CHARACTERIZATION, HOST RANGE AND MANAGEMENT OF SWEET POTATO FEATHERY MOTTLE VIRUS

BY

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THESIS
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Department of Plant Pathology
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Dedicated to my beloved father

Late Shri. M. Lajapathy

DECLARATION

I hereby declare that this thesis entitled **"Characterization, host range and management of sweet potato feathery mottle virus"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,
14.12.2001

M. L. JEEVA

CERTIFICATE

Certified that this thesis entitled **"Characterization, host range and management of sweet potato feathery mottle virus"** is a record of research work done independently by **Mrs. M. L. Jeeva** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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LIST OF ABBREVIATIONS

AAP	Acquisition access period
AG	Approach grafting
BAP	Benzyl amino purine
BCIP	p-toluidine salt of 5-bromo-4 chloro-3-indolyl phosphate
BOD	Biological oxygen demand
BSA	Bovine serum albumin
CIP	International Potato Center
CLS	Chlorotic leaf spot
CPMV	Cowpea mosaic virus
CTCRI	Central Tuber Crops Research Institute
DAC-ELISA	Direct antigen coating Enzyme linked immunosorbent assay
DAP	Days after planting
DAS-ELISA	Double antibody sandwich Enzyme linked immunosorbent assay
DEP	Dilution end point
DIECA	Sodium diethyl dithiocarbamate
DM	Dry matter copntent
DN	Double node
EDTA	Ethylene diamine tetraacetate
ELISA	Enzyme linked immunosorbent assay
EM	Electron microscopy
FAO	Food and agriculture organization
HR	Highly resistant
HS	Highly susceptible
IAP	Inoculation access period
IC	Internal cork
LIV	Longevity *in vitro*
MS	Moderately resistant
NAA	Naphthalene acetic acid
NBPGR	Natioal Bureau of Plant Genetic Resources
NBT	p-nitro blue tetrazolium
NCM-ELISA	Nitro cellulose membrane Enzyme linked immunosorbent assay
OD-phenol	Orthodihydroxy phenol
PAL	Phenylalanine ammonia lyase
PBS	Phosphate buffered saline
PBS –T	Phosphate buffered saline with 0.05 % Tween-20
PC	Poochedichuvala
PDI	Per cent disease index
PO	Peroxidase
PPO	Polyphenol oxidase
PS	Pink spot
PVP	Poly vinyl pyrrolidone

R	Resistant
RC	Russet crack
RS	Ring spot
S	Susceptible
SB	Sree Bhadra
SBMV	Southern bean mosaic virus
SDS	Sodium dodecyl sulphate
SG	Side grafting
SPFM	Sweet potato feathery mottle
SPFMV	Sweet potato feathery mottle virus
SN	Single node
SR	Sree Rethna
SPV-A	Sweet potato virus-A
SPVD	Sweet potato virus disease
SPV-N	Sweet potato virus-N
TBS	Tris buffered saline
TIP	Thermal inactivation point
TN	Triple node
TTBS	Tris buffered saline with 0.05 % Tween-20
VT	Vavvathooki
WG	Wedge grafting

INTRODUCTION

1. INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is widely grown throughout the tropics and warm temperate regions of the world. It ranks seventh in the world in importance as a food crop and in terms of production with 129 million tonnes in 1998 (FAO, 1998). In developing countries it ranks fourth in importance after rice, wheat and corn (Karyeija *et al.*, 1998).

The tubers are used as a subsidiary food whereas the vines form an excellent source of green fodder for cattle. In some countries the tips of vines are used as vegetable. In sweet potato starch is the most important root dry matter, which provides energy. It is an excellent source for industrial food products and alcoholic beverages in Japan and China (Padmaja, 2000). In India the crop is grown in 19 states/ union territories. The major area (70 per cent) under the crop is confined to the states of Orissa, Bihar and Uttar Pradesh, contributing 74 per cent of production. The annual production reported in 1998 from India was 1.2 million tonnes from an area of 0.14 million hectare (FAO, 1998). Sweet potato has tremendous potential as a food crop and it ranks first among cultivated crops in the developing countries in terms of edible energy produced per unit area per unit time (194 Mj /ha/day) (Lila Babu, 2000).

Several pathogens are known to cause diseases in sweet potato plants. Among them, the diseases caused by viruses are of worldwide economic importance. More than 15 diseases caused by viruses and virus- like

pathogens have been reported in sweet potato. Sweet potato feathery mottle virus (SPFMV) is one of the five viruses infecting sweet potato which has been detected and confirmed independently. Sweet potato mild mottle virus (SPMMV), sweet potato chlorotic stunt virus (SPCSV), sweet potato latent virus (SPLV) and cucumber mosaic virus (CMV) are the other four viruses recorded on the crop (Karyeija *et al.*, 1998).

SPFMV is a member of the genus Potyvirus of the family Potyviridae with single stranded RNA as its genome. This virus causes disease in sweet potato wherever the crop is grown. In India the occurrence of SPFMV was reported from different places (Daines and Martin, 1964; Thankappan and Nair, 1990; Kumar *et al.*, 1993).

Leaf symptoms caused by SPFMV- infection on sweet potato are generally mild and transient but may include pink spot, ring spot, feathering, chlorotic leaf spot, mosaic and puckering, especially on older leaves. Economic loss may be associated with external cracking and internal corkiness, making the tuberous roots unmarketable. However, there are only few reports of this form of loss. Instead, SPFMV has economical impact as a component cause of the complex malady known as sweet potato virus disease (SPVD) (Geddes, 1990). This is more severe than the infection caused by SPFMV alone.

Preliminary investigations carried out at Central Tuber Crops Research Institute, Thiruvananthapuram indicated the presence of SPFM symptoms in sweet potato in Kerala. Most of the accessions in the germplasm collection of

CTCRI and plants in the farmers fields are infected by SPFMV. Detailed information on symptomatology and biological properties of the virus infecting sweet potato in Kerala is not available. Details on these aspects are necessary for formulating effective disease management strategies.

The unique importance of sweet potato as a subsidiary source of food in situation of food shortage and the economic impact of SPFM in this crop reported from other countries are the major reasons for taking up the study on the following aspects of the disease.

- Survey on the occurrence of the disease
- Symptomatology
- Transmission of the virus
- Physiological changes in sweet potato as a result of infection by SPFMV
- Biochemical changes caused by SPFMV- infection.
- Biometrical changes due to SPFMV- infection
- Characterization of the virus
- Host range of the virus
- Management of the disease using different methods

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Sweet potato feathery mottle virus (SPFMV) is one of the eight viruses that have been detected, identified and confirmed independently in sweet potato. SPFMV is a very important and widely disseminated of all known sweet potato viruses all over the world. This virus disease has been reported in all tropical and subtropical countries and in the warm places of temperate countries (Fuentes and Salazar, 2000). The sweet potato feathery mottle (SPFM) is also known as sweet potato russet crack, sweet potato internal cork, sweet potato chlorotic leaf spot and sweet potato virus A. The SPFM usually occurs as a mixed infection with other viruses. This virus was first reported by Doolittle and Harter (1945) in the United States of America and in Kenya, Tanzania and Uganda (Sheffield, 1957), when it was named as sweet potato vein clearing virus. Thankappan and Nair (1990) conducted a survey in Kerala and reported the occurrence of symptoms like ring spots and chlorotic spots in sweet potato fields. Kumar *et al.* (1991) identified the occurrence of SPFM in India.

2.1 Survey

During a survey of virus diseases of tuber crops, a mosaic disease of sweet potato was observed for the first time in India (Mahto and Sinha, 1978). Moyer *et al.* (1980) conducted a survey of commercial sweet potato fields in North Carolina (USA) and found two widely distributed strains of FMV on

cultivar Jersey, viz., one common strain caused only the foliar symptoms and the other RC - FMV which caused russet crack symptoms on the roots.

Chiu *et al.* (1982) found that among 320 sweet potato varieties in Taiwan, 285 were affected with virus diseases. In a survey of wild *Ipomoea* spp. as potential reservoirs of SPFMV in Louisiana (USA), *I. trichocarpa* was found as the perennial reservoir of SPFMV (Clark *et al.*, 1986).

Cohen *et al.* (1988) reported that in Israel, the double infection of sweet potatoes with SPFMV and cucumber mosaic virus (CMV) caused severe stunting and yellowing of plants. A survey conducted in Venezuela on viral infection of sweet potato showed that the diseased plants exhibited vein clearing and chlorotic spot on the leaves. Based on host range, morphology, physical properties and transmission it was concluded that sweet potato feathery mottle virus was the causal agent (Olivero *et al.*, 1989).

SPFMV was identified at eight locations in Ontario (Canada) in 1988 with moderate to high incidence of infection (Stobbs *et al.*, 1991). The International Potato Center (CIP), Lima, Peru conducted a survey in southern Peru, Argentina and Kenya and reported that 8.34% and 27.6% of the samples were infected with SPFMV in Peru and Kenya respectively and SPFMV was observed to be the most prevalent virus in Argentina (CIP, 1992).

Surveys on sweet potato diseases were conducted during 1989 - 1997 in five districts of Kerala, viz., Kasaragod, Kannur, Malappuram, Palakkad and Thiruvananthapuram besides CTCRI farm. Among the symptoms of viral diseases it was observed that chlorotic leaf spot, ring spot, feathering,

puckering, mosaic, leaf curl, leaf cupping, yellow netting, interveinal chlorosis and vein fasciation occurred in varying intensities (Thankappan *et al.*, 1998). In China, Shang Youfen *et al.* (1999) found that the infection rate of viral diseases in sweet potato ranged from 5 - 40 % when grown in the field. They also observed that the main symptoms were purple spots, ring spots, feathery mottle and chlorotic spots.

2.2 Symptomatology

Quite early SPFMV was used to denote the symptoms of leaf spotting and vein banding in sweet potato (Doolittle and Harter, 1945). Nusbaum (1945) reported that when SPFMV infected sprouts were transplanted in the field, the older leaves showed an indistinct pale yellow or green mottling after four to six weeks of normal growth as soon as the vine growth began to decline. Later on, a purple discolouration appeared at the margins of these discoloured areas and within a few days clearly defined purple ring spots appeared. As these spots coalesced, the leaves became chlorotic, eventually showing a dull bronze mottling and they soon fell off. When the leaves showed mottling, internal cork symptom was also invariably found in the roots. Miller (1955) reported that under field conditions, particularly in bright summer, the symptoms of feathery mottle virus in sweet potatoes became partially masked and only in the shaded portions of the plant, definite symptoms could be observed. Loebenstein and Harpaz (1960) reported that symptoms of the ring spot viruses were masked when temperature reached 26-28^{0}C in Jerusalem, Israel. Alconero (1972) reported that yellow spots occurred in cultivars with no apparent purple pigmentation or presence of

anthocyanin. Only purple rings developed in those having intense pigmentation and both these symptoms were seen in cultivars of slight purple colour. He also noticed that the symptoms of virus- infected sweet potato were conspicuous under direct light than under low light intensity in a glass house. SPFM disease was characterized by necrotic lesions on pencil roots of cv. Jersey. In cross protection trials, sweet potatoes infected by feathery mottle virus (FMV) were protected from appearance of russet crack (RC) symptom. Hence it was suggested that RC was caused by a strain of FMV (Campbell *et al.*, 1974)

Moyer and Kennedy (1978) reported that the symptoms of SPFM consisted of chlorotic leaf spot and vein mottling in *Ipomoea batatas* cv. Georgia Red. Moyer *et al.* (1980) observed that foliar symptoms in cultivars Jersey and Jewel of *I. batatas* were chlorotic vein banding and chlorotic spotting for both the strains of FMV and RC -FMV. They also found that during summer season of 1976, sweet potato in North Carolina showed symptoms such as chlorotic spots with or without purple borders, chlorotic vein banding and feathering. Moyer and Cali (1985) reported that foliar symptoms are generally mild and transient but may include vein clearing, feathering, chlorotic leaf spot, and spots with purple border particularly on older leaves. They concluded that symptoms on roots might include external cracking and internal necrosis depending on the cultivar.

Olivero *et al.* (1989) found that the SPFMV- infected plants showed vein clearing and chlorotic leaf spot on the leaves. Kumar *et al.* (1991) confirmed the association of SPFMV with the symptoms like faint chlorotic

leaf spot, distinct chlorotic leaf spot followed by expanding ring spots (RS) and the chlorotic leaf spot and RS which diffuse to cover entire leaf lamina. Stobbs *et al.* (1991) reported that the symptoms like chlorotic leaf spot often with purple borders and mild stunting of plants without root abnormalities were associated with SPFMV- infected sweet potato plants. It was suggested by Usugi *et al.* (1994) that a filamentous virus (designated VC) causing vein clearing in *I. nil* and RC in some Japanese sweet potato cultivar was a new strain of SPFMV. They indicated it as SPFMV-S (severe strain of FMV).

Fuentes and Salazar (2000) reported that certain cultivars infected with SPFMV showed the symptoms of vein clearing, mild and distinct chlorotic leaf spot and chlorotic leaf spot occasionally bordered by pigmented tissues. They also observed internal cork (IC) or external RC in /on roots of some cultivars.

2.3. Transmission

2.3.1 Through planting material

Sweet potato is usually vegetatively propagated by using vine cuttings of mature plants. SPFMV is perpetuated from one cropping cycle to the next through cuttings from infected plants and through the discarded vines which can easily establish, especially when the land is left fallow. This may also result in perpetuation of SPFMV between cropping cycles (Karyeija *et al.*, 1998). Field planting without any selection favoured the long and persistent method of transmission of internal cork virus (a strain of SPFMV) in sweet potato.

Sometimes reduction in SPFMV- infection in field plants may occur due to the absence of the virus in some of the cuttings taken from infected sweet potato plants (Nielson, 1981; Esbenshade and Moyer, 1982; Gibson *et al.*, 1997). Cadena – Hinojosa and Campbell (1981b) reported that SPFMV was unevenly distributed in the plant.

Geddes (1990) reported that the propagation of the crop by cuttings and the continuous presence of insect vectors make "viruses", the most important pathogens of sweet potato in Africa. Thankappan and Nair (1990) reported that symptoms of viral infection such as leaf roll, ringspot, chlorotic leafspot and yellow netting could be carried over to the next season through infected cuttings and indicated that the primary spread of the disease was through infected cuttings.

Different types of symptoms of viral infection, viz., ringspot, chlorotic leaf spot, mosaic, leaf curl, leaf cupping, yellow netting and fan leaf from different accessions of sweet potato germplasm at CTCRI, were maintained as virus culture through vegetative propagation (Thankappan *et al.*, 1998). Walkey (1991) reported that infection by most of the viruses is completely systemic and any propagule, viz., tubers, bulbs, corms etc are likely to be infected. The transmission of sweet potato virus diseases occurred through unharvested roots in the field (Karyeija *et al.*, 1998)

2.3.2 Sap transmission

Sap transmission trials with sweet potato viruses from sweet potato to sweet potato were by and large, unsuccessful. Clerk (1960) found that

attempts of mechanical transmission of sweet potato vein clearing virus into healthy shoots of sweet potato by using infected sap and celite, and infected sap with celite buffered by phosphate were not successful. Loebenstein and Harpaz (1960) reported that they were unable to transmit the sweet potato ring spot virus by mechanical means. The RC virus was not mechanically transmitted from sweet potato to sweet potato cv. Jersey Orange (Daines and Martin, 1964).

Hollings *et al.* (1976) reported that the sweet potato sap contained powerful inhibitors, which prevented the successful sap transmission to test plants. Cali and Moyer (1981) found that the common strain of FMV could not be transmitted to *I. batatas* cv. Jersey by mechanical means. Moyer and Kennedy (1978) could transmit the virus from *I. nil* to *I. batatas,* varieties, Jersey and Georgia red.

2.3.3 Insect transmission

Many insects, viz., thrips, jassids, aphids etc have been tested as probable vectors of sweet potato viruses. Loebenstein and Harpaz (1960) found that the sweet potato ring spot virus was readily transmitted by *Myzus persicae* (Sulzer) but not by the tobacco whitefly (*Bemisia tabaci* Gennadius).

The cotton aphid (*Aphis gossypii* Glover), groundnut aphid (*Aphis craccivora* Koch.) and green peach aphid (*Myzus persicae*) were reported as the efficient vectors of SPFMV (Schaefers and Terry, 1976; Moyer and Kennedy, 1978; Karyeija *et al* .,1998). Liao *et al.* (1979) observed that SPV - A was transmitted by *M. persicae* in a non- persistent manner. Neinhaus

(1980) reported that the feathery mottle syndrome of sweet potato was associated with an aphid- transmissible virus.

In laboratory studies in North Carolina, the RC - Strain of SPFMV (RC- FMV) was transmitted by *A. gossypii* in a non- persistent manner (Kennedy and Moyer, 1982). Stobbs *et al.* (1991) reported that FMV was transmitted in a non- persistent manner by *M. persicae.*

Feo *et al.* (2000) found that chlorotic dwarf of sweet potato is caused by the synergistic combination of two aphid- transmitted viruses, viz., SPFMV and sweet potato mild speckling virus, with a whitefly- transmitted virus (serologically related to sweet potato chlorotic stunt virus (SPCSV)). Gibson *et al.* (2000) also reported that sweet potato virus disease is caused by the dual infection by the aphid -borne SPFMV and the whitefly- borne SPCSV.

2.3.4 Graft transmission

Grafting is used as the most successful method of transmission of sweet potato viruses. Campbell *et al.* (1974) reported the formation of necrotic lesions in sweet potato cv. Jersey, after graft inoculation of virus- free plants with russet crack infected plants. Hahn *et al.* (1981) successfully used a core - graft transmission method for rapid screening for resistance to sweet potato virus disease complex.

Nielsen (1981) found that when the plants infected with sweet potato internal cork virus were graft inoculated to virus- free variety of sweet potato, they could successfully transmit the virus. Rossel (1983) reported that approach grafting of sweet potato plants to the test clone pre- infected with

the whitefly- transmitted virus resulted in symptom appearance. Brazilian isolate of SPFMV- infected sweet potato was transmitted by grafting to sweet potato. (Pozzer *et al.* , 1995).

2.3.5 Seed transmission

Majority of the plant viruses are not transmitted through seeds. Liao *et al.* (1979) observed that sweet potato yellow spot virus was not transmitted through the seeds. Cadena- Hinojosa and Campbell (1981a) showed that the seedling raised from seeds of SPFMV- infected *I. incarnata* plants did not develop any symptoms of viral infection.

The absence of seed transmission of SPFMV in sweet potato was also reported by Wolters *et al.* (1990). The seed transmission of SPFMV was unsuccessful through the seeds of *I. setosa* (Pozzer *et al.*, 1995) and *I. batatas* (Karyeija *et al.*, 1998) ,collected from SPFMV- infected plants .

2.4 Physiological Changes

The decrease in photosynthesis and an increase in respiration rates have been reported in virus- infected plants by many authors (Gates and Gudauskas, 1967; Jensen, 1968; Tu and Ford, 1968; Tu *et al.*, 1968; Leal and Lastra, 1984; Penazio and Roggero, 1999 and Herbers *et al.*, 2000). In papaya, mosaic symptoms caused by papaya ring spot potyvirus type P did not change the photosynthesis, respiration and transpiration rates significantly. However, the leaves with yellowing could not photosynthesize, but could respire and transpire at reduced rates (Gonzalez- Fernandez *et al.*, 1994).

Increase in photosynthetic rate and decrease in transpiration rate were observed by Du Xihua *et al.* (1999) in virus- eliminated sweet potato cultivars, Xushu No 18, Lushu No.7 and 8 and Beijing 553 when compared with the infected plants. Fuentes and Salazar (2000) found that smaller chloroplasts due to SPFMV associated SPVD caused yield reduction in sweet potato.

Decrease in net photosynthetic rate and evaporative rate were reported due to PVY infection in tobacco (Guo Xingqi *et al.*, 2000).

2.5 Biochemical changes

2.5.1 Carbohydrate and their fractions

An increase in total sugar content was reported by many workers in virus- infected plants (Sarkar *et al.*, 1989 ; Sohal and Bajaj ,1993 ; Prasad *et al* ., 1992; Sarma *et al* ., 1995). Singh and Singh (1984) observed that the southern bean mosaic virus (SBMV) infection decreased the total sugar and starch in cowpea cultivars. They also found that both SBMV and CPMV reduced the carbohydrate fractions, viz., total sugars, reducing sugars, non reducing sugars and starch in cowpea cv Pusa Dofsali. A significant reduction in starch content was observed in french bean leaves infected with bean common mosaic poty virus (Ravinder *et al.*, 1989). Radhika (1999) reported that the carbohydrate content increased in the leaves of resistant variety of cowpea (C0.6) but decreased in susceptible variety (Sharika) infected with black eye cowpea mosaic virus.

2.5.2 Chlorophyll

Reduction in total chlorophyll, chlorophyll a and chlorophyll b due to virus infection was reported in french bean (Ravinder *et al.*, 1989), cucurbits (Chakraborthy *et al.*, 1994), bhendi (Sarma *et al.*, 1995), soybean (Dantre *et al.*, 1996) and chilli (Singh *et al.*, 1998). A significant reduction in the leaf chlorophyll content of sweet potato cultivar TIS 1499 was found associated with SPVD- infection (Hahn, 1979). Olivero and Oropeza (1985) reported that the chlorophyll content of the plants grown from infected cuttings showing symptoms of SPFMV was low when compared to healthy plants.

2.5.3 Phenolic compounds

Total phenol content was reported to be high in virus- infected leaves of many plants (Chakraborthy *et al.*, 1994; Sarma *et al.*, 1995; Dantre *et al.*, 1996; Banerjee and Kalloo, 1998 and Srivatsava and Tiwari, 1998).

Higher quantities of phenolic compounds were recorded in hypersensitive cowpea leaves infected with tobacco ring spot virus (Sastry and Nayudu, 1988). Sohal and Bajaj (1993) reported that mung bean yellow mosaic virus- infection resulted in increased total phenol in both resistant and susceptible varieties. They also found that the OD -phenol and flavanol showed an increase in the resistant varieties when compared to susceptible ones.

Resistant cultivars had higher contents of phenol, OD- phenol and flavanol due to cotton leaf curl virus infection when compared to susceptible varieties (Gurdeep Kaur *et al.*, 1998). Radhika (1999) reported that there was

not much change in phenol content in both resistant and susceptible varieties of cowpea infected with black eye cowpea mosaic virus.

2.5.4 Protein

Increased protein due to virus infection was reported in bhendi (Sarma *et al.*, 1995), cowpea (Yadav and Sharma, 1987; Patil and Sayyad, 1991; Radhika, 1999) and tomato (Banerjee and Kalloo, 1998).

Singh and Suhag (1982) reported a decrease in protein content in mung bean and urd bean leaves infected with yellow mosaic viruses, mung urd yellow mosaic virus 1 and mung urd yellow mosaic virus 2.

2.5.5 Enzymes

Changes in the defense related enzymes as a result of infection by different viruses have been reported in many plants (Batra and Kuhn, 1975; Wagih and Coutts, 1982; Rathi *et al.*, 1986). Khatri and Chenulu (1970) found that the peroxidase (PO) activity increased due to cowpea mosaic virus infection in cowpea in both resistant and susceptible varieties. They have also observed that the activity was higher in susceptible variety than in resistant.

Studies on biochemical changes induced by bell pepper dwarf mosaic virus (BPDMV) in *Capsicum annuum* L. revealed increased levels of polyphenol oxidase activity in virus- infected leaves (Jitendra Pal *et al.*, 1989). Behl and Chowfla (1991) reported that activities of peroxidase (PO)

and polyphenol oxidase (PPO) activity were induced by necrotic strain of PVY in tomato in susceptible and resistant varieties.

Biochemical changes in leaves infected with mungbean yellow mosaic virus revealed that both resistant and susceptible varieties showed decreased phenyl alanine ammonia lyase (PAL), PO and PPO activity (Sohal and Bajaj, 1993).

Hot pepper plants systemically infected with hot pepper necrotic strain (HPNS) of TMV showed higher PO activity in infected as compared to healthy plants. It was found that PO activity preceded necrosis. There was a decline in PO activity between 49 and 63 days after inoculation (Savic *et al.*, 1997). Radhika (1999) reported that the activities of defence related enzymes like PO, PPO and PAL were higher in a cowpea variety (CO 6) resistant to black eye cowpea mosaic virus, ten days after inoculation as compared to the susceptible.

2.6 Biometrical studies

2.6.1 Vine and leaf characters

Hahn (1979) reported that there was a marked reduction in the leaf area, vine length, fresh and dry weight of stem and leaf in sweet potato cultivar TIS 1499 infected with sweet potato virus disease (SPVD).

There was a reduction in the weight of fresh stem and leaf by 7 % and 24 %, respectively, in sweet potato cultivars Tainung 57 and Tainung 63 infected by a virus complex, SPV- A and SPV- N (Liao *et al.*, 1983).

Olivero and Oropeza (1985) reported a severe reduction in the leaf area, number of leaves per plant, vine length and leaf yield in the plants infected with SPFMV. A study conducted by Yang *et al.* (1993) revealed that the vine length, fresh weight and number of roots per plant were greater in healthy when compared to SPVD- infected plants. An increase in weight of the leaf and vine was observed in healthy plants of sweet potato when compared to diseased (Kano and Nagata, 1999). Ma Daifu *et al.* (2000) reported that the SPVD free sweet potato showed vigorous vine growth and enhanced dry matter accumulation.

2.6.2 Changes in tuber characters

Increased yield in virus- free sweet potato plants was reported by many workers (Over de Lindon and Elliott, 1971; Joubert *et al.*, 1979 ; Yang *et al.*, 1993; Green *et al* ., 1992; Gibson *et al* ., 1997 ; Karyeija *et al.* , 1998).

Nome and Docampo (1976) found that the mean tuber yield of 25 healthy sweet potato plants was 1. 40 kg and it was only 0. 24 kg from 25 plants infected with sweet potato vein mosaic. A drastic reduction in the average weight of tubers, number of tubers per plant, yield and quality of the tubers was also observed by them in the infected plants when compared to healthy.

Hahn (1979) reported that the total and marketable fresh tuber yields were reduced by 78 % and 76% respectively in SPVD- affected plants. He also found that tuber dry matter content was not influenced by infection. There was a marked reduction in the weight of fleshy root by 33% and 31 %

respectively, in the sweet potato cultivars Tainung 57 and Tainung 63 infected by a virus complex (Liao *et al.*, 1983). Olivero and Oropeza (1985) observed 43.6 % reduction in fresh tuber yield of sweet potato plants grown from SPFMV- infected cuttings.

A yield reduction of 90 % was reported in clone 502, highly susceptible to SPVD complex (Ngeve, 1990). Ngeve and Bouwkamp (1991) observed a drastic reduction in tuber yield by 56 - 90 % in plants showing SPVD symptoms. They also found that the total and marketable tuber numbers were also reduced by the disease.

In Venezuela, symptomless, apparently healthy plants of cultivar USR P1 - 20 yielded twice as that of plants with SPFMV symptoms (Pozzer *et al.*, 1995). Karyeija *et al.* (1998) reported that economic loss may be associated wth external cracking and internal corkiness making the tuberous roots unmarketable. Liao *et al.* (1982) found that there was no difference in protein, sugar and starch contents of tubers in the diseased plants infected by a virus complex (SPV- A and SPV- N).

2.7 Characterization

2.7.1 Purification

Purification is essential for characterization and identification of viruses.

SPFMV was purified by many workers (Campbell *et al.*, 1974; Cadena - Hinojosa and Campbell, 1981a; Nakashima *et al.*, 1993; Pozzer *et al.*, 1995).

Moyer and Kennedy (1978) reported that the infectivity of partially purified SPFMV preparations obtained from infected *I. nil* leaves was attained by 0.05 M sodium borate buffer (pH 8.0) with the addition of the chelating agent DIECA (0.01 M) and n- butanol (8ml / 100 ml of buffer).

SPFMV- infected *I .nil* leaves were used for purifying the virus by many workers (Moyer and Kennedy, 1978; Cali and Moyer, 1981; Usugi *et al*; 1994; Zhu *et al*; 1994). Cali and Moyer (1981) reported that urea and Triton X - 100 were essential in the resuspension buffer to suspend the final pellet and prevent aggregation of virions formed during purification of RC strain of SPFMV.

Cohen *et al.* (1988) purified the virus from infected sweet potato leaves using low speed centrifugation followed by cesium chloride gradient centrifugation. Purification of a Peruvian isolate of SPFMV was done by Nakashima *et al.* (1993) by using polyethylene glycol precipitation, ultra centrifugation on a sucrose cushion and cesium chloride gradient centrifugation.

2.7.2 Serology

Many workers have succeeded in producing antiserum against SPFMV. (AVRDC, 1984; CIP, 1989; Abad and Moyer, 1992; Usugi *et al.*, 1994).

Moyer and Kennedy (1978) produced antiserum to a purified preparation of SPFMV which had a titre of 1: 1024 in microprecipitin test and used it in enzyme linked immunosorbent assay (ELISA).

Antisera were prepared for four isolates of aphid- transmitted sweet potato viruses, viz., feathery mottle, russet crack, internal cork and chlorotic leaf spot by Cadena- Hinojosa and Campbell (1981a). They produced antiserum by injecting rabbits intramuscularly with purified virus at 8- 10 day intervals and the rabbits were bled by cardiac puncture ten days after last injection.

Cali and Moyer (1981) administered the purified SPFMV common strain virus subcutaneously and also intramuscularly to Newzealand white rabbits at five day intervals for six weeks for the production of high titre virus specific antiserum.

2.7.3 Serological detection

Moyer and Kennedy (1978) performed double diffusion tests and found protein bands at an antiserum dilution of 1 : 16 with SPFMV infected extracts and no precipitin lines in healthy extracts. A dot - blot immunobinding assay was developed by Moyer (1986) for the detection of SPFMV in sweet potato. They reported that relatively low titre and irregular virus distribution and the high concentration of interfering substances such as phenolics and latex in sweet potato tissue had prevented the adoption of DAS - ELISA.

Abad and Moyer (1992) performed membrane immunobinding assay and direct blotting of tissue sap for the detection of SPFMV. Hammond *et al.* (1992) used polyclonal and monoclonal antibodies to examine the serological relationships among three filamentous viruses, viz., SPFMV, sweet potato

mild mottle virus and sweet potato latent virus of sweet potato and found that there was no significant relationship between them.

Li *et al.* (1992) prepared hybridomas which produced monoclonal antibodies for the detection of SPFMV and successfully performed NCM - ELISA to detect the virus.

Makeshkumar *et al.* (1999) performed NCM – ELISA for the detection of different sweet potato viruses including SPFMV in CTCRI germplasm.

2.7.4 Physical Properties

Physical properties of SPFMV were studied by many virologists. Moyer and Kennedy (1978) reported the physical properties of SPFMV in *I. nil* sap, viz., thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) as 60-65^0 C, 10^{-3}- 10^{-4} and <12 hours, respectively. The same properties were found by Olivero *et al.* (1989) in a virus-infected sweet potato sap and based on this finding they reported that SPFMV was the causal agent.

Usugi *et al.* (1994) found that the infectivity of a new strain of SPFMV that causes RC of fleshy roots of some Japanese cultivars of sweet potato was lost at a dilution between 1: 10, 000 and 1: 100, 000 after storage for one day at 20^0 C and the TIP was between 50 - 60 ^{0} C.

Pozzer *et al.* (1995) and Brunt *et al.* (1997) reported the same DEP (10^{-3} - 10^{-4}) and TIP (60 - 65^0 C) but different LIV as < 1 h and 7- 12 h, respectively, for SPFMV infecting sweet potato.

2.7.5 Electron Microscopy

The particles of RC -FMV were reported as flexuous rods of 800 nm in length (Campbell *et al.*, 1972) and FMV as 810 - 860 nm in length (Moyer and Cali, 1985). Hammond *et al.* (1974) isolated flexuous rod particles of 700 nm size from the foliage of sweet potato plants, produced from roots with internal hardening or hard core.

Moyer and Kennedy (1978) found 830 - 850 nm long flexuous rods in electron microscopic observation of leaf dip preparation as well as purified preparation of SPFMV negatively stained with 2% phosphotungstic acid (pH 7.0).

Cadena- Hinojosa and Campbell (1981 b) reported that FMV was readily detected by serologically specific electron microscopy in partially purified virus preparation from infected *I. incarnata* and *I. batatas* leaves with symptoms and the virus was absent in leaves from plants without symptoms. They also reported that normal lengths of the flexuous particles of the isolates of four aphid- transmitted sweet potato viruses, viz., feathery mottle virus, russet crack virus, internal cork virus and chlorotic leaf spot virus were 829$\pm$ 38, 834 $\pm$ 39, 839 $\pm$ 38 and 845 $\pm$ 32 nm, respectively.

SPFMV in Venezuela was reported as flexuous rods of 840 nm long (Olivero *et al.*, 1989). Kumar *et al.* (1991) detected SPFMV through electron microscopy (leaf dip preparation) and immunosorbent electron microscopy in infected germplasm accessions which showed chlorotic spots, ring spot, and feathering symptoms. Usugi *et al.* (1994) performed the serologically specific

electron microscopy of SPFMV using protein A-gold and observed the filamentous flexuous virus particles.

Attempts were made to view the causal viral particles associated with different symptoms such as feathering, ring spot, chlorotic spot, leaf curl, leaf cup and mosaic under EM employing the leaf dip preparation method using 2% uranyl acetate for staining. (Thankappan *et al.*, 1998). They observed long flexuous rod shaped virus particles of about 750 nm size, only in leaf samples exhibiting feathering, ring spot, chlorotic spot and mosaic.

2. 8 Host range

Host range studies provide useful information on the survival and spread of viruses. Campbell *et al.* (1972) reported that the sweet potato russet crack virus caused foliar symptoms on *I. setosa, I. nil* and *I. tricolor* similar to those by SPFMV and was mechanically transmissible to hosts of the family Convolvulaceae.

The host range of SPFMV and the symptoms on *Ipomoea* spp. susceptible to SPFMV were studied by Moyer and Kennedy (1978). They inoculated 27 species of *Ipomoea* with the sap extracted in 0.05 M potassium phosphate buffer (pH 7.2) containing 0.01 M DIECA and observed that only eight species, viz., *I. nil, I. setosa, I. tricolor, I. lacunosa, I. hederaceae, I. purpurea, I. wrightii, I. batatas* cv. Georgia Red and *I. batatas* 'Jersey' were infected. They also found that *Chenopodium amaranticolor, Vigna unguiculata, Datura stramonium, Nicotiana tabacum* Burley 21 and *N. glutinosa* were non - hosts. While working on the FMV strain of SPFMV Moyer *et al.*

(1980) and Cali and Moyer (1981) also found that it did not infect *Chenopodium* spp. Further they have reported that the symptoms on *I. batatas* cultivars. Jersey, Jewel and other *Ipomoea* spp. were chlorotic vein banding and chlorotic spots for both FMV and RC strains.

Clark *et al.* (1986) found that a wild species of *Ipomoea*, viz., *I. trichocarpa* was a potential reservoir of SPFMV in Louisiana. Among 14 *Ipomoea* spp. and three *Merremia* spp. collected from sweet potato fields of Venezuela and inoculated with SPFMV, ten species of *Ipomoea, viz., I. nil, I. tiliaceae, I. fistulosa, I. alba, I. hederaceae, I. carnea, I. purpurea, I. tricolor, I. wrightii and I. setosa* showed the symptoms of infection (Olivero and Trujillo- Pinto, 1989).

Usugi *et al.* (1994) observed that *Ipomoea* spp. showed different types of symptoms like vein banding , chlorotic spots, vein chlorosis and mosaic and only chlorotic spots were seen in *C. amaranticolor* and *C. quinoa* when they were inoculated with severe strain of SPFMV. They have also found that *N. tabacum* (White Burley, Samsun, Havana, Xanthi), *N. benthamiana, N. glutinosa, N. debney and Datura stramonium* are non- hosts to the virus.

Pozzer *et al.* (1995) found that the host range of SPFMV was limited to species of Convolvulaceae and it was transmissible by mechanical inoculation. It was also reported that the host range of SPFMV was restricted to the family Convolvulaceae *(Ipomoea* spp.) and Chenopodiaceae *(C. amaranticolor* and *C. quinoa*) and some strains infect species of Solanaceae (Karyeija *et al.*, 1998; Fuentes and Salazar, 2000).

2.9 Management of the disease

2.9.1 Screening for resistance

The reaction of sweet potato varieties to internal cork range from highly susceptible to highly resistant and resistance to this virus disease is readily transmitted to sweet potato progeny (Nielsen and Pope, 1960).

The reaction of sweet potato seedlings to the RC strain of FMV was investigated by Arrendell and Collins (1986). They reported that among 501 open pollinated seedlings from nine parental clones inoculated with this virus under greenhouse conditions, 64 per cent showed foliar symptoms, root symptoms or both.

Results of the screening trials conducted in Delhi with the sweet potato accessions in the CIP germplasm collection for immunity to SPFMV showed that out of 1641 accessions, 30 were free from SPFMV infection (CIP, 1989). When the different accessions in the germplasm collection of CIP were inoculated with four isolates and three strains of SPFMV, 13 were found resistant and these accessions when exposed to field conditions showed that four maintained resistance in the field. Three sweet potato clones, viz., Nemantee, Jewel and IITA Tis 2498 were found resistant to C and C-1 strains of SPFMV (CIP, 1991).

