TOMATO MOSAIC TOBAMOVIRUS : CHARACTERISATION, SERODIAGNOSIS, SEED TRANSMISSION AND HOST RESISTANCE IN TOMATO (*Lycopersicon esculentum* Mill.)

UAS, UNIVERSITY LIBRARY
DHARWAD

SHOBA CHERIAN

DEPARTMENT OF PLANT PATHOLOGY

UNIVERSITY OF AGRICULTURAL SCIENCES

BANGALORE

1996

TOMATO MOSAIC TOBAMOVIRUS : CHARACTERISATION, SERODIAGNOSIS, SEED TRANSMISSION AND HOST RESISTANCE IN TOMATO (*Lycopersicon esculentum* Mill.)

SHOBA CHERIAN

Thesis submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfilment of the requirements
for the award of the Degree of

Doctor of Philosophy

IN

PLANT PATHOLOGY

BANGALORE FEBRUARY 1996

U. A. S.
University Library
DHARWAD.

Acc. No. TH- 6366

DEPARTMENT OF PLANT PATHOLOGY

UNIVERSITY OF AGRICULTURAL SCIENCES

BANGALORE

CERTIFICATE

This is to certify that the thesis entitled "**TOMATO MOSAIC TOBAMOVIRUS: CHARACTERISATION, SERODIAGNOSIS, SEED TRANSMISSION AND HOST RESISTANCE IN TOMATO (Lycopersicon esculentum Mill.)**" submitted in partial fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY (PLANT PATHOLOGY)** to the University of Agricultural Sciences, Bangalore is a record of research work carried out by **Ms.SHOBA CHERIAN**, under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

Bangalore.
February 22, 1996.

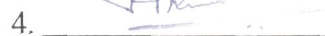
V.MUNIYAPPA
MAJOR ADVISOR

APPROVED BY: Chairman :
(V.MUNIYAPPA) 5/6/96

Members : 1.
(H.R.REDDY)

2.
(H.S.SAVITHRI)

3.
(K.P.RAMAPRASANNA) 5/6/96

4. 
(K.S.RAVI)

ACKNOWLEDGEMENT

My grateful acknowledgements are due to the guide and chairman of my advisory committee, **Dr. V.Muniyappa**, Professor and Head, Department of Plant Pathology, University of Agricultural Sciences, Bangalore for his valuable advise and untiring courtesy extended throughout the period of this investigation and thesis presentation. He has been my constant source of inspiration throughout the entire course of my research.

I am extremely grateful to **Dr.H.S.Savithri,** Professor, Department of Biochemistry, Indian Institute of Science, Bangalore for serving as a member of my advisory committee and for providing facilities at the Institute for nucleic acid characterisation of the virus.

I humbly acknowledge the efforts of the members of my advisory committee **Dr.H.R.Reddy**, Retired Professor and Head, Department of Plant Pathology, UAS, Bangalore; **Dr.K.P.Ramaprasanna**, Professor and Head, Department of Seed Science and Technology, UAS, Bangalore and **Dr.K.S.Ravi**, Assistant Professor, Department of Biotechnology, UAS, Bangalore for their constant support and valuable suggestions during the course of this study.

I am thankful to **Dr.J.Nagaraju**, Deputy Director, and **Dr.Awasthi**, Seri Biotech Research Laboratory, Bangalore for providing ultra centrifuge facilities required for purification of the virus.

The electron microscope studies were successful with the valuable aid of **Dr.Y.Ramamohan**, Senior Scientific Officer, Department of Neuropathology, NIMHANS, Bangalore.

Dr.K.P.Vasanth Shetty, Director, Indo-American Hybrid Seeds, Bangalore and **Mr.Subba Rao**, **Mr.Giri** of Mahyco Seed Company need special mention for having supplied some of the tomato leaf and seed samples required for this investigation.

I acknowledge in a special way the support extended to me by the faculty and staff of the Department of Plant Pathology, UAS, Bangalore. The cooperation extended by the fellow students in the Department of Plant Pathology, UAS, Bangalore and Department of Biochemistry, IISc., Bangalore will always be cherished.

I proudly mention the unfailing support of my parents and members of my family, especially my brother, ***C.Alexander****, for their patience, support and encouragement during the course and completion of my. doctoral study.*

A special note of thanks to Mr.Manjunath, M.N.Enterprises, R.T.Nagar, Bangalore for helping me in typing the thesis.

Bangalore.
February22, 1996.

[SHOBA CHERIAN]

CONTENTS

CHAPTER	TITLE	PAGE NO.
I	**INTRODUCTION**	1
II	**REVIEW OF LITERATURE**	3
III	**MATERIAL AND METHODS**	29
IV	**EXPERIMENTAL RESULTS**	58
4.1.	Local lesion assay	58
4.2.	Standardisation of penicillinase based enzyme linked immunosorbent assay (PNC-ELISA) in comparision with local lesion assay on N.glutinosa for detection of ToMV in tomato	58
4.3.	ELISA based surveys in tomato fields for TOMV infection	58
4.4.	Symptomatology	63
4.5.	Purification of the virus	63
4.5.1.	Spectrophotometry and determination of concentration of ToMV in purified preparations	63
4.6.	Electron microscopy	64
4.7.	SDS - polyacrylamide gel electrophoresis (SDS- PAGE) for molecular weight determination of virus protein	64

D. I. (PGS)

4.8.	Agarose gel electrophoresis for molecular weight determination of virus RNA	64
4.9.	Production of polyclonal antibodies to ToMV	64
4.9.1.	Determination of antiserum titre	65
4.10.	Purification of γ globulins and conjugation of enzyme with γ globulin	65
4.11.	Detection of ToMV in infected tomato and other hosts by using ToMV antiserum	65
4.12.	Physical properties of ToMV	68
4.13.	Determination of seed borne nature of ToMV	71
4.13.1.	Detection of ToMV in seeds	71
4.13.2.	Growing on test to find out ToMV transmission from seed to plant	71
4.13.3.	Detection of externally borne ToMV on tomato seeds	75
4.13.4.	Effect of TSP and other treatments on ToMV	75
4.13.5.	Detection of ToMV in tomato seed lots of different sizes.	75
4.13.6.	Detection of seed borne infection of ToMV in commercial tomato seeds by ELISA	79
4.14.	Testing of plant parts for the presence of ToMV	79

4.14.1.	Detection of ToMV in different parts of tomato fruits	79
4.15.	Host range	84
4.15.1.	Natural host plants of ToMV	84
4.16.	Indexing of field samples of tomato	84
4.17.	Varietal screeing	96
4.17.1.	Grafting of Lycopersicon esculentum cv. Arka Vikas on CMV sel. INRA (L.peruvianum)	96
4.17.2.	Testing CMV Sel. INRA (L.peruvianum) under field conditions	103
4.18.	Interactive effects of TLCV and ToMV in tomato plants carrying their mixed infection	103
V	**DISCUSSION**	108
VI	**SUMMARY**	115
VII	**REFERENCES**	118

LIST OF TABLES

TABLE	TITLE	PAGE NO.
1.	Members of the tobamovirus group	4
2.	Dissimillarities between tobacco mosaic and tomato mosaic viruses	7
3.	Reported host range of TMV by natural infection	19
4.	Host range of TMV on artificial inoculation	21
5.	Comparison of local lesion numbers and lesion density produced by ToMV on N.glutinosa	59
6.	Penicillinase based enzyme linked immunosorbent assay (PNC-ELISA) in comparison with local lesion assay for detection of ToMV	60
7.	ELISA based survey for detection of ToMV in tomato samples	61
8(a).	Determination of titre of antiserum	66
8(b).	Determination of titre of conjugate	67
9.	Dilution end point of ToMV	69
10.	Thermal inactivation point of ToMV	70

11.	Longevity in vitro of ToMV	72
12.	Determination of seed borne infection of ToMV in tomato and tobacco.	73
13.	Detection of ToMV infection in nursery seedlings of tomato	74
14.	Detection of externally borne ToMV in tomato seeds	76
15.	Effect of TSP and other treatments on ToMV infection	77
16.	Effect of TSP in eliminating ToMV from tomato seeds	78
17.	Detection of ToMV in tomato seed samples of various sizes	80
18.	Detection of seed borne infection of ToMV in commercial tomato seeds	81
19.	Detection of ToMV in different parts of tomato plants	82
20.	Detection of ToMV in different parts of tomato fruits	83
21.	Host range of ToMV	85
22.	Indexing of different host plants showing mosaic like symptoms for the presence of ToMV	89

23. Testing of tomato samples for ToMV infection 91

24. Indexing of tomato samples from Chikkaballapur for ToMV infection 92

25. Testing of tomato samples for TLCV and ToMV 95

26. Performance of Indian tomato cultivars and hybrids to ToMV 97

27. Reaction of F1 tomato hybrids and crosses to ToMV 99

28. Reaction of Lycopersicon species to ToMV 100

29. Testing Lycopersicon species agianst ToMV infection in ELISA 101

30. Grafting infected Arka vikas on L.peruvianum CMV Sel. INRA 102

31. Reaction of CMV Sel. INRA (L.peruvianum) grafts to tomato mosaic tobamovirus 104

32. Mean plant height of tomato plants carrying mixed infection of TLCV and ToMV. 105

33. Mean No. of leaves of tomato plants Carrying mixed infection of TLCV and ToMV 107

LIST OF FIGURES

Figure	Title	Between pages
1.	Tomato Mosaic Virus culture	29-30
2.	ToMV on tomato cv. Arka Vikas showing mosaic with pointed leaves	29-30
3.	Necrotic local lesions induced by ToMV on Nicotiana glutinosa	30-31
4.	Detection of ToMV using Penicillinase ELISA	59-60
5.	ToMV on N.tabacum cv. samsun showing light and dark green mottling	63-64
6.	ToMV on tomato cv. Arka Vikas (a) showing mosaic symptoms compared with healthy leaf (b) mosaic symptoms with pointed leaves	63-64
7.	Ultraviolet absorption spectrum of purified ToMV from infected N.tabacum cv. Samsun	63-64
8.	Electron micrograph showing rigid rod shaped particles of ToMV in purified preparation (a) 36000x magnification (b) 95000x magnification (c) 180000x magnification	64-65
9.	Electrophoresis of ToMV coat protein in 10 per cent polyacrylamide gel stained with coomassie brilliant blue	64-65
10.	Ultraviolet absorption spectrum of purified ToMV-RNA	64-65

11.	Agarose gel electrophoresis of ToMV-RNA	64-65
12.	Double Gel diffusion test	68-69
13.	Double Gel diffusion test	68-69
14.	Graph showing dilution end point and thermal inactivation point of ToMV	70-71
15.	Graph showing longevity in vitro of ToMV	72-73
16.	Mild mosaic symptoms produced by ToMV on Capsicum annuum cv. G-4	88-89
17.	Local lesions produced by ToMV on Capsicum annuum cv. california wonder	88-89
18.	Chlorotic local lesions produced by ToMV on Chenopodium amaranticolor	88-89
19.	Necrotic local lesions produced by ToMV on Datura metel	88-89
20.	ToMV on Datura stramonium showing necrotic local lesions (a) Small circular spots (b) Enlarged spots	88-89
21.	Chlorotic local lesions produced by ToMV on Gomphrena globosa	88-89
22.	Necrotic local lesions produced by ToMV on N.glutinosa	88-89
23.	Necrotic local lesions produced by ToMV on N.rustica	88-89

24. Necrotic local lesions produced by ToMV on N.sylvestris 88-89

25. Necrotic local lesions produced by ToMV on N.tabacum var. CTRI special 88-89
(a) Size of lesions 24 hrs after inoculation
(b) Size of lesions 48 hrs after inoculation

26. Necrotic local lesions produced by ToMV on N.tabacum var. L1158 88-89

27. Necrotic local lesions produced by ToMV on N.tabacum var. Riwaka-1 88-89

28. Necrotic local lesions produced by ToMV on N.tabacum var. white burley 88-89

29. Systemic infection produced by ToMV on N.benthamiana 88-89

30. Systemic infection produced by ToMV on N.tabacum var. Jayasri 88-89
(a) Green blisters
(b) Malformed leaf

31. Systemic infection produced by ToMV on N.tabacum var. samsun 88-89

32. Systemic infection produced by ToMV on N.tabacum var. xanthi 88-89

33. Systemic infection produced by ToMV on Physalis floridana 88-89

34. Tomato cv. Vaishali, showing mosaic symptoms induced by ToMV — 98-99

35. Tomato cv. Rupali, showing mosaic symptoms induced by ToMV — 98-99

36. Tomato cv. NS-815, showing mosaic symptoms induced by ToMV — 98-99

37. Tomato cv. NS-893, showing mosaic symptoms induced by ToMV — 98-99

38. Established graft of CMV Sel. INRA (L.peruvianum) on stock of ToMV infected Arka Vikas — 102-103

39. Established graft of CMV Sel. INRA (stock) with infected Arka Vikas as scion — 102-103

40. CMV Sel. INRA (L.peruvianum) planted in the field following sap inoculation — 102-103

41. Interactive effects of TLCV and ToMV in tomato plants inoculated with TLCV followed by ToMV — 107-108

42. Interactive effects of TLCV and ToMV in tomato plants inoculated with ToMV followed by TLCV. — 107-108

IMPORTANT LIST OF ABBREVIATIONS

A	-	Absorbance
ALP	-	Alkaline phosphatase
BSA	-	Bovine Serum Albumin
BTB	-	Bromo thymol blue
DAC	-	Direct antigen coating
DAS	-	Double antibody sandwich
DEP	-	Dilution end point
E	-	Extinction coefficient
ELISA	-	Enzyme linked immuno sorbent assay
LIV	-	Longevity in vitro
mA	-	Milli amperes
mμ	-	Milli microns
nm	-	Nanometer
M	-	Molar
N	-	Normal
PBST	-	Phosphate buffer saline + tween
PEG	-	Poly ethylene glycol
PNC	-	Penicillinase
RNA	-	Ribonucleic acid
RPM	-	Revolutions per minute
SDS-PAGE	-	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TIP	-	Thermal inactivation point
TLCV	-	Tomato leaf curl virus
TMV	-	Tobacco mosaic virus
ToMV	-	Tomato mosaic virus
TSP	-	TSP
ul	-	Microlitre
° C	-	Degree celisius

INTRODUCTION

I. INTRODUCTION

Tomatoes which are grown both in home gardens and commercially are one of the worlds most popular vegetables. They are good sources of vitamins A and C and can help alleviate deficiencies of these vitamins in developing countries like India (Villareal, 1980).

The area under tomatoes in the world is about 28.96 million hectares with an annual production of 692.87 million tonnes, the average yield being around 24,328 Kg per ha (Anon, 1992a). Tomato is grown throughout India, occupying an area of about 0.42 m.ha producing 5 million tonnes of fruits with a productivity of 11,905 Kg/ha (Anon, 1992a). The crop occupies an area of 0.02 m.ha in Karnataka accounting for 0.43 million tonnes of fruits.

Tomato is affected by a number of diseases causing substantial losses in yields. Besides fungal, bacterial and mycoplasmal infections it is also affected by a large number of viral diseases (Anon, 1983). Among the viral diseases, tomato leaf curl and tomato mosaic are destructive diseases of tomato in India (Tewari et al, 1972; Saikia and Muniyappa, 1989). Tomato mosaic virus (ToMV) is known to cause upto 59.0 per cent reduction in weight of tomato fruits (Broadbent, 1964; Giri and Mishra, 1990).

Tomato mosaic virus is a definite member of the tobamo virus group (Francki et al., 1985). The disease is characterised by mosaic mottling and marked reduction in size of tomato leaves. Fruits from infected plants are also reduced in size (Miller and Thornberry, 1958; Broadbent, 1964; Hari Prasada Rao and Reddy, 1971).

Though, this disease has been known for quite a long period, the review of literature on Indian tobamoviruses reveal considerable work regarding symptomatology, host range, biophysical properties, transmission and management of the virus. Relatively few reports are available regarding field surveys, seed transmission, purification and serology (Reddy et al., 1969; Nariani et al., 1970, Kamra and Dubey, 1975). References are lacking regarding protein or nuclei acid characterisation of Indian tobamoviruses. Therefore, the present study was undertaken to characterise the local isolate of this virus.

The objectives of the study were,

1. Purification of tomato mosaic tobamovirus.
2. Production of polyclonal antiserum.
3. Characterisation of viral protein and nucleic acid.
4. Study of host range of tomato mosaic virus.
5. Seed transmission and detection of seed borne infection of ToMV in the commercial tomato seeds by ELISA.
6. Testing Lycopersicon species, commercial cultivars and germplasm for resistance.
7. Interactive effects of TLCV and ToMV in tomato plants carrying their mixed infection and
8. Screening field samples for ToMV infection.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The literature pertaining to studies on different aspects of tomato mosaic tobamovirus disease has been reviewed and is presented below.

Many viruses infect tomato crop (Lycopersicon esculentum) (Avgelis, 1986). The occurence of tomato mosaic virus on tomato was first reported from India by Das and Raychaudhuri, (1953). Broadbent (1964) reported yield losses due to tomato mosaic virus to be as much as 23 per cent. Among the virus diseases affecting tomato crop, tomato mosaic virus is an important disease (Tewari et al., 1972). Giri and Mishra (1990) have studied the effect of tomato mosaic virus on tomato crop. The virus was found to cause 34.02 per cent and 59.77 per cent reduction in the number and weight of tomato fruits respectively. Several other workers have also reported the natural occurence of tomato mosaic virus on tomato (Miller, 1953; Miller and Thornberry 1958; Ramakrishnan et al., 1964a; Silva et al., 1986; Gardner & Kendrick, 1992; Rao and Reddy, 1971). Sharma et al., (1984) have recognised mosaic and leaf curl as limiting factors in the successful production of tomatoes. Review articles on tobamoviruses have been published (Francki et al., 1985; Van Regenmortel, 1981).

The name tobamovirus is derived from the type member of the group, tobacco mosaic virus (TMV). The virus particles are rigid rods 18nm in diameter, and generally have one modal length of 300nm. They are built from a single species of protein subunit of molecular weight about 17.6×10^{-3} arranged in a helix; each particle contains a single molecule of positive sense, single stranded RNA of molecular weight about 2×10^{6}. (Francki et al., 1985).

True Tobamoviruses.

Definite members of the group have very stable particles that usually occur in high concentrations in their host plants. They are easily mechanically transmissible, and are naturally transmitted by contact and incidental wounding. There seem to be no efficient natural vectors. Seed transmission sometimes occurs (Francki et al., 1985).

The number of isolates and strains of tobamoviruses that have been studied and named is very large. Many of the viruses have been described under different names

Table 1 : Members of the tobamovirus group.

Sl. No.	Virus	Natural Hosts	Key Reference
1.	Cucumber green mottle mosaic virus (CGMMV)	Cucurbits	Hollings et al., 1975
2.	Cucumber virus 4 (CV4)	Cucurbits	Gibbs, 1975
3.	Frangipani mosaic virus (FMV)	Frangipani	Varma & Gibbs, 1978
4.	Odontoglossum ringspot virus (ORSV)	Orchids	Paul, 1975
5.	Ribgrass mosaic virus (RMV)	Plantago	Oshima & Harrison, 1975
6.	Sammon's opuntia virus (SOV)	Cacti	Sammoms & Chessin, 1961 Brandes & Chessin, 1965
7.	Sunnhemp mosaic virus (SHMV)	Leguminosae	Kassanis & Varma, 1975
8.	Tobacco mosaic virus (TMV)	Tobacco	Zaitlin & Israel, 1975
9.	Tobacco mosaic U_2 virus (T_2MV)	Solanaceae	Gibbs, 1975
10.	Tomato mosaic virus (ToMV)	Tomatoes	Hollings & Huttinga, 1976
11.	Youcai mosaic virus (YMV)	Crucifers	Chang et al., 1964

referred to as strains of TMV. All the tobamoviruses have very similar physical properties but vary in the primary structure of their RNA and coat protein as well as in their host range. As approved by the International Committee on taxonomy of plant viruses in 1990, eleven distinct tobamoviruses have been described (Table.1)(Francki et al., 1985).

Two well characterised strains of tomato mosaic virus (ToMV) subgroup are the dahlemense and Y-TAMV (yellow tomato atypical mosaic virus) strains (Knight et al., 1962). Most strains isolated from infected tomato plants all over the world were very similar in amino acid composition and were serologically related (Wang and Knight, 1967; Pelham, 1972; Dawson et al., 1975). Although TMV vulgare can infect tomato, the strains found to infect tomato crops in the field are nearly always members of the tomato mosaic virus group (Van Regenmortel, 1981). Isolates that belong to the group of tomato mosaic virus strains are differentiated from TMV by the fact that they produced local lesions but no systemic invasion in Nicotiana sylvestris (Wang and Knight, 1967; Van Regenmortel, 1981).

Knight et al., (1962) have isolated a distinctive strain of tobacco mosaic virus isolated from tomato. They studied the strain under study through amino acid analysis; investigating the nature of carboxy-terminal (C-terminal) residues and amino terminal (N-terminal) groups of the strain proteins; serological tests and analysis of viral nucleic acid. The new strain (Y-TAMV) produced milder symptoms on tomato than the type strain of TMV. Five peptides were observed to be distinct between Y-TAMV and TMV. While Y-TAMV had C-terminal serine, TMV had threonine as the C-terminal residue. They also found that antiserum against Y-TAMV, completely absorbed with TMV, had residual antibodies left capable of giving precipitates with the homologous antigen.

Vandewalle and Siegel (1976) have assessed the nucleotide sequence similarities between the genomes of strains of tobacco mosaic virus by a competition hybridisation test. Two strains included in the study were dahlemense strain of tomato mosaic virus and Y-TAMV. These strains differed from the U_1 strain of TMV in 42 out of the 158 amino acid positions. The extent of homology between the nucleic acid of U_1-TMV strain and that of the RNAs of the test strains were measured by determining the extent to which an excess of a test strain RNA would interfere with the annealing of

radiolabeled U_1 RNA (Group A) to its complementary strand. Results showed that the dahlemense and Y-TAMV did not compete in the annealing reaction, indicating a distinct dissimilarity in nucleotide sequence from that of U_1 RNA. However, competition hybridisation experiments revealed a close homology between RNA of Y-TAMV and dahlemense strain were classified as Group C. These differences between TMV and ToMV are summarized in Table 2.

2.1. **Symptomatology.**

Rao and Reddy (1971) described the symptoms on tomato infected with mosaic virus disease. The naturally infected tomato plants in the field showed mosaic symptoms as dark green islands surrounded by light green areas and reduced leaf size. In artificially inoculated plants, the disease appeared 2 weeks after inoculation on young foliage as mosaic mottling with light green areas surrounded by dark green islands. The leaf size was severely reduced and the infected plants produced normal fruits, but they were reduced in size compared to those from healthy plants. The symptoms described by Miller and Thornberry (1958) and Avgelis (1986) also resembled the symptoms reported by Rao and Reddy (1971). Taylor et al., (1969) have reported internal browning of tomato fruits following ToMV inoculation.

Broadbent (1964) has found that leaves of infected plants were sometimes scorched and lower leaves died earlier than those of healthy plants.

2.2. **Purification of the virus.**

Numerous techniques have been used in the purification of TMV (Miller and Thornberry, 1958; Hebert, 1963; Steere, 1964; Leberman, 1966; Gooding and Hebert, 1967; Reddy et al., 1969; Shankar et al., 1971; Bald and Terry Suzuki, 1974; Nariani et al., 1977; Asselin and Zaitlin, 1978; Sandhu and Chohan, 1978). Gooding and Hebert (1967) have purified TMV from Nicotiana tabacum leaves using a simple technique which is a modification of the polyethylene glycol (PEG) technique reported by Hebert

Table 2. Dissimilarities between Tobacco mosaic virus and Tomato mosaic virus.

Character	TMV	ToMV	Reference
1. Amino acid composition			
Lys	2	2	Wang & Knight, 1967
His	0	0	
Arg	11	9	
Asp	18	17	
Thr	16	17	
Ser	16	15	
Glu	16	19	
Pro	8	8	
Gly	6	6	
Ala	14	11	
Cys	1	1	
Val	14	15	
Met	0	1	
Ile	9	7	
Leu	12	13	
Tyr	4	5	
Phe	8	8	
Trp	3	3	
Total	158	158	
2. Capsid protein			
C-terminus	Threonine	Serine	Vandewalle & Siegel, (1976)
N-terminus	Not acetylated	Acetylated	
3. Serological cross reaction			
	SDI		
TMV Antiserum			
ToMV Antigen	1.2[a]		
ToMV Antiserum			
TMV Antigen	1.1		Van Regenmortel,1975
4. Nucleic acid homology	Group A	Group C	Vandewalle & Siegel, (1976)
5. Differential host			
<u>Nicotiana sylvestris</u>	Systemic	Local	Van Regenmortel, 1971

SDI - Serological differentiation index values represent the number of two fold dilution steps seperating homologous from heterologous titers.

(1963). The virus was extracted in 0.5M Na_2HP0_4 - KH_2P0_4 buffer p^H 7.2 containing 1 per cent 2 - mercaptoethanol using 1g of tissue/ml of buffer. After straining 8ml of butanol/100 ml extract was added while stirring and then centrifuged at 10,000g for 30min. To the supernatant, 4.0g of PEG (mol.wt. 6000) /100 ml was added while stirring and after the PEG dissolved, the suspension was centrifuged at 10,000g for 15 min. Pellet was resuspended in 20 ml of 0.01M Po_4 buffer (p^H 7.0) /100 ml initial extract and clarified at 10,000g for 15min. The supernatant was further purified by a second precipitation with PEG using 0.4g of NaCl and 0.4g of PEG for each 10ml of virus suspension.

Bald and Suzuki (1974) have purified TMV by extracting infected tissue using 18 ml of butanol, 0.8g of bentonite, 0.3g of sodium sulfite and 200ml of water per 100g of tissue. After centrifuging at 10,000g for 10min, the clarified supernatant was centrifuged at 17,000 rpm for 90min. The pellet was resuspended in distilled water, 1/10 the original volume and centrifuged at low speed. To every 20ml of supernatant, 1.2ml of 7 per cent n-butanol was added and centrifuged at high speed for 90 min. Pellet was resuspended in distilled water, followed by a low speed centrifugation.

Shankar et al., (1971) have purified the virus from mosaic affected bottle gourd through three methods.

1. **Butanol - centrifugation method** : Infected leaves were mixed with 1.25 per cent wt/volume of 0.2M phophate buffer, p^H 7.6 (cold) containing 0.1 per cent thioglycollic acid. After squeezing through a muslin cloth, butanol was added at 8.5 per cent. The mixture was stored overnight in a fridge at 8°C and then centrifuged at 12,500 rpm for 25 mins. The supernatant was centrifuged at 30,000 rpm for 120 mins, to pellet the virus. The pellet was suspended in 0.2M phosphate buffer (p^H 7.6) and subjected to one more cycle of low and high speed centrifugation. This pellet was then suspended in o.85 per cent saline and centrifuged at 5000 rpm for 15 mins to get a purified virus preparation that is highly opalescent and infective. Nariani et al., (1970), have also used this butanol-centrifugation method to purify southern sunnhemp mosaic virus.

2. **Ether carbon tetrachloride method.**

In this method each 100g leaf material was mixed in 200ml of 0.1M phosphate buffer (p^H 7.0) containing 25ml each of cold diethyl ether and carbon tetrachloride. After stirring for 15 mins, suspension was centrifuged at 6,000 rpm for 20 mins and the aqueous phase was collected by means of a syringe. This was centrifuged at 30,000 rpm for 120 mins to pellet the virus. The pellets were suspended in M/20 phosphate buffer (p^H 7.6) and centrifuged at 5000 rpm for 15 mins to remove the suspended impurities.

3. **Use of thioglycollic acid and borax and borate buffer.**

The sap expressed from infective leaf material was stored for 11 days in the freezer at - 10°C, thawed and thioglycollic acid (1ml of 1 per cent solution per 10ml sap) and borax (2ml of 0.05M solution per 10ml sap) added. The mixture was centrifuged at 10,000 rpm for 10 mins. The virus was sedimented from the supernatant fluid by centrifugation at 30,000 rpm for 120 mins. The pellets were suspended in 0.2M borate buffer (p^H 8.2) and the suspension centrifuged at 10,000 rpm for 3 mins. The virus was again sedimented from the supernatant by centrifugation at 30,000 rpm for 120 mins. The final pellets were taken in 0.02 M borate buffer (p^H 8.2) at the rate of 0.5ml per tube and centrifuged at 10,000 rpm for 3 mins.

They observed that the virus purified by the butanol centrifugation gave the highest precipitation end point (1:32,768) followed by ether carbon tetrachloride method (1:16,384) and the use of thioglycollic acid with borax and borate buffer (1:18,192). Compared to the first two methods, the virus purified by the third method was slightly greenish.

Asselin and Zaitlin (1978) have purified TMV from tobacco leaves by essentially employing the method of Gooding and Hebert (1967). After the first precipitation with PEG, and centrifugation at low speed, the supernatant was subject to sucrose density gradient centriguation for further concentration and elimination of host material. SW 28 rotor tubes were filled with 6, 9, 9 and 9 ml of 10, 20, 30 and 40 per cent sucrose and left in the refrigerator a day previous to loading virus. 5ml of virus was

loaded onto gradient tubes and centrifuged at 28,000 rpm for 2-3 hours. Light scattering band is collected and absorbance measured at 260nm. This was then diluted in buffer and pelleted at 36,000 - 60,000 rpm for 1-3 hrs. The pellet was dissolved in an ml of buffer.

Asselin et al., (1984) have outlined a procedure of getting pure TMV isolates by following Polyacrylamide Gel Electrophersis (PAGE) after virus purification.

2.3. **Electron Microscopy.**

Kamra and Dubey (1975) have studied the particle morphology of a TMV strain from chilli by electron microscopy. Purified virus suspension (1:30) was mounted on formvar coated grids and allowed to dry. The grids were shadow cast with palladium at 40° angle. For negative staining the virus suspension was mounted on carbon coated grids and stained for 30 seconds with 1.0 per cent PTA adjusted to three different p^H viz., 6.0, 6.5 and 7.2 with 1N KoH. The best staining was obtained at p^H 6.0. The virus particles were rigid rods varying in lengths from 100 to 411nm. The modal length of the virus was found to be 230nm. Similar electron microscope determination of size and shape of sunnhemp mosaic tobamovirus was done by De (1951). Electron microscopy of leaf dip preparations of mosaic infected banana leaf sap revealed rod shaped virus particles similar to TMV. They measured about 300nm in length (Singh, 1988).

Uyemoto and Humner (1973) found that the virus particles of TMV can be clearly stained with uranyl acetate and they appeared as rigid rods when seen through the electron microscope. Similar observations have been made by Hibi et al., (1973) with respect to TMV.

Horne et al., (1976) have stained TMV with freshly prepared solution of 0.5 per cent or 2.0 per cent uranyl acetate in glass distilled water and recorded the electron micrographs on a JEOL, TEM 100B instrument fitted with a pointed filament and 'cool beam' gun system. High resolution micrographs showed the protein structure units in the rigid rods.

Sigurgerssoni and Stanley (1947) have reported that electron micrographs, prepared by the shadow casting technique of the freshly expressed juice and tobacco mosaic diseased turkish tobacco plants showed that most of the rod shaped particles measured 15 by 280mμ in size. A number of particles of double this length were also seen. They were formed by the end to end union of the particles.

Nariani et al., (1977) have reported a mosaic disease on musk melon. The electron micrographs of the purified preparation of the virus particles were found to be rigid rods measuring 280mμ x 15mμ. Niazi et al., (1973) have seen long rigid rods measuring 318-325nm x 17nm when virus samples of TMV infecting sunnhemp was examined in the electron microscope.

Verma et al., (1972) have also reported a rod shaped virus causing mosaic of Solanum khasianum measuring 304mu in length and 15mu in breadth when seen using a electron microscope.

Bos (1975) have made electron micrographs of crude sap preparations of tobacco mosaic virus with 50-200 particles per photograph. They found that calculated average lengths turned out to differ upto 4 per cent according to the site photographed and calculated average lenghts also appeared to depend on potentiometer settings required for focussing, thus with TMV they could only determine relative particle sizes.

Ball and Brakke (1968) have used leaf dip serology for electron microscopic identification of TMV. They found that the test required very small amounts of antiserum and the reaction of the virus rod and antibodies are directly observed.

2.4. **Molecular weight of virus coat protein.**

Stanley (1935) first isolated a protein possessing the properties of TMV from infected tobacco plants.

Hill and Shepherd (1972) have estimated the molecular weights of the coat proteins of 15 plant viruses by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. When run on a 10 per cent gel, tobacco mosaic virus coat proteins had a estimated molecular weight of 15,600 ± 600.

Scalla et al., (1976) have detected two high molecular weight proteins associated with tobacco mosaic virus (TMV) - infected tobacco plants analysed by electrophoresis in sodium dodecyl sulphate (SDS) - polyacrylamide gels. A protein of 150,000 daltons and a smaller protein of 130,000 daltons were detected in the gels. The larger protein was not detectable in healthy plants but the second smaller protein was seen in extracts of both healthy and infected plants. Francki et al.,1985 have reported that the TMV genome produces four proteins: 165K, 130K, 30K and the coat protein. The coat protein with type TMV has 158 residues and a molecular weight of 17,604.

Lammelli (1970) has described a method of gel electrophoresis using which many hitherto unknown proteins could be found in bacteriophage T4. The seperation gel contained 0.375 M Tris-HCl (p^H 8.8) 0.1 per cent SDS, 0.025 per cent TEMED and ammonium per sulphate. The stacking gel of 3 per cent acrylamide contained 0.125 M Tris HCl and 0.1 per cent SDS.

Electrophoresis was carried out with a current of 3 mA per gel (about 7 hr). The proteins were stained for 1 hr. at 37°C with a 0.1 per cent Coomassie brillant blue solution made up freshly in 50 per cent TCA. Bands were seen after destaining in 7 per cent acetic acid.

Molecular weight of coat proteins of several viruses have been determined by SDS-PAGE (Hedrick and Smith, 1968; Wang and Knight, 1967; Bem and Murant, 1979; Wray et al., 1981; Hogue and Asselin, 1984).

2.5. Molecular weight determination of virus RNA.

Gierer and Schramm (1956) have isolated RNA from tobacco tissue infected with tobacco mosaic virus by centrifuging with an equal amount of water saturated phenol. The RNA had a sedimentation constant of 12-18S, compared with 180S for tobacco mosaic virus.

Rejinders et al., (1974) have determined the molecular weights of a number of plant viral RNAs by gel electrophoresis under denaturing conditions. RNA was extracted using phenol-cresol method described by Rejinders et al., (1973). The RNA

was then subject to polyacrylamide gel electrophoresis. Assuming a linear relationship between electrophoretic mobility and log (molecular weight), a mean molecular weight of 2.07 x 10^6 was calculated for TMV RNA. This value of $2.07 \pm 0.06 \times 10^6$ daltons for the molecular weight of TMV RNA was in agreement with the molecular weights of TMV RNA determined by Boedkter (1960) and Siegel et al., (1973). Murant and Taylor (1978) have also estimated molecular weight of several viral RNA's by polyacrylamide gel electrophoresis under denaturing conditions.

Torrance and Harrison (1981) have extracted RNA from virus particle by five different methods-Pronase-SDS method; two phase phenol method; SDS/Phenol method; sodium perchlorate method and ammonium sulphate method. Aqeous polyacrylamide gels (2.4 per cent) were prepared and used for electrophoresis as described by Bem and Murant (1979). In this method tobacco mosaic virus RNA of molecular weight 2 x 10^6 served as a marker RNA species.

Murant et al., (1972; 1981) have estimated molecular weight of plant virus RNA by agarose gel electrophoresis and they have reported that electrophoresis of undenatured RNA in polyacrylamide gels, although commonly used to estimate molecular weight, may give incorrect values because the RNA molecules do not have hydrodynamically equivalent conformations. They have denatured RNA molecules by heating at 50°C in 1M-glyoxal, 50 per cent dimethyl sulphoxide and their molecular weight estimated by elctrophoresis in a 0.75 per cent agarose gel. TMV RNA with 6340 nucleotides and a molecular weight of 2.19 x 10^6 was used as a standard in this electrophoresis. The molecular weight of TMV RNA has been determined by polyacrylamide gel electrophoresis. It was found that the linear relation between log (molecular weight) and electrophoretic mobility was independent within limits of salt or gel concentration. The apparent molecular weight of TMV RNA was found to vary betwen 2.2 x 10^6 and 2.5 x 10^6 (Loening, 1967; Loening, 1969).

Several other workers have also determined the nucleic acid molecular weight by denaturing the nuclei acid and then subjecting the denatured nucleic acid to electrophoresis (Bhalla and Sehgal, 1973; Hansen, 1976; Torrance and Harrison, 1981).

2.6. Serology..

2.6.1 Production of antiserum.

Reddy et al., (1969) have produced antiserum against TMV purified from infected Nicotiana tabacum leaves. Purified virus was mixed with Freund's adjuvant in 1:1 proportion and in each rabbit 3 injections of such preparation were given in the large muscle of the hind leg, first two injections, 10 days apart, and a third 3 or 4 weeks after the second injection. The rabbits were bled 10 days after the final injection and this antiserum had a titre of 1/3200 as determined by precipition tube test. Noordam (1973) has given the procedure for agar gel diffusion tests.

Anand and Sahambi, (1965) have produced antiserum to TMV affected sunnhemp leaves by mixing 0.5mg of purified virus with an equal volume of 2N saline and injected intravenously into marginal vein of ear of an albino rabbit. Four such injections were given at an interval of 3 days and then the animal bled 15 days after the last injection. Four such bleedings after the last injection at weekly intervals yielded about 50ml of clarified antiserum.

2.6.2. Purification of r globulin and prepartion of enzyme conjugate.

Clark and Adams (1977) have given the procedure for purificatin of r globulin by precipitating in saturated ammonium sulphate solution. The OD280 of purified r globulin is measured and strength of r globulin adjusted to read approximately 1.40D (about 1mg/ml). They have described the single step glutaraldehyde method for conjugation of r globulin with enzyme. Accordingly 1ml (= 5mg) enzyme precipitate is dissolved in 1ml of purified r globulin. 0.06 per cent fresh glutaraldehyde is added and left for 4 hours at room temperature. After dialysing 3 times against 500ml PBS to remove glularaldehyde, Bovine Serum Albumin (BSA) is added to about 5mg/ml and stored at + 4°C.

A similar procedure is also described by Clark and Bar Joseph (1984).

2.6.3. **Ouchterlony agar gel diffusion test.**

Reddy et al., (1969) have performed this test in petri plates of 9 cm diameter wherein a central well surrounded by 5 wells were made by using a cork borer of 6mm diamter. Serial two fold dilutions of tobacco mosaic virus antigen was tested against pre immune serum. Of the different serological tests employed, precipitin ring test could detect smaller quantities of antigen than agar gel diffusion tests.

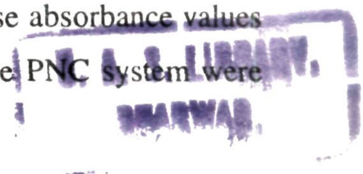
Serological studies on naturally occuring strains and nitrous acid induced mutants of tobacco mosaic virus have been made through immuno - diffusion tests (Van Regenmortel, 1965, 1967; 1975).

Scott (1974) has discussed how gel diffusion tests can be interpreted and report that gel diffusion alone is not enough to know if isolates are serologically identical or not.

Four tobamoviruses infecting cultivars of pepper were identified through immuno - diffusion tests by Wetter (1984). They could identify the different isolates from pepper (Capsicum) in immunodiffusion tests with either TMV, ToMV or PMMV (Pepper mild mottle virus). The three viruses were easily distinguished by a strong spur formation.

2.6.4. **Enzyme linked immunosorbent assay (ELISA).**

Sudarshana and Reddy (1989) have standardised a penicillinase (PNC) - based enzyme linked immunosorbent assay to detect maize mosaic virus (MMV) in sorghum leaf extracts, peanut mottle virus (PMV) in peanut leaf extracts and tomato spotted wilt virus (TSWV) in peanut leaf extracts. Rabbit F_c - specific antibodies conjugated with PNC were used in the assay. Sodium salts of penicillin-G at 0.5 - 1.0mg/ml and Bromothymol Blue (BTB) at 0.1-0.2 mg/ml were found to be suitable as substrate - indicator mixtures for PNC - based ELISA. The colour change from blue to greenish yellow to deep yellow was indicative of a positive reaction. These absorbance values were measured at 620nm. They found that the sensitivity of the PNC system were



TH- 6566

comparable to those of the alkaline phosphatase (ALP) and horseradish peroxidase (HRP) systems in detecting Maize mosaic virus, Peanut mottle virus, and Tomato spotted wilt virus. Moreover the Penicillinase conjugate could be used at a greater dilution than those of the ALP and HRP conjugate and the BTB substrate mixture was stable for atleast 3 weeks at 4°C. The PNC-based ELISA proved a less expensive means for assaying plant viruses by ELISA because penicillin is readily available at a substantially lower cost than p-nitrophenyl phosphate, the commonly used substrate for ALP in plate ELISA.

Sela et al., (1984) found the end point of TMV detection by ELISA to be 5-10mg per assay. In comparison, the dot hybridisation assay detected 2.5 pg of TMV - RNA and was about twice as sensitive as ELISA.

Koening and Paul (1982) have compared the variations of ELISA with respect to their ability to detect and differentiate serologically related plant viruses. They found that the broadest range of serologically related viruses were detected by an indirect ELISA on unprecoated plates (DAC-ELISA) DAC-ELISA enabled Koening (1981) to detect cross reactions among a wider range of serologically related viruses in the tymo, tombus, como, tobamo, potex, carla and poty virus groups.

Direct antigen coating and protein A coating (PAC) forms of indirect ELISA were standardized and compared with the double antibody sandwich (DAS) form of direct ELISA for their usefulness in the detection of three peanut viruses: peanut mottle virus (PMV), tomato spotted wilt virus (TSWV) and Indian peanut clump virus (IPCV). While the peanut viruses could be detected in peanut seeds and tissue at 1:1000 dilution using PAC and DAS procedure, the DAC method detected the viruses at a 1:10,000 dilution in buffer (Hobbs et al., 1987).

Lommel et al., (1982) have found that indirect ELISA proved more sensitive than double antibody sandwich ELISA for detection of carnation mottle and canation ringspot virus in crude plant extracts.

TMV has been detected in tobacco plants and gladiolus plantlets by means of sandwich-ELISA by Fernandez et al., (1988) and using indirect ELISA by Cai, (1985) respectively. Van Regenmortel and Burckard (1980) have detected a wide spectrum of tobacco mosaic virus strains by indirect ELISA.

2.7. **Physical properties of ToMV.**

Sandhu and Chohan (1978) have studied physical properties of tobacco mosaic virus from Chilli (Capsicum annuum). The virus could retain its infectivity at 90° C and its DEP was beyond 1:1,000,000. Nariani et al, (1977) have reported a mosaic disease of musk melon (Cucumis melo L.) which was found to be infective when heated to 90°C for 10 minutes, at a dilution of 1:100,000 and also after storage for 60 days at room temperature.

Rao and Reddy (1971) have studied the physical properties of a local isolate of TMV infecting tomato. The virus was found to withstand a dilution upto 10^{-3}. The thermal inactivation point of the virus was between 50-55°C and TMV could be recovered from the virus extracts stored at room temperature (25-30°C) upto 133 days of storage, which was the longest period tested.

Kamra and Dubey (1975) have studied biophysical properties in vitro of a mosaic disease of chilli. They found that the virus remained infective after heating to 85°C for 10 mins but was rendered non-infective at 90°C. It had a dilution end point between 1:100,000 and 1:200,000. The longevity of the virus in crude extract was beyond 16 weeks at 5°C.

Ramakrishnan et al (1964b) have reported a mosaic of greengram (Phaseolus aureus) which was not inactivated even after heating for 10 mins at 90°C and even at a dilution of 1:100,000.

Several other workers have also studied the physical properties of tobamoviurses infecting Sunnhemp (Crotolaria juncea) (Niazi et al., 1973;), Solanum khasianum (Verma et al., 1972); Passion flower (Passiflora caerulea) (Mali and Vyanjane, 1980); Pigeon pea (Cajanus cajan) (Singh and Pandey, 1979); banana (Singh, 1988) and bottle gourd (Shankar et al., 1971).

2.8. **Host range.**

TMV has been reported to occur on several host plants. The host plants known to be infected by TMV naturally are listed in Table 3. along with the reference.

The virus has been transmitted by mechanical sap inoculation onto a number of host plants as listed in Table.4.

2.9. **Local lesion assay.**

Nicotiana glutinosa is reported as a local lesion host of tobacco mosaic virus. Local lesion assay is described as a measure of the concentration of TMV (Holmes, 1929). Tewari et al., (1972) have used this local lesion method, a measure of active virus, to screen tomato varieties for resistance to tomato mosaic virus.

Datura stramonium and N.glutinosa have been used as local lesion hosts of TMV by Taylor et al., (1961) to study transmission of TMV in tomato seed.

Verma et al., (1972) have used N.glutinosa to study the physical properties of a virus causing mosaic of Solanum khasianum.

2.10. **Seed transmission.**

The transmission of tobacco mosaic virus (TMV) in seeds from infected tomato plants has been reported (John and Sova, 1955; Taylor et al., 1961, Broadbent, 1965a; Bos, 1977) and the location of the virus in infected tomato seed has been studied by Taylor et al., (1961). They have tested for contaminating virus on seed coats by washing 20 seeds for 5 minutes in 1 ml of p^H 7 phosphate buffer and inoculating the washings onto local lesion assay hosts (N.glutinosa and Datura stramonium). This study has revealed that the location of TMV in tomato seeds from infected plants is in and on the seed coat and in a small percentage of endosperms but not in embryos. Seed coat virus was eliminated by acid extraction or washing in trisodium phosphate (TSP).

Table 3. Host range of TMV by natural infection.

Sl. No. Common Name	Scientific Name	Systemic/ Local lesion	Reference
1. Banana	Musa paradisiaca	S	Singh (1988)
2. Bell pepper	Capsicum annuum	S	Alagianalingam & Ramakrishnan (1972)
3. Bottle gourd	Lagenaria siceraria	S	Shankar et al., (1971)
4. Brinjal	Solanum melongena	S	Mitra & Majumdar (1980)
5. Cape gooseberry	Physalis peruviana	S	Capoor & Sharma (1965)
6. Chilli	Capsicum furtescens	S	Sri Ram & Shukla (1975-76) Kamra & Dubey (1975)
7. Chilli	Capsicum annuum	S	Verma et al., (1964)
8. Cornflower	Centaurea cyanus	S	Aslam et al., (1985)
9. Cowpea	Vigna unguiculata	-	Mali et al., (1979)
10. Datura	Datura ferox	L	Kandaswami & Ramakrishnan (1964)
11. Dahlia	Dahlia variabilis	S	Nagarajan & Summanvar (1973)
12. Eucalyptus	Eucalyptus citriodora	-	Sastry et al., (1971)
13. French beans	Phaseolus vulgaris	-	Nagaich & Vashisth (1963)

14.Greengram	Vigna aureus	S	Ramakrishnan et al., (1964b)
15.Musk melon	Cucumis melo	S	Nariani et al., (1977)
16.Passion flower	Passiflora caerulea	S	Mali & Vyanjane (1980)
17.Petunia	Petunia hybrida	S	Verma et al., (1964).
18.Phlox	Phlox	-	Khatri & Chenulu (1966)
19. Pigeon pea	Cajanus cajan	S	Singh & Pandey (1979)
20.Sunnhemp	Crotolaria juncea	S	Niazi et al., (1973)
21.Tobacco	Nicotiana sylvestris	-	Sachidananda (1966)
22.Tobacco	Nicotiana tabacum	S	Verma et al., (1964)
23.Tobacco	N.plumbaginifolia	L	Krishna et al., (1982)
24.Tomato	Lycopersicon esculentum	S	Rao & Reddy (1971)
25. -	Digitalis lanata	-	Sastry & Khan (1971)
26. -	Physalis minima	S	Verma & Chowdhury (1984)
27. -	Solanum khasianum	S	Verma et al., (1972)
28. -	Vernonia cinerea	S	Mariappan et al., (1970)

Table 4. Host range of TMV on artificial inoculation.

Sl. No.	Common Name	Scientific Name	Systemic/ Local lesion	Reference
1.	Bell pepper	Capsicum annuum	L	Rao and Reddy (1971)
2.	Beet root	Beta vulgaris	L	Verma et al., (1972)
3.	Black gram	Vigna mungo	S	Mali & Vyanjane (1980)
4.	Brinjal	Solanum melongena	L	Rao and Reddy (1971)
5.	Cassia	Cassia tora	L	Capoor (1961)
6.	Chenopodium	Chenopodium amaranticolor	L	Singh (1969)
7.	Chenopodium	C.quinoa	L	Mali & Vyanjane (1980)
8.	Chenopodium	C.murale	S	Ramakrishnan et al., (1964a)
9.	Chenopodium	C. album	L	Verma et al., (1972)
10.	Chenopodium	C.ambrosioides	L	Verma et al., (1972)
11.	Chilli	Capsicum sp.	S	Sastry et al., (1971)
12.	Cluster bean	Cyamopsis tetragonaloba	L	Raychaudhari et al., (1962)
13.	Cowpea	Vigna unguiculata	S	Mali & Vyanjane (1980)
14.	Datura	Datura	S	Phatak & Verma (1970)
15.	Datura	Datura innoxia	L	Thakur & Sastry (1971)
16.	Datura	Datura stramonium	L	Mali & Vyanjane (1980)
17.	Datura	Datura metel	L	Kamra & Dubey (1975)

18. Field bean	Dolichos lablab	S	Mali & Vyanjane (1980)
19.French bean	Phaseolus vulgaris	S	Singh & Pandey (1979)
20.Green gram	Vigna aureus	S	Singh & Pandey (1979)
21.Groundnut	Arachis hypogaea	L	Niazi et al., (1973)
22.Musk melon	Cucumis melo	S	Singh (1988)
23.Pea	Pisum sativum	L	Mali & Vyanjane (1980)
24.Pigeon pea	Cajanus cajan	S	Singh & Pandey (1979)
25.Potato	Solanum tuberosum	S	Verma et al., (1972)
26.Soybean	Glycine max	S	Mali & Vyanjane (1980)
27.Sunnhemp	Crotolaria juncea	L	Niazi et al., (1973)
28.Tobacco	Nicotiana glutinosa	L	Murthy (1982)
29.Tobacco	Nicotiana sylvestris	S	Mali & Vyanjane (1980)
30.Tobacco	Nicotiana tabacum	S	Mali & Vyanjane (1980)
31.Tobacco	Nicotiana rustica	L	Mali & Vyanjane (1980)
32.Tobacco	Nicotiana paniculata	S	Sastry et al., (1971)
33.Tobacco	Nicotiana glauca	L	Singh & Pandey (1979)
34.Tomato	Lycopersicon esculentum	S	Rao and Reddy (1971)
35.Tomato	Lycopersicon esculentum	S	Mali & Vyanjane (1980)
36.Tulasi	Ocimum sanctum	L	Nagarajan & Summanvar (1973)
37. -	Canavalia ensiformis	L	Mali & Vyanjane (1980)

38. -	Crotolaria sericea	S	Raychaudhari et al., (1962)
39. -	Desmodium	S	Singh & Pandey (1979)
40. -	Dolichos biflorus	L	Ramakrishnan et al., (1964a)
41. -	Gomphrena globosa	L	Verma et al., (1972)
42. -	Glycine soja	S	Singh & Pandey (1979)
43. -	Gynura aurantica	S	Singh (1988)
44. -	Hyocyamus niger	S	Sastry et al., (1971)
45. -	Gynandropsis pentaphylla	S	Verma et al., (1972)
46. -	Heliotropium supinum	S	Verma et al., (1972)
47. -	Kochia indica	S	Verma et al., (1972)
48. -	Nicandra physaloides	S	Sastry et al., (1971)
49.Lima bean	Phaseolus lunatus	S	Singh & Pandey (1979)
50. -	Physalis angulata	S	Sastry et al., (1971)
51. -	Physalis floridana	S	Rao and Reddy (1971)
52. -	Solanum khasianum	S	Ismail et al., (1979)
53. -	Solanum gilo	S	Verma et al., (1972)
54 -	Solanum incanum	S	Verma et al., (1972)
55. -	Solanum integrifolium	S	Verma et al., (1972)
56. -	Solanum khasianum	S	Verma et al., (1972)
57. -	Solanum nigrum	L	Rao and Reddy (1971)

58.	-	Solanum sisymbrifolium	S	Verma et al., (1972)
59.	-	Trianthema portulacastrum	L	Raychaudhari et al., (1962)
				Verma and Singh (1973)
60.	-	Vicia faba	L	Ramakrishnan et al., (1964a)
61.	-	Vigna sinensis	L	Niazi et al., (1973)

Broadbent (1965a) has studied the seed transmission of TMV. He found that the percentage of seed infection differed with tomato cultivar and time of infection. The seed usually carried TMV externally, in low concentrations but a quarter also carried it within the testa or the endosperm. Fewer, necrotic blackened seeds were obtained from late infected plants than from those infected earlier and more necrotic seeds were infected with TMV. Soaking perviously cleaned seeds in 10 per cent Teepol solution for 2 hr, or in 10 per cent TSP solution for 20 min inactivated external virus. Several other workers have also worked on the epidemiology and seed borne nature of tomato mosaic. (Alexander, 1960; John and Sova, 1955; Crowley, 1959; Broadbent, 1963; Bennett, 1969; Phatak, 1974; Kwaje and Young, 1979; Mc Guire et al., 1979; Broadbent & Fletcher, 1963; Broadbent et al., 1965; Bewley and Corbett, 1930). Alexander (1960) also reported that TSP treatment of seed reduced the amount of tomato mosaic virus on tomato seed. Broadbent (1965 a, b) have detected tomato mosaic in tomato root and soil in which infected plants were grown. Habib (1986) have also detected ToMV in middle leaves and root extracts of tomato 3 weeks after inoculation. Broadbent (1963) has reported that the best way to prevent contamination from TMV-infected tools is to dip and scrub them in 3 per cent TSP solution.

Gooding (1975) has studied the effectiveness of soaking tomato mosaic virus infected tomato seed in different combinations of tri-sodium ortho phosphate and sodium hypochlorite. He has found that soaking seeds in a 1 per cent aqeous solution of tri sodium orthophosphate for 15 mins and then in 0.525 per cent sodium hypochlorite for 30 mins was the most effective treatment for the inactivation of the virus and it also did not reduce seed germination.

Of several detergents, TSP was reported as a strong inhibitor of TMV by Taniguichi (1976). Benoit (1977) and Benoit and Maury (1976) have studied transmission of TMV through tobacco seed. They have found that infection in tobacco seed is confined to the nucellar layer of seed coat, neither endosperm nor embryo being infected. The virus in the seed coat or associated debris persist in soil and cultural practices causing plant injury transferred the source of infection to the plant. Storing infected seeds in a cool place for 3-6 months before sowing followed by immersion in 10 per cent tri sodium phosphate and washing helped eliminate infection present in seeds.

Tobacco mosaic virus (TMV) was recovered from 10 to 36 commercial tomato seeds by Gooding and Suggs (1976) indicating that infected seed should be recognised as a possible inoculum source when outbreaks of TMV occur.

2.11. **Field surveys in tomato growing areas.**

Avgelis (1986) have surveyed six main tomato producing areas of the western coast and northern area near Rethimno in Greece. Samples were collected from tomato plants suspected of being infected with tomato mosaic. In the lab, they were checked for the presence of the virus by grinding in 0.1M phosphate buffer p^H 7.2 and rubbing the extract onto celite dusted leaves of Nicotiana glutinosa, Nicotiana rustica and Datura stramonium (indicator plants). They found that plants with virus symptoms were mainly concentrated in plastic houses and ToMV was detected in about 77 per cent of the samples.

A survey of diseases of glasshouse tomato crops in Yorkshire and Lancashire (England) was undertaken in 1974 and 1975. Eighty one tomato crops were assessed for ToMV in 1974 and 50 in 1975. Tomato mosaic symptom were seen in 73 and 66 per cent of the crops in each year respectively (Fletcher et al., 1977; Fletcher and Harris, 1979).

Plants of 13 species (9 families) with symptoms suggesting virus infection were sampled during a survey in Bangladesh in 1986-87 (Akanda et al., 1991). They employed DAS-ELISA for diagnosis with 11 anti virus sera. Eight viruses were detected, one being TMV. A similar survey for the presence of ToMV on commercial crops of glasshouse tomato has been reported by Brisson et al., (1983).

2.12. **Occurence of TLCV and ToMV in tomato.**

Dhanju and Varma (1986) have reported a complex disease on tomato with widespread occurence in Haryana (India). Symptoms include crinkling, dwarfing, yellowing and premature withering of leaves together with stunting and profuse branching

of plants. They found that this disease was caused by a combined infection by two viruses viz., TMV and TLCV. the TMV infection came from seed and was followed by TLCV infection transmitted by Bemisia tabaci.

Interaction studies of ToMV and other pathogens and their effect on tomato have also been reported like the interaction study between ToMV and Melodoigyne incognita by Alam et al., (1990) and Goswami and Chenulu (1974).

2.13. **Varietal resistance**

Tewari et al., (1972) have screened some cultivated and wild tomato varieties for TMV resistance in India. Commercial tomato varieties Pusa Ruby, Sioux, Best of All, Pusa Early Dwarf and accessions of wild species L.pimpinellifolium, L.peruvianum, L.hirsutum and L.glandulosum were included in the testing programme. Compared to Pusa Ruby (standard variety for comparison), among the wild species L.peruvianum and L.hirsutum were the most resistant species. According to the mean value of the local lesions, L.esculentum varieties were graded in the order of Sl.120, Pusa red Plum, Sioux, Pusa Early dwarf, Best of All and Pusa Ruby.

Tomato varieties Punwee, VFT - 8, VFN - Bush and hybrids F38 X Sel.152, Kalchi x Punjab Chhrhara and Ronita x Punjab Tropic were apparently found free from mosaic infections when Sharma et al., (1984) studied the reaction of some tomato varieties and hybrids to ToMV. Mayee et al., (1977) have screened one hundred lines of tomato germplasm agaisnt TMV - tomato strain prevalent in Punjab State through sap inoculation. Ten lines, collected from outside the country produced no symptoms and no lesions on assay host (Chenopodium amaranticolor) and were grouped as resistant. Another 23 lines exhibited a moderately susceptible reaction. But a large collection were found to be susceptible and it included a majority of the recommended and cultivated varieties. Mayee et al., (1974 a,b) also screened different genotypes of tomato against leaf curl and mosaic in 1973.

Among 61 varieties of tomato plants tested by Rao and Reddy (1971), only American Culture 63 G4 63 was found to be completely resistant. All the other varieties

were grouped as susceptible. Resistance to ToMV in tomato has also been studied by Pelham (1966); Poonam and Varma (1976) and Silva et al., (1986). Similarly, several chilli varieties have been screened for resistance against tobacco mosaic tobamovirus by Sangar et al., (1988), Ramakrishnan et al., (1965) and Tewari (1982).

MATERIAL AND METHODS

Fig. 1

Fig. 2

III. MATERIAL AND METHODS

The experiments were conducted at the Hebbal campus of the University of Agricultural Sciences, Bangalore during 1994-96. The studies include standardization of penicillinase based enzyme linked immunosorbent assay (PNC-ELISA) for detection of ToMV, purification of the virus, protein and nucleic acid characterisation, production of polyclonal antibodies, survey for incidence of ToMV in and around Bangalore, determination of seed borne nature of the virus, host range, TLCV-ToMV interaction studies and screening tomato genotypes for ToMV resistance.

3.1. Virus culture

ToMV culture used in the present investigation was collected from naturally infected tomato (cv. Gotya) field in Kolar district of Karnataka State during the end of 1994 and established in greenhouse at UAS, Bangalore. The established culture (Fig.1 & 2) was confirmed as ToMV by PNC-ELISA using antiserum raised against ToMV. The isolate also produced local lesions on N.sylvestris confirming it to be ToMV. This culture (local isolate) was maintained on Nicotiana tabacum cv samsun by sap inoculation in an insect proof glasshouse.

3.2. Sap transmission

Preparation of sap

For all experimental purposes, the virus was extracted from Nicotiana tabacum cv samsun leaves in 0.07 M phosphate buffer p^H 7.0. The infected leaves were ground with a pestle and mortar. Buffer was added at the rate of 2 ml per gram of tissue. The crushed tissue was filtered through a muslin cloth and the filtrate used for mechanical inoculation.

Fig.1.Tomato Mosaic Virus culture

Fig.2.ToMV on tomato cv. Arka Vikas showing mosaic with pointed leaves

Inoculation

600 mesh carborundum powder was spread uniformly on the top 2-3 leaves. Prepared sap was then smeared uniformly with desired pressure using a cotton pad. After inoculation, excess sap and carborundum were washed out with tap water and kept in a insect proof glasshouse for symptom development.

Composition of Phosphate buffer 0.07 M

$Na_2HPo_4\ 2H_20$ 12.45g/L

KH_2Po_4 9.53g/L

Monobasic was added to dibasic and adjusted to p^H 7.0

3.3. **Local lesion assay**

Nicotiana glutinosa on which ToMV produces characteristic local lesions was used as a indicator host (Fig.3). To compare the lesions produced on N.glutinosa leaves of various sizes, total lesions on a leaf were counted and divided by the area of that leaf to yield the parameter lesions per unit area. Lesions were expressed per square centimetre (lesions/cm^2).

3.4. **Penicillinase based enzyme linked immunosorbent assay (PNC-ELISA) for detection of virus.**

A direct antigen coating method (DAC-ELISA) described by Hobbs et al., (1987) and modified by Sudarshana and Reddy (1989) was used to detect ToMV in the leaf and seed samples of host plants. Rabbit Fc - specific globulins prepared in goat (Sigma, USA) and conjugated to pencillinase enzyme was used.

Fig. 3. Necrotic local lesions induced by ToMV on Nicotiana glutinosa

Fig. 3

The following steps were used for ToMV detection using PNC-ELISA.

Materials

1. ELISA polystyrene microplate (Corning, Laxbro)
2. ToMV specific polyclonal antiserum
3. Adjustable multichannel micropipettes (range 1 - 20 µl; 100 to 1000 µl)
4. Buffers
5. Enzyme labelled conjugate and
6. Substrate mixture

Buffers and solutions used in ELISA

1. Carbonate buffer (Coating buffer)

Sodium carbonate	1.59g
Sodium hydrogen carbonate	2.93g
Distilled water	1000ml
p^H	9.6

2. Phosphate buffer saline (PBS)

Disodium hydrogen orthophosphate	1.44g
Potassium dihydrogen orthophosphate	0.20g
Potassium chloride	0.20g
Sodium chloride	8.00g
Distilled water	1000ml
p^H	7.4

3. PBS Tween (PBS-T)

1000 ml PBS + 0.05percent Tween 20

4. Antibody buffer

PBS-T	100ml
Polyvinyl pyrolidone (PVP)	2.0g
Ovalbumin	0.2g

5. Substrate mixture

NaoH 0.2M	50ml
Bromothymol blue	20mg
Benzyl penicillin (Sodium)	2.0mg/ml
p^H	7.2

3.4.1. **Detection of ToMV in the host plant**

Antigen Preparation

Host leaves infected with ToMV were harvested and tissue was ground in coating buffer (1g in 10ml carbonate buffer) with the help of pestle and mortar. The extract was immediately filtered through a layer of muslin cloth and the extract was taken in a watchglass. The required quantity was added to wells of ELISA plates.

Steps involved in PNC-ELISA

1. ELISA plates were coated with 100 µl crude extracts of test antigen to each well and incubated overnight at 4°C after covering with lid.

2. After overnight incubation, the plates were washed in PBS-T buffer with three quick washes and three washes with three minutes interval. The plates were dried by tapping over a tissue paper pad.

3. Crude antiserum was diluted in antibody buffer and was cross absorbed with healthy leaf extracted in antibody buffer. For cross absorption the healthy tissue was ground in antibody buffer (1g tissue / 20 ml antibody buffer) and crude antiserum was diluted in healthy leaf extract and suspended for 45 minutes at room temperature before use. 100µl of diluted antiserum was added to each well and incubated for 3 hours at room temperature.

4. Plates were washed in PBS-T as in step-2.

5. Penicillinase labelled anti rabbit F_c specific IgG conjugate was diluted (1:1000) in antibody buffer and 100 µl of this was added to each well. Plates were incubated for three hours at room temperature (25 to 30° C).

6. Plates were washed in distilled water with 0.05percent Tween-20 and dried using tissue paper pad.

7. To each well 150µl of the substrate mixture was dispensed. Substrate mixture: Dissolve 20mg Bromo Thymol Blue (BTB) in 50 ml 0.2 M NaoH and neutralised with alkali by adding concentrated HCl dropwise. The volume was made upto 100 ml using distilled water. Sodium pencillin G at the rate of 2 mg per ml was added (the p^H was adjusted to 7.2 using either 0.01M to 0.1M HCl or NaOH before use). The plates were incubated at room temperature (25 to 30° C) for further reactions.

Observations : The reactions were observed for 30 min to 2 hr and recorded the results either visually or by measuring the loss in absorbance at 620 nm.

Results : The blue colour of the substrate mixture first turns to bright green, then light orange yellow and finally to yellow colour. A green colour indicates weak positive and orange yellow colour indicated strong positive. The reactions can be quantified by measuring the loss of absorbance at 620 nm. The results were also visually scored as positive (+) and negative (-).

3.5. Purification of the virus

Buffers

Phosphate buffer 0.1M

$Na_2HPO_4 2H_2o$ 17.79g/L

KH_2PO_4 13.61g/L

Monobasic was added to dibasic to adjust p^H 7.2.

Extraction buffer

To the phosphate buffer, add

1 per cent 2-mercaptoethanol

Pellet dissolving buffer

0.01 M phosphate buffer p^H 7.0

Sample collection

ToMV infected samsun leaves exhibiting characteristic symptoms were harvested 3 weeks after inoculation. 100g of leaf tissue was processed each time.

Procedure

a) The method described by Asselin and Zaitlin (1978) was employed here.

1. 100g of infected leaves (frozen overnight) was homogenized using a mechanical blender in extraction buffer (2ml / 1gm tissue). 20ml chloroform was added slowly while grinding.

2. Extract was filtered through cheese cloth and clarified by centrifugation at 10,000 rpm for 20 min at 4°C

3. Polyethylene glycol (PEG) 6,000 (4 per cent final concentration) and NaCl (final concentration 0.2M) were added to supernatant and stirred continuously for 3 hrs at 5°C

4. The precipitate was centrifuged at 10,000 rpm for 20 min at 5°C

5. PEG precipitate was dissolved in 0.01M phosphate buffer p^H 7.0 (0.5ml/g of tissue) and left in refrigerator (5°C) overnight.

6. Suspension was centrifuged at 10,000 rpm for 10 min at 5°C

7. Resulting supernatant was precipitated again by adding 4 per cent polyethylene glycol (PEG, mol. wt. 6000) and 0.2 M sodium chloride, stirred continuously for 3 hrs at 5°C.

8. This precipitate was pelleted at 10,000 rpm for 20 min at 5°C.

9. PEG precipitate was redissolved in 100ml of 0.01M phosphate buffer p^H 7.0 and left at 5°C overnight.

10. Suspension was centrifuged at 10,000 rpm for 10 min at 5°C. Supernatant was centrifuged at 36,000 rpm for one and a half hour. The pellet was dissolved in 25ml of 0.01M phosphate buffer.

11. 5ml of Supernatant was layered on each gradient column prepared by layering 6, 9, 9 and 9 ml of 100, 200, 300 and 400g/L of sucrose in phosphate buffer 0.07M p^H 7 kept at 4°C overnight. Then it was centrifuged at 25,000 rpm for 3 hr in SW 28 rotor.

12. The light scattering zone was siphoned out, diluted with buffer and centrifuged at 36,000 rpm for 1 1/2 hrs.

13. Final pellet was then resuspended in 2ml of 0.01 M phosphate buffer p^H 7.0.

14. Dissolved pellet was centrifuged at 10,000 rpm for 10 min and supernatant was collected.

3.5.1. Spectrophotometry and determination of concentration of purified virus.

ToMV purified from freshly infected N.tabacum cv. samsun tissue was used for ultra violet (u.v.) absorption studies in a spectrophotometer. The final pellet dissolved in 2ml buffer was diluted to 1:10, 1:100 and 1:1000. The u.v. absorption of the dilution were recorded at 260 and 280 nm. The concentration of the purified virus in 1ml was calculated by using the formula $E^{0.1\%}_{260nm} = 3.1$ or A260/3.1 = Concentration of virus (mg/ml) where 3.1 is the specific extinction coefficient for TMV. The A260/A280 ratio was also calculated for the purified virus.

3.6. Electron microscopy

To examine the virus from purified preparations in the electron microscope, a drop of virus preparation was placed on the carbon coated grids and allowed to settle for 2-3 minutes. The excess of sample was removed by using blotting paper. A small drop of 1 per cent uranyl acetate, was placed on it and allowed to react for 2-3 min. Alternatively a drop of virus was placed on carbon coated grid and allowed for 1-2 min. followed by staining with 8-10 drops of 1 per cent uranyl acetate. The excess stain was drained off by touching a blotting paper strip to the edge of the grid. The grids were dried for 15 to 30 min and examined under JEOL 100CX electron microscope. The photographs of the virus particles were taken and size of the particles was calculated by the following formula:

$$\text{Actual size of a particle in nm} = \frac{\text{Size of the particle in the electron micrograph (mm)}}{\text{Magnification}}$$

3.7. Sodium dodecyl sulphate - Polyacrylamide gel electrophoresis (SDS-PAGE) for molecular weight determination of virus protein.

The SDS-PAGE was performed by following the method of Lammeli (1970) and Hogue and Asselin (1984). A 10 per cent gel was used with SDS discontinuous buffer system with stacking gel p^H 6.8 and resolving gel p^H 8.8 and tris-glycine (p^H 8.3) as electrode buffer. The preparation of reagents and electrophoretic procedures followed are given below.

Reagents and gel preparation for SDS-PAGE:

2X Sample buffer

0.0025 M Tris-HCl p^H 6.8	0.76g
2percent SDS	2 g
5percent 2-mercaptoethanol	5 ml
10percent Sucrose	10g
0.002 per cent Bromophenol blue	0.002g
Distilled water	100ml

Mix sample 1:1 with 2X sample buffer and boil for 5 minutes.

Acrylamide stock

Acrylamide	30.0g
Bisacrylamide	0.8g

Dissolved in distilled water and made upto 100 ml. The solution was filtered and stored at 4°C in amber coloured bottle.

Stacking gel buffer

0.5 M Tris-HCl p^H 6.8	6.05g
Distilled water	100 ml

Filter and store at 4°C

Resolving gel buffer

3M Tris-HCl p^H 8.8	36.3g

Distilled water	100 ml

Filter and store at 4°C

X10 Reservoir buffer stock

0.25 M Tris	30.25g
1.92 M Glycine p^H 8.3	144.15g
SDS	10.0g
Distilled water	1000ml

Ammonium persulphate

1.5 per cent solution in distilled water	0.15g/10ml

10percent SDS

Ten grams of SDS dissolved in water with gentle stirring and volume made upto 100 ml with distilled water.

Resolving gel 10 per cent	**For 40 mls**
Acrylamide stock	13.3 ml
Resolving gel buffer	5.0 ml
Distilled water	19.3 ml
Ammonium persulphate	2.0 ml
SDS 10percent solution	0.4 ml

TEMED	0.02 ml

Fill gel to about 1 cm below the position of the comb and overlay with saturated butanol.

Stacking gel 3.75percent

Acrylamide	2.5 ml
Stacking gel buffer	5.0 ml
Distilled water	11.3 ml
SDS	0.2 ml
Ammonium persulphate	0.8 ml
TEMED	0.015 ml

Stain

Coomassie Brillant blue R 250 (0.1 per cent)

Coomassie blue	100 mg
Methanol	50 ml
Acetic acid	20 ml
Distilled water	50 ml

Coomassie blue is dissolved in methanol and mixed with acetic acid and distilled water. Filter before use.

Destaining solution

Methanol	30 ml
Acetic acid	10 ml
Distilled water	60 ml

Preparation of separating and stacking gel

Using 1mn spacers the vertical slab gel unit was assembled in the casting mode. The resolving gel solutions were mixed according to the recipe in a conical flask, and the gel solution was poured quickly into the mold after addition of ammonium persulphate and TEMED solutions and overlayered with saturated butanol. The gel polymerised within 20-30 min after which butanol layer was poured off.

The stacking gel solution was prepared according to the recipe and overlayered on the resolving gel to the brim. A 10 well comb was inserted quickly, taking care not to trap any air bubbles and the gel was allowed to set for 30 min.

Sample and molecular weight standards

The purified ToMV and the mixture of seven molecular weight protein standards (Sigma stock No.SDS-7, Dalton Mark VII-L) were heated for five min. in a boiling water bath. The protein markers used (with molecular weights in parenthesis) were : Bovine allumin (66,000); Egg albumin (45,000); Glyceraldehyde - 3 - P - dihydrogenase (36,000); Carbonic anhydrase (29,000); Tripsinogen Bovine Pancreas (24,000); Trypsin inhibitor (20,100) and lactalbumin (14,200).

Loading and running of gels:

The comb was slowly removed from the gel, clamped to the vertical slab gel electrophoretic unit with the notched plate facing the upper reservoir. The reservoirs were filled with the tank buffer. 20 µl of ToMV sample and 5 µl of standard protein markers were loaded into the wells seperately. The two reservoirs were connected to power supply with the cathode connected to the upper reservoir and electrophoretic run performed at 50mA until the dye reaches the bottom of the gel.

The gel was then carefully transferred from the mold into a tray containing

Coomassie Brilliant Blue for one hour, removed and kept in destaining solution until destaining was complete and transferred to gel storage solution. The photograph of the gel was taken and the migration distance of protein band and tracking dye was tabulated.

The migration value (abscissa) were plotted against the know molecular weight of marker proteins (ordinate) on semi-logarithmic paper and the molecular weight of ToMV coat protein was estimated from calibration curve.

3.8. **Agarose gel electrophoresis for molecular weight determination of virus RNA**:

RNA was isolated and Agarose gel electrophoresis was performed by following the procedure of Murant et al., (1981) and Torrance and Harrison, 1981.

3.8.1 **Isolation of viral RNA from purified ToMV**

3.8.1.1 **Reagents**: All solutions to be prepared in DEPC water.and autoclaved at 15-20 lb pressure for 20 min to minimise RNase contamination.

Distilled water containing 0.1percent diethylpyrocarbonate (DEPC)

Distilled water	1000 ml
DEPC	1.0 ml

Dispense in aliquots and autoclave. Store at 4°C.

10percent Sodium dodecyl sulphate (SDS)

SDS	1.0g
Sterile distilled water	10ml

(Store the solution at room temperature)

Ethylenediamine tetraacetic acid, sodium salt (Na_2EDTA).

200 mM Na_2EDTA (372.24) 3.722g

Distilled water 50 ml

Adjust p^H to 8. Autoclave and store at 4°C

2.5M Sodium acetate

Sodium acetate 10.25 g

Distilled water 50.0 ml

Adjust p^H to 5.5 with acetic acid, autoclave and store at 4°C

Absolute ethanol stored at - 20 deg.C

Chloroform isoamylalcohol (24:1, v/v)

Chloroform 24 ml

Isoamylalcohol 1 ml

RNA dissociation buffer

0.05M Sodium citrate buffer

0.2 per cent SDS

0.05 per cent EDTA

Phenol+Chloroform (1:1, V/V)

Water saturated phenol 50ml

Chloroform isoamylalcohol 50ml

3.8.1.2 **Procedure**

1. An equal volume of RNA dissociation buffer was added to 250 ul of purified virus. This was incubated at 55 degree C for 10 min.

2. An equal volume of Phenol+Chlofoform (1:1 ratio) was added and vortexed for 1 to 2 mins.

3. Centrifuge at 10,000 rpm, 10 min. at 4°C. Upper aqueous phase was collected with a micro pipette into another eppendorff tube. Reextraction was done by addition of equal volume of chloroform to the aqueous phase and centrifuging at 10,000 rpm for 10 min.

4. To the upper aqueous phase, sodium acetate (1/10 volume) was added, shaken & then 2.5 volumes of cold ethanol was added. The preparation was stored for one hour in liquid nitrogen for precipitation of nucleic acid. This was centrifuged at 10,000 rpm for 10 min.

5. The pellet was washed with 70 per cent ethanol and centrifuged at 10,000 rpm for 10 min.

6. The pellet was dried at 37 degree C for 10 to 15 mins. and the final dried pellets dissolved in a minimal volume of DEPC treated sterile water.

7. The final RNA preparation was subjected to spectral analysis and run on a agarose gel.

3.8.2. **Determination of molecular weight of RNA**

TBE buffer, p^H 8.3.

Tris (0.089 M)	5.389g
Boric acid (0.089 M)	2.751g
EDTA (0.002 M)	0.074g
Distilled water	500 ml

Sample buffer

TBE buffer, p^H 8.3	80 ml
Glycerol (20 per cent)	2.0 ml
Bromophenol blue (0.1 per cent)	100 ul

Ethidium bromide (1mg/1ml)

Dissolve 1mg of ethidium bromide in 1ml of distilled water and store at 4°C

3.8.2.1 **Sample preparation**

1. The RNA precipitate was dissolved in minimum volume of sample buffer.

3.8.2.2 **Agarose gel electrophoresis**

1. Gel boats were prepared by covering the edges of a mould with tape and were placed on a level surface. Comb is set in gel boat and melted 1 per cent agarose solution (50°C) was poured into the gel boat without trapping air bubbles.

2. Comb was removed after solidification of gel. Tape was removed and boat transferred onto horizontal electrophoresis unit. TBE buffer with 2 ul ethidium bromide (stock of 10 mg/ml) was poured into tanks (buffer level was 1-2 mm above the gel surface).

3. Sample virus RNA and marker RNA (Physalis mottle virus RNA of 6 Kb and Sesbania mosaic virus RNA of 4.5 Kb) were loaded to separate wells (5 ul/well). Samples were run at 50 volts for 1 hr at room temperature.

4. Bands were observed using a U.V. transilluminator and gels photographed.

3.9. Production of polyclonal antibodies.

For immunising rabbits each time ToMV purified from 100g freshly infected N.tabacum cv. samsun leaves were used. New Zealand white rabbits were given three intramuscular injections each with 1ml purified virus emulsified with an equal volume of Freund's incomplete adjuvant and one intravenous injection without adjuvant. Four bleedings were made by cutting the marginal vein of the ear at weekly intervals starting from one week after the final injection. Serum was seperated after keeping the blood for 2 hr at room temperature followed by keeping at 4°C in a refrigerator overnight.

3.9.1. Determination of antiserum titre

Two fold dilutions of antiserum viz., 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 and 2048 were prepared in PBST-PVP-OA and tested using agar gel double diffusion test using the procedure described in 3.11.1 and pencillinase based-DAC ELISA using the procedure described in 3.4 to determine the antiserum titre.

3.10. Purification of γ globulins and conjugation with enzyme

γ globulin was purified from the antiserum by following the procedure of Clark and Adams (1977).

3.10.1. **Purification of γ globulins**

Procedure

1. To 1 ml of antiserum, 9 ml distilled water was added.

2. To this 10 ml saturated ammonium sulphate solution was added and left for 60 min. at room temperature.

3. This was centrifuged to collect the precipitate and the precipitate dissolved in 2 ml 1/2 strength PBS.

4. It was dialysed 3 times against 500 ml 1/2 - strength PBS.

5. OD_{280} was measured and strength of γ globulin was adjusted to read approximately 1.4OD (about 1mg/ml). This was stored in eppendorff tubes in the freezer.

3.10.2. **Conjugation of enzyme with γ globulin**

The γ globulin was conjugated with penicillinase enzyme and used in ELISA. Conjugation was done following the procedure of Clark and Adams (1977).

Procedure

1. Five mg penicillinase enzyme precipitate was dissolved in 1ml 0.01M PO_4 buffer and centrifuged at 10,000 rpm for 15 min.

2. 1ml of r globulin was added to the enzyme supernatant. This was dialyzed 2 times against 500 ml PBS (1 hr. apart).

3. Fresh glutaraldehyde solution was added to 0.06percent final concentration and mixed well.

4. This was left for 4 hrs at room temperature. A yellow brown colour developed.

5. After dialyzing 3 times against 500 ml PBS to remove glutaraldehyde, the conjugate was stored at + 4°C in eppendorff tubes.

3.11. Detection of ToMV in infected tomato and tobacco by using ToMV antiserum.

3.11.1 Ouchterlony agar double diffusion test.

Agar gels were prepared in plastic petri dishes by pouring 15ml of 0.8percent agar (Difco) prepared in PBS. Sodium azide was added to the molten agar. At the centre of each plate wells were cut in the agar by using a gel cutter with 6 tubes of 4mm diameter at 4mm apart. The bottom of the wells were sealed by pouring a drop of molten agar. The petri plates were then incubated at room temperature for diffusion of antigen and antiserum. In the central well 20 µl drops of ToMV antiserum was placed. In the outer wells 20 ul drops of sap extracted from healthy tobacco, diseased tobacco, dilutions of purified antigen of ToMV and buffer were placed.

3.11.2. Enzyme - linked Immunosorbent Assay (ELISA).

ELISA was also used to detect presence of ToMV in infected tomato and tobacco using antiserum and conjugate produced against ToMV. ToMV was detected both through penicillinase based direct DAS and indirect DAC ELISA. Procedure for indirect DAC ELISA was as described in 3.4. For direct DAS ELISA, the buffers used were prepared as given in 3.4. The protocol was as follows:

1) ELISA plates were coated with 100 µl of purified ToMV globulin in carbonate buffer, pH 9.6.

2) Plates were incubated for three hours at room temperature and washed in PBST buffer with three quick washes and three washes of three min. interval. The plates were dried by tapping over a tissue paper pad.

3) 100 ul crude extracts of test antigen were added to each well and incubated overnight at 4° C.

4) Plates were washed as in step 2 and blocked with dried milk (5g per 100ml PBST-PVP) for 30 min.

5) Milk was poured out, plates tapped dry and coated with 100µl of penicillinase conjugate (produced in rabbits), 1:1000 dilution.

6) After three hours incubation at room temperature, plates were washed in PBST and 150µl of substrate mixture (BTB + Penicillin-G, pH 7.2) was added to each well.

7) Plates were incubated at room temperature and the colour change measured both visually and as loss in absorbance at 620nm.

3.12. ELISA based survey in tomato fields for ToMV infection.

During field survey ToMV infected samples and suspected samples were collected. At each location from each field, 25 to 30 samples of infected leaves were collected. The leaf samples collected were packed in polythene bags to avoid drying during transit. The samples were labelled giving the details of sample number, location and date of collection. All the samples were brought to laboratory and stored at 4°C until further processing. These samples were then tested by ELISA.

3.13. Symptomatology

Symptoms produced by ToMV on Nicotiana tabacum cv. Samsun and tomato cv. Arka Vikas following sap inoculation in the glasshouse are described.

3.14. Physical properties

3.14.1 **Dilution end point determination**

Eight test tubes were labelled as undiluted, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. Using a pipette, 9 ml of phosphate buffer (0.07M) was added to each of the test tube. In a pastle and mortar 1 gram of ToMV infected samsun leaf was ground with 9 ml of phosphate buffer and the contents squeezed through a muslin cloth. The clear extract was poured in the test tube labelled as 10^{-1}. Using a 1 ml pipette an ml of sap from the first tube (10^{-1} dilution) was transferred to the second tube to give dilution 10^{-2}. After mixing thoroughly, 1 ml of the 10^{-2} was added to the next tube to obtain dilution 10^{-3}. This was repeated upto the dilution 10^{-7}.

Another 1g of ToMV infected samsun leaf was ground with 1 ml of phosphate buffer and pressed through a muslin cloth. This filtrate represented the sample in the undiluted test tube.

Each dilution was inoculated on two N.glutinosa leaves and the leaves were separately labelled. The dilutions were also tested in ELISA.

3.14.2. **Thermal inactivation point determination**

Eight test tubes were labelled as control, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C and 100°C. Five grams of ToMV infected samsun leaves were ground in 25 ml of Po_4 buffer and pressed through a cheese cloth. Using a pipette 2ml of this extract was added to each of the labelled test tubes. Water is heated in a bath. When the water in the bath reached the lowest temperature (40°C), the tube labelled 40°C was put in it. A thermometer is placed close to the tube and at the same level to monitor the temperature. After 10 mins, this test tube was removed from the bath, cooled in running water, and placed in the test tube rack.

After heating the bath to the next temperature (50°C), a second tube was treated in the same way as above. After all tubes have been treated, they were inoculated on two leaves of N. glutinosa and separately labelled. Unheated sample served as a control.

3.14.3. **Longevity in vitro determination**

100g ToMV infected samsun leaves were weighed and ground in Po_4 buffer (1 part leaf: 5 parts buffer). After filtering through a cheese cloth, the extract was stored in 2 brown bottles at room temperature.

A second set of 100 infected leaves were ground in buffer, squeezed through a muslin cloth and stored in a brown bottle in a refrigerator (5°C)

A third set of 100 infected leaves were put into brown paper covers (5 leaves/cover) and stored in the cupboard.

From the first & second set, inoculations were made to a leaf of N.glutinosa once a week. From the third set, 1g of leaf was ground in 5 ml buffer, filtered and inoculated onto a leaf of N.glutinosa once every week from the commencement of this experiment (27.5.94)

3.15. **Determination of seed borne nature of ToMV in tomato seeds.**

Seed samples

Seeds of tomato hybrids F1 Fiona, Gotya, Rupali, Ramya were collected from infected plants in fields from Kolar, Yelahanka, Chintamani and Chikkaballapur while seeds of cultivar Arka Vikas, Pusa Ruby, Rashmi and Vaishali were collected from plants infected by inoculation under glass house conditions. Commercial seeds tested included varieties Pusa Ruby and Arka Vikas from Pro Agro Seeds, Gotya and S-41 from Mahyco seeds. Seeds of tobacco varieties Jayasri, Samsun and Xanthi (systemically infected) were collected from plants infected by inoculation under glass house conditions.

Standardization of ELISA and local lesion assay for detection of ToMV in tomato seeds.

Seed samples were assayed for ToMV by grinding them with 0.07M PO_4 buffer, 1ml per 100 seeds in a mortar. The resulting suspension was then inoculated over a N.glutinosa leaf with a finger which was thoroughly washed in 10 per cent TSP in between inoculations. This suspension was also tested in microtitre plates using penicillinase based DAC-ELISA.

3.15.1. **Detection of ToMV in tomato seeds.**

Tomato seeds (Gotya, F1 Fiona, Rupali, Ramya, Arka Vikas, Pusa Ruby, Rashmi, Vaishali) were extracted by squeezing the pulp from fruits of infected plants onto a nylon mesh and drying them. 100 such seeds of the previously mentioned varieties were crushed in 1 ml of phosphate buffer p^H 7.0 using a glass pastle and mortar. The crushed extract was inoculated onto Nicotiana glutinosa using a glass rod or finger which was thoroughly washed in 10 per cent tri-sodium orthophosphate in between inoculations to avoid any possible contamination (Taylor et al., 1961; Broadbent, 1965a). Seed extract was also tested in ELISA.

0.1g of seeds of the tobacco varieties Jayasri, Samsun and Xanthi were also crushed in an ml of buffer and tested on N.glutinosa and in ELISA as done in the case of tomato seed.

3.15.2. **Growing on test to find out ToMV transmission from seed to plant.**

The seeds of the varieties F1 Fiona, Rupali, Arka Vikas, Rashmi and Pusa Ruby (collected from infected plants) were transplanted into polythene covers filled with sterilized planting medium (soil mixed with compost). They were maintained undisturbed in a insect free glasshouse for symptom expression. At the end of six weeks, the plants were indexed on N.glutinosa for the presence of ToMV and also tested in ELISA.

3.15.3. **Location of the virus in tomato seed**

The seed borne nature of ToMV was studied by washing one set of 100 seeds collected from infected plants of F1 Fiona and Rupali in 5 ml of 10 per cent TSP for 20 mins while another set was washed in phosphate buffer p^H 7.0 for 20 minutes. After rinsing in distilled water, both the washed seeds and respective seed washings were inoculated onto N.glutinosa.

3.15.4. **Freeing infected seeds from virus**

Since literature and our work suggest TSP can eliminate ToMV from tomato seeds an experiment was conducted to know the effect of different concentrations and quantities of trisodium orthophosphate, sodium carbonate, and sodium bicarbonate on ToMV. Different quantities of phosphate buffer, distilled water and leaf ground without any buffer served as controls.

In each case, 1g of ToMV infected samsun leaf was ground with 1 and 5 ml of 1, 5 & 10 per cent TSP; 1 and 5 ml of 1, 5 and 10 per cent Na_2Co_3; 1 and 5 ml of 1, 5 and 10percent $NaHCo_3$; 1 and 5 ml of 0.07 MPo_4 buffer p^H 7.0 and 1 and 5 ml of distilled water and 1g of samsun infected leaf was ground as such.

Further, the seeds were treated with 10 per cent TSP for 15 mins for its ability to eliminate the virus from tomato seeds. For this 2 sets of 100 infected tomato seeds each of the varieties Arka Vikas, Pusa Ruby and Rupali were washed in a 10 per cent TSP soaking treatment for 15 mins, rinsed well in distilled water and then ground in 1 ml of 0.07 MPo_4 buffer and indexed by inoculation on N.glutinosa.

3.15.5. **Detection of ToMV in tomato seed lots of various sizes**

The presence of ToMV in seed samples of various sizes was detected by crushing and testing several seed lots (collected from infected plants) containing 100, 50, 20, 5 and 1 seed both through ELISA and local lesion assay.

3.15.6. **Detection of seed borne infection of ToMV in commercial tomato seeds by ELISA.**

To detect seed borne infection of ToMV in seeds of commercial tomato varieties, 5 lots of 100 seeds each of the varieties Pusa Ruby and Arka Vikas from Pro Agro seeds, 5 lots of 100 seeds each of Gotya and S-28 from Mahyco seeds were crushed in 2 ml of coating buffer and the suspension obtained after squeezing through muslin were tested in ELISA. 5 lots of 100 seeds each of the four varieties Pusa Ruby, Arka Vikas, S-28 and Gotya were also crushed in an ml of 0.07M PO_4, p^H 7.0 and the suspensions inoculated onto N.glutinosa.

3.16. **Testing plant parts for the presence of ToMV**

Artificially inoculated tomato plants of the cv. Arka Vikas were maintained in the glasshouse. Six weeks after inoculation, different parts of the plant viz., petals, sepals, stalk, stem, leaf and root were crushed in $P0_4$ buffer p^H 7.0 and indexed on Nicotiana glutinosa. The soil in which the infected plants were grown was also tested.

Fruits collected from these infected plants were washed in 10 per cent TSP and then the skin, juice and pulp of such fruits were tested through local lesion assay and ELISA.

3.17. **Host range**

The study was undertaken to determine the host range of this virus. Eighty host plants were raised through seeds in the insect proof glass house and were inoculated with ToMV through sap. The plants were maintained in an insect proof glasshouse for expression of symptoms. Plants which exhibited the symptoms after inoculation were recorded. These plants were also indexed onto N.glutinosa to confirm that the symptoms

were produced by ToMV. Plants which did not exhibit any symptoms even after 8 weeks of inoculation were also indexed on N.glutinosa to test whether they were symptomless carriers.

3.17.1. Indexing different host plants showing mosaic like symptoms

Different host plants (crops and weeds) showing any mosaic like symptoms were collected from field of Main Research Station, University of Agricultural Sciences, Hebbal, fields in UAS, GKVK and farmers fields. The leaf samples collected were packed in polythene bags and tested in the laboratory both on N.glutinosa and in ELISA to detect the presence of ToMV.

3.18. Indexing of field samples of tomato

During field survey conducted in Bangalore North, Bangalore South, Chikkaballapur, Chintamani, Kolar, Shimoga, and Yelahanka, tomato samples were collected at the rate of 25-30 samples from each field. 170 suspected samples from a field in South Delhi were also indexed. The leaf samples collected were packed in polythene bags and tested in the laboratory both on N.glutinosa and in ELISA (1014 samples from 13 fields).

From a couple of fields (Bangalore South and Chintamani) all plants from every tenth row in a field were sampled to know incidence of the virus.

3.19. Varietal screening

In the glasshouse, fifty five tomato hybrids, cultivars and breeding lines and eighteen accessions belonging to Lycopersicon cheesmanii; L.glandulosum; L.hirsutum; L.peruvianum and L.pimpinellifolium were evaluated for resistance against ToMV. Two weeks old seedlings were planted in the polythene bags and inoculated with infectious

sap from samsun. Symptoms produced by the virus were noted weekly upto 6 weeks after inoculation, following which the plants were indexed by back inoculation on Nicotiana glutinosa and tested in ELISA.

3.19.1. **Grafting of L.esculentum cv. Arka Vikas on CMV sel.INRA (L.peruvianum).**

Side Grafting: The CMV Sel. INRA (L.peruvianum) twigs were cut into 'v' shape and these scions were inserted into the slant cuts (1.5 cm) made on the stock plants of infected Arka Vikas. The grafted portion was firmly tied with parafilm strip and then scion was covered with polythene cover and grafted plants were kept in shade for establishment of the graft and were then transferred to insect proof cages. Grafting was also done using infected Arka Vikas as scion and CMV Sel.INRA as stock.

Top grafting: Terminal portion of the CMV Sel.INRA plants were removed and a longitudinal cut (2 cm) was made, where a diseased twig of Arka Vikas (with compatible cut) was inserted. Scion and stock were then tied firmly with parafilm strip and covered with a polythene bag. Plants were kept in shade until the grafts were established and then transferred to insect proof cages. Grafting was also done using infected Arka Vikas as stock and INRA as scion. The total no. of grafts made & no. of grafts established were recorded in each case. Eight and twelve weeks after grafting, leaves of CMV Sel.INRA from the established grafts were indexed on N.glutinosa and tested in ELISA.

3.19.2 **Testing CMV Sel.INRA under field conditions**

One hundred seedlings of CMV Sel.INRA (L.peruvianum) were planted in polythene covers, sap inoculated with ToMV and then transferred to the field under open condition and grown for several months. Once a month, leaves from these plants were indexed on N.glutinosa.

3.20. **Interactive effects of TLCV and ToMV on tomato growth.**

Seedlings of tomato cv Arka Vikas were planted in polythene bags and used in this experiment. Twenty treatments were maintained and for each treatment ten plants were used. The treatments included uninoculated tomato, ToMV inoculated alone, ToMV+ TLCV simultaneously, ToMV+ after 24 h TLCV, ToMV+ after 1 wk TLCV, ToMV+ after 2 wk TLCV, ToMV + after 4 wk TLCV, ToMV + after 6 wk TLCV, ToMV + after 8 wk TLCV, TLCV alone, TLCV + after 6 hr ToMV, TLCV + after 24 hr ToMV, TLCV + after 1 wk ToMV, TLCV + after 2 wk ToMV, TLCV + after 4 wk ToMV, TLCV + after 6 wk ToMV and TLCV + after 8 wk ToMV. At the end of the experiment, plants from each treatment were indexed on N.glutinosa to know if TLCV had any effect on ToMV concentration in the plants.

When the seedlings were 15 days old, 80 plants were inoculated with ToMV by sap transmission and another 80 plants were inoculated with TLCV by whitefly transmission. 10 plants were left uninoculated to serve as control. All the three sets of plants were maintained separately in insect proof glasshouses. Following the first inoculation with ToMV or TLCV at regular intervals as required by the particular treatment, inoculations were made with ToMV or TLCV.

Days taken for symptom production (ToMV and TLCV) were recorded in each treatment, Plant height and No. of leaves/plant were recorded at regular intervals (2, 4, 6 and 10 weeks after first inoculation).

3.20.1. **Whitefly transmission**

Whitefly transmission was made by using Bemisia tabaci. About 200-500 adults of Bemisia tabaci maintained on healthy cotton plants (Gossypium hirsutum Cv. Lakshmi) were collected from the rearing cages and released into PVC tubes in which TLCV infected branch was inserted previously and allowed to feed for 24 hours as acquisition access period. These viruliferous whiteflies were then released onto healthy tomato seedlings, which were to be inoculated with TLCV. They were allowed to feed

for 24 hours as inoculation access period. After inoculation the whiteflies were removed and sprayed with 0.1 per cent Triazophos (35EC) to kill all the whiteflies. Inoculated plants were kept in an insect proof glasshouse for symptom production.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

4.1. **Local lesion assay** : Leaves of Nicotiana glutinosa when inoculated with ToMV produced local lesions 48 hours after inoculation (Fig.3). It was found that when the same inoculum of ToMV obtained from N.tabacum cv. samsun was inoculated to N.glutinosa leaves of different sizes, they produced widely varying lesion numbers. But when lesions / cm^2 was calculated in each case, it almost accounted to the same lesion density (Table.5).

4.2. **Standardization of penicillinase based enzyme linked immunosorbent assay (PNC - ELISA) in comparison with local lesion assay on N.glutinosa for detection of ToMV in tomato.**

PNC - ELISA was standardized and successfully used to detect ToMV in tomato plants, seeds and other hosts using antiserum produced against ToMV (Fig.4). ELISA results (Table.6) showed that ToMV could be detected in infected leaf extracts with host dilution of 10^{-1} (1.14) to 10^{-7} (0.054) using an antiserum of dilution 1:1000. In comparison, ToMV infected leaf extract diluted to 10^{-6} produced local lesions on N.glutinosa (0.06 lesions/cm^2).

4.3. **ELISA based survey in tomato fields for ToMV virus infection.**

The field surveys conducted over several locations mainly around Bangalore during 1994-96, showed that the incidence of ToMV ranged from 0 to 100 per cent. While the incidence was very high in some fields like in Chikkaballapur where out of total of 334 samples tested, 299 were infected and another field in Chintamani where 62 out of 65 samples indexed were ToMV positive (Table 7). In total out of 1229 samples tested from different fields, 688 were infected with ToMV (Avinash; Cross B; F1 Fiona, Gaurav; Gotya; LIHB - 230; Namdhari 893; Namdhari 1096; Naveen; Pusa Ruby; Ramya; Rashmi; Rasika; Red Globe; Rupali; S-28; S-41; Sadabahar; Vaishali).

Table 5: Comparison of lesion number and lesion density produced by ToMV on N.glutinosa.

Leaf No.	Leaf area (cm^2)[a]	Lesions/leaf	Lesions/cm^2
1	46.50	320	6.88
2	27.45	182	6.63
3	52.68	341	6.51
4	30.87	188	6.09

[a] leaf area will be different for different leaves.

Fig.4.Detection of ToMV using Penicillinase ELISA

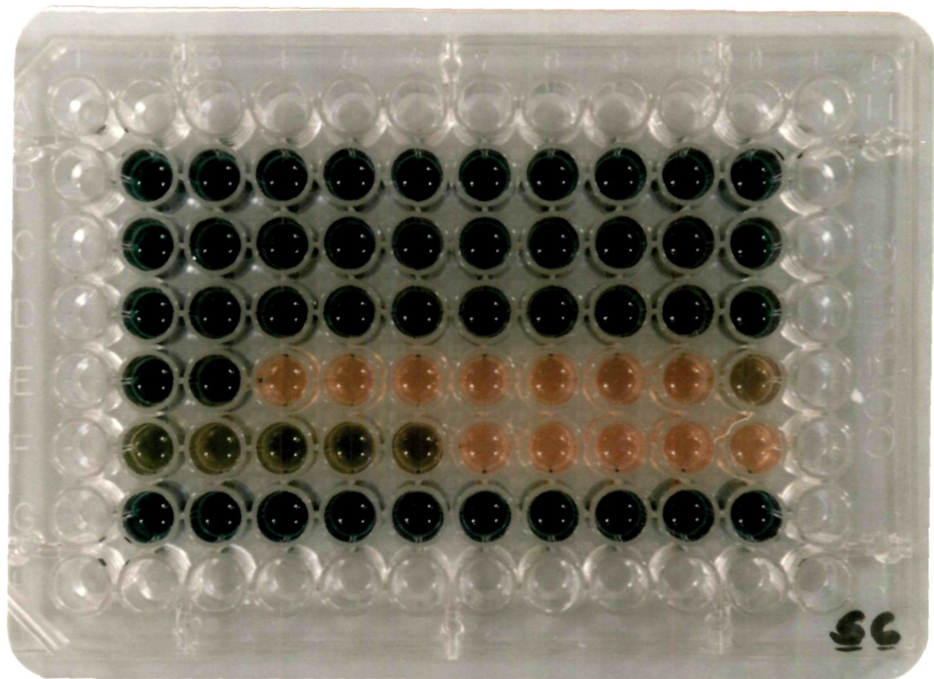


Fig. 4

Table 6: Penicillinase based enzyme linked immunosorbent assay (PNC-ELISA) in comparison with local lesion assay for detection of tomato mosaic virus.

Antigen dilution		Absorbance readings[a,b]	*N.glutinosa* Lesions/cm^2
Infected	10^{-1}	1.139	7.37
	10^{-2}	1.130	5.45
	10^{-3}	1.118	4.15
	10^{-4}	1.117	2.30
	10^{-5}	0.856	0.43
	10^{-6}	0.215	0.06
	10^{-7}	0.054	0.00
	10^{-8}	0.005	0.00
Healthy	10^{-1}	0.011	0.00

[a] Mean absorbance values of five wells at 620 nm obtained after deducting the mean absorbance value of buffer control.

[b] Absorbance values were considered positive if they yielded OD values twice greater than the mean optical density of wells coated with healthy plant extracts.

Table 7: ELISA based survey for detection of ToMV in tomato samples.

Place of Collection	Variety/Hybrid /Breeding line	No. of Samples tested	No. of samples infected
Bangalore South	Breeding lines	267	226
Kolar	F1, Fiona	41	16
Kolar	Gotya	5	4
Kolar	Pioneer Seeds	32	0
Kolar	Rashmi	36	1
Kolar	Avinash	35	0
Bylakare	NS 386	46	8
Bangalore North	Pusa Ruby	11	0
Badakanapalya	Pusa Ruby	6	6
Chikkaballapur	Varieties and Tomato breeding lines	328	279
Shimoga	Pusa Ruby	15	0
Talagavare Chintamani	Ramya	65	62
Thimmasandra Chintamani	S-28	12	8

Thimmasandra Chintamani	S-41	16	0
Yelahanka	Rupali	20	20
Haldenahalli Anekal,	Real Shakthi	25	15
Haldenahalli Anekal,	- [a]	12	8
Chettahalli Anekal,	Real Shakthi	19	8
Chettahalli Anekal,	- [a]	14	0
Chokkandally	- [a]	6	1
Malur	- [a]	6	0
Hoskote	- [a]	6	0
Sarjapur	- [a]	6	0
Channasandra	Tomato lines	10	0
Hoskote		5	0
South Delhi	Pusa Ruby, Rashmi, Rupali & Breeding lines	170	2
		1229	688

[a] - variety unkown.

4.4. **Symptomatology**

The tomato mosaic virus disease on tomato and tobacco cv. samsun are characterised by light and dark green mottling on the leaves (Fig.5 & 6). In tomato, the leaflets of affected leaves are usually distorted, puckered and smaller than normal. Sometimes the leaflets appear pointed and become pale green. Fruits are also reduced in size. Less severely infected tomato plants produced very mild mottling symptoms on the leaves. The ToMV infected tomato plants collected during field surveys showed characteristic mottling and distortion of the top leaves.

4.5. **Purification of the virus.**

ToMV was successfully purified from systemically infected N.tabacum cv. samsun leaves 2 weeks after inoculation. The light scattering zone was observed in sucrose density gradient tubes at the distance of 5 cm from the bottom. In all the preparations about 2 cm wide light scattering zone was consistently seen at this position. This zone was highly infectious on N.tabacum cv. Samsun (10/10 plants infected) and produced 7.42 lesions/cm^2 on N.glutinosa. The purified virus had A260/A280 ratios close to 1.2.

4.5.1. **Spectrophotometry and determination of concentration of ToMV in purified preparations.**

For spectrophotometry, the final 1ml purified virus (ToMV) was serially diluted, 1:10, 1:100 and 1:1000. The u.v. absorption at 260 and 280 nm was measured. The average A260/A280 ratio was 1.2. Average yield of the virus was .80 mg/ml or 80 mg/100g of tissue (average of 8 purification).

Fig.5.ToMV on N.tabacum cv. samsun showing light and dark green mottling

Fig.6.ToMV on tomato cv. Arka Vikas

(a) showing mosaic symptoms compared with healthy leaf

(b) mosaic symptoms with pointed leaves

Fig. 5

Fig. 6 a

Fig. 6 b

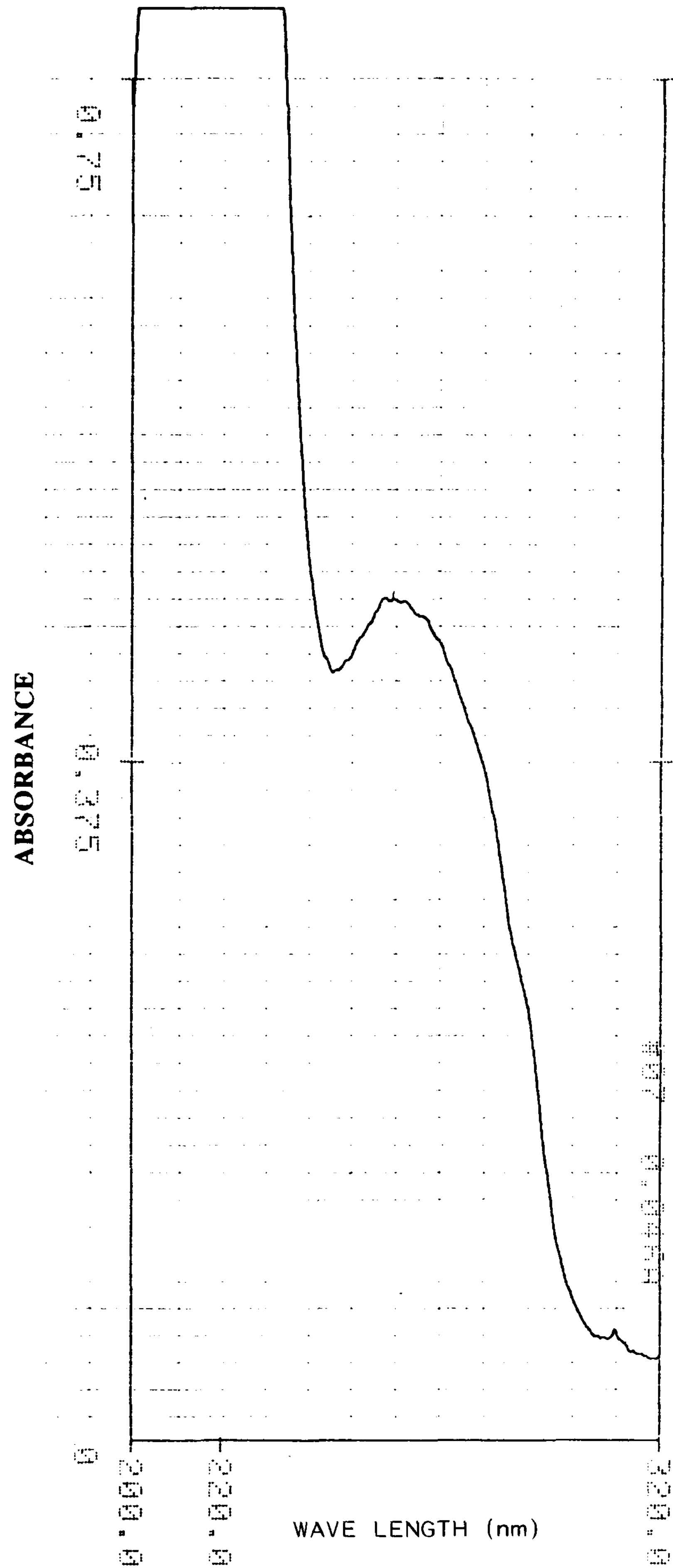


Fig. 7. Ultraviolet absorption spectrum of purified ToMV from infected N.tabacum cv. Samsun

4.6. **Electron microscopy.**

Large number of rod shaped particles of ToMV, 300nm x 18nm size were observed in the electron microscope when the final purified preparations of the virus were stained with 1 per cent aqeous uranyl acetate (Fig.8).

4.7. **SDS - polyacrylamide gel electrophoresis (SDS-PAGE) for molecular weight determination of virus protein.**

Polyacrylamide gel electrophoresis of ToMV coat protein in SDS revealed that ToMV consists of a protein of molecular weight 18,000 daltons (Fig.9). The purified virus produced a single band close to the marker, Trypsin inhibitor (mol.wt.20,100). The molecular weight value for viral coat protein was calculated from the regression lines obtained by plotting the mobility of standard proteins against the log of their molecular weight.

4.8. **Agarose gel electrophoresis for molecular weight determination of virus RNA.**

The virus RNA was scanned between 200 to 300nm using a u.v. spectrophotometer. It produced a peak at 260nm (Fig.10).

Agarose gel electrophoresis of ToMV RNA revealed that ToMV consists of a RNA which moved the same distance as Physalis mottle virus RNA which is a 6Kb RNA (Fig.11).

4.9. **Production of antiserum against ToMV.**

Antiserum was successfully produced in rabbit against ToMV by giving 3

Fig.8. Electron micrograph showing rigid rod shaped particles of ToMV in purified preparation
(a) 36000x magnification

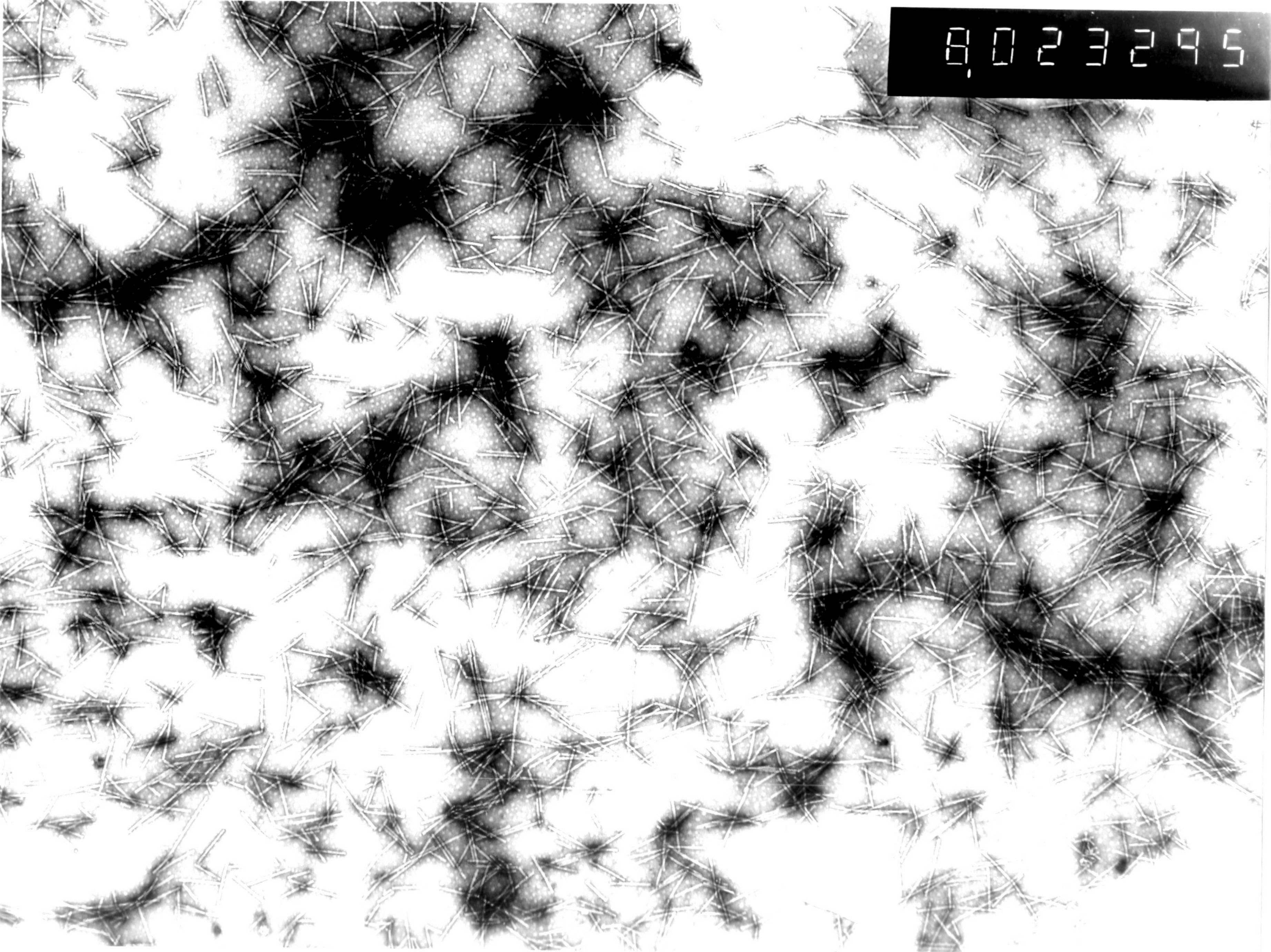


Fig. 8 a

Fig.8. Electron micrograph showing rigid rod shaped particles of ToMV in purified preparation
(b) 95000x magnification

Fig. 8 b

Fig.8. Electron micrograph showing rigid rod shaped particles of ToMV in purified preparation (c) 180000x magnification

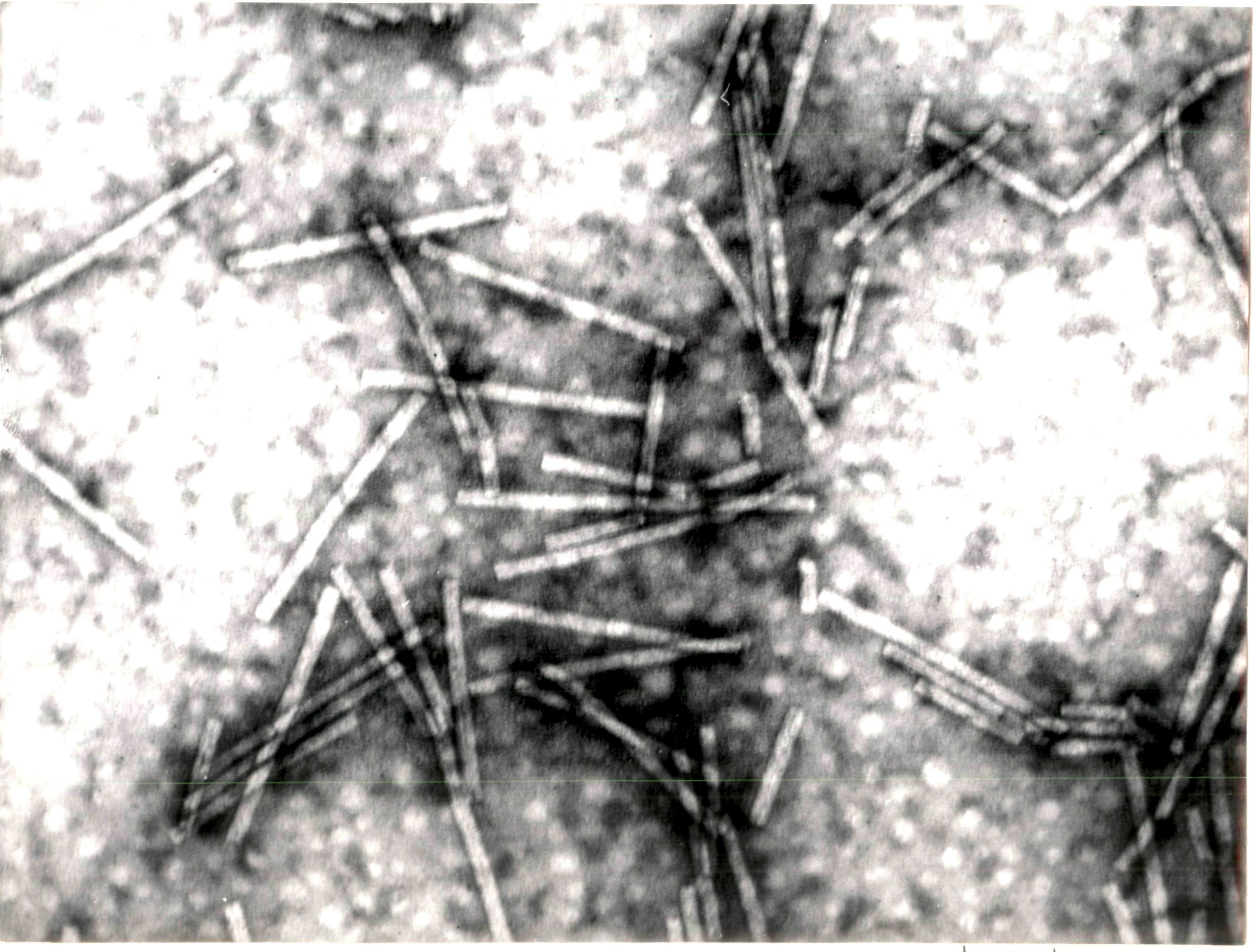


Fig. 8 C

9. Electrophoresis of ToMV coat protein in
10 per cent polyacrylamide gel stained with
coomassie brilliant blue

(a) Lane 1: Markers Type VII-L (Sigma)
Lanes 2,3,4,5: ToMV Coat Protein
2,3: 10 ul (1:100 dilution)
4: 10 ul (1:10 dilution)
5: 5 ul (1:10 dilution)

(b) Closer view of the gel

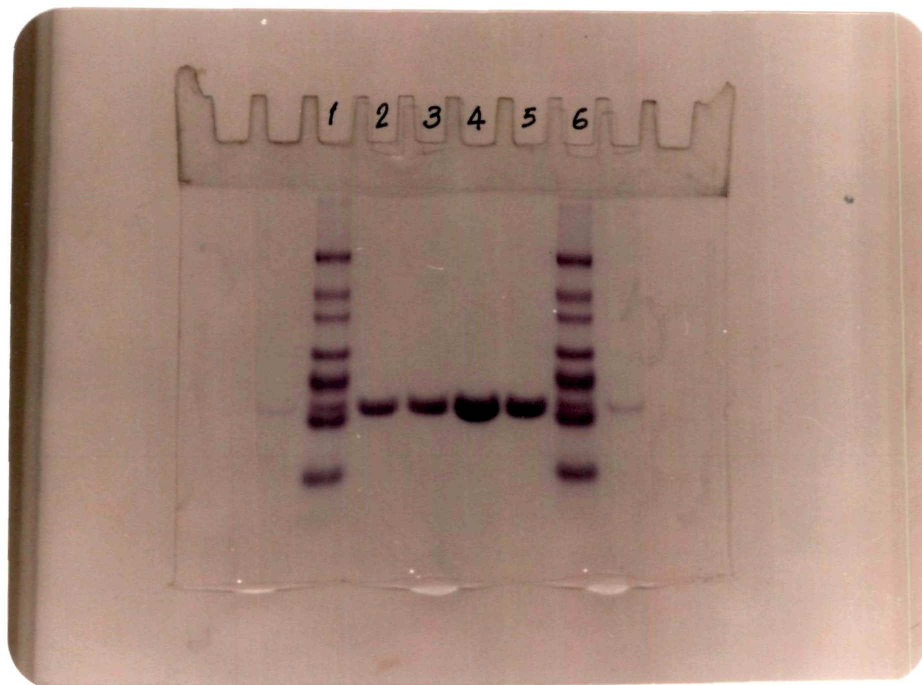


Fig. 9 a

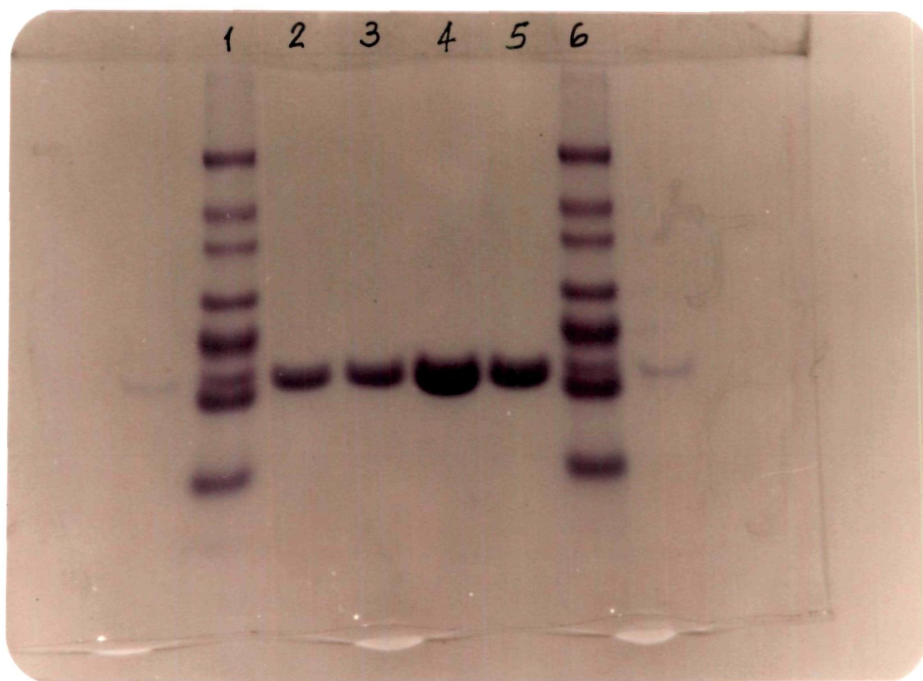


Fig. 9 b

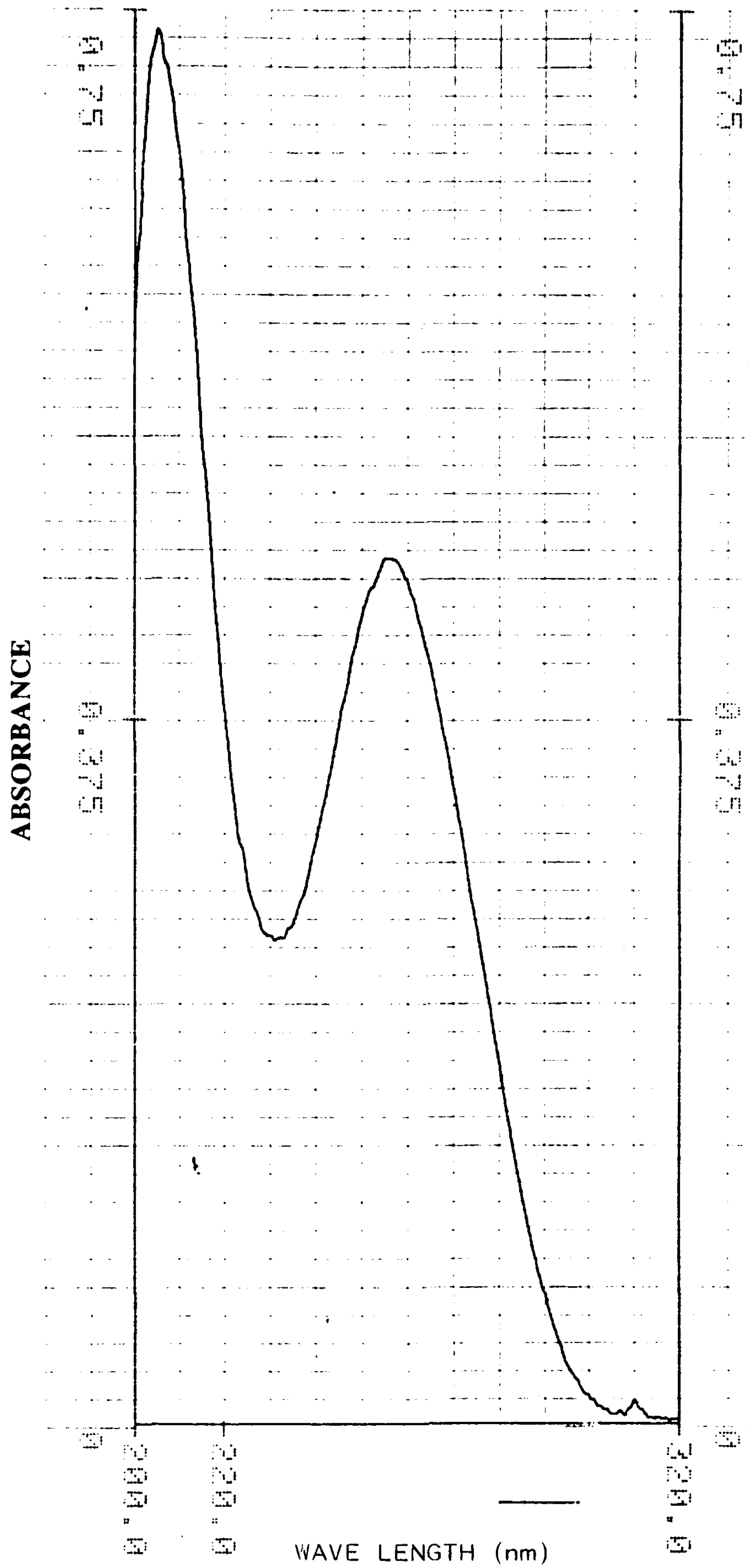


Fig. 10. Ultraviolet absorption spectrum of purified ToMV-RNA

11. Agarose gel electrophoresis of ToMV-RNA

(a) ToMV RNA run on a agarose gel

(b) ToMV RNA run with markers on the gel

Lane 1: SMV RNA (4.5 Kb)

Lane 2: ToMV RNA

Lane 3: PhMV RNA (6.2 Kb)

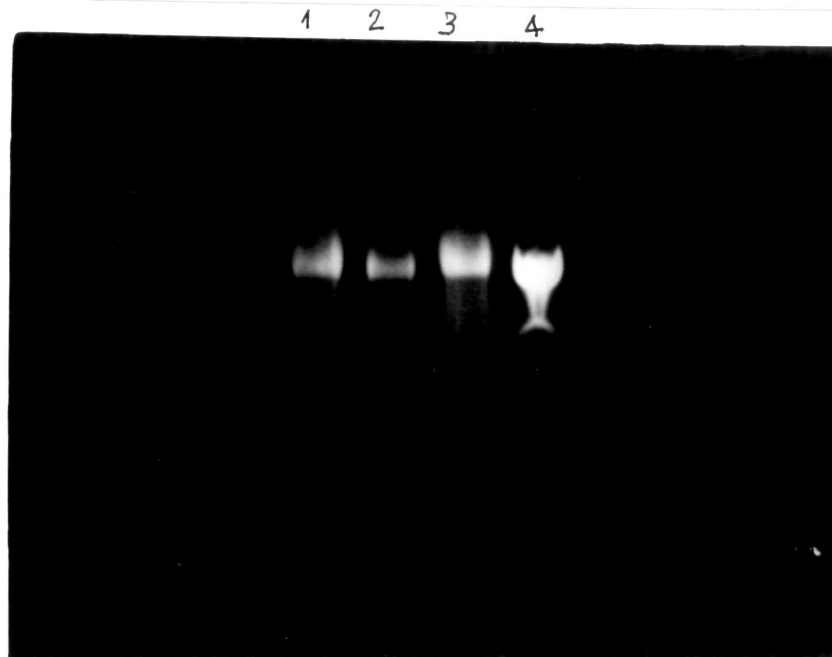


Fig. 11 a

Fig. 11 b

intramuscular and 1 intravenous injections with the purified virus. The rabbit was bled 4 times at weekly intervals starting from 1 week after the last injection. The serum was seperated by keeping blood for 2 hrs at room temperature followed by keeping at 4°C in a refrigerator overnight.

4.9.1. **Determination of titre of antisera.**

Antiserum prepared against standard purified preparation of ToMV reacted with crude extract of ToMV and purified virus to give titres of 1/8,000 and 1/32,000 in penicillinase based DAS - ELISA (Table.8a). The antiserum gave a titre of 1/4096 in gel diffusion test when tested with purified virus (ToMV).

4.10. **Purification of γ globulin and conjugation of enzyme with γ globulin**

γ glolubin was purified from the antiserum using the procedure of Clark and Adams (1977) and absorbance reading was taken in spectrophotometer at 280 nm and adjusted to 1 mg/ml (1.40D = 1mg/ml).

1 ml (=1mg) purified γ glolulin was successfully conjugated with penicillinase enzyme (1 ml = 5 mg) precipitate and using this conjugate and the homologous antiserum, ToMV was detected in ToMV infected samples of tomato and tobacco. The Conjugate gave a titre of 1/2000 when tested in penicillinase based DAS ELISA using ToMV antiserum of dilution 1:1000(Table.8b).

4.11. **Detection of ToMV in infected tomato and other hosts by using ToMV antiserum.**

4.11.1. **Ouchterlony agar gel double diffusion test.**

This test successfully detected ToMV in the purfied preparation and crude

Table 8a. Determination of titre of antisera.

Crude sap		Purified virus	
Dilution of antisera [a]	Absorbance values [b]	Dilution of antisera [a]	Absorbance values [b]
1:1000	1.680	1:1000	3.1395
1:2000	1.669	1:2000	3.0775
1:4000	1.608	1:4000	3.0035
1:8000	0.710	1:8000	3.0585
1:16,000	0.119	1:16,000	3,0175
Healthy	0.090	1:32,000	1.1715
		1:64,000	1.1365
		1:1,28,000	0.0235
		Healthy	0.192

[a] Tested in ELISA.

[b] Twice healthy is considered postive.

Table 8b. Determination of titre of conjugate.

Dilution of conjugate [a]	Absorbance values [b]
1:500	3.1165
1:1000	3.0575
1:2000	2.3065
1:4000	0.396
1:8000	0.1225
1:16,000	0.040
Healthy	0.192

[a] Tested in DAS - ELISA with antiserum dilution of 1:1000.

[b] Twice healthy is considered postive.

extract from tobacco and tomato infected with ToMV. Clear precipitation bands could be seen (Fig.12 & 13) in purified preparation while crude extracts produced faint bands within 24 hours.

4.11.2. **Enzyme linked immunosorbent assay (ELISA)**

Using ELISA, ToMV was detected in crude extracts of tobacco and tomato infected with ToMV. Purified preparations gave a very strong reaction in ELISA.

4.12. **Physical properties of ToMV**

Dilution end point

The dilution end point of ToMV was found to be between 10^{-6} and 10^{-7} (Table 9 & Fig.14).

While undiluted sap produced 7.37 and 8.32 lesions/cm^2 on N.glutinosa, the highest reacting dilution of 10^{-6} produced 0.06 and 0.11 lesions/cm^2 on the local lesion host. Dilutions 10^{-7} and 10^{-8} produced no local lesions.

Thermal inactivation point of ToMV

The thermal inactivation point of ToMV was found to be 100°C (Table.10 & Fig.14). The infectivity was completely lost only after boiling the sap at 100°C for 10 min. At 90°C, it produced 0.31 and 0.27 lesions/cm^2 in the two replications respectively.

Longevity in vitro

All three treatments namely crude sap maintained at room temperature, crude

Fig.12. Ouchterlony double Gel diffusion test

Central well `a': ToMV antiserum

wells 1: Purified ToMV undiluted 30ul + 3ul 1% SDS

2: Purified ToMV (1/10) 30ul + 3ul 1% SDS

3: Purified ToMV (1/100) 30ul + 3ul 1% SDS

4: Sap from ToMV infected tomato 30ul + 3ul 1% SDS

5: Sap from healthy tomato

6: Buffer

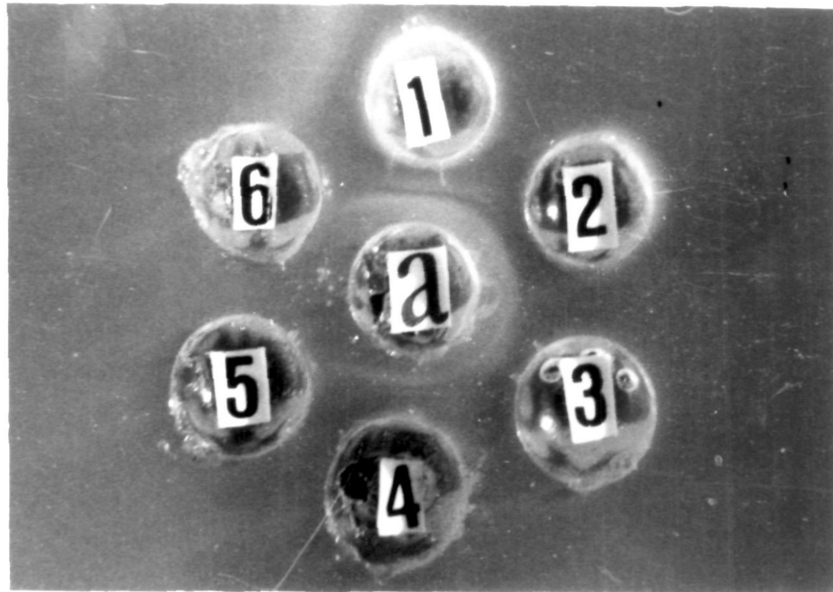


Fig. 12

Fig.13. Ouchterlony double Gel diffusion test
Central well `a': ToMV antiserum
wells 1-2: Purified ToMV undiluted 20ul + 2ul 1% SDS
3-4: Sap from healthy tomato
5-6: Buffer

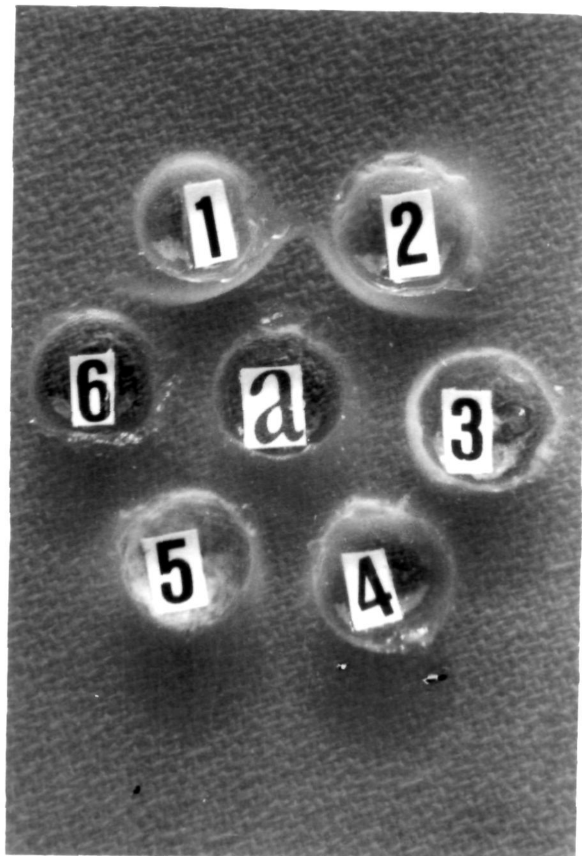


Fig. 13

Table 9: Dilution end point of ToMV

Dilution of Crude sap	*N. glutinosa* Lesions / cm^2		
	RI	RII	MEAN
Undiluted	7.37	8.32	7.85
10^{-1}	6.63	6.97	6.80
10^{-2}	5.45	5.49	5.47
10^{-3}	4.15	4.25	4.20
10^{-4}	2.30	3.10	2.70
10^{-5}	0.45	0.91	0.68
10^{-6}	0.06	0.11	0.09
10^{-7}	0	0	0
10^{-8}	0	0	0

Table 10. Thermal inactivation point of ToMV.

Temperature (°C)	N.glutinosa (Lesions/cm²)		
	RI	RII	Mean
Control	9.05	10.09	9.20
40	8.18	8.74	8.90
50	7.42	7.98	7.70
60	5.95	6.87	6.41
70	5.59	5.80	5.70
80	1.98	3.09	2.54
90	0.31	0.27	0.29
98	0	0	0

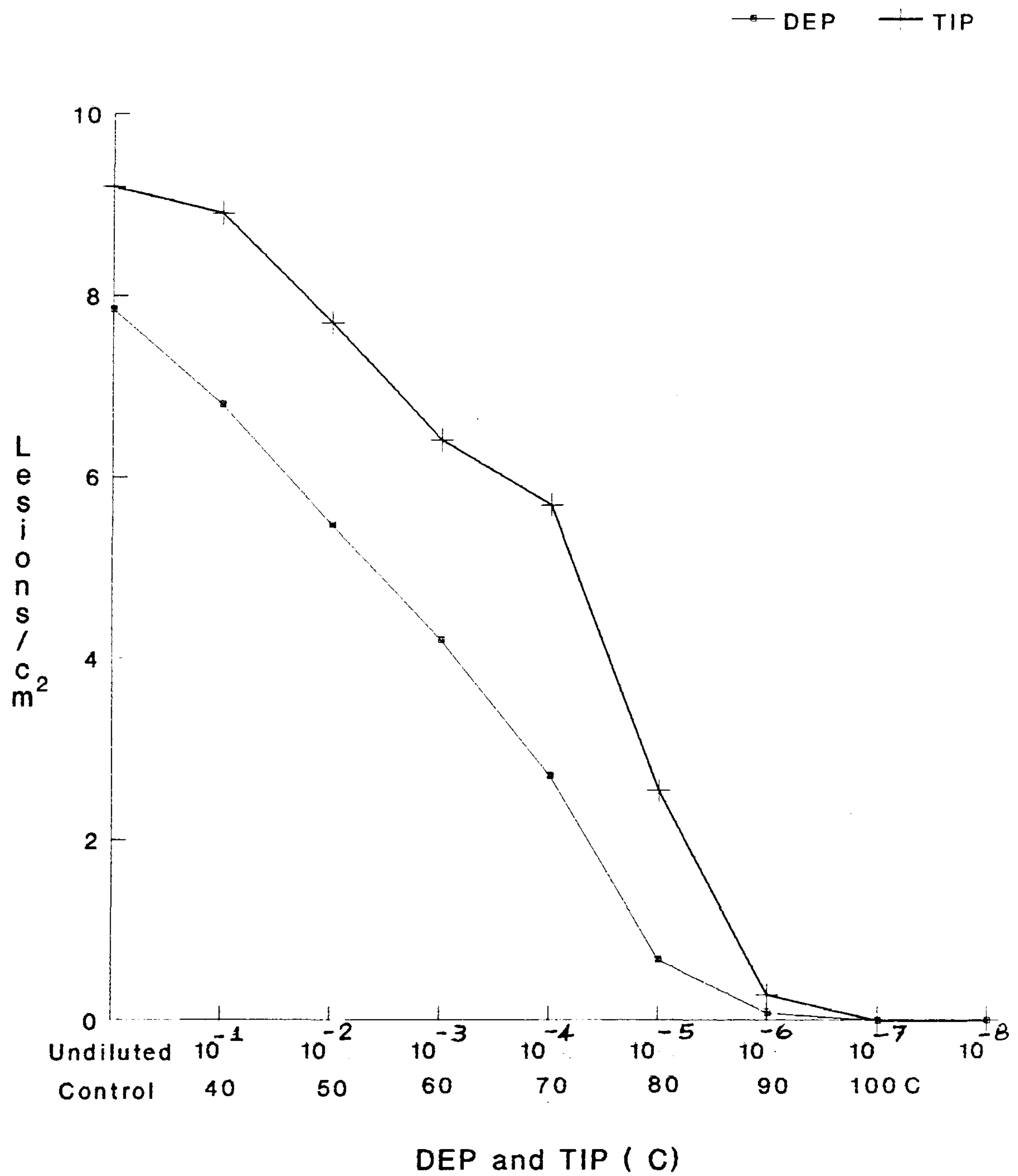


Fig 14: Graph showing dilution end point and thermal inactivation point of ToMV

sap maintained in fridge and dried leaves produced local lesions on N.glutinosa even after 539 days of ageing (Table.11 & Fig.15). At the start of the experiment crude sap at room temperature, crude sap at 5°C and dried leaves produced 10.02, 9.91 and 11.67 lesion/cm^2 respectively while 539 days later they produced 0.27, 0.38 and 0.22 lesions/cm^2 respectively.

4.13. **Determination of seed borne nature of ToMV.**

4.13.1. **Detection of ToMV in seeds.**

The data presented in Table.12 shows the presence of ToMV in seeds extracted from fruits of ToMV infected plants (both natural infection and inoculated) of cultivars Arka Vikas, F1Fiona, Pusa Ruby, Ramya and Rupali when tested both on N.glutinosa and in ELISA.

Tobacco seeds collected from N.tabacum cv. Samsun & Jayasri seedlings systematically infected by ToMV also produced local lesions on N.glutinosa and tested positive in ELISA.

4.13.2. **Growing on test to find out ToMV transmission from seed to plant.**

Seeds of cultivars Arka Vikas, F1Fiona, Pusa Ruby, Rupali and Rashmi were collected from infected tomato plants. The results indicated that 2/65 seedlings of Arka Vikas, 11/198 seedlings of F1 Fiona, 5/89 seedlings of Pusa Ruby, 10/150 seedlings of Rupali and 3/62 seedlings of Rashmi were found to be infected. This accounted for a per cent seed transmission ranging from 3 per cent in Arka Vikas to 6 per cent in Rupali (Table.13).

Table 11. Longevity in vitro of ToMV.

Treatment (Ageing in days)	LIV of crude sap[a] at room temp.	LIV of crude[a] sap at 5°C	LIV of [a] dried leaves
Control	10.33	9.91	11.67
6	9.33	9.09	9.14
10	8.59	9.14	8.24
13	8.42	8.91	7.23
23	7.11	9.33	7.13
27	6.68	9.09	7.96
37	5.95	7.62	5.78
48	5.34	8.93	3.18
63	4.18	6.47	3.48
75	2.40	5.50	2.16
87	2.18	4.37	2.19
111	1.31	3.79	1.13
135	2.12	4.01	1.26
159	2.01	3.79	1.22
189	2.18	3.55	1.13
217	1.31	2.50	0.98
226	1.18	2.14	1.02
294	0.35	0.49	0.27
345	0.28	0.34	0.16
382	0.36	0.23	0.22
418	0.44	0.26	0.32
443	0.29	0.49	0.32
468	0.29	0.39	0.32
539	0.27	0.38	0.22

[a] Lesions/cm^2 on N.glutinosa

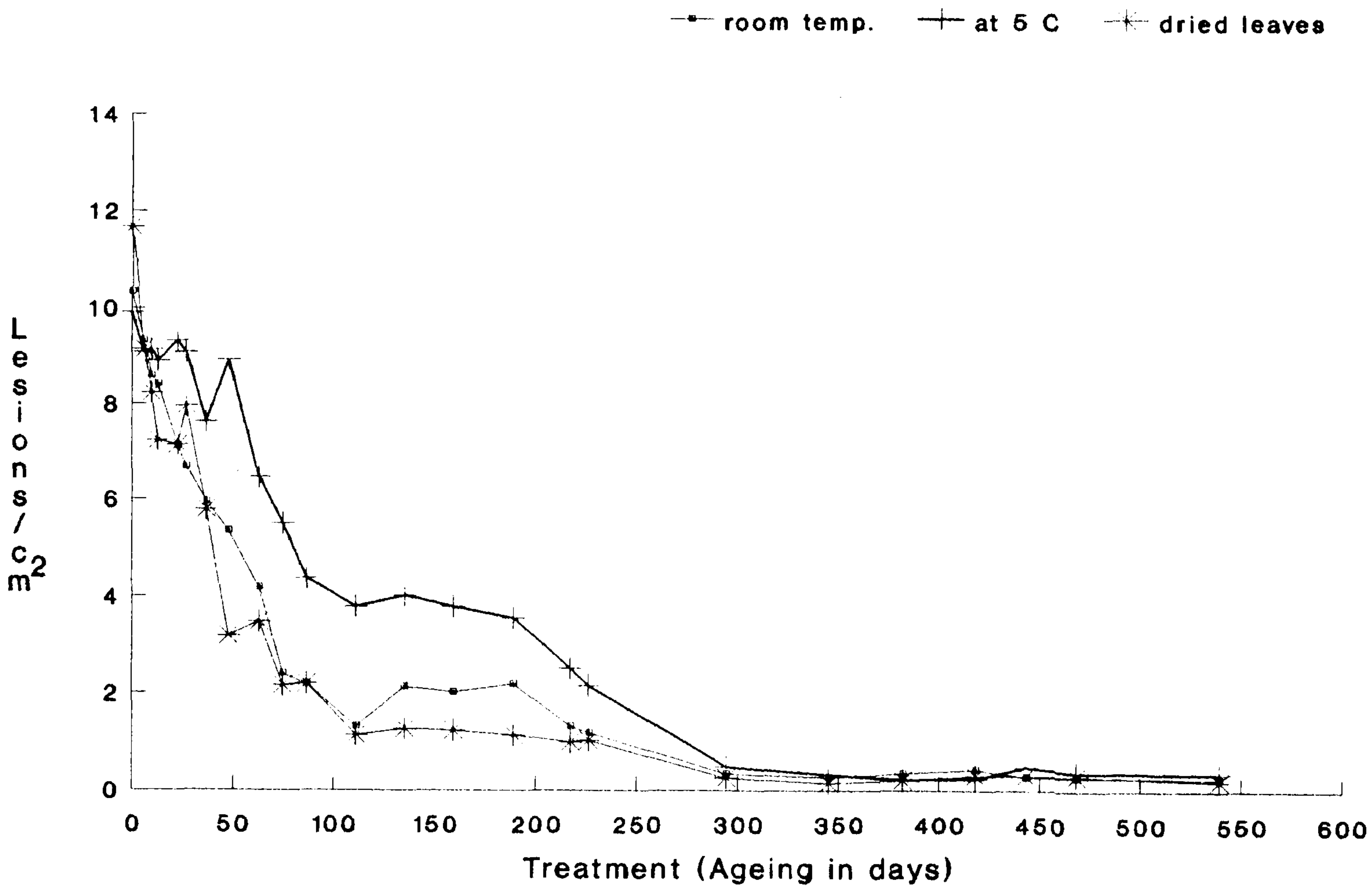


Fig. 15: Graph showing longevity in vitro of ToMV

Table 12. Determination of seed brone infection of ToMV in tomato and tobacco.

Variety /Hybrid	Place of collection	Nature of infection	Qty. of seeds tested	Local lesion assay Lesions/cm^2	Reaction in ELISA
Tomato					
Arka Vikas	Hebbal	Inoculated	100	0.20	1.130
F1 Fiona	Kolar	Natural	100	0.14	0.987
Pusa Ruby	Hebbal	Inoculated	100	0.04	0.560
Rupali	Yelahanka	Natural	100	0.20	1.150
Ramya	Chintamani	Natural	100	0.19	1.105
Tobacco					
Jayasri	Hebbal	Inoculated	0.1g	0.03	0.487
Samsun	Hebbal	Inoculated	0.1g	0.02	0.420
Xanthi	Hebbal	Inoculated	0.1g	0.05	0.565

Table 13. Detection of ToMV in nursery seedlings of tomato

Variety/[a] Hybrid	No of seeds[b] sown	No of seedlings transplanted	Local lesion assay	
			No. of seedlings infected	Percentage infection
Arka Vikas	80	65	2	3.08
F1Fiona	200	198	11	5.56
Pusa Ruby	100	89	5	5.62
Rupali	150	140	10	6.67
Rashmi	100	62	3	4.84

[a] Seeds collected from infected plants

[b] Seeds sown in sterilized soil.

4.13.3. **Detection of externally borne ToMV on tomato seeds.**

The presence of ToMV in the phosphate buffer washings and on the washed seeds (collected from infected plants) revealed the presence of ToMV on the surface of tomato seeds (Table.14). However, when a second set of the same seeds were washed in TSP, virus could be detected by ELISA in TSP washed seeds but not in and seed washings and no virus could be detected by local lesion assay in both TSP washed seeds & seed washings showing that TSP can inactivate externally borne ToMV.

4.13.4. **Effect of Tri Sodium phosphate amd other treatments on ToMV.**

Since TSP was found to eliminate ToMV (Table.15) this test was conducted to know the ability of different concentrations of TSP, Na_2Co_3 and $NaHCo_3$ to inactivate ToMV. 1g of infected samsun leaf when ground with 1 and 5ml of 10 per cent TSP and 5ml of 5 per cent TSP produced no lesions on N.glutinosa. The next effective treatment was 5ml of 1 per cent TSP followed by 5ml of 5 per cent Na_2Co_3, 5ml of 5 per cent Na_2Co_3, 1ml of 5 per cent TSP and 1ml of 1 per cent TSP which produced 0.08, 0.29, 0.32, 0.46 and 0.49 lesions/cm^2 on N.glutinosa respectively. In general, different concentrations and quantities of Na_2Co_3 were more effective in inactivating ToMV compared to $NaHCo_3$.

4.13.4.1. **Effect of TSP in eliminating ToMV from tomato seeds.**

The most effective treatment from Table.16 (10 per cent TSP) was checked for its ability to eliminate ToMV from infected tomato seeds. Infected seeds of Arka Vikas, Rupali and Pusa Ruby after soaking in 10 per cent TSP for 15 mins produced 0 lesions/cm^2 on N.glutinosa (Table.16).

4.13.5 **Detection of ToMV in tomato seed lots of different sizes.**

Results obtained from indexing seed samples (collected from infected tomato

Table 14. Detection of externally borne ToMV in tomato seeds.

Treatment	Variety/[a] Hybrid	No of seeds tested[b]	*N.glutinosa* Lesions/cm^2 Washed seeds	*N.glutinosa* Lesions/cm^2 Seed Washings	ELISA Washed seeds	ELISA Seed Washings
0.07MPO_4 buffer	F1 Fiona	50	1.63	0.07	+	+
p^H 7.0 soaked for 20min	Rupali	50	1.49	0.10	+	+
10% TSP	F1 Fiona	100	0.00	0.00	+	-
soaked	Rupali	100	0.00	0.00	+	-
20 min.	Pusa Ruby	100	0.00	0.00	+	-

[a] All seeds washed in distilled water after soaking treatments.

[b] 100 seeds crushed in 1ml buffer.

TSP = Trisodium phosphate.

Table 15. Effect of TSP and other treatments on ToMV infection.

Treatment	No.of lesions/leaf	N.glutinosa Lesions/cm^2
1. 1g infected samsun + 1% TSP 1ml	16	0.49
2. 1g infected samsun + 1% TSP 5ml	2	0.08
3. 1g infected samsun + 5% TSP 1ml	11	0.46
4. 1g infected samsun + 5% TSP 5ml	0	0
5. 1g infected samsun + 10% TSP 1ml	0	0
6. 1g infected samsun + 10% TSP 5ml	0	0
7. 1g infected samsun + 1% Na_2Co_3 1ml	52	1.86
8. 1g infected samsun + 1% Na_2Co_3 5ml	39	1.23
9. 1g infected samsun + 5% Na_2Co_3 1ml	80.	2.14
10. 1g infected samsun + 5% Na_2Co_3 5ml	8	0.29
11. 1g infected samsun + 10% Na_2Co_3 1ml	102	3.05
12. 1g infected samsun + 10% Na_2Co_3 5ml	9	0.32
13. 1g infected samsun + 1% $NaHCo_3$ 1ml	296	7.42
14. 1g infected samsun + 1% $NaHCo_3$ 5ml	301	6.54
15. 1g infected samsun + 5% $NaHCo_3$ 1ml	206	5.25
16. 1g infected samsun + 5% $NaHCo_3$ 5ml	90	1.93
17. 1g infected samsun + 5% $NaHCo_3$ 5ml	91	1.90
18. 1g infected samsun + 10% $NaHCo_3$ 5ml	25	0.42
19. 1g infected samsun + Po_4 buffer p^H7.0 1ml	252	8.69
20. 1g infected samsun + Po_4 buffer p^H7.0 5ml	299	7.92
21. 1g infected samsun + Distilled water 1ml	319	7.34
22. 1g infected samsun + Distilled water 5ml	312	6.86
23. 1g infected samsun	310	8.37

Table 16. Effect of TSP [a] in eliminating ToMV from tomato seeds.

Variety	No.of seeds tested	No.of sets tested	*N.glutinosa* Lesions/cm^2
Pusa Ruby	100	2	0
Arka Vikas	100	2	0
Rupali	100	2	0
Control[b]	100	1	1.7

[a] Soaking treatment with 10% TSP for 15 mins.

[b] 100 untreated seeds served as the check.

plants) of various sizes on N.glutinosa revealed that even a single infected seed can be seperately crushed, inoculated and virus detected both through N.glutinosa and in ELISA. Out of 20 sets of single seeds of Ramya and F1Fiona tested, virus was detected in 11 & 8 sets respectively (Table.17).

Use of 50 and 100 seeds in all the 3 varieties (Ramya, F1 Fiona & Rupali) collected from infected plants always reacted in ELISA and produced local lesions on N.glutinosa.

4.13.6. **Detection of seed borne infection of ToMV in commercial tomato seeds.**

Out of 5 sets of 100 seeds each of the commercial tomato varieties tested, ToMV was detected in all 5 sets of variety Gotya and 2 sets of the variety S-28 (Table.18) when indexed on N.glutinosa and tested in ELISA.

4.14. **Testing plant parts for the presence of ToMV.**

Leaves, petals, sepals, stalk, stem, roots and fruits of ToMV inoculated tomato plants (cv. Pusa Ruby) produced local lesions when indexed on N.glutinosa (Table.19). Even the soil in which the infected plants were grown when indexed for ToMV produced an average of 0.37 lesions/cm^2. Leaves had the highest virus concentration and produced an average of 6.59 lesions/cm^2 (average from five plants of Pusa Ruby).

4.14.1. **Detection of ToMV in different parts of tomato fruits.**

When different parts of fruits collected from infected plants were tested, ToMV could be detected in the skin, juice, pulp and seeds of all tested frutis of the varieties F1 Fiona, Pusa Ruby, Ramya, Rupali, Rashmi and Vaishali (Table.20). In each variety five fruits were tested.

Table 17. Detection of ToMV in tomato seed samples of various sizes.

Variety/ Hybrid	Sample size (No. of seeds)	No. of sets tested	No.of sets infected
Ramya	100	5	5
	50	10	10
	25	10	10
	5	10	7
	1	20	11
F1 Fiona	100	5	5
	50	10	10
	25	10	9
	5	20	7
	1	20	8
Rupali	100	5	5
	50	5	5
	25	5	4
	5	5	2
	1	5	1

Table.18: Detection of seed borne infection of ToMV in commercial tomato seeds

Variety	Source	No. of seeds tested	No of sets tested	No infected[a]
Pusa Ruby	Pro Agro seeds	100	5	0
Arka Vikas	Pro Agro seeds	100	5	0
Gotya	Mahyco Seeds	100	5	5
S-28	Mahyco Seeds	100	5	2

[a] tested both on N.glutinosa and in ELISA

Table 19. Detection of ToMV in different parts of tomato plants.

Plant part indexed	N.glutinosa Avg. Lesions/cm^2 [a]
Top leaves	6.59
Stem	0.67
Root	0.65
Flower stalk	0.57
Petals	0.29
Sepals	0.25
Upper leaf petiole	0.23
Soil	0.37

[a] Each value is the average from five plants of tomato var. Pusa Ruby.

Table 20. Detection of ToMV in different parts of tomato fruits.

Variety/ Hybrid	Place of Collection	Nature of infection	Average Lesions/cm² [a] Fruit part indexed			
			Skin	Juice	Pulp	Seed
F1 Fiona	Kolar	Natural	0.56	0.14	0.22	0.14
Pusa Ruby	Huskur Gate	Natural	0.61	0.38	0.87	NT[b]
Ramya	Chintamani	Natural	2.04	0.36	0.87	0.19
Rupali	Yelahanka	Natural	0.95	0.89	1.44	0.20
Rashmi	Hebbal	Inoculated	2.54	1.66	1.09	NT
Pusa Ruby	Hebbal	Inoculated	0.60	0.48	0.09	0.04
Vaishali	Hebbal	Inoculated	0.88	1.58	1.68	NT

[a] Each value is the average from five fruits in a variety

[b] Not tested.

4.15. **Host range**

The results of the host range studies are presented in Table.21. Out of 80 plant species inoculated, 18 species took ToMV infection. The infected species were Capsicum annuum var. G-4; Capsicum annuum var. California wonder; Chenopodium amaranticolor; Chenopodium murale; Datura metel; Datura stramonium; Nicotiana benthamiana; N.glutinosa; N.rustica; N.sylvestris; N.tabacum var. CTRI special; N.tabacum var. Jayasri; N.tabacum var. L1158; N.tabacum var. Riwaka-1; N.tabacum var. samsun; N.tabacum var. white burley; N.tabacum var. Xanthi; Physalis floridana; Solanum tuberosum (Fig.16-33).

4.15.1. **Indexing different host plants showing various mosaic like symptoms in the field for the presence of ToMV**

Out of 409 samples of 34 plant species showing different mosaic symptoms tested, ToMV was detected in 6/11 tobacco samples from Shimoga; 8/68 samples of Chilli from Kolar; and 2 out of 3 leaf samples of Chilli from Kolar (Table.22).

4.16. **Indexing of field samples of tomato**

Out of 267 tomato samples from Bangalore South, 226 produced local lesions on N.glutinosa and tested positive in ELISA (Table.23).

None of the samples of the varieties; Pusa Ruby; S-41, (Thimmasandra, Chintamani) were infected with ToMV. Tomato samples collected from two fields in Anekal fields in Hoskote and a field each in Sarjapur & Channasandra were found to be healthy (Table.6). 4 of 5 samples of the variety Gotya from Kolar, 8/46 samples of NS386, 6/6 samples of Pusa Ruby from Badakanapalya were infected with ToMV. Of 328 varieties and tomato breeding lines from Nath seeds (Chikkaballapur), 279 were infected with ToMV (Table.24). Of 170 tomato samples from IAHS farm, 129 were infected with TLCV but only two of them indexed positive for ToMV (Table.25). From the remaining fields indexed, 16/41 tomato samples of F1 Fiona (Kolar), 1/36 samples

Table.21: Host range of ToMV

Test plants	Common Name	Cultivar	Plants infected Plants inoculated	Symptom description	N.glutinosa Lesions/cm^2	ELISA
Abelmoschus esculentus	Bhendi	P.K.	0/20	-	0	-
Abutilon	-	-	0/20	-	0	
Acalypha indica	-	-	0/20	-	0	-
Ageratum conyzoides	-	-	0/20	-	0	-
Althaea rosea	Hollyhock	-	0/20	-	0	-
Amaranthus spp.	Amaranthus	R-104(Suwarna)	0/20	-	0	-
	Amaranthus	Sabbaggi	0/20	-	0	-
Arachis hypogaea	Groundnut	ICGS-76	0/20	-	0	-
		K-134	0/20	-	0	-
Balsam impatiens	Balsam	-	0/20	-	0	-
Brassica nigra	Mustard	Bhavani	0/20	-	0	-
Brassica oleracea	Knol Khol		0/20	-	0	-
Cajanus cajan	Redgram	TTB 7	0/20	-	0	-
Capsicum annuum	Chilli	G-4	10/20	Systemic	0.08	+
Capsicum annuum	Capsicum	California wonder	20/20	Necrotic Local lesions	0.09	+
Catheranthus roseus	Periwinkle	-	0/10	-	0	-
Celosia cristata	Cocks Comb	-	0/20	-	0	-
	Celosia	-	0/20	-	0	-
Chenopodium amaranticolor	Chenopodium	-	20/20	Chlorotic local lesion	0.84	+
Chenopodium murale	Chenopodium	-	10/20	Systemic	0.08	+
Cicer arietinum	Bengal gram	Annigeri Bheema	0/20	-	0	-
Citrullus vulgaris	Water melon	Peta Negra	0/20	-	0	-
		Sugar baby	0/20	-	0	-
Croton bonplandianum	Weed	-	0/20	-	0	-
Crotolaria juncea	Sunnhemp	-	0/20	-	0	-
Cucurbita moschata	Pumpkin	Poinsett Calypso Mamoth	0/20	-	0	-
Cucumis sativus	Cucumber	Green long	0/20	-	0	-

Cyamopsis tetragonaloba	Cluster bean	-	0/20	-	0
Datura metel	Datura	-	20/20	Necrotic Local lesions	-
Datura stramonium	Datura	-	10/10	Necrotic Local lesions	-
Daucaus carota	Carrot		0/20	-	0
Delonix regia	Gulmohar	-	0/20	-	0
Dolichos lablab	Field bean	HA 3	0/20	-	0
Echinochloa frumentacea	Barnyard millet	VL-29	0/20	-	0
		K-1	0/20	-	0
Eleusine coracana	Ragi	PR-202	0/20	-	0
		HR-9 11	0/20	-	0
		Indaf-5	0/20	-	0
		HRC 505-2	0/20	-	0
		Indaf-8	0/20	-	0
Eclipta alba	-	-	0/20	-	0
Euphorbia geniculala	Weed	-	0/20	-	0
Gomphrena globosa	Bachelors button	-	10/10	lesions	0[a]
Glycine max	Soybean	-	0/20	-	0
Gossypium barbadense	Cotton	-	0/20	-	0
Guizotia abyssinica	Niger	Niger-66	0/20	-	0
Gypsophila sp.	Gypsophila	-	0/20	-	0
Helianthus annus	Sunflower	Modern	0/20	-	0
		BSH-1	0/20	-	0
		KBSH-1	0/20	-	0
		Hybrid	0/20	-	0
Linaria sp.	Linaria mixed	-	0/20	-	0
Luffa acutangula	Ridge gourd		0/20	-	0
Manihot esculenta	Cassava	M-4	0/10	-	0
Malvastrum coramandalianum	-	-	0/20	-	0
Momordica charantia	Bitter Gourd		0/20	-	0
Nicotiana benthamiana	Tobacco	-	6/10	Systemic	3.2
Nicotiana glutinosa	Tobacco	-	-	Necrotic local lesion	0[a]
Nicotiana rustica	Tobacco	-	-	-	0
Nicotiana tabacum	Tobacco	Samsun	50/50	Systemic	8.45
		Jayasri	50/50	Systemic	7.85
		Xanthi	50/50	Systemic	7.67

Nicotiana tabacum	Tobacco	White burley		Necrotic local lesion	0*	+
		Riwaka-1		Necrotic local lesion	0*	+
		L1158		Necrotic local lesion	0*	+
		CTRI Special		Necrotic local lesion	0*	+
Panicum miliaceum	Proso millet	SVL-20	0/20	-	0	-
		GPUP-10	0/20	-	0	-
Panicum miliare	Little millet	Co-2	0/20	-	0	-
		Bau-4	0/20	-	0	-
Parthenium hysterophorus	Weed	-	0/20	-	0	-
Paspalum sorbiculatum	Kodo millet	GPUK-3	0/20	-	0	-
Phaseolus lunatus	Lima bean	-	0/20	-	0	-
Phaseolus calcaratus	Rice bean	-	0/20	-	0	-
Phaseolus vulgaris	French bean	S-9	0/20	-	0	-
		Top Crop	0/20	-	0	-
Physalis floridana	-	-	20/20	Systemic	3.89	+
Pisum sativum	Peas	-	0/20	-	0	-
Portuloca sp.	Portuloca	-	0/20	-	0	-
Raphanus sativus	Radish		0/20	-	0	-
Ricinus communis	Castor	Aruna	0/20	-	0	-
Sesamum indicum	Seasmum	-	0/20	-	0	-
Setaria italica	Navanae	Arjuna	0/20	-	0	-
		SIA326	0/20	-	0	-
Solanum melongena	Brinjal	-	0/20	-	0	-
Solanum nigrum	-	-	0/20	-	0	-
Solanum tuberosum	Potato	-	3/10	Symptom less carrier	0.32	+
Trichosanthes anguina	Snake Gourd		0/20	-	0	-
Trigonella foenum - graecum	Menthi	-	0/20	-	0	-
Vigna aureus	Greengram	LGG461	0/20	-	0	-
		PS16	0/20	-	0	-
Vigna mungo	Blackgram	T-9	0/20	-	0	-
		K3	0/20	-	0	-

Vigna unguiculata	Cowpea	KBC 1	0/20	-	0	-
		TVX944-02E	0/20	-	0	-
		C-152	0/20	-	0	-
Zea mays	Maize	-	0/20	-	0	-
Zinnia elegans	Zinnia	-	0/20	-	0	-

* Though these plants showed necrotic local lesions, when indexed on N.glutinosa no lesions were produced.

Fig.16. Mild mosaic symptoms produced by ToMV on Capsicum annuum cv. G-4

Fig.17. Local lesions produced by ToMV on Capsicum annuum cv. california wonder

Fig.18. Chlorotic local lesions produced by ToMV on Chenopodium amaranticolor

Fig. 16

Fig. 17

Fig. 18

Fig.19. Necrotic local lesions produced by ToMV on _Datura metel_

Fig.20. ToMV on _Datura stramonium_ showing necrotic local lesions

(a) Small circular spots

(b) Enlarged spots

Fig. 19

Fig. 20 a

Fig. 20 b

Fig.21. Chlorotic local lesions produced by ToMV on *Gomphrena globosa*

Fig. 21

Fig. 21

Fig.22. Necrotic local lesions produced by ToMV on *N.glutinosa*

Fig.23. Necrotic local lesions produced by ToMV on *N.rustica*

Fig.24. Necrotic local lesions produced by ToMV on *N.sylvestris*

Fig. 22

Fig. 23

Fig. 24

25. Necrotic local lesions produced by ToMV on *N.tabacum* var. CTRI special

(a) Size of lesions 24 hrs after inoculation

(b) Size of lesions 48 hrs after inoculation

Fig. 25 a

Fig. 25 b

Fig.26. Necrotic local lesions produced by ToMV on *N.tabacum* var. L1158

Fig.27. Necrotic local lesions produced by ToMV on *N.tabacum* var. Riwaka-1

Fig.28. Necrotic local lesions produced by ToMV on *N.tabacum* var. white burley

Fig. 26

Fig. 27

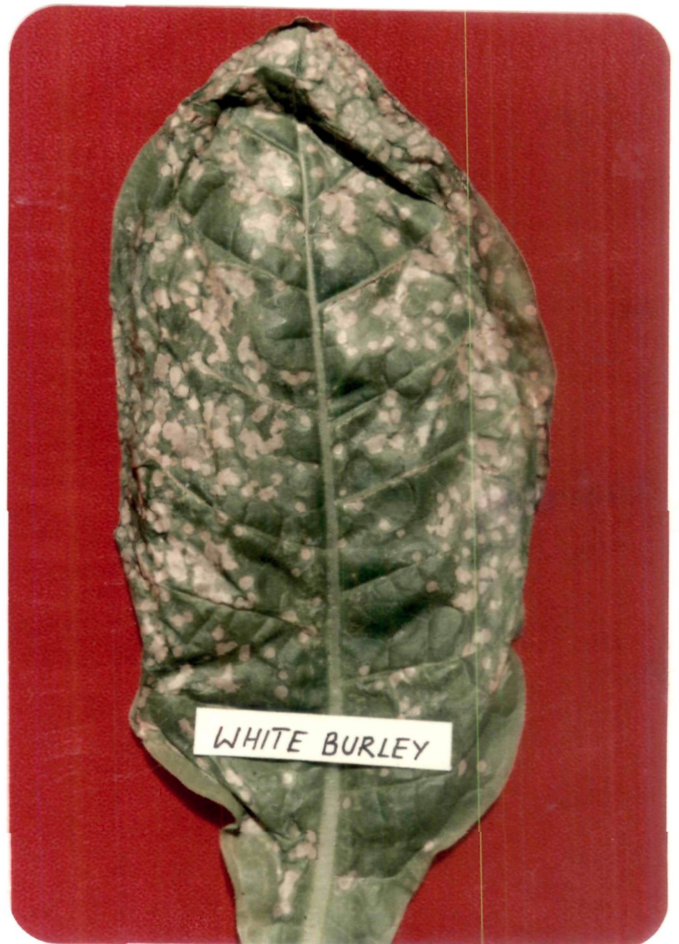


Fig. 28

29. Systemic infection produced by ToMV on
N.benthamiana

30. Systemic infection produced by ToMV on
N.tabacum var. Jayasri

(a) Green blisters

(b) Malformed leaf

Fig. 29

Fig. 30 b

Fig. 30 a

Fig.31. Systemic infection produced by ToMV on *N.tabacum* var. samsun

Fig.32. Systemic infection produced by ToMV on *N.tabacum* var. xanthi

Fig.33. Systemic infection produced by ToMV on *Physalis floridana*

Fig. 31

Fig. 32

Fig. 33

Table 22. Indexing of different host plants showing mosaic like symptoms for the presence of ToMV.

Place of collection	Host plant	No. of samples tested	No. of samples infected
Hebbal	Chilli	253	·0
MRS, Hebbal	Crop plants & weeds	26	0
	Pigeon pea		
	Cassava		
	Malvastrum		
	French bean		
	Sunnhemp		
	Broadbean		
	Cassia sp.		
	Groundnut		
	Chilli		
	Cowpea		
	Lucerne		
	Burlaria		
	Crossandra		
	Amorphaphallus		
	Amaranthus		
	Sunflower		
	Maize		
	Euphorbia		
	other weeds		
Hoskote	Papaya	2	0
	Chilli		
Chettyhalli	Cucurbit	1	0

Kanmangala	Chow chow	2	0
Devanahalli	Papaya	2	0
	Sunnhemp	3	0
	Solanum nigrum	1	0
	Crossandra	1	0
Netherlands	Ficus	2	0
	Cattleya orchid		
Hebbal	Weed	1	0
Shimoga	Tobacco	11	6
Anekal Tq.	Chilli	15	0
Anekal Tq.	French bean	10	0
Anekal Tq.	Marigold	5	0
Chintamani, Kolar Dist.	Water Melon	5	0
Kolar	Chilli	68	8
UAS, GKVK	Sunnhemp	3	2

[a] Tested on *N.glutinosa* and in ELISA

Table 23: Testing of tomato samples for tomato mosaic virus infection

Sl.No.	Breeding line[a]	No. of plants tested	No. of plants infected
1.	A22	12	12
2.	C36	13	8
3.	K6	26	18
4.	K21	22	22
5.	K31	22	22
6.	K32	23	19
7.	K33	22	7
8.	K34	25	25
9.	K35	24	21
10.	K36	24	19
11.	K37	23	22
12.	K38	31	31
		267	226

[a] Tomato leaf samples from experimental farm, IAHS, Chennasandra, Bangalore South.

Table 24. Indexing of tomato samples from Chikkaballapur for ToMV infection

Variety/Breeding line	No.of plants tested	No.of samples infected with ToMV
Varieties		
Avinash	5	5
Cross B	5	5
Gaurav	5	5
LIHB-230	5	5
Namdhari 893	5	5
Namdhari 1096	5	5
Naveen	7	4
Navodaya	6	6
Rashmi	5	5
Rasika	5	5
Rad Glove	5	5
Rupali	5	5
Sadabahar	5	5
Vaishali	5	5
Breeding Lines		
NTH10	9	4
NTH11	2	0
NTH63	12	12
NTH64	5	5
NTH65	5	5
NTH68	5	5
NTH76	5	5
NTH80	5	5

NTH84	6	6
NTH88	5	5
NTH89	5	5
NTH94	5	5
NTH95	5	5
NTH98	5	5
NTH146	5	5
NTH147	5	5
NTH224	5	5
NTH226	5	5
NTH236	6	6
NTH237	3	1
NTH238	6	6
NTH239	6	6
NTH241	3	3
NTH243	5	5
NTH244	5	5
NTH245	9	4
NTH246	2	2
NTH248	5	5
NTH249	4	4
NTH250	4	4
NTH251	3	3
NTH255	5	5
NTH272	6	6
NTH273	5	1
NTH274	5	3
NTH275	5	2
NTH277	5	0
NTH278	5	5
NTH280	3	2
NTH281	5	5
NTH283	5	0
NTH284	5	0
NTH285	6	6

NTH286	5	3
NTH287	6	6
NTH288	5	0
NTH289	5	5
NTH291	5	1
NTH292	5	3
NTH295	7	6
NTH296	4	2
NTH297	6	5
NTH298	5	5
NTH299	5	1
NTH300	5	5
NTH301	5	5
NTH302	5	5
NTH303	5	5
NA110	5	5
NA293	4	2
NA301	5	5
NA342	4	4
NA501	5	5
NA601	6	6
NA701	5	5
NA901	5	4
	328	279

Table 25. Testing of Tomato samples for TLCV and TOMV

Tomato line	No of samples	No of samples infected with	
		TLCV[a]	ToMV[b]
Pusa Ruby R_1	16	16	0
Pusa Ruby R_2	17	17	0
Rashmi	18	18	0
Rupali	19	19	0
TMH 9410	17	14	0
TMH 9414	19	6	0
TMH 9423	19	4	0
TMH 9429	16	16	0
TMH 94103	17	9	0
Tomato lines	12	9	2
	170	129	2

[a] based on symptomatology

[b] indexed on N.glutinosa

of Rashmi (Kolar), 62/65 samples of Ramya (Chintamani), 20/20 samples of Rupali (Yelahanka), 5/25 and 8/19 samples of Real Shakti from two fields in Anekal were infected with ToMV when tested in ELISA and indexed on N.glutinosa.

4.17. **Varietal Screeening**

Fifty five tomato hybrids, cultivars and breeding lines and eighteen accessions belonging to Lycopersion cheesmanii L.glandulosum; L.hirsutum; L.peruvianum and L.pimpinellifolium, were evaluated for resistance against tomato mosaic tobamovirus (ToMV) (Tables.26 to 29). Of the 41 Indian tomato cultivars and hybrids screened against ToMV, none were found resistant when indexed on N.glutinosa and tested in ELISA. 8 of these cultivars were highly susceptible and produced between 16-25 lesions/sq.cm. (Fig.34-37) while 26 of the tomato lines produced between 6-15 lesions/cm^2. Entries NS-167, S-18, IAHS88-2, IAHS88-1, NS-1094, Hissar Anmol, NS386,Abha and Alok produced less than 5 lesions/cm^2. ToMV was detected in all the cultivars when tested using ELISA (Table.26).

Of the 15 F1 tomato hybrids and crosses tested, F1 Top 21, F1 Tyking and Pimhertylc 91 recorded less than 2 lesions/cm^2 on N.glutinosa (Table.27). Of the Lycopersicon species, the accession CMV Sel. INRA (L.peruvianum) was resistant to ToMV. It produced 0 lesions/cm^2 on N.glutinosa (Table.28). When these 18 accessions of Lycopersicon were tested in penicillinase ELISA and the results analysed by obtaining the absolute absorbance values by subtracting the average OD values of duplicate wells from the buffer average, it was found that only CMV Sel. INRA gave a negative reaction in ELISA also (Table.29).

4.17.1. **Grafting of Lycopersicon esculentum cv. Arka Vikas on CMV Sel. INRA (L.peruvianum).**

Of 200 grafts made using inoculated CMV Sel. INRA and infected Arka Vikas only 15 of them established successfully (Table.30; Fig.38 & 39).

Table 26: Performance of Indian tomato cultivars and hybrids to Tomato Mosaic Virus (ToMV)

Cultivar/ Hybrid	Source of Seeds	N.glutinosa Lesions/cm^2	ELISA
Vaishali	IAHS	22.41	2.040
PSCL - 3	Pro Agro Seeds	18.29	1.980
Rajani	IAHS	18.12	1.885
Pusa Ruby	IIHR	17.23	1.678
S - 37	Mahyco Seeds	17.14	1.667
S - 68	Mahyco Seeds	16.96	1.573
Arka Vikas	IIHR	16.37	1.575
Naveen	IAHS	16.23	1.581
Maharaja	National Agro Seeds	15.85	1.610
S - 22	Mahyco Seeds	15.69	1.595
S - 25	Mahyco Seeds	15.45	1.578
S - 28	Mahyco Seeds	15.22	1.523
Paramamitra	Heritage Seeds	15.16	1.519
Krishna	Sungrow Seeds	14.91	1.509
PSCL - 4	Pro Agro Seeds	14.86	1.510
NS 815	Namdhari Seeds	14.83	1.507
PSCL - 2	Pro Agro Seeds	14.38	1.510
Rupali	IAHS	14.14	1.503
S - 16	Mahyco Seeds	14.01	1.498
S - 41	Mahyco Seeds	13.86	1.492
Maitri	Century Seeds	13.53	1.489
PSCL - 1	Pro Agro Seeds	13.47	1.482
Nath Amruth	Nath Seeds	13.15	1.479
S - 72	Mahyco Seeds	12.63	1.461
Rashmi	IAHS	12.28	1.452
Lerica	Sandoz Seeds	12.24	1.398
S - 29	Mahyco Seeds	12.20	1.450
NS 893	Namdhari Seeds	11.66	1.419
Karnataka	IAHS	11.48	1.426
Sheetal	IAHS	11.30	1.410
Mangala	IAHS	10.96	1.400
IAHS 88-3	IAHS	10.79	1.395
NS 167	Namdhari Seeds	9.74	1.393

S - 18	Mahyco Seeds	6.22	1.278
IAHS 88-2	IAHS	4.39	1.198
IAHS 88-1	IAHS	2.33	1.096
NS 1094	Namdhari Seeds	2.09	0.968
Hissar Anmol	India	1.14	0.960
NS 386	Namdhari Seeds	0.88	0.686
Abha	IIHR	0.64	0.592
Alok	IIHR	0.52	0.610

34. Tomato cv. Vaishali, showing mosaic symptoms induced by ToMV

35. Tomato cv. Rupali, showing mosaic symptoms induced by ToMV

36. Tomato cv. NS-815, showing mosaic symptoms induced by ToMV

37. Tomato cv. NS-893, showing mosaic symptoms induced by ToMV

Fig. 34

Fig. 35

Fig. 36

Fig. 37

Table 27. Reaction of F_1 tomato hybrids and crosses to ToMV.

Hybrid/Line	Origin	*N.glutinosa* Lesions/cm^2	ELISA
Progress No.1	France	8.91	1.328
F_2 Fiona	France	6.49	1.310
Duotylc 90	France	6.41	1.305
$R_1$24 - 1- 2	UAS, B'Lore	5.95	1.198
F_1 Fiona	France	5.84	1.192
Pertylc 91	France	5.51	1.095
Lignon C_{20}-5-M_1	France	5.34	1.009
Octolyc 90	France	4.63	0.960
Pimpertylc 93	France	4.57	0.927
$R_1$21/6/1	UAS, B'Lore	3.82	0.860
F_1 Jackal	France	3.78	0.780
F_1 Top 21	France	1.89	0.740
F_1 Tyking	France	1.28	0.795
Pimpertylc 91	France	0.72	0.500

Table 28. Reaction of Lycopersicon species to ToMV.

Species	No.of Plants Indexed/ No.of Plants Inoculated	Avg.Lesions/ cm^2
L.hirusutum		
LA 1777	10/20	0.88(49)
PI 390658	10/20	2.22(111)
PI 390659	10/18	4.52(272)
L.pimpinellifolium		
874	10/20	3.75(146)
974	10/20	4.84(320)
874/SS	10/20	2.32(114)
hirsute	10/20	4.74(191)
LA121	10/20	2.82(124)
LA1335	10/20	1.83(137)
LA1478	10/20	8.32(471)
LA1582	10/20	3.65(200)
V-14	10/20	4.27(212)
V-13-MSO-1	10/20	3.61(156)
L.cheesmanii		
LA1401	10/20	8.19(430)
L.peruvianum		
CMV Sel. INRA	20/40	0(0)
PI 127830	10/20	3.22(213)
L.esculentum		
PI 367956	10/20	1.98(141)

Table 29. Testing Lycopersicon species against ToMV in ELISA.

Species	O.D.	Absoulute Absorbance value [a]	Reaction
L.hirsutum			
LA1777	1.477	0.545	+
PI 390658	1.184	0.838	+
PI 390659	1.241	0.782	+
L.pimpinellifolium			
874	0.550	1.472	+
974	0.401	1.621	+
874/SS	0.286	1.736	+
hirsute	0.691	1.331	+
LA121	0.301	1.721	+
LA1478	0.379	1.643	+
LA1582	0.294	1.728	+
V-14	0.541	1.481	+
L.peruvianum			
CMV Sel. INRA	2.000	0.022	-
PI 127830	0.229	1.793	+
L.esculentum			
PI 367956	1.058	0.965	+
Healthy	1.845	0.177	+

[a] Absolute Absorbance values were obtained after deducting the absorbance value of Buffer control (2.0223).

Table.30: Grafting infected Arka Vikas (L.esculentum) on L.peruvianum CMV Sel. INRA

Stock	Scion	No. of Grafts made	Type of Grafting	No. of grafts established
CMV Sel. INRA	Arka Vikas	50	Top grafting	11
CMV Sel. INRA	Arka Vikas	50	Side grafting	3
Arka Vikas	CMV Sel. INRA	50	Top grafting	1
CMV Sel. INRA	Arka Vikas	50	Side grafting	0

Fig.38. Established graft of CMV Sel. INRA (L.peruvianum) on stock of ToMV infected Arka Vikas

Fig.39. Established graft of CMV Sel. INRA (stock) with infected Arka Vikas as scion

Fig.40. CMV Sel. INRA (L.peruvianum) planted in the field following sap inoculation

Fig. 38

Fig. 39

Fig. 40

The stock and scion of these established grafts were indexed on N.glutinosa and tested using ELISA 6 weeks after grafting. Arka Vikas as stock or scion produced local lesions on N.glutinosa but CMV Sel. INRA never took ToMV infection (Table.31).

4.17.2. **Testing CMV Sel. INRA (L.peruvianum) under field conditions.**

Hundred plants of CMV Sel. INRA were raised in polythene covers, sap inoculated with ToMV two times and then planted out in the field. None of the hundred plants produced any lesions on N.glutinosa and ToMV could not be detected using ELISA in these plants even 2 months after planting out in the field (Fig.40).

4.18. **Interactive effects of TLCV and ToMV in tomato plants carrying their mixed infection.**

Seven plants of tomato cultivar Arka Vikas were included in each treatment.

4.18.1. **Days for symptom production.**

The data regarding days taken for symptom production has shown that tomato seedlings have taken infection at all stages of their growth (Table.32). However, as the age of the crop increases, the incubation period also increased in the seedlings.

4.18.2. **Plant height**: The height of the tomato plants were recorded in cms. It was 19.35, 41.28, 87.21 and 121.51 cms, 30, 45, 60 and 95 days after sowing (DAS) in the control plants. Plants inoculated with TLCV and ToMV simultaneously recorded the lowest height on all the observation dates (39cms. after 95 days of sowing). The difference in height between plants of the other treatments varied only in a range of 1-3cms, 30 DAS. 95 DAS, it was found that the maximum height was in control plants (121.51cms)

Table 31. Reaction of CMV Sel INRA (L.peruvianum) grafts to tomato mosaic tobamovirus.

Graft No.	Stock	Scion	N.glutinosa Lesions/cm^2	
			INRA [a,b]	Arka Vikas [a]
1.	Arka Vikas	INRA	0	2.86
2.	INRA	Arka Vikas	0	2.32
3.	INRA	Arka Vikas	0	2.94
4.	INRA	Arka Vikas	0	2.46
5.	INRA	Arka Vikas	0	2.83
6.	INRA	Arka Vikas	0	2.22
7.	INRA	Arka Vikas	0	2.35
8.	INRA	Arka Vikas	0	3.09
9.	INRA	Arka Vikas	0	3.11
10.	INRA	Arka Vikas	0	2.69
11.	INRA	Arka Vikas	0	3.25
12.	INRA	Arka Vikas	0	2.66
13.	INRA	Arka Vikas	0	3.68
14.	INRA	Arka Vikas	0	3.29
15.	INRA	Arka Vikas	0	3.01

[a] Indexed 8 weeks after grafting.

[b] Indexed 8 and 12 weeks after grafting.

Table 32 : Average Plant height (in cm) of tomato[a] plants carrying mixed infection of TLCV and ToMV.

Treatment	Age of the crop in days				Days for symptom production	
	30	45	60	95	TLCV	ToMV
Ceck	19.3571	41.2857	87.2143	121.5143	-	-
TLCV alone	14.0714	21.5000	39.3571	49.1857	10	-
TLCV + ToMV simultaneously	11.6429	16.6429	23.0000	39.0000	10	8
TLCV + 24 hr ToMV	13.3571	18.7143	34.1429	41.4286	10	8
TLCV + 1 wk ToMV	13.7143	18.8571	37.2143	42.5714	10	9
TLCV + 2 wk ToMV	14.7857	20.0000	41.4286	43.2143	10	10
TLCV + 4 wk ToMV	15.0000	20.9286	41.6429	44.9286	10	12
TLCV + 6 wk ToMV	15.3571	20.5714	40.6429	46.5000	10	14
TLCV + 8 wk ToMV	14.9286	20.6429	61.7857	48.6429	10	15
ToMV alone	16.7143	29.2857	75.4286	115.4286	10	8
ToMV + TLCV simultaneously	12.5714	19.1429	30.2143	41.1429	10	8
ToMV + 24 hr TLCV	13.2143	19.5714	30.8571	40.3571	10	8
ToMV + 1 wk TLCV	14.6429	21.2857	43.2143	51.1429	10	8
ToMV + 2 wk TLCV	15.5714	27.0000	50.2143	52.2143	10	8.
ToMV + 4 wk TLCV	16.5714	30.9286	60.9286	67.1429	12	8
ToMV + 6 wk TLCV	16.2857	30.2143	71.8571	85.5000	12	8
ToMV + 8 wk TLCV	17.1429	30.8571	72.5714	117.7857	16	8
SEm±	0.3117	0.7672	0.7334	1.1046		
CD (5%)	0.8639	2.1266	2.0329	3.0618		

[a] cultivar Arka Vikas

followed by inoculation with ToMV + 8 wk TLCV (117.79cms) and ToMV alone (115.43cms). In comparison, plants inoculated with TLCV followed by ToMV 8 wks later and TLCV alone produced highly stunted plants measuring 48.64cms and 49.19 cms respectively, while plants inoculated with both the viruses simultaneously were 39.00cms tall (Table.32; Fig 41 & 42).

4.18.3. **Number of leaves**: As far as number of leaves were concerned, the difference between the treatments were insignificant 30 DAS. Even 45, 60 and 95 DAS, the difference between the treatments was not much. However plants inoculated with both the viruses simultaneously had the lowest number of leaves (Table 33). However, when the plants were indexed on N.glutinosa, there was no significant difference between the treatments with respect to the no. of lesions they produced (Table.33).

Table 33 : Mean No. of leaves of tomato[a] plants carrying mixed infection of TLCV and ToMV.

Treatment	Age of the crop in days				*N.glutinosa* Lesions/cm^2
	30	45	60	95	
Check	4.2857	8.1429	13.8571	20.5714	0
TLCV alone	3.8571	7.7143	12.1429	15.8571	0
TLCV + ToMV simultaneously	3.7143	6.5714	8.7143	15.7143	3.04
TLCV + 24 hr ToMV	3.8571	7.4286	10.8571	16.0000	2.62
TLCV + 1 wk ToMV	4.0000	7.0000	11.8571	16.1429	3.25
TLCV + 2 wk ToMV	3.8571	7.5714	12.0000	16.4286	2.86
TLCV + 4 wk ToMV	4.1429	8.1429	11.5714	17.1429	2.78
TLCV + 6 wk ToMV	3.8571	7.7143	12.1429	16.7143	3.42
TLCV + 8 wk ToMV	4.1429	7.4286	12.7143	16.8571	2.60
ToMV alone	4.1429	7.5714	12.4286	17.2857	3.22
ToMV + TLCV simultaneously	3.7143	7.4286	13.2857	16.1429	2.68
ToMV + 24 hr TLCV	3.8571	7.7143	13.2857	17.0000	2.82
ToMV + 1 wk TLCV	4.0000	7.2857	13.4286	17.4286	2.75
ToMV + 2 wk TLCV	4.1429	7.5714	13.2857	17.5714	3.08
ToMV + 4 wk TLCV	4.1429	8.0000	13.4286	18.2857	3.21
ToMV + 6 wk TLCV	4.2857	7.8571	13.2857	18.2785	3.36
ToMV + 8 wk TLCV	4.1429	8.0000	13.2857	19.1429	
SEM +	0.1800	0.2254	0.3093	0.3329	
CD	0.4900	0.6249	0.8572	0.9229	

[a] cultivar Arka Vikas
[b] each value is the average of five tomato plants from each treatment

Fig.41. Interactive effects of TLCV and ToMV in tomato plants inoculated with TLCV followed by ToMV

Fig.42. Interactive effects of TLCV and ToMV in tomato plants inoculated with ToMV followed by TLCV.

Fig. 41

Fig. 42

DISCUSSION

V. DISCUSSION

Tomato mosaic tobamovirus (ToMV) is an important disease of tomato in India. The natural occurence of ToMV was observed on tomato in India for the first time by Das and Raychaudhuri (1953). Although this disease is existing in the country for several decades threatening tomato cultivation, no systematic efforts have been made to study the characterisation and diagnosis of the disease.

The present investigations therefore, were undertaken on purification of the virus, production of antiserum, characterisation of viral protein and nucleic acid, diagnosis through ELISA, seed transmission, host range, interaction studies (TLCV and ToMV) and varietal screening.

Tomato mosaic virus disease on tomato and N.tabacum cv. samsun are characterised by the appearance of light and dark green mottling on the leaves. In tomato, the leaflets of affected leaves are usually distorted, puckered and smaller than normal. Fruits are also reduced in size. These symptoms were similar to those described by Rao and Reddy, 1971; Miller and Thornberry, 1958; Broadbent; 1964 and Avgelis, 1986.

The ELISA based survey conducted to assess the prevalence of ToMV in tomato growing areas revealed that ToMV was present in most of the tomato growing areas around Bangalore. The highest per cent incidence was almost 100 in some of the fields surveyed like for instance, several hybrids from Badakanapalya and Bangalore North (100); Yelahanka, Bangalore (100); Chintamani (95.38); Chikkaballapur (85.06) and Bangalore South (84.64). Similar results have been obtained in surveys conducted by Avgelis (1986); Fletcher et al., 1977 and Fletcher and Harris, 1979. This information obtained through survey is important as tomato seeds are produced in many of these areas and unless the seeds are harvested from plants tested to be virus free, one cannot be sure of freedom from ToMV.

Serological diagnostic methods are preferred for virus detection and identification due to its sensitivity and conveinence when large number of samples are

to be tested. Several new techniques have been reviewed by Torrance and Jones (1981) and have suggested ELISA method for large scale routine testing. The two enzymes that have been largely used in ELISA for detection of plant viruses are alkaline phosphatase (ALP) and horse radish peroxidase (HRP) (Clark and Bar-Joseph, 1984). The penicillinase based ELISA described by Sudarshana and Reddy (1989) is a less expensive means for assaying plant viruses by ELISA as penicillin is readily available at a substantially lower cost than p-nitrophenyl phosphate, the commonly used substrate for ALP-ELISA. The present ELISA results (Table 5) showed that the virus could be detected at higher dilution of antigen ($1:10^{-7}$) at antiserum dilution of 1:1000. In addition, N.glutinosa also detected the virus upto 10^{-6} dilution of infected leaf extract showing that the two diagnostic procedures together would ensure detection of even a single infected sample out of the total number of samples as reported by Rao and Reddy, 1971.

However, in local lesion assay, it was always necessary to calculate lesions per unit area because the number of lesions produced by a leaf is proportional to the area inoculated as is evident from the results of table 4. Therefore to obtain comparable results lesions/cm^2 has always been calculated.

ToMV was purified from systemically infected N.tabacum cv. samsun leaves. The yield of the virus was 80mg/100g tissue (undiluted virus). Using this procedure partially or fully, other workers have also purified the virus with a yield of 1 gm/1000g tissue. (Gooding and Hebert, 1967; Steere, 1964; Asselin and Zaitlin, 1978; Reddy et al., 1969; and Nariani et al., 1977). A260/A280 value of the virus was found to be 1.2. This ratio for TMV is equal to 1.23 according to Noordam (1973). The above workers have observed rigid rod shaped particles varying in length from 100-411 nm (Kamra and Dubey, 1975b) and 318-325 nm by Niazi et al., 1973. Width recorded was 17 nm. The virus purified in this study was also observed in the electron microscope. They measured 300 x 18 nm in size.

Antiserum was produced againt ToMV at Department of Plant Pathology, UAS, Bangalore. The antiserum had a titre of 1: 8000 in ELISA using crude extract as antigen (ToMV). The antiserum reacted with purified virus (ToMV) to give a titre of 1:4096 in gel diffusion test. Since ELISA is used as a diagnostic tool to detect virus

from crude extract of infected plant samples and double diffusion readily reacted with purified virus, the highest reacting dilution of the antiserum (antiserum titre) was detected using crude sap in ELISA and purified virus in double gel diffusion test. Antiserum reacted with purified virus and crude extract (from infected plants) in gel diffusion tests. However, the bands were very faint for crude extract. Reddy et al., 1969 have produced antiserum in rabbits against TMV and the antiserum had a titre of 1/3200 as determined by precipitin tube test. In gel diffusion test, they observed clear precipitation bands when ToMV antigen was tested against its homologous antiserum.

But, by using the antiserum in pencillinase based DAC-ELISA, the virus could be successfully detected in crude extracts of infected tomato and tobacco. Using this penicillinase ELISA Sudarshana and Reddy (1989) have also detected maize mosaic virus (MMV) in sorghum leaf extracts, peanut mottle virus (PMV) and Tomato spotted wilt virus (TSWV) in peanut leaf extracts. Thus since double diffusion test produced very faint bands with crude extract and also required high amounts of antiserum, ELISA which is very economical and highly sensitiive in detecting the virus even from crude extracts(diluted upto 10^{-7}), it is a preferred serological test over double diffusion test.

From the antiserum, γ globulins were purified by ammonium sulphate precipitation method and conjugated with penicillinase enzyme by the glutaradelhyde method. These γ globulins and enzyme conjugate were used for detecting ToMV in infected samples of tobacco and tomato using direct DAS-ELISA (Clark and Adams, 1977).

Polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS-PAGE) of ToMV revealed that the virus had a single major protein component of molecular weight 18000 D. Francki et al., (1985) have also reported that the coat protein of TMV has 158 residues and a molecular weight of 17,600.

Agarose gel electrophoresis of ToMV RNA revealed that ToMV consists of a RNA of molecular weight 6KB. This is in agreement with the results of Rejinders et al., (1973; 1974); Boedkter, 1960; Siegel et al., 1973; Murant and Taylor, 1978 and Bem and Murant, 1979. However attempts regarding protein and nucleic acid characterization of Indian tobamoviruses are lacking.

Studies on the properties of ToMV in crude extract showed that it had a dilution end point (DEP) of 10^{-7}, thermal inactivation point (TIP) of 90° C and longevity in vitro (LIV) beyond 540 days (for crude sap maintained at room temperature; crude sap maintained in fridge and dried infected leaves). However crude sap and dried leaves at room temperature seems to be significantly infectious upto 100 days while sap at 4 deg. C seems infectious upto 226 days. These results agree closely to that reported by Sandhu and Chohan (1978) for TMV in chilli (Capsicum annum), which had a TIP of 90° C; and DEP of 10^{-7}. Similar observations have been made by Nariani et al., (1977); Kamra and Dubey, 1975; and Ramakrishnan et al., (1964b).

Seed transmission provides a very effective means of introducing virus to a crop at an early stage giving randomised foci of infection throughout the field. The presence of ToMV on tomato seed and its transmission through seed showed that seeds from infected plants serve as an important factor in the spread of this virus (John and Sova, 1955). It is of great significance especially with a virus like ToMV, because once introduced the virus can further spread by mechanical means. Thus use of tomato seeds freed from ToMV is a must in cultivation of tomato. While there are reports that TMV in tomato is not seed transmitted (Gardner and Kendrick, 1992), several investigators have reported various amounts of seed transmission (Bewley and Corbett, 1930; Alexander, 1960; Taylor, 1961; Broadbent, 1965a). Present results showed that ToMV could be detected through ELISA on tomato seeds collected from infected plants and a 3-6 per cent seed transmission was recorded through growing on test (Shoba Cherian and Muniyappa, 1995).

ToMV was detected in different parts of the infected tomato plants (leaves, petals, sepals, stalk, stem, petiole, roots) and in the skin, juice and pulp of fruits from infected plants. Broadbent (1965a,b) and Habib (1986) have also detected ToMV in leaves and root extracts of tomato 3 weeks after inoculation. It was found that most ToMV is carried externally on the surface of tomato seeds as has also been reported by John and Sova (1955). ToMV was detected in all the sets of 50 and 100 tomato seeds collected from infected plants (Table 17). Thus to detect if commercial seed lots were contaminated with ToMV, 5 lots of 100 seeds of each variety were tested in ELISA and presence of ToMV in them was successfully detected. This makes it necessary for seed

companies and farmers to suitably treat their seed lots before use. A soaking treatment of infected tomato seeds in 10 per cent tri sodium phosphate for 20 mins freed the seeds from ToMV. Thus seed treatment with TSP can effectively eliminate and inactiviate the virus and seeds can be treated in this manner prior to planting. This is in agreement with the results of other investigators like Taylor (1961) and Broadbent (1965a). Use of treated seeds is also important because of the rapidly increasing international exchange of seed which has contributed to spread of economically important diseases (Bos, 1977). As part of crop improvement programme, lot of germplasm is being introduced into India and chances are fairly high that new strains of the virus may be introduced into our country through seeds. And since the particles of this virus are stable, even if a small per cent of the particles are released, they manage to get dispersed by various means and infect a new crop at a distant location.

Host range studies of ToMV revealed that it infected 18 out of the 80 host species tested. These host species have been reported to take ToMV infection by other workers (Mali and Vyanjane, 1980; Singh, 1988; Kamra & Dubey (1975); Rao and Reddy (1971); Verma et al., (1972), etc). But some of the hosts reported to take ToMV infection by previous workers (Table 2) did not get infected during this study in spite of repeated inoculations. Cultivated crops among them include Lablab purpureus, Glycine max, Vicia faba, Vigna mungo, Pisum sativum, Vigna unguiculata, Vigna aureus, Arachis hypogaea, and Solanum melongena. This is because some of the earlier workers have reported host range based solely on symptomatology without supporting it with tests like local lesion assay and ELISA. Moreover, in some cases the specific varieties of each host suspectible to the virus have not been mentioned. It was also found during this study that of the several different hosts tested only a couple of hosts showing mosaic like sympotoms in nature produced local lesions on N.glutinosa confirming the presence of the virus.

Of the 41 Indian tomato cultivars and hybrids screened against ToMV, none were found resistant when indexed on N.glutinosa and tested in ELISA. Mayee et al., (1977) also reported that a large collection of the recommended and cultivated tomato varieties were found to be susceptible to ToMV.

Of the 15 F_1 tomato hybrids and crosses tested, 3 of them produced a very low lesion density (< 2 lesions/cm^2) on N.glutinosa. Among the 18 accessions of Lycopersicon tested, the accession CMV Sel. INRA (L.peruvianum) was resistant to ToMV. Tewari et al., 1972 also screened several tomato lines against ToMV and found that some accessions of L.peruvianum and L.hirsutum were the most resistant species. The established grafts of CMV Sel. INRA on infected Arka Vikas also tested free of ToMV, confirming the resitance in this accession and this resistance can be employed in breeding programmes.

Interaction studies of ToMV and TLCV in tomato plants carrying their mixed infection revealed that plants inoculated with both the viruses were the ones which showed maximum reduction in plant height and TLCV had a greater effect in reducing plant heights compared to ToMV. The two viruses together had a synergistic effect in reducing the height of inoculated tomato plants. Such synergistic effects of ToMV with Meloidogyne incognita in tomato has been reported by Alam et al., (1990) and Goswami & Chenulu (1974).

Conclusion: The present studies on ToMV revealed that the mean incidence of this virus in the tomato growing areas around Bangalore was about 55.98 per cent. Penicillinase based ELISA was standardized and used in the detection of ToMV in host and seeds.

The physical properties of virus revealed it is a highly stable virus. In addition since it is found to be seed borne in nature, unless seeds are suitably treated with tri sodium phosphate, the virus once introduced can further spread by mechanical means. The results also revealed the existence of resistance in a wild accession CMV Sel. INRA (L.peruvianum) and this can be of great value in breeding for disease resistance in tomato.

More significant contributions are the production of antiserum, purification of r globulin and preparation of enzyme conjugate against ToMV for diagnosis by employing ELISA. This sensitive technique detected virus infection in crude sap of infected plants collected from field and also glass house inoculated plants. When field

samples of tomato were tested for ToMV, even apparently healthy looking tomato plants also showed positive reaction in ELISA. Mol. Wt. determination of protein and nucleic acid has helped characterisation and comparison of the local isolates with those reported in other countries.

Further several field samples of tomato can be collected and differentiated into TMV and ToMV based on reaction on N.sylvestris. c-DNA probes and monoclonal antibodies can be produced for ToMV and tested with the various isolates to know the differences between them at genomic level. The differences listed between TMV and ToMV by other workers can thus be confirmed.

SUMMARY

VI. SUMMARY

1. Penicillinase based enzyme linked immunosorbent assay (DAC-ELISA & DAS-ELISA) were standardized and successfully used to detect tomato mosaic virus (ToMV) in host and seeds using antiserum raised against ToMV. While ToMV could be detected in infected leaf extracts with host dilution of 10^{-7} using ELISA, local lesion assay (N.glutinosa) detected the virus in dilutions of upto 10^{-6}.

2. The survey to assess the incidence of tomato mosaic virus (ToMV) in tomato growing areas around Bangalore during kharif and rabi 1994-96 revealed that the incidence varied from 0 to 100 per cent. The mean incidence of 55.98 per cent was observed. ELISA was used during survey of ToMV disease in 25 tomato growing fields around Bangalore. Of 1229 tomato leaf samples collected from these fields, 688 samples reacted in ELISA.

3. Infected tomato and N.tabacum cv. Samsun plants showed light and dark green mottling on the leaves. In tomato, the leaflets of infected leaves showed distortion, puckering and were smaller then normal. Fruit size was also reduced.

4. ToMV was purified from systemically infected N.tabacum cv. Samsun. The average yield of ToMV was 80 mg/ml(undiluted virus). $^{A}260/^{A}280$ ratios of purified preparations of the virus was very close to 1.2. The purified virus was successfully transmitted to hosts of ToMV by mechanical inoculation. Electron microscopy of purified preparations of ToMV showed numerous rigid rod shaped particles measuring 300 x 18 nm in size.

5. Antiserum was produced in rabbits against ToMV. This antiserum had a titre of 1:32000 in ELISA and 1:4096 in gel diffusion test. **r** globulins were purified from the produced antiserum by ammonium sulphate precipitation method and conjugated with pencillinase enzyme by the gultaraldehyde method. Using the produced r globulins and conjugate ToMV was detected in infected samples of tobacco and tomato.

6. Agar gel double diffusion tests using ToMV antiserum successfully detected ToMV in purified preparations and crude leaf extracts of infected tobacco and tomato plants. ELISA, also detected ToMV in crude leaf extracts of infected tobacco and tomato plants.

7. Polyacrylamide gel electrophoresis (SDS-PAGE) of ToMV coat protein revealed that the virus coat protein has a molecular weight of 18KD.

8. Agarose gel electrophoresis of ToMV RNA revealed that ToMV consists of a RNA of molecular weight of 2 x 10^6 daltons.

9. Study of the physical properties of ToMV in crude sap revealed that it had a dilution end point between 10^{-6} and 10^{-7}, thermal inactivation point between 90°C-100°C and longevity in vitro of beyond 540 days (for crude sap maintained at room temperature; crude sap maintained in fridge and dried infected leaves).

10. ToMV was detected by ELISA and local lesion assay (on N.glutinosa) in seeds of tomato varieties Arka Vikas, F_1 Fiona, Pusa Ruby, Ramya and Rupali and in seeds of tobacco varieties Samsun, Jayasri and Xanthi. Per cent seed transmission ranged from 3 per cent in Arka Vikas to 6 per cent in Rupali (tested by growing on test).

11. ToMV was detected in washed tomato seeds and phosphate buffer washings through ELISA and local lesion assay, an indication of external seed borne nature of the virus. Soaking treatment of infected tomato seeds in 10 per cent tri sodium phosphate for 15 mins eliminated ToMV from tomato seeds. ToMV was detected in all seed lots (collected from infected tomato plants) containing 50 seeds and above. They were detected in several of the commercial seed lots tested.

12. ToMV could be detected in all infected plant parts viz., top leaves, stem, root, flower stalk, petals, sepals, leaf petiole and soil (in which infected plants are grown). ToMV could be detected in the skin, juice, pulp and seeds of tomato fruits collected from infected plants.

13. Host range studies of ToMV revealed that out of 80 plant species tested, 18 host species took ToMV infection. Of them hosts which took systemic infection are Capsicum annuum var G-4, Chenopodium murale, Nicotiana tabacum, var Samsun, Jayasri and Xanthi, Physalis floridana and Solanum tuberosum. The local lesion hosts include Capsicum annuum var. California wonder, Chenopodium amaranticolor, Datura metel, Datura stramonium and Nicotiana tabacum var. White burley, Riwaka-1, L-1158, CTRI

special, Nicotiana glutinosa and Nicotiana sylvestris. Natural infection of ToMV was detected through ELISA and local lesion assay in tomato, tobacco and chilli samples.

14. Out of the tomato samples indexed from tomato fields, a higher per cent incidence was recorded from a field in Bangalore South (84.64 per cent); Chikkaballapur (85.96 per cent), Kolar (44.44 per cent); Badakanapalya, Bangalore North (100 per cent), Chintamani (95.38 per cent), Yelahanka, Bangalore (100 per cent) and two fields in Anekal (60 and 42.11 per cent).

15. Of 55 tomato hybrids, cultivars and breeding lines and eighteen accessions belonging to Lycopersicon cheesmanii, L.glandulosum, L.hirsutum, L.pervianum and L.pimpinellfolium evaluated for resistance against ToMV, only one accession, CMV sel. INRA (L.peruvianum) was resistant to ToMV. It produced 0 lesions/cm^2 on N.glutinosa and ToMV could not be detected in it through ELISA. Twelve of the tested entries produced less than 5 lesions/cm^2 on the local lesion host. Established grafts of CMV sel.INRA (L.peruvianum) on infected Arka Vikas also tested free of the virus 6 weeks after grafting.

16. Interaction studies of ToMV and TLCV in tomato plants carrying their mixed infection revealed that plants inoculated with both these viruses simultaneously were highly stunted and produced comparitively lesser leaves. TLCV had a greater effect in reducing plant height compared to ToMV.

REFERENCES

VII. REFERENCES

Akanda,A.M., Tsuno,K. and Wakimoto,S., 1991, Serodiagnosis of viruses infecting some crops of Bangladesh. J.Faculty.Agri., **35** : 121-129.

Alagianagalingam,M.N. and Ramakrishnan,K., 1972, Rhizosphere microflora of tobacco mosaic virus infected Capsicum annum L. Indian J.Microbiology, **12** : 23-26.

Alam,M.M., Samad,A. and Anwer,S., 1990, Interaction between tomato mosaic virus and Meloidogyne incognita in tomato. Nematologia Mediterranea, **18** : 131-133.

Alexander,L.J., 1960, Inactivation of TMV from tomato seed (Abstr.) Phytopathology, **50** : 627.

Anand,G.P.S. and Sahambi,H.S., 1965, Serological relationship of sunnhemp mosaic virus with some other mosaic viruses commonly occuring in Delhi. Indian Phytopath., **18** : 204-205.

Anon, 1983, Pest control in tropical tomatoes. Centre for overseas pest research, London, 130 pp.

Anon, 1992a, FAO year book. Vol.46. Food and Agricultural Organisation of the United Nations, Rome, 281 pp.

Aslam,M., Srivastