurbita pepo var. melopepo (L.) Alef.cv. Patty Pan.

20. Lagenaria siceraria (Mol.) Stndl. cv. Pusa Summer Prolific Long.

21. Luffa acutangula Roxb. cv. J. Long.

22. Luffa cylindrica (L.) Roem. cv. Pusa Chikni.

23. Momordica charantia L. cv. Coimbatore White Long.

24. Trichosanthes anguina L. cv. Green Long.

VI. Labiateae :

25 Ocimum basilicum L.

VII. Leguminosae :

26. Arachis hypogaea L. cv. SB-XI

27 Cassia occidentalis L.

28. Cicer arietinum L. cv. BDN-9-3

29. Glycine max (L.) Merr. cv. MACS-13.

30. Vicia faba L.

31. Vigna mungo (L.) Hepper cv. Sindhkeda-1-1.

32. Vigna radiata (L.) Wilcz. cv.J-781.

33. Vigna unguiculata (L.) Walp. cv. C-152.

VIII. Malvaceae :

34. Abelmoschus esculentus (L.) Moench. cv.Pusa Sawani.

IX Solanaceae :

35. Capsicum annuum L. cv. NP-46-A.

36. Datura stramonium L.

37. Lycopersicon esculentum Mill. cv. Pusa Ruby

38. Nicotiana glutinosa L.

39. Nicotiana rustica L.

40. Nicotiana tabacum L. cv. White Burley.

41. Solanum melongena L. cv. Pusa Purple Long.

3.6 Indexing of field infected cucurbitaceous plant species for the presence of PRSV :

Field grown cucurbitaceous plant species like bittergourd (Momordica charantia L.), bottlegourd (Lagenaria siceraria Standl.), ridgegourd (Luffa acutangula Roxb.), snakegourd (Trichosanthes anguina L.), and

spongegourd (Luffa cylindrica (L.) Roem.), showing mosaic symptoms and watermelon (Citrullus lanatus (Thumb) Matsum and Nakai.) showing mosaic, vein-banding and leaf distortion symptoms were indexed on healthy seedlings of indicator plants of papaya cv. Washington and summersquash cv. Patty Pan using aphid vector (A. gossypii) for detecting the presence of PRSV.

Adult apterate forms of A. gossypii, after having given a pre-acquisition fasting of 1 hour, were fed on young symptomatic leaves of test cucurbitaceous plant species for 5 minutes and then transferred in batches of 30 per plant to a set of 10 healthy seedlings each of papaya (cv. Washington) and summersquash (cv. Patty Pan) indicator plants for an inoculation feeding of 4 hours. Subsequently, aphids were killed by spraying 0.02 per cent dimethoate insecticide and the inoculated plants were maintained in an insect- free glass house and observed for symptom development. Healthy seedlings of papaya and summersquash, similarly exposed to non-viruleferous aphids served as controls.

3.7 Physical properties :

The studies on physical properties viz., thermal inactivation point (TIP), dilution end point (DEP) and longevity in vitro (LIV) of 10 PRSV isolates were carried

out as per the procedures outlined by Bos *et al.* (1960), using papaya leaves (cv. Washington) infected with different isolates of PRSV as virus source and summersquash (*C. pepo* var. *melopepo*) cv. Patty Pan as an assay host.

3.7.1 Thermal inactivation point (TIP) :

Standard leaf extracts of isolates of PRSV were prepared in 1:1 ratio of leaf tissue to 0.1 M phosphate buffer (pH 7.0) containing 0.1 per cent DIECA. Aliquots of 2 ml standard extracts of 10 PRSV isolates were pipetted into a set of nine glass test-tubes per isolate and were subjected separately to different temperature treatments from 40 to 80°C with an interval of 5°C for 10 minutes in a thermostatic water bath. After temperature treatment, cold water was runover outside the tubes to cool the contents. All the treated extracts were then indexed on summersquash assay host by conventional leaf-rub method using carborundum (800 mesh) as an abrasive. A set of 10 plants were inoculated with each PRSV isolate-temperature treatment combinations. The cotyledons of assay host (summersquash) plants, similarly inoculated with untreated standard extracts of different PRSV isolates were used as controls. Later, the test plants were washed and maintained in an insect-free glass house. Observations were recorded with respect to number of

plants infected and the percentage infectivity was calculated, based on 10 test plants.

3.7.2 Dilution end point (DEP):

This experiment was conducted with a view to determine upto what extent the sap could be infective when subjected to a serial dilutions. The standard leaf extracts of 10 PRSV isolates were prepared in 1:1 ratio of tissue to buffer (0.1 M phosphate buffer of PH 7.0 containing 0.1 per cent DIECA) and were further diluted in ten fold (X^{-10}) series, from undiluted, 10^{-1} to 10^{-7}. A set of 10 seedlings of an assay host at cotyledon stage were inoculated separately with dilution treatments of PRSV isolates starting with the highest dilution and ending up with the crude leaf extract. The healthy seedlings of an assay host inoculated with undiluted or standard extracts of PRSV isolates served as controls. Immediately after inoculation, the test plants were washed and maintained in an insect- free glass house. Observations were recorded isolate wise, with respect to number of plants infected by each dilution treatment.

3.7.3 Longevity in vitro (LIV) :

In order to know the longevity of the virus in crude sap, an experiment of aging in vitro was carried out. The leaf extracts of PRSV isolates were prepared in 1:1 ratio of

tissue to buffer (neutral 0.1 M phosphate buffer containing 0.1 per cent DIECA), were kept at room temperature (27 to 30°C) in rubber-stoppered tubes. Different treatments of LIV included the period ranging from 0 to 16 hours with an interval of 2 hours between treatments. Sap extracts of PRSV isolates stored for different periods, were, then, inoculated separately onto a set of healthy seedlings of an assay host using conventional leaf-rub method. Each set of assay host used for inoculation comprised of 10 healthy seedlings. Subsequently, the assay host seedlings were washed and maintained in an insect-free glass house. Observations were recorded isolate -wise with respect to number of plants infected by different LIV treatments.

3.8 Serology :

The serological relationship of the virus isolates under investigation were tested with fifteen viruses viz., "cucumber mosaic virus" (CMV), " tobacco ringspot virus" (TRSV), " potato virus X" (PVX), "bean common mosaic virus" (BCMV), "bean yellow mosaic virus" (BYMV), "blackeye cowpea mosaic virus" (BICMV), " cowpea aphid-borne mosaic virus" (CAMV), "datura distortion mosaic virus" (DDMV), " papaya ringspot virus" (PRSV)," peanut mottle virus" (PnMV),"potato virus Y" (PVY), " soybean mosaic virus"(SMV),

"watermelon mosaic virus-1" (WMV-1), "watermelon mosaic virus-2" (WMV-2) and " wisteria vein mosaic virus" (WVMV) belonging to four taxonomic virus groups of cucumo, nepo, potex and poty, using serological techniques like, " drop precipitin test on slides" and "SDS (sodium dodecyl sulphate)- immunodiffusion test". Details pertaining to the source and titre of antisera used in these tests are furnished below :

Sr. No.	Virus group/ antiserum	Source and titre
I.	Cucumovirus group :	
1.	Cucumber mosaic virus (CMV), isolate CPM	Czechoslovakia, titre not mentioned.
II.	Nepovirus group :	
2.	Tobacco ringspot virus(TRSV)	Denmark, titre= 1:32.
III.	Potexvirus group :	
3.	Potato virus X (PVX)	CPRI., Simla, titre not mentioned.
IV.	Potyvirus group :	
4.	Bean common mosaic virus(BCMV), isolate TPM-6.	Czechoslovakia; titre not mentioned.
5.	Bean yellow mosaic virus (BYMV), isolate FMV-1.	Czechoslovakia, titre= 1:1024.
6.	Blackeye cowpea mosaic virus (BlCMV)	USA., titre not mentioned.

Sr. No.	Virus group/ antiserum	Source and titre
7.	Cowpea aphid-borne mosaic virus (CAMV), Nigerian isolate	IITA., Ibadan, Nigeria, titre= 1:1024.
8.	Datura distortion mosaic virus (DDMV)	MAU., Parbhani, titre= 1:1024.
9.	Papaya ringspot virus (PRSV) Hawaiian isolate	USA., titre not mentioned.
10.	Peanut mottle virus (PnMV), peanut isolate	IGRISAT., Hyderabad titre= 1:800.
11.	Potato virus Y (PVY)	Torino, Italy, titre= 1:2048.
12.	Soybean mosaic virus (SMV), Nigerian isolate	IITA, Ibadan, Nigeria, titre not mentioned.
13.	Watermelon mosaic virus-1(WMV-1)	Torino, Italy, titre= 1: 256.
14.	Watermelon mosaic virus-2(WMV-2)	Torino, Italy, titre not mentioned.
15.	Wisteria vein mosaic virus (WVMV)	Torino, Italy, titre=1:1024.

3.8.1 Drop precipitin test on slides :

For this test, drops of virus isolates (antigens) in crude leaf extracts were mixed directly with drops of specific antiserum on separate, clean microscopic glass slides (Bercks et al., 1972). These slides were then incubated under high humidity for few hours at about 20°C and examined under a microscope at about 60X. The diluent

used for antigens and antisera was phosphate buffered saline (PBS) (0.1 M neutral phosphate buffer + 0.15 M Nacl). The antiserum dilution used was 1:4 and that of antigen was 1:10. Healthy sap from test plants, normal serum and PBS were included as controls. Observations were recorded for precipitation reaction for each antigen-antiserum combination.

3.8.2 SDS- immunodiffusion test :

SDS (sodium dodecyl sulphate)- immunodiffusion test was carried out as per the procedure outlined by Yeh et al. (1984) and Purcifull et al. (1984), using crude sap extracts of PRSV isolates. The test was carried out in clean, sterilised, 9 cm flat bottom Petri dishes. The agar medium consisting of 0.8 per cent non-nutrient agar, 1 per cent sodium azide and 0.5 per cent SDS was prepared in PBS (0.1 M phosphate buffer (pH 7.0) + 0.15 M Nacl), by autocalving at 15 pounds pressure per square inch. Then 20 ml of agar medium was pipetted into Petri dishes, ensuring a complete coverage of bottom area and allowed to solidify. Using a sterile-cork borer, one central well and six peripheral wells of 5 mm diameter and 6 mm apart were cut and the plugs were removed with the help of a pipette attached to a suction pump. The bottom of the wells were sealed by placing a drop of melted agar medium, using

a capillary tube, so as to prevent the leaching of antiserum and antigen to the bottom of the Petri dish.

Antigens (crude sap extracts of 10 PRSV isolates viz., AKL, AUR, LTR, NAN, OSB, PBN(HG), PBN(FF), PUN-HC.c.p., PUN. c.p. and THA were prepared by grinding separately 1 g each of freshly harvested, virus-infected leaf tissues of summersquash (cv. Patty Pan) in 1 ml of PBS containing 5 per cent ascorbic acid. These samples, after being filtered through muslin cloth were further diluted in the proportions of 1:2, 1:4, 1:8 and 1:16 and used immediately.

The antisera used in this test were"papaya ringspot virus" (PRSV), " watermelon mosaic virus-1" (WMV-1) and "watermelon mosaic virus-2"(WMV-2). Sap from healthy leaves of summersquash (cv. Patty Pan,), PBS and normal serum were used as control. Antiserum was pipetted in the central well and antigen (in serial dilutions) and controls were pipetted in peripheral wells. The results of the SDS-immunodiffusion test were recorded by observing the Petri dishes under stereobinocular microscope, with its lamp turned upwards and illuminating the dishes from below and the lines of precipitation if any were examined from above.

3.9 Electron microscopy :

The electron microscopy of the virus under investigation was accomplished by " leaf-dip preparations" and "ultrathin sections" of young papaya leaves systemically infected by PRSV isolate (PBN-FF).

3.9.1 Leaf-dip preparations :

Leaf-dip preparations of PRSV isolate (PBN-FF) were made following the procedures of Brandes (1957). For leaf-dip preparations, freshly harvested young leaves of papaya, preferably 12 days after virus inoculation by an aphid vector (A. gossypii) and showing vein-clearing symptoms were cut into small strips. Freshly cut surfaces of several of such strips were quickly dipped in distilled water droplets and held until the droplets on clean glass slides turned light green, then, carbon-coated formvar copper grids (300 mesh) were allowed to float on these droplets for 5 minutes. Later, the excess leaf extract was absorbed carefully with the help of blotter paper and the grids were stained with 2.0 per cent phosphotungstic acid (PTA), pH 6.5. The grids, were, then, air-dried and examined in a Philips 201C model, transmission electron microscope at 60 KV. The particle sizes were estimated by comparing their projected micrographs with that of a diffraction gratings of the same magnification. About 100

particles were measured to determine the normal and modal lengths. The normal particle length was calculated as the mean value of the main maximum. The values for main maximum and standard error (S.E.), was calculated following procedures outlined by Chandel (1972). The most frequently occurring value was treated as modal length. The data was presented in the form an histogram as outlined by Tomlinson (1964).

3.9.2 Ultrathin sectioning :

The main objective of ultrathin sectioning was to study the cytoplasmic cylindrical inclusions (CCI) produced by the virus under investigation and also to assign it to a particular virus group based on its inclusion morphology. Spurr's epoxy resin blocks embeded with infected leaf tissue pieces of papaya were prepared and used for thin sectioning, as per the procedure outlined by Hayat (1970). For block making, 100 per cent Spurr's resin medium was prepared from mixing 10 g of 4-vinylcyclohexenedioxide (VCD), 8 g of diglycidyl ether of polypropylene glycol (DER-736), 26 g of nonenyl succinic anhydride (NSA) and 0.4 g of dimethylaminoethanol (DMAE). Infected leaf tissues for this purpose were collected from young systemically infected leaves, showing vein-clearing symptoms preferably 12 days after inoculation by an aphid vector (A. gossypii).

Tissues from infected papaya leaves were cut into pieces of 1 to 2 mm square. These tissue pieces were, then, fixed in 3 per cent glutaraldehyde in 0.1 M phosphate buffer, pH 6.8 for 3 hours at $4^{o}C$. Following fixation, tissues were washed in 3 changes of 10 minutes each in 0.1 M phosphate buffer, pH 6.8 on a gyrating shaker and were post-fixed in 1 per cent osmium tetraoxide (OSO_4) in 0.1 M phosphate buffer, pH 6.8 for 3 hours at $4^{o}C$. Subsequently, tissues were washed in distilled water for 10 minutes on a gyrating shaker and were dehydrated for 30 minutes at room temperature in a graded series of acetone i.e. 30, 50, 70, 90 and 100 per cent. Following dehydration, tissues were subjected to two separate overnight storage series of infiltration; the first one in 100 per cent acetone + 100 per cent Spurr's resin and the second one in 100 per cent resin alone. Immediately after infiltration, tissues were embeded in 100 per cent freshly prepared Spurr's resin. Tissue embeding was done in beam capsules and flat plastic moulds, filled with Spurr's resin and care was taken to orient the tissues in proper planes. Then capsules and flat plastic moulds containing the tissue in Spurr's resin were polymerised in a hot-airoven at $65^{o}C$ overnight. After polymerisation, blocks were separated from beam capsules and plastic moulds. Blocks were, then trimmed suitably to expose the tissues, with the help of a hand trimmer.

Ultrathin sections were cut at 60 to 90 nm using LKB Ultratome III- 8800 with glass knives. Sections were pickedup on carbon-coated copper grids (300 mesh) and stained with saturated solution of uranyl acetate for 15 minutes followed by 2 per cent lead citrate for 5 minutes. The grids were air-dried and examined in a Philips 201C model, transmission electron microscope at 60 KV.

Similarly blocks prepared from healthy papaya tissue using Spurr's resin were cut and sections were used to compare with the sections of infected tissues as a part of control treatments.

3.10 Screening of Caricaceae for resistance to PRSV :

Sixteen cultivars of Carica papaya viz., Co-1, Co-2, Co-3, Co-4, Co-5, Co-6, Coorg Honey Dew, Honey Dew, Pant Papaya-1, Pusa Delicious, Pusa Dwarf, Ranchi, Solo, Solo Hawaii, Sunrise Solo and Washington; C. cauliflora and an interspecific hybrid between C. cauliflora and C. papaya were screened in order to locate a source of resistance to PRSV. Test plants of papaya cultivars, an interspecific hybrid and C. cauliflora were raised from healthy seeds sown in earthen-pots containing steam- sterilised soil, sand and compost (2:1:1) mixture and maintained in an insect-free glass house. Twenty healthy-50 day old seedlings of each test cultivars, an interspecific hybrid and

C. cauliflora were inoculated with PRSV (PBN-FF isolate) by conventional leaf-rub method using 0.1 M phosphate buffer (pH 7.0) containing 0.1 per cent DIECA as an extraction buffer and carborundum (800 mesh) as an abrasive. "Papaya ringspot virus" (PRSV-PBN-FF) maintained on Washington cultivar of papaya was used as source of virus inoculum. After inoculation, all the test plants were washed with water and maintained in an insect-free glass house. Healthy seedlings of test cultivars, an interspecific hybrid and C. cauliflora inoculated with distilled water and carborundum served as controls. All the test plants were regularly observed for symptom development. Observations were also recorded with respect to incubation period, number of plants infected, symptoms and plant height.

For assessing the varietal reaction, the following disease rating was adopted.

Immune (I): Inoculated test cultivars not developing any symptoms of PRSV and virus also not recovered by back indexing on summersquash (cv. Patty Pan) test plants.

Tolerant (T): Inoculated test cultivars not developing any symptoms of PRSV but virus was recovered by back indexing on summersquash test plants.

Susceptible (S): Inoculated test cultivars showing all the characteristic symptoms of PRSV and virus also recovered readily by back indexing on summersquash test plants.

RESULTS

4. RESULTS

4.1 Disease incidence and field symptoms :

The results of surveyes made in 10 papaya plantations in nine localities of Thane, Aurangabad, Beed, Latur, Nanded, Osmanabad, Parbhani, Pune and Akola representing different regions of Maharashtra State viz., Konkan, Marathwada, Vidharbha, and Western Maharashtra, for the incidence of "papaya ringspot virus" (PRSV) are given in Table-1. The results indicated that the incidence of PRSV was found to range from 53 to 100 per cent during winter months of 1985-86.

The plants of recently introduced papaya cultivars viz., Co-1, Co-2, Co-3, Co-4, and Co-5 and an interspecific hybrid (*C. cauliflora* x *C. papaya*) showed 100 per cent infection by PRSV in the plantation from the Pune locality. Cent per cent incidence of PRSV was also found on plants of Washington cultivar in plantations from localities of Parbhani, Pune and Beed districts, whereas the plants of same cultivar from the plantation in the locality from Aurangabad district recorded lower disease incidence (62 per cent). Comparatively, low disease incidence was recorded on plants of different local cultivars in plantations from localities of Parbhani, Nanded and Akola

Table 1: Incidence of papaya ringspot virus (PRSV) on papaya in different regions of Maharashtra.

Sr. No.	Place of survey	No.of orchards surveyed	Age of plants (in months)	Variety	Plant population	No.of plants infected by PRSV	% diseased plants	Intercrops	Crops grown in the vicinity.
I.	KOKANKAN								
I.	KONKAN								
1.	Pilar, Thane(District)	1	8	Local	911	911	100.00	Bhe,Cp, Rg.	Ban, Bri, Bhe
II.	MARATHWADA								
2.	Panewade,Aurangabad(District)	1	12	Washington	50	31	62.00	Bhe,Cab, Cp.	S.cane, Sor.
3.	Parli, Beed(District)	1	12	Washington	30	30	100.00	Bhe,Cab, Bog.	S.cane, Baj.
4.	Lakhnewadi,Latur(District)	1	12	Local	65	49	75.38	Bhe,Cp, Clb.	Bhe,Cp, Brin,Tom, Chi, S.cane, Sor.
5.	Khandhar, Nanded(District)	1	12	Local	400	352	88.00	Cp,Bhe, Clb.	Cot, S.cane, Sor.
6.	Tuljapur,Osmanabad(District)	1	12	Co-1	810	810	100.00	Bhe,Cp,Cab, Clb.	Cot, S.cane, G.nut, Sor.
7.	Parbhani,Parbhani(District)	2	11	Washington	58	58	100.00	Bhe,Cp,Cab, Clb.	-
		Garden-2	12	Local	450	237	52.66	Chi,Bhe,Bog, Rg.	Rg,Clb,Cot and Calotropis gigantea on field bunds.
III.	VIDHARBHA								
8.	Akola,Akola(District)	1	12-24	Local	25	15	60.00	-	Nursery plants
IV.	WESTERN MAHARASHTRA								
9.	Ganesh Khind, Pune	1	12	Interspecific hybrid	52	52	100.00	-	Fruit crops like pomegranate, mango, guava and vegetable crops like brinjal, tomato and bhendi.
				Co-1	52	52	100.00	-	
				Co-2	52	52	100.00	-	
				Co-3	52	52	100.00	-	
				Co-4	52	52	100.00	-	
				Co-5	39	39	100.00	-	
				Sole	52	52	100.00	-	
				Washington	52	52	100.00	-	

Ban = banana, Baj = bajra, Bhe = bhendi, Bog = bottlegourd, Bri = brinjal, Cab = cabbage, Chi = chillies, Clb = clusterbeans. Cot = cotton, Cp = coupea, G.nut = groundnut, Rg = ridgegourd, S.cane = sugarcane, Sor = sorghum, Tom = tomato, Wm = watermelon.

PLATE - I

Fig. 1 : Field-grown PRSV infected papaya plant with symptoms of mosaic on leaves.

PLATE
I

Fig.1

districts. The plants of a local cultivar of papaya grown in the locality of Thane district, Co-1 cultivar grown in locality of Latur district and that of Solo cultivar grown at Pune recorded 100 per cent infection by PRSV.

Observations made for the colonisation of aphids on papaya plants in papaya plantations in different localities revealed the absence of aphid colonisation. However, intercrops and crops grown in the vicinity were found to be colonised by different species of aphids. Crops like okra, bottlegourd, ridgegourd, watermelon, brinjal, chillies and cotton were found to be colonised by the melon aphid, Aphis gossypii. The green peach aphid, Myzus persicae was found to be colonising on cabbage. Calotropis gigantea, a common weed in fields was found to be infested by the oleander aphid, Aphis nerii. Leguminous crops like cowpea and groundnut were found to be colonised by the black legume aphid, Aphis craccivora and clusterbeans by Acyrthosiphon pisum, the pea aphid. Graminaceous hosts like pearl-millet and sorghum were found to be colonised by Melanaphis sacchari, the sugarcane aphid and Rhopalosiphum maidis, the corn-leaf aphid.

The field infected papaya plants by PRSV showed characteristic symptoms on foliage, petiole, stem and fruits. The foliage symptoms included mosaic (Plate-I,

PLATE - II

Fig. 2 : Field grown PRSV-infected papaya plant displaying severe distortion and shoestringing symptoms on papaya leaves.

Fig. 3 : Field grown PRSV-infected papaya plant showing green to dark green spots and streaks on stem and petioles.

PLATE
II

Fig.2

Fig.3

PLATE - III

Fig. 4 : PRSV- infected papaya fruit showing water-soaked, dark green, circular spots and rings on the rind.

Fig. 5 : PRSV- infected papaya fruit exhibiting large rings and line patterns on the rind.

PLATE
III

Fig.4

Fig.5

Fig.1), severe distortion and shoestringing (Plate-II, Fig.2). Stem and petiole symptoms included light green to dark green, water-soaked spots and streaks (Plate-II, Fig.3). Fruits from infected papaya trees exhibited water-soaked, dark green, circular spots (Plate-III, Fig.4) and rings which often coalesced to form large rings and line patterns (Plate-III, Fig.5).

4.2 Isolates :

Ten isolates of PRSV, one each from Konkan and Vidharbha, two from Western Maharashtra and six from Marathwada regions were collected, named and abbreviated after the localities from which these were collected. These included AKL, AUR, LTR, NAN, OSB, PBN(FF), PBN(HG), PUN.HC.c.p., PUN-C.p., and THA. These isolates were transmitted initially on diagnostic hosts like Washington papaya and summersquash cv. Patty Pan by mechanical sap inoculation. Subsequently, these isolates were maintained on Washington papaya and summersquash plants either by mechanical sap inoculation or aphid inoculation and were used for further studies. The reactions of diagnostic hosts to each of the 10 isolates are shown in Table -2. No apparent differences in reactions of diagnostic hosts to 10 virus isolates were noticed. On papaya cv. Washington, these isolates reacted only systemically producing symptoms of chlorosis, vein-clearing, mottling, mosaic, blistering,

Table 2: Reactions of test plants of papaya and summersquash to different isolates of papaya ringspot virus (PRSV).

Sr. No.	Isolates of PRSV	Origin of isolates		Reactions of test plants*	
		District	Region	Papaya cv. Washington	Summersquash cv. Patty Pan
1.	AKL	Akola	Vidharbha	Chl, Vc, Mot, Mo, Bl, Ld, Ss.	Vc, Pvchl, Mo, Ld.
2.	AUR	Aurangabad	Marathwada	Chl, Vc, Mot, Mo, Bl, Ld, Ss.	Vc, Pvchl, Mo, Ld.
3.	LTR	Latur	Marathwada	Chl, Vc, Mot, Mo, Bl, Ld, Ss.	Vc, Pvchl, Mo, Ld.
4.	NAN	Nanded	Marathwada	Chl, Vc, Mot, Mo, Bl, Ld, Ss.	Vc, Pvchl, Mo, Ld.
5.	OSB	Osmanabad	Marathwada	Chl, Vc, Mot, Mo, Bl, Ld, Ss.	Vc, Pvchl, Mo, Ld.
6.	PBN(FF)	Parbhani	Marathwada	Chl, Vc, Mot, Mo, Bl, Ld, Ss.	Vc, Pvchl, Mo, Ld.
7.	PBN (HG)	Parbhani	Marathwada	Chl, Vc, Mot, Mo, Bl, Ld, Ss.	Vc, Pvchl, Mo, Ld.
8.	PUN-HC.c.p.	Pune	Western Maharashtra	Chl, Vc, Mot, Mo, Bl, Ld, SS	Vc, Pvchl, Mo, Ld.
9.	PUN-C.P.	Pune	Western Maharashtra	Chl, Vc, Mot, Mo, Bl, Ld, Ss.	Vc, Pvchl, Mo, Ld.
10.	THA	Thane	Konkan	Chl, Vc, Mot, Mo, Bl, Ld, Ss.	Vc, Pvchl, Mo, Ld.

*/= Chl= chlorosis, Ld= leaf distortion, Mo= mosaic, Mot = mottling, Pvchl= patchy veinal chlorosis, Bl = blistering, Ss = shoestringing, Vc= vein-clearing.

severe leaf distortion and shoestringing, while on summersquash cv. Patty Pan, the systemic foliage symptoms caused included vein-clearing, patchy veinal chlorosis, mosaic and leaf distortion (Table -2).

4.3 Transmission :

4.3.1 Mechanical transmission :

The results on mechanical transmission of 10 PRSV isolates from papaya to papaya (cv. Washington) are presented in Table-3 . It is evident from the data given in the table that all the ten PRSV isolates viz., AKL, AUR, LTR, NAN, OSB, PBN(FF), PBN(HG), PUN-HC.c.p., PUN-C.p. and THA were readily transmitted from papaya to papaya by mechanical means using conventional leaf-rub method with (0.1 M phosphate (pH 7.0) containing 0.1 per cent DIECA) and extraction buffer. All the 10 PRSV isolates reacted systemically without any apparent differences in the symptom expression and also in the sequence of symptom appearance and incubation period. The level of mechanical transmission recorded for different isolates was as high as 100 per cent. In all the seedlings of papaya, which were inoculated with different isolates, the initial symptoms of chlorosis and vein-clearing appeared on young leaves 14 days after virus inoculation (Plate-IV, Figs. 6 and 7A). The subsequent symptoms observed were mottling, mosaic (Plate-IV, Fig.7B), blistering (Plate-V, Fig.8) different

Table 3: Mechanical transmission of PRSV isolates from papaya to papaya using 0.1 M phosphate buffer, pH 7.0 containing 0.1 per cent DIECA.

Sr. No.	Isolates	No.of plants infected/no. of plants inoculated	% transmission	Incubation period in(days)	Symptoms observed*
1.	AKL	20/20	100.00	14	Chl, Vc, Mot, Mo, Bl, Ld, Ss.
2.	AUR	20/20	100.00	14	Chl, Vc, Mot, Mo, Bl, Ld, Ss.
3.	LTR	20/20	100.00	14	Chl, Vc, Mot, Mo, Bl, Ld, Ss.
4.	NAN	20/20	100.00	14	Chl, Vc, Mot, Mot, Bl, Ld, Ss.
5.	OSB	20/20	100.00	14	Chl, Vc, Mot, Mot, Bl, Ld, Ss.
6.	PBN(HG)	20/20	100.00	14	Chl, Vc, Mot, Mo, Bl, Ld, Ss.
7.	PBN(FF)	20/20	100.00	14	Chl, Vc, Mot, Mo, Bl, Ld, Ss.
8.	PUN-HC.c.p.	20/20	100.00	14	Chl, Vc, Mot, Mo, Bl, Ld, Ss.
9.	PUN-C.p.	20/20	100.00	14	Chl, Vc, Mot, Mo, Bl, Ld, Ss.
10.	THA	20/20	100.00	14	Chl, Vc, Mot, Mo, Bl, Ld, Ss.

*/= Bl = blistering, Chl = chlorosis, Ld = leaf distortion, Mo = mosaic, Mot = mottling, Ss = shoestinging, Vc = vein-clearing.

PLATE - IV

Fig. 6 : PRSV- infected papaya plant in glass house showing initial symptoms of chlorosis and vein-clearing on newly developing leaves upon mechanical sap and aphid inoculations.

Fig. 7 : PRSV- infected papaya leaves displaying vein-clearing (A) mottling and mosaic (B) symptoms.

PLATE
IV

Fig.6

Fig.7

PLATE - V

Fig. 8 : PRSV-infected papaya plant exhibiting symptoms of blistering on leaves

Fig. 9 : PRSV- infected papaya plant displaying symptoms of mosaic, blistering and distortion of leaves.

PLATE
V

Fig.8

Fig.9

PLATE - VI

Figs. 10 : PRSV- infected papaya plants displaying slight distortion of leaves.

Fig. 11 : PRSV- infected papaya plants displaying moderate distortion of leaves.

PLATE
VI

Fig.10

Fig.11

PLATE - VII

Fig. 12 : PRSV-infected papaya plant displaying severe distortion symptoms on leaves.

Fig. 13 : PRSV-infected papaya plants showing extremely severe distortion of leaves (shoestringing).

PLATE
VII

Fig.12

Fig.13

PLATE VIII

Figs. 14 : PRSV-infected papaya leaves showing mosaic and varying degree of distortion and shoestringing.

PLATE
VIII

Fig.14

PLATE IX

Fig. 15 : PRSV-infected papaya plants showing twisted petioles and assuming a "spring-like" appearence.

PLATE
IX

Fig.15

PLATE - X

Fig. 16 : An healthy petiole of papaya plant (A); petiole from PRSV- infected papaya plant showing brownish streaks (B).

Fig. 17 : An healthy stem of papaya plant (A); stem from PRSV- infected papaya plant exhibiting dark green water-soaked spots and streaks (B).

PLATE
X

Fig.16

Fig.17

PLATE - XI

Fig. 18 : PRSV- infected papaya plants displaying severe stunting (in foreground). In the background unionculated healthy tall papayaplant.

PLATE
XI

Fig.18

degrees of leaf distortion (Plates V to VII, Figs. 9 to 12) and shoestringing (Plates-VII and VIII, Figs. 13 and 14). These symptoms were evident in a sequence of a time gap of 1 to 2 weeks. In some papaya cultivars like Co-1, Co-2, Co-3, Pusa Delicious, Pusa Dwarf and Solo, petioles of infected plants showed characteristic twisting and assumed "spring-like" appearance (Plate-IX, Fig.15). All the infected plants showed premature leaf shedding leading to denuding of stem bearing a few or cluster of small thread-like leaves at the top. Green to dark green or brown spots or streaks with a water-soaked appearance were evident on petioles (Plate-X, Fig.16B) and stems (Plate-X, Fig.17B) of infected plants 20 to 25 days after virus inoculation. All the infected plants exhibited retarded growth and were markedly stunted (Plate-XI, Fig.18).

During summer months, masking of disease symptoms was observed and plants which showed severe symptoms during winter months, produced apparently normal leaves during summer. These plants, however, again showed severe disease symptoms with the onset of monsoon and fall in temperatures. In all the diseased plants, symptoms disappeared during the month of April and remained symptomless till the first fortnight of July. In all the symptomless plants, the revival of symptoms was evident from the second fortnight of July and by the first fortnight of August, all such plants exhibited severe leaf distortion and shoestringing

foliage symptoms and water-soaked spots or streaks on petioles and stems.

4.3.2 Aphid transmission of ten PRSV isolates :

The results of aphid transmission tests carried out for ten PRSV isolates are presented in Table-4a and graphically in Fig.(19). The data presented in table and graph indicated that all the 10 PRSV isolates were readily transmitted from papaya to papaya (cv. Washington) in a non-persistent manner by 8 aphid species. All the 8 aphid species viz., Acyrthosiphon pisum, Aphis craccivora, A. gossypii, A. nerii, Melanaphis sacchari, Myzus persicae, Rhopalosiphum maidis and Uroleucon compositae, were found to be the vector of 10 PRSV isolates i.e. AKL, AUR, LTR, NAN, OSB, PBN(FF), PBN (HG), PUN-HC.c.p., PUN-C.p and THA. However, the vector efficiency was found to vary with the species. The green peach aphid, Myzus persicae was found to be the most efficient vector, transmitting all the isolates of PRSV to the extent of 100 per cent. The other vectors of PRSV isolates in order of merit with respect to efficiency included the cotton aphid, Aphis gossypii (ave.90.15 per cent), the oleander aphid, A. nerii (ave. 84.50 per cent) and the safflower aphid, U. compositae

Table 4a: Transmission of ten papaya ringspot virus (PRSV) isolates by eight aphid species

Sr. No.	Aphid species	No.of aphid/ test plant.	No.of test plants /aphid species	Incu-bation period (in days)	PRSV isolates- % transmission										Average % trans-mission
					AKL	AUR	LTR	NAN	OSB	PBN (HG)	PBN (FF)	PUN-HC-c.p.	PUN-C.P.	THA	
1.	Acyrthosiphon pisum(Harris)	30	20	12	60	80	60	40	60	60	60	40	60	65	58.50
2.	Aphis craccivora Koch.	30	20	12	40	80	40	40	60	60	60	60	60	40	54.00
3.	Aphis gossypii Glover	30	20	12	100	90	90	85	90	95	85	90	80	100	90.15
4.	Aphis nerii Boyer De Fonscolombe.	30	20	12	100	100	80	90	85	100	80	80	60	70	84.50
5.	Melanaphis(= Longiunguis) sacchari(Zehnter).	30	20	12	60	40	40	60	40	40	40	40	40	60	46.00
6.	Myzus persicae(Sulzer)	30	20	12	100	100	100	100	100	100	100	100	100	100	100.00
7.	Rhopalosiphum maidis (Fitch)	30	20	12	40	40	40	40	40	80	60	40	40	50	47.00
8.	Uroleucon(=Dactynotus)	30	20	12	80	60	60	100	80	100	100	80	60	60	78.00

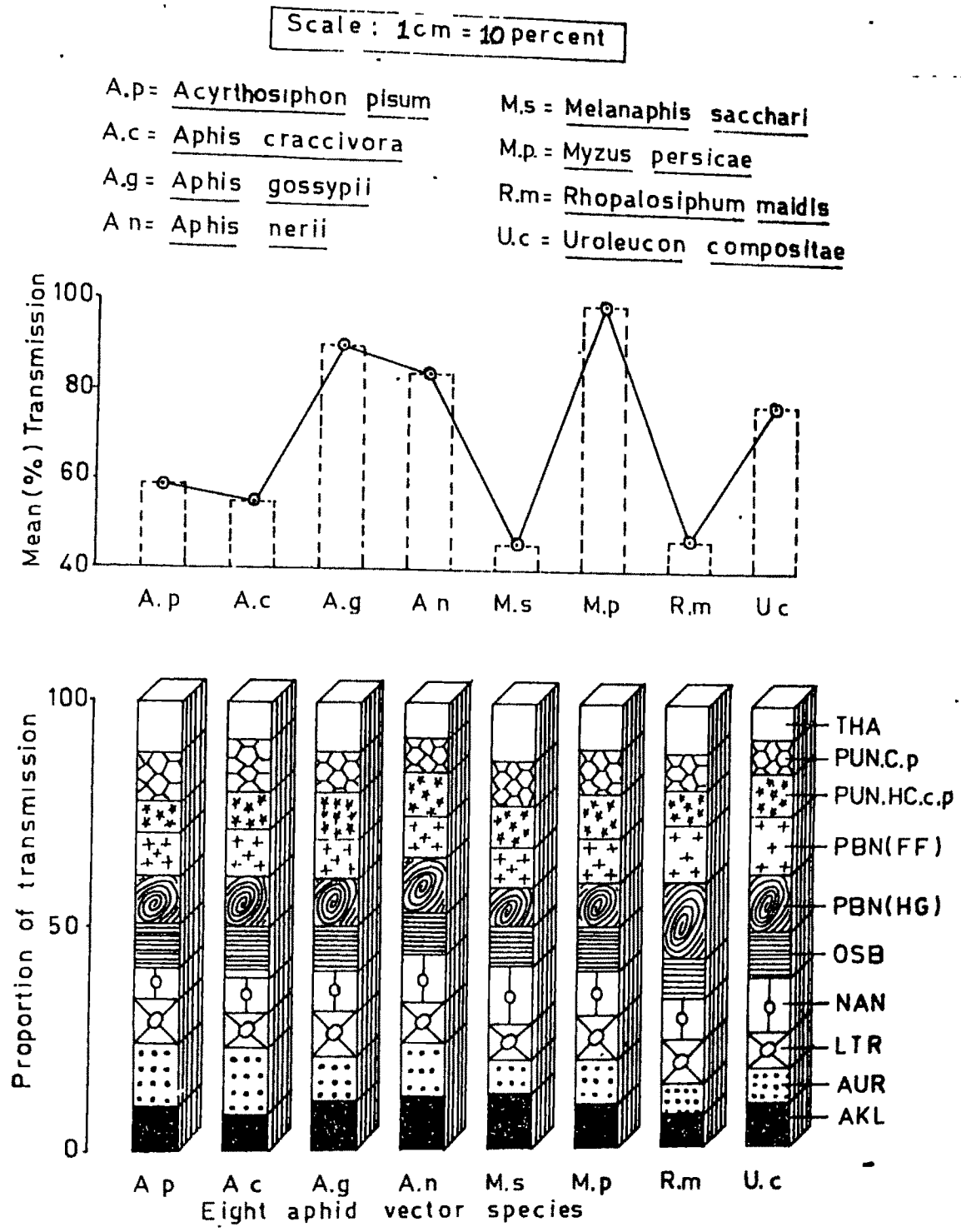

Fig.19. EFFICIENCY AND PROPORTION OF TRANSMISSION OF TEN ISOLATES OF PRSV BY EIGHT APHID VECTOR SPECIES

(ave. 78 per cent). The other four aphid species viz., the pea aphid, A. pisum (ave. 58.5 per cent), the black legume aphid, A. craccivora (ave. 54 per cent), the maize aphid, R. maidis (ave. 47 per cent) and the sugarcane aphid, M. sacchari (ave. 46 per cent) were comparatively less efficient vectors.

Moreover, slight variation was found with respect to incubation periods. For instance, in case of transmission involving 7 aphid species viz., A. pisum, A. craccivora, A. gossypii, A. nerii, M. sacchari and U. compositae, all the 10 PRSV isolates caused initial foliar symptoms of chlorosis and vein-clearing on the test papaya seedlings within 12 days after virus inoculation, whereas, in case of R. maidis, all the isolates produced initial foliar symptoms of chlorosis and vein-clearing on test papaya seedlings within 11 days after virus inoculation (Table-4a). The symptoms produced on test plants upon aphid inoculation, were identical with those that were produced on test plants upon mechanical sap inoculation (Table-4b). However, incubation period for initial symptom development was comparatively shorter in case of aphid inoculation of test plants (11-12 days) (Table-4a) than by mechanical sap inoculation of test plants (14 days) (Table-3).

Table 4b: Reactions of Washington papaya test plants upon aphid inoculations to ten PRSV isolates.

Sr. No.	Aphid species	Isolates of PRSV-and reactions of test plant (papaya cv. Washington)*									
		AKL	AUR	LTR	NAN	OSB	PBN(HG)	PBN(FF)	PUN-HC-c.p.	PUN-C.p.	TNA
1.	Acyrthosiphon pisum(Harris)	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss.
2.	Aphis caraccivora (Koch).	Chl,Vc,Mot, Mo,Bl, Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss
3.	Aphis gossypii Glover.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.
4.	Aphis parii Beyer De Fenscolombe.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl;Vc,Mot, Mo,Bl,Ld,Sss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.
5.	Melanaphis (=Longiunquis) sacchari (Zehntr.).	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, MoBl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.
6.	Myzus persicae (Sulzer)	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss.
7.	Rhopalosiphum maidis (Fitch.).	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo, Bl,Ld,Ss
8.	Uroleucon (=Dactynotus) compositae (Theobald)	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss.	Chl,Vc,Mot, Mo, Bl,Ld,Ss	Chl,Vc,Mot, Mo;Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss	Chl,Vc,Mot, Mo,Bl,Ld,Ss.

*/= Bl = blistering, Chl = chlorosis, Ld = leaf distortion, Mo = mosaic, Mot = mottling, Ss = sheestringing, Vc = vein-clearing.

4.3.3 Transmission of PRSV using different parts of infected papaya plant as a source of inoculum :

The results of transmission of PRSV-PBN(FF) isolate, from different infected tissues, employing mechanical sap and aphid inoculations are shown in Table-5. The data given in the table revealed that the virus could be easily transmitted from different virus source tissues viz., leaf, petiole, stem and fruit, to healthy papaya seedlings by both mechanical sap inoculation and by two aphid vector species viz., Aphis gossypii and Myzus persicae. Of the four virus source tissues tested, infected leaf was found to be the best source of virus inoculum for PRSV transmission by mechanical sap and aphid (A. gossypii and M. persicae) inoculations, followed by fruit, stem and petiole. However, differences were found to exist among the virus source tissues with respect to percentage transmission under both the methods of inoculation. For instance, in case of leaf as a source of virus inoculum, the level of transmission was found to be as high as 100 per cent when inoculations were made through infected sap and viruleferous aphid species of M. persicae, while it was only 80 per cent with an aphid vector species of A. gossypii. In case of petiole as a source virus inoculum, the level of transmission by mechanical sap inoculation was at par (60 per cent) with

that of a inoculation by aphid vector species of M. persicae, while it was comparatively low for an aphid vector species of A. gossypii (50 per cent). When stem was used as virus source inoculum, a low transmission value was recorded in case of mechanical sap inoculation (45 per cent) as compared to inoculations by aphid vector species like M. persicae (80 per cent) and A. gossypii (60 per cent). There were no significant differences among PRSV transmission values between sap (75 per cent) and two aphid vector species viz., A. gossypii (70 per cent) and M. persicae (80 per cent), when fruit was used as a source for virus inoculum. The incubation period of the virus was also found to vary with the methods of inoculation used for the transmission of virus from different source tissues. Thus, upon mechanical sap inoculation, virus from different source of tissues, the incubation period was found to be slightly longer (14 days) as compared to a shorter incubation period (12 days) resulting from the inoculation by aphid (A. gossypii and M. persicae) vector species. All the test plants, upon mechanical sap and aphid inoculations produced characteristic symptoms of PRSV viz., leaf distortion and shoestringing,(Table-5).

Table 5: Transmission of PRSV (PBN-FF isolate) by mechanical sap and aphid inoculations of test plants using different infected papaya tissues as source of inoculum.

Sr. No.	Source of virus inoculum	No. of test plants inoculated	Methods of inoculation and transmission characteristics						Characeristic symptoms on test plants (papaya cv. Washington.
			Mechanical sap inoculation		Aphid inoculation				
					Aphis gossypii		Myzus persicae		
			Incubation period (in days)	% transmission.	Incubation period (in days)	% transmission.	Incubation period (in days)	% transmission.	
1.	Leaf	20	14	100	12	80	12	100	Leaf distortion, shoestringing.
2.	Petiole	20	14	60	12	50	12	60	Leaf distortion, shoestringing.
3.	Stem	20	14	45	12	60	12	80	Leaf distortion, shoestringing.
4.	Fruit	20	14	75	12	70	12	80	Leaf distortion, shoestringing.

4.3.4 Virus-vector relationship :

4.3.4.1 Efficiency of different morphological forms of the aphids in PRSV transmission :

The results on the efficiency of morphological forms of A. gossypii and M. persicae in PRSV (isolate PBN-FF) transmission are shown in Table-6a and represented graphically (Fig.20). It is revealed from the table and graph that various morphological forms viz., nymphs, alate adults and apterate adults of A. gossypii and M. persicae, were capable of transmitting PRSV. However, there existed significant differences in the level of transmission between different morphological forms. For instance, apterate adults of A. gossypii (90 per cent) and M. persicae (100 per cent) were most efficient in transmitting PRSV than alate adults and nymphs (Table-6a) (Fig.20).

4.3.4.2 Influence of the number of aphids in PRSV transmission :

The results on the influence of number of aphids on PRSV (isolate PBN-FF) transmission are shown in Table-6b and also presented graphically (Fig.21). It is revealed from the table and graph that a single aphid of A. gossypii and M. persicae was able to transmit PRSV to the extent of 20 per cent. The percentage transmission was, however, found to increase with an increase in the number of

Table 6a: Transmission of PRSV(isolate PBN-FF) using morphological forms of two aphid vectors.

Sr. No.	Form of aphid	No.of aphids /test plant	No.of test plants /aphid species	Aphid species and % transmission	
				Aphis gossypii	Myzus persicae
1.	Alate adult	30	20	40	45
2.	Apterate adult	30	20	90	100
3.	Nymph	30	20	25	35

Table 6b: Effect of different number of aphids on transmission of PRSV (isolate PBN-FF).

Sr. No.	No.of aphids /test plant	No.of test plants /aphid species	Aphid species and transmission characteristics			
			Aphis gossypii		Myzus persicae	
			No.of plants infected	% infection.	No.of plants infected	% infection.
1.	1	20	4	20	4	20
2.	5	20	4	20	8	40
3.	10	20	8	40	16	80
4.	15	20	12	60	16	85
5.	20	20	16	80	20	100
6.	25	20	18	90	20	100
7.	30	20	20	100	20	100
8.	35	20	20	100	20	100
9.	40	20	20	100	20	100

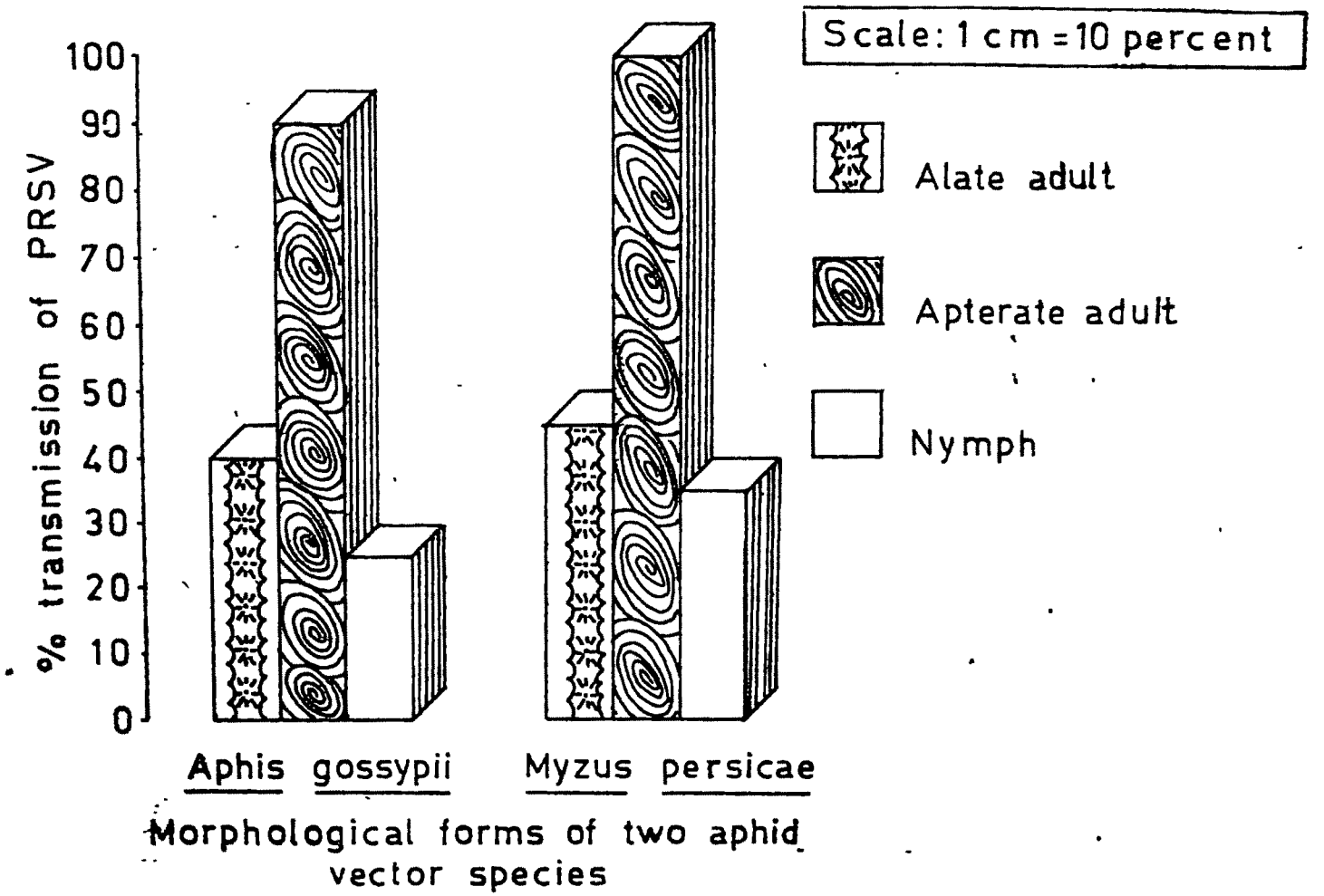

Fig.20. EFFICIENCY OF MORPHOLOGICAL FORMS OF TWO APHID SPECIES IN THE TRANSMISSION OF PRSV ISOLATE PBN (FF)

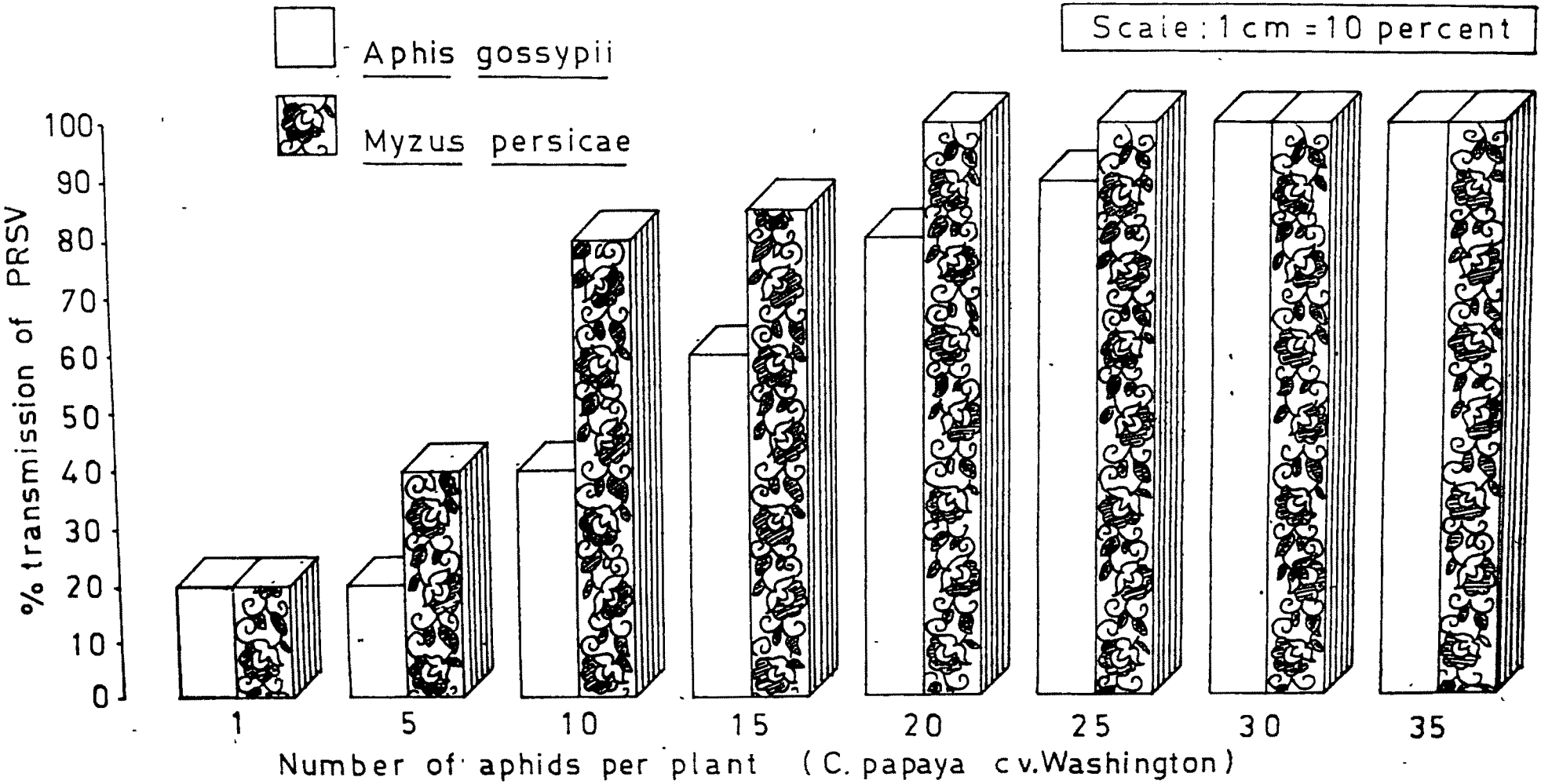

Fig.21. INFLUENCE OF THE NUMBER OF APHIDS OF Aphis gossypii and Myzus persicae ON PRSV TRANSMISSION

viruleferous aphids. The number of aphids required to achieve 100 per cent transmission varied with the species of aphids. For instance, in case of M. persicae only 20 aphids per plant were required to obtain a 100 per cent transmission level, whereas in case of A. gossypii, 30 aphids were required to obtain 100 per cent transmission. No apparent decline in PRSV transmission, however, was found, in case, the number of aphids exceeded 30 per test plant, in both these aphid (A. gossypii and M. persicae) vector species.

4.3.4.3 Effect of pre-acquisition fasting periods given to aphid vector species on PRSV transmission :

The results on the effect of pre-acquisition fasting periods given to A. gossypii and M. persicae on PRSV (isolate PBN-FF) transmission are presented in Table-6c and graphically in (Fig.22). It is revealed from the table and graph that pre-acquisition fasting period was shown to increase the efficiency of transmission over control i.e. the aphid vectors without any pre-acquisition fasting periods. Maximum transmission of 90 per cent and 100 per cent was obtained in case of A. gossypii and M. persicae respectively, when the aphids were subjected to 1 hour pre-acquisition starvation. It was further found to decline progressively after 1 hour pre-acquisition fasting period till it came equal to no-fasting treatment, at 6 hours.

Table 6c: Effect of different pre-acquisition fasting periods on the efficiency of vector species in PRSV(isolate PBN-FF) transmission.

Sr. No.	Fasting (hours)	No.of aphids /test plant	No.of test plants /aphid species	Aphid species-% transmission	
				Aphis gossypii	Myzus persicae
1.	0	30	20	10	20
2.	0.5	30	20	60	70
3.	1.0	30	20	90	100
4.	2.0	30	20	80	90
5.	3.0	30	20	60	80
6.	4.0	30	20	50	60
7.	5.0	30	20	40	40
8.	6.0	30	20	20	30

Table 6d: Effect of different acquisition feeding periods on the efficiency of vector species in PRSV(isolate PBN-FF) transmission.

Sr. No.	Acquisition feeding (minutes)	No.of aphids /test plant	No.of test plants /aphid species.	Aphid species-% transmission	
				Aphis gossypii	Myzus persicae
1.	1	30	20	20	30
2.	2	30	20	45	50
3.	5	30	20	85	100
4.	10	30	20	75	80
5.	15	30	20	65	70
6.	30	30	20	60	60
7.	60	30	20	50	40
8.	120	30	20	30	30

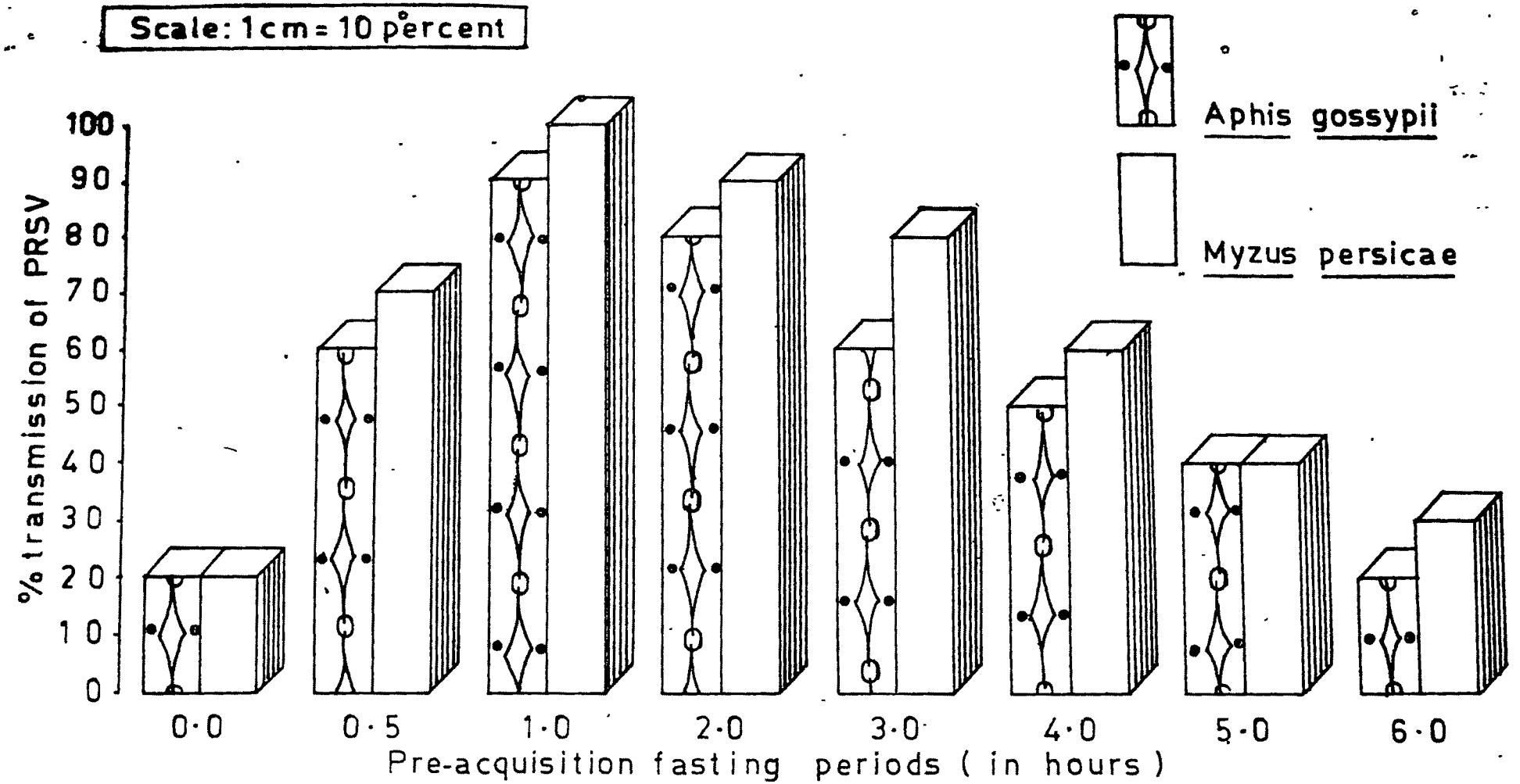

Fig.22. EFFECT OF PRE-ACQUISITION FASTING PERIODS ON PRSV TRANSMISSION

4.3.4.4 Effect of different acquisition feeding periods given to aphid vectors species on PRSV transmission :

The results on the effect of different acquisition feeding periods given to A. gossypii and M. persicae for testing their efficiency in PRSV (isolate- PBN-FF) transmission are presented in Table-6d and graphically in (Fig.23). It is revealed from the table and graph that the aphid vector species acquired the virus from infected source within 1 minute. The rate of transmission recorded for 1 minute acquisition feeding was as low as 20 and 30 per cent respectively for A. gossypii and M. persicae. However, the transmission efficiency of vectors was found to increase with an increase in acquisition feeding. It was highest at an acquisition feeding of 5 minutes for both A. gossypii (85 per cent) and M. persicae (100 per cent). Further, increase in acquisition feeding time was observed to be responsible for decrease in virus transmission by both the aphid species.

4.3.4.5 Effect of different inoculation feeding periods given to aphid vector species on PRSV transmission :

The results of the different inoculation feeding periods given to aphid vectors for testing their efficiency in PRSV (isolate PBN-FF) transmission are presented in Table-6e and graphically in (Fig.24). It is revealed from

Table 6e: Effect of different inoculation feeding periods on the efficiency of aphid vectors in PRSV(isolate PBN-FF) transmission.

Sr. No.	Inoculation feeding (minutes)	No. of aphids/ test plant	No. of test plants/ aphid species	Aphid species-% transmission	
				Aphis gossypii	Myzus persicae
1.	1	30	20	25	30
2.	2	30	20	50	50
3.	5	30	20	55	60
4.	10	30	20	70	80
5.	15	30	20	85	95
6.	30	30	20	70	75
7.	60	30	20	60	60
8.	120	30	20	40	45

Table 6f: Persistence of the PRSV(isolate PBN-FF) in two aphid vector species.

Sr. No.	Transmission feeding (minutes)	No. of tests conducted.	Aphid species and serial transmission*									
			Aphis gossypii Series of plants					Myzus persicae series of plants				
			1	2	3	4	5	1	2	3	4	5
1.	1	1	+	-	-	-	-	+	-	-	-	-
		2	+	-	-	-	-	+	+	-	-	-
		3	+	-	-	-	-	-	+	-	-	-
2	2	1	+	-	-	-	-	+	+	-	-	-
		2	+	-	-	-	-	+	-	-	-	-
		3	+	-	-	-	-	+	-	-	-	-

*/ = (+) = test plants infected by aphid inoculation;

(-) = test plants not infected by aphid inoculations.

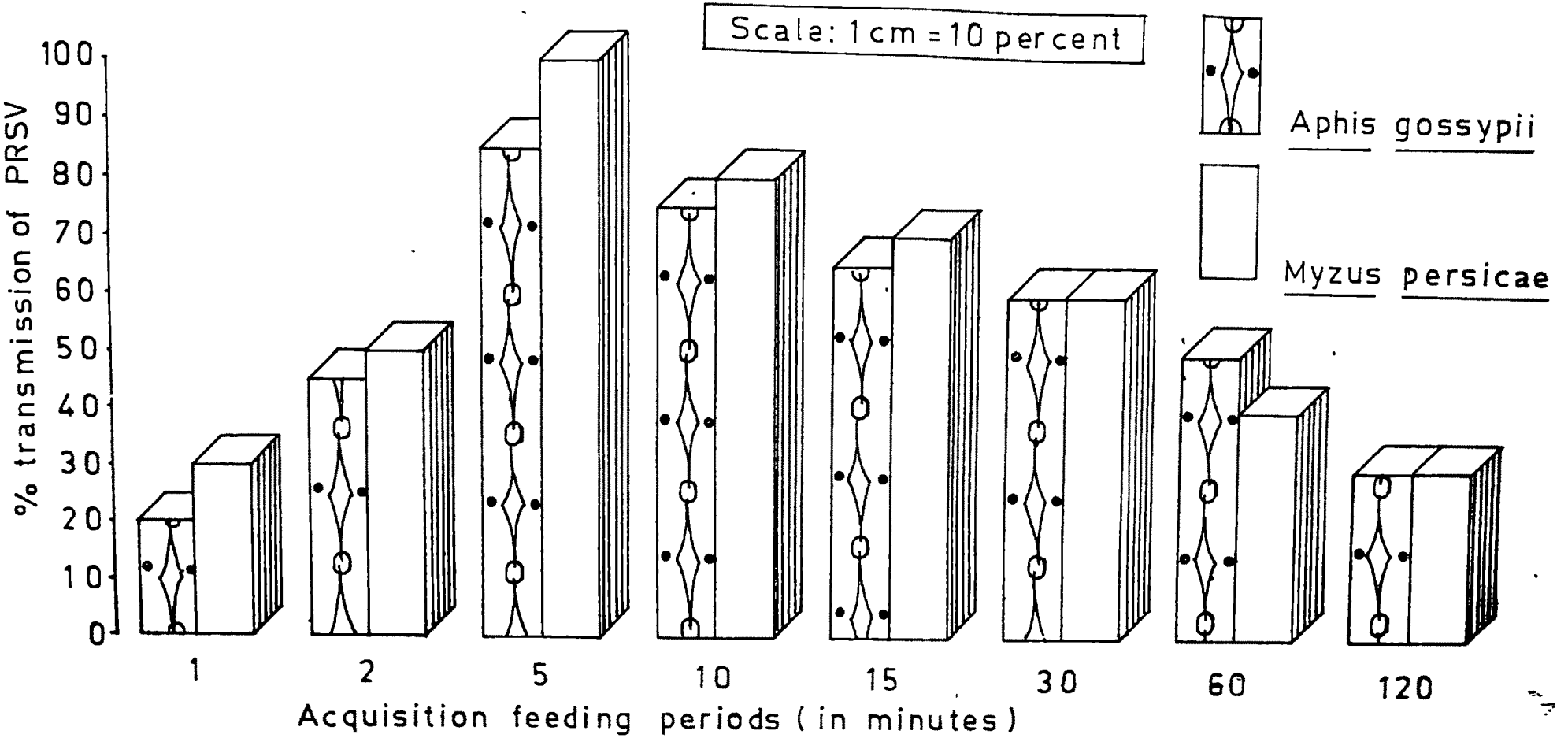

Fig.23. EFFECT OF DIFFERENT ACQUISITION FEEDING PERIODS ON THE TRANSMISSION OF PRSV

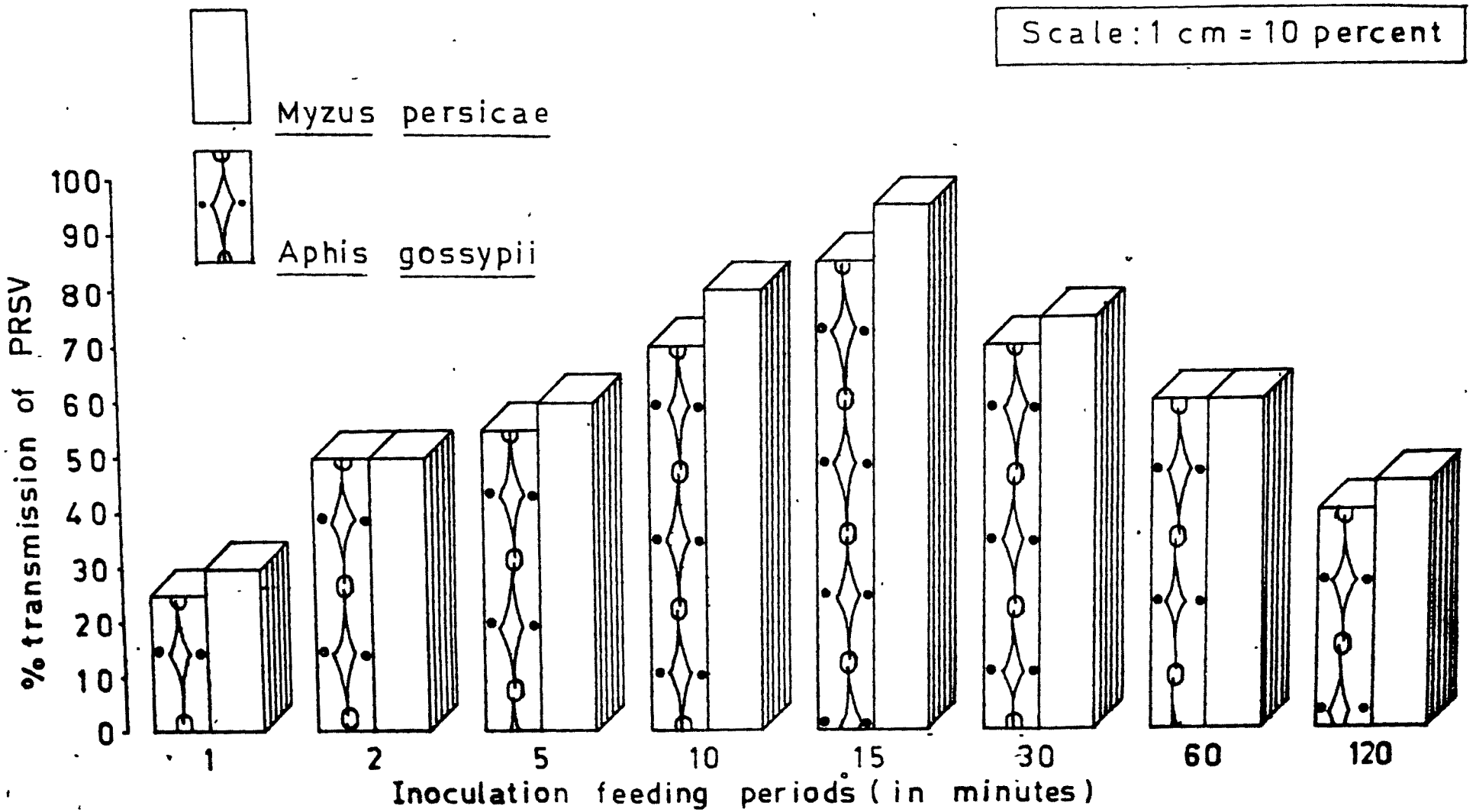

Fig.24. EFFECT OF DIFFERENT INOCULATION FEEDING PERIODS ON THE TRANSMISSION OF PRSV

the table and a graph that 1 minute feeding on test plant was sufficient to initiate infection by both the species of aphids, A. gossypii (25 per cent) and M. persicae (30 per cent). However, the percentage infectivity increased with prolonged inoculation feeding periods on test plant. An inoculation feeding of 15 minutes virtually resulted in a higher rate of virus transmission in both the aphid vector species i.e. A. gossypii (85 per cent) and M. persicae (95 per cent) and found to be optimum inoculation feeding period for aphid vector species in PRSV transmission. There was, however, no increase in the percentage transmission by further increase in the inoculation feeding periods.

4.3.4.6 Persistence of PRSV in the aphid vector species :

The results on persistence of PRSV (isolate PBN-FF) in the aphid vector species are presented in Table-6f. The data given in the table indicated that in both inoculation feeding periods, the viruleferous aphids of A. gossypii and M. persicae caused infection upto second test plant series. However, the shorter inoculation feeding of 1 minute, had no infection on the first test plant series, whereas feeding for 2 minutes had positive transmission on first and second test plant series in case of M. persicae. M. persicae was able to infect 3 out of 5 healthy plants of second series, but A. gossypii did not infect any plant after it had fed on the first series of test plants. The

aphids of A. gossypii and M. persicae, were, therefore, unable to retain the virus beyond initial feeding of few minutes. This indicates that the PRSV being transmitted by aphid vectors in the non-persistent manner.

4.3.5 Seed transmission :

The results of PRSV transmission through the seeds of papaya, using " growing-on" and "indicator" inoculation tests are displayed in Table-7. It is revealed from the table that PRSV was not found to be seed-borne in papaya, even after 2,200 seeds extracted from virus-infected fruits were tested in five lots. Furthermore, none of the seedlings grown from 4,400 seeds originated from infected and healthy fruits, showed the symptoms of the virus and the virus was also not recovered in pooled samples. Moreover, infections of fruits by PRSV did not reduce seed germination significantly (Table-7).

4.3.6 Graft transmission :

The results on graft transmission test (Table-8) indicated that PRSV (isolate PBN-FF) could be successfully transmitted from infected to healthy papaya plants by "approach-grafting". The extent of transmission was as high as 100 per cent and initial foliar symptoms of the disease like chlorosis and vein-clearing were evident on the scions

Table 7: Growing-on and indicator inoculation tests for detection of PRSV in seeds of papaya

Seed lots	No.of seeds germinated/ no.of seeds sown.	% germination	% reduction in germination.	No.of seedlings with virus symptoms.	% seedlings with virus symptoms.	Virus recovery on test plants	
						Papaya cv. Washington	Summer-squash cv. Patty Pan
Lot-I							
a. Seeds from healthy fruits.	473/500	94.60		0*	0	-**	-
b. seeds from infected fruits	466/500	93.20	1.40	0	0	-	-
Lot-II							
a. Seeds from healthy fruits.	378/400	94.50		0	0	-	-
b. Seeds from infected fruits.	364/400	92.50	1.70	0	0	0	-
Lot-III							
a. Seeds from healthy fruits.	465/500	93.00		0	0	-	-
b. Seeds from infected fruits	457/500	90.14	2.86	0	0	-	-
Lot-IV							
a. Seeds from healthy fruits.	381/400	95.25		0	0	-	-
b. Seeds from infected fruits.	378/400	94.50	0.75	0	0	-	-

Contd...

Table 7: Contd.

Seed lots	No.of seeds germinated/ no.of seeds sown.	% germination.	% reduction in germination.	No.of seedlings with virus symptoms.	% seedlings with virus symptoms.	Virus recovery on test plants	
						Papaya cv. Washington.	Summer-squash cv. Patty-Pan.
Lot- V							
a. Seeds from healthy fruits	376/400	94.00		0	0	-	-
b. Seeds from infected fruits	365/400	91.25	2.75	0	0	-	-

*/ = 0 = seedling free from symptoms of PRSV.

**/ =(-)= negative recovery.

(healthy plants) within 25 to 60 days after grafting (Table-8). The symptoms produced by grafting on test plants were similar to those produced on test plants by mechanical sap inoculation and aphid inoculation tests.

4.3.7 Dodder transmission :

The results on dodder transmission of PRSV (isolate PBN-FF) are presented in Table-9. From the data depicted in Table-9 it is obvious that PRSV could be transmitted from infected to healthy papaya plants by dodder (Cuscuta species). The dodder transmission of PRSV was as high as 80 per cent with an incubation period 25 to 35 days after the establishment of dodder contacts. Symptoms produced on test plants by dodder transmission were similar to those produced on test plants by mechanical sap, aphid and graft inoculations.

4.4 Host range :

The results of host range studies of 10 PRSV isolates using mechanical sap and aphid inoculations are presented in Table-10. Of the 41 test plant species, belonging to nine different families, PRSV isolates infected 14 plant species belonging to 3 families of Caricaceae, Chenopodiaceae and Cucurbitaceae. Therefore, the host range of the virus was intermediate. Two plant species from Chenopodiaceae were only the local lesion hosts of the PRSV

Table 8: Graft transmission of papaya ringspot virus(PRSV)

No.of plants grafted	No.of plants infected	% transmission	Incubation period (in days)	Symptoms on graft inoculated plants.
20	20	100.00	25 to 60	Chlorosis, vein-clearing mosaic, blistering, distortion and shoestinging on leaves and water-soaked green to darkgreen streaks on petioles and stems.

Table 9: Dodder (Cuscuta species) transmission of papaya ringspot virus (PRSV)

No.of dodder unions established.	No.of plants infected.	% transmission	Incubation period (in days)	Symptoms on dodder inoculated plants.
20	16	80.00	25 to 35	Chlorosis, vein-clearing, mosaic, blistering, distortion and shoestringing on leaves and water-soaked, green to darkgreen streaks on petioles and stems.

isolates. Twelve plant species in Caricaceae and Cucurbitaceae were invaded systemically by 10 PRSV isolates. Moreover, the results in Table-10 also indicated that apparently no differences were found among 10 PRSV-isolates with respect to host range and reactions of 14 susceptible hosts. The details of the reactions of susceptible hosts are described as under :

I. Caricaceae :

1) Carica cauliflora

C. cauliflora reacted only systemically to all the isolates of PRSV, without producing any visible symptoms, by both mechanical sap and aphid inoculation of virus isolates (Table-10). However, virus was recoverable from non-inoculated leaves by back indexing on indicator hosts. All the 10 PRSV isolates caused symptomless infection in C. cauliflora (Table-10).

2) Carica papaya. cv. Washington

Washington cultivar of papaya reacted systemically to all the ten PRSV isolates. The incubation period of ten isolates slightly varied with the method of inoculation. Thus, in case of mechanical sap inoculation, all the isolates exhibited initial symptoms of chlorosis and vein-clearing within 14 days after virus inoculation, whereas all the isolates exhibited initial symptoms of

Table 10: Reactions of 41 test plants upon mechanical sap and aphid inoculations to 10 PRSV isolates.

Sr. No.	Host	No.of test plants/ isolate	% infection (Ave.of all isolates)	Incubation period (in days)	Isolates of PRSV and Host reactions*										REMARKS
					AKL	AUR	LTR	NAN	OSB	PBN (HG)	PBN (FF)	PUN-HC-c.p.	PUN-C p.	THA	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
I.	Amaranthaceae:														
1.	Celosia argentia L.	10	-	-	-	-	-	-	-	-	-	-	-	-	
2.	C. plumosa L.	10	-	-	-	-	-	-	-	-	-	-	-	-	
3.	Gomphrena globosa L.	10	-	-	-	-	-	-	-	-	-	-	-	-	
II.	Caricaceae :														
4.	Carica cauliflora Jacq.	10	-	-	Lt.	Lt.	Lt.	Lt.	Lt.	Lt.	Lt.	Lt.	Lt.	Lt.	
5.	C. papaya L. cv. Washington.	10	100 M 90.5	14 M 12 A	S 100M 100A	S 100 90	S 100 90	S 100 90	S 100 90	S 100 95	S 100 85	S 100 90	S 100 80	S 100 100	
III.	Chenopodiaceae :														
6.	Beta vulgaris L.	10	-	-	-	-	-	-	-	-	-	-	-	-	Members of Chenopodiaceae were tested only by mechanical sap inoculation.
7.	Chenopodium album Red	10 (leaves)	-	-	-	-	-	-	-	-	-	-	-	-	
8.	C. amaranticolor Coste. et. Reyn.	10 (leaves)	1.40 M (Lesions)	13 M	L M (2.0*)	L (1.5)	L (1.0)	L (1.0)	L (1.0)	L (1.5)	L (2.0)	L (1.0)	L (1.5)	L (1.0)	
9.	C. murale L.	10 (Leaves)	-	-	-	-	-	-	-	-	-	-	-	-	
10.	C. quinoa Willd.	10	2.00	14 M	L M (1.5*)	L (2.0)	L (2.0)	L (2.5)	L (2.0)	L (2.0)	L (2.5)	L (1.5)	L (2.0)	L (2.0)	
IV	Compositae :														
11.	Zinnia elegans Jacq.	10	-	-	-	-	-	-	-	-	-	-	-	-	

Table 10 Contd.

1.	2.	3	4	5	6	7	8	9	10	11	12	13	14.	15	16
V.	Cucurbitaceae :														
12.	Benincase hispida Cogn. cv. Co-1.	10	44/52	18/16	S 40/40	S 40/40	S 30/60	S 40/50	S 30/50	S 60/60	S 50/60	S 60/50	S 50/60	S 40/50	
13.	Citrullus lanatus(Thumb) Matsum & Nakai. cv.Sugar Baby	10	70/61	24/22	S 80/40	S 40/80	S 60/40	S 80/60	S 80/60	S 60/60	S 50/70	S 80/60	S 90/60	S 80/50	
14.	C. lanatus var.fistulosus Duth & Full,cv.Arka Tinda	10	34/41	11/10	S 40/40	S 40/50	S 40/30	S 20/30	S 40/40	S 40/50	S 20/40	S 20/50	S 40/30	S 40/50	
15.	Cucumis melo L. cv. Arka Jeet.	10	56/63	15/12	S 40/50	S 60/60	S 40/80	S 40/70	S 80/60	S 80/80	S 60/70	S 60/60	S 40/50	S 60/50	
16.	C. sativus L. cvs.Banglore Dwarf.	10	80/62	13/72	S 100/80	S 80/70	S 80/80	S 100/50	S 80/50	S 60/70	S 80/60	S 80/60	S 80/50	S 100/70	
	Banglore Special White	10	75/70	16/13	S 80/60	S 60/50	S 60/60	S 60/80	S 80/70	S 100/90	S 80/70	S 50/80	S 100/70	S 60/70	
	Local	10	75/50	15/12	S 50/60	S 80/50	S 80/40	S 50/60	S 70/60	S 60/50	S 70/50	S 60/40	S 80/50	S 60/50	
17.	Cucurbita maxima Duchense cv. Arka Suryamukhi	10	53/55	20/16	S 40/40	S 60/50	S 40/60	S 40/60	S 80/40	S 60/70	S 50/50	S 60/50	S 40/60	S 60/70	
18.	C. pepo L. cv. Arka Chandan	10	67/72	11/16	S 60/80	S 40/80	S 60/80	S 80/60	S 80/60	S 60/80	S 50/70	S 80/80	S 90/60	S 80/70	
19.	C. pepo var. melopepo (L.)	10	99/85	13/12	S 100/90	S 80/100	S 80/90	S 80/80	S 100/90	S 100/80	S 100/80	S 80/70	S 80/80	S 80/90	

Contd...

Table 10 Contd..

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
20.	Lagenaria siceraria (Mol) Stndl. cv. Pusa Summer Prolific Long.	10	-	-	-	-	-	-	-	-	-	-	-	-	
21.	Luffa acutangula Roxb. cv. J. Long.	10	49/56	20/18	S 50/60	S 70/40	S 50/60	S 60/50	S 60/70	S 60/70	S 50/60	S 30/50	S 40/50	S 40/50	
22.	L. Cylindrica (L.) Reem. cv. Pusa Chikni.	10	-	-	-	-	-	-	-	-	-	-	-	-	
23.	Momordica charantia L. cv. Coimbatore White Long.	10	-	-	-	-	-	-	-	-	-	-	-	-	
24.	Trichosanthes anguina L. cv. Green Long.	10	59/59	15/12	S 80/50	S 80/80	S 40/60	S 60/70	S 60/60	S 60/60	S 50/50	S 40/50	S 60/50	S 60/60	
VI.	Labiateae :														
25.	Ocimum basilicum L.	10	-	-	-	-	-	-	-	-	-	-	-	-	
VII.	Leguminosae :														
26.	Arachis hypogaea L. cv.SB-XI	10	-	-	-	-	-	-	-	-	-	-	-	-	
27.	Cassia occidentalis L.	10	-	-	-	-	-	-	-	-	-	-	-	-	
28.	Cicer arietinum L. cv. BDN 9-3	10	-	-	-	-	-	-	-	-	-	-	-	-	
29.	Glycine max (L.) Merr. cv. MACS-13	10	-	-	-	-	-	-	-	-	-	-	-	-	
30.	Vicia faba L.	10	-	-	-	-	-	-	-	-	-	-	-	-	

Contd...

Table 1C Contd...

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
31.	Vigna mungo (L.) Hepper. cv. Sinakheda-1-1	10	-	-	-	-	-	-	-	-	-	-	-	-	
32.	V. radiata (L.) Wilcz cv. J-781	10	-	-	-	-	-	-	-	-	-	-	-	-	
33.	V. unguiculata (L.) Walp cv. C-152	10	-	-	-	-	-	-	-	-	-	-	-	-	
VIII.	Malvaceae :														
34.	Abelmoschus esculentus (L.) Moench. cv. Pusa Sawani	10	-	-	-	-	-	-	-	-	-	-	-	-	
IX	Solanaceae :														
35.	Capsicum annuum L. cv. NP-46-A.	10	-	-	-	-	-	-	-	-	-	-	-	-	
36.	Datura stramonium L.	10	-	-	-	-	-	-	-	-	-	-	-	-	
37.	Lycopersicon esculentum Mill. cv. Pusa Ruby	10	-	-	-	-	-	-	-	-	-	-	-	-	
38.	Nicotiana glutinosa L.	10	-	-	-	-	-	-	-	-	-	-	-	-	
39.	N. rustica L.	10	-	-	-	-	-	-	-	-	-	-	-	-	
40.	N. tabacum L. cv. White Burley	10	-	-	-	-	-	-	-	-	-	-	-	-	
41.	Solanum melongena L. cv. Pusa Purple Long.	10	-	-	-	-	-	-	-	-	-	-	-	-	

*/ = M = mechanical inoculation; A = aphid inoculation

Figures in parenthesis with asterick = average number of local lesions/leaf.

L = local; Lt = latent; (-) = no infection, S = systemic.

chlorosis and vein-clearing within 12 days after virus inoculation by aphid vector. The percentage infectivity of ten PRSV isolates also differed depending on the type of virus inoculation methods employed. For instance, aphid inoculation of virus resulted into 80 to 100 per cent infectivity (ave. 90.5 per cent), whereas it was as high as 100 per cent in case of virus inoculation by mechanical sap inoculation (Table-10). In spite of differences in per cent infectivity and incubation periods, there appeared a striking similarity in symptomatology and sequence of symptom development on test plants inoculated by mechanical sap as well as by aphid vector species. The systemic foliar symptoms recorded for 10 PRSV isolates included chlorosis and vein-clearing, mottling, mosaic, blistering, distortion and shoestringing. Symptoms like green to darkgreen, water-soaked spots and streaks on petioles and stems were also evident on the infected plants. All the infected plants were markedly stunted (Plates-IV to XI, Figs. 6 to18).

II. Chenopodiaceae :

3. Chenopodium amaranticolor :

C. amaranticolor was infected by all the 10 PRSV isolates inciting chlorotic local lesions surrounded by reddish borders on the inoculated leaves within 16 days after virus isolate inoculations (Plate-XII, Fig.25).

Table 11: Effect of source of virus inoculum on the number of local lesions developed on mechanically inoculated leaves of Chenopodium species.

Sr. No.	Virus source/ Chenopodium species	PRSV isolates and particulars of local lesion on Chenopodium species									
		ANL	AUR	LTR	NAN	CSB	PBN(HG)	PBN(FF)	PUN-HC-c.p.	PUN-C.p.	THA
I.	Source = Papaya cv. Washington										
1.	C. amaranticolor										
a.	Types of lesions	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL
b.	Ave.no.of lesions/leaf	2.0	1.5	1.0	1.0	1.0	1.5	2.0	1.0	1.5	1.0
c.	Incubation period(in days)	16	16	16	16	16	16	16	16	16	16
2.	C. quinoa										
a.	Type of lesions	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL
b.	Ave.no.of lesions/leaf	1.5	2.0	2.0	2.5	2.0	2.0	2.5	1.5	2.0	2.0
c.	Incubation period(in days)	11	11	11	11	11	11	11	11	11	11
II.	Source = Summersquash cv. Patty Pan										
3.	C. amaranticolor										
1.	Type of lesions	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL
b.	Ave.no.of lesions/leaf	4.0	3.0	4.0	3.0	2.5	3.5	4.0	2.0	2.5	3.5
c.	Incubation period(in days)	16	16	16	16	16	16	16	16	16	16
4.	C. quinoa										
a.	Type of lesions	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL
b.	Ave.no.of lesions/leaf	6.8	4.7	4.3	5.5	5.7	6.3	8.0	7.5	6.6	7.3
c.	Incubation period(in days)	11	11	11	11	11	11	11	11	11	11

PLATE - XII

Fig. 25 : Symptoms of chlorotic local lesions on inoculated leaves of Chenopodium amaranticolor caused by PRSV.

Fig. 26 : Symptoms of chlorotic local lesions on inoculated leaves of Chenopodium quinoa elicited by PRSV.

PLATE
XII

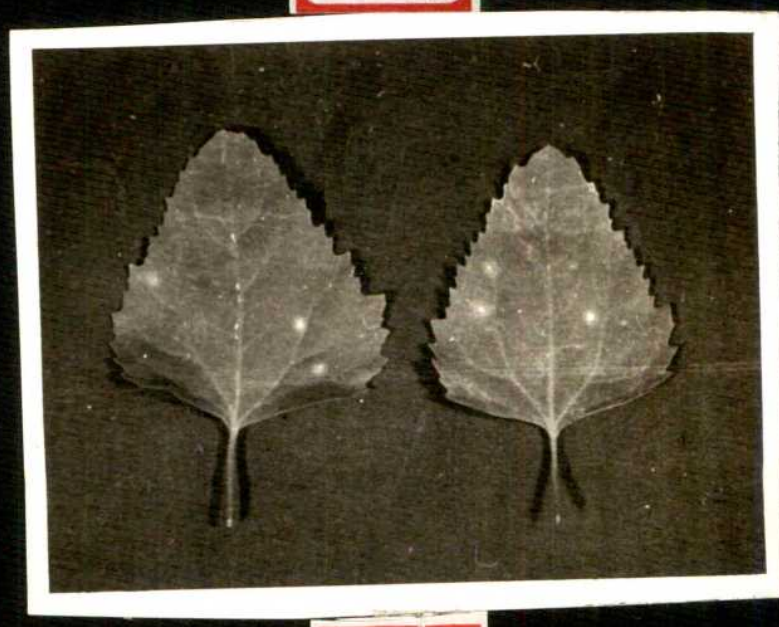

Fig.
25

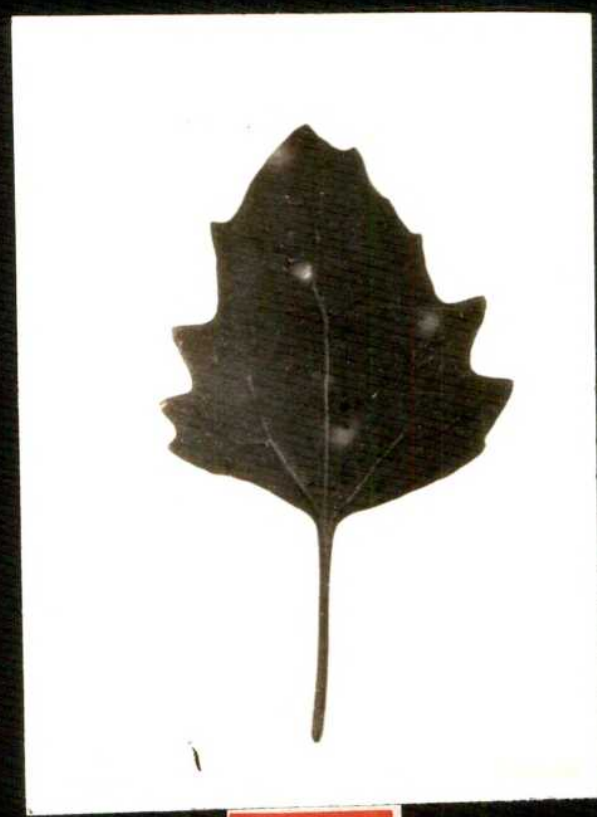

Fig.
26

4. Chenopodium quinoa :

C. quinoa was also infected by all the 10 PRSV isolates producing local symptoms in the form of chlorotic lesions (Plate-XII, Fig.26) within 11 days after virus isolate inoculations.

It was observed that the local lesions produced on C. amaranticolor and C. quinoa were fewer and highly inconsistent and erratic, when papaya was used as a virus source for ten PRSV isolates. However, average number of lesions/leaf of C. amaranticolor and C. quinoa were found to increase with slight consistency, when summersquash cv. Patty Pan was used as virus source for ten PRSV isolates (Table-11). The average number of lesions/leaf of C. amaranticolor and C. quinoa were found to range from 1 to 2 and from 1.5 to 2.0 respectively when papaya leaves were used as source of virus inoculum. When summersquash leaves were used as virus inoculum source, the corresponding values of lesions/leaf showed an increase and ranged from 2 to 4 in case of C. amaranticolor and 4.3 to 8.0 in case of C. quinoa (Table-11).

III. Cucurbitaceae :

5. Ashgourd (Benincasa hispida cv. Co-1):

All the 10 PRSV isolates induced only systemic symptoms on ashgourd with an infectivity level ranging

PLATE - XIII

Fig. 27 : Symptoms of patchy veinal chlorosis and mosaic on non-inoculated leaves of Benincase hispida incited by PRSV

Fig. 28 Symptoms of vein-clearing, mosaic and distortion on non-inoculated leaves of Citrullus lanatus elicited by PRSV.

PLATE
XIII

Fig.
27

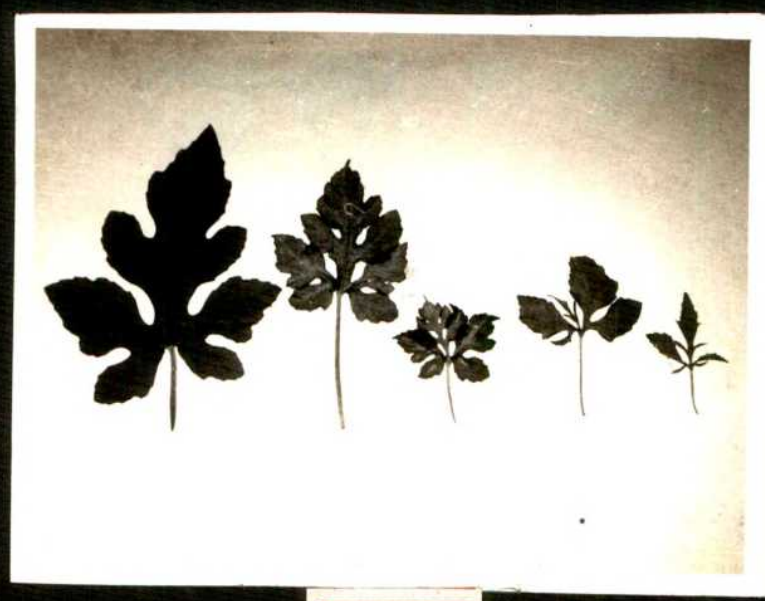

Fig. 28

from 30 to 60 per cent (ave. 44 per cent) and 40 to 80 per cent (ave. 52 per cent) for mechanical sap and aphid inoculations respectively. Apparently no difference in symptomatology was observed on test plants of this host when inoculated with ten PRSV isolates by mechanical sap inoculation and viruleferous aphids. The systemic foliar symptoms induced on this host by ten PRSV isolatesinlcuded vein-clearing, patchy veinal chlorosis and mosaic (Plate-XIII, Fig.27). However, incubation periods of PRSV isolates varied with method of inoculation. In case of mechanical sap inoculation, the incubation period of ten isolates on this host was slightly extended (18 days) as compared to inoculation by viruleferous aphids (16 days) (Table-10).

6. Watermelon (Citrullus lanatus cv. Sugar Baby) :

Sugar Baby cultivar of watermelon reacted systemically to all the PRSV isolates when inoculated through conventional leaf-rub method and viruleferous aphids. Symptoms produced on this host in response to mechanical sap and aphid inoculations by different isolates were similar. These included vein-clearing, mosaic and leaf distortion (Plate-XIII, Fig.28). But incubation period and percentage infectivity of PRSV isolates varied with the methods of inoculation. In case of mechanical sap inoculation, time taken for the production of initial symptoms of vein-clearing

was slightly longer (24 days) as compared to aphid inoculation (22 days). Similarly, aphid inoculation of different isolates has resulted in lower rate of infectivity (ave. 61 per cent) as compared to sap inoculation of isolates (ave. 70 per cent) (Table-10).

7. Roundgourd (*Citrullus lanatus* var. *fistulosus* cv. Arka Tinda):

Roundgourd cultivar, Arka Tinda when inoculated with 10 PRSV isolates using mechanical sap and aphid inoculations, reacted only with systemic symptoms of vein-clearing, mosaic and distortion (Plate-XIV, Fig.29). However, there existed some variation in the incubation period and percentage infectivity of PRSV isolates, by two methods of inoculations. For instance, in case of mechanical inoculation, the incubation period of PRSV isolates was 11 days, whereas it wasonly 10 days in case of aphid inoculation of PRSV isolates. Similarly, the percentage infectivity of roundgourd varied with the method of inoculation. It was higher in case of aphid inoculation of PRSV isolates (ave. 41 per cent) as compared to mechanical sap inoculation (ave. 34 per cent) (Table-10).

PLATE - XIV

Fig. 29 : Symptoms of vein-clearing, mosaic and distortion on non-inoculated leaves of C. lanatus var. fistulosus caused by PRSV.

Fig. 30 : Symptoms of vein-clearing, mosaic and disstortion on non-inoculated leaves of Cucumis melo elicited by PRSV.

PLATE
XIV

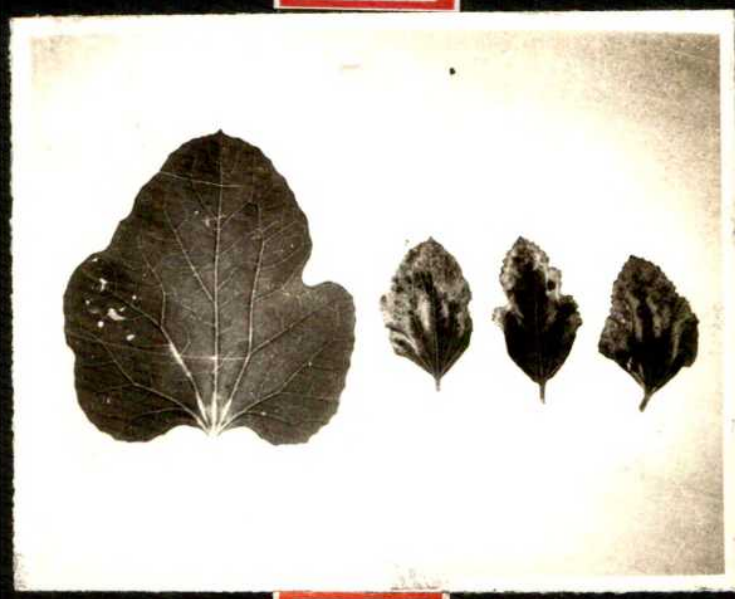

Fig.29

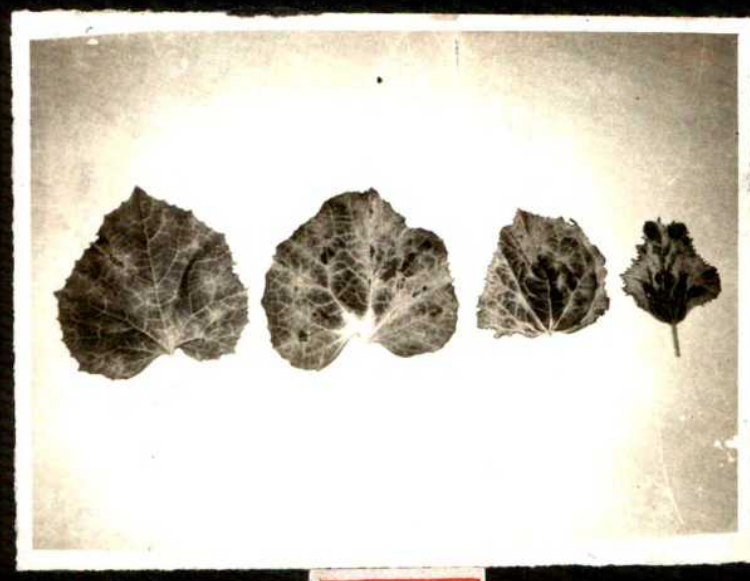

Fig.30

8. Muskmelon (Cucumis melo cv. Arka Jeet) :

Test plants of muskmelon (cv. Arka Jeet) reacted systemically to all the 10 PRSV isolates upon mechanical sap and aphid inoculations. In both the methods of inoculations, the systemic foliar symptoms induced by PRSV isolates were similar. These included vein-clearing, mosaic and distortion (Plate -XIV, Fig.30). Test plants of C. melo displayed higher level of infectivity to ten PRSV isolates (ave. 63 per cent) upon aphid inoculation as compared to ten PRSV isolate inoculation by mechanical means (ave. 56 per cent). In case of mechanical sap inoculation, the incubation period of ten PRSV isolates on C. melo was found to be 15 days, while in case of aphid inoculation it was only 12 days (Table-10).

9. Cucumber (Cucumis sativus cvs. Banglore Dwarf, Bangalore Special White and Local :

All the three cultivars of cucumber viz., Bangalore Dwarf, Banglore Special White and Local reacted systemically upon sap and aphid inoculation of PRSV isolates. No apparent differences were evident in the symptomatology. In Banglore Dwarf and Banglore Special White cultivars all the 10 PRSV isolates caused vein-clearing, mottling and mosaic, while in local cultivar, the symptoms of vein-clearing and mosaic (Plate-XV, Fig.31) were induced. But the incubation period

PLATE - XV

Fig. 31 : Symptoms of vein-clearing and mosaic on non-inoculated leaves of Cucumis sativus cv. Local incited by PRSV.

Fig. 32 : Symptoms of vein-clearing and mosaic on non-inoculated leaves of Cucurbita maxima elicited by PRSV.

PLATE
XV

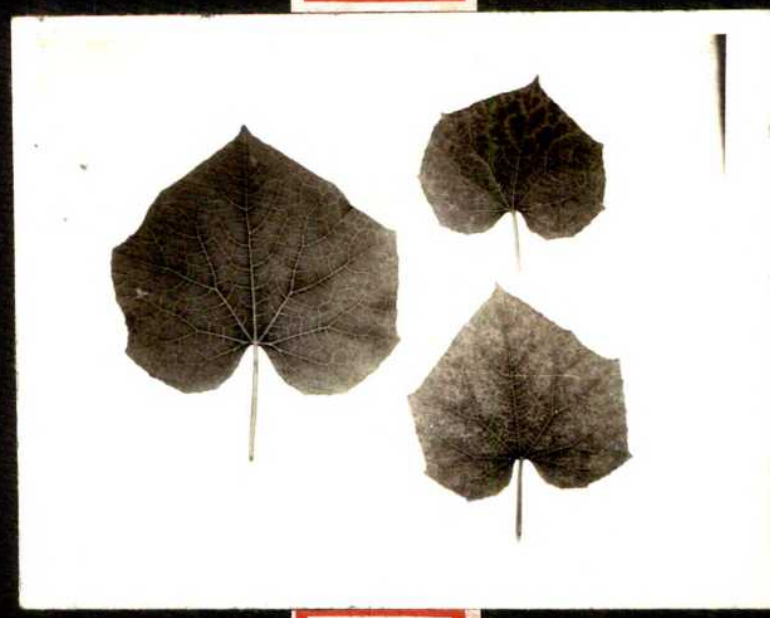

Fig.31

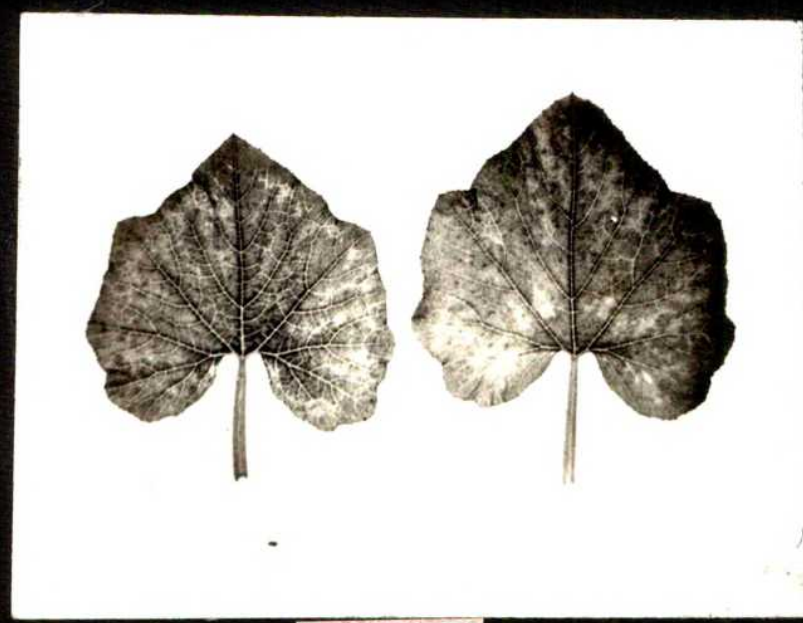

Fig.32

and level of infectivity of ten PRSV isolates varied with the method of inoculation. Thus, in case of mechanical inoculation, the initial symptoms of vein-clearing appeared within 13, 16 and 15 days after virus inoculation on Banglore Dwarf, Banglore Special White and Local cultivars respectively, while in case of aphid inoculation, the initial symptoms of vein-clearing appeared 12 days after virus inoculation on Banglore Dwarf and Local and 13 days after virus inoculation on Banglore Special White. High rate of infectivity of PRSV isolates was observed on Banglore Dwarf (ave. 80 per cent) followed by Banglore Special White (ave. 75 per cent) and Local (ave. 66 per cent) by mechanical sap inoculation, while the corresponding values of infectivity were comparatively low on Banglore Dwarf (ave. 62 per cent), Banglore Special White (ave. 70 per cent) and Local (ave. 50 per cent) by aphid inoculation (Table-10).

10. Wintersquash (Cucurbita maxima cv. Arka Suryamukhi) :

This host reacted systemically to all the 10 PRSV isolates, with an infectivity level ranging from 40 to 80 per cent (ave. 53 per cent) and 40 to 70 per cent (ave. 55 per cent) respectively in case of mechanical sap and aphid inoculations. The initial symptoms of vein-clearing induced by PRSV isolates on C. maxima appeared 20 and 16 days after virus inoculation by sap and aphid respectively. All the PRSV isolates induced systemic infection without any apparent

variation in symptomatology, under both the methods of inoculation (Table-10). The sequence of systemic symptoms induced by PRSV isolates on Arka Suryamukhi cultivar of wintersquash included vein-clearing followed by mosaic (Plate-XV, Fig.32).

11. Pumpkin (Cucurbita pepo cv. Arka Chandan):

Pumpkin cultivar, Arka Chandan was found to react systemically to all the ten PRSV isolates with an infectivity level ranging from 60 to 90 per cent (ave. 67 per cent) in case of sap inoculation, and 60 to 80 per cent (ave. 72 per cent) in case of aphid inoculation. The initial symptoms of vein-clearing caused by ten PRSV isolates on C. pepo were evident within 16 and 11 days after virus inoculations by mechanical sap and aphid inoculation methods respectively. All the PRSV isolates induced only systemic foliar symptoms of vein-clearing, patchy veinal chlorosis and mosaic on newly developed true leaves (Table-10) (Plate-XVI, Fig.33).

12. Summersquash (Cucurbita pepo var. melopepo cv. Patty Pan):

Summersquash cultivar, Patty Pan reacted systemically to all the 10 PRSV isolates by both the methods of inoculation. The systemic foliar symptoms included vein-clearing, patchy veinal chlorosis, mosaic and distortion (Plate- XVI,Fig.34). The level of infectivity by ten PRSV isolates on summersquash varied from 80 to 100 per cent (ave. 89 per cent) in case of

PLATE - XVI

Fig. 33 : Symptoms of vein-clearing, patchy veinal chlorosis and mosaic on non- inoculated leaves of Cucurbita pepo incited by PRSV

Fig. 34 : Symptoms of vein-clearing, patchy veinal chlorosis on non-inoculated leaves of Cucurbita pepo var. melopepo elicited by PRSV.

PLATE
XVI

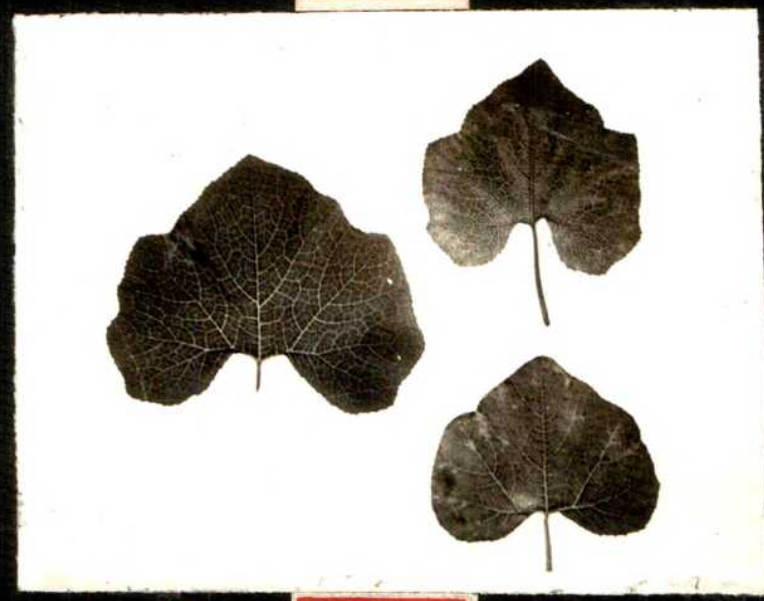

Fig.33

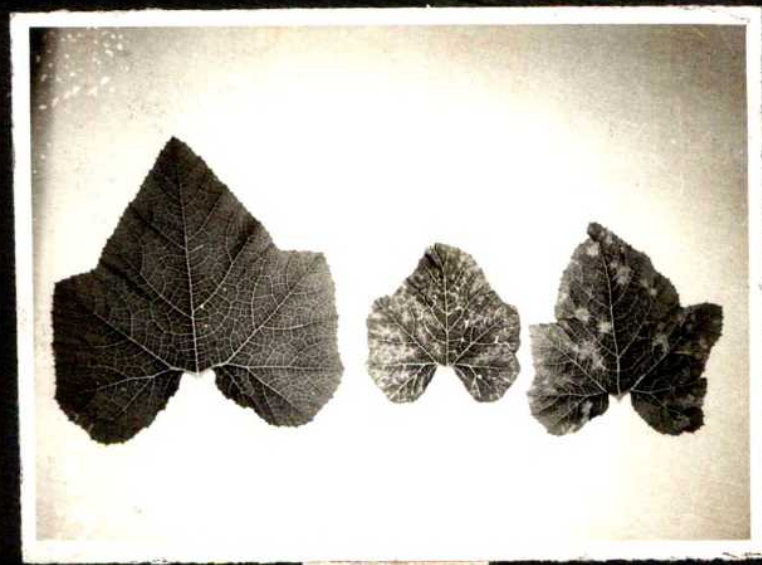

Fig.34

sap inoculation, while in case of aphid inoculation it was found to range from 70 to 100 per cent (ave. 85 per cent). The incubation period of PRSV isolates varied with method of inoculation. It was 12 days in case of aphid inoculation and 13 days in case of mechanical sap inoculation(Table-10).

13. Ridgegourd (Luffa acutangula cv. J. Long) :

The test plants of ridgegourd (cv. J. Long) reacted systemically to all ten PRSV isolates with an infectivity level ranging from 30 to 70 per cent (ave. 49 per cent) in case of sap inoculation and 40 to 70 per cent (ave. 56 per cent) in case of aphid inoculation. The incubation period of PRSV isolates on L. acutangula following mechanical sap inoculation was 20 days, and it was only 18 days following aphid inoculation (Table-10). The systemic foliar symptoms induced by 10 PRSV isolates on ridgegourd were similar and included chlorotic spotting and mosaic (Plate-XVII, Fig. 35).

14. Snakegourd (Trichosanthes anguina cv. Green Long):

Snakegourd cultivar, Green Long was infected by all the 10 PRSV isolates to the extent of 40 to 80 per cent (ave. 59 per cent) by both sap and aphid inoculation (Table-10). All the PRSV isolates induced systemic foliar symptoms of vein-clearing and mosaic (Plate-XVII, Fig.36) on newly developed leaves of this host within 15 days of

PLATE - XVII

Fig. 35 : Symptoms of chlorotic-spotting and mosaic on non-inoculated leaves of *Luffa acutangula* elicited by PRSV.

Fig. 36 ; Symptoms of mosaic on non-inoculated leaves of *Trichosanthes anguina* caused by PRSV.

PLATE
XVII

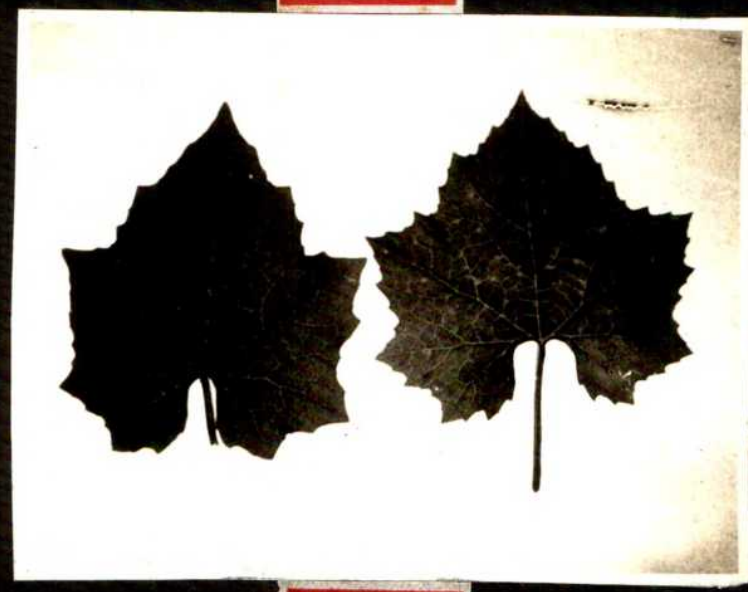

Fig.35

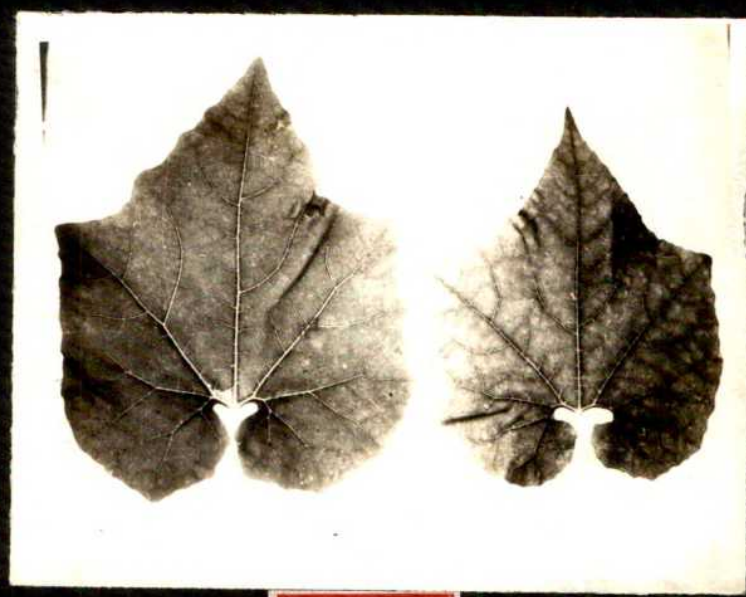

Fig.36

virus inoculation by mechanical sap and 12 days in case of aphid inoculations (Table-10).

The following 27 plant species viz., Abelmoschus esculentus, Arachis hypogaea, Beta vulgaris, Capsicum annuum, Cassia occidentalis, Celosia argentea, C. plumosa, Chenopodium album, C. murale, Cicer arietinum, Datura stramonium, Glycine max, Gomphrena globosa, Lagenaria siceraria, Luffa cylindrica, Lycopersicon esculentum, Momordica charantia, Nicotiana glutinosa, N. rustica, N. tabacum, Ocimum basilicum, Solanum melongena, Vicia faba, Vigna mungo, V. radiata, V. unguiculata, and Zinnia elegans were not infected by 10 PRSV isolates and even the virus was not recovered by back indexing on test plants. These plants species were, therefore, common non-hosts of ten PRSV isolates (Table-10).

4.5 Indexing of field infected cucurbitaceous plant species for the presence of PRSV :

The results of indexing of field infected cucurbitaceous plant species for the presence of PRSV are shown in Table-12. It is revealed from the table that watermelon was the only cucurbitaceous host, that was naturally infected by PRSV. The PRSV could not be detected or recovered from other cucurbitaceous plant species although

Table 12: Indexing of field infected cucurbitaceous plant species for detecting the presence of PRSV.

Sr. No	Host	Field symptoms	Indicator hosts* Papaya cv. Washington		Summersquash cv. Patty Pan	
			Recovery	Symptoms	Recovery	Symptoms.
1.	Bittergourd	Mo	-	-	-	-
2.	Bottlegourd	Mo	-	-	-	-
3.	Ridgegourd	Mo	-	-	-	-
4.	Snakegourd	Mo	-	-	-	-
5.	Spongegourd	Mo	-	-	-	-
6.	Watermelon	Vc,Mo,Ld	**	Vc, Mo, Bl, Ld, Ss.	+	Vc, Pvch, Mo, Ld.

*/ = (-) = negative recovery; (+) = positive recovery

Bl = blistering; Ld = leaf distortion; Mo = mosaic,

Pvch = patchy veinal chlorosis; Ss = shoestrining;

Vc = vein-clearing.

displayed virus-like symptoms under field conditions and were not the natural hosts of the PRSV. Indexing results also indicated that watermelon was a natural host of the PRSV and could serve as a reservoir host for its subsequent spread onto papaya, since this host was also found to be naturally infested by aphid species during surveys conducted for PRSV pre-valance in the State of Maharashtra.

4.6 Physical properties :

4.6.1 Thermal inactivation point (TIP)

The results on TIP of 10 PRSV isolates are shown in Table-13a. It is evident from the data given in the table that the assay host plants became infected by all the 10 PRSV isolates at temperatures upto 60°C but not at 65°C. However, the percentage infectivity decreased progressively as the temperatures were increased.

4.6.2 Dilution end point (DEP) :

The results on DEP of different PRSV isolates are displayed in Table-13b. It is revealed from the table that all the PRSV isolates were inactivated between the dilutions of 10^{-3} to 10^{-4}, but not 10^{-5} dilutions. However, the percentage infectivity decreased progressively as the dilutions were increased.

Table 13a: Thermal inactivation point of 10 PRSV isolates

Sample No.	Exposure temperature (°C)	No.of plants inoculated/ isolate	Isolates of PRSV									
			AKI	AUR	LTR	NAN	OSB	PBN(HG)	PBN(FF)	PUN-HC-c.p.	PUN-C.P.	THA
1.	Untreated (Control)	10	10[a]/100[b]	10/100	10/100	10/100	10/100	10/100	10/100	10/100	10/100	10/100
2.	40	10	10/100	9/90	10/100	10/100	10/100	9/90	10/100	10/100	9/90	10/100
3.	45	10	8/80	8/80	7/70	8/80	7/70	8/80	7/70	8/80	8/80	7/70
4.	50	10	6/60	6/60	5/60	6/60	5/50	6/60	5/50	6/60	6/60	5/50
5.	55	10	4/40	4/40	3/30	4/40	3/30	4/40	3/30	4/40	4/40	3/30
6.	60	10	1/10	2/20	1/10	1/10	1/10	2/20	1/10	1/10	1/10	1/10
7.	65	10	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
8.	70	10	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
9.	75	10	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
10.	80	10	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

a/b = no.of plants infected/ percentage infectivity.

0/0 = i no infection.

Table 13b: Dilution end point of 10 PRSV isolates.

Sample No.	Dilution	No.of plants inoculated/ isolates	Isolates of PRSV									
			AKL	AUR	LTR	NAN	OSB	PBN(HG)	PBN(FF)	PUN-HC-c.p.	PUN-C.P.	THA
1.	Undiluted sap (control)	10	$10^a/100^b$	10/100	10/100	10/100	10/100	10/100	10/100	10/100	10/100	10/100
2.	10^{-1}	10	8/80	7/70	8/80	8/80	8/80	8/80	8/80	7/70	8/80	8/80
3.	10^{-2}	10	6/60	4/40	5/50	6/60	5/50	4/40	6/60	4/40	4/40	5/50
4.	10^{-3}	10	2/20	1/10	1/10	2/20	1/10	1/10	1/10	2/20	1/10	1/10
5.	10^{-4}	10	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
6.	10^{-5}	10	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
7.	10^{-6}	10	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
8.	10^{-7}	10	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
9.	1											

a/b = no. of plants infected/ percentage infectivity

0/0 = no infection.

4.6.3 Longevity in vitro (LIV) :

The results of LIV of different PRSV isolates are presented in Table-13c. It is revealed from the table that the virus isolates were viable upto 10 but not 12 hours at room temperature (27 to 30°C). The assay host inoculated plants became infected upto 10 but not 12 hours aging at room temperature. Percentage infectivity decreased progressively as the aging of the sap was increased at room temperature.

4.7 Serology :

4.7.1 Drop perciptin test on slides :

The results of this test are presented in Table-14. It is evident from the table that all the 10 virus isolates under study showed positive reaction to antisera of only three potyviruses viz.,"papaya ringspot virus" (PRSV) "watermelon mosaic virus-1" (WMV-1) and "watermelon mosaic virus-2" (WMV-2), but not to the members of other poty, potex, cucumo or nepovirus groups including BCMV, BYMV, BlCMV, CAMV, CMV, DDMV, PnMV, PVX, PVY, SMV, TRSV, and WVMV. These results clearly indicated that all the 10 virus isolates were serologically related to PRSV, WMV-1 and WMV-2, definitive members of potyvirus group. No serological relations of any of the 10 virus isolates was observed with healthy sap from papaya, summersquash, PBS or normal serum.

Table 13c: Longevity *in vitro* of 10 PRSV isolates

Sample No.	Inoculation, hours after storage	No.of plants inoculated/ isolates	Isolates of PRSV									
			AKL	AUR	LTR	NAN	OSB	PBN(HG)	PBN(FF)	PUN-HC-c.p.	PUN-C.P.	THA
1.	Immediately after extraction of the sap (control)	10	10[a]/100[b]	10/100	10/100	10/100	10/100	10/100	10/100	10/100	10/100	10/100
2.	2	10	8/80	8/80	9/90	8/80	9/90	8/80	8/80	9/90	9/90	8/80
3.	4	10	6/60	6/60	7/70	6/60	6/60	6/60	7/70	7/70	6/60	6/60
4.	6	10	4/40	4/40	4/40	5/50	4/40	5/50	4/40	4/40	5/50	4/40
5.	8	10	3/30	2/20	2/20	3/30	2/20	3/30	2/20	2/20	3/30	2/20
6.	10	10	1/10	2/20	1/10	1/10	2/20	1/10	1/10	1/10	2/20	1/10
7.	12	10	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
8.	14	10	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
9.	16	10	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

a/b = no.of plants infected/ percentage infectivity

0/0 = no infection.

Table 14: Serological relationship of 10 PRSV isolates with 15 viruses belonging to four different taxonomic groups

Sr. No.	Test antisera	Reaction of test antigens (PRSV isolates)*									
		AKL	AUR	LTR	NAN	OSB	PBN(HG)	PBN(FF)	PUN-HC-C.P.	PUN-C.P.	THA
I.	Cucumovirus group :										
1.	Cucumber mosaic virus (CMV)	-	-	-	-	-	-	-	-	-	-
II.	Nepovirus group :										
2.	Tobacco ringspot virus (TRSV)	-	-	-	-	-	-	-	-	-	-
III.	Potexvirus group :										
3.	Potato virus-X (PVX)	-	-	-	-	-	-	-	-	-	-
IV	Potyvirus group :										
4.	Bean common mosaic virus (BCMV)	-	-	-	-	-	-	-	-	-	-
5.	Bean yellow mosaic virus (BYMV)	-	-	-	-	-	-	-	-	-	-
6.	Blackeye cowpea mosaic virus(BLCMV)	-	-	-	-	-	-	-	-	-	-
7.	Cowpea aphid borne mosaic virus(CAMV)	-	-	-	-	-	-	-	-	-	-
8.	Datura distortion mosaic virus(DDMV)	-	-	-	-	-	-	-	-	-	-
9.	Papaya ringspot virus(PRSV)	+	+	+	+	+	+	+	+	+	+
10.	Peanut mottle virus (pnMV)	-	-	-	-	-	-	-	-	-	-
11.	Potato virus Y (PVY)	-	-	-	-	-	-	-	-	-	-
12.	Soybean mosaic virus (SMV)	-	-	-	-	-	-	-	-	-	-
13.	Watermelon mosaic virus-1(WMV-1)	+	+	+	+	+	+	-	+	+	+
14.	Watermelon mosaic virus-2(WMV-2)	+	+	+	+	+	+	+	+	+	+
15.	Wisteria vein mosaic virus (WVMV)	-	-	-	-	-	-	-	-	-	-

*/ = (+) = serological reaction positive.
(-) = serological reaction negative.

4.7.2 SDS- immunodiffusion test :

The serological relationship of the 10 virus isolates under study was also tested against the antisera of three poty viruses viz., " papaya ringspot virus"(PRSV) "watermelon mosaic virus-1" (WMV-1) and "watermelon mosaic virus-2" (WMV-2) using SDS- immunodiffusion test. The results of these tests showed the development of single line of precipitation within 14 hours after incubation between the antisera of PRSV (Plate- XVIII, Fig.37), WMV-1 and WMV-2 and the test antigens i.e. 10 PRSV isolates. Serologically, all the 10 virus isolates were indistinguishable since the line of precipitation was continuous. Neither PBS, normal serum nor the healthy sap from papaya or summersquash gave any reaction, with PRSV antiserum.

4.8 Electron microscopy :

4.8.1 Leaf-dip preparations :

The data of length distribution and frequency of virus particles is shown in Table-15 and in histogram (Fig.38). The leaf-dip preparations from papaya (cv. Washington) infected with PRSV-PBN(FF) isolate, consistently revealed the presence of elongated, flexuous, rod-shaped virus particles and also particles in loose aggregates (Plate-XIX, Fig.39). The particles were, however,

Table 15: Length distribution and frequency of papaya ringspot virus (PRSV- PBN(FF) particles

Class interval (nm)	Particle frequency	Mid-values (nm)	Remarks
686- 720	3	703	
721- 755	18	738	Normal length = 777 ± 4 nm
756- 790	48	773	
791- 825	24	808	Modal length = 775 ± 4 nm
826- 860	4	843	
861- 895	2	878	
896- 930	1	913	

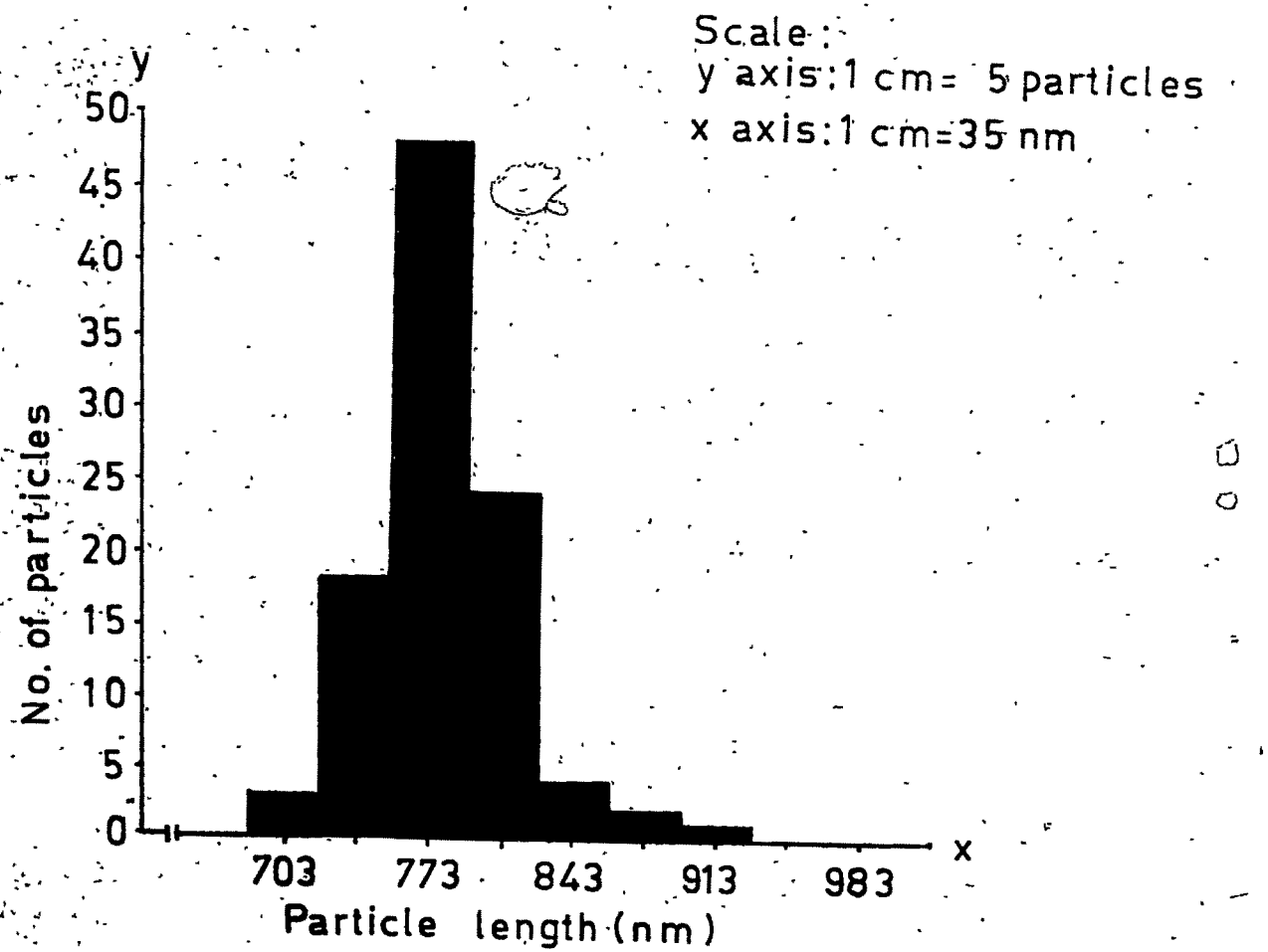

Fig.38. HISTOGRAM OF PARTICLE LENGTH DISTRIBUTION OF PAPAYA RINGSPOT VIRUS (PRSV) ISOLATE PBN (FF)

PLATE - XVIII

Fig. 37 : Sodium dodecyl sulphate (SDS)-immunodiffusion test illustrating serological relationship different virus isolates (antigens in peripheral wells viz., 1= PRSV PBN(FF); 2 = PRSV-AKL; 3 = PRSV-THA; 4 = PRSV-PUN-H.C. c.p.; 5 = PRSV-AUR; 6 = PRSV-LTR) with papaya ringspot virus (PRSV) antiserum to Hawaiian isolate in the Central Well (P).

PLATE
XVIII

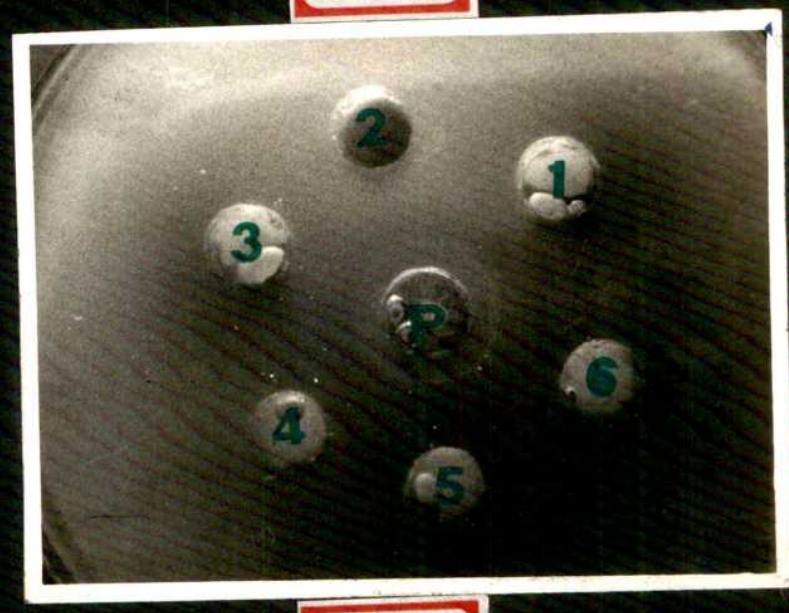

Fig.37

PLATE - XIX

Fig. 39. : Electron micrograph of leaf-dip preparation of PRSV-infected papaya leaf showing virus particles in loose aggregates.

PLATE
XIX

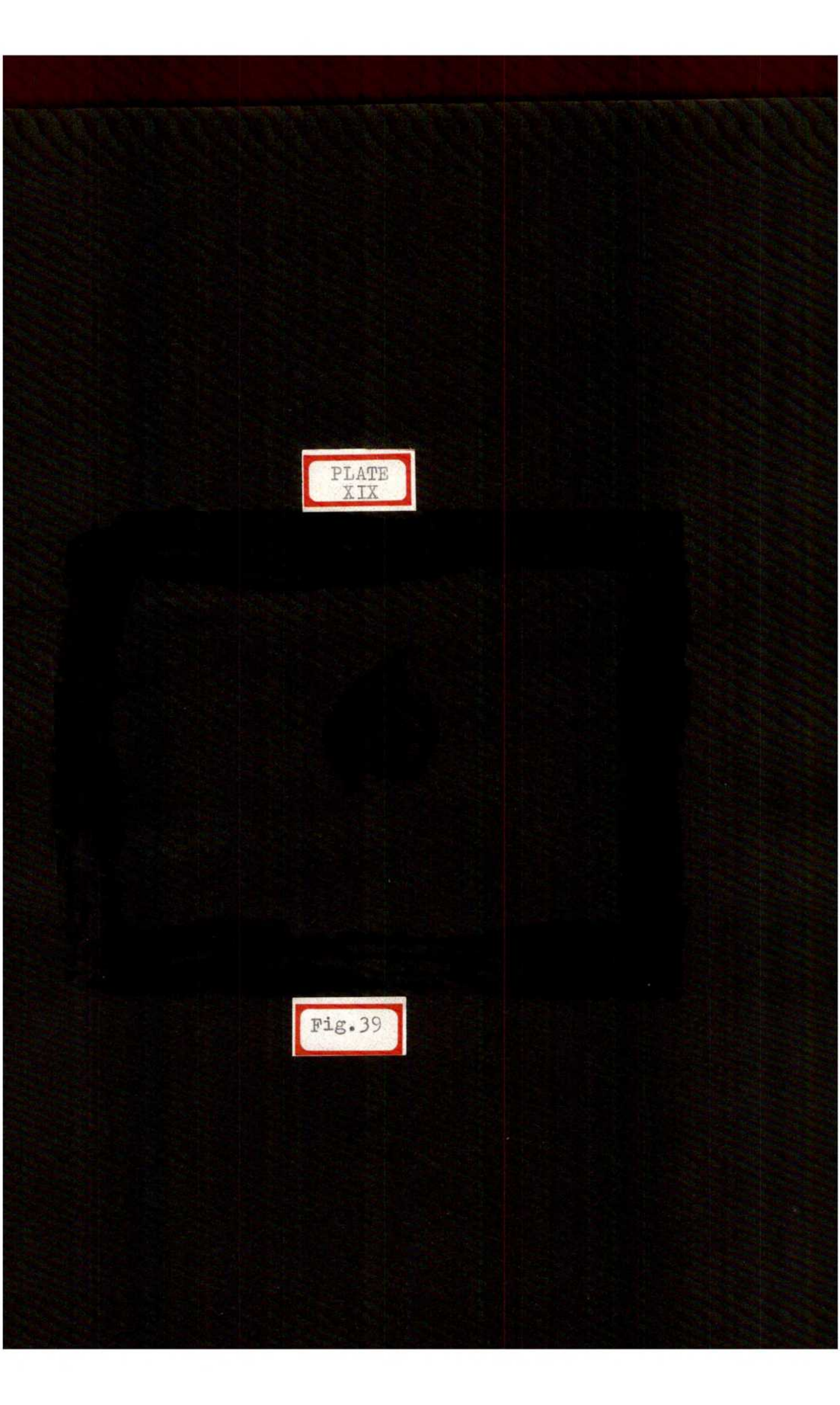

Fig.39

not present in dip preparation from healthy leaf tissues. The histogram (Fig.38) shows a maximum between 686 to 930 nm and a small number of non-specific particles with shorter length (below 680 nm) and longer length (above 950 nm) have been deleted from the histogram (Fig.38). The means of main maximum was used for calculation of the normal particle length and it was 777 ± 4 nm. The modal (most frequently occuring value) length was 775 ± 4 nm (Table-15). The results of this study showed that the virus under investigation belonged to the "potyvirus group".

4.8.2 Ultrathin sections :

Ultrathin sections of papaya leaf tissues infected by PRSV (PBN-FF isolate) displayed numerous cytoplasmic cylindrical inclusions (CCI) consisting of "pinwheels" and "scrolls" (Plate-XX, Fig.40) and brush-like (Plate-XX,Fig.41) and hair-like virus aggregates (VA). The CCI and VA were evident only in the cytoplasm of the infected cells. The results of this study clearly indicated that the virus under investigation belonged to a sub-division-I of the "potyvirus group".

4.9 Resistance to PRSV in Caricaceae :

The data on the reactions of 16 varieties of C. papaya, and a C. cauliflora and an interspecific hybrid (C. cauliflora x C. papaya) to "papaya ringspot virus"(PRSV)

Table 16: Reaction of *Carica cauliflora*, *C. papaya* cultivars and an interspecific hybrid to PRSV(isolate PBN-FF).

Sr. No.	*Carica* spp.,/ varieties.	No.of plants inoculated/ no.of plants infected.	% infectivity	Incubation period (in days)	Symptoms*	Ave.height of 20 healthy plants (in cm)	Ave.height of 20 diseased plants(cm)	% reduction in plant height.	Rating**
I.	*Carica cauliflora* Jacq.	0/20	-	-	-	77.81	70.41	9.05	T
II.	*Carica papaya* L.								
1.	Co-1	20/20	100.00	15	Chl,Vc,Mot,Mo,Bl,Ld,Ss,Pt.	65.80	34.00	48.32	S
2.	Co-2	18/20	90.00	16	Chl,Vc,Mot,Mo,Bl,Ld,Ss Pt.	59.40	38.60	35.01	S
3.	Co-3	20/20	100.00	15	Chl,Vc,Mot,Mo,Bl,Ld,Ss,Pt.	58.80	30.50	48.12	S
4.	Co-4	16/20	80.00	15	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	99.90	48.40	51.55	S
5.	Co-5	20/20	100.00	16	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	87.50	51.40	41.25	S
6.	Co-6	18/20	90.00	15	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	75.20	44.30	41.09	S
7	Coorg Honey Dew	20/20	100.00	16	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	87.30	46.80	46.39	S
8.	Honey Dew	16/20	80.00	15	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	93.20	58.40	37.33	S
9.	Pant papaya-1	20/20	100.00	15	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	74.40	41.00	44.89	S
10.	Pusa Delicious	18/20	90.00	15	Chl,Vc,Mot,Mo,Bl,Ld,Ss,Pt.	99.70	54.60	45.23	S
11.	Pusa Dwarf	18/20	90.00	15	Chl,Vc,Mot,Mo,Bl,Ld,Ss,Pt.	62.70	28.10	55.18	S
12.	Ranchi	20/20	100.00	15	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	97.10	46.90	51.18	S
13.	Sole	18/20	80.00	15	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	98.70	46.10	53.29	S
14.	Sole Hawaii	16/20	80.00	16	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	100.00	53.90	46.20	S
15.	Sunrise Sole	18/20	80.00	16	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	91.40	45.20	50.54	S
16.	Washington	20/20	100.00	14	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	75.90	37.60	50.46	S
III.	Hybrid(*C. cauliflora* x *C. papaya*)	19/20	95.00	14	Chl,Vc,Mot,Mo,Bl,Ld,Ss.	89.00	46.50	47.75	S

*/ = Bl= blistering, Chl = chlorosis, Ld = leaf distortion, Mo = mosaic, Mot = mottling, Pt = petiole twisting, Ss = shoestringing, Vc = vein-clearing

**/ = S = susceptible, T = Tolerant.

PLATE- XX

Fig. 40 : Electron micrograph of Ultrathin section of papaya leaf tissue infected by PRSV showing "pinwheels"(PW) and " scrolls"(SC).

PLATE
XX

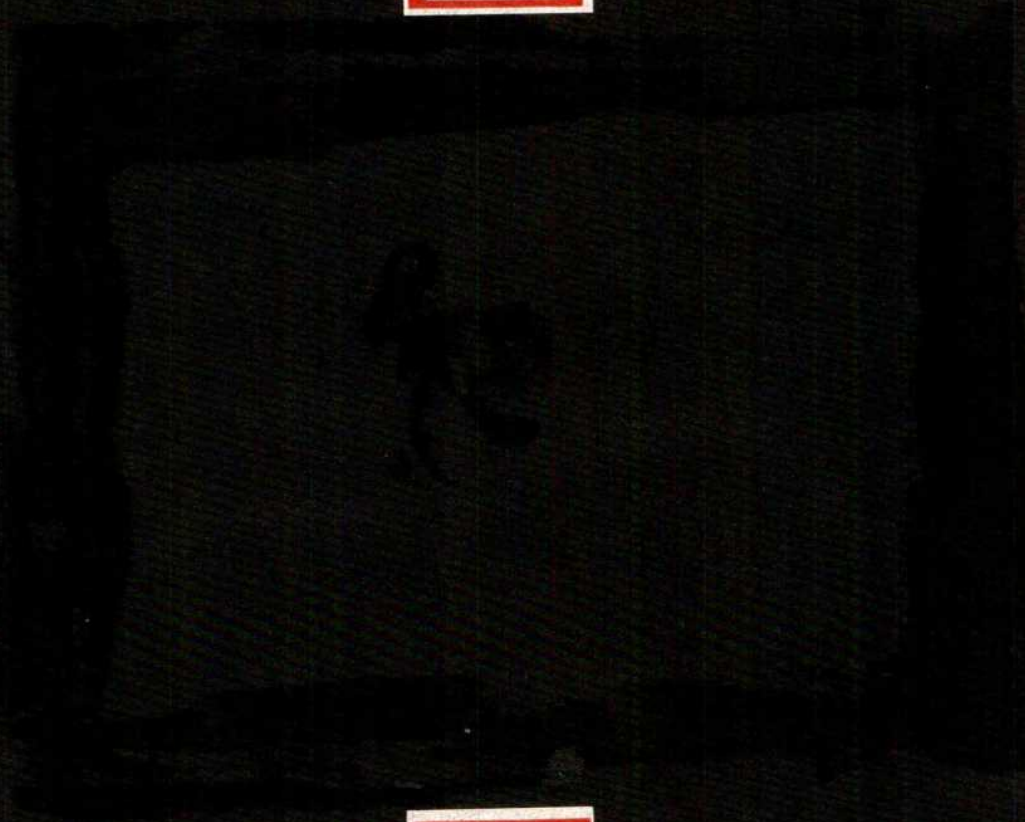

Fig. 40

PLATE - XXI

Fig. 41 : Electron-micrograph of ultrathin section of papaya leaf tissue infected by PRSV showing virus agreegates (VA).

PLATE
XXI

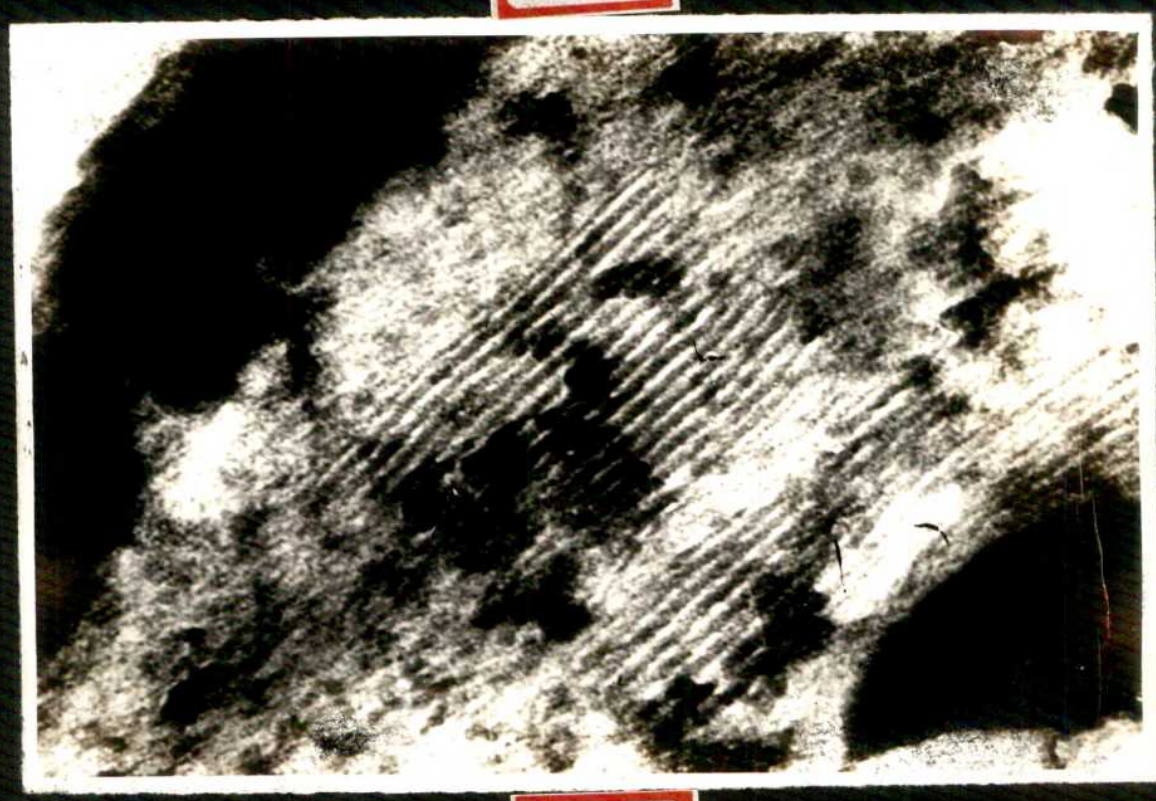

Fig.41

is presented in Table-16. The data given in the table revealed that all the 16 varieties of C. papaya and the interspecific hybrid tested, were rated as susceptible based on the severity of foliage symptoms, height-reduction and percentage infectivity. Percentage infectivity of 16 varieties of C. papaya and an interspecific hybrid to PRSV was found to range from 80 to 100 . Carica cauliflora was infected by PRSV without producing any obvious symptoms. C. cauliflora, was, therefore, rated as tolerant to PRSV (Table-16).

The data on the effect of PRSV on plant height is also presented in Table-16. The data revealed that PRSV caused reduction in plant height in all the 16 test varieties of C. papaya and in the interspecific hybrid. However, the percentage reduction in plant height significantly varied among varieties and the interspecific hybrid and in general it was found to range from 35.01 and 55.18 per cent. Highest reduction in plant height was observed in Pusa Dwarf (55.18 per cent), and lowest (35.01 per cent) in Co-2. papaya varieties. PRSV infection, however, did not reduce plant height of C. cauliflora significantly.

DISCUSSION

5. DISCUSSION

5.1 Disease incidence and field symptoms :

In the present investigation, the PRSV has been found prevalent in different regions of Maharashtra State on Carica papaya cultivars; Carica cauliflora and on an interspecific hybrid (C. cauliflora x C. papaya) grown at Ganesh Khind Gardens in Pune. The symptoms induced by PRSV under field conditions were strikingly similar at all the locations. The occurrence of PRSV on papaya has also been reported from other states of India like Uttar Pradesh and Rajasthan (Khurana, 1974; Sureka et al., 1977). Other aphid transmitted, incompletely characterised papaya virus diseases resembling PRSV for some properties have also been reported from other states of India like Bihar (Mishra and Jha, 1955; Varma, 1971), Madhya Pradesh (Garga, 1963), Haryana (Varma, 1971), Punjab (Cheema and Reddy, 1985a,b), Uttar Pradesh (Singh, 1969a,b; Bhaskar, 1983) and Andhra Pradesh (Susan John, 1985). The "mosaic virus disease" of papaya reported from Pune by Capoor and Varma (1948, 1958) is not available for comparison since the original culture of the same is lost. However, virus isolates collected from the same locality (Ganesh Khind Gardens, Pune) and used in the present investigation have given indications that in fact these authors were

dealing with PRSV only. This valuable confirmation has established that papaya in Maharashtra is exclusively infected by PRSV, but not by "papaya mosaic virus" (PMV), a member of "potexvirus group". The PRSV is reported to occur on papaya at an alarmingly high proportion, limiting its profitable cultivation in different parts of the world. These include Africa (Kulkarni and Sheffield, 1968), Brazil (Barbosa and Paguio, 1982b), the Caribbean countries (Story and Halliwell, 1969), Colombia (Sanchez de Luque et al., 1980), USA: Florida (Conover, 1962; Cook and Zettler, 1970; Wan and Conover, 1983), and Hawaii (Ishii and Holtzmann, 1963); South America (Herold and Weibel, 1962) and Taiwan (Wang et al., 1978).

Papaya plants grown in different plantations were not found to be infested by aphids in the present investigation, as also has been reported from Cuba, Puerto Rico (Schaefers, 1969), USA:(Florida) (Wan and Conover, 1983), India (Capoor and Varma, 1958; Garga, 1963; Varma, 1971) and Taiwan (Hsieh and Hwang, 1986). However, Jensen (1949b) has reported that Myzus persicae could breed and colonise on papaya plants. According to him, the population of M. persicae in the fields in Hawaii fluctuated with the season, weather conditions and with the abundance of natural enemies. He found that cool spring months were more

favourable than the warmer, drier summer months for population build up. He also found the correlation between the vector population and disease spread in the papaya plots maintained in Manoa Valley in Hawaii. The high incidence of PRSV in papaya plantations of Maharashtra State reflects that field spread of PRSV has been taking place. However, the role played by the aphids that infest weeds, intercrops and crops grown in the vicinity of papaya plantations in spreading PRSV remain largely undetermined. It is because of the fact that PRSV has narrow and restricted host range and outside Caricaceae, it is infecting only some members of Cucurbitaceae. Among these also, only watermelon has been found to be infected spontaneously in the present investigation. Inspite of the fact that watermelon was also found to be infested by an aphid vector species, it remains yet to be established upto what extent watermelon can play a role in the epidemiology of the PRSV by way of acting as a reservoir host both for the virus and the vector.

The aphids that infest weeds, intercrops and neighbouring crops of papaya plantations in Maharashtra State have also shown to be vectors of the PRSV in the present investigation. However, these aphids have not been found to colonise on papaya plants in any of the surveyed localities of Maharashtra, as such their role in field spread of PRSV from papaya to papaya remains largely undetermined, though some workers predict that the PRSV spread within and to new papaya

plantations takes place during aphid migration periods (Varma, 1971; Wan and Conover, 1983; Hsieh and Hwang, 1986).

For field symptomatology the present virus disease on papaya resembles "leaf deformation" of Bangladesh (Chatterji, 1943), " PRSV" of Brazil (Barbosa and Paguio, 1982a), " Type A virus disease" (Torres and Giacometti, 1986) and " PRSV" (Sanchez de Luque and Martinez Lopez, 1976a,b) of Colombia; " Cotorro mosaic" of Cuba (Acuna and Zayas, 1940, 1946), "papaya decline viruses" of East Africa (Kulkarni, 1970), "DRV", " FMRV" and " PRSV" of Florida (Conover, 1964a,b; de Bokx, 1965; Wan and Conover, 1983), " PRSV" of Gilbert Islands (Sharmuganathan, 1980); " a new virus disease of papaya" (Parris, 1938), " PRSV" (Holmes et al., 1940; Jensen, 1949a) and "PMV" (Ishii and Holtzmann, 1963) of Hawaii; "PMV" (Capoor and Varma, 1948, 1958; Mishra and Jha, 1955; Garga, 1963; Singh, 1969b; Varma, 1971), "DRV" and PRSV" (Khurana, 1974; Khurana and Bhargava, 1970)," PLRV" (Singh, 1969a) and " PRSV" (Sureka et al., 1977; Yemewar and Mali, 1980; Susan John, 1985) of India; "PRSV" of Nigeria (Lana, 1980), " Shredded leaf" of Pakistan (Farqui et al., 1972), "PMV" of Puerto Rico (Adsuar, 1947a)," PMV" of Queensland (Simmonds, 1965),"PRSV" of Sri Lanka (Rajapakse and Herath, 1982) and "PRSV" of Vietnam (Brcak and Pozdena, 1976). However, the

symptoms like "hook" or U-shaped" spots on stem described in case of " Cotorro mosaic" of Cuba (Acuna and Zayas, 1946), "Phyllody" described in case of Venezuelan isolate of PRSV (de la Rosa and Lastra, 1983) and "apocarpy" and "double papaya" formation in case of "DRV" (Khurana and Bhargava, 1970) and " PRSV" (Sureka et al., 1977) were not found associated with any of the ten PRSV isolates from Maharashtra.

5.2 Virus identity andexistence of strains :

All the 10 isolates viz., AKL, AUR, LTR, NAN, OSB, PBN(FF), PBN(HG), PUN-H.C.c.p, PUN-C.p and THA were identified as"type-P" isolates of "papaya ringspot virus" (PRSV), a member of "potyvirus group"(Purcifull, 1972; Hollings and Brunt, 1981; Purcifull et al., 1984) based on particle shape and size, inclusion morphology, host range, serology, aphid transmissibility, and physical properties in crude sap. Strains of PRSV have been reported from other countries (Conover, 1964a,b; Story and Halliwell, 1969; Chang, 1979; Gonsalves and Ishii, 1980) based on severity of symptoms they induce. In the present investigation, no strainal differences were evident, since all the 10 isolates induced identical symptoms on papaya and other diagnostic hosts. Therefore, these isolates belong to the same biological group. Yeh et al. (1984) while comparing nine

isolates of PRSV from different geographical origins also found no differences in severity of symptoms they induced on papaya and concluded that all the nine isolates belonged to the same biological group.

The PRSV prevalent in Maharashtra typically induced "pinwheels" and "scrolls" but not "laminated aggregates" in infected host cells. Therefore, the PRSV in the present investigation was assigned to sub-division-I of "potyvirus group" (Edwardson, 1974; Edwardson et al., 1984; Matthews, 1982) for its inclusion morphology.

5.3 Transmission :

5.3.1 Mechanical transmission :

All the 10 PRSV isolates under study were found readily transmitted by mechanical means. PRSV and PRSV-like isolates have been reported to be transmitted by mechanical means elsewhere (Parris, 1938; Adsuar, 1947a; Capoor and Varma, 1948, 1958; Conover, 1962, 1964a,b; Ishii and Holtzmann, 1963; de Bokx, 1965; Zettler et al., 1968; Story and Halliwell, 1969; Singh, 1969a; Kulkarni, 1970; Cook and Milbrath, 1971; Sanchez de Luque and Martinez Lopez 1976a,b, 1977; Sureka et al., 1977; Lana, 1980; Yemewar and Mali, 1980; Rajapakse and Herath, 1981,1982; Barbosa and Paguio, 1982a,b; Bhaskar, 1983; de la Rosa and Lastra, 1983; Yeh et al., 1984).

The symptoms induced by the present PRSV isolates on papaya were similar or identical to those reported for PRSV isolates elsewhere (Parris, 1938; Jensen, 1946, 1947, 1949a; Adsuar, 1947a; Capoor and Varma, 1948, 1958; Conover, 1962, 1964a,b; Herold and Weibel, 1962; Ishii and Holtzmann, 1963; de Bokx, 1965; Story and Halliwell, 1969; Singh, 1969a,b; Kulkarni, 1970; Varma, 1971; Khurana,1974; Khurana and Bhargava, 1971; Brcak and Pozdena, 1976; Sanchez de Luque and Martinez Lopez, 1976a,b; Sureka et al., 1977; Lana, 1980; Yemewar and Mali, 1980; Bhaskar, 1983; de la Rosa and Lastra, 1983; Wan and Conover, 1983; Yeh et al., 1984; Susan John, 1985). However, the symptoms like petioles assuming "spring-like" appearance in some cultivars of papaya viz., Co-1, Co-2, Co-3, Pusa Delicious, Pusa Dwarf and Solo observed in the present studies have not been reported elsewhere.

Masking of disease symptoms during summer months (April to June) evident in the present studies have also been reported for PRSV isolates like DRV, FMRV, PMV or PRSV- type strain elsewhere (Jensen, 1949a; Conover, 1962, 1964a,b; Garga, 1963; Cook, 1972; Sureka et al., 1977; Yeh et al., 1984).

5.3.2 Aphid transmission :

Eight aphid species viz., Acyrthosipon pisum, Aphis craccivora, A. gossypii, A. nerii, Melanaphis sacchari, Myzus persicae, Rhopalosiphum maidis and Uroleucon compositae have been found to be the vectors of the 10 PRSV isolates under study, transmitting them in the non-persistent manner. These eight aphid species except M. sacchari have also been reported as vectors of PRSV elsewhere (Capoor and Varma, 1948, 1958; Jensen, 1949a,b; Martorell and Adsuar, 1952, Pontis Videla, 1953; Ishii and Holtzmann, 1963; Garga, 1963; Conover, 1964a,b; Kralovic, 1967; Gabrovska et al., 1967; Zettler et al., 1968; Marin, 1969; Schaefers, 1969; Singh, 1969a,b; Higa and Namba, 1971; Khurana and Bhargava, 1971; Varma, 1971; Sanchez de Luque and Martinez Lopez, 1976a,b, 1977; Lana, 1980; Yemewar and Mali, 1980; Rajapakse and Herath, 1981, 1982; Wang, 1981; Barbosa and Paguio, 1982a; Bhaskar, 1983: Hwang and Hsieh, 1984; Cheema and Reddy, 1985a,b; Susan John, 1985; Dake, 1986). M. sacchari has not hitherto been recorded as a vector of PRSV.

Amongst eight aphid species, Myzus persicae has been found efficient vector in transmitting the present PRSV isolates. Similar results have also been reported

by other workers (Capoor and Varma, 1948, 1958; Jensen, 1949a,b; Schaefers, 1969; Rajapakse and Herath, 1981, 1982).

5.3.3 Transmission of PRSV using different parts of infected papaya plant as a source of inoculum :

In the present studies, it has been found that besides virus infected leaves, it was also possible to use petiole, stem or fruit tissues as a source of inoculum in virus transmission by mechanical means or by aphid vectors. So far virus infected leaf tissue has been extensively used as a source of virus inoculum in transmission tests (Capoor and Varma, 1948, 1958; Jensen, 1949b; Conover, 1964a,b; Schaefers 1969; Singh, 1969a,b; Story and Halliwell, 1969; Khurana and Bhargava, 1971; Varma, 1971, Sanchez de Luque and Martinez Lopez, 1976a,b; Sureka et al., 1977; Lana, 1980; Yemewar and Mali, 1980; Rajapakse and Herath, 1981, 1982; Wang, 1981; Bhaskar, 1983; Susan John, 1985; Dake, 1986). But other virus infected tissues like roots or fruits as a source of inoculum in transmission studies have been used by few workers (Jensen, 1949b; Story and Halliwell, 1969; Lana, 1980). Information in respect to transmission of PRSV from infected stem and petioles is not available in the literature surveyed.

5.3.4 Virus-vector relationship :

5.3.4.1 Efficiency of different morphological forms of aphids in PRSV transmission :

It has been indicated in the present study that apterate adults of A. gossypii and M. persicae were more efficient in the transmission of PRSV (isolate PBN-FF) than alate adults and nymphs. Similar results have been reported by Singh (1972c) and Bhaskar (1983) while working with PMV and Aphis gossypii.

5.3.4.2 Influence of the number of aphids in PRSV transmission :

It has been found in the present study, that infectivity percentage increased progressively with the increase in the number of aphids and the maximum number required for achieving cent per cent transmission varied with the aphid species used in the transmission tests. Similar trend in percentage infectivity as a result of aphid transmission has been reported by few other workers (Singh, 1971c, 1972c; Cheema and Reddy, 1985b).

5.3.4.3 Effect of pre-acquisition fasting periods given to aphid vector species on PRSV transmission :

In the present investigation, pre-acquisition fasting of one hour given to aphid vector species viz., A. gossypii and M. persicae, has resulted in the increase of

vector efficiency in the transmission of PRSV at least by 7 to 8 times. Similar results have also been reported by few workers (Singh, 1971c, 1972c; Cheema and Reddy, 1985b) between PRSV isolates and aphid vector species viz., A. gossypii, M. persicae and R. maidis.

5.3.4.4 Effect of different acquisition feeding periods given to aphid vector species on PRSV transmission :

In the present investigation, acquisition feeding period of 5 minutes has been found optimum for vector species (A. gossypii and M. persicae) to transmit PRSV more efficiently. Lower and higher acquisition feeding times have resulted into low level of virus transmission. Similar acquisition feeding periods for PRSV transmission have also been reported by other workers (Jensen, 1949a, Schaefers, 1969; Singh, 1971c, 1972c; Wang, 1981; Cheema and Reddy, 1985b).

5.3.4.5 Effect of different inoculation feeding periods given to aphid vector species on PRSV transmission :

In the present investigation, it has been found that for A. gossypii and M. persicae, an inoculation feeding of 15 minutes resulted in maximum virus transmission. The results of this investigation are in concurrence with those of Singh (1971c, 1972c) and Cheema and Reddy (1985b).

5.3.4.6 Persistence of PRSV in aphid vector species :

The results of this study have indicated that A. gossypii and M. persicae have lost the virus more rapidly when fed on the series of test plant thereby indicating a non-persistent type of virus-vector relationship. Similar results have been reported for PMV and A. spiraecola in Puerto Rico (Adsuar, 1947c); "PMV" and A. gossypii, M. persicae and R. maidis in India (Capoor Varma, 1958; Singh, 1972c; Cheema and Reddy, 1985b), and "PRSV" and M. persicae in Hawaii (Jensen, 1949a).

5.3.5 Seed transmission :

PRSV has not been found to be seed-borne in papaya in the present investigation. Seed transmission of PRSV in papaya have also not been detected by other workers (Ishii and Holtzmann, 1963; Conover, 1964a; Wang et al., 1978).

5.3.6 Graft transmission :

PRSV has been found to be transmitted by "approach-grafting" in the present investigation. Similar results have been reported by other workers for some of the PRSV isolates elsewhere (Adsuar, 1947a; Capoor and Varma, 1948, 1958; Garga, 1963).

5.3.7 Dodder transmission :

PRSV has also been found to be transmitted by dodder (Cuscuta species) in the present investigation. No information is available on the dodder transmission of PRSV in the literature surveyed.

5.4 Host range :

Papaya ringspot virus (PRSV) have been reported to have intermediate host range mostly confined to the members of three families viz., Caricaceae, Chenopodiaceae and Cucurbitaceae (Purcifull, 1972; Wang et al., 1978; Yeh et al., 1984) as has been the case with the present virus isolates. Chenopodium amaranticolor and C. quinoa have also been found to be the local lesion hosts of the present PRSV isolates as has been reported elsewhere (Kulkarni, 1970; Cook and Milbrath, 1971; Sureka et al., 1977; Wang et al., 1978; Chang, 1979; Lana, 1980; Yemewar Mali, 1980; Rajapakse and Herath, 1982; Bhaskar, 1983; Yeh et al., 1984; Susan John, 1985). However, the local lesion development on these hosts was found to be erratic when papaya rather than summersquash tissue formed the source of virus inoculum. This might be due to low concentration of virus in papaya tissue (Yeh et al., 1984). Carica cauliflora has been reported to be resistant to PRSV by some of the workers (Malaguti et al., 1957;

de Zerpa, 1958, 1962, 1967; Vasudeva, 1959; Capoor and Varma 1961; Horovitz and Jimenez, 1967; Mekako and Nakasone, 1975). C. cauliflora has been found to be susceptible to all the 10 PRSV isolates in the present investigation, as has been reported by some workers from Florida (Conover, 1964a,b) and Hawaii (Cook and Milbrath, 1971).

"Type-P" isolates but not "type-W" isolates of PRSV have been reported to infect papaya (Carica papaya) (Milne and Gorgan, 1969; Purcifull and Hiebert, 1979; Yeh et al., 1984). All the 10 PRSV isolates in the present investigation have been found to infect papaya host.

A Florida isolate of PRSV and an aphid transmissible PMV from India have been reported to infect ridgegourd (Luffa acutangula) (Capoor and Varma, 1958; Milne et al., 1969; Yeh et al., 1984) as has been the case with the present PRSV isolates.

Some of the "type-P" isolates of PRSV have been found to induce prominent mosaic and leaf distortion, whereas others induce mild mottle on pumpkin (Cucurbita pepo) (Gonsalves and Ishii, 1980). "Type-W" isolates have been found typically inducing foliar symptoms of mosaic, darkgreen blisters and distortion, whereas stripe mosaic and distortion have been reported for Guadeloupe strain on pumpkin (Purcifull et al., 1984). All the 10 PRSV

isolates in the present studies have been found to induce prominent mosaic but not mild mottle symptoms on the young leaves of the pumpkin.

Other cucurbitaceous hosts of the present PRSV isolates included ashgourd (Benincasa hispida), watermelon (Citrullus lanatus), roundgourd (C. lanatus var. fistulosus), muskmelon (Cucumis melo), wintersquash (Cucurbita maxima), summersquash (C. pepo var. melopepo) and snakegourd (Trichosanthes anguina). For instance, Benincasa hispida (Yemewar and Mali, 1980), Citrullus lanatus (Capoor and Varma, 1948; 1958; Ishii and Holtzmann, 1963; Conover, 1964a,b; Namba and Kawanishi, 1966; Zettler et al., 1968; Sanchez de Luque and Martinez Lopez, 1976b, 1977; Wang et al., 1978; Bhaskar, 1983; Yeh et al., 1984; Susan John, 1985), C. lanatus var. fistulosus (Capoor and Varma, 1948, 1958), C. melo (Capoor and Varma, 1948; 1958; Conover, 1964a,b; Sanchez de Luque and Martinez Lopez, 1976b, 1977), C. sativus (Capoor and Varma, 1948, 1958; Ishii and Holtzmann, 1963; Conover, 1964a,b; Namba and Kawanishi, 1966; Zettler et al., 1968; Lana, 1980; Yemewar and Mali, 1980; Rajapakse and Herath, 1982; Bhaskar, 1983; Yeh et al., 1984; Susan John, 1985), Cucurbita maxima (Capoor and Varma, 1958; Ishii and Holtzmann, 1963; de Bokx, 1965; Zettler et al., 1968; Story and Halliwell 1969; Cook and Zettler, 1970; Cook and Milbrath, 1971; Lopez Pinto, 1972; Sanchez de Luque and

Martinez Lopez, 1976b, 1977; Sureka *et al.*, 1977; Yemewar and Mali, 1980; Rajapakse and Herath, 1982; Wan and Conover, 1983; Bhaskar, 1983), *C. pepo* var. *melopepo* (Capoor and Varma, 1948, 1958; Herold and Weibel, 1962; Conover, 1964a,b; de Bokx, 1965; Zettler *et al.*, 1968; Story and Halliwell, 1969; Sureka *et al.*, 1977; Lana, 1980; Yemewar and Mali, 1980; Barbosa and Paguio, 1982a; de la Rosa and Lastra, 1983) and *Trichosanthes anguina* (Capoor and Varma, 1948, 1958; Bhaskar, 1983) have also been reported as PRSV hosts elsewhere.

The non-hosts of the present PRSV isolates have also been reported as non-hosts of PRSV isolates elsewhere. These include *Lagenaria siceraria* (Sureka *et al.*, 1977; Yemewar and Mali, 1980; Susan John, 1985), *Luffa cylindrica* (Capoor and Varma, 1948, 1958; Conover, 1964a,b) and *Momordica charantia* (Capoor and Varma, 1948, 1958; Ishii and Holtzmann, 1963; Conover, 1964a,b; Sanchez de Luque and Martinez Lopez, 1976b, 1977; Yemewar and Mali, 1980; Susan John, 1985).

5.5 Indexing of field infected cucurbitaceous plant species for the presence of PRSV :

Of the various cucurbits indexed, only watermelon has been found to be infected by PRSV spontaneously in the present investigation. Some of the cucurbits have been reported as natural hosts of PRSV elsewhere(Wang *et al.*, 1978).

5.6 Physical properties :

5.6.1 Thermal inactivation point (TIP) :

The present PRSV isolates have been found to be inactivated between 60 and 65°C and in this respect they resemble PRSV isolates reported from East Africa (Kulkarni, 1970), Puerto Rico (Adsuar, 1947c), Uttar Pradesh, India (Singh, 1969b), and Colombia (Sanchez de Luque and Martinez Lopez, 1976b).

5.6.2 Dilution end point (DEP)

The present PRSV isolates have been found to be inactivated between the dilutions of 10^{-3} to 10^{-4}. In this respect they resembled PRSV isolates from Dominican Republic (Story and Halliwell, 1969), Florida (Conover, 1964a,b), India (Capoor and Varma, 1948, 1958; Sureka et al., 1977; Yemewar and Mali, 1980; Bhaskar, 1983), Nigeria (Lana, 1980), Puerto Rico (Adsuar, 1947c), Venezuela (Lopez Pinto, 1972) and Taiwan (Wang et al., 1978; Chang, 1979).

5.6.3 Longevity in vitro (LIV)

The present PRSV isolates have been found to be viable upto 10 but not 12 hours at room temperature (27 to 30°C). The values for LIV of the present PRSV isolates resemble PRSV isolates reported from India (Yemewar and Mali,

1980; Bhaskar, 1983), Nigeria (Lana, 1980) and Taiwan (Wang et al., 1978).

5.7 Serological relationship :

All the present PRSV isolates have been found to be serologically related to antisera of "papaya ringspot virus" (PRSV), "watermelon mosaic virus-1" (WMV-1) and "watermelon mosaic virus-2" (WMV-2). All the 10 virus isolates in the present investigation have been identified as the "type-P" isolates of PRSV, based on the particle and inclusion morphology, aphid transmissibility and serological relationship with PRSV. Since, PRSV has been shown to be serologically very closely related, if not identical to "watermelon mosaic virus-1" (WMV-1) and distantly related to "watermelon mosaic virus-2"(WMV-2)(Webb and Scott, 1965; Milne and Grogan, 1969; Purcifull and Hiebert, 1979; Russo et al., 1979; Gonsalves and Ishii, 1980; Barbosa and Paguio, 1982; Yeh et al., 1984), it is possible, therefore, to get a positive serological reaction of the present PRSV isolates to that of a WMV-1 and WMV-2 besides PRSV. In fact, recently "papaya ringspot virus" (PRSV) and "watermelon mosaic virus-1" (WMV-1) have been grouped under one name of "papaya ringspot virus" (PRSV) with "type-P and "W" isolates as suggested by Lovisolo (1980) and Yeh et al. (1984), based on similarities in properties like serological, biological, cytological and in resistance mechanisms in cucurbits (Purcifull et al., 1984).

5.8 Particle and inclusion morphology :

The particle morphology and ability to induce cytoplasmic cylindrical inclusions (CCI) consisting of "pinwheels" and "scrolls" in the host cells, places the present virus in potyvirus groups (Hollings and Brunt, 1981; Matthews, 1982). Edwardson (1974) has placed both type-P and W isolates of PRSV to subgroup-I, because they typically induced "pinwheels" and " scrolls" but not "laminated aggregates" in host cells as has been the case with present PRSV isolate.

Themodal length of the present PRSV isolates is similar to PRSV isolates reported from Brazil (Barbosa and Paguio, 1982a), Dominican Republic (Herold and Weibel, 1962), East Africa (Kulkarni, 1970), Florida (de Bokx, 1965; Zettler et al., 1968; Smith, 1972), Fugian (Ko et al., 1979), India (Khurana, 1974; Dake, 1986), Nigeria (Lana, 1980), Venezuela (de la Rosa and Lastra, 1983), Vietnam (Brcak and Pozdena, 1976) and Taiwan (Chen, 1984). Besides cytoplasmic cylindrical inclusions (CCI) consisting of "pinwheels" and " scrolls", the present PRSV prevalent in Maharashtra State also induced the formation of brush-like and hair-like aggregates of virus particles in the cytoplasm of the infected cells. The presence of similar cytoplasmic cylindrical inclusions (CCI) and virus aggregates (VA) in

the cytoplasm of the infected cells have also been reported for PRSV isolates from Brazil (Barbosa and Paguio,1982a), Dominican Republic (Story and Halliwell, 1969), Florida (Zettler et al., 1968; Edwardson et al., 1984), South China (Wu et al., 1983) and Taiwan (Chen, 1984).

5.9 Resistance to PRSV in Caricaceae :

Although tolerant selections of and their cross combinations have been identified (Conover, 1976; Conover and Litz, 1978), resistance to PRSV has not been found to occur within Carica papaya (Cook and Zettler, 1970; Conover, 1976; Conover and Litz, 1978; Wang et al., 1978). Some species of Carica including C. cauliflora, however, have been reported to be resistant to PRSV (Malaguti et al., 1957; de Zerpa, 1958, 1962, 1967; Vasudeva, 1959; Capoor and Varma, 1961; Horovitz Jimenez, 1967, Mekako and Nakasone, 1975). On the contrary, C. cauliflora has been reported as susceptible to PRSV by few workers (Conover, 1964a,b; Cook and Milbrath,1971) as has been the case with the present PRSV isolate of Maharashtra State. Therefore, use of C. cauliflora is not warranted in crossing programmes with C. papaya aimed at developing a resistant plant material to PRSV. In the present investigation, all the 16 varieties of C. papaya and an interspecific hybrid have been found susceptible and

C. cauliflora tolerant to PRSV. The present PRSV isolate has also been found to reduce plant height to an extent of 55 per cent. Reduction in plant height, though not quantified, have also been reported by earlier workers (Parris, 1938; Chatterji, 1943; Capoor and Varma, 1948, 1958; Jensen, 1949a; Ishii and Holtzmann, 1963; Garga, 1963; Conover, 1964a,b; Varma, 1971; Sanchez de Luque and Martinez Lopez, 1976a; Yemewar and Mali, 1980; Barbosa and Paguio, 1982a; Susan John, 1985).

SUMMARY

6. SUMMARY

All the 10 virus isolates collected on papaya from different localities of Marathwada, Vidharbha, Konkan and Western Maharashtra were identified as "type-P" isolates of "papaya ringspot virus" (PRSV), a definitive member of subdivision-I of the "potyvirus group", based on particle shape and size, inclusion morphology, aphid transmissibility, serological relationship, host range and physical properties in crude sap.

Natural infections of papaya by PRSV characterised by foliage symptoms consisting of mosaic mottling, severe distortion and shoestringing; streaking on petioles and stem and rings on fruits was prevalent in all the nine districts surveyed. The disease incidence varied from 53 to 100 per cent in different papaya plantations. The plants of recently introduced papaya cultivars like Co-1, Co-2, Co-3, Co-4 and Co-5 and an interspecific hybrid (*Carica cauliflora* x *C. papaya*) showed 100 per cent infection by PRSV. None of the surveyed localities displayed infestation of papaya plants by aphids. However, aphid species like *Aeyrthosiphon pisum*, *Aphis craccivora*, *A. gossypii*, *A. nerii*, *Melanaphis sacchari*, *Myzus persicae* and *Rhopalosiphum maidis* were found to infest intercrops, weed hosts and also crops grown in the vicinity of papaya

plantations. It remains to be established whether or not these aphid species play an important role in the spread of PRSV within or outside papaya plantations in Maharashtra.

All the 10 PRSV isolates were readily transmitted by mechanical means from papaya to papaya and to other hosts. With the use of extraction buffer, the level of transmission was 100 per cent. The symptoms produced on papaya test plants upon mechanical sap inoculation were similar to those found on plants spontaneously infected by the virus in papaya plantations.

Similarly, all the 10 PRSV isolates were readily transmitted by eight aphid species viz., Acyrthosiphon pisum (58.5 per cent), Aphis craccivora (54 per cent), A. gossypii (90.15 per cent), A. nerri (84.5 per cent), Melanaphis sacchari (46 per cent), Myzus persicae (100 per cent), Rhopalosiphum maidis (47 per cent) and Uroleucon compositae (78 per cent) in a non-persistent manner from papaya to papaya. Of these, M. persicae was found to be the most efficient vector. Melanaphis sacchari has been recorded as a new vector of PRSV.

Infected leaf was found to be the best source for virus transmission by mechanical sap and aphid inoculations as compared to fruit, petiole and stem.

Studies on virus-vector relationship indicated that the apterate () adults of A. gossypii and M. persicae were more efficient vectors of PRSV than nymphs and alate adults. A pre-acquisition fasting of one hour; acquisition feeding of 5 minutes and an inoculation feeding of 15 minutes were found to be optimum for achieving maximum transmission of PRSV by aphid vector species viz., A. gossypii and M. persicae. The virus was lost from the vectors within brief inoculation feedings (1-2 minutes) and thus virus-vector relationship was found to be non-persistent type.

Besides mechanical and aphid transmissibility, the virus was also found to be transmitted by grafting (approach-grafting) and by dodder (Cuscuta species), but not through seeds of papaya.

The virus had an intermediate host range and could infect 14 plant species belonging to Caricaceae, Chenopodiaceae and Cucurbitaceae families. Besides Carica papaya and C. cauliflora, the virus hosts included Chenopodium amaranticolor, C. quinoa, watermelon(Citrullus lanatus), roundgourd (C. lanatus var. fistulosus), muskmelon (Cucumis melo), cucumber (Cucumis sativus), pumpkin (Cucurbita pepo), summersquash (C. pepo var. melopepo), wintersquash (C. maxima), ashgourd (Benincasa hispida),

ridgegourd (Luffa acutangula) and snakegourd (Trichosanthes anguina). The virus non-hosts included Abelmoschus esculentus, Arachis hypogaea, Beta vulgaris, Capsicum annuum, Cassia occidentalis, Celosia argentea, C. plumosa, Chenopodium album, C. murale, Cicer arietinum, Datura stramonium, Glycine max, Gomphrena globosa, Lagenaria siceraria, Luffa cylindrica, Lycopersicon esculentum, Momordica charantia, Nicotiana glutinosa, N. rustica, N. tabacum, Ocimum basilicum, Solanum melongena, Vicia faba, Vigna mungo, V. radiata, V. unguiculata and Zinnia elegans. Watermelon was found to be infected spontaneously by PRSV.

The physical properties of the virus included TIP between 60 and 65°C, dilution end point (DEP) between 10^{-3} and 10^{-4} and longevity in vitro between 10 and 12 hours at room temperature (28-30°C).

All the 10 PRSV isolates were found to be serologically related to three potyviruses viz., "papaya ringspot virus" (PRSV), "watermelon mosaic virus-1" (WMV-1) and "watermelon mosaic virus-2" (WMV-2).

The virus was characterised by flexuous, filamentous, rod-shaped particles having a normal and modal lengths of 777 ± 4 nm and 775 ± 4 nm respectively. The virus induced " pinwheels" and " scrolls" but not " laminated aggregates" in the cytoplasm of the host cell. The virus

was also found to induce brush-like and hair-like virus aggregates besides inclusions in the cytoplasm of the host cell.

All the 16 cultivars of Carica papaya and an interspecific hybrid (C. cauliflora x C.papaya) were found to be susceptible to PRSV. However, C. cauliflora was found to be tolerant to PRSV. In susceptible cultivars the virus was also found to reduce plant height (upto 55 per cent) significantly.

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