Kumar *et al.* (1991) screened 48 germplasm collections of NBPGR, New Delhi which were maintained under greenhouse conditions for virus infection and reported that 38 developed faint chlorotic spot and mild mottle

symptoms. It was confirmed as SPFMV through electron microscopy and immunosorbent electron microscopy.

Heisswolf *et al.* (1994) reported that the mother stocks of four sweet potato cultivars (Rojo Blanco. Resisto, Lo - 323 and Red abundance) were free from SPFMV but 11 per cent of the same cultivars were infected with the virus when screened by grafting with *I. setosa*.

It was reported that in field trials with 23 sweet potato cultivars at four locations in Nigeria, resistance to SPVD was identified a rapid method of screening by core- grafting of roots (Karyeija *et al.*, 1998).

Thankappan and Nair (1990) reported that among 789 germplasm entries screened at CTCRI thoroughly for two seasons for the presence of symptom of viral infection, 24 lines exhibited typical ring spots and eight lines chlorotic leaf spots. They did not find any symptoms on tuber in the lines tested.

Thankappan *et al.* (1998) screened 846 sweet potato accessions maintained in germplasm collection at CTCRI for various diseases based on symptoms. They found ring spot and feathering symptoms in 373 accessions, chlorotic spots in 343, puckering in 15 and mosaic in 23 entries.

2.9.2 Thermotherapy

In 1962, Hildebrand reported that among 100 selections affected by sweet potato internal cork virus stored at 38 ^{0} C for three months, 63 yielded

some virus- free cuttings. The remaining 37 when heat-treated again at 38^0C for six months, virus- free cuttings were obtained from 31 of them.

Hildebrand (1964) observed that varieties of sweet potato showed difference in the effect of heat on internal cork virus infection. He also found that continuous exposure of the planting materials to 38^0C for three months was ineffective in some varieties and the cuttings of the varieties Heartogold and Puerto Rico were virus- free after heat treatment at the same temperature for five months and six months, respectively.

In China, Liao and Chung (1979) found that a virus causing yellow spotting of sweet potato in a variety Tainung 63 was not eliminated from 50 mm long terminal shoots which were kept in a growth chamber at 38 - 42 ^{0}C for 30 - 90 days. Liao *et al.* (1982) reported that the duration of constant heat treatment of plants infected with sweet potato virus A and sweet potato virus N at 38^0 C was positively correlated with the number of virus- free seedlings obtained by shoot tip culture.

Gama (1988) also obtained virus-free plants of 15 sweet potato cultivars by heat treatment at 30 - 37 ^{0} C combined with meristem tip culture.

Heat treatment of the stem tip explants up to 2.5 cm below the meristems of rooted stem cuttings were reported to be free of sweet potato yellow dwarf virus (Green and Lo, 1989).

2.9.3 Induction of systemic resistance using chemicals

Application of chemicals for the induction of systemic resistance in various plants to virus diseases was reported by many workers (Coutts and Wagih, 1983; Caner *et al.*, 1985; Kovalenko *et al.*, 1993). Exogenous application of salicylic acid increased the resistance of several plants to many types of pathogens (Enyedi *et al.*, 1992)

Salicylic acid inhibited the systemic multiplication of alfalfa mosaic virus in Samsun NN. tobacco (Van Huijsduijnen *et al.*, 1986).

White *et al.* (1986) reported that the chemicals salicylic acid, manganese chloride and barium chloride induced resistance in Xanthi-nc tobacco leaves to tobacco mosaic virus infection.

Salicylic acid at a concentration of 10 mM induced resistance in cucumber to cucumber mosaic virus by reducing the concentration of virus particles by 83.3% (Nabila, 1999).

Jayashree *et al.* (1999) found that among the virus inhibitory chemicals tested for their efficacy in controlling pumpkin yellow vein mosaic virus in pumpkin (cv. CO-2), acetyl salicylic acid recorded the least virus transmission followed by barium chloride.

Bioassay of chemicals, viz., salicylic acid, manganese chloride and barium chloride to evaluate their efficacy in reducing the symptoms caused by cowpea aphid-borne mosaic virus in *Chenopodium amaranticolar* and cowpea,

revealed that the post inoculation treatment of manganese chloride gave maximum inhibition of the symptoms (Radhika, 1999).

Pun *et al.* (2000) reported that among salicylic acid, manganese chloride and barium chloride sprayed exogenously on bhendi plants infected with bhendi yellow vein mosaic virus, barium chloride was most effective in reducing the symptoms followed by acetyl salicylic acid (200 ppm) and salicylic acid (500 ppm) which registered a reduction of 75 and 73.3 per cent respectively, over control. The induction of resistance due to the application of chemicals might be due to the production of PR-proteins (Malamy *et al.*, 1990; Yalpani *et al.*, 1991)

2.9.4. Meristem culture

Meristem culture is the most effective method of eliminating viruses from sweet potato plants.

Meristem tip culture of sweet potato plants was performed by many workers as a method of elimination of viruses from planting materials (Nome and Salvadores, 1980; Frison and Ng, 1981; Kuo *et al.*, 1985; Love *et al.*, 1987; Green *et al.*, 1992., Mukherjee *et al.*, 1993; Nair and Govindankutty, 1998., Shang Youfen *et al* ., 1999).

Alconero *et al.* (1975) developed complete plants of sweet potato from meristematic tips (0.4 - 0.8mm) of axillary shoots of ten sweet potato cultivars within 20 - 50 days in a modified Murashige and Skoog (MS) medium supplemented with kinetin : IAA in the ratio of 0.5: 0.2.

A nutrient medium that could support the meristem culture of sweet potato was MS + inositol (100 mg)+ thiamine HCl (2 mg) + NAA (2 mg) and a pH of 5.7 for shoot initiation and MS + Inositol (100 mg) + thiamine HCl (2 mg)+ Sucrose (30 g) + agar (7 g) without benzyl amino purine (BAP) and naphthalene acetic acid (NAA) for multiplication/ propagation (Govindankutty, 2000).

2.9.5 Storage

Hildebrand (1968) reported that storage of sweet potato plants at 38 ^{0}C for three months was ineffective in eliminating internal cork virus. But they reported that similar exposure for six months would eliminate the virus from mother plants and the plants of 'Heartogold' variety appeared virus free after a treatment of five months at the same temperature.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The studies on sweet potato feathery mottle (SPFM) detailed herein were carried out during 1998-2001. The experiments in laboratory, glass house and pot culture were conducted at College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram which is situated at 8.5^0 north latitude, 76.9^0 east longitude at an altitude of 29 metres above mean sea level. The survey was conducted in the farmers' fields (Plate 1) in Thiruvananthapuram District, Kerala having an area of 2192 sq km with 40 ha under cultivation of sweet potato.

3.1 Source and maintenance of SPFMV culture

Vine cuttings of three varieties, viz., Sree Bhadra, Sree Rethna and Sree Vardhini released by CTCRI and two local varieties, viz., Vavvathooki and Poochedichuvala from farmers' field which showed typical symptoms of SPFMV, viz., chlorotic leaf spot, pink spot, ring spot and feathering served as the initial virus source. The culture was maintained by periodical planting of cuttings from the source plants and the cuttings (20- 25 cm length) were planted in earthen pots of 30 cm diameter. The growth medium was 1:1:1 pot mixture comprising of sand, soil and cowdung. These plants were used for further studies.

Plate 1 sweet potato- view in farmer's field

3.2 Survey

A detailed survey was conducted in the major sweet potato growing areas of Thiruvananthapuram district. The stratified three stage random sampling was adopted in the survey. Krishibhavans (extension unit of Department of Agriculture- Government of Kerala) having more than two hectares under sweet potato cultivation were identified. The cultivation was prevalent in the irrigated low land areas of Kalliyoor, Vizhinjam, Venganoor, Balaramapuram, Pallichal and Kottukal Krishibhavans as summer rice fallow crop during January- April.

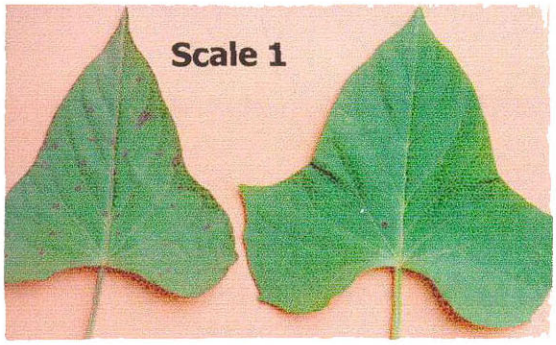
From each Krishibhavan, two localities and from each locality two farmers were selected at random. Observation was recorded from one holding of each farmer in four quadrats of 3 m x 1 m size. In all, ninety six plots were selected. The data were collected after one month of planting and at the time of harvest.

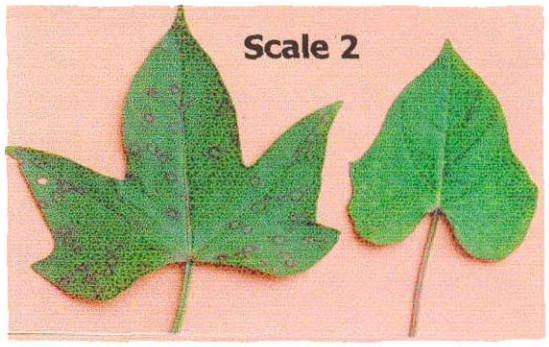
3.2.1 Varietal reaction

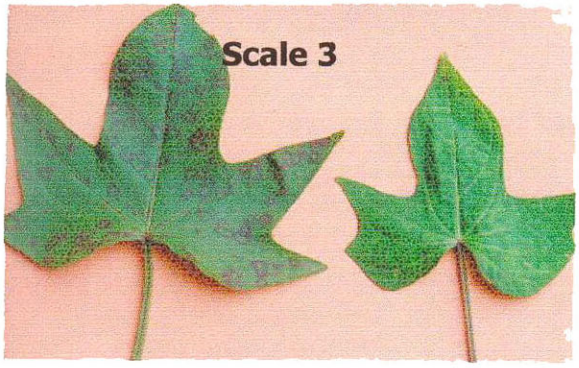
3.2.1.1 Disease intensity

The percent disease index (PDI) of all the local varieties were calculated by taking ten plants from each variety using 0-4 scale (Plate 2).

The PDI was calculated after Mayee and Datar (1986) using 0-4 scale as described below.







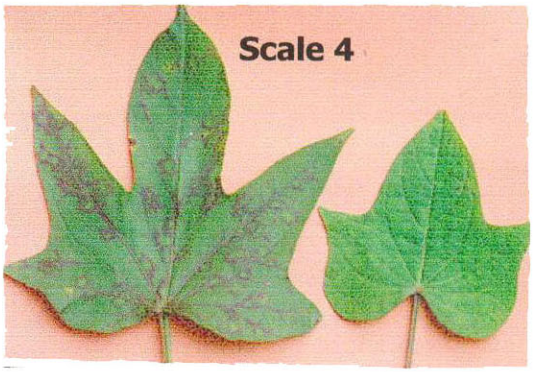


Plate 2 0-4 scale for scoring SPFMV

0- No symptom

1-Faint chlorotic leaf spot (CLS)/ pink spot (PS)

2- Distinct chlorotic spot /ring spot (RS)/ faint mosaic

3- Ring spot and slight feathering/ distinct mosaic

4- Severe feathering/ distinct mosaic and puckering.

$$PDI = \frac{\text{Sum of grades of each leaf}}{\text{Number of leaves assessed}} \times \frac{100}{\text{Maximum grade}}$$

The varieties were also tested using NCM-ELISA for the presence of virus.

3.2.1.2 Procedure for NCM-ELISA (Nitrocellulose membrane – enzyme- linked immunosorbent assay)

The test was carried out as per the protocol standardized by CIP (2000), using the CIP NCM-ELISA KIT.

The leaf samples were collected in polythene bags. A composite sample from each plant to be tested was made by taking one leaf each from the top, middle and bottom levels.

From each leaf sample a disc of approximately 1 cm diameter was cut by keeping the leaf within the polythene bag. Using a small test tube, the leaf disc was cut by exerting pressure outside the polythene bag. The remaining part of leaf was discarded. The leaf discs were ground with 3 ml of extraction buffer inside the bag using round-bottomed glass test tube. The bag was kept for 30-45 minutes at room temperature (28± 4^{0}C) till the plant sap got separated.

A dry sheet of filter paper was spread on a table and a nitrocellulose membrane was placed on the top of it. A grid (1.5 cm x 1.5 cm) was drawn on the 7 cm x 6 cm NCM (pore size 0.45 μm) with pencil. Using a sterile pasteur pipette, a drop (15-20 μl) of the clear supernatant from the bag was placed on to the centre of the square on the NCM. Sterile pipettes were used to spot the samples and the spots were dried using a hair dryer.

The NCM was immersed in 30 ml of the blocking buffer for one h. It was transferred to Tris-buffered saline (TBS) for a brief wash. The TBS was removed and the antibody solution, prepared by mixing 0.1 ml of polyclonal antibodies of SPFMV(CIP- NCM ELISA kit) with 30 ml of antibody buffer was added to the petri dish. Then the membrane was incubated overnight in a sealed petri dish on a shaker at 50 rpm at a temperature of 28 ± 4^0 C. The antibody solution was removed and the membrane was washed with constant agitation in 30 ml of TBS-Tween (T-TBS) (0.5 ml of Tween-20/l of TBS) for three minutes and the washing was repeated four times.

The T-TBS was removed and the membranes were dried between layers of blotting paper on a flat surface and pressed gently to remove any excess solution. The membrane was placed in a conjugate solution for one hour. The conjugate solution was prepared by mixing the alkaline phosphatase conjugated goat anti rabbit IgG (1:1000) with 30 ml conjugate buffer.

The conjugate solution was removed and the membrane was washed in 30-ml of T-TBS for three minutes and the washing was repeated four times.

The NBT (p-nitro blue tetrazolium) BCIP (p-toluidine salt of 5-bromo-4 chloro-3-indolyl phosphate) substrate solution was added to the membrane in the petri dish and incubated for one hour at room temperature.

Membranes were then washed three times by immersing them in distilled water for ten minutes. The water was changed three times. The membrane was placed on dry filter paper and dried. It was observed for positive reactions, i.e., appearence of bluish purple spots.

The composition of buffers and solutions are given in Appendix I.

3.2.2 Types of foliar symptoms

Observation was made on different types of symptoms developed in different varieties

3.2.3 Disease incidence

Disease incidence was calculated by recording the number of plants infected out of the total plants in each plot.

3.2.4 Disease intensity on leaves

The PDI was calculated for individual plots as described in 3.2.1.1. The data were analysed statistically.

3.2.5 Presence of vectors

Observation was made in each plot for the presence of insect vectors.

3.2.6 Tuber symptoms

The tubers of different varieties were observed for the presence of symptoms during harvest.

3.3 Symptomatology

3.3.1 Natural condition (without shade)

Studies on symptomatology were carried out using four varieties of sweet potato, viz., Sree Bhadra (SB), Sree Rethna (SR), Vavvathooki (VT) and Poochedichuvala (PC). The vine cuttings (20-25 cm length) of these varieties were planted in earthen pots (30 cm diameter) to study the different types of symptoms. The plants were observed for the appearance of the disease and intensity was scored at different intervals, viz., 30, 60, 90 and 105 days after planting. Nine cuttings were planted for each variety at the rate of three cuttings per pot. All the plants were pruned after 90 days. The PDI was calculated as under 3.2.1.1 and analysed statistically.

3.3.2 Under different intensities of shade

Effect of different intensities of shade on symptomatology was studied in two varieties of sweet potato, viz., Sree Rethna and Vavvathooki using shade nets providing 0, 25, 50 and 75 per cent shade. The vine cuttings of these varieties were planted in earthen pots of 20 cm diameter to study the expression of symptoms in different shades. The plants were observed for the appearance of the disease and disease intensity was recorded after 30 and 60 days of planting using 0-4 scale. Twelve cuttings were planted for each

variety at the rate of three cuttings per pot. The PDI was calculated as mentioned earlier. The sprouting time, first appearance of symptoms and major symptoms were also observed.

3.4 Transmission

3.4.1 Through vine cuttings

Single node (SN), double node (DN) and triple node (TN) cuttings of the four different varieties, viz., Sree Bhadra, Sree Rethna, Vavvathooki and Poochedichuvala of infected sweet potato plants were planted in earthen pots to study the transmission of SPFMV through vine cuttings. Nine cuttings were taken for each treatment and planted at the rate of three cuttings per pot. The development and nature of symptoms were observed regularly.

3.4.2 Tuber transmission

Tubers were collected from four varieties, viz., Sree Bhadra, Sree Rethna, Vavvathooki and Poochedichuvala of sweet potato plants infected by SPFMV and planted in earthen pots to observe the nature and development of symptoms after sprouting in the plants. Four tubers were planted in each of three pots from each variety and the development of symptom was observed and recorded.

3.4.3 Sap transmission

3.4.3.1 Transmission from sweet potato to sweet potato

Leaves of Sree Rethna exhibiting symptoms of SPFMV served as the virus source. Mechanical transmission of the virus was attempted using the

sap extracted with different buffers such as 0.1 M sodium borate buffer (pH 8.0), 0.05 M and 0.1 M potassium phosphate buffer (pH 7.2) with and without sodium diethyl dithiocarbamate (DIECA), 0.1 M sodium phosphate buffer (pH 7.2), 0.1 M citrate buffer (pH 7.2) and 0.1M Tris buffer (Tris(hydroxymethyl)amino methane) (pH 7.2) (Appendix II). Twenty cuttings were planted in five pots at the rate of four in each pot. Fifteen-day-old healthy sweet potato plants (variety Celopia) were used for the study.

For inoculation, one part of leaf tissue was homogenized with one part of cold buffer (w/v) using a chilled mortar and pestle. The homogenate was filtered through a thin layer of absorbent cotton to remove large cellular debris and maintained in the chilled condition. Leaves at the middle portion (fifth to eighth leaf) of the plant were inoculated. Prior to inoculation, leaves were uniformly dusted with carborundum powder (600 mesh). Test plants were inoculated with the forefinger moistened with the inoculum, by gently rubbing on the upper surface of the leaves. The inoculated leaves were washed after ten seconds with distilled water. The plants were observed daily for the development of symptoms up to two months.

3.4.3.2 Transmission from *Ipomoea nil* to sweet potato

The SPFMV- infected *I. nil* leaves were used as the virus source. The procedure for sap extraction and inoculation was similar to that employed in the previous experiment. Potassium phosphate buffer 0.05 M (pH 7.2) alone was used for sap extraction. The sap of SPFMV- infected *I. nil* was inoculated

on to ten sweet potato plants of the variety, Sree Rethna. The percentage infection and development of symptoms were recorded.

3.4.4 Insect transmission

Insect transmission studies were conducted using three species of aphids, viz., *Aphis craccivora* Koch, *A. gossypii* Glover, and *Pentalonia nigronervosa* Coquerel and one species of whitefly, *Bemisia tabaci* Gennadius (Plate 3).

3.4.4.1 Mass culture of aphid species

The three species of aphids, *A. craccivora*, *A. gossypii* and *P. nigronervosa* were reared on cowpea (*Vigna unguiculata*), brinjal (*Solanum melongena*) and banana (*Musa paradisiaca*), respectively. The potted plants were placed inside insect proof cages and the pure culture of the insects were released upon the plants for their multiplication. Old plants inside the cages were replaced with healthy young plants from time to time.

3.4.4.2 Mass culture of *B. tabaci*

Pure culture of *B. tabaci* was raised and maintained on brinjal plants. The potted plants were placed inside the insect proof cages and pure culture of *B. tabaci* was released for multiplication. Old plants were replaced with healthy young plants from time to time.

3.4.4.3 Handling of aphids

The aphids were handled using moistened camel hair brush (No: zero) and care was taken not to cause injury to the stylet.

Aphis craccivora

Aphis gossypii

Pentalonia nigronervosa

Bemisia tabaci

Plate 3 Insect species used for transmission studies

3.4.4.4 Handling of whiteflies

An aspirator consisting of a glass tube (30 cm length and 0.5 cm diameter) and a rubber tube (45 cm length and 0.5 cm diameter) was used for handling of whiteflies. By turning the leaves slightly upwards, the whiteflies were gently sucked into the glass tube of the aspirator. Whiteflies, thus collected, were subsequently used either for acquisition or inoculation feeding.

3.4.4.5 Acquisition and inoculation of the virus

For aphid transmission, groups of 20 aphids were starved for one hour and allowed five min of acquisition access period (AAP) on detached infected sweet potato leaves and then allowed an inoculation access period (IAP) of 24 h on 15-day-old healthy sweet potato plants (varieties, Sree Vardhini and Sree Rethna), *I. nil* and *I. setosa* plants.

For whiteflies, AAP of 24 h was given on 40 to 60 -day-old diseased sweet potato plants and later they were transferred to healthy sweet potato plants for inoculation feeding. The IAP was 24 h.

After IAP, the insects were killed by spraying the plants with 0.1 % Nuvacron. Per cent transmission and nature of symptoms were recorded. The plants were observed for the development of symptoms.

3.4.5 Graft transmission

Two varieties, Sree Bhadra and Sree Rethna were selected as diseased stocks and three types of grafting, viz., approach grafting, side grafting and

wedge grafting were performed with the variety, Celopia, as healthy scion. Parafilm was used to wrap the grafted portion. The grafted plants were covered with polythene bags for ten days to maintain humidity for successful union.

Per cent transmission, nature and development of symptoms were recorded.

3.4.6 Seed transmission

Seeds were collected from SPFMV infected *I. batatas* varieties, Sree Bhadra (250 Nos) and Sree Rethna (90 Nos) *I.nil* (375 Nos) and *I. setosa* (65 Nos) plants. Scarification of the seeds was done by immersing the seeds in concentrated sulphuric acid (98%) to a depth of 0.5 cm. After 15 min, the acid was poured off, the seeds rinsed three to four times with tap water and the peel remaining attached to the seeds was physically removed.

The scarified seeds were kept in sterilized petri dishes in which six layers of ordinary moistened filter paper was placed. After keeping the seeds, they were again covered with another layer of moistened filter paper on the seeds. Excess water was drained. After germination (four days) seedlings were planted in earthen pots (10 cm) and observed for the development of symptoms up to three months. The samples were collected randomly from the seedlings which do not show the symptom and tested through NCM- ELISA (3.2.1).

3.5 Physiological changes

The healthy and SPFMV- infected cuttings of Sree Rethna and Sree Vardhini were used for this experiment. Four cuttings in each of the healthy and infected plants of the two varieties were planted at the rate of one cutting per pot.

The photosynthetic CO_2 uptake, respiration rate and transpiration were measured using LCA 4 (ADC UK) portable CO_2 analyzer after 45 days of planting. The dark respiration rate was recorded after keeping the plants in dark for 30 min. The data were statistically analysed to find out whether there is any significant difference between infected and healthy plants.

3.6 Biochemical studies

Biochemical analyses of the leaves of two susceptible varieties (Sree Rethna and Vavvathooki) and a tolerant variety (Sree Vardhini) were carried out.

The cuttings of healthy and SPFMV- infected plants of all the varieties were planted in earthen pots of 35 cm diameter. Nine cuttings of each variety were planted at the rate of three cuttings per pot and kept in insect proof house.

Leaf samples were taken at 15, 30 and 45 day intervals after planting. Samples were taken from fifth to tenth leaf of the vine. Biochemical analyses were done to estimate the changes in total carbohydrates, starch, total sugars, chlorophyll, total phenols, OD-phenols, flavanols, protein and activities of

defense related enzymes like peroxidase, polyphenol oxidase and phenyl alanine ammonia lyase. Three replications were maintained for each sample.

3.6.1 Estimation of total carbohydrates

Total carbohydrate content was estimated by anthrone method (Hodge and Hofreiter, 1962). One hundred milligrams of leaf sample was weighed in to a boiling tube and hydrolysed with five ml of 2.5 N HCl by keeping it at 100^{0}C in a water bath and then cooled to room temperature. The hydrolysate was neutralised with solid sodium carbonate ($Na_2 Co_3$) until the effervescence ceased. Later the volume was made up to 100 ml with distilled water and centrifuged at 10,000 rpm for ten min. To 0.5 ml of the supernatant, 0.5 ml of distilled water and four ml of anthrone reagent (200 mg anthrone in 100 ml of ice cold 95 per cent H_2SO_4) were added. The reaction mixture was heated for eight min at 100^{0}C in a water bath and cooled rapidly. The colour of the solution became green to dark green, the absorbance of which was determined at 630 nm using a Systronics UV-VIS spectrophotometer 118. Amount of carbohydrate present was calculated from standard curve prepared using D-glucose and expressed in terms of milligrams of glucose equivalent per gram of leaf tissue on fresh weight basis.

3.6.2 Estimation of starch

One hundred milligrams of leaf sample was ground with 25 ml of 80 per cent ethanol and the extract was heated in boiling tube in a water bath at 80-85 ^{0}C for 10 min. It was then centrifuged at 5000 rpm for 15 min

and the supernatant was decanted into a beaker. The extraction was repeated twice and supernatant was pooled and saved to determine the total sugars.

Five ml of water was added to the residue left in the centrifuge tubes followed by 6.5 ml of perchloric acid (52%). The contents were constantly stirred for five minutes with a glass rod and occasionally for the next 15 min. Twenty ml of water was added to this and centrifuged. The supernatant was decanted into 100 ml volumetric flask. Again the extraction was repeated by adding five ml each of water and perchloric acid, stirring occasionally for the next 30 min. The contents of the tube were transferred into a volumetric flask and made up to 100 ml with water and filtered through filter paper. The first few ml of the filtrate was discarded and the rest was diluted to a known volume and the sugar was analysed with anthrone reagent as in carbohydrate estimation. The sugar content was calculated in terms of glucose equivalent and a factor of 0.9 was used to convert the values of glucose to starch.

3.6.3 Estimation of total sugars

Total sugar content was determined by anthrone method (Hodge and Hofreiter, 1962).

Two hundred microlitres of the saved supernatant (3.6.2) was evaporated using water bath at 80^0 C and one ml of water was added to dissolve the sugars. Four ml of anthrone reagent was added and the reaction mixture was heated at 100^0C for eight min in a water bath and cooled rapidly. The absorbance of the green coloured solution was determined at 630

nm using spectrophotometer. D- glucose was used as standard. Total sugar content was expressed in terms of milligrams per gram fresh weight basis.

3.6.4 Estimation of chlorophyll

Chlorophyll was estimated by the method described by Arnon (1949). One gram of leaf sample (W) was finely cut and ground in a mortar with 20 ml of 80 per cent acetone. The homogenate was centrifuged at 5000 rpm for five min and the supernatant was transferred to a 100 ml volumetric flask. The above procedure was continued till the residue became colourless. The final volume (V) in volumetric flask was made up to 100 ml. Absorbance of the solution at 645 (A_{645}) and 663 (A_{663}) nm was determined using spectrophotometer against the solvent (80 per cent acetone) as blank. The chlorophyll content was calculated using the following equations and expressed as milligrams of chlorophyll per gram of leaf tissue.

Total chlorophyll = 20.2 (A_{645}) + 8.02 (A_{663}) x V/ 1000 x W.

Chlorophyll a = 12.7 (A_{663}) - 2.69 (A_{645}) x V/1000 x W.

Chlorophyll b = 22.9 (A_{645})- 4.68 (A_{663}) x V/ 1000 x W.

3.6.5 Estimation of phenolics

Leaf sample (200 mg) was extracted in 7.5 ml of 80 per cent ethanol. The ethanolic extract was heated at 100^{0}C in a water bath for 10 min. After cooling, the extract was centrifuged at 10,000 rpm for 10 min. The volume of the supernatant was made up to 7.5 ml with 80 per cent ethanol

which was used further for estimating total phenols and ortho -dihydroxy phenols (OD-phenols) and flavanol.

3.6.5.1 Total Phenols

Total phenol content was estimated following the procedure of Bray and Thorpe (1954). To one ml of the ethanol extract, five ml of distilled water and 250 µl of IN Folin-Ciocalteau reagent were added. After incubating the mixture at room temperature for 30 min , one ml of saturated sodium carbonate (Na_2CO_3) solution was added and the mixture was further incubated for 15 min. The reaction lead to the development of blue colour. The intensity of which was determined at 725 nm against blank using a spectrophotometer. The phenol content was expressed in catechol equivalents mg g^{-1} of leaf tissue on fresh weight basis.

3.6.5.2 OD-phenols

OD-phenol content of the ethanol extract was estimated by employing the method of Mahadevan (1966). Arnow's reagent was prepared by dissolving 10 g of sodium nitrite ($NaNo_2$) and 10 g of sodium molybdate (Na_2 Mo O_4) in 100 ml of distilled water. The reagent was stored in a brown bottle in a cool dark place.

To one ml of the ethanol extract, one ml of 0.5 N Arnow's regent, 10 ml of distilled water and two ml of 1 N NaOH were added. A reagent blank was maintained without the extract. Soon after the addition of the alkali, pinkish yellow colour developed. The absorbance of the solution was determined at 515 nm using the spectrophotometer. The OD-phenol content

was expressed in catechol equivalents mg g^{-1} of leaf tissue on fresh weight basis.

3.6.5.3 Estimation of flavanols

Flavanol content was estimated by following the procedure of Swain and Hillis (1959). One ml each of alcohol extract taken in two boiling tubes (A&B) was evaporated to 0.1 ml and made up to two ml with distilled water. Four ml of vanillin reagent (one g of recrystallised vanillin dissolved in 100ml of 70 % concentrated sulphuric acid) was added rapidly within 10-15 seconds to A and four ml of sulphuric acid to B. Blank was prepared in another boiling tube C containing four ml vanillin regent and two ml of water. The contents of tube A and tube B were kept in cold water to keep its temperature below 35 ^{0}C. The tubes were kept at room temperature (28-30^{0}C) for exactly 15 min. The absorbance of the contents of tubes A, B and C were determined at 500 nm against 47 per cent sulphuric acid (tube D) in spectrophotometer. The absorbance values of the contents of tubes B and C were subtracted from that of A. Alternatively the absorbance of the contents from tubes (A+D) against (B+C) was determined. The flavanol content was calculated using a standard curve prepared with phloroglucinol.

3.6.6 Estimation of protein

Total soluble protein content was estimated as per the procedure described by Bradford (1976). One gram of leaf sample was homogenized in ten ml of 0.1 M sodium acetate buffer (pH 4.7) (Appendix III) and centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was saved for estimation of

soluble protein. The reaction mixture consisted of 0.5 ml enzyme extract, 0.5 ml distilled water and five ml diluted (five times) dye solution. The absorbance was read at 595 nm in a spectrophotometer against reagent blank. Bovine serum albumin was used as the protein standard. The protein content was expressed as microgram albumin in equivalent of soluble protein per gram on fresh weight basis.

3.6.7 Estimation of defense related enzymes

3.6.7.1 Estimation of peroxidase (PO)

Peroxidase activity was determined according to the procedure described by Srivastava (1987).

Leaf sample of 200 mg was homogenized in one ml of 0.1 M sodium phosphate buffer (pH 6.5) (Appendix II) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenization was done at 4^0C using a chilled mortar and pestle. The homogenate was filtered through a muslin cloth and centrifuged at 5000 rpm for 15 min at 4 ^{0}C. The supernatant was used as the enzyme extract for the assay of PO activity.

The reaction mixture consisting of one ml 0.05 M pyrogallol, and 50 µl enzyme extract was taken in both reference and sample cuvettes, mixed and kept in a spectrophotometer and the reading was adjusted to zero at 420 nm. The enzyme reaction was started by adding one ml of one per cent hydrogen peroxide (H_2O_2) into sample cuvettes and change in absorbance was measured at 30 seconds interval and expressed as change per minute per gram on fresh weight basis.

3.6.7.2 Estimation of polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) activity was determined as per the procedure given by Mayer *et al.* (1965). The enzyme extract was prepared as per the procedure given for the estimation of peroxidase.

The reaction mixture contained one ml of 0.1 M sodium phosphate buffer (pH 6.5) and 50 µl of enzyme extract. The cuvettes were placed in a spectrophotometer and absorbance set to zero. The reaction was started after adding one ml of 0.01 M catechol. The changes in absorbance were recorded at 495 nm and the PPO activity was expressed as changes in the absorbance of the reaction mixture per gram on fresh weight basis.

3.6.7.3 Estimation of phenylalanine ammonia lyase (PAL)

PAL activity was analysed based on the procedure described by Dickerson *et al.* (1984). The enzyme extract was prepared by homogenizing one gram leaf sample in five ml of 0.1 M sodium borate buffer (pH 8.8) (Appendix III) containing a pinch of PVP, using a chilled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 20 min, at 4^0C. The supernatant was used for the assay of PAL activity. The reaction mixture contained three ml of 0.1M sodium borate buffer (pH 8.8), 0.2 ml enzyme extract and 0.1 ml of 12mM L- Phenylalanine prepared in the same buffer. The blank contained three ml of 0.1M borate buffer (pH 8.8) and 0.2 ml enzyme extract. The reaction mixture and blank were incubated at 40 ^{0}C for 30 min and reaction was stopped by adding 0.2 ml of 3 N hydrochloric acid (HCl). The absorbance was determined at 290 nm in a spectrophotometer.

PAL activity was expressed as microgram of cinnamic acid produced per minute per gram on fresh weight basis.

3.7 Biometrical studies

3.7.1 Vine and leaf characters

The healthy and infected cuttings of Sree Rethna and Vavvathooki were planted in earthen pots. Ten cuttings for observation and three cuttings for destructive sampling to estimate dry matter content were planted. Observations on number of vines, length of vine, length of internode, number of leaves, leaf area and drymatter content were recorded at 30, 60 and 90 days after planting. Dry matter content also was estimated at three intervals. The total biomass was recorded at the time of harvest. The Biometrical observations were carried out as follows.

3.7.1.1 Number of vines

Total number of vines per plant was recorded.

3.7.1.2 Length of vine

Length of three vines from each plant was measured.

3.7.1.3 Length of internode

Eight internodes from each of three vines per plant were measured.

3.7.1.4 Number of leaves

Total number of leaves of each plant was recorded

3.7.1.5 Leaf area

Four leaves at random from each of three vines were taken per plant to measure leaf area using a Model 3100 area meter.

3.7.1.6 Total Biomass

Total weight of the vines and leaves together was recorded at the time of harvest for each plant after 110 days of planting.

3.7.1.7 Dry matter content

Dry matter (DM) content of the vine and leaves together was estimated by taking 100 g of sample from each plant by destructive sampling. The samples were cut into small pieces and kept in an oven at 80^0C in a paper envelope for five days, at the end of which a constant weight was reached. Per cent dry matter was calculated as follows.

$$DM = \frac{\text{Final weight}}{\text{Initial weight}} \times 100$$

3.7.2 Tuber characters

3.7.2.1 Number of tubers and tuber weight

The total number of tubers and total tuber weight were recorded for each plant at the time of harvest after 110 days of planting.

3.7.2.2 Colour and shape of tubers

The difference in colour and shape of the tubers of healthy and SPFMV infected plants were observed.

3.7.2.3 Size of tubers

The tuber size was recorded for the biggest three tubers from each plant in terms of length and girth.

3.7.2.4 Dry matter content

The dry matter content of the tubers was estimated by taking 100 g of tuber sample from pooled one-kilogram tubers for each treatment. Three replications were maintained. The dry matter content was estimated as mentioned in 3.7.1.7 for sweet potato vines and leaves.

3.7.2.5 Quality of tubers

3.7.2.5.1 Culinary quality

The tuber samples were taken from healthy and infected plants of both the varieties, viz., Sree Rethna and Vavvathooki and cooked in a container with water. Equal time of cooking was maintained for all the samples.

The method reported by Prema *et al.* (1975) for cassava was modified and followed for conducting organoleptic tests. After cooking, the samples were given to different consumers, viz., staff, students, labourers and housewives to record the flavour, flesh colour, consistency, sweetness and

overall taste of the flesh of the boiled sweet potatoes by giving a proforma (Appendix IV) with four grades, viz., excellent, good, medium or poor.

The opinions of the clientele are pooled and the results are presented on the change in culinary quality if any, due to the infection of SPFMV.

3.7.2.5.2 Quantity of nutrients

Total carbohydrates, total sugars, starch and protein contents of the tubers were estimated by using the methods explained under 3.6.

3.8 Characterization of the virus

3.8.1 Purification

Purification of the virus was carried out as per the protocol developed by Moyer and Kennedy (1978) with slight modification.

Systemically- infected *I. nil* leaves were homogenized with 0.05 M sodium borate buffer (pH 8.0) containing 0.01 M DIECA (1:3, w/v) using a chilled mortar and pestle. To the homogenate n-butanol was added dropwise (7.5 ml/100 ml buffer) and stirred with a sterile glass rod for 30 min. The homogenate was centrifuged at 5000 rpm for 15 min at 4^0C in a refrigerated high speed centrifuge (Hettich EBA. 12 R). The aqueous phase was collected and the virus was precipitated by the addition of 4 per cent polyethylene glycol (PEG 6000) and 0.2 M sodium chloride (NaCl) initially by stirring for 15 min and then incubating at 4^0C for an additional 90 min. The precipitate was pelleted at 10,000 rpm in a refrigerated table- top micro centrifuge at 4^0C (Hettich MIKRO

24-48 R) for 30 min. The pellet was resuspended in 0.05 M borate buffer (pH 8.0) containing 0.005 M EDTA and 0.05 M urea and clarified by centrifugation at 5000 rpm for five min to remove insoluble particles. The final suspension was dialysed overnight against one litre of 0.005 M borate buffer at 4 ^{0}C. This was concentrated by keeping in a sucrose pack. Infectivity assay was performed by using healthy seven- day- old *I. nil* plants.

The concentrated virus preparation was used as antigen source for immunization of rabbits.

3.8.2 Serology

3.8.2.1 Antiserum production

New Zealand female white rabbits of 12-month age were injected intramuscularly with one ml of the purified virus, emulsified with an equal volume of Freund's incomplete adjuvant (Difco) at weekly intervals. The rabbits were bled through the marginal ear vein 10 days after the last injection. The blood was collected and allowed to coagulate. The coagulated blood clot was loosened with the help of a sterilized glass rod and samples were kept overnight at 4 ^{0}C. The antiserum was pipetted out using a micropipette and dispensed into one ml vials. Sodium azide (0.2%) was added to the clarified serum to prevent microbial contamination. They were filled in vials, sealed, labelled and stored at -20^{0}C.

3.8.2.2 Serodiagnosis

3.8.2.2.1 Ouchterlony agar double-diffusion tests

Serological petri dishes were used for conducting the test. The petri dishes were coated with a thin layer (one mm) of 0.8 per cent agarose containing 0.85 per cent sodium chloride, 0.5 per cent sodium dodecyl sulphate (SDS) and 1.0 per cent sodium azide and allowed to solidify. Then the plates were placed on a marked paper, showing the position of wells, at six equidistant positions at 60^0 angle around the centre point and five mm distance from the outer edge of the central well. Tubes of five mm diameter were placed in each position without disturbing the lower layer. Above this layer, melted agarose was again added to a thickness of three mm. After solidification, tubes were gently removed to form wells on the agarose gel. The wells were labelled from 1 to 7. The central well (no. 1) of each plate received 30 µl of undiluted antiserum through a micropipette. The surrounding wells 2 and 4 and 6 and 7 received 30 µl of extracts of infected *I. nil* and *I. batatas* cultivar, Sree Rethna, respectively. Well 3 received healthy plant sap and well 5 the extraction buffer. Petri dishes were incubated in humid condition in a glass jar and examined for the appearance of characteristic precipitin bands. Three replications were maintained.

3.8.2.2.2 Enzyme linked immunosorbent assay (ELISA)

3.8.2.2.2.1 Direct antigen coating - ELISA (DAC-ELISA)

DAC-ELISA for the detection of SPFMV in the following samples was done by using the polyclonal antibodies received from Dr. R.W. Gibson, Natural Resources Institute, University of Greenwich, UK. The procedure described by Huguenot *et al.* (1992) was followed for the detection.

Samples used for the detection

1. *I. batatas* varieties, Sree Rethna, Sree Vardhini and Vavvathooki (both healthy and infected).

2. *I. nil* (both healthy and with yellow netting symptom)

3. *I. setosa* (healthy and those showing mosaic and chlorotic leaf spot symptoms separately).

4. Aphids - *A. craccivora* (before and after acquisition access period (AAP).

Leaves were taken as samples for items 1,2 and 3.

The samples were ground in coating buffer (1:5 w/v) (Appendix I). The homogenate was centrifuged at 5000 rpm for ten min at 4 ^{0}C. The samples were then added at the rate of 100 µl into Nunc immunological plates and incubated for three h. The treatments were replicated thrice. Antiserum (100 µl) at 1:1000 dilution in PBS-T was added and incubated overnight at 4^{0}C. The plates were washed with PBS-T and then treated with 100 µl of

alkaline phosphatase linked antirabbit immunoglobulin diluted in PBS-T (1:10,000 v/v) and incubated for two h at 37^0 C. Wells were washed with PBS-T as before. The substrate p-nitrophenyl phosphate was added to each well (100 µl/well) (Appendix V) and readings were taken after incubating it for one h at room temperature. Reaction was stopped by adding 50 µl of 4 % NaOH. The absorbance was measured at 405 nm in an ELISA reader (MULTISKAN MS).

3.8.2.2.2.2 Nitrocellulose membrane- ELISA (NCM-ELISA)

The seedlings developed from seeds of *I. batatas, I. nil* and *I. setosa* and meristem cultured plants without symptoms and the samples tested in DAC-ELISA were used for NCM-ELISA (3.2.1.2).

3.8.3 Physical properties

Potassium phosphate buffer (0.05 M, pH 7.2) containing 0.01 M DIECA(Appendix II) was used to extract the sap in all the three tests.

3.8.3.1 Dilution end point (DEP)

The systemically infected *I. nil* leaves (five g) were homogenized with the buffer (1:1 w/v). The homogenate was strained through a thin layer of absorbent cotton. Serial dilutions of the crude sap, viz., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were made as follows. Nine ml of buffer was taken in each of five test tubes. One ml of crude sap was transferred to the first tube with 9 ml buffer to get a dilution of 10^{-1}. After mixing uniformly one ml of 10^{-1} dilution was transferred to the next tube containing nine ml buffer to get 10^{-2} dilution. This process was continued till a dilution of 10^{-5} was obtained. *I. nil* plants

were inoculated at cotyledonary stage with the above five dilutions separately. Ten plants were inoculated with each dilution. Plants inoculated with undiluted sap served as control.

The plants were kept under insect proof conditions and the per cent infection was recorded in each treatment ten days after inoculation.

3.8.3.2 Thermal inactivation point (TIP)

TIP was measured by using standard sap of infected *I. nil* leaves (one g ml^{-1} of buffer). Two ml aliquot was incubated for ten min at 40, 45, 50, 55, 60, 65 and 70^{0}C. After the incubation period the samples were removed and cooled immediately. The sample kept at room temperature served as control. The samples were used to inoculate *I. nil* seedlings at the rate of ten plants for each treatment. Observations on the percentage of infected plants were taken ten days after inoculation.

3.8.3.3 Longevity i*n vitro* (LIV)

LIV was also determined by using standard sap. Samples of two ml aliquot were incubated at room temperature and at 8^{0}C for 48 h. Inoculation was done at 0, 2, 4, 6, 8, 10, 24 and 48 h of preparing the standard sap. The sample inoculated at 0 h was treated as control. Ten *I. nil* plants were used to receive each treatment. The percentages of plants infected were recorded after ten days.

3.8.4 Electron microscopy (EM)

The indicator host *I. nil* and sweet potato (*I. batatas*) varieties Sree Rethna and Sree Bhadra were used for electron microscopic studies.

Different buffers, viz., Tris buffer (pH 7.2), 0.05 M potassium phosphate buffer (pH 7.2) (Appendix II) and phosphate buffered saline (pH 7.4) (Appendix I) were used to extract the sap. Uranyl acetate (2%), phosphotungstic acid (2%) and ammonium molybdate (5%) were used for staining.

3.8.4.1 Negative staining method

Leaf homogenate was prepared by grinding 50-100 mg leaf sample with 50-200 µl of the buffer using glass homogenizer. One small drop of the tissue homogenate was placed on a carbon coated copper grid and was allowed to dry for two min. The grid was washed with drops of distilled water and the excess water was drained using a strip of filter paper. The grid was immediately stained with five drops of stain. Excess stain was drained, air dried and examined in Zeiss (West Germany) electron microscope at Central Plantation Crops Research Institute, Regional station, Kayamkulam, Kerala.

3.8.4.2 Petiole dip method

Leaf dip method developed by Brandes (1957) was modified and used for the detection of the virus in petiole under EM.

The petiole was cut and the droplet exuded from the cut end was touched on the grids, allowed to dry for two min and stained separately as mentioned above. All the grids were examined in electron microscope.

3.8.4.3 Shape and size

The shape of the virus particles was observed and the size was calculated using ultrastructure size calculator.

3.9 Host range

Host range studies were carried out in 25 species of plants belonging to the families of Convolvulaceae, Chenopodiaceae, Solanaceae, Asteraceae, Capparidaceae, Rubiaceae, Fabaceae and Amaranthaceae (Table.1).

Plants were grown in small earthen pots of 15 cm diameter in insect proof condition. SPFMV- infected sweet potato leaves were homogenated with three volumes of 0.05 M potassium phosphate buffer (pH 7.2) with 0.01 M DIECA (Appendix II), strained through muslin cloth and inoculated on to the plants one week after germination. Carborundum powder (600 mesh) was used as the abrasive. Uninoculated healthy plants were kept as control.

After 15 days of inoculation, the number of plants infected and the nature of symptoms were recorded.

Table 1 Plant species used for SPFMV host range studies

Sl. No	Plant species	Family
1.	*Acalypha indica* L.	Euphorbiaceae
2.	*Borreria sticta* L.	Rubiaceae
3.	*Chenopodium amaranticolor* Coste and Acyn.	Chenopodiaceae
4.	*Cleome viscosa* L.	Capparidaceae
5.	*Datura metel* L.	Solanaceae
6.	*Datura stramonium* L.	Solanaceae
7.	*Emelia sonchifolia* Dc.	Asteraceae
8.	*Euphorbia hirta* L.	Euphorbiaceae
9.	*Gomphrena globosa* L.	Amaranthaceae
10.	*Ipomoea carnea* Jacq.	Convolvulaceae
11.	*I. nil* (Linn.) Roth.	Convolvulaceae
12.	*I. muricata* Jacq.	Convolvulaceae
13.	*I. setosa* Kev.	Convolvulaceae
14.	*I. tricolor* Cav.	Convolvulaceae
15.	*Nicotiana benthamiana* L.	Solanaceae
16.	*N.glutinosoa* L	Solanaceae
17.	*N. tabacum* L.	Solanaceae
18.	*N. tabacum* L. (Burley 21)	Solanaceae
19.	*N. tabacum* L. (Havana)	Solanaceae
20.	*N. tabacum* L. (Samsun)	Solanaceae
21.	*Synedrella nodiflora* L.	Asteraceae
22.	*Solanum nigrum* L.	Solanaceae
23.	*Tridax procumbens* L.	Asteraceae
24.	*Vernonia cinerea* Edgw.	Asteraceae
25.	*Vigna unguiculata* (L.) Walp.	Fabaceae

3.10 Management of the disease

3.10.1 Screening

The sweet potato germplasm collection of 848 accessions at CTCRI, Sreekariyam, Thiruvananthapuram were screened for their reaction to SPFMV based on the symptoms. Scoring was done using a 0-4 scale. Based on the scoring, per cent disease index (PDI) was calculated (3.2.1). The PDI was catergorised into five groups as follows and the varietal reaction was assessed based on the intensity of the external visual symptoms.

0 - Highly resistant

1-25 - Resistant

26-50 - Moderately susceptible

51-75 - Susceptible

76-100- Highly susceptible.

3.10.2 Thermotherapy

3.10.2.1 Hot water treatment

Vine cuttings of infected Sree Rethna and Vavvathooki were treated at different temperatures, viz., 34, 36, 38, 40, 42, 44, 46 and 48^{0}C in hot water bath for 5, 10 and 15 min. The treated cuttings were planted in earthen pots. Ten cuttings were used for each treatment. Cuttings kept at room temperature were treated as controls.

The sprouting percentage, appearance of initial symptoms, major symptoms and the number of plants free from the symptoms were recorded. PDI was calculated using 0-4 scale and the formula mentioned earlier (3.2.1.1).

3.10.2.2 Dry heat

Vine cuttings of Vavvathooki were incubated at 37^0C and 39^0C in a B.O.D incubator up to eight days, separately. Ten cuttings were sampled everyday from incubation up to eight days and planted in earthen pots. The development of symptoms was observed. After 45 days of planting per cent infection was also observed. The PDI was calculated as described under 3.2.1.1.

3.10.3 Induction of systemic resistance using chemicals

3.10.3.1 Bio- assay on indicator host

The effects of application of different chemicals such as barium chloride, manganese chloride and salicylic acid were evaluated on the indicator host, *I. nil*. Both pre and post- inoculation treatments were done with different concentrations, viz., 50,100 and 150 mg l^{-1}.

In pre- inoculation treatment, the chemicals were sprayed 24 h prior to mechanical inoculation of the virus. In post- inoculation treatment, they were sprayed 24 h after inoculation. The treated plants were kept in insect proof condition.

Infected *I. nil* leaves were homogenized with 0.05 M potassium phosphate buffer (pH 7.2) (1:1 w/v) with 0.01 M DIECA (Appendix II), strained through cotton and inoculated at cotyledonary stage.

The number of plants infected in each treatment was recorded for evaluating the efficacy of chemicals. Control plants were maintained without application of the chemicals.

3.10.4 Meristem tip culture

3.10.4.1 Plant material

Tubers from infected Sree Rethna and Vavvathooki were planted in earthen pots under insect proof condition. After two weeks, the sprouts were grown sufficiently to remove the terminal bud together with a short stem section from each. Rapidly growing vegetative buds were taken as meristem donors. The buds were collected in clean plastic bags.

3.10.4.2 Preparation of sterile tissue

The buds were thoroughly washed in tap water and disinfected by soaking them in two per cent "labolene" solution for 15 minutes and again washed with distilled water three to four times to remove any trace of the solution. Finally the buds were left in sterilized water in a beaker. These buds were then taken to a laminar flow chamber and soaked in 0.1 per cent mercuric chloride for one minute, followed by 70

sterile filter paper for absorbing excess water just before dissection. Scalpel and forceps (two sets) were dipped in alcohol inside a glass container after autoclaving them.

3.10. 4.3 Dissection of buds and explant preparation

A disinfected bud was placed under a dissection microscope using the forceps. The stem was held steadily while young leaves were removed by bending them away from the stem with the back of the scalpel blade.

The underlying leaf primordia were then removed by inserting the tip of the scalpel into the base of each primordium and flicking the tip of the scalpel away from the stem axis. Thus the apical dome was left with two or three of the youngest leaf primordia. They were removed by scraping them with the cutting edge or back edge of the scalpel blade.

After removing all leaf primordia using the scalpel, a slicing cut was made at the base of the dome by holding the stem. When the cut penetrated about half way through the tissue beneath the dome, by a lifting motion the dome was separated from the support and it adhered to the blade. Sometimes a second cut was also given on the opposite side of the dome for complete removal. The dissection was carried out as quickly as possible to avoid drying. The meristem was then placed on the surface of induction medium (approximately 10 ml) in tissue culture tubes.

3.10.4.4 Incubation of cultures

Following excision and inoculation, the cultures were placed in a incubation room at a temperature of 24 ± 4^{0}C, giving a photoperiod of 16 h.

3.10.4.5 Induction medium

Murashige and Skoog medium (Murashige and Skoog, 1962) with Naphthalene acetic acid (NAA) 0.1 mg l^{-1} and 6-benzyl adenine (BA) 0.5 mg l^{-1} was used for induction. A shoot, 1-2 cm in length, often with a small basal callus, without roots developed from explant was used for regeneration.

3.10.4.6 Regeneration medium

Each shoot of about 1-2 cm in length with a small basal callus on the induction medium was then transferred to the regeneration medium (zero-MS) and kept for 4-5 weeks. After the development of root and small shoot, the regenerated plants were transferred for establishment.

The composition of media and the method of media preparation are given in the Appendix VI.

3.10. 4.7 Hardening

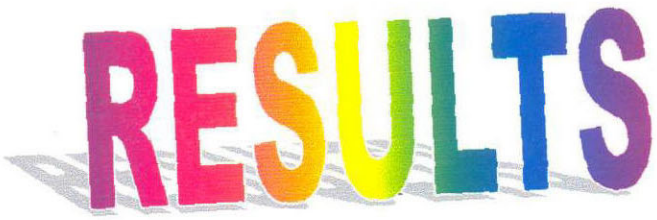
Regenerated plantlets were planted in small disposable plastic cups having 1 : 1 v/v in mixture of sterile sand and soil. The plants were covered with polythene bags with a hole for watering, for one week. The covers were removed and the plantlets were exposed to full sunlight in insect proof condition.

The plants were observed daily for symptom development. Leaf samples were taken at random from 30 -day- old plants, which did not show the symptom of SPFM and tested for the presence of SPFMV through NCM-ELISA (3.2.1.1).

3.10.5 Storage of vine cuttings

The SPFMV- infected cuttings (20 cm) were taken from Sree Rethna and Vavvathooki. The cuttings were kept at room temperature (28 $+4^0$C) and planted after 7, 15, 20 and 30 days of storage. The cuttings planted on the day of taking them were kept as control. In each interval ten cuttings were planted in earthen pots of 20 cm diameter.

The sprouting time, percentage of sprouting and appearance of first symptom were recorded. The PDI was calculated by scoring the plants, 45 days after planting using 0-4 scale (3.2. 1.1).


RESULTS

4. RESULTS

4.1 Survey

Survey was conducted in the area under six Krishibhavans of Thiruvananthapuram district, viz., Kalliyoor, Vizhinjam, Venganoor, Balaramapuram, Pallichal and Kottukal having sweet potato cultivation.

In each Krishibhavan, two localities and from each locality two farmers were selected. From each farmer's field, four plots of 3 m x 1 m size were randomly selected. Data on varietal reaction, types of foliar symptoms, disease incidence, disease intensity, presence of vectors and tuber symptoms were recorded from each plot.

4.1.1 Varietal reaction

The local varieties used by the farmers were Celopia, Kottaramchuvala, Pappadavella, Peanivella, Poochedichuvala and Vavvathooki (Plate 4). All these varieties were infected by SPFMV except Kottaramchuvala, which did not show any symptoms in the field. There was no positive reaction in this variety when tested through NCM-ELISA using CIP- NCM-ELISA kit. Scoring varieties for SPFM was done using 0-4 scale in ten plants for each cultivar and the PDI was calculated (Table 2 and Fig.1). The PDI varied between varieties.

Poochedichuvala

Celopia

Kottaramchuvala

Vavvathooki

Plate 4 Sweet potato varieties in farmers' fields

4.1.2 Types of foliar symptoms

Different types of SPFM symptoms were observed on the varieties (Table 2). Chlorotic leaf spot, pink spot, ring spot, feathering and combination of two or more of these were the major symptoms observed in the field. Fading of symptoms like ring spot and feathering was also observed in some varieties, viz., Pappadavella, Peanivella and Vavvathooki.

4.1.3 Disease incidence

Out of the total 96 plots surveyed, 100 per cent incidence was recorded in 54 plots whereas no incidence was noted in 15 plots (Table 3). In the remaining 27 plots 1-25 per cent, 26-50 per cent, 51-75 per cent and 76-99 per cent disease incidence were observed in 2, 12, 8 and 5 plots respectively (Fig.2). Variation was also observed between farmers' fields, localities and Krishibhavans.

4.1.4 Disease intensity

In each plot, 25 plants were selected at random and scoring for SPFM was done using 0-4 scale. PDI was calculated and presented in the Table 4. The results showed that the PDI was significantly different between plots (Table 4) Krishibhavans, localities and farmers' fields (Table 5) .The PDI of SPFM disease, viz., 0-25, 26-50, 51-75 and 76-100 was observed in 34, 8, 47 and 7 plots respectively (Fig.3).

Table 2 Symptoms and intensity of SPFM in different varieties of sweet potato in farmers' fields

Sl. No	Varieties	*Per cent disease index	Symptoms
1	Celopia	15	PS,RS, Slight feathering
2	Kottaramchuvala	0	No symptoms
3	Pappadavella	89	CLS,PS,RS , feathering
4	Peanivella	47	PS,RS, feathering
5	Poochedichuvala	16	CLS, slight feathering
6	Vavvathooki	93	CLS,PS,RS,feathering

*Mean of ten replications
CLS – Chlorotic leaf spot PS - Pink spot RS - Ring spot

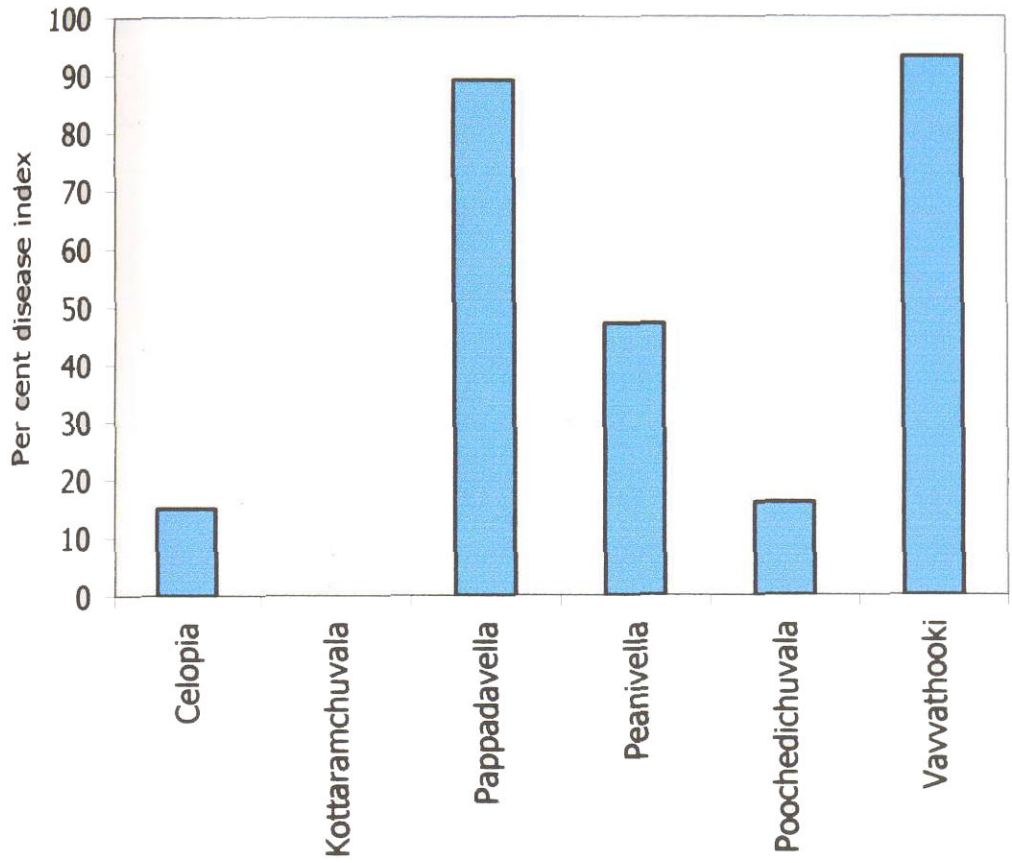


Fig.1. SPFM intensity in different varieties of sweet potato in farmers' fields

Table 3 Incidence of SPFM of sweet potato in farmers' fields

Sl. No	Treatments	Per cent disease incidence Plots			
		1	2	3	4
1.	$K_1 L_1 F_1$	62.5	100	50	100
2.	$K_1L_1 F_2$	30	0	72	73
3.	$K_1L_2F_1$	0	0	100	100
4.	$K_1L_2F_2$	0	0	100	100
5.	$K_2 L_1 F_1$	0	100	30.3	0
6.	$K_2 L_1 F_2$	100	100	100	100
7.	$K_2 L_2F_1$	0	40	88	100
8.	$K_2 L_2F_2$	100	87	53.33	83.33
9.	$K_3 L_1 F_1$	19	0	100	100
10.	$K_3 L_1 F_2$	100	100	100	100
11.	$K_3 L_2F_1$	0	28.6	87.9	100
12.	$K_3 L_2F_2$	100	100	100	100
13.	$K_4 L_1 F_1$	100	53.13	100	100
14.	$K_4 L_1 F_2$	100	100	100	100
15.	$K_4 L_2F_1$	84.6	0	46.4	100
16.	$K_4 L_2F_2$	100	100	100	100
17.	$K_5 L_1 F_1$	40	100	0	100
18.	$K_5 L_1 F_2$	0	100	45.45	100
19.	$K_5 L_2F_1$	100	43.33	0	100
20.	$K_5 L_2F_2$	100	100	100	100
21.	$K_6 L_1 F_1$	67.7	100	46.43	100
22.	$K_6 L_1 F_2$	100	100	54.8	0
23.	$K_6 L_2F_1$	35.7	53	100	100
24.	$K_6 L_2F_2$	9.68	32.29	100	100

K- Krishibhavan L – Locality F- Farm

Table 4 Intensity of SPFM in the farmers' fields in six Krishi bhavans

Sl. No.	Treatments	*Per cent disease index Plots			
		1	2	3	4
1.	$K_1 L_1 F_1$	26.35 (5.23)	7.69 (2.95)	62.05 (7.94)	56.41 (7.58)
2.	$K_1 L_1 F_2$	10.99 (3.46)	49.32 (7.09)	13.72 (3.84)	0 (1)
3.	$K_1 L_2 F_1$	7.92 (2.99)	75 (8.72)	73.43 (8.63)	84.31 (9.24)
4.	$K_1 L_2 F_2$	57.74 (7.66)	62.76 (7.98)	0 (1)	0 (1)
5.	$K_2 L_1 F_1$	65.77 (8.17)	8.63 (3.10)	0 (1)	0 (1)
6.	$K_2 L_1 F_2$	60.95 (7.87)	63.83 (8.05)	65.94 (8.18)	63.67 (8.04)
7.	$K_2 L_2 F_1$	7.9 (2.98)	48.58 (7.04)	68.80 (8.35)	0 (1)
8.	$K_2 L_2 F_2$	52.48 (7.31)	43.70 (6.69)	8.91 (3.15)	63.24 (8.01)
9.	$K_3 L_1 F_1$	3.23 (2.06)	63.13 (8.01)	60.45 (7.84)	60.25 (7.83)
10.	$K_3 L_1 F_2$	53.85 (7.41)	55.32 (7.50)	71.13 (8.49)	67.10 (8.25)
11.	$K_3 L_2 F_1$	62.53 (7.97)	64.30 (8.08)	57.99 (7.68)	0 (1)
12.	$K_3 L_2 F_2$	63.42 (8.03)	75.03 (8.72)	64.78 (8.11)	0 (1)
13.	$K_4 L_1 F_1$	80.53 (9.03)	10.76 (3.43)	75.76 (8.76)	58.98 (7.74)
14.	$K_4 L_1 F_2$	59.86 (7.80)	64.22 (8.08)	58.08 (7.69)	79.75 (8.99)
15.	$K_4 L_2 F_1$	12.81 (3.72)	0 (1)	4.88 (2.42)	67.01 (8.25)
16.	$K_4 L_2 F_2$	62.26 (7.95)	75.78 (8.76)	60.37 (7.83)	63.62 (8.04)
17.	$K_5 L_1 F_1$	6.06 (2.66)	72.26 (8.56)	0 (1)	0 (1)
18.	$K_5 L_1 F_2$	54.85 (7.47)	74.09 (8.67)	6.82 (2.80)	78.12 (8.89)
19.	$K_5 L_2 F_1$	42.28 (6.58)	9.08 (3.17)	0 (1)	78.74 (8.93)
20.	$K_5 L_2 F_2$	70.20 (8.44)	66.81 (8.23)	54.56 (7.49)	42.94 (6.63)
21.	$K_6 L_1 F_1$	12.15 (3.63)	73.27 (8.62)	5.90 (2.63)	61.41 (7.9)
22.	$K_6 L_1 F_2$	61.24 (7.89)	48.27 (7.02)	10.51 (3.39)	0.51 (1.23)
23.	$K_6 L_2 F_1$	9.29 (3.21)	11.45 (3.53)	74.63 (8.70)	44.59 (6.75)
24.	$K_6 L_2 F_2$	1.81 (1.68)	5.87 (2.62)	78,19 (8.90)	73.25 (8.62)

*Mean of 25 replications Figures in the parentheses are square root transformed means

K - Krishibhavan L – Locality F- Farmer P - Plot

$CD_{(0.01)}$ K X L X F X P – 0.48

Table 5 : Intensity of SPFM in farmers' fields at different localities and Krishibhavans

Locality	Farmer	*Per cent disease index					
		K_1	K_2	K_3	K_4	K_5	K_6
L_1	F_1	38.12 (6.25)	18.6 (4.43)	46.77 (8.16)	56.51 (7.58)	19.58 (4.54)	38.18 (6.26)
	F_2	18.51 (4.42)	63.60 (8.04)	61.85 (7.93)	65.68 (8.15)	53.47 (7.38)	30.13 (5.58)
Mean (L x K)		28.31 (5.41)	41.10 (6.49)	54.31 (7.44)	61.00 (7.87)	36.53 (6.13)	34.16 (5.93)
L_2	F_1	60.16 (7.82)	31.32 (5.69)	46.21 (6.87)	21.18 (4.70)	32.53 (5.79)	34.99 (6.0)
	F_2	30.13 (5.58)	42.08 (6.56)	50.80 (7.20)	68.51 (8.34)	58.63 (7.72)	39.71 (6.38)
Mean (L x K)		45.15 (6.79)	36.70 (6.14)	48.51 (7.04)	43.35 (6.66)	45.58 (6.82)	37.39 (6.20)
Mean (Krishibhavan)		36.73 (6.14)	38.9 (6.32)	51.41 (7.24)	52.18 (7.29)	41.06 (6.48)	35.78 (6.06)

* Mean of four plots Figures in the parentheses are square root transformed means

CD ($_{0.01}$) - Krishibhavan (K) -0.21 L x K -0.34 L x F x K - 0.48

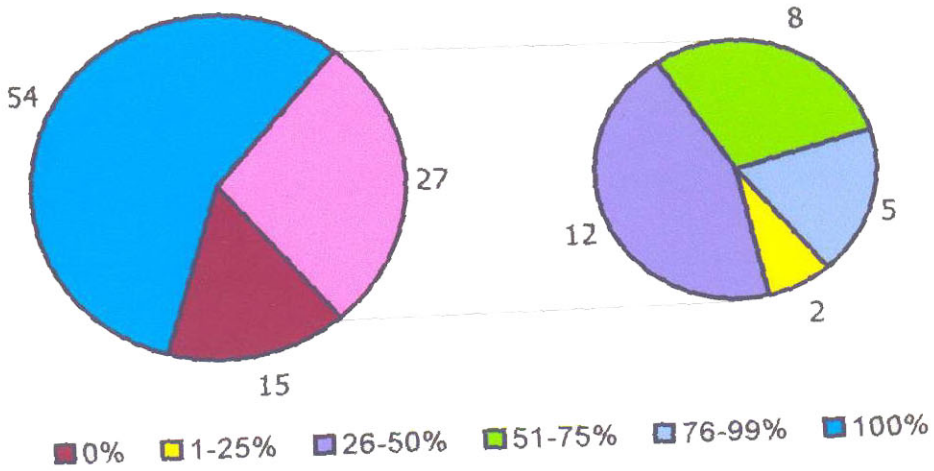


Fig. 2. SPFM incidence in farmers' fields

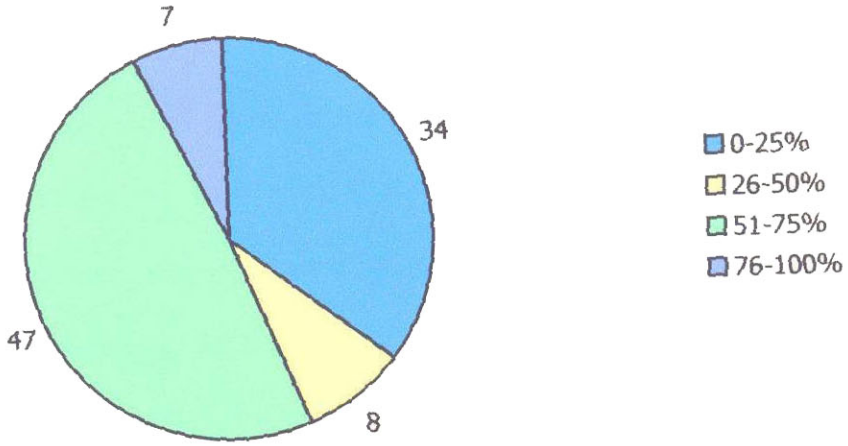


Fig. 3. SPFM intensity in farmers' fields

4.1.5 Presence of vectors

No vectors especially aphids/ whiteflies were present in any of the sweet potato fields surveyed. Tortoise beetles and pumpkin beetles were commonly found in all the fields.

4.1.6 Tuber symptoms

The tubers were examined during harvest for the presence of symptoms. The tubers of the variety Vavvathooki showed constrictions. However, there were no healthy plants available in the field for comparison.

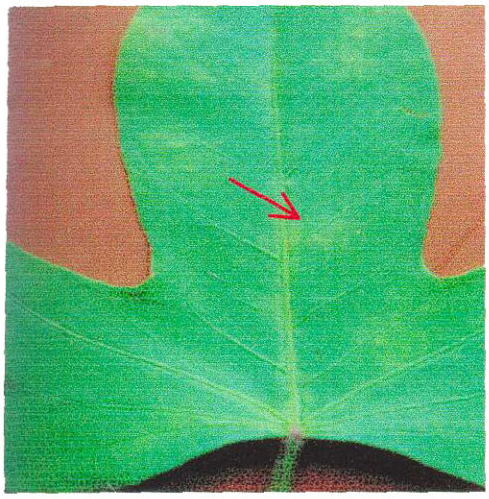
4.2. Symptomatology

4.2.1 Natural condition (without shade)

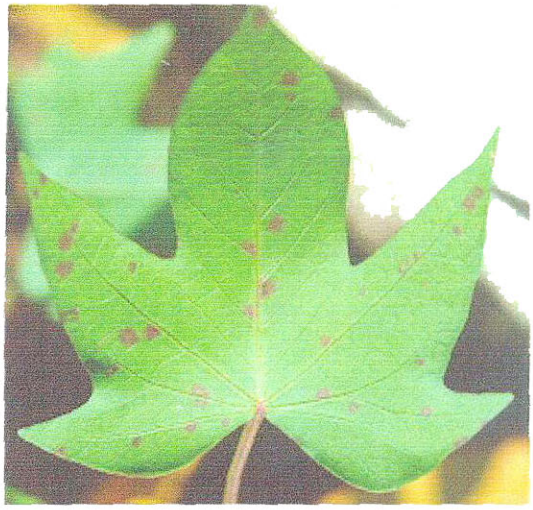
The symptoms observed were pink spot alone, ring spot alone, pink spot and ring spot, chlorotic leaf spot alone, pink spot and chlorotic leaf spot, ring spot and chlorotic leaf spot, pink spot, ring spot and chlorotic leaf spot, feathering alone, ring spot and feathering and feathering followed by yellowing (Plate 5). Among them pink spot, ring spot, chlorotic leaf spot and feathering were the major symptoms observed in all the varieties except in Poochedichuvala where only feathering and faint chlorotic leaf spot appeared. Fading of the symptom was also observed following the appearance of ring spot and also after feathering.

Generally, 15-20 days after planting, the middle leaves showed indistinct pale yellow specks or spots or indistinct pale yellow mottling. Later, a purple pigmentation was found on the specks or around the margins of the chlorotic leaf spots. In another 3-4 days, clearly defined purple ring

Chlorotic leaf spot



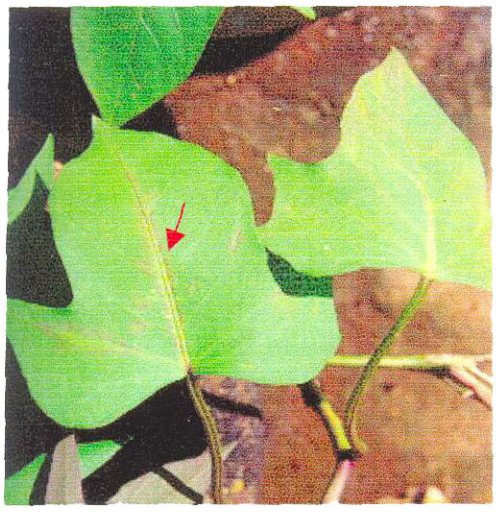
Pink spot



Ring spot

Feathering

Fading



Senescence

Plate 5 Different types of SPFM symptoms

spots were formed and diffused into expanded feathering. After two weeks these symptoms got faded in many leaves. Chlorosis appeared followed by pale purple feathering. The per cent disease index (PDI) was different between varieties and intervals (Fig.4). The PDI was highest in all varieties at 60 days after planting and the values were 69.54, 91.71, 68.18 and 8.98 in Sree Bhadra (SB), Sree Rethna (SR), Vavvathooki (VT) and Poochedichuvala (PC) respectively. This was followed by PDI at 30 days, 90 days and 105 days after planting (Table 6). Among the varieties SR recorded the highest PDI followed by VT, SB and PC.

Root symptoms like external cracking and internal corkiness were not observed in any of the varieties except some constrictions in VT. Statistical analysis revealed that the PDI was significantly different between the varieties SR and SB, VT and SR but not between SB and VT. The PDI at 105 days after planting was significantly different from other intervals viz., 30, 60 and 90. But the PDI was not significantly different between 30, 60 and 90 days after planting.

4.2.2 Under different intensities of shade

Sweet potato vine cuttings of the varieties Sree Rethna and Vavvathooki were planted in different intensities of shade, viz., 0, 25, 50 and 75 per cent. The development of symptoms was observed and disease intensity was scored from 30 and 60- day-old plants and the PDI were calculated.

All the cuttings planted under different intensities of shade sprouted within 2-3 days. The initial symptoms and the major symptoms in each shade intensity are presented in the Table 7.

Table 6 SPFM intensity on different varieties of sweet potato

Sl. No.	Varieties	Per cent disease index (PDI)* Days after planting				Mean ** (Varieties)
		30	60	90	105	
1.	Sree Bhadra (SB)	63.27 (8.02)	69.54 (8.40)	62.84 (7.99)	27.86 (5.37)	55.88 (7.54)
2.	Sree Rethna (SR)	85.24 (9.29)	91.71 (9.63)	78.83 (8.93)	74.31 (8.68)	82.52 (9.14)
3.	Vavvathooki (VT)	63.55 (8.03)	68.18 (8.32)	47.70 (6.98)	45.32 (6.80)	56.19 (7.56)
4.	Poochedichuvala (PC)	0 (1.0)	8.98 (3.16)	7.31 (6.80)	0 (1.0)	4.07 (2.25)
	Mean (Intervals)**	53.02 (7.35)	59.60 (7.78)	49.17 (7.08)	36.80 (6.15)	

*Mean of nine replications
Figures in the parentheses are square root transformed means
** Significant at CD $_{(0.01)}$ level

$CD_{(0.01)}$ Varieties(V) 0.87 Intervals(I) 0.87 V x I 1.77

Table 7 Effect of different intensities of shade on symptom development of SPFM in sweet potato varieties , Sree Rethna and Vavvathooki

Sl. No.	Shades	Days after planting for first appearance of symptoms		Initial symptoms		Major symptoms
		SR	VT	SR	VT	
1	0	16-20	16-20	CLS,PS	CLS	CLS,PS,RS, F
2	25	16-20	20-25	CLS,PS	CLS	CLS,PS,RS, F
3	50	20-30	20-30	CLS,PS	CLS	CLS,PS,RS, F
4	75	20-30	30-35	CLS,PS	CLS	RS,F

SR - Sree Rethna, VT - Vavvathooki
CLS - Chlorotic leaf spot , PS - Pink spot , RS - Ring spot , F – Feathering

The statistical analysis of the data indicated that the different intensities of shade had significantly influenced the disease intensity (Table 8 and Fig.5). In general, a declining trend in PDI was observed as the percentage of shade was increased. The PDI in the variety Sree Rethna, viz., 88.20, 36.81 and 22.92 and in Vavvathooki, viz., 74.47, 9.03 and 16.67 were observed 60 days after planting at 25, 50 and 75 per cent shades while in plants kept without shade the PDI were 93.77 and 83.33 in Sree Rethna and Vavvathooki, respectively. The data recorded 30 days after planting also showed a similar trend.

The effect of shade on PDI was significantly different at various shade intensities in different intervals in both the varieties.

4.3 Transmission

4.3.1 Through vine cuttings

Attempts to study the transmission through different types of sweet potato vine cuttings revealed that all plants developed from single node (SN) , double node (DN) and triple node (TN) showed symptoms of SPFMV infection in all the varieties tried. The first appearance of the symptom was 12-20 days after planting in the case of plants developed from DN and TN cuttings except the variety Poochedichuvala, whereas in plants from SN cuttings it was after 25 days (Table 9). The prominent symptoms observed from the plants were chlorotic leaf spot, pink spot, ring spot and feathering.

Table 8 Effect of different intensities of shade on per cent disease index of SPFM in sweet potato varieties , Sree Rethna and Vavvathooki

Sl. No	Varieties	*Per cent disease index								Mean
		30 DAP				60 DAP				
		S1	S2	S3	S4	S1	S2	S3	S4	
1	Sree Rethna	54.86 (7.47)	64.88 (8.12)	34.02 (5.71)	23.62 (4.96)	93.77 (9.73)	88.20 (9.44)	36.81 (6.15)	22.92 (4.89)	52.39 (6.80)
2	Vavvathooki	92.36 (9.66)	36.10 (6.09)	6.94 (2.82)	0 (1)	83.33 (9.18)	74.47 (8.69)	9.03 (3.17)	16.67 (4.20)	39.86 (6.39)
	Mean (S x I)	73.61 (8.64)	50.49 (7.18)	20.48 (4.63)	11.8 (3.58)	88.55 (9.46)	81.34 (9.07)	22.92 (4.89)	19.80 (4.86)	
	Mean (Intervals)	39.09 (6.33)				53.15 (7.36)				

* Mean of twelve replications DAP Days after planting
Figures in the parentheses are square root transformed means
S1 - 0% shade S2- 25% shade S3- 50% shade S4- 75% shade

$CD_{(0.01)}$

Shades(S)	0.78**
Varieties(V)	0.52**
Intervals(I)	0.52**
S×I	1.01**
S×V×I	1.43**

** Significant at $CD_{(0.01)}$

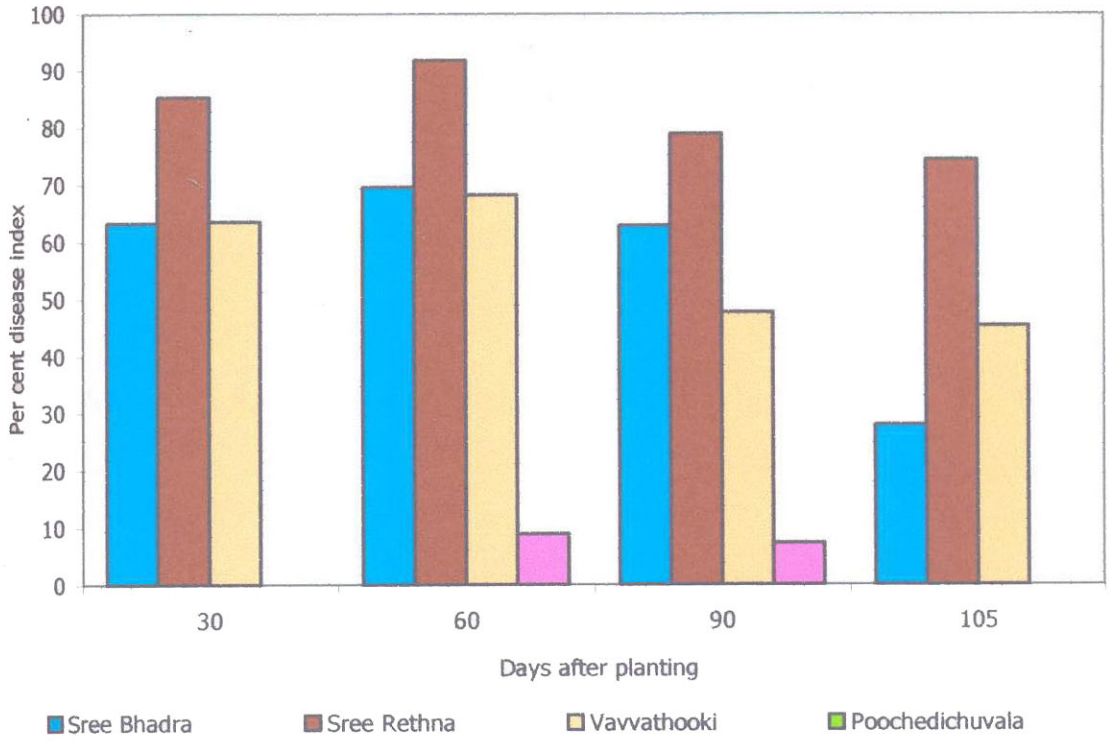


Fig.4. Per cent diseae index of SPFM on different varieties of sweet potato

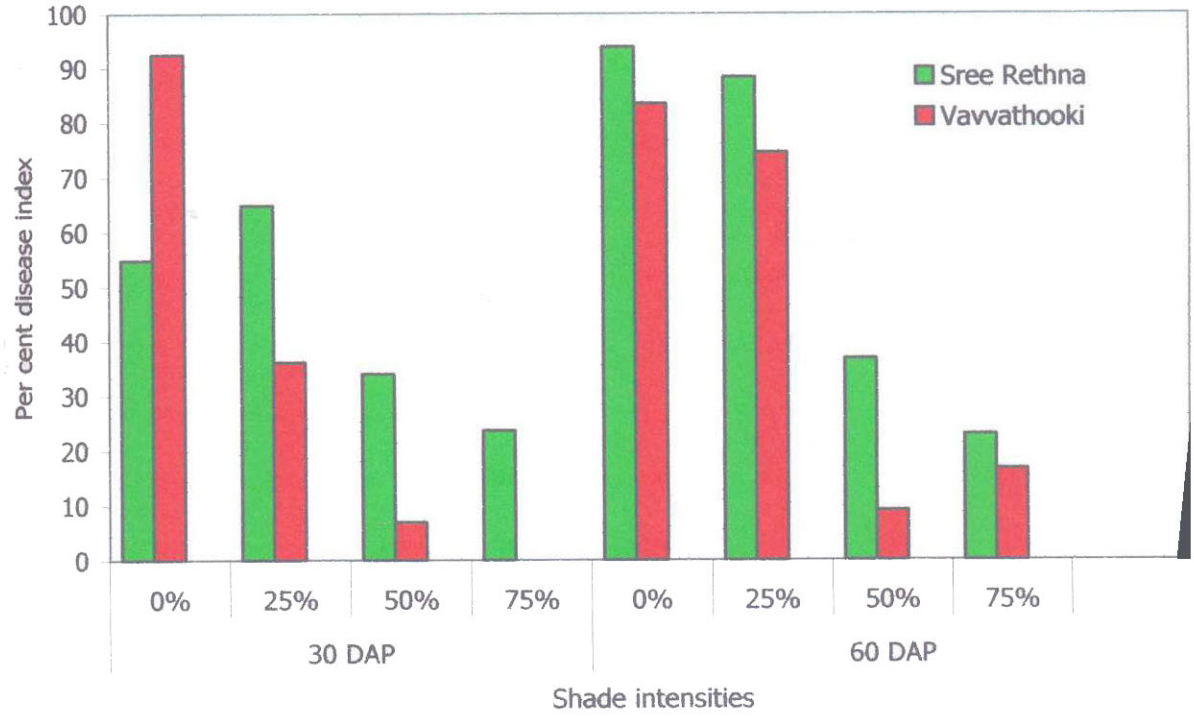


Fig.5. Effect of different intensities of shade on per cent disease index of SPFM in sweet potato varieties, Sree Rethna and Vavvathooki

Table 9 Effect of different types of vine cuttings on symptom development of SPFM in four different varieties of sweet potato plants

Sl. No.	Varieties	Days after planting for Initial appearance of symptoms			Initial symptoms			Major symptoms
		SN	DN	TN	SN	DN	TN	
1.	Sree Bhadra	30-40	15-20	15-20	CLS,PS	CLS,PS	CLS,PS	CLS ,PS, RS,F
2.	Sree Rethna	25-30	15-20	15-20	CLS,PS	CLS,PS	CLS,PS	CLS ,PS, RS,F
3.	Vavvathook i	25-40	12-18	12-18	CLS,PS	CLS,PS	CLS,PS	CLS ,PS, RS,F
4.	Poochedichu ala	30	25-30	35-30	CLS	CLS	CLS	CLS ,PS, RS,F

CLS – Chlorotic leaf spot PS – Pink spot RS- Ring spot F - Feathering
SN – Single node DN – Double node TN – Triple node

4.3.2 Tuber transmission

SPFMV is transmitted through infected tubers. The symptoms were similar to that developed from vine cuttings. The symptoms were first observed 12-20 days after sprouting in Sree Bhadra, Sree Rethna and Vavvathooki but after 25-30 days in Poochedichuvala. Plants from SPFMV-infected tubers generally showed the major symptoms like pink spot, ring spot, chlorotic leaf spot and feathering (Table 10).

4.3.3 Sap transmission

4.3.3.1 Transmission from sweet potato to sweet potato

Mechanical transmission studies of SPFMV between sweet potato plants demonstrated that none of the methods tried was effective in transmitting the virus. Hence, SPFMV was not mechanically transmissible from sweet potato to sweet potato with the methods tried in this study.

4.3.3.2 Transmission from *Ipomoea nil* to sweet potato

In this study, 20% of the sweet potato plants of the variety Sree Rethna were infected when inoculated with the SPFMV infected *I. nil* sap. The first appearance of the symptom was after 15 days of inoculation and only feathering was observed. (Table 11).

4.3.4 Insect transmission

Insect transmission studies were carried out using three aphid species, viz., *A. craccivora*, *A. gossypii* and *P. nigronervosa* and the whitefly, *B. tabaci* to find out the efficient vector of SPFMV.

Table 10 SPFM symptom development in four varieties of sweet potato plants raised from tubers

Sl. No.	Varieties	DAP for first appearance of symptoms	Initial symptom	Major symptoms
1.	Sree Bhadra	15-20	CLS, PS	CLS, PS, RS, F
2.	Sree Rethna	13-20	CLS, PS	CLS, PS, RS, F
3.	Vavvathooki	12-15	CLS, PS	CLS, PS, RS, F
4.	Poochedichuvala	25-30	CLS	CLS and F

CLS-Chlorotic leaf spot PS- Pink spot RS - Ring spot F - Feathering
DAP Days after planting

Table 11 Transmission of SPFMV from *I.nil* to sweet potato

Sl. No.	Number of plants		Per cent transmission	Symptoms
	Inoculated	Infected		
1	10	2	20	Feathering, fading
2	10	1	10	Feathering, fading
3	10	3	30	Feathering, fading
Mean	10	2	20	

The observation showed that *A. craccivora* gave the highest percentage of transmission viz., 40, 40 and 60 followed by *A. gossypii* (20, 20 and 30) in *I. batatas* variety Sree Rethna, *I. nil* and *I. setosa,* respectively. *P. nigronervosa* and *B. tabaci* did not transmit the virus (Table 12 and Fig.6). The first appearance of symptom was between 20 to 30 days in the case of both the species of aphids in all the *Ipomoea* spp. tried (Table 13).

The symptoms developed on plants after insect transmission were chlorotic leaf spot, ring spot and feathering in *I. batatas*, chlorotic leaf spot, mosaic, distortion, puckering and vein clearing in *I. nil*, and chlorotic leaf spot, mosaic, vein banding, ring spot and distortion in *I. setosa*

4.3.5 Graft transmission

Approach grafting (AG), side grafting (SG) and wedge grafting (WG) were successful in transmitting the virus with different efficiency (Fig.7 and plate 6). Details of the trial are presented in the Table 14.

Highest percentages of transmission of 90 and 100 were through AG in Sree Bhadra and Sree Rethna varieties, respectively. These were followed by WG (80% and 60%) and SG (60% each) in Sree Bhadra and Sree Rethna. The symptom expression was also earlier in AG (I5-23 d) followed by WG (20-25 d) and SG (32-40 d) in both the varieties. The major symptoms observed were chlorotic leaf spots, ring spots and feathering.

Plate 6 SPFMV transmission through wedge grafting

Table 12 Transmission efficiency of SPFMV by different vectors to various host species

Sl. No.	Vectors	Per cent transmission								
		I. batatas var. Sree Rethna			*I. nil*			*I. setosa*		
		No. of plants		Per cent transmission	No. of plants		Per cent transmission	No. of plants		Per cent transmission
		Inoculated	Infected		Inoculated	Infected		Inoculated	Infected	
1.	*A. craccivora*	15	6	40	10	4	40	10	6	60
2.	*A. gossypii*	15	3	20	10	2	20	10	3	30
3.	*P.nigronervosa*	15	0	0	10	0	0	10	0	0
4.	*B. tabaci*	15	0	0	10	0	0	10	0	0

Table 13 Aphid transmission and SPFM symptom development in three species of *Ipomoea*.

Sl. No.	Host plants	DAP for first appearance of symptoms	Initial symptoms	Major symptoms
1.	*I. batatas* var. Sree Rethna	25-30	CLS	CLS ,RS, F
2.	*I. nil*	21-25	CLS	CLS, Mosaic, distortion, puckering ,vein clearing
3.	*I. setosa*	20-25	CLS	CLS,mosaic, vein banding, RS, distortion

CLS - Chlorotic leaf spot RS - Ring spot F - Feathering

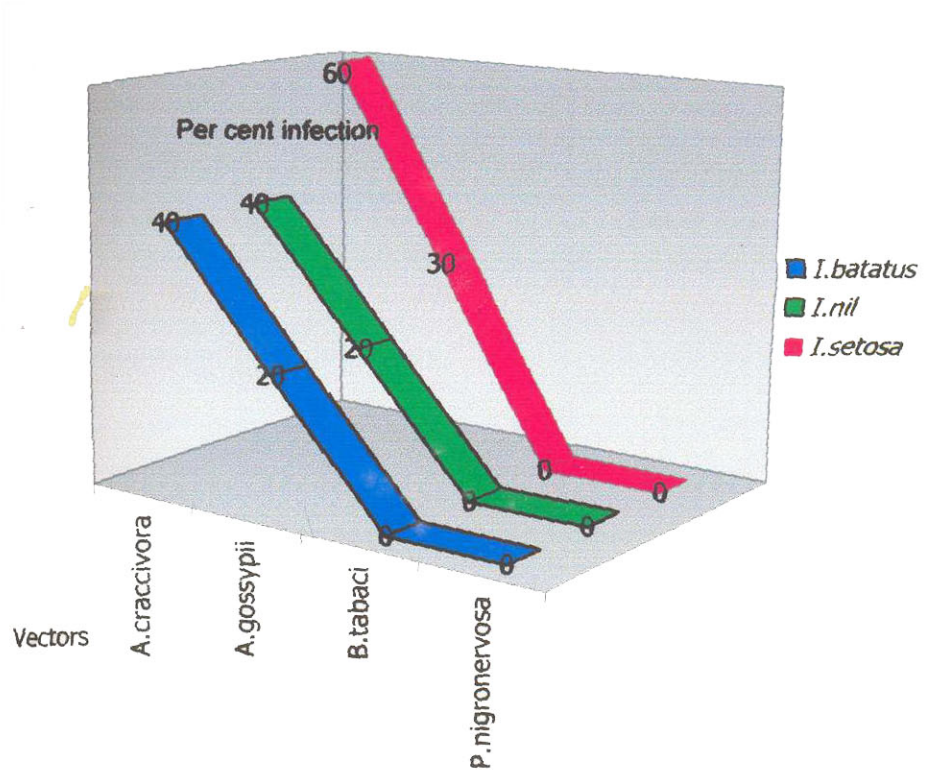


Fig. 6. Transmission efficiency of SPFMV by different vectors to various host species

Table 14 SPFMV transmission in sweet potato plants (varieties, Sree Bhadra and Sree Rethna) through different types of grafting

Sl. No	Types of grafting	Varieties	No. of grafts made	Successful grafts	Virus infected	Per cent transmission
1	Approach grafting	Sree Bhadra	15	14	13	90
		Sree Rethna	15	15	15	100
2	Side grafting	Sree Bhadra	20	12	7	60
		Sree Rethna	20	10	6	60
3	Wedge grafting	Sree Bhadra	15	12	10	80
		Sree Rethna	15	11	7	60

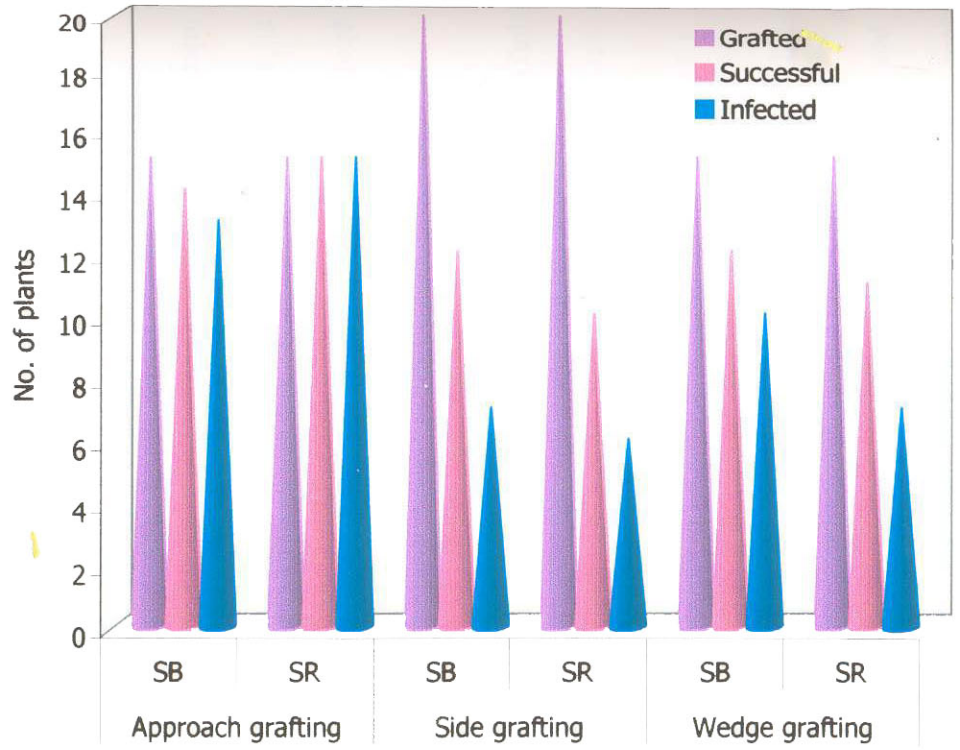


Fig.7. Transmission of SPFMV through different types of grafting in sweet potato varieties, Sree Bhadra(SB) and Sree Rethna(SR)

4.3.6 Seed transmission

Seedlings of *I. batatas* varieties, Sree Bhadra and Sree Rethna, *I. nil* and *I. setosa* were raised in the order of 135, 53, 350 and 58 numbers respectively. They were observed for development of symptoms up to three months. None of the seedlings showed the symptoms of SPFM (Table 15). When leaf samples were taken at random from the seedlings, which did not show the symptoms and tested through NCM - ELISA, none of them showed positive reaction to SPFM.

4.4 Physiological changes

The data pertaining to photosynthesis, respiration and transpiration were statistically analysed to find out whether there was any significant physiological change in the sweet potato plants due to SPFMV- infection.

The results showed that the effect of SPFMV- infection on photosynthesis was highly significant, i.e., 18.74 μ mol $m^{-2}s^{-1}$ and 6.33 μ mol $m^{-2}s^{-1}$ in the case of healthy and infected respectively in Sree Rethna, but in a tolerant variety, Sree Vardhini, it was not significant (Table 16 and Fig.8).

While comparing the varieties, in healthy plants, photosynthesis was significantly high in the susceptible variety, Sree Rethna (18.74 μmol $m^{-2}s^{-1}$) whereas in infected plants the tolerant variety Sree Vardhini showed higher rate of photosynthesis (9.78 μmol $m^{-2}s^{-1}$) than Sree Rethna. No significant changes were observed due to SPFMV- infection in respiration and transpiration in both the varieties.

Table 15 Seed transmission test for SPFMV

Sl. No	Names of plants	Number of seeds		Per cent germination	Per cent transmission	Reaction in NCM ELISA
1	*I. batatus* (Sree Bhadra)	250	135	47	0	–
2	*I. batatus* (Sree Rethna)	90	53	58	0	–
3	*I. setosa*	75	58	72	0	–
4	*I. nil*	375	350	93	0	–

Table 16 Physiological changes caused by SPFM in sweet potato varieties, Sree Rethna and Sree Vardhini

Sl. No	Varieties	* Physiological changes		
		Photosynthesis (μ mol m^{-2} s^{-1})	Respiration (mol m^{-2} s^{-1})	Transpiration (mol m^{-2} $s^{-1)}$
1	Sree Rethna H	18.74	2.11	1.91
	I	6.33	2.36	2.23
2	Sree Vardhini H	10.70	4.80	3.18
	I	9.78	2.91	3.47
	CD (0.01)	2.53	NS	NS

H- Healthy, I- Infected

* Mean of nine replications
NS - Non significant

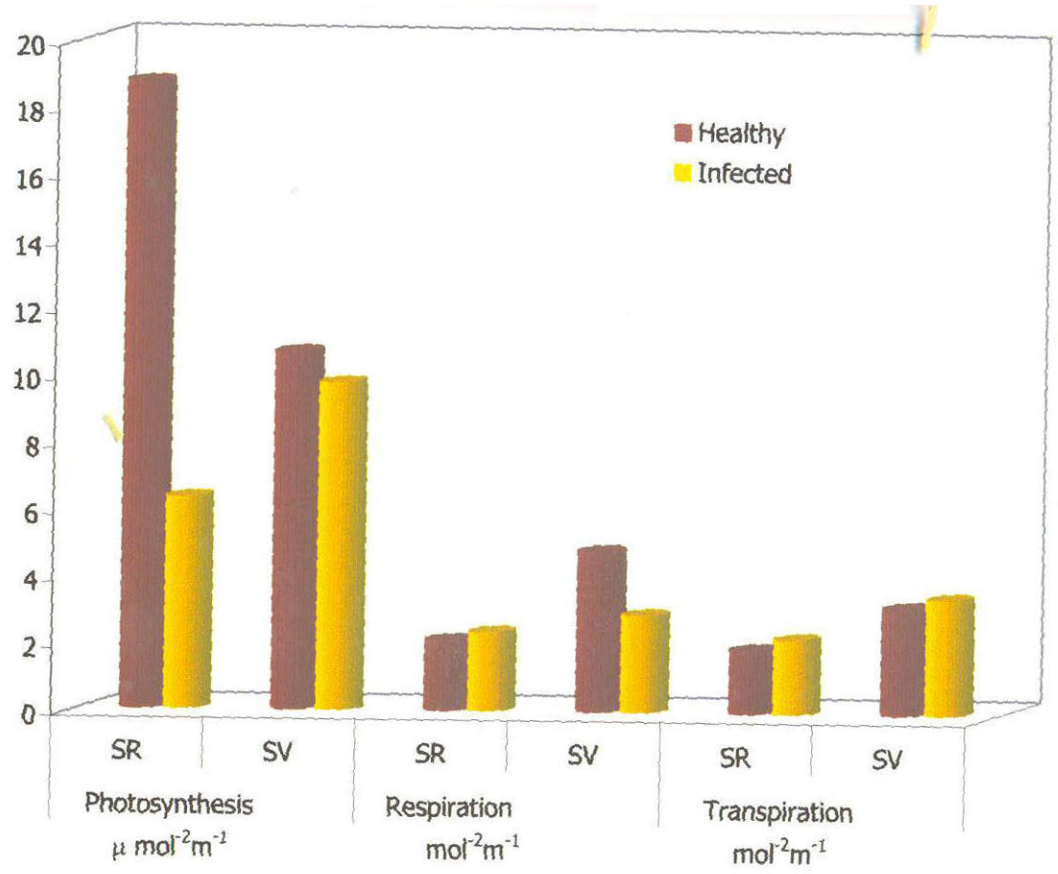


Fig. 8. Effect of SPFM on the physiology of sweet potato varieties,Sree Rethna and Sree Vardhini

4.5 Biochemical studies

Changes in the total carbohydrates, starch, total sugars, chlorophyll, total phenols, OD-phenols, flavanols, proteins and defense related enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonia lyase were studied in healthy and SPFMV- infected sweet potato plants. Two susceptible varieties, Sree Rethna and Vavvathooki and one tolerant variety, Sree Vardhini were used for the study.

4.5.1 Total carbohydrates

Statistical analysis of the data revealed that there was a significant reduction in the total carbohydrate contents of the susceptible varieties, Sree Rethna and Vavvathooki due to SPFMV infection at 30 days and 30 and 45 days after planting respectively. The tolerant variety, Sree Vardhini did not show any change due to the infection at all the three intervals (Fig. 9). The reduction was 8.96, 10.99 and 2.86 mg g^{-1} in Sree Rethna, Vavvathooki and Sree Vardhini respectively 30 days after planting (Table 17).

4.5.2 Starch

It was observed that the SPFMV infection caused significant reduction in starch content in susceptible varieties such as Sree Rethna and Vavvathooki but not in the tolerant variety Sree Vardhini (Table 17 and Fig.9).

The amounts of reduction in the infected plants were 12.34, 10.00 and 1.67 mg g^{-1} in Sree Rethna, Vavvathooki and Sree vardhini, respectively, 30

days after planting. The highest reduction was noted in Sree Rethna and Vavvathooki 30 days after planting.

4.5.3 Total sugars

The data on total sugar content of the leaves of healthy and SPFMV-infected plants of viz., Sree Rethna, Vavvathooki and Sree Vardhini did not show significant variation (Table 17).

4.5.4 Total chlorophyll

The data on total chlorophyll content of healthy and infected leaves showed that SPFMV- infection caused significant reduction in chlorophyll content in all the three varieties at different intervals, except in Vavvathooki and Sree Vardhini at 15 days after planting.

The maximum reduction was observed 30 days after planting in Sree Rethna and Sree Vardhini, viz., 0.66 and 0.48 mg g^{-1} whereas in Vavvathooki it was 45 days after planting. The reduction was 0.64 mg g^{-1}. The trend showed that the reduction was more in susceptible varieties than the tolerant variety (Table 18 and Fig.10).

4.5.4.1 Chlorophyll 'a'

The data showed that the SPFMV infection significantly reduced the chlorophyll 'a' content in three varieties at different intervals except Vavvathooki and Sree Vardhini at 15 days after planting (Table 18 and Fig.10).

Table 17 Effect of SPFMV infection on total carbohydrates, starch and total sugars in sweet potato plants

Sl. No	Varieties	* Total Carbohydrates (mg g^{-1}) DAP			* Starch mg g^{-1} DAP			* Total sugars (mg g^{-1}) DAP		
1		15	30	45	15	30	45	15	30	45
	Sree Rethna H	35.38	47.56	34.14	34.33	38.67	26.00	6.00	8.33	7.67
	I	37.95	38.60	30.14	30.33	26.33	19.67	7.50	8.01	9.33
		(+2.57)	(-8.96)	(-4.00)	(-4.00)	(-12.34)	(-6.33)	(+1.50)	(-0.32)	(+1.66)
		NS	**	NS	**	**	**	NS	NS	NS
2	Vavvathooki H	33.13	44.67	41.33	24.33	34.67	33.67	8.33	9.33	7.33
	I	33.00	33.68	31.33	23.00	24.67	21.33	8.00	8.33	9.00
		(-0.13)	(-10.99)	(-10.00)	(-1.33)	(-10.00)	(-12.34)	(-0.33)	(-1.00)	(+1.67)
		NS	**	**	NS	**	**	NS	NS	NS
3	Sree Vardhini H	33.78	44.00	33.13	26.00	33.67	23.33	8.00	9.00	8.67
	I	33.15	41.14	32.76	24.00	32.00	22.33	8.33	8.46	9.33
		(-0.63)	(-2.86)	(-0.37	(-2.00)	(-1.67)	(-1.00)	(+0.33)	(-0.54)	(+0.66)
		NS	NS	NS	NS	NS	NS	NS	NS	NS
		$CD_{(0.01)}$ 5.28			$CD_{(0.01)}$ 3.71					

* Mean of three replications ** Significant at CD (0.01), NS-Non significant
DAP- Days after planting, H- Healthy, I- Infected
Figures in the parentheses are values increase (+)/ decrease (-) over healthy

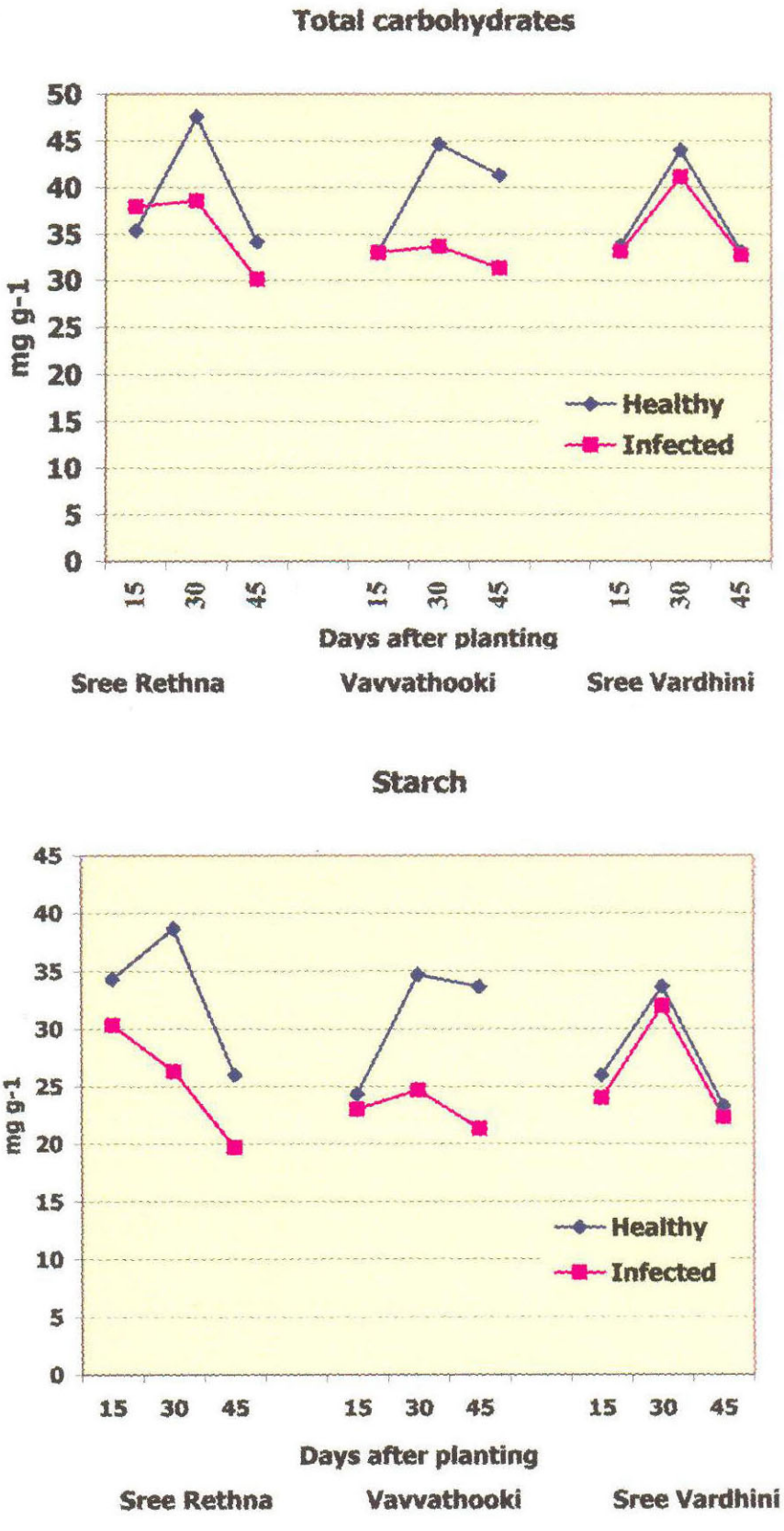
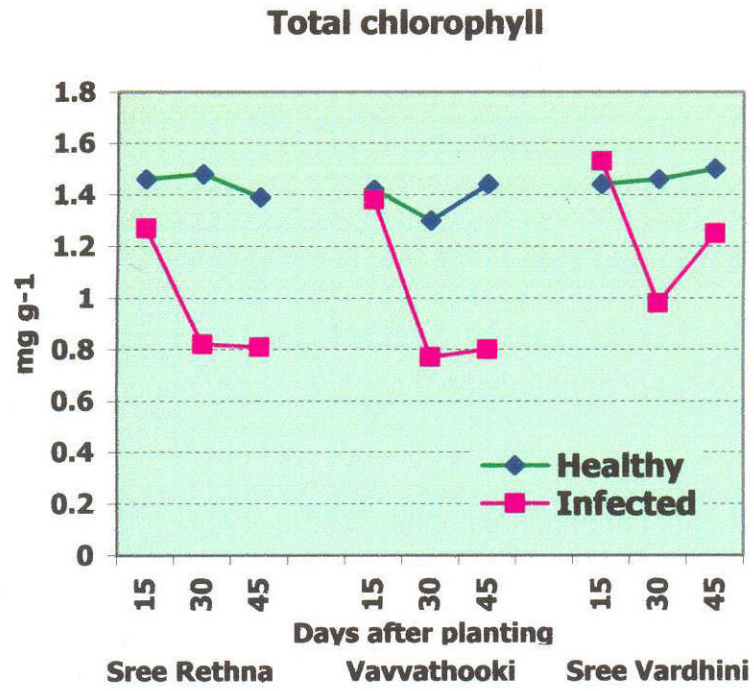


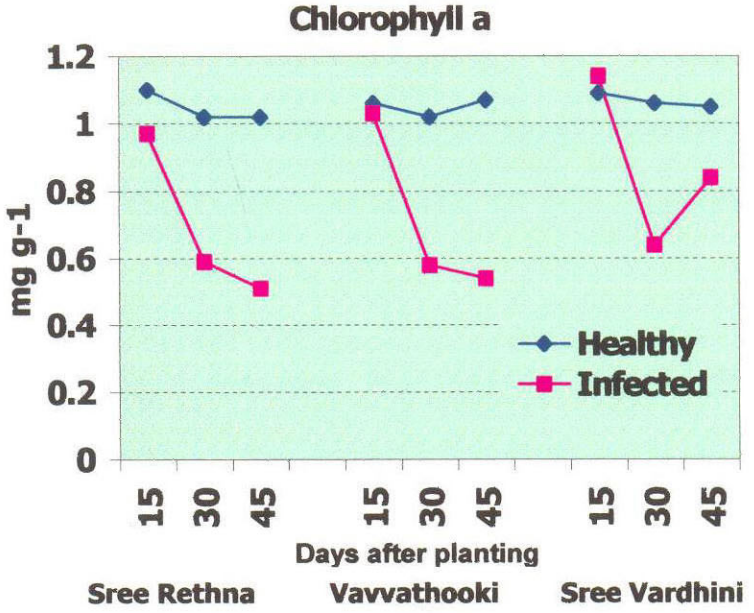
Fig. 9. Effect of SPFM on total carbohydrates and starch content in sweet potato leaves

Table 18. Effect of SPFMV –infection on the total chlorophyll, chlorophyll 'a' and chlorophyll 'b' in sweet potato plants

Sl. No	Varieties	♦Total chlorophyll (mg g^{-1}) Days after planting			♦Chlorophyll 'a' (mg g^{-1}) Days after planting			♦ Chlorophyll 'b' (mg g^{-1}) Days after planting		
1	Sree Rethna	15	30	45	15	30	45	15	30	45
	H	1.46	1.48	1.39	1.10	1.02	1.02	0.36	0.38	0.36
	I	1.27	0.82	0.81	0.97	0.59	0.51	0.30	0.18	0.30
		(-0.19)	(-0.66)	-0.58)	(-0.13)	(-0.43)	(-0.51)	(-0.06)	(-0.20)	(-0.06)
		*	*	*	*	*	*	**	**	**
2	Vavvathooki H	1.42	1.30	1.44	1.06	1.02	1.07	0.36	0.36	0.36
	I	1.38	0.77	0.80	1.03	0.58	0.54	0.34	0.19	0.26
		(-0.04)	(-0.53)	(-0.64)	(-0.03)	(-0.44)	(-0.53)	(-0.02)	(-0.17)	(-0.10)
		NS	*	*	NS	*	*	NS	**	**
3	Sree Vardhini H	1.44	1.46	1.50	1.09	1.06	1.05	0.45	0.46	0.45
	I	1.53	0.98	1.25	1.14	0.64	0.84	0.43	0.34	0.41
		(+0.09)	(-0.48)	(-0.25)	(+0.05)	(-0.42)	(-0.21)	(-0.02)	(-0.12)	(-0.04)
		NS	*	*	NS	*	*	NS	**	NS
		CD (0.05) 0.1314			CD (0.05) 0.0990			CD (0.01) 0.0619		

♦ Mean of three replications * Significant at CD $_{(0.05)}$. ** significant at CD $_{(0.01)}$.
NS-Non significant. DAP- Days after planting, H- Healthy, I. Infected
Figures in the parenthesis are values increase (+)/ decrease (-) over healthy





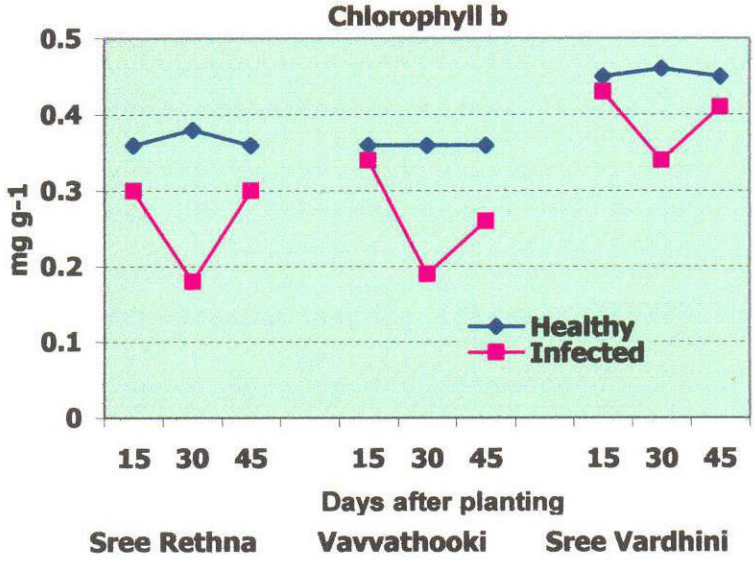


Fig. 10. Effect of SPFM on chlorophyll content in sweet potato leaves

The highest reduction was observed 45 days after planting in Sree Rethna and Vavvathooki. The reductions were 0.51 and 0.53 mg g^{-1} respectively. This was followed by reductions at 30 and 15 days after planting. In the case Sree Vardhini the highest reduction was observed 30 days after planting, i.e., 0.42 followed by 45 and 15 days after planting.

The observations showed that the reduction was more in Vavvathooki followed by Sree Rethna and the least in the tolerant variety Sree Vardhini.

4.5.4.2 Chlorophyll 'b'

The SPFMV- infected plants of all the varieties, generally showed significant reduction in chlorophyll 'b' except Vavvathooki at 15 days after planting and Sree Vardhini at 15 and 45 days after planting (Table 18 and Fig.10).

The highest reduction was observed in Sree Rethna 30 days after planting followed by Vavvathooki and Sree Vardhini. The reduction was more at 30 days after planting followed by 45 days. The reduction after 15 days of planting was significant only in Sree Rethna. The reduction after 30 days of planting was 0.20, 0.17 and 0.12 mg g^{-1} in Sree Rethna, Vavvathooki and Sree Vardhini respectively.

4.5.5 Phenolics

The data revealed that the effects of SPFMV infection on the total phenol, OD-phenol and flavanol contents were not significant in any variety at all the intervals (Table 19).

Table 19 Effect of SPFMV -infection on total phenols, OD- phenols and flavanols in sweet potato plants

Sl. No	Varieties	* Total phenols (mg g^{-1}) Days after planting			* OD- Phenols (mg g^{-1}) Days after planting			* Flavanol s(mg g^{-1}) Days after planting		
1	Sree Rethna	15	30	45	15	30	45	15	30	45
	H	1.86	3.65	3.34	1.05	1.57	1.62	0.25	0.20	0.19
	I	1.98	4.08	3.75	1.09	1.43	1.44	0.18	0.16	0.17
		(+0.12)	(+0.43)	(+0.41)	(+0.04)	(-0.14)	(-0.18)	(-0.07)	(-0.04)	(-0.02)
		NS	NS	NS	NS	NS	NS	NS	NS	NS
2	Vavvathooki H	1.91	1.89	2.79	1.02	1.28	1.22	0.25	0.19	0.16
	I	2.10	2.25	2.76	1.01	1.47	1.43	0.19	0.19	0.14
		(+0.19)	(+0.36)	(-0.03)	(-0.01)	(+0.19)	(+0.21)	(-0.06)	(0)	(-0.02)
		NS	NS	NS	NS	NS	NS	NS	NS	NS
3	Sree Vardhini H	2.45	3.55	3.57	1.39	1.63	1.67	0.22	0.19	0.16
	I	2.62	3.81	3.80	1.31	1.95	1.85	0.20	0.19	0.17
		(+0.17)	(+0.26)	(+0.23)	(-0.08)	(+0.32)	(+0.18)	(-0.02)	(0)	(+0.01)
		NS	NS	NS	NS	NS	NS	NS	NS	NS

* Mean of three replications. NS- Non significant
DAP- Days after planting, H- Healthy, I.-Infected
Figures in the parenthesis are values increase (+) / decrease (-) over healthy.

4.5.6 Protein

The protein contents of the infected plants of the two susceptible and one tolerant variety were also not significantly differed from healthy plants at all the three intervals (Table 20).

4.5.7 Defense related enzymes

4.5.7.1 Peroxidase

The data on peroxidase activity of healthy and SPFM infected sweet potato plants revealed that the variation between them was statistically not significant in all the three varieties studied at the three intervals (Table 20).

4.5.7.2 Polyphenol oxidase

The differences in polyphenol oxidase were highly significant in both the susceptible varieties observed at 45 days after planting whereas at 15 and 30 days after planting and also in tolerant variety the differences were not significant (Table 20 and Fig.11). The reduction in the change due to SPFM infection was higher in the variety Vavvathooki followed by Sree Rethna and Sree Vardhini. The reduction in the OD values were 0.35, 0.27 and 0.19 respectively, in the above varieties at 45 DAP.

4.5.7.3 Phenylalanine ammonia lyase

The differences in phenylalanine ammonia lyase activity between healthy and infected plants were statistically not significant in any of the three varieties at all the three intervals (Table 20).

Table 20 Effect of SPFMV infection on protein, peroxidase, polyphenol oxidase and Phenylalanine ammonia lyase in sweet potato plants

Sl. No	Varieties	Protein (mg g^{-1}) Days after planting			Peroxidase (change in OD value g^{-1} m^{-1}) Days after planting			Polyphenol oxidase (change in OD value g^{-1} m^{-1}) Days after planting			Phenylalanine ammonia lyase (µg g^{-1}) Days after planting		
		15	30	45	15	30	45	15	30	45	15	30	45
1	Sree Rethna H	42.24	52.97	45.23	0.47	2.47	2.83	0.79	0.30	0.62	925	968	905
	I	37.37	45.70	39.35	0.55	2.67	2.90	0.76	0.32	0.35	92.3	975	854
		(-4.87)	(-7.27)	(-5.88)	(+0.08)	(+0.20)	(+0.07)	(-0.03)	(+0.02)	(-0.27)	(-2.00)	(+7.00)	(-51)
		NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS
2	Vavvathooki H	34.32	43.36	37.16	37.16	2.35	2.66	0.62	0.16	0.64	1040	1060	902
	I	28.06	35.60	30.32	30.32	3.00	3.10	0.51	0.40	0.29	1046	1036	930
		(-6.26)	(-7.76)	(-6.84)	(-6.84)	(+0.65)	(+0.44)	(-0.11)	(+0.24)	(-0.35)	(+6.00)	(-24.00)	(+28.00)
		NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS
3	Sree Vardhini H	51.48	63.80	54.95	1.22	2.90	1.92	0.72	0.65	0.43	1163	1118	918
	I	54.66	68.66	58.62	1.96	2.98	2.63	0.90	0.74	0.24	1170	1176	1013
		(+3.18)	(+4.86)	(3.67)	(+0.74)	(+0.08)	(+0.71)	(+0.18)	(+0.09)	(-0.19)	(+7.00)	(+58)	(+95)
		NS	NS	NS	NS	NS	NS	NS	NS	NS			
								CD (0.01) 0.27					

* Mean of three replications. ** significant at CD (0.01) NS- Non significant
H- Healthy, I.-Infected
Figures in the parenthesis are values increase (+) / decrease (-) over healthy.

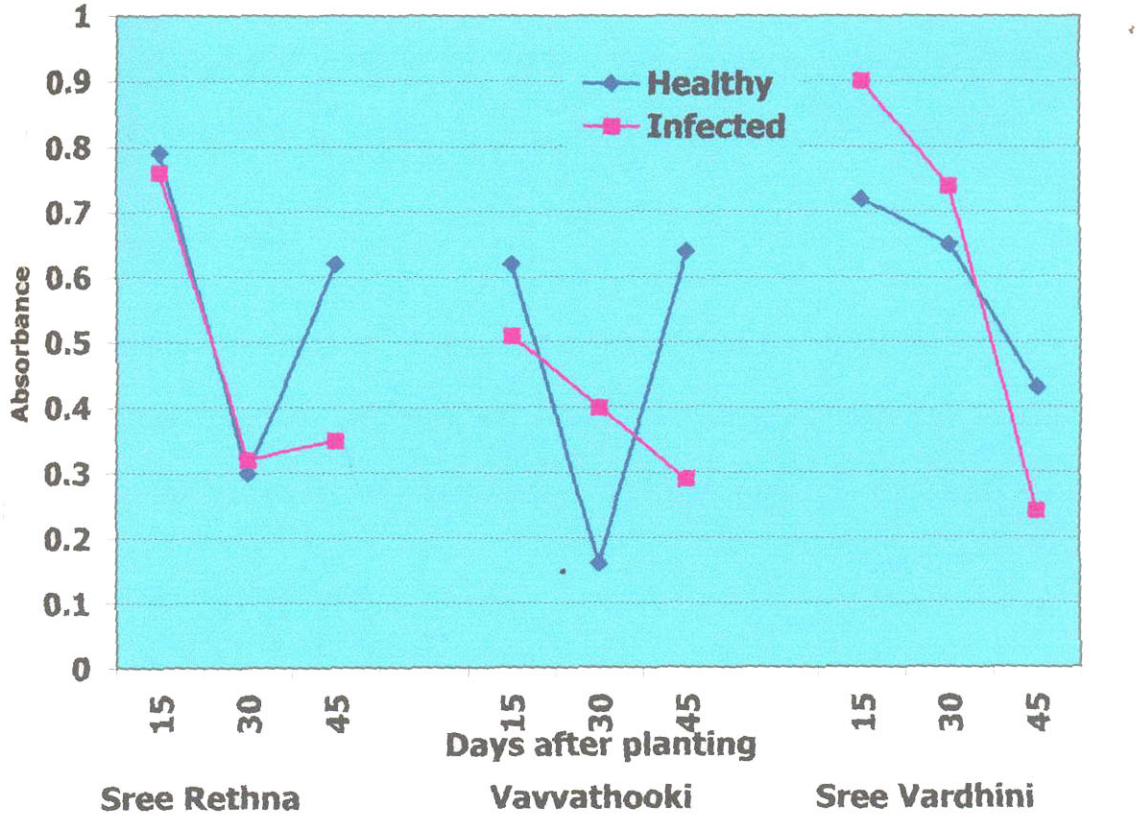


Fig. 11. Effect of SPFM on the activity of polyphenol oxidase in sweet potato leaves

4.6 Biometrical studies

4.6.1 Vine and leaf characters

4.6.1.1 Number of vines

The results (Table 21) indicated that the SPFMV- infection did not significantly reduce the number of vines in both the varieties, viz., Sree Rethna and Vavvathooki in all the three periods of observation.

4.6.1.2 Length of vine

The statistical analysis of the data revealed that the effect of SPFMV-infection on the length of vine was not significant when compared to healthy plants in both the varieties, viz., Sree Rethna and Vavvathooki. (Table 21).

4.6.1.3 Length of internode

The internode length was significantly influenced by the SPFMV-infection in the variety Vavvathooki(Table 21) .

The internode length was significantly high in the infected plants of the variety Vavvathooki at all the three times of observations, viz., 30, 60 and 90 DAP. The increase in lengths was 0.45, 0.84 and 0.67 cm in the respective DAP in infected plants (Fig.12). However, in the variety Sree Rethna, the internode length was not significantly influenced by SPFMV- infection.

Table 21 Effect of SPFMV- infection on number of vines, length of vines and length of internodes in Sree Rethna and Vavvathooki varieties of sweet potato

Sl. No.	Varieties		*Number of vines Days after planting			*Length of vine (cm) Days after planting			*Length of internode(cm) Days after planting		
			30	60	90	30	60	90	30	60	90
1.	Sree Rethna	H	3	5	7	34.83	56.77	59.77	2.44	2.24	2.26
		I	2	5	7	42.71	57.10	57.05	2.16	1.97	1.96
			(-1)	(0)	(0)	(+7.88)	(+0.33)	(+2.72)	(-0.28)	(-0.27)	(-0.30)
			NS	NS	NS	NS	NS	NS	NS	NS	NS
2.	Vavvathooki	H	4	9	13	23.84	20.99	23.27	1.48	1.30	1.39
		I	5	8	12	29.90	26.51	27.69	1.93	2.14	2.06
			(-1)	(-1)	(-1)	(+6.06)	(+5.52)	(+4.42)	(+0.45)	(+0.84)	(+0.67)
	CD (0.05)		NS	NS	NS	NS	NS	NS	0.341	0.341	0.341

H – Healthy, I – Infected *Mean of ten replications, NS – Non significant
Figures in the parentheses are values of decrease (-)/ increase(+) over healthy.

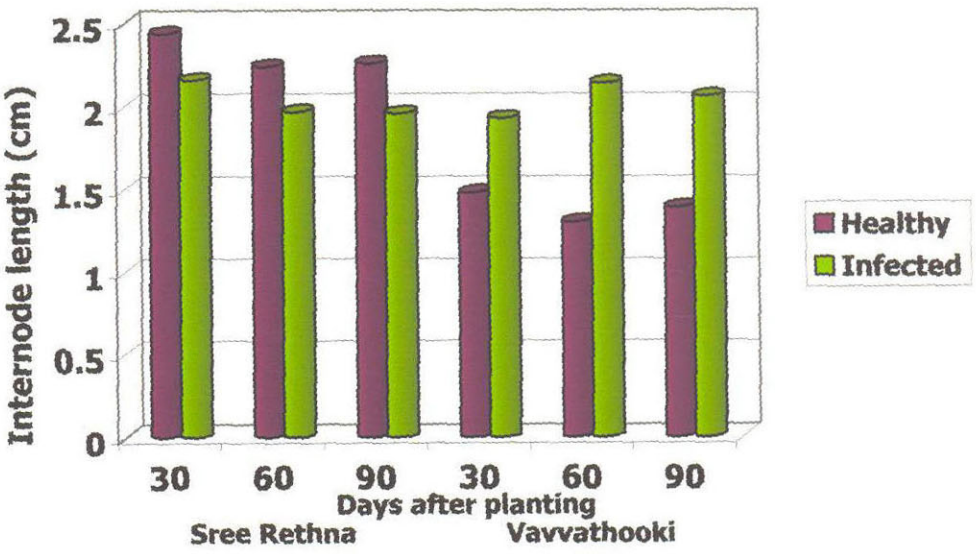


Fig.12. Effect of SPFMV- infection on length of internode in Sree Rethna and Vavvathooki varieties of sweet potato

4.6.1.4 Number of leaves

The SPFMV- infected plants showed no significant difference in total number of leaves when compared to healthy plants in both the varieties in all the three periods of observation (Table 22).

4.6.1.5 Leaf area

No significant difference was observed in leaf area between healthy and infected plants of both the varieties in all the three times of observation (Table 22).

4.6.1.6 Total Biomass

The data (Table 23) showed that SPFMV- infection did not significantly reduce the biomass of sweet potato plants in Sree Rethna and Vavvathooki.

4.6.1.7 Dry matter content

There was no significant difference in the dry matter content between healthy and infected plants in Sree Rethna and Vavvathooki (Table 23).

4.6.2 Tuber characters

4.6.2.1 Number of tubers and tuber weight

The results showed that there was no significant difference in the total weight of tubers in SPFMV- infected and healthy sweet potato plants of both the tested varieties (Table 23).

Table 22 Effect of SPFMV- infection on total number of leaves and leaf area of sweet potato varieties, Sree Rethna and Vavvathooki

Sl. No.	Varieties	*Total number of leaves Days after planting			*Leaf area (cm^2) Days after planting		
		30	60	90	30	60	90
1	SreeRethna Healthy	44	97	109	32.64	30.13	29.28
	SreeRethna Infected	36	99	99	31.82	30.42	29.81
		(-8.00)	(+2.00)	(-10.00)	(-0.82)	(+0.29)	(+0.53)
		NS	NS	NS	NS	NS	NS
2	Vavvathooki Healthy	102	108	107	20.81	20.80	25.04
	Vavvathooki Infected	96	110	113	22.38	21.15	23.52
		(-6.00)	(+2.00)	(+6.00)	(+1.57)	(+0.35)	(-1.52)
		NS	NS	NS	NS	NS	NS

NS - Non significant
* Mean of ten replications

Table 23 Effect of SPFMV- infection on yield parameters and drymatter of sweet potato vines and tubers of two varieties, Sree Rethna and Vavvathooki

Sl. No.	Varieties		Vine				Tuber				
			**Drymatter			*Total biomass (g)	*Number	*Weight (g)	*Size (cm)		**Drymatter (g)
			30 DAP	60 DAP	90 DAP				Length	Girth	
1.	Sree Rethna	H	18.00	18.75	21.01	54.00	5	161	9.76	10.03	23.33
		I	17.67	18.14	21.70	66.00	5	172.5	10.40	11.05	23.00
			(-0.33)	(-0.61)	(+0.69)	(-12.00)	(0)	(+11.50)	(+0.64)	(+1.02)	(-0.33)
	CD (0.05)		NS	NS	NS	NS	NS	NS	NS	NS	NS
2.	Vavvathooki	H	16.33	18.76	19.47	73.00	4	163	15.02	10.62	28.00
		I	17.33	19.10	20.42	65.00	5	155	19.96	8.12	22.67
			(+1)	(+0.34)	(-0.85)	(-8.00)	(+1.00)	(-8.00)	(+4.94)	(-2.50)	(-5.33)
	CD (0.05)		NS	NS	NS	NS	NS	NS	3.59	1.49	3.10

NS- Non significant
*Mean of ten replications **Mean of three replications DAP Days after planting

4.6.2.2 Colour and shape of tubers

The observation showed that there was no difference in colour between healthy and infected tubers in both the varieties. However, constrictions were observed in the tubers of the variety, Vavvathooki, infected with SPFM (Plate 7).

4.6.2.3 Size of tubers

In the variety Sree Rethna the infected plants showed no significant differences in the length and girth of the tubers when compared to the healthy plants, while in the variety Vavvathooki there were significant differences in these tuber characters (Fig. 13). The increase in tuber length (4.94 cm) and decrease in tuber girth (2.50 cm) were observed in infected plants of the variety Vavvathooki (Table 23).

4.6.2.4 Drymatter content

The data pertaining to the effect of SPFMV- infection on the drymatter content of sweet potato tubers showed that in the variety Sree Rethna, the drymatter content was not significantly influenced by SPFMV- infection whereas in Vavvathooki there was significant reduction (5.33 %) in SPFMV-infected plants (Table 23 and Fig.13).

4.6.2.5 Quality of tubers

4.6.2.5.1 Culinary quality

The opinion of the consumers on different culinary qualities of cooked tubers of healthy and SPFMV- infected plants is presented as the number of persons suggesting each category, viz., excellent, good, moderate and poor (Table 24). The study indicated that variation in flavour, sweetness, colour

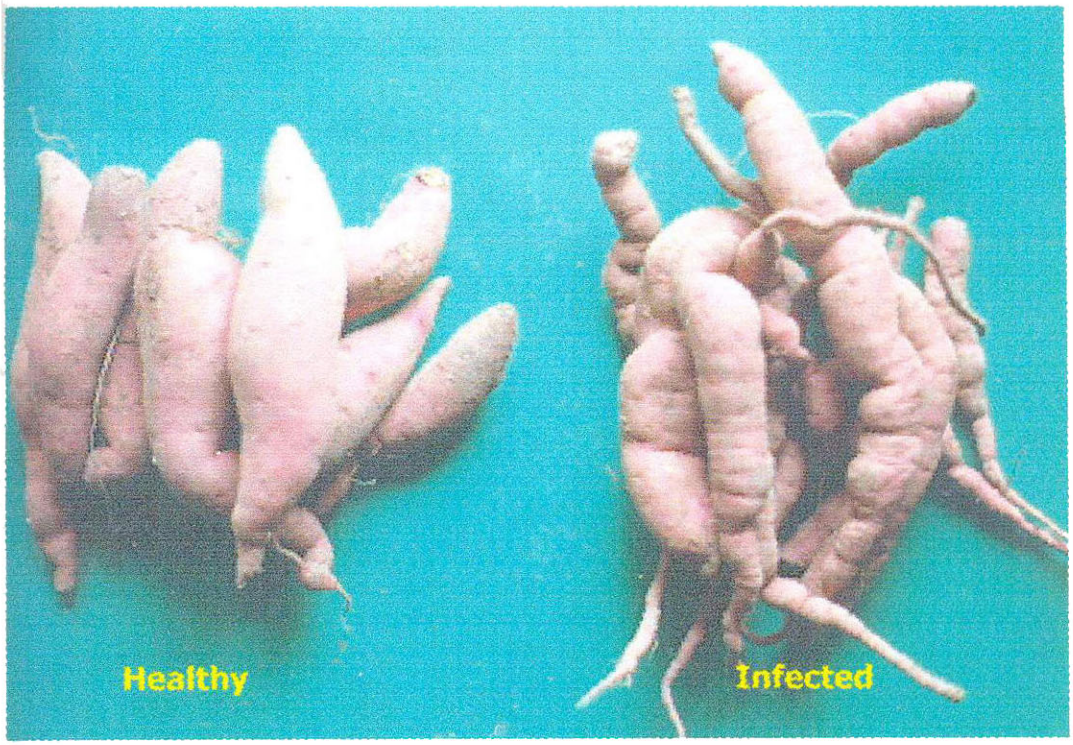


Plate 7 Constrictions due to SPFMV- infection

Table 24 Effect of SPFMV- infection on culinary qualities of sweet potato tubers of Sree Rethna and Vavvathooki

Sl. No	Quality characters		Number of consumers							
			Sree Rethna				Vavvathooki			
			E	G	M	P	E	G	M	P
1	Flavour	H	-	18	12	-	3	12	15	-
		I	-	20	10	-	3	11	16	-
2	Consistency	H	-	22	8	-	4	17	9	-
		I	-	18	12	-	-	10	20	-
3	Sweetness	H	-	24	6	-	5	11	14	-
		I	-	22	8	-	4	10	16	-
4	Colour	H	-	21	9	-	8	18	4	-
		I	-	18	12	-	6	16	8	-
5	Over all taste	H	8	17	5	-	6	19	5	-
		I	9	18	3	-	4	18	8	-

Category: E- Excellent, G- Good, M- Moderate, P- Poor
H- Healthy, I- Infected

and also the over all taste of the tubers was not much in both the varieties between healthy and infected. The variation was more in consistency. The consistency of the tubers from healthy plants of the variety Sree Rethna was categorized as good by 22 and as moderate by 8 persons while the corresponding figures were 18 and 12 in the tubers from infected plants.

In the variety Vavvathooki, the consistency of the tubers was categorized as excellent, good and moderate by 4, 17 and 9 consumers in healthy and 0, 10 and 20 respectively in infected tubers.

4.6.2.5.2 Quantity of nutrients

4.6.2.5.2.1 Total Carbohydrates

The data on the effect of SPFMV- infection on total carbohydrate content of the tubers of Sree Rethna and Vavvathooki showed that the disease reduced the total carbohydrate content in tubers significantly. The reduction was 25.75 and 25.00 mg g^{-1} in Sree Rethna and Vavvathooki, respectively (Table 25 and Fig.14).

4.6.2.5.2.2 Starch

A similar trend as in total carbohydrates was observed on starch content also (Table 25 and Fig.14). The reduction in the starch content was 27.75 and 24.00 mg g^{-1} in the case of Sree Rethna and Vavvathooki, respectively. It was noted that the reduction was more in Sree Rethna than Vavvathooki.

Table 25 Effect of SPFMV infection on total carbohydrates, starch, sugars and proteins in the sweet potato tubers

Sl. No.	Varieties		*Quality changes			
			Total carbohydrates (mg g^{-1})	Starch (mg g^{-1})	Total sugars (mg g^{-1})	Proteins (mg g^{-1})
1.	Sree Rethna	H	280.00	222.00	44.25	66.50
		I	254.25	194.25	43.75	62.32
			(-25.75)	(-27.75)	(-0.50)	(-4.18)
			**	**	NS	NS
2.	Vavvathooki	H	247.00	191.50	41.75	58.26
		I	222.00	167.50	39.75	54.82
			-25.00	-24.00	-2.00	-3.44
			**	**	NS	NS

* Mean of four replications
** Significant at CD $_{(0.01)}$ NS – Non significant
Figures in the parentheses are values of decrease (-) over healthy.

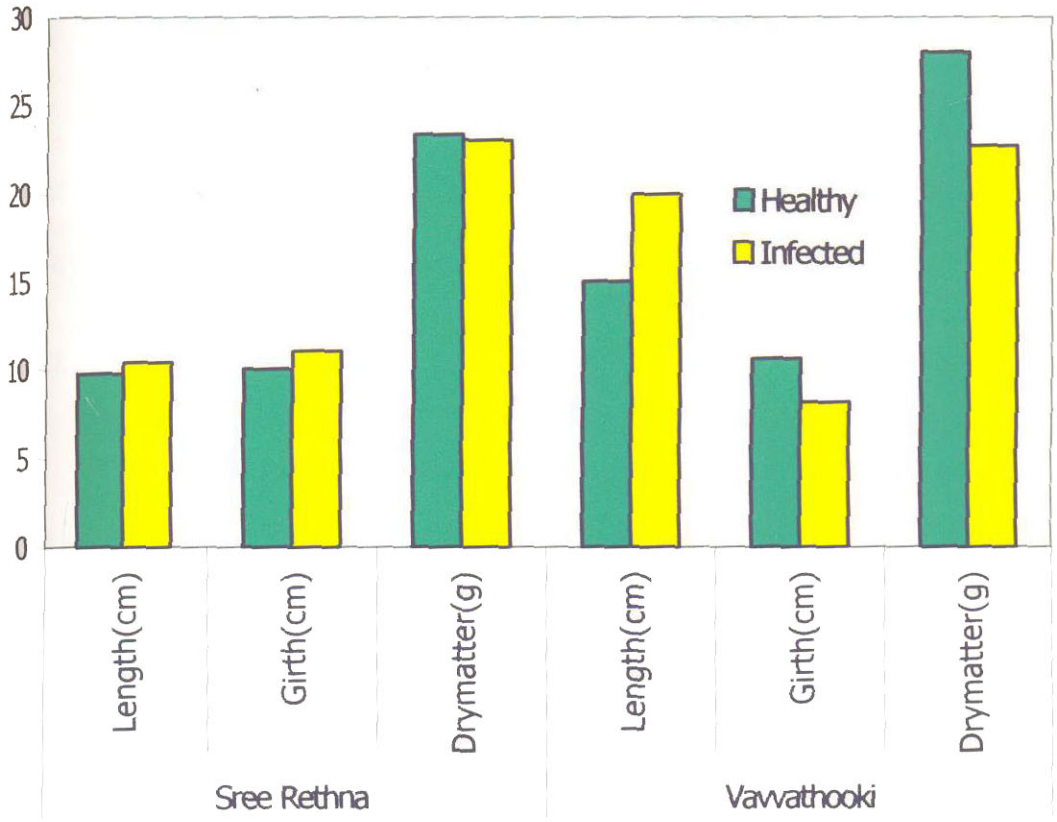


Fig.13.Effect of SPFMV- infection on sweet potato tuber characters

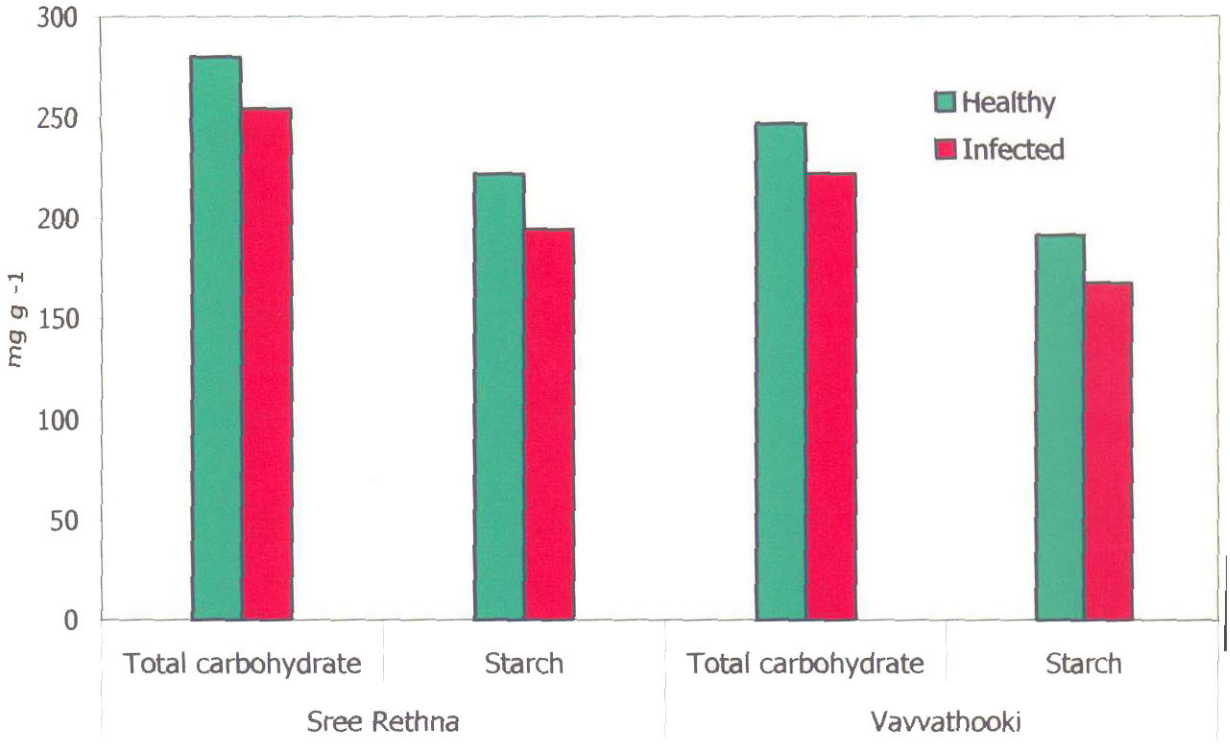


Fig. 14. Effect of SPFMV- infection on total carbohydrates and starch content in sweet potato tubers

4.6.2.5.2.3 Total sugars

The results indicated that the variations in the total sugar contents of the tubers from healthy and infected plants of the varieties Sree Rethna and Vavvathooki were not statistically significant (Table 25).

4.6.2.5.2.4 Protein

The protein content of the tubers from healthy SPFMV- infected plants of the varieties Sree Rethna and Vavvathooki did not vary significantly (Table 25).

4.7 Characterization of the virus

4.7.1 Purification

After purification, the diluted (1:100, v/v) virus preparation was inoculated on to *I. nil* seedlings to test their infectivity. The inoculated plants showed mosaic and distortion symptoms after six days in the first true leaf. The percentage of infection was 90 when 30 plants were inoculated in three trials. The results are presented in the Table 26.

4.7.2 Serodiagnosis

4.7.2.1 Ouchterlony agar double diffusion tests

The agar double diffusion test was performed with partially clarified leaf extracts of SPFMV infected *I. nil* and *I. batatas* against SPFMV polyclonal antiserum (3.8.2). Precipitin band was formed between the antiserum and the wells with the infected samples. There was no precipitin

Table 26 Per cent infection and symptom development due to purified SPFMV inoculation in *I. nil* seedlings

Items of observation	Results
Number of plants infected out of 30	27
Per cent infection	90
First appearance of symptom	6-10 days
Initial symptom	Faint mosaic
Major symptoms	Mosaic, distortion, vein banding and chlorotic leaf spot

band between the antiserum and the wells with the healthy and buffer control samples. These results showed that the antibodies of SPFMV were present in the antiserum.

4.7.2.2 Enzyme- linked immunosorbent assay (ELISA)

4.7.2.2.1 Direct antigen coating – ELISA (DAC- ELISA)

Polyclonal antibodies of SPFMV from Dr. R. W. Gibson was used in this test. The antigen-antibody reaction resulted as yellow colour and thus showed positive reaction in SPFMV- infected *I. batatas* varieties, Sree Rethna, Sree Vardhini and Vavvathooki, *I. nil, I. setosa* and also in aphids after acquisition access period (AAP). This indicated the presence of SPFMV in all the above samples.

The absorbance was read in an ELISA reader. The data indicated the variations in virus titre between different samples. The absorbance values were low in all the healthy samples (Table 27 and Fig.15).

The *I. setosa* with chlorotic leaf spot symptoms showed highest absorbance (0.955) followed by *I. nil* with yellow netting symptom (0.798) and *Aphis craccivora* after AAP (0.522). Least absorbance was read with *I. batatas* healthy sample. In *I. batatas*, *I. nil* and *I. setosa* all the healthy samples showed less absorbance than infected. Similarly in *A. craccivora,* sample of aphids before AAP showed less absorbance than that after AAP.

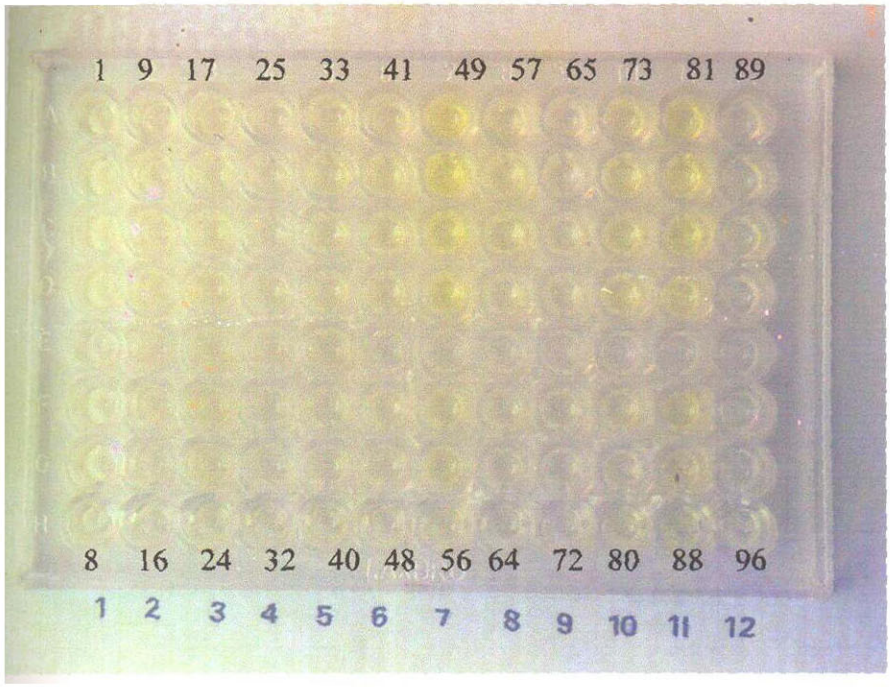


Plate 8a. Detection of SPFMV through DAC-ELISA

Sl. No.	Samples
9 -12	Sree Rethna (H)
13-16	Sree Vardhini(H)
17-20	Vavvathooki(H)
21-24	*I.nil*(H)
25-28	*I. setosa* (H)
29-32	*A. craccivora*(B)
49-52	*I.setosa*(I)
53-56	*I.nil*(I)
73-76	*A.craccivora*(A)
77-80	Sree Vardhini(I)
81-84	Vavvathooki(I)
85-88	Sree Rethna(I)

H- Healthy, I- Infected
B- Before acquisition,
A- After acquisition

Table 27 Absorbance of SPFMV- infected samples at 405 nm in ELISA reader

Sl.No.	Sample	* Absorbance at 405 nm
1	SR (H)	0.009
2	SR (I)	0.415
3	SV (H)	0.003
4	SV (I)	0.078
5	VT (H)	0.012
6	VT (I)	0.679
7	*I.nil* (H)	0.022
8	*I.nil* (I)	0.798
9	*I.setosa* (H)	0.019
10	*I.setosa* (I)	0.955
11	*A.craccivora* (B)	0.029
12	*A.craccivora* (A)	0.522

* Mean of four replications
SR - Sree Rethna SV - Sree Vardhini VT - Vavvathooki
H-Healthy I-Infected B-Before acquisition A-After acquisition

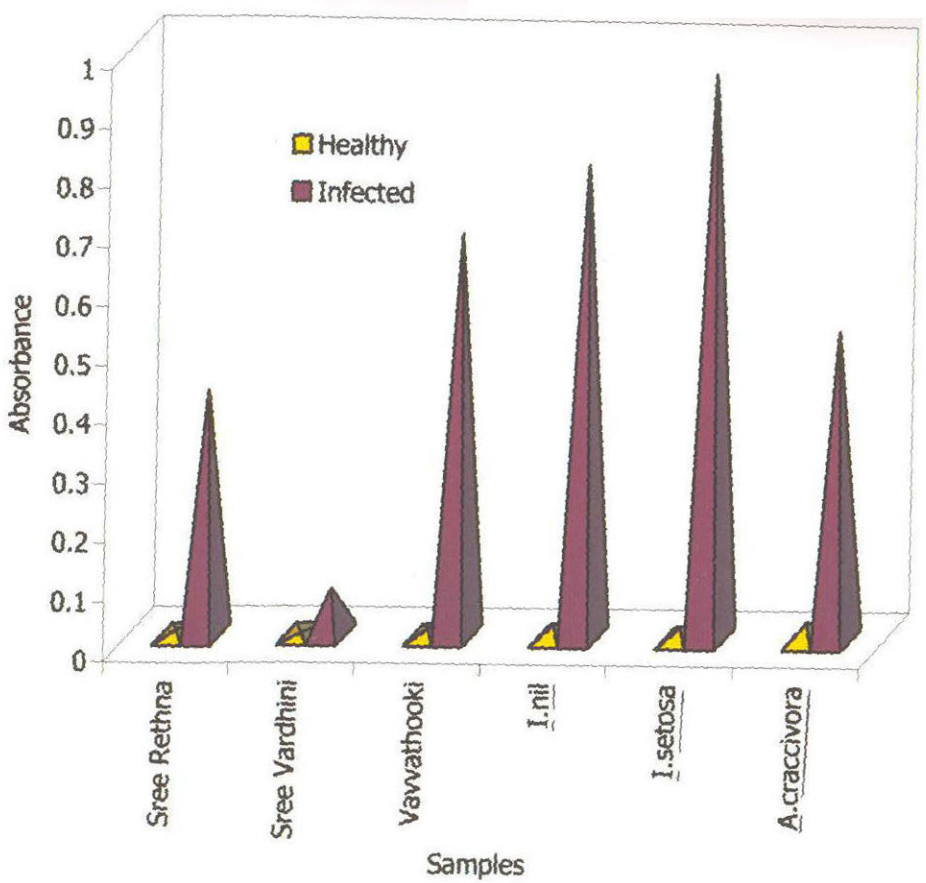


Fig. 15. Absorbance of SPFMV infected samples at 405 nm in ELISA reader

4.7.2.2.2 Nitrocellulose membrane- ELISA (NCM- ELISA)

The polyclonal antibodies to SPFMV from CIP was used for this study. Here also all the SPFMV- infected leaf samples and *A. craccivora* after AAP showed purple coloured spots in the NCM which showed that these samples contained SPFMV (Plate 8b). The reaction of all the samples are given in the Table 28.

4.7.3 Physical properties

4.7.3.1 Dilution end point (DEP)

It is evident from the data (Table 29 and Fig.16) that the DEP of the virus was between 10^{-3} and 10^{-4}. The per cent infection reduced as the dilution increased (Fig. 16). Among the different dilutions, viz., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}, the highest per cent infection was observed in 10^{-1} (70) dilution followed by 10^{-2} (50) and 10^{-3} (20). The infectivity was lost at 10^{-4} and 10^{-5} dilutions.

4.7.3.2 Thermal inactivation point (TIP)

The virus inoculum was subjected to different temperatures. The infectivity decreased as the temperature increased. The highest percentage of infection was observed in samples kept at room temperature (28 $\pm^0$C) as control followed by those kept at 40, 45, 50, 55 and 60^{0}C in water bath. (Fig.16). The infectivity was lost at 65^{0}C. The data indicated that the TIP of the virus was between 60 and 65^{0}C (Table 30 and Fig.15).

Plate 8b Detection of SPFMV through NCM-ELISA

Sl. No.	Samples
1 ,2	*I. batatas* (seeds)
6,7	*I. nil*(seeds)
9,10	*I. setosa*(seeds)
28,29	Sree Rethna (H)
39,40	Sree Vardhini (H)
52,53	Vavvathooki (H)
54,55	Kottaramchuvala
17,18	*A. craccivora* (B)
5,8	Sree Vardhini(I)
13,15	*I. setosa* (chlorotic leaf spot)
25,27	*I. nil*(yellow netting)
37,39	*A. craccivora* (A)
61,63	Sree Rethna(I)
60,72	Vavvathooki(I)

H- Healthy, I- Infected
B- Before acquisition,
A- After acquisition

Table 28 Reaction of different samples with polyclonal antibodies of SPFMV in NCM- ELISA

Sl. No	Samples	Reaction	
		H/ B	I/ A
I	Seed		
1	*I. batatas*	_	
2	*I. nil*	_	NA
3	*I. setosa*	_	
II	Leaves		
1	*I..batatus*		
1.1	Sree Rethna	_	+
1.2	Sree Vardhini	_	+
1.3	Vavvathooki	_	+
1.4	Kottaramchuvala	_	NA
1.5	*I.nil*(yellow netting)	NA	+
1.6	*I.setosa* (chlorotic leaf spot)	NA	+
III	*A. craccivora*	_	+

NA Not tried . + positive - negative

H – Healthy
B – Before acquisition
I – Infected
A – After acquisition

Table 29 Dilution end point of SPFMV

Sl. No.	Dilution	Number of plants infected out of ten	Per cent infection
1	10^{-1}	7	70
2	10^{-2}	5	50
3	10^{-3}	2	20
4	10^{-4}	-	-
5	10^{-5}	-	-
6	Undiluted	9	90

Table 30 Thermal inactivation point of SPFMV

Sl. No	Temperature (^{0}C)	Number of plants infected out of ten	Per cent infection
1	40	7	70
2	45	6	60
3	50	4	40
4	55	3	30
5	60	1	10
6	65	-	0
7	70	-	0
8	Room temperature (28± 4^0 C)	7	70

4.7.3.3 Longevity *in vitro* (LIV)

The infectivity of the sap was lost between 6 and 8 h of incubation at room temperature (28 ± 4^{0}C) and 10 h and 12 h under refrigerated condition (8^{0}C) (Table 31 and Fig.16). In general it was found that the infectivity decreased as the period in storage increased.

Highest per cent infection was observed in control i.e., the sap which was inoculated at 0 h followed by 2, 4, and 6 h when the sap was stored at room temperature. Under refrigerated condition (8^{0}C) also the same trend was found but the infectivity was retained up to 10 h of storage.

4.7.4 Electron microscopy

In the electron microscopic studies the leaves of SPFMV infected *I. nil* and sweet potato (*I. batatas*), varieties Sree Rethna and Sree Bhadra were used by extracting the sap in different buffers and using three different stains as mentioned under 3.8.4.

4.7.4.1 Negative staining method

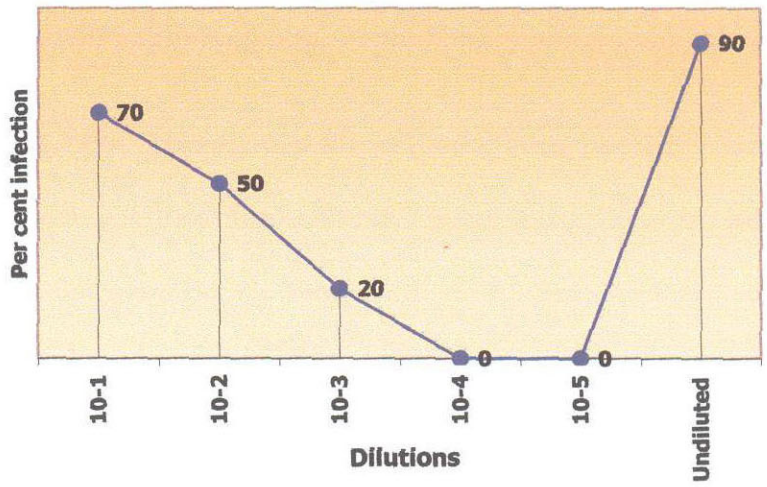
The leaf homogenates were prepared by grinding the samples with three different buffers separately, viz., phosphate buffered saline, potassium phosphate buffer and Tris HCl buffer and stained with phosphotungstic acid, uranyl acetate and ammonium molybdate, individually. The virus particles were seen only in the case of *I. nil* sap extracted in all the three buffers and stains. No particles could be seen from the *I. batatas* sample by this method.

Table 31 Longevity *in vitro* of SPFMV

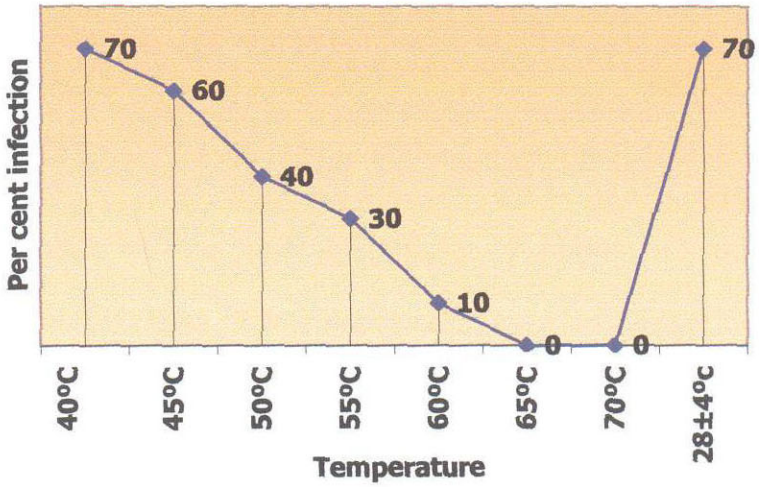
Sl. No	Storage period (h)	Number of plants infected out of ten		Per cent infection	
		RT	RC	RT	RC
1	0	8	9	80	90
2	2	8	8	80	80
3	4	5	8	50	80
4	6	3	7	30	70
5	8	-	5	-	50
6	10	-	2	-	20
7	12	-	-	-	-
8	24	-	-	-	-
9	28	-	-	-	-

RT- Room temperature (28 $\pm 4^0$C)
RC- Refrigerated condition (8^0 C)

Dilution end point



Thermal inactivation point



Longevity *in vitro*

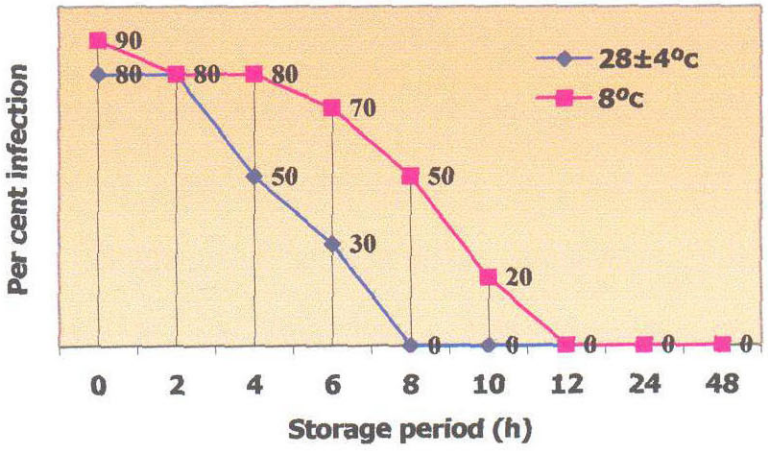


Fig. 16 Physical properties of SPFMV

4.7.4.2 Petiole dip method

In this method, the virus particles were seen in all the samples of *I. nil* and *I. batatas* using all the three stains. More particles were observed in this method than in the negative staining method. Among the three stains used, 2% ammonium molybdate gave good contrast.

4.7.4.3 Shape and size

The particles were long flexuous rods. The size ranged from 658-785 nm with an average of 748 nm (plate 9).

4.8 Host range

Host range studies with 25 species of plants belonging to different families from the vicinity of sweet potato field revealed that one weed species *Emilia sonchifolia* from Asteraceae, three species from Convolvulaceae, viz., *I. muricatum*, *I. nil* and *I. setosa,* four from Solanaceae, *D. stramonium*, *N. benthamiana*, *N. tabacum* (Burley 21), *N. tabacum* (Havana) were infected by SPFMV (Plate 10).

I. muricatum showed chlorotic leaf spot, mosaic, leaf distortion, and puckering 20 days after inoculation. The infected *I. nil* plants showed initially mosaic symptoms five days after inoculation, followed by leaf distortion and vein banding. Vein clearing was observed after 20 days. The vein banding was limited to primary and secondary veins.

I. setosa plants showed initially mosaic symptom after ten days followed by chlorotic leaf spot and vein banding. Vein banding was mostly

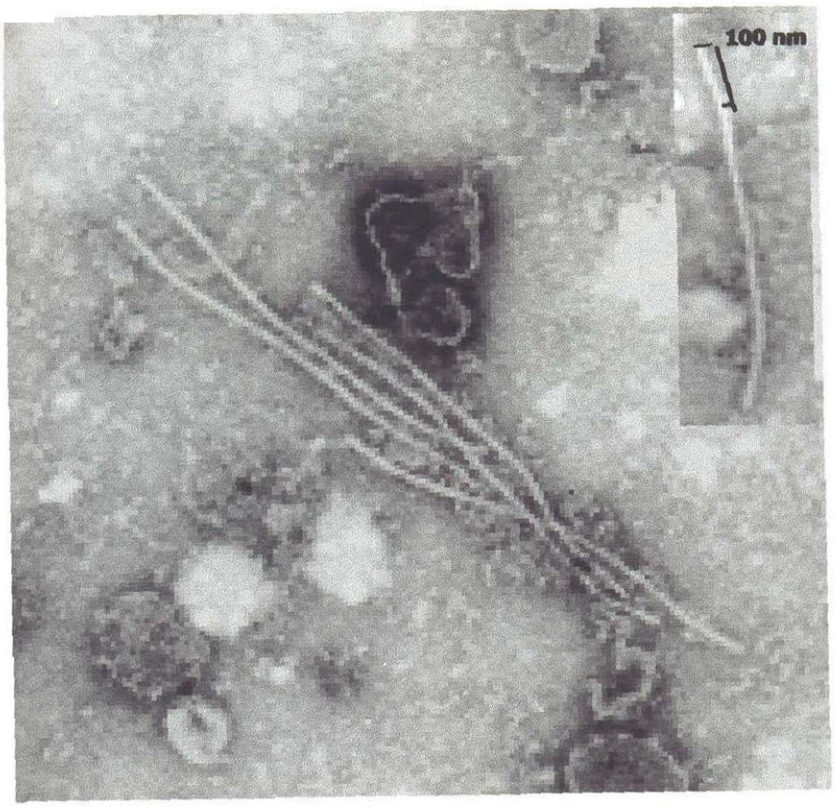


Plate 9 SPFMV particles

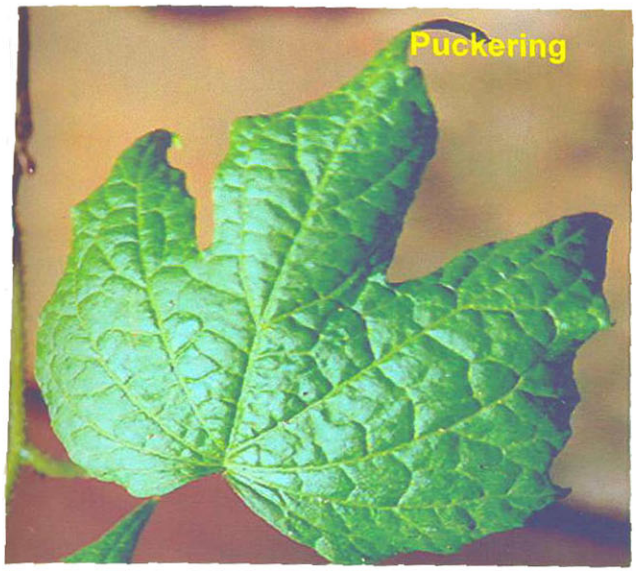
Datura stramonium

Ipomoea nil

I. nil

Ipomoea setosa

I. setosa



I. setosa

Plate 10 Susceptible host species to SPFM

limited to the primary veins and in some leaves to secondary veins, which is accompanied by mild distortion. In older leaves, purple borders appeared around CLS when kept exposed to direct sunlight. *N. benthamiana* showed mosaic symptoms and leaf distortion in the younger leaves, 12 days after inoculation. *N. tabacum* (Burley 21) and *N. tabacum* (Havana) showed mosaic and puckering symptoms 12 and 15 days after inoculation respectively. The per cent transmission and the symptoms developed in the susceptible species of SPFMV are presented in Table 32.

4.9 Management of the disease

4.9.1 Screening for resistance

Among 848 accessions of sweet potato germplasm screened for their reaction to SPFM based on visual symptoms at CTCRI, 708 showed different types of SPFM symptoms.

Major symptoms of SPFM observed in the germplasm, were chlorotic leaf spot, ring spot, pink spot, feathering, mosaic and puckering. Among these, the chlorotic leaf spot was predominant (501) followed by mosaic (310), puckering (305) ring spot (251), feathering (245) and pink spot (235) (Fig.17).

A database was prepared using the PDI and the category of individual accessions, for necessary retrieval. The data revealed that among the total accessions, 140, 183, 153, 190 and 182 accessions were highly resistant, resistant, moderately susceptible, susceptible and highly susceptible to the disease respectively (Fig.18). Accession numbers along with PDI and reaction are given in the Table 33.

Table 32 Host range of SPFMV and symptoms of infection in plant species

Sl. No.	Plant species	Number of plants infected/ inoculated	Per cent transmission	Symptoms and number of days after which symptom appeared							
				CLS	LD	LC	MO	PU	RS	VB	VC
1	*Datura stramonium*	5/15	33	-	20-25	-	15-17	-	-	-	-
2	*Emilia sonchifolia*	14/20	70	-	-	25-30	-	-	-	-	-
3	*I. muricatum*	13/15	87	12	20	-	15	20		-	-
4	*I. nil*	9/10	90	-	10	-	5	-		15	20
5	*I.setosa*	10/12	83	12	20	-	10	15	40	15	30
6	*Nicotiana benthamiana*	8/15	53	-	20	-	12	-	-	-	-
7	*N. tabacum* (Burley 21)	1/10	10	-	-	-	12	12	-	-	-
8	*N. tabacum* (Havana)	2/10	20	-	-	-	15	15	-	-	-

CLS Chlorotic leaf spot, **LD** Leaf distortion, **LC** Leaf curl, **MO** Mosaic,
PU Puckering , **RS** Ring spot , **VB** Vein banding , **VC** Vein clearing

Table 33 Percent disease index and varietal reaction of sweet potato accessions

HR		HR		HR		R		R		R		R		MS		MS		MS	
Acc. No	PDI	Acc. No	PDI	Acc. No	PDI	Acc. No	PDI	Acc. No	PDI	Acc. No	PDI	Acc. No	PDI	Acc. No	PDI	Acc. No	PDI	Acc. No	PDI
S1	0.00	S466	0.00	S98	0.00	S787	1.66	S493	8.33	S214	15.00	S1210	20.00	S1019	30.00	S459	38.33	S909	46.60
S102	0.00	S472	0.00	S99	0.00	S145	1.67	S948	8.33	S304	15.00	S1257	20.00	S1028	30.00	S1120	38.33	S1175	46.66
S112	0.00	S488	0.00	S462	0.00	S284	1.67	S1067	8.33	S1217	15.00	S1015	21.33	S1034	28.33	S163	36.66	S604	45.00
S136	0.00	S494	0.00	S930	0.00	S341	1.67	S1256	8.33	S1273	15.00	S629	21.60	S1047	31.66	S755	40.00	S479	48.33
S142	0.00	S499	0.00	S1032	0.00	S382	1.67	S1031	8.33	S36	16.66	S1025	21.66	S1066	28.33	S8	36.67	S890	45.00
S148	0.00	S513	0.00	S1072	0.00	S68	3.33	S617	9.60	S106	16.66	S54	21.67	S1138	30.00	S1265	38.33	S331	46.67
S154	0.00	S515	0.00	S1073	0.00	S134	3.33	S53	10.00	S486	16.66	S56	21.67	S1139	30.00	S1266	38.33	S478	46.67
S155	0.00	S333	0.00	S1088	0.00	S144	3.33	S80	10.00	S823	16.66	S258	21.67	S1176	26.66	S707	35.00	S1027	43.33
S17	0.00	S527	0.00	S1118	0.00	S149	3.33	S114	10.00	S78	16.67	S283	21.67	S1184	30.00	S999	39.90	S1280	46.67
S183	0.00	S540	0.00	S1122	0.00	S176	3.33	S117	10.00	S82	16.67	S975	21.70	S119	31.66	S334	40.00	S777	48.30
S184	0.00	S545	0.00	S1150	0.00	S231	3.33	S191	10.00	S167	16.67	S579	23.30	S1190	30.00	S48	39.99	S701	48.30
S185	0.00	S335	0.00	S944	0.00	S267	3.33	S390	10.00	S175	16.67	S648	23.30	S1231	28.33	S70	36.67	S836	45.00
S19	0.00	S547	0.00	S1156	0.00	S274	3.33	S1070	10.00	S194	16.67	S682	23.30	S1255	31.66	S641	41.60	S504	48.33
S193	0.00	S562	0.00	S1196	0.00	S276	3.33	S1119	10.00	S240	16.67	S716	23.30	S133	28.33	S47	36.66	S544	45.00
S203	0.00	S565	0.00	S1201	0.00	S277	3.33	S1141	10.00	S292	16.67	S808	23.30	S16	28.33	S850	36.60	S506	45.00
S204	0.00	S577	0.00	S945	0.00	S533	3.33	S1030	10.00	S1033	16.67	S1005	23.30	S174	30.00	S14	38.33	S1083	45.00
S207	0.00	S580	0.00	S1221	0.00	S533	3.33	S822	11.60	S581	18.30	S9	23.33	S197	26.67	S1173	35.00	S131	45.00
S208	0.00	S582	0.00	S1249	0.00	S998	3.33	S21	11.66	S647	18.30	S23	23.33	S236	33.33	S1276	41.66	S41	50.00
S215	0.00	S588	0.00	S1251	0.00	S1086	3.33	S34	11.66	S671	18.30	S37	23.33	S24	33.33	S1147	41.66	S1163	48.33
S216	0.00	S605	0.00	S1252	0.00	S1262	3.33	S108	11.66	S49	18.33	S156	23.33	S245	30.00	S91	38.33	S690	46.60
S219	0.00	S607	0.00	S1259	0.00	S1283	3.33	S471	11.66	S129	18.33	S288	23.33	S250	33.33	S217	41.67	S41	50.00
S222	0.00	S620	0.00	S1260	0.00	S28	5.00	S1002	11.66	S139	18.33	S424	23.33	S27	30.00	S395	36.67	S1017	45.00
S224	0.00	S655	0.00	S1261	0.00	S57	5.00	S87	11.67	S170	18.33	S1008	23.33	S29	26.67	S709	35.00	S1224	43.33
S227	0.00	S668	0.00	S1267	0.00	S109	5.00	S124	11.67	S202	18.33	S1179	23.33	S328	33.33	S256	41.67	S69	50.00
S230	0.00	S670	0.00	S1268	0.00	S182	5.00	S178	11.67	S260	18.33	S1230	23.33	S362	33.33	S492	41.67	S71	50.00
S238	0.00	S713	0.00	S1272	0.00	S525	5.00	S187	11.67	S285	18.33	S33	25.00	S379	26.67	S1207	35.00	S140	45.00
S244	0.00	S717	0.00	S1284	0.00	S867	5.00	S246	11.67	S346	18.33	S132	25.00	S38	26.67	S795	35.00	S59	45.00
S263	0.00	S727	0.00	S1285	0.00	S1269	5.00	S310	11.67	S483	18.33	S199	25.00	S4	28.33	S684	36.60	S501	45.00
S286	0.00	S728	0.00	S1286	0.00	S618	6.60	S465	11.67	S484	18.33	S391	25.00	S44	30.00	S1180	36.67	S1021	45.00
S299	0.00	S322	0.00	S1287	0.00	S873	6.60	S903	13.30	S1048	18.33	S420	25.00	S440	30.00	S221	38.33	S691	46.60
S3	0.00	S758	0.00	S1046	0.00	S141	6.66	S118	13.33	S1076	18.33	S1071	25.00	S448	31.67	S710	41.60	S726	48.33
S308	0.00	S762	0.00	KK	0.00	S570	6.66	S125	13.33	S1174	18.33	S1227	25.00	S470	31.66	S689	40.00	S213	48.33
S309	0.00	S785	0.00	S1288	0.00	S983	6.66	S189	13.33	S1193	18.33	S1277	25.00	S480	26.67	S598	36.60	S298	45.00
S311	0.00	S888	0.00	S940	0.00	S1093	6.66	S218	13.33	S1271	18.33	S731	18.33	S491	33.33	S1250	41.67	S332	50.00
S313	0.00	S911	0.00	S680	0.00	S10	6.67	S241	13.33	S463	19.99	S942	6.66	S529	26.66	S150	35.00	S507	43.33
S319	0.00	S353	0.00	S946	0.00	S89	6.67	S295	13.33	S162	20.00	S96	18.33	S572	31.66	S696	40.00	S254	48.33
S55	0.00	S361	0.00	S949	0.00	S165	6.67	S318	13.33	S200	20.00	S1270	21.66	S58	30.00	S788	36.70	S1044	45.00
S61	0.00	S369	0.00	S950	0.00	S172	6.67	S339	13.33	S354	20.00	S101	23.33	S589	31.60	S127	40.00	S702	48.30
S62	0.00	S374	0.00	S959	0.00	S188	6.67	S526	13.33	S398	20.00	S733	13.33	S597	33.30	S750	41.66	S856	48.33
S64	0.00	S376	0.00	S965	0.00	S363	6.67	S553	13.33	S416	20.00			S6	30.00	S105	36.67	S863	45.00
S66	0.00	S377	0.00	S969	0.00	S1022	6.67	S729	13.33	S489	20.00			S81	33.33	S1223	41.66	S1212	48.33
S72	0.00	S383	0.00	S971	0.00	S832	8.30	S959	13.33	S632	20.00			S821	33.30	S1010	41.66	S859	48.33
S75	0.00	S385	0.00	S980	0.00	S993	8.30	S1084	13.33	S740	20.00			S880	26.60	S469	34.99	S316	43.33
S77	0.00	S389	0.00	S992	0.00	S18	8.33	S1087	13.33	S741	20.00			S923	33.33	S1258	41.67	S407	50.00
S88	0.00	S431	0.00	S1000	0.00	S123	8.33	S1183	13.33	S748	20.00			S973	28.30	S600	36.60	S381	45.00
S90	0.00	S444	0.00	S1004	0.00	S179	8.33	S1218	13.33	S803	20.00					S1085	33.33	S52	43.33
S322	0.00			S1023	0.00	S198	8.33	S1007	14.00	S1060	20.00					S1243	33.33	S152	43.33
						S487	8.33	S164	15.00	S1151	20.00					S872	33.37	S275	43.33

S Acc. No	PDI	S Acc. No	PDI	S Acc. No	PDI	S Acc. No	PDI	HS Acc. No	PDI	HS Acc. No	PDI	HS Acc. No	PDI	HS Acc. No	PDI
S736	50.00	S1181	58.33	S621	65.00	S986	71.66	S1035	78.33	S1095	81.66	S1278	87.00	S1135	98.00
S708	51.60	S1182	58.33	S628	65.00	S1013	71.66	S1097	76.66	S880	80.00	S849	85.00	S732	91.66
S742	51.66	S1188	58.33	S645	65.00	S1078	71.66	S11	76.67	S953	80.00	S920	85.00	S1096	91.66
S858	51.66	S1222	58.33	782	65.00	S1157	71.66	S1128	78.33	S1148	81.66	S270	87.98	S643	98.30
S2	51.67	S1233	58.33	S816	65.00	S1211	71.66	S1166	78.33	S1192	81.66	S602	88.30	S831	98.30
S615	51.67	S1165	59.99	S1040	65.00	S537	71.67	S1186	78.33	S406	81.67	S630	88.30	S853	98.33
S39	51.67	S12	60.00	S1125	65.00	S1100	71.67	S1195	78.33	S1098	81.67	S870	88.30	S854	98.33
S235	51.67	S26	60.00	S1241	65.00	S1124	71.67	S1215	76.67	S1123	80.00	S1146	85.00	S1203	91.67
S450	51.67	S31	60.00	S749	66.33	S976	71.70	S1228	76.66	S887	80.00	S866	85.00	S878	91.66
S1263	53.00	S495	60.00	S851	66.60	S817	73.10	S1247	78.00	S1153	80.00	S886	85.30	S1290	91.67
S653	53.30	S503	60.00	S916	66.60	S813	73.30	S1282	78.33	S1114	81.67	S877	88.30	S1132	98.33
S692	53.30	S592	60.00	S738	66.66	S834	73.30	S1289	76.66	S922	80.00	S885	85.00	S1064	91.66
S721	53.30	S593	60.00	S751	66.66	S896	73.30	S347	80.00	S712	83.30	S1274	88.33	S735	78.33
S723	53.30	S794	60.00	S797	66.66	S917	73.30	S402	76.67	S1018	80.00	S954	85.00	S1219	91.66
S778	53.30	S1062	60.00	S1003	67.77	S936	73.30	S543	76.67	S1057	80.00	S1090	85.00	S1129	91.67
S481	53.33	S1089	60.00	S625	68.00	S223	73.33	S559	78.33	S747	81.66	S1059	86.66	S661	96.60
S1024	53.33	S1177	60.00	S595	68.30	S262	73.33	S561	78.33	S837	81.66	S1204	86.67	S926	96.60
S1137	53.33	S1229	60.00	S616	68.30	S508	73.33	S564	79.99	S1216	81.67	S1092	88.33	S657	100.00
S563	54.99	S590	61.60	S646	68.30	S1074	73.33	S571	79.99	S1171	83.00	S1264	88.33	S1039	100.00
S779	54.99	S912	61.60	S660	68.30	S1094	73.33	S585	76.60	S348	80.00	S1012	83.33	S827	90.00
S349	55.00	S913	61.60	S784	68.33	S1279	73.33	S606	78.30	S584	81.60	S650	86.60	S635	91.70
S380	55.00	S925	61.60	S789	68.33	S567	74.99	S609	76.60	S456	80.00	S1113	83.33	S879	90.00
S644	55.00	S43	61.66	S855	68.33	S574	74.99	S65	78.33	S746	81.66	S1036	86.66	S1133	95.00
S695	55.00	S524	61.66	S862	68.33	S745	74.99	S693	78.30	S686	81.60	S763	86.60	S893	93.30
S765	55.00	S568	61.66	S1121	68.33	S396	75.00	S714	78.30	S820	81.60	S807	86.60	S497	93.33
S1136	55.00	S764	61.66	S915	69.70	S451	75.00	S754	76.66	S681	80.00	S651	85.00	S1038	90.00
S1185	55.00	S941	61.66	S314	70.00	S610	75.00	S757	76.66	S688	80.00	S667	85.00	S1281	90.00
S1189	55.00	S984	61.66	S365	70.00	S706	75.00	S761	78.33	S962	81.66	S1205	86.67	S759	96.66
S1245	55.00	S1168	61.66	S626	70.00	S773	75.00	S772	78.33	S978	81.66	S891	86.70	S1202	96.67
S662	56.00	S1232	61.66	S654	70.00	S811	75.00	S793	78.33	S1091	81.66	S1170	87.00	S1134	98.00
S587	56.60	S1240	61.66	S786	70.00	S839	75.00	S805	76.66	S753	80.00	S775	85.00	S711	91.60
S601	56.60	S1155	61.67	S864	70.00	S845	75.00	S806	76.66	S768	80.00	S842	85.00	S828	91.60
S573	56.66	S1248	62.00	S1077	70.00	S868	75.00	S812	78.30	S840	81.60	S843	86.60	S1220	93.33
S575	56.66	S1246	63.00	S1082	70.00	S943	75.00	S814	78.30	S846	81.60	S752	86.66	S1235	93.33
S955	56.66	S523	63.30	S1169	70.00	S952	75.00	S825	76.60	S512	80.00	S1140	83.33	S890	90.00
S958	56.66	S703	63.30	S1275	70.00	S961	75.00	S826	76.60	S549	80.00	S1029	84.99	S910	90.00
S367	56.67	S809	63.30	S586	71.60	S1026	75.00	S830	78.30	S848	81.60	S876	86.66	S634	95.00
S500	56.67	S815	63.30	S611	71.60	S1030	75.00	S838	78.30	S894	81.60	S882	86.66	S642	95.00
S1191	58.00	S835	63.30	S612	71.60	S1099	75.00	S875	76.66	S804	80.00	S844	85.00	S939	91.60
S705	58.30	S22	63.33	S685	71.60	S1152	75.00	S883	78.77	S1117	81.67	S881	88.30	S1238	98.33
S970	58.30	S412	63.33	S704	71.60	S699	93.30	S900	78.30	S902	81.60	S884	86.66	S658	95.00
S25	58.33	S535	63.33	S774	71.60	S730	68.33	S928	76.60	S623	80.00	S608	85.00	S935	90.00
S419	58.33	S737	63.33	S802	71.60	S739	68.33	S931	78.30	S566	81.66	S990	86.66	S934	95.00
S482	58.33	S1237	63.33	S829	71.60	S93	70.00			S933	83.30	S1291	88.33		
S1079	58.33	S510	63.34	S914	71.60	S663	70.00			S967	83.30	S871	89.90		
S1131	58.33	S196	65.00	S560	71.66	S734	71.66			S760	83.33	S92	90.00		
S1178	58.33	S315	65.00	S781	71.66	S1001	58.33			S780	83.33	S505	90.00		
		S340	65.00	S857	71.66					S874	83.33	S594	90.00		

HR Highly resistant R Resistant M Moderately susceptible S Susceptible HS Highly susceptible
Acc. No Accession number PDI per cent disease index

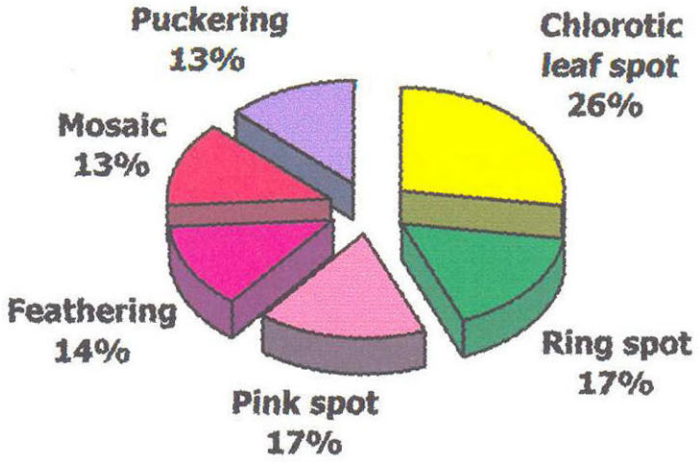


Fig. 17 Percentage of accessions with different types of SPFM symptoms

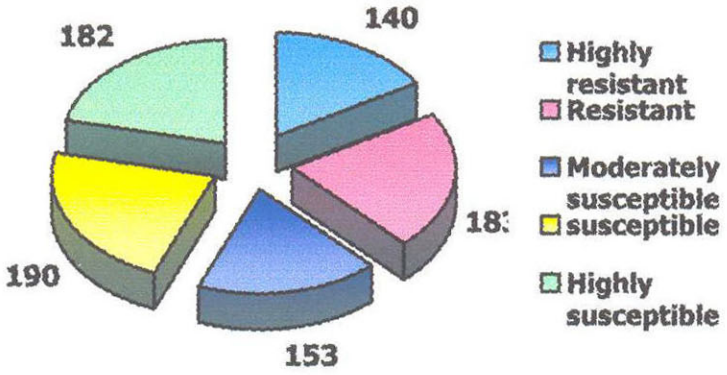


Fig.18 Reaction of sweet potato accessions to SPFMV

4.9.2 Thermotherapy

4.9.2.1 Hot water treatment

The PDI of SPFM in sweet potato varieties, Sree Rethna and Vavvathooki treated in hot water at different temperatures (3.10.2.1) for different periods of time (5, 10 and 15 min) were calculated. All the treated and untreated plants showed the symptoms of SPFM.

The results indicated that in hot water treatment the temperature did not influence the PDI in both the varieties, Sree Rethna and Vavvathooki. The period of treatment in each temperature had also not significantly influenced the PDI (Table 34a and 34b).

Generally the cuttings treated at different temperatures at various periods of time, viz., 5, 10, 15 min had sprouted on second or third day. The percentage of sprouting was 100 up to 46 ^{0}C and it was reduced at 48^{0}C when treated for 10 min in Sree Rethna and sprouting was completely inhibited in Vavvathooki even when treated for 5 min. Further increase had completely inhibited the sprouting in Sree Rethna also. The appearance of first symptom was between 13 and 18 days of planting in Sree Rethna when treated at 34^{0}C for 5, 10 and 15 min and it were 15 to 20 days for vavvathooki. When treated at 48^{0}C for 10 min the time taken for the appearance of first symptom was 25 to 30 in Sree Rethna (Table 35).

Major symptoms observed were the chlorotic leaf spot, pink spot, ring spot and feathering in both the varieties. In general all the sprouted cuttings treated in hot water showed symptoms of SPFM.

Table 34 a Effect of hot water treatment of sweet potato (variety, Sree Rethna) vine cuttings for different periods of time on SPFM

Sl.No.	Temperature (°C)	*Per cent disease index			Mean
		Time intervals (min)			
		5	10	15	
1	34	68.26 (8.32)	67.28 (8.26)	56.87 (7.61)	64.14 (8.06)
2.	36	76.53 (8.81)	65.44 (8.15)	56.87 (7.61)	66.28 (8.19)
3.	38	63.55 (8.03)	65.44 (8.15)	76.53 (8.81)	68.51 (8.33)
4.	40	73.00 (8.60)	70.54 (8.46)	70.81 (8.47)	71.45 (8.51)
5.	42	75.76 (8.76)	69.12 (8.37)	75.85 (8.79)	73.58 (8.63)
6.	44	74.30 (8.68)	67.58 (8.28)	75.85 (8.79)	72.58 (8.58)
7.	46	74.54 (8.69)	58.59 (7.71)	73.00 (8.75)	68.71 (8.35)
8.	48	57.66 (7.59)	100 (•)	-	-
9.	Control (28 ± 4°C)	70.95 (8.48)	-	-	-

Non significant

* Mean of ten replications - No figures (cuttings did not sprout)

• Statistical analysis was not done (No sufficient replication since cuttings did not sprout)
Figures in the parentheses are square root transformed means

Table 34 b Effect of hot water treatment of sweet potato (variety, Vavvathooki) vine cuttings for different periods of time on SPFM

Sl. No.	Temperature (°C)	*Per cent disease index			Mean
		Time intervals (min)			
		5	10	15	
1	34	84.57 (9.25)	75.52 (8.75)	73.30 (8.62)	77.80 (8.87)
2.	36	72.39 (8.57)	66.44 (8.21)	65.96 (8.18)	68.26 (8.32)
3.	38	72.66 (8.58)	72.75 (8.59)	65.96 (8.18)	70.45 (8.45)
4.	40	72.57 (8.58)	79.14 (8.95)	79.08 (8.95)	76.93 (8.83)
5.	42	71.49 (8.51)	72.59 (8.58)	71.13 (8.49)	71.74 (8.53)
6.	44	68.89 (8.36)	69.98 (8.42)	84.79 (9.26)	74.55 (8.69)
7.	46	74.54 (8.69)	73.80 (8.65)	69.90 (8.42)	72.75 (8.59)
8.	48	-	-	-	-
9.	Control 28 ± 4°C	85.56 (9.30)			

Non significant

* Mean of ten replications

Figures in the parentheses are square root transformed means

- No figures (cuttings did not sprout)

Table 35 Effect of hot water treatment on SPFM symptom development

Sl. No.	Treatment and period	Sprouting percentage		Sprouting time (days)		Appearance of first symptom (days)	
		SR	VT	SR	VT	SR	VT
1	34°						
	5	100	100	2	2	13-18	15-20
	10	100	100	2	2	13-20	15-20
	15	100	100	2	2	13-20	15-20
2	36°						
	5	100	100	3	2	15-20	15-20
	10	100	100	2	2	15-20	15-20
	15	100	100	2	2	15-20	15-20
3	38°						
	5	100	100	2	2	15-20	15-20
	10	100	100	2	2	15-20	15-20
	15	100	100	2	2	15-20	15-20
4	40°						
	5	100	100	2	2	15-20	15-20
	10	100	100	3	2	15-20	15-20
	15	100	100	3	2	15-20	15-20
5	42°						
	5	100	100	2	2	15-20	15-20
	10	100	100	2	2	15-20	15-20
	15	100	100	2	2	15-20	15-20
6	44°						
	5	100	100	2	2	15-20	15-20
	10	100	100	2	2	15-20	15-20
	15	100	100	2	2	15-20	15-20
7	46°						
	5	100	100	3	3	15-20	15-20
	10	100	100	3	3	15-20	15-20
	15	100	100	4	5	25-30	15-20
8	48°						
	5	100	-	3	-	23-25	-
	10	20	-	8	-	25-30	-
	15	-	-	-	-	-	-

SR Sree Rethna VT Vavvathooki

4.9.2.2 Dry heat

The results showed that all the sprouted plants whose cuttings were exposed to 37^0C and 39^0C at different days of incubation were infected with SPFMV.

It was found that incubation at both 37^0C and 39^0C did not significantly influence PDI of SPFM (Table 36). The number of days of incubation also did not have significant effect on PDI at both the temperatures.

Sprouting percentage was 100 in samples kept for all the different incubation periods at 37^0C except on the eighth day at which it was 50. At 39^0 C the sprouting percentage was 100 up to the second day of incubation, on the third day it was reduced to 60 and from fourth day onwards there was no sprouting (Table 37).

4.9.3 Induction of systemic resistance using chemicals

4.9.3.1 Bio-assay on *I. nil* plants

The data pertaining to the effect of chemicals such as barium chloride, manganese chloride and salicylic acid at different concentrations, viz., 50 mg, 100 mg and 150 mg l^{-1} on SPFMV- infection are presented in the Table 38 and Fig.19.

Table 36 Effect of dry heat treatment of vine cuttings of sweet potato (Vavvathooki) on SPFM

Days of incubation	*Per cent disease index (PDI)	
	37°C	39°C
0	90.37 (9.56)	87.55 (9.41)
1	91.72 (9.63)	86.91 (9.38)
2	78.53 (8.92)	82.40 (9.13)
3	77.96 (8.89)	77.26 (8.85)
4	86.02 (9.33)	-
5	92.36 (9.66)	-
6	76.89 (8.83)	-
7	68.51 (8.34)	-
8	95.78 (9.84)	-
Mean	94.77 (9.79)	83.53 (9.19)

Non significant

* Mean of ten replications

\- No figures (cuttings did not sprout)

Figures in the parentheses are square root transformed means

Table 37 Effect of dry heat treatment on sprouting and symptom development of SPFMV in sweet potato plants (Vavvathooki)

Days of incubation	Sprouting percentage		Sprouting time (days)		Appearance of first symptom(days)	
	37°C	39°C	37°C	39°C	37°C	39°C
0	100	100	2-3	2-3	12-15	12-15
1	100	100	2-3	2-3	12-15	12-15
2	100	100	2-3	2-3	12-15	12-15
3	100	60	2-3	6-8	12-15	20-30
4	100	0	2-3	-	12-15	-
5	100	0	2-3	-	12-15	-
6	100	0	2-3	-	12-15	-
7	100	0	5-6	-	15-20	-
8	50	0	5-8	-	25-30	-

4.9.3.1.1 Pre-inoculation treatment

When the chemicals were sprayed 24 h prior to inoculation of the virus, barium chloride at 150 mg l^{-1} concentration showed highest per cent decrease of infection (60 %) over control, followed by barium chloride 100 mg l^{-1} and manganese chloride 150 mg l^{-1} (50% each) and barium chloride 50 mg l^{-1} and manganese chloride 100 mg l^{-1} (30 % each). The least difference was observed in manganese chloride 50 mg l^{-1} and salicylic acid 150 mg l^{-1} (20 % each). Salicylic acid 50 mg and 100 mg l^{-1} did not show any effect on the infection.

4.9.3.1.2 Post- inoculation treatment

The treatment of chemicals on *I. nil* plants, 24 h after inoculation revealed that barium chloride 150 mg l^{-1} was most effective with 80 per cent decrease over control followed by barium chloride 100 mg l^{-1} (70%) and 50 mg l^{-1} (60%), manganese chloride 150 mg l^{-1} (30%), 100 mg l^{-1} and 50 mg l^{-1} (20% each). The least reduction was by salicylic acid 150 mg l^{-1} (10%). The salicylic acid at 100 mg l^{-1} and 50 mg l^{-1} had not showed any reduction in percent infection.

4.9.4 Meristem culture

The meristem tip was excised and inoculated on to MS medium with hormones for shoot induction. A shoot, 1-2 cm in length with a basal callus was developed within 25-30 days from the meristem tip. Each one of the shoots thus developed was then transferred to the regeneration medium for the

development of root and shoot. The development of root and shoot occurred within 4-5 weeks (Plate 11).

After regeneration, the plantlets, with shoot and root, were planted in small disposable cups for hardening and development. These plants were kept under observation for three months for the development of symptoms (Plate 12).

It was found that 85 and 96 per cent of meristem derived plants were free from SPFM disease in sweet potato varieties, Sree Rethna and Vavvathooki, respectively (Table 39). In diseased plants the feathering symptoms were observed after 30 days of planting.

Leaf samples were taken from ten plants each from 30-day-old Sree Rethna and Vavvathooki which did not show SPFM symptoms. The samples were tested for the presence of SPFMV through NCM- ELISA. The results showed no positive reaction in any of the samples, which indicated absence of the virus.

4.10.5 Storage of vine cuttings

The results indicated that the storage of cuttings did not significantly influence the PDI of SPFM when compared to control (Table 40). The sprouting of the cuttings was reduced when planted 15 and 20 days after storage and there was no sprouting after 30 days in the variety Sree Rethna. In Vavvathooki the sprouting was completely arrested after storage for 15 days. The sprouting time was generally increased as the storage period increased. Appearance of first symptom was also not influenced by the storage period (Table 41).

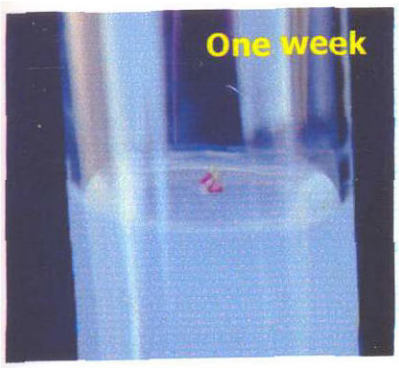
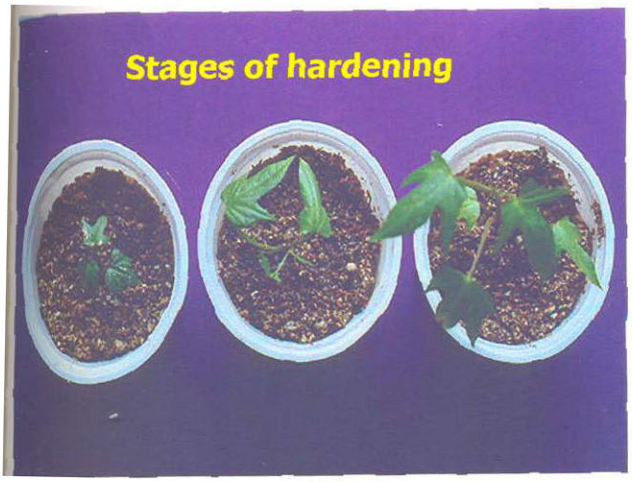


PLATE 11 Meristem culture



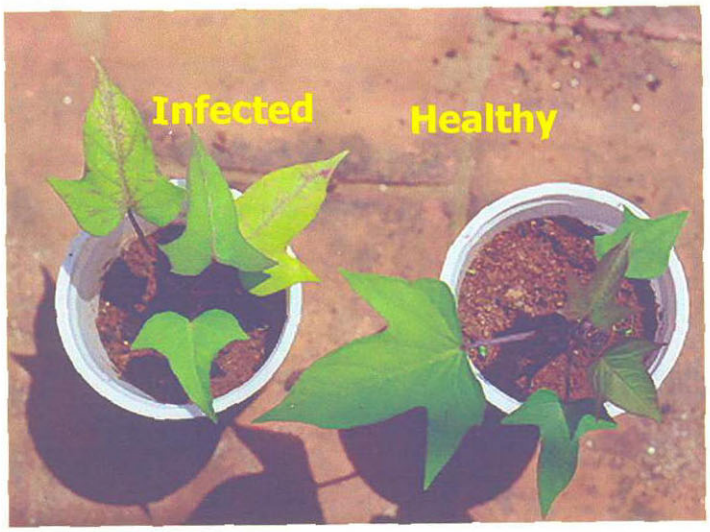


Plate 12 Hardening of meristem cultured plants

Table 38 Effect of application of chemicals on infection of SPFMV in *I. nil*

Sl. No.	Chemical	Concentration (mg l^{-1})	Per cent infection	
			Pre-inoculation	Post-inoculation
1	Barium chloride	50	70 (30)	40 (60)
2	Barium chloride	100	50 (50)	30 (70)
3	Barium chloride	150	40 (60)	20 (80)
4	Manganese chloride	50	80 (20)	80 (20)
5	Manganese chloride	100	70 (30)	80 (20)
6	Manganese chloride	150	50 (50)	70 (30)
7	Salicylic acid	50	100 (0)	100 (0)
8	Salicylic acid	100	100 (0)	100 (0)
9	Salicylic acid	150	80 (20)	90 (10)
10	Control	-	100	100

Figures in the parentheses are per cent decrease over control

Table 39 : Percentage of SPFM free sweet potato plants through meristem culture

Sl.No.	Varieties	No. of plants developed			Percentage of disease free plants
		Total	Healthy	Diseased	
1.	Sree Rethna	85	72	13	85
2.	Vavvathooki	55	53	2	96

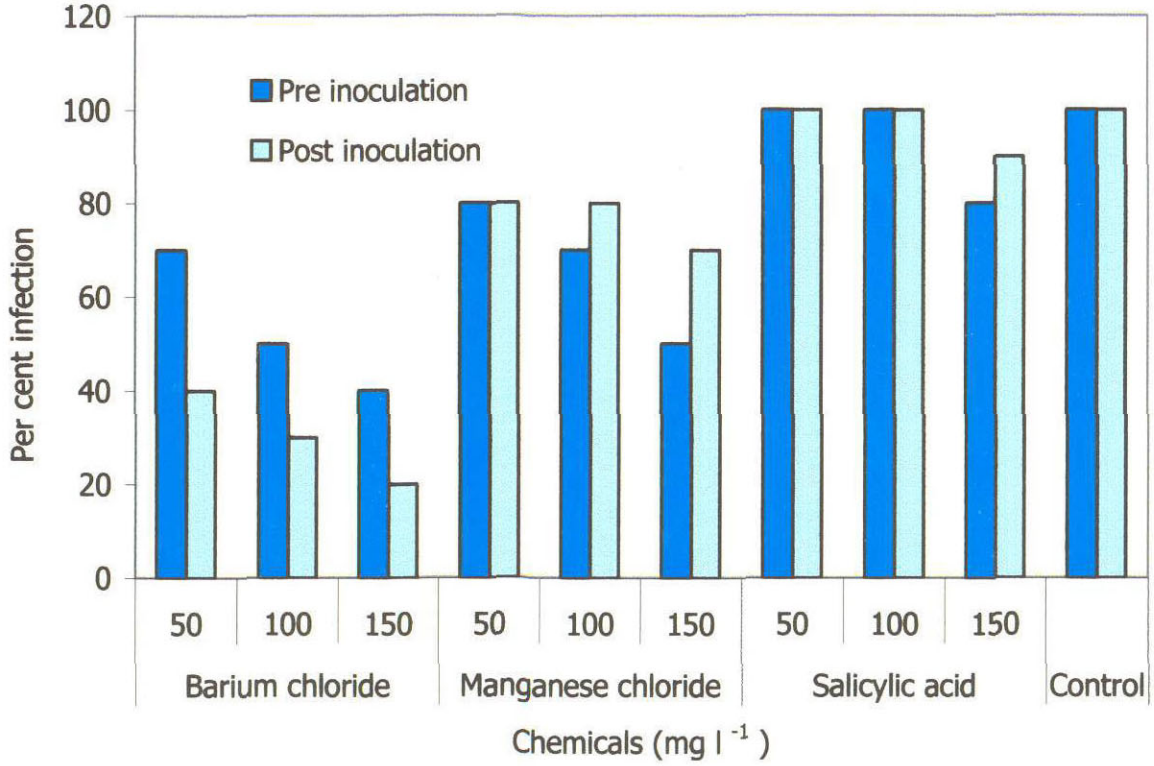


Fig. 19 Effect of application of chemicals on infection of SPFMV in *I.nil*

Table 40 Effect of storage of cuttings on SPFM symptom development in sweet potato

Sl.No.	*Per cent disease index (PDI)			Mean
	Intervals (Days after storage)	Varieties		
		Sree Rethna	Vavvathooki	
1.	0	32.56 (5.79)	57.32 (7.64)	44.94 (6.78)
2.	7	41.12 (6.49)	51.52 (7.25)	46.32 (6.88)
3.	15	70.00 (•)	-	-
4.	20	54.16 (•)	-	-
Mean		49.46 (7.10)	54.42 (7.44)	-

Non significant

* Mean of ten replications - No figures (cuttings did not sprout)

• Statistical analysis was not done (not sufficient replication since cuttings did not sprout)

Figures in the parentheses are square root transformed means

Table 41 Effect of storage of cuttings on sprouting and symptom development

Sl. No.	Varieties and storage period		Sprouting time (days)	Percentage of sprouting	Percentage of infection	Appearance of first symptom (days)
1.	SREE RETHNA	0	2	100	100	12-20
2.		7	2	100	100	16-20
3.		15	4	50	100	16-20
4.		20	6	20	100	15-20
5.		30	N.Sp	0	-	-
6.	VAVVATHOOKI	0	2	100	100	13-20
7.		7	2	100	100	15-20
8.		15	N.Sp	0	-	-
9.		20	N.Sp	0	-	-
10.		30	N.Sp	0	-	-

N.Sp – No sprouting

DISCUSSION

5. DISCUSSION

Sweet potato is a crop with a great potential as a subsidiary food crop. The tubers and the leaves are rich in carbohydrates, besides a few vitamins and minerals. The crop gives high yield within a considerably short period of time with low inputs even on marginal lands. Sweet potato is affected by many diseases caused by fungi, bacteria, viruses and phytoplasmas. More than 12 different viruses belonging to seven different taxonomic groups have been reported to infect the crop. Sweet potato feathery mottle is one of the important virus diseases that occurs wherever sweet potato is grown.

5.1 Survey

Survey of sweet potato fields at Thiruvananthapuram district revealed that SPFM is widely prevalent. Different symptoms like chlorotic leaf spot, pink spot, ring spot, fading of symptoms and combination of two or more of these were observed. This is in agreement with the earlier report of Thankappan *et al.* (1998). They reported these types of symptoms in sweet potato fields during a survey in five districts of Kerala and confirmed that they were due to SPFMV- infection through electron microscopic studies and enzyme-linked immunosorbent assay .

In the present investigation 0- 100% variation in disease incidence and intensity was observed between Krishibhavans, fields and plots. In the farmers' fields at Palakkad district, variation in the incidence of symptoms of SPFM, viz., chlorotic leaf spot, ring spot and feathering were observed by

Makeshkumar *et al.* (1999). The tubers of virus infected plants did not show any symptom except some constrictions in Vavvathooki. But no healthy plants were available in the field for comparison. Since the crop is vegetatively propagated it was observed that both healthy and infected plants were not available for even a single variety in any of the farmers' fields. This might be due to the planting of infected/ healthy cuttings in rotation without any selection from the farmers' own field or nearby fields. In a previous study no tuber symptoms were observed by Thankappan and Nair (1990).

SPFM was reported by several scientists from other countries also (Moyer *et al.*, 1980; Chiu *et al.*, 1982; Cohen *et al.*, 1988 and CIP, 1992).

In a previous study, it was reported that FMV isolate of SPFMV did not cause "russet crack" symptom on tubers whereas the russet crack(RC) isolate did (Cadena Hinojosa and Campbell, 1981a). Further, Usugi *et al.* (1994) suggested two strains,viz., severe strain (SPFMV –S) which caused russet crack in tuber and vein clearing in leaves and ordinary strain (SPFMV – O) which did not produce symptom in tuber. The similarity of symptoms in the leaves and absence of russet crack in the tubers indicate that the virus in the present study might be related to FMV isolate/ SPFMV –O.

SPFMV has been reported to be transmitted by *Aphis craccivora*, *A. gossypii* and *Myzus persicae* (Karyeija *et al.*, 1998). But such insects which are the known vectors of viruses were not present in any of the sweet potato fields surveyed in the present studies. This indicates that in Kerala the spread of SPFM is mainly through planting of infected cuttings.

5.2 Symptomatology

Symptoms like chlorotic leaf spot, pink spot, ring spot and combination of all these symptoms were observed and the observations were almost similar to that noticed by Shang Youfen *et al.* (1999). They had also observed chlorotic spots, purple spots, ring spots and feathery mottle as main symptoms of virus infection.

These types of symptoms were observed by many workers in India (Kumar *et al.*, 1991; Thankappan and Nair, 1990; Thankappan *et al.*, 1998; Makeshkumar *et al.*, 1999) and in other countries (Moyer and Kennedy, 1978; Moyer *et al.*, 1980; Cadena Hinojosa and Campbell, 1981a; Olivero *et al.*, 1989; Stobbs *et al.*, 1991 and Yang *et al.*, 1993).

The PDI was not significantly different between 30, 60 and 90 DAP. However, the highest disease intensity in the present study was observed at 60 days after planting followed by 30, 90 and 105 days. No tuber symptoms except the constrictions in the variety Vavvathooki were observed. In the earlier work of Thankappan and Nair (1990) also, the symptoms in the leaves were noticed as prominent during the active growth period between 30 and 60 days after planting. They also observed the fading of symptoms during later period and symptoms of any kind were not detected by them in the tubers.

The varieties, Sree Bhadra, Sree Rethna and Vavvathooki which had intermediate leaf colour/ purple pigmentation showed chlorotic leaf spot, pink spot, ring spot and feathering whereas the variety Poochedichuvala which does not have purple pigmentation exhibited only chlorotic leaf spot and slight feathering. This finding is in agreement with the observation by

Alconero (1972). He reported that yellow leaf spot occurred on cultivars with no apparent purple pigment and both purple and yellow leaf spots were present in cultivars of intermediate colour.

Results on symptom expression under different intensities of shade indicated a declining trend in the intensity of symptom as the intensity of shade increased. Severe symptom expression or intensity was observed when plants were grown without shade followed by 25, 50 and 75 per cent shade. This result is in agreement with the finding of Alconero (1972) with other sweet potato viruses. He noticed that the symptoms of virus- infected sweet potato plants were more conspicuous under direct light than under low light intensity in a glass house. But Miller (1955) reported that under field conditions, particularly in bright summer weather, the symptoms of feathery mottle virus in sweet potatoes in Puerto Rico, Georgia and Texas in USA became partially masked and only in the shaded portions of the plant, definite symptoms could be observed. This finding might have been due to the differences in variety of sweet potato, strain of the virus and temperature in that region.

5.3 Transmission

5.3.1 Through planting material

Transmission through different types of vine cuttings, viz., single node, double node and triple node was studied. The results indicated that all the plants developed from different types of cuttings showed symptoms of SPFMV- infection in all the four varieties. However, the first appearance of symptoms was delayed in the case of plants developed from single node

cuttings as it took more than 25 days after planting whereas in other cuttings, it was discernible at 12-20 days.

The variability in SPFMV concentration in sweet potato shoots is well documented (Cadena- Hinojosa and Campbell, 1981 b and Moyer *et al.*, 1980). The former reported that SPFMV was unevenly distributed in the plant and therefore the presence of virus could be confirmed only when the infected plants start showing symptoms. The transmission of SPFMV through vine cuttings was reported by several workers (Geddes, 1990; Karyeija *et al.*, 1998; Thankappan *et al.*, 1998). They have also reported that vegetative propagation provides sweet potato viruses with an efficient mechanism of perpetuation and dissemination.

Hildebrand (1968) reported that field planting without any selection favoured the long and persistent method of transmission of internal cork virus (a strain of SPFMV) in sweet potato.

Esbenshade and Moyer (1982) reported that not all the cuttings taken from virus infected sweet potato plants caused symptoms when grafted on *I. setosa*. It was further discussed by Gibson *et al.* (1997) that this might be due to a restricted distribution in resistant plants, which lead to elimination of the virus in some cuttings.

In contradiction to the above findings, results of the present experiment revealed that all the cuttings were found to be infected with the virus. This difference might be due to the extent of susceptibility of the varieties used and the distribution of the virus in the entire shoot.

5.3.2 Tuber transmission

Results of the present investigations on tuber transmission indicated that all the plants developed from tubers obtained from different varieties of SPFMV- infected sweet potato plants expressed the symptoms of SPFM.

This is in agreement with the finding of Walkey (1991), since infection by most of the viruses is completely systemic and any propagule, viz., tuber, bulb, corm etc. is likely to be infected.

Hildebrand (1958) reported that the SPFMV in the field is usually revealed in the sprouted growth from the infected roots. The transmission through unharvested roots in the field was also reported by Karyeija *et al.* (1998).

5.3.3 Sap transmission

Sap transmission of SPFMV from sweet potato to sweet potato using different buffers was unsuccessful in the present study which is similar to the observations of several workers reported earlier (Loebenstein and Harpaz, 1960; Daines and Martin, 1964; Hollings *et al.*, 1976; Cali and Moyer, 1981).

However, the virus could be transmitted from infected *I. nil* to sweet potato. Similarly, Moyer and Kennedy (1978) also could transmit the virus from *I. nil* to *I. batatas*, varieties, Jersey and Georgia Red. The failure of sap transmission from sweet potato to sweet potato might be due to the presence of some powerful inhibitors (Hollings *et al.*, 1976). The effect of virus inhibitors had been the subject of extensive reviews of Bawden (1954) and Loebenstein (1972). Such inhibitors include enzymes and polysaccharides.

The absence of such inhibitors in *I. nil* could be the reason for the successful transmission of the virus from *I. nil* to sweet potato and not vice-versa.

5.3.4 Insect transmission

Insect transmission studies were conducted with the aphids *Aphis craccivora*, *A. gossypii* and *Pentalonia nigronervosa* and the whitefly, *Bemisia tabaci*. The results revealed that *A. craccivora* and *A. gossypii* transmitted the virus from sweet potato to sweet potato, *I. nil* and *I. setosa* with varying efficiencies, although *A. craccivora* was more efficient. *A. craccivora,* *A. gossypii* and *M. persicae* were reported as efficient vectors of SPFMV (Schaefers and Terry, 1976; Moyer and Kennedy, 1978; Kennedy and Moyer 1982; Karyeija *et al.*, 1998). *A. gossypii* was also reported as the most efficient vector followed by *M.persicae* and *A.craccivora* (Moyer and Kennedy, 1978) in transmitting SPFM.

The aphid *P. nigronervosa* and whitefly *B. tabaci* did not transmit the virus in the present study. *P. nigronervosa* was used for the first time because this aphid is very much prevalent in Kerala and is the vector of the virus causing Katte disease of cardamom as well as banana bunchy top. Moyer and Kennedy (1978) also did not get SPFMV transmission through other aphid species, viz., *Lipaphis crysimi*, *Rhopalosiphum padi* and *R. maydis*. Similarly the transmission was reported as unsuccessful with the whitefly, *B. tabaci* (Loebenstein and Harpaz, 1960)

5.3.5 Graft transmission

In graft transmission of SPFMV from sweet potato to sweet potato all the three types of grafting, viz., approach grafting, side grafting and wedge grafting were successful in transmitting the virus with different grades of efficiency in two varieties. It has been reported that SPFMV was graft transmissible (Pozzer *et al.*, 1995).

Rossel (1984) found that the symptomless sweet potato plants were infected when approach grafted with SPVD-infected clones. Graft transmission of other strains of SPFMV was also successfully performed by different workers (Campbell *et al.* (1972), Daines and Martin(1964) (russetcrack) and Nielson(1981) (internal cork)).

5.3.6 Seed transmission

The seedlings of *I. batatas*, varieties, Sree Bhadra and Sree Rethna, *I. setosa* and *I. nil* were raised from seeds collected from SPFMV- infected plants. None of the seedlings showed symptoms and the random samples of seedlings tested with NCM-ELISA revealed negative reaction with SPFMV polyclonal antibodies.

Results of earlier study of Liao *et al.* (1979) revealed that SPV-A (earlier name for SPFMV) was not transmitted through the seeds of *I. batatas*. Seed transmission of SPFMV was also reported unsuccessful in *I. setosa* (Pozzer *et al.*, 1995) and *I. incarnata* (Cadena Hinojosa and Campbell, 1981b). The findings of the present studies are in conformity with the above observation.

5.4 Physiological changes

In the present investigation, photosynthesis was significantly low in SPFMV- infected plants of the susceptible variety Sree Rethna. No significant changes were found in respiration and transpiration in SPFMV-infected plants.

The decrease in photosynthesis and an increase in respiration rates had been reported in many virus- infected plants by other workers (Tu and Ford, 1968; Leal and Lastra, 1984; Penazio and Roggero, 1999; Herbers *et al.*, 2000).

Gonzalez-Fernandez *et al.* (1994) reported that photosynthesis, respiration and transpiration rates did not change significantly due to mosaic symptoms caused by papaya ring spot potyvirus type P. In general, the present result is comparable with the above finding.

Du Xihua *et al.* (1999) reported that photosynthesis increased and transpiration decreased in virus- eliminated sweet potato cultivars when compared with the SPVD- infected plants. Guo Xingqi *et al.* (2000) reported decreased photosynthetic and evaporative rates due to PVY- infection in tobacco.

Reduced photosynthesis in the SPFMV- infected plants might be due to the reduction in chlorophyll resulted from symptoms like chlorotic leaf spot, pink spot, ring spot and feathering. Fuentes and Salazar (2000) reported that the yield reduction in SPVD- infected sweet potato plants was associated with reduced photosynthesis.

5.5 Biochemical changes

Infection by pathogens is normally accompanied by some biochemical changes in the host plants (Diener, 1963). Such changes may be different in susceptible and resistant reactions of the hosts and may vary with virulence of the pathogen.

5.5.1 Total Carbohydrates

Total carbohydrate content was significantly reduced in both the susceptible varieties, Sree Rethna and Vavvathooki at 30 and 45 days after planting. The reduction was not significant in the tolerant variety, Sree Vardhini. Studies on the changes in total carbohydrates due to SPFMV - infection had not been conducted earlier. However, decreased total carbohydrate content due to virus- infection in various plants was reported earlier (Ramiah, 1978, Thind *et al.*, 1996 and Srivatsava and Tiwari, 1998)

Narayanasamy and Ramakrishnan (1966) suggested that reduction in the carbohydrate content might occur due to the breakdown of carbohydrates in virus- infected plants.

In the present study, it was observed that the starch content was significantly reduced due to SPFMV- infection. This may be the reason for the reduction in the total carbohydrate contents of infected plants of susceptible varieties.

5.5.2 Starch

It was observed that the SPFMV- infection caused significant reduction in starch content in the case of highly susceptible varieties such as Sree Rethna and Vavvathooki but not in tolerant variety Sree Vardhini.

The result of the present study is in agreement with the finding of Ravinder *et al.* (1989) who found that starch content significantly decreased in french bean leaves infected with the bean common mosaic potyvirus.

Similarly decrease in starch content in many other virus- infected plants was also reported by other workers (Singh and Singh, 1984; Singh and Suhag, 1982; Thind *et al.*, 1996).

The lower amount of starch in virus- infected plants might be due to the decrease in rate of net photosynthesis (Khatri and Chenulu, 1970). This might be the reason for the starch reduction in the infected plants in the present study .

5.5.3 Total sugars

The total sugar content was not significantly affected in SPFMV-infected plants in the present study.

An increase in the content of total sugars in infected plants was reported by many workers in other crops (Ravinder *et al.*, 1989; Prasad *et al.*, 1992; Sohal and Bajaj, 1993; Sarma *et al.*, 1995).

Generally the overall increase in total sugars might be due to the inhibition of translocation of sugars (Ahmed *et al.*, 1986). In SPFMV-

infected plants the translocation might not have been affected because the flow in phloem was not inhibited. Reduction in total sugar was also reported earlier due to viral- infection. (Dantre *et al.*, 1996; Banerjee and Kalloo, 1998). This was reported to be due to the high rate of respiration coupled with a decline in photosynthesis activity in infected tissue (Diener, 1963). Since no change in respiration was observed in SPFMV- infected plants this could be the reason for the unaltered total sugar content in such plants.

5.5.4 Chlorophyll

In the present study, total chlorophyll, chlorophyll 'a' and chlorophyll 'b' were significantly reduced in infected plants. Olivero and Oropeza (1985) also recorded low chlorophyll content in the plants grown from SPFMV-infected cuttings when compared with healthy cuttings.

The reduction in total chlorophyll, chlorophyll a and chlorophyll b content was reported by many other workers (Leal and Lastra, 1984; Sarma *et al.*, 1995; Dantre *et al.*, 1996; Thind *et al.*, 1996; Singh *et al.*, 1998). The reduction in chlorophyll content may be due to increased chlorophyllase activity as observed by Ramiah *et al.* (1972) and Ahmed *et al.* (1986).

5.5.5 Phenolics

Several factors contribute to disease resistance and susceptibility in plants and the total phenols and ortho-dihydroxy phenols have been often correlated with disease resistance. But in the present studies it was found that there were no significant differences in the contents of total phenols, OD-phenols and flavanols between infected and healthy plants.

Increase in total phenols in virus infected plants was reported by many workers (Sarma *et al.*, 1995; Thind *et al.*, 1996; Banerjee and Kalloo, 1998; Srivatsava and Tiwari, 1998). It was reported that resistant cultivars of cotton showed higher contents of total phenols, OD phenols and flavanols as a result of infection by cotton leaf curl virus when compared to susceptible varieties (Gurdeep Kaur *et al.*, 1998).

But Radhika (1999) reported that there was no significant change in the total phenol content in both resistant and susceptible varieties of cowpea plants infected with black eye cowpea mosaic virus. Results of the present studies are in agreement with this finding.

The SPFMV- infection might not have contributed to an increase in the activity of hexose-mono phosphate shunt pathway which produced intermediates required for synthesis of phenolic compounds. The less concentration and the less virulent strain of the virus could be the reasons for this.

5.5.6 Proteins

The protein contents of all the infected seedlings of the two susceptible and one tolerant varieties of sweet potato were not significantly different from those of the healthy ones.

The increased (Patil and Sayyad, 1991; Banerjee and Kalloo, 1998; Radhika, 1999) and decreased (Singh and Suhag, 1982; Thind *et al.*, 1996; Sarma *et al.*, 1995) protein contents in virus infected plants were reported by earlier workers. Increased protein content might be due to the production of

new PR proteins and decrease might be due to degradation of proteins in the host (Uritani, 1971). These might not have occurred significantly in the SPFMV- infected plants because of the low concentration as well as the less virulent strain of the virus.

5.5.7 Defense related enzymes

Defense related enzymes are reported to play important role in the induction of resistance (Dasgupta, 1988). The changes in activities of PAL and PO were not significant in both the susceptible and resistant varieties in the present study, whereas the activity of PPO was reduced in the SPFMV-infected plants of susceptible varieties. Sohal and Bajaj (1993) reported that there was an increase in PAL and PO activity due to mungbean yellow mosaic virus in both susceptible and resistant varieties tested. They also found that PPO activity decreased in susceptible varieties but not in resistant variety. Results of the present studies agree only in the case of PPO with the above report. Farkas *et al.* (1960) reported that increased PPO had been found mostly in infected plants with necrotic lesions. In such case there will be a decrease in phenols and accumulation of quinines. This caused simultaneous necrosis and cell death. They had also suggested that the increase was to a lesser extent in systemic hosts. Appearance of necrotic local lesions is usually accompanied by an enhancement of host respiration (Merrett and Bayley,1969).

Increase in respiration or oxygen uptake and necrotic lesion have not been observed in the present study. It was also reported that PPO action may

be the result of decompartmentalization or injury and not the cause of cell death (Loebenstein, 1972) .

5.6 Biometrical studies

5.6.1 Vine and leaf characters

In general, changes in morphological and biometrical characters in a plant may lead to change in the yield of that plant. In the present study changes in different morphological characters of sweet potato vines and leaves due to SPFMV- infection were studied. The results indicated a significant increase in length of internode in SPFMV- infected plants of Vavvathooki but not Sree Rethna. There was no significant difference in other characters such as number of vines, length of vine, number of leaves, leaf area, dry matter and total biomass.

The increased internode length in Vavvathooki plants might be due to hyperauxiny or production of auxins like IAA (Indole acetic acid) and IBA (Indole butyric acid) in the host by the virus- infection as suggested by Dasgupta (1988). He reported that a number of virus diseases, which caused abnormal increase in the size of plant parts thus indicating the involvement of growth regulators.

Contrary to the present findings, Olivero and Oropeza (1985) reported a severe reduction in the leaf area, number of leaves per plant, vine length and leaf yield in plants infected with SPFMV. Kano and Nagata (1999) also reported that the length and weight of the stem and stem diameters were more

in the plants developed from virus- free cuttings than in the SPFMV- infected plants.

However, Kantack and Martin (1958) found that foliage production by virus- free and SPFMV- inoculated sweet potato plants was similar. The present results are in agreement with this finding.

The reduction in the biometrical characters reported by the earlier workers might be due to the difference in the variety used and their susceptibility or variation in the strain of the virus studied or due to mixed infections in the samples studied.

5.6.2 Tuber characters

Earlier reports on yield losses caused by SPFMV -infection were few and unsatisfactory because most of the experiments were laid out to determine the general benefit or usefulness of virus- free planting material without considering the yield loss caused by SPFMV alone. Hence an attempt was made to study the effect of infection on the yield of tubers under pot culture trials using two varieties, viz., Sree Rethna and Vavvathooki.

The results clearly indicated that SPFMV- infection did not significantly affect either the number of tubers produced by the plant or the quantity of tubers. The girth and dry matter content were significantly affected in Vavvathooki. The length of the tuber was significantly increased in infected plants. In a yield loss trial in South Africa, a virus- free clone of cultivar Impala yielded three times more than the infected plants (Joubert *et al.*, 1979). Another green house trial in Uganda showed that virus-free plants

recorded twice the yield of graft inoculated SPFMV infected plants (Gibson *et al.*, 1997).

In the present study, the carbohydrate content and generally the biometrical characters of the foliage were not found to be adversely affected by SPFMV- infection. This may be the reason for the non-significant yield difference between healthy and SPFMV- infected plants. Though the decrease in the girth of tuber was significant in SPFMV- infected sweet potato plants in Vavvathooki, the increase in the length might have compensated for the weight of the tubers without a significant reduction in yield.

The observations repeatedly indicated that no tuber symptoms such as internal cork or russet crack reported elsewhere were noticed in this locality. However, tubers from diseased plants of the variety Vavvathooki had constrictions that were probably never observed earlier in India, which needs further confirmation. The absence of russet crack and internal cork symptoms on tubers of SPFMV- infected sweet potato varieties suggests that virus strains causing these symptoms were probably not present in this locality.

5.6.3 Quality of tubers

5.6.3.1 Culinary quality

The studies on the effect of SPFMV- infection on the culinary quality of tubers revealed marked variations in flavour, sweetness, colour and also the overall taste of the tubers were not observed between healthy and infected tubers in both the varieties tested. However, comparatively more variation was observed in the consistency of the tubers.

Nusbaum (1945) reported dark, hard corky spots on the tuber flesh due to internal cork virus. Brass (1946) further found that though the corky area was not affecting the flavour and consistency of surrounding tissues, the infected tubers were not preferred by the consumers. Daines and Martin (1964) observed fine cracks in the skin and extension of outer cortex due to russet crack virus. In the present work, such symptoms were not observed. However, the difference in the consistency of tubers observed in the present study might be due to the constrictions in the tubers. Karyeija *et al.* (1998) reported that economic loss might be associated with external cracking and internal corkiness, making the tuberous roots unmarketable. In the present work since the flesh of the tuber is not affected due to SPFMV- infection and no external cracking was observed, the effect of SPFM on culinary quality of tubers is negligible.

5.6.3.2 Quantity of nutrients

The total carbohydrate and starch contents of tubers are reduced due to SPFMV- infection in both the susceptible varieties but not the total sugar and protein contents. Liao *et al.* (1982) reported that tuber quality of sweet potato cultivars, Tainung 57 and Tainung 63 infected by a virus complex of SPV. A and SPV. N were not affected. They found that there was no difference in protein, sugar and starch contents of tubers in the diseased plants compared to those in healthy plants. The reduction in starch and carbohydrate contents observed in the present study might be due to the difference in the varietal response and the virus studied. The reduction in carbohydrate and starch in tubers might be due to the reduced photosynthesis and chlorophyll content in

leaves as reported in the present study. Starch is the most important component in sweet potato in root dry matter which provides energy and forms an excellent source for food products and animal feed in many countries other than India. Alcoholic beverages are also made from this starch in Japan and China (Padmaja, 2000). Thus, the reduced starch content due to infection apart from affecting the nutritive value will also reduce the economic value of the tubers.

5.7 Characterization of the virus

5.7.1 Purification and serology

It is necessary to separate the virus particles from the host tissues, concentrate *in vitro* and purify to produce antiserum for serological studies.

SPFMV was purified in the present work by following the method of Moyer and Kennedy (1978) with slight modification, using *I. nil* plants infected by SPFMV. Since, *I. nil* plants are systemically infected by SPFMV and are having high concentration of the virus, it was used for purification. This host plant was used by many other workers also for purification of SPFMV (Cali and Moyer, 1981; Usugi *et al.*, 1994; Zhu *et al.*, 1994).

Walkey (1991) suggested that for purifying a virus, it should multiply to a high concentration in the selected host and the host should be systemically infected. Hence, *I. nil* was selected as suitable host for multiplication and purification of the virus. Since no local lesion host was known, the purified virus was inoculated on to *I. nil* seedlings and the

infection was confirmed. The infectivity tests of the virus using *I. nil* seedlings was also done earlier by Moyer and Kennedy (1978).

Antiserum was produced in the present study by injecting New Zealand white rabbits with the purified virus as per the procedure by Van Roegenmortel (1982). Walkey (1991) reported that intramuscular injections by mixing the purified virus preparation with Freund's incomplete adjuvant and bleeding 10 to 14 days after the last injection and storing serum with sodium azide 0.02 to 0.1 per cent was best for antiserum production. The same method was followed in the present study also. The antiserum of SPFMV was produced by many earlier workers also (Abad and Moyer, 1992; Moyer and Kennedy, 1978; Cadena Hinojosa and Campbell, 1981a; Usugi *et al.*, 1994).

In the present study, agar double diffusion test was conducted and the precipitin band was formed between the SPFMV- infected samples of the leaf extracts of *I. nil* and *I. batatas* and the antiserum but not between healthy samples. This has confirmed that the antibodies for SPFMV were present in the antiserum.

Moyer and Kennedy (1978) also reported that protein bands were observed in double diffusion tests at an antiserum dilution of 1:16 with SPFMV- infected extracts and no precipitin lines with healthy extracts.

ELISA is one of the very sensitive tests for detection and identification of viruses. DAC-ELISA was performed with various samples using polyclonal antibody of SPFMV. Polyclonal antibody of SPFMV obtained from CIP

showed positive reaction only with infected *I. batatas, I. nil* and *I. setosa* samples and also in aphids after acquisition access period.

International Institutions like International potato centre (CIP) and Asian vegetable research development centre, generally used DAS- ELISA to determine healthy and SPFMV- infected plants of sweet potato in different studies. DAS-ELISA was performed by Esbenshade and Moyer (1982) and Hammond *et al.* (1992) also for the detection of other viruses.

DAC-ELISA was not carried out earlier for sweet potato viruses. However, it was carried out for many potyviruses (Ndiaye *et al.*, 1993; Bashir and Hampton, 1995; Radhika, 1999). In the present study, DAC-ELISA was preferred to detect the virus because the antibody requirement for this test was less than that in DAS- ELISA.

NCM- ELISA is also an important immunoassay, which is useful in the detection of viruses in large number of samples. The requirement of sample quantity is also less. This technique is widely used for screening and detecting sweet potato viruses (CIP, 1989; Gibson *et al.*, 1997; Karyeija *et al.*, 1998).

CIP had developed a NCM - ELISA kit along with positive control for comparison. This helped for the detection of SPFMV in different samples in the present study. The infected samples alone showed positive reaction. Karyeija *et al.* (1998) suggested that the improved sensitivity of ELISA might be useful as an effective method for screening large number of samples to be supplied as virus free planting materials for managing sweet potato viruses.

5.7.2 Physical properties

Physical properties like dilution end point (DEP), thermal Inactivation point (TIP) and longevity *in vitro* (LIV) were studied for SPFMV.

The results indicated that DEP was between 10^{-3} to 10^{-4}, TIP 60-65^{0}C and LIV, 6-8 h at 28± 4°C and 10-12 h at 8°C. Similar results were obtained by Moyer and Kennedy (1978) and Olivero *et al.* (1989) and Brunt *et al.* (1997) for SPFMV. In addition to symptomatology, these findings also further confirmed that the virus used in the present study is SPFMV.

5.7.3 Electron microscopy

In the present study, flexuous rod shaped particles of size ranging from 658-785 nm with an average length of 748 nm were observed to be associated with the samples tested. This was in agreement with the previous report of Makeshkumar *et al.* (1999). They also observed long flexuous rod shaped particles of 750 nm size in samples exhibiting chlorotic leaf spot, ring spot, feathering and mosaic. This again confirms that the virus in the present study is a strain of SPFMV.

Virus particles were not seen when samples from the leaf lamina were used in negative staining. But the samples prepared from the petiole showed more particles with good contrast possibly due to a lower concentration of the virus in the lamina and presence of high mucilage resulting in reduced clarity. Gibbs and Padovan (1993) also reported that in membrane immunobinding assay, the samples taken from petiole gave more reliable results than those from lamina.

5.8 Host range

Host range studies were conducted to find out the best indicator, assay and collatèral hosts and the mode of survival of the virus. The results indicated that the host range was generally limited to Convolvulaceae and Solanaceae and to a lesser extent to Asteraceae. Karyeija *et al.* (1998) also reported that SPFMV was mostly restricted to plant species belonging to Convolvulaceae. Pozzer *et al.* (1995) also reported that the host range of SPFMV was limited to Convolvulaceae and Chenopodiaceae.

The results of the present host range studies indicated that *I. muricatum*, *I. nil*, *I. setosa*, *D. stramonium*, *E. sonchifolia*, *N. tabacum* (Burley 21) and *N. tabacum* (Havana) were infected by SPFMV and showed different types of symptoms.

I. setosa and *I. nil* were reported by many workers as best indicator hosts for SPFMV (Moyer and Kennedy, 1978;; Olivero and Trujillo, 1989, Usugi *et al.*, 1994) and the results of the present studies also are in agreement with these reports.

Sap transmission of SPFMV to *C. amaranticolor* was unsuccessful in the present studies. Similar findings were reported by Moyer and Kennedy (1978) and Cali and Moyer (1981). *I. muricatum*, a highly proteinaceous vegetable crop and some of the weed species nearby the sweet potato fields were also tested for the first time. In the present study *I. muricatum* and *E. sonchifolia*, a weed species showed infection of SPFMV. This is the first report of these plant species as collateral hosts of SPFMV.

In the present investigation, no local lesion host could be identified. All the susceptible hosts identified in this study were systemically infected.

5.9 Management

5.9.1 Screening for resistance

Screening of sweet potato germplasm accessions at CTCRI, Thiruvananthapuram for resistance against SPFMV was done based on symptom expression under field conditions and accessions without any visible symptom were identified. The database, prepared with PDI and its categorization is useful to retrieve information on the reaction of individual germplasm accession to SPFMV.

Screening for resistance was done by several workers in many sweet potato growing countries against different strains of SPFMV (Nielsen and Pope, 1960; Arrendell and Collins, 1986; Heisswolf *et al.*, 1994; Karyeija *et al.*, 1998).

Among the total 848 accessions screened in the present work, 140 were free from any of the SPFM symptom. From this, further selection could be made by virus indexing through immuno assay and grafting with indicator host and green house trials. International Potato Centre at Lima, Peru also had identified some lines like Nemantee, Jewel and IITA Tis 2498 resistant to C and C1 isolates of SPFMV (CIP, 1991). The screening trials were extensively being done at CIP for the selection of immune/ resistant plants from germplasm (CIP, 1989).

Screening of germplasm at CTCRI for SPFM was done earlier by Thankappan *et al.* (1998) based on visible symptoms. The different types of

symptoms in each accession were observed by them. In the present study, scoring was done for SPFM in each accession. A scale was developed based on all the known symptoms and PDI was calculated as explained in 3.2.1.1 for individual accession. The accessions were categorized into different groups as highly resistant, resistant, moderately susceptible, susceptible and highly susceptible based on the PDI. This is the first attempt of scoring which will be useful to future workers for varietal screening.

5.9.2 Thermotherapy

In general, when an infected plant is exposed to temperature between 30 to 40^0 C, the virus replication is inhibited and the young shoots continue to grow (Walkey, 1991) which do not necessarily show symptoms.

Hot water treatment between 34 to 48^0 C for varying duration, viz., 5, 10 and 15 min was tried in sweet potato cuttings of Sree Rethna and Vavvathooki to eliminate the virus from them. But none of the treatments could eliminate the virus or inhibit the symptom development in the treated cuttings. This might be because of the failure of the treatment with the particular range of temperature to arrest the virus multiplication. This is corroborated by the present finding that the SPFMV has a higher TIP of about 65^0 C which is lethal to the sweet potato vines as indicated by the observation that cuttings treated at or above 46 to 48^0 C did not sprout at all.

Most of the earlier work regarding hot water treatment in sweet potato is in tissue culture for treating the explants, viz., meristem along with short stem and shoot tip. No work has yet been conducted on hot water treatment of sweet potato cuttings anywhere else.

However, Mirla *et al.* (1986) reported that sugarcane mosaic potyvirus was eliminated when treated with hot water for four days at 55, 56.5 and 57.5^0 C. In the United States, Quarantine unit, Department of Agriculture also had done hot water treatment of sugarcane clones to eliminate pests and pathogens especially sugarcane mosaic caused by a potyvirus (Hurtt, 1996).

In the present study, up to 46^0 C, the sprouting of the cutting was not affected whereas at 48^0 C it was arrested completely in Vavvathooki probably due to the tenderness of the vines. The delay in the appearance of symptoms after the heat treatment may be due to the delay in sprouting and development of plants as well as partial inactivation of virus particles.

Dry heat treatment at 37 and 39^0 C was also not successful in eliminating the SPFMV. The sprouting of the cuttings was not affected when incubated up to eight and three days at 37^0 C and 39^0 C respectively. Further increase in incubation time affected both the sprouting time and the appearance of first symptom.

Both hot water and dry heat treatment in the present study were not successful in eliminating the virus. The higher temperature levels tried, affected the sprouting of the cuttings and therefore the heat therapy could not be successfully employed in the disease control programmes for SPFMV.

5.9.3 Induction of systemic resistance using chemicals

Resistance can be induced in plants against various pathogens including viruses as a result of various treatments including chemicals (Ham merschmidt and Kuc, 1995).

In the present investigation, the chemicals barium chloride, manganese chloride and salicylic acid, at three different concentrations, viz., 50, 100 and 150 mg l^{-1} were tested to induce resistance against SPFMV in *I. nil* plants. The results indicated that barium chloride at the concentration of 150 mg l^{-1} showed highest inhibition followed by manganese chloride and salicylic acid. The concentrations of the chemicals were directly proportional to the inhibition of the disease. Similar results were obtained by Pun *et al.* (2000) and reported that maximum inhibition of bhendi yellow vein mosaic virus was by barium chloride followed by acetyl salicylic acid.

The induction of resistance was also reported in pumpkin plants against yellow vein mosaic by using salicylic acid and barium chloride (Jayashree, 1999). Radhika (1999) reported that manganese chloride induced resistance in cowpea against black eye cowpea mosaic virus effectively.

However, the present results on salicylic acid are contrary to the previous reports because the least inhibition was observed in salicylic acid application even at the highest concentration used (150 mg g^{-1}). The other two concentrations, viz., 50 and 100 mg g^{-1} were not effective in inhibiting the virus.

In plants displaying acquired systemic resistance, several proteins accumulate. Kassanis (1981) called them resistance-associated proteins. Others (Pierpoint *et al.*, 1981; Van Loon, 1983) preferred to call them pathogenesis related proteins (PR). The induction of resistance due to the application of these chemicals also might be due to the production of PR-proteins as suggested by Malamy *et al.* (1990); and Yalpani *et al.* (1991).

They reported that the induction of resistance is due to the induced expression of a set of genes associated with the synthesis of PR-proteins.

5.9.4 Meristem culture

Meristem tip culture was attempted in the present study to find out its possible use in eliminating the disease from planting materials. The results revealed that about 85 and 96 per cent of meristem derived plants were free from the disease symptoms in Sree Rethna and Vavvathooki, respectively. The diseased meristem derived plants showed only feathering symptoms. NAA 0.1 mg l^{-1} and BAP 0.5 mg l^{-1} were used for induction with M.S medium and M.S medium without harmones was used for regeneration. The plants were ready for hardening within 40-50 days. A similar combination was used earlier by other workers also (Alconero *et al.*, 1975; Love *et al.*, 1987).

Meristem culture was successfully employed by many workers in sweet potato to eliminate viruses (Green *et al.*, 1992; Mukherjee *et al.*, 1993; Nair and Govindankutty, 1998; Shang Youfen *et al.*, 1999). In general the number of virus-free plantlets produced was inversely proportional to the size of the tip (Walkey, 1991).

The present study indicates that meristem culture technique could be utilized successfully for mass production of disease-free plants. However, utmost care must be taken to take the meristem and to rogue out the meristem derived diseased plants.

5.9.5 Storage of vine cuttings

Studies on the effect of storage of vine cuttings on disease control revealed that storing vines at room temperature 28 ± 4^0C for 15-20 days was not effective in eliminating the virus from the vines.

The sprouting of vines was completely arrested after 15-20 days of storage in Sree Rethna and 15 days in Vavvathooki. Storage for a lesser period did not render the vines virus- free. The results were similar to those of heat treatment. No work had been conducted anywhere else by earlier workers regarding storage of sweet potato vines at room temperature to inhibit viruses.

However, Hildebrand (1968) reported that storage of sweet potato plants at 38 ^{0}C for three months was ineffective in eliminating internal cork virus. But they reported that similar exposure for six months resulted in the elimination of the virus from mother plants and the plants of 'Heartogold' variety appeared virus-free after five months treatment at the same temperature. In the present study, cuttings were used instead of whole plants whose sprouting was affected as explained earlier and that could be the reason for the failure.

The various aspects of studies on SPFM in the present work will throw a light for the future work as follows:

- The survey and studies on symptoms will be useful for planning further work

- The scoring scale developed for screening of SPFM and transmission through aphids, grafting and sap of *I. nil* to sweet potato could be utilized in future for various studies.

- The effect of SPFM on biochemical and biometrical changes of sweet potato vine, leaf and tubers will be a basis for further assessment of economic loss due to the disease.

- Purification, characterization and other serological studies will be useful for developing a diagnostic kit for the detection of the disease.

- The quick detection of SPFMV using the petiole of infected leaves could be used further for the electron microscopic studies of all sweet potato viruses.

- The identified indicator hosts could be further used for various bio-assays and for the multiplication of the virus.

- The screening of sweet potato accessions done and data-base prepared will be much useful for further selection of lines, resistant to SPFMV.

- The chemical, barium chloride identified in this study for induction of resistance could be utilized for the management of SPFM in sweet potato.

- The meristem culture could be adopted for the mass production of virus-free planting materials.

SUMMARY

6. SUMMARY

A survey in six Krishibhavans of Thiruvananthapuram district revealed that SPFM is widely prevalent in sweet potato plants in the region. Local varieties cultivated by the farmers show variation in the intensity of the disease. Different types of symptoms such as chlorotic leaf spot, pink spot, ring spot, feathering and combination of two or more of these were observed. Incidence and intensity of the disease varied significantly between plots, fields, localities and Krishibhavans. No insect vectors were found in the farmers' fields. No tuber symptoms except constrictions in the variety Vavvathooki were observed.

Similar types of symptoms as observed during the survey were recorded in four different varieties which were subjected to detailed investigation. The disease intensity varied between varieties and intervals of observation. The highest intensity was at 60 days after planting. Among the varieties Sree Rethna recorded highest disease intensity followed by Vavvathooki, Sree Bhadra and Poochedichuvala.

Different intensities of shade had a significant influence on the disease intensity. A declining trend in disease intensity was observed as the percentage of shade was increased.

The virus was transmitted through different types of vine cuttings and tuber. Sap transmission was successful only through sap from infected *I. nil* to *I. batatas*. The insects *A. craccivora* and *A. gossypii* non-persistently transmitted the virus from *I. batatas* to *I. batatas*, *I. nil* and *I. setosa*. *A.*

craccivora was a more efficient vector than *A. gossypii*. *Pentalonia nigronervosa* and *Bemisia tabaci* did not transmit the virus.

Different types of grafting, viz., approach grafting, side grafting and wedge grafting could transmit the virus from sweet potato to sweet potato. There was no transmission through the seeds of *I. batatas*, *I. nil* and *I. setosa* collected from highly SPFMV- infected plants.

Significant reduction was observed in photosynthesis due to SPFMV-infection in Sree Rethna, the highly susceptible variety and not in Sree Vardhini, the tolerant variety of sweet potato. The respiration and transpiration were not significantly affected by SPFMV infection.

Total carbohydrates, starch and polyphenol oxidase of leaves affected by SPFMV were decreased in Sree Rethna, Vavvathooki, and not in Sree Vardhini whereas total chlorophyll, chlorophyll 'a', chlorophyll 'b' were found reduced in all the three varieties. Total sugars, total phenols, OD-phenols, flavanols, protein and enzymes, peroxidase and phenylalanine ammonialyse were not affected by SPFMV infection.

The infection did not reduce the number of vines, length of vine, number of leaves, leaf area, biomass and leaf and stem dry matter in Sree Rethna and Vavvathooki. The length of internode alone was increased due to the virus infection in Vavvathooki.The mean number of tubers, total tuber weight and colour were not affected due to SPFMV infection. However, constrictions were found in the tubers of infected Vavvathooki plants.

An increase in length and decrease in girth of tubers were observed in infected plants of Vavvathooki. Dry matter content of tubers was also reduced in the tubers of this variety.

Culinary quality of the tubers did not vary much between healthy and infected plants, except the consistency of the tubers in infected plants. The starch and total carbohydrate contents were reduced in the tubers due to infection in both the susceptible varieties, viz., Sree Rethna and Vavvathooki but not the total sugars and protein.

Purification of SPFMV was done from infected *I. nil* plants and antiserum was produced. Agar double diffusion test was conducted and the presence of antibodies in the antiserum was confirmed. DAC-ELISA and NCM- ELISA were also performed and the presence of virus was detected in the infected *I. batatas*, *I. nil*, *I. setosa* and in *A. craccivora* after acquisition of the virus.

Physical properties of the virus in the crude sap of infected *I. nil* were studied and the dilution end point (DEP) was between 10^{-3} and 10^{-4}, thermal inactivation point (TIP) was between 60 and 65^0C and longevity *in vitro* (LIV) was 6 to 8h at 28 ± 4^0C and 10 to 12h at 8^0C.

Electron microscopy of the virus particles showed that the virus was a long flexuous rod measuring approximately 748 nm in length. The sample prepared from petiole was more effective than leaves for electron microscopic studies since it contained more number of virus particles.

Screening of germplasm accessions at CTCRI, Thiruvananthapuram revealed that out of 848 accessions, 708 showed different types of SPFM

symptoms such as chlorotic leaf spot (501), mosaic (310), puckering (305), ring spot (251), feathering (245) and pink spot (235). A data base was prepared and it showed that 140, 183, 153, 190 and 182 accessions were highly resistant, resistant, moderately susceptible, susceptible and highly susceptible to the disease, respectively.

Hot water treatment at various temperatures from 34 to 48^{0}C for different periods of time (5, 10 and 15 min) and dry heat at 37 and 39^{0}C for different days of incubation were not effective in reducing the incidence of the disease. The percentage of sprouting was affected by hot water treatment at 48^{0}C for 10 min and dry heat at 37^0 C for eight days and 39^{0}C for three days.

Pre and post inoculation spray of chemicals such as barium chloride, manganese chloride and salicylic acid induced resistance in *I. nil* plants and reduced the SPFMV infection. Barium chloride was more effective followed by manganese chloride and salicylic acid. The per cent infection was inversely proportional to the concentration of the chemicals. Salicylic acid at 100 mg l^{-1} and 50 mg l^{-1} had not showed any reduction in infection.

Through meristem tip culture, 85 and 96 per cent of SPFM symptom free plants were produced in two varieties of sweet potato, viz., Sree Rethna and Vavvathooki, respectively.

Storage of cuttings at room temperature did not influence the intensity of SPFM. The sprouting was reduced in both the varieties after 15 days of storing.

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*** Originals not seen**

APPENDICES

APPENDIX-I

Buffers used in NCM-ELISA

1 TBS (Tris buffered saline)

Tris base	2.423 g (0.02 M)
Sodium chloride	29.5 g (0.5 M)
Sodium azide	0.2 g (0.02%)

Dissolve in 950 ml distilled water. Adjust pH to 7.5 with concentrated (5 N) HCl and bring to volume of 1 litre with distilled water. Two litres are needed to process 2-4 membranes. This buffer should be prepared before starting the test and is stable for four weeks in the refrigerator (4°C).

2 Extraction buffer

Ethylene diamine tetra acetate(EDTA)	2.423 g (0.02 M)
TBS	100 ml

Mix well. EDTA takes a long time to dissolve. This buffer should be prepared before starting the test and is stable for four weeks in the refrigerator (4°C).

3 Blocking buffer

Bovine serum albumin(BSA)	3 g (3%)
TBS	100 ml

Add TBS slowly to BSA and mix well. 5% Carnation non-fat dry milk or 0.15% casein, technical (Sigma C-0376) or 0.15% Casein, purified (Sigma C-5890) can be used instead of BSA. (The casein does not dissolve completely, but blocks adequately)

4 Antibody buffer

Polyvinylpyrrolidone	3 g (3%)
BSA	0.2 g (0.2%)
TBS	100 ml

Mix PVP-40 and BSA. Add TBS in small amounts and mix well

5 Antibody solution

Add polyclonal antibodies to the antibody buffer to obtain the appropriate IgG dilution. Prepared before use but can be kept at 4°C and reused once within one week.

6 Washing buffer (TTBS : Tris buffered saline with 0.05% Tween-20)

TBS	1 litre
Tween-20	0.5 ml

Mix well

7 Conjugate solution

Add anti-rabbit IgG-alkaline phosphatase conjugate to the antibody buffer to obtain the appropriate dilution. Prepared before use but can be kept at 4°C and reused once within one week.

8 Colour development buffer

Tris base	6.05 g (0.1 M)
Sodium chloride	2.92 g (0.1 M)
Magnesium chloride	0.51 g (5 MM)
Sodium azide	0.1 g (0.02%)

Dissolve Tris and NaCl in 450 ml distilled water
Adjust pH to 9.5 with concentrated (5 N) HCl.
Add $MgCl_2$ and NaN_3
Bring the volume to 500 ml with distilled water. This buffer is stable for four weeks in the refrigerator (4°C). Before use this buffer should be brought to room temperature.

9 Colour development solution

NBT	11 mg
BCIP	5.5 mg
N, n-dimethyl formamide	100 µl
Sodium azide	0.1 g (0.02%)

Dissolve NBT in 32 ml colour development buffer stirring for 20 minutes and protect from light (cover with aluminium foil). Apart dissolve BCIP in 100 µl n, n-dimethyl formamide. Immediately before use, add the BCIP solution dropwise to the NBT solution while stirring. This solution is not stable.

This colour development solution can be prepared from stock solution of NBT and BCIP as follows :

A. Stock solution NBT : 333.5 mg in 5 ml of 70% n, n-dimethyl formamide.
B. Stock solution BCIP : 166.5 5 mg in 5 ml of n, n-dimethyl formamide

Add 160 µl of A to 32 ml of the colour development buffer, mix well, while stirring add drop wise 160 µl of B and mix well again.
Stock solution A and B can be kept for at least two months at 4°C

APPENDIX - II

Buffers used in sap transmission

1 0.1 M Sodium borate buffer (pH 8.0)

A. Boric acid (0.2 M) 1.237 g / 100 ml
B. Borax $Na_2B_4O_7$-$10H_2O$ 1.907 g /100 ml

50 ml of A mixed with 4.9 ml of B, diluted to a total of 200 ml

2 Potassium phosphate buffer

A. 0.1 M Potassium dihydrogen phosphate 6.084 g / 500 ml
B. 0.1 M Dipotassium hydrogen phosphate 8.079 g / 500 ml

0.1 M (pH 7.2) – 28 ml of A mixed with 72 ml of B
0.05 M (pH 7.2)– 28 ml of A mixed with 72 ml of B, diluted to a total of 200 ml

(0.01 M DIECA) – 0.225 g / 100 ml

3 0.1 M Sodium phosphate buffer (pH 7.2)

A. 0.1 M Sodium phosphate monobasic anhydrous 5.999 g / 500 ml
B. 0.1 M Sodium phosphate dibasic dihydrate 8.899 g / 500 ml

28 ml of A mixed with 72 ml of B

4 0.1 M Citrate buffer (pH 6.2)

A. 0.1 M Citric acid 2.101 g / 100 ml
B. 0.1 M Sodium citrate 2.940 g / 100 ml

1.6 ml of A mixed with 18.4 ml of B

5 0.1 M Tris buffer (pH 7.2)

Tris 24.23 g / 1000 ml

22.5 ml of 0.2 N HCl mixed with 25 ml of Tris , diluted to a total of 50 ml

APPENDIX - III

Buffers used in biochemical analysis

1 0.1 M Sodium acetate buffer (pH 4.7)

Stock solutions

A. 0.2 M solution of acetic acid (11.55 ml in 1000 ml)
B. 0.2 M solution of sodium acetate (16.49 g $C_2 H_3O_2Na$ in 1000 ml)

22.7 ml of A mixed with 27 ml of B, diluted to a total of 100 ml

2 0.1 M Sodium phosphate buffer (pH 6.5)

Stock solutions
As in appendix II

68 ml of A mixed with 32 ml of B

3 0.1 M Sodium borate buffer (pH 8.8)

Stock solutions
As in appendix II

50 ml of A mixed with 30 ml of B, diluted to a total of 200 ml

APPENDIX - IV

Proforma to evaluate culinary quality

Sl.No	Characters	A	B
1.	Flesh colour		
2.	Flavour		
3.	Consistency		
4.	Sweetness		
5.	Overall taste		

E - Excellent G - Good M – Moderate P - Poor
A, B - Sample

APPENDIX - V

Buffers used in Characterization

1 Purification

1.1 0.05 M Sodium borate buffer (pH 8.0)

Stock solutions

As in appendix II

3 ml of A mixed with 7 ml of B, diluted to a total of 40 ml

2 DAC- ELISA

2.1 Phosphate buffered saline (PBS - pH 7.4)

Sodium chloride	-	8.0 g
Potassium dihydrogen phosphate	-	0.2 g
Disodium hydrogen phosphate	-	1.1 g
Potassium chloride	-	0.2 g
Sodium azide	-	0.2 g
Water	-	1000 ml

0.5 ml Tween 20 (0.05%)

2. 2 Coating buffer (pH 9.6)

Sodium carbonate	-	1.59 g
Sodium bicarbonate	-	2.93 g
Sodium azide	-	0.2 g
Water	-	1000 ml

2.3 Substrate solution (pH 9.8)

Diethanolamine	-	97 ml
Sodium azide	-	0.2 g
Water	-	800 ml

Add HCl to give pH 9.8

3 Electron microscopy

3.1 Tris HCl buffer pH 8.0 (0.1 M)

Tris – 24.23 g/ 1000 ml

12.5ml of 0.2 N HCl mixed with 25 ml of Tris diluted to 50 ml

3.2 0.05 M Potassium phosphate buffer (pH 7.2)

As in appendix II

3.3 Phosphate buffered saline pH 7.4

As in appendix V (2.1)

*Distilled water should be used to dissolve the chemicals and to make up the buffer

APPENDIX VI
Stock solutions for MS basal medium

Sl. No.	Constituents	Amount of chemical taken (mg)	Amount mg l^{-1}	Solution ml l^{-1}
1.	Stock solution I (250 ml 10 X)			
	$MgSO_4$ 7 H_2O	3700	370	25 ml
	KH_2PO_4	1700	170	
	KNO_3	19000	1900	
	NH_4NO_3	16500	1650	
2.	Stock solution II (100 ml 20 X)			
	$CaCl_2$ 2 H_2O	8800	440	5 ml
3.	Stock solution III (100 ml – 100 X)			
	H_3BO_3	620	6.2	1 ml
	$MnSO_4$. 4 H_2O	1690	16.9	
	$ZnSO_4$. 2 H_2O	860	8.6	
	$Na_2M_0O_4$. 2 H_2O	25	0.25	
	KI	83	0.83	
4.	Stock solution IV (100 ml – 20 X)			
	$FeSO_4$ $_{7H}$2O	556	27.8	5 ml
	Na_2 EDTA. 2 H_2O	746	37.3	
5.	Stock solution V (250 ml – 500 X)			
	Cu SO_4. 5 H_2O	12.5	0.025	.5 ml
	CO Cl_2. 6 H_2O	12.5	0.025	
6.	Stock solution VI (100 ml-100X)			
	Thiamine HCl	10	0.1	1 ml
	Pyridoxin HCl	50	0.5	
	Nicotinic acid	50	0.5	
	Glycine	200	2	

Myoinositol – 100 mg
Sucrose – 30 g
Agar – 7.5 g

* Dissolve $FeSO_4$. 7 H_2O and Na_2 EDTA 2 H_2O separately in 45 ml distilled water by heating and constant stirring. Mix the two solutions adjust the pH to 5.5 and add distilled water to makeup the final volume to 100 ml.

All the prescribed stock solutions, chemicals and hormones were taken in a beaker and the volume was made up to 1 litre. The agar was dissolved in the medium by melting before autoclaving.

CHARACTERIZATION, HOST RANGE AND MANAGEMENT OF SWEET POTATO FEATHERY MOTTLE VIRUS

BY

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ABSTRACT OF A THESIS
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ABSTRACT

A survey revealed that sweet potato feathery mottle is widely prevalent in the farmers' fields of sweet potato at Thiruvananthapuram district of Kerala and the types and intensity of symptoms varied between varieties. Chlorotic leaf spot, pink spot, ring spot and feathering are the major symptoms observed. No vectors were present and no tuber symptoms were found except tuber constrictions in one variety, Vavvathooki. In symptomatology studies also, similar types of symptoms with various combinations were observed. Initial symptom was found after 15 days and the highest intensity between 30 to 60 days after planting in pots. The intensity of symptoms got reduced as the intensity of shade was increased. The virus was readily transmitted through single node, double node and triple node vine cuttings, tubers and three types of graftings, viz., approach grafting, side grafting and wedge grafting. It was non-persistently transmitted by *Aphis craccivora*, *A. gossypii* and through sap from infected *I. nil* to *I. batatas*. However, sap transmission from *I. batatas* to *I. batatas* was not achieved. There was no transmission either through seed or through *Pentalonia nigrônervosa* and *Bemisia tabaci*. Photosynthesis was reduced only in the susceptible variety Sree Rethna due to leaf infection but respiration and transpiration were not affected in both the susceptible and tolerant varieties. The infection caused a reduction in the content of total carbohydrates, starch, total chlorophyll, chlorophyll'a' and chlorophyll'b' and polyphenoloxidase activity in leaves of susceptible varieties, Sree Rethna and Vavvathooki. In leaves, total sugars, total phenolics, protein, peroxidase and phenylalanine ammonia lyase activities were not affected by the infection.

There was no significant change in number of vines, length of vine, number of leaves, leaf area, total biomass and drymatter caused by SPFMV- infection except an increase in internode length. There was no colour change in tubers. Tubers of Vavvathooki plants infected with SPFMV showed constrictions, increase in length and reduction in girth. In tubers, quantities of total carbohydrates, starch and drymatter were reduced while protein and sugars were not affected due to infection. The virus was purified and infectivity was tested. The antiserum produced was confirmed by agar double diffusion test for the presence of antibodies. Detection of SPFMV in samples was done through DAC-ELISA and NCM-ELISA. The dilution end point (DEP) of SPFMV was found to be between 10^{-3} and 10^{-4}, thermal inactivation point (TIP) between 60 and 65^{0}C and longevity *in vitro* between 6 and 8 h at room temperature (28± 4^{0}C) and 10 and 12 h at 8^{0}C. The petiole sample was ideal in electron microscopy for quick detection of SPFMV. The particles were long flexuous rods measuring approximately 748 nm in length. In host range studies, among 25 plant species from nine families, eight species, three from Convolvulaceae, four from Solanaceae and one weed species from Asteraceae were found to act as collateral hosts of the virus. Hot water treatment between 34 to 42^{0}C for 5, 10 and 15 min and dry heat incubation of cuttings at 37^0 and 39^{0}C for different periods were not effective in inhibiting the virus. In the induction of resistance, the chemical barium chloride (0.015%) showed highest per cent inhibition of infection in *I. nil* plants followed by manganese chloride and the least by salicylic acid. Through meristem culture 85 and 96 per cent of virus free sweet potato plants were produced from the two varieties, Sree Rethna and Vavvathooki. Storage of vine cuttings for disease management was unsuccessful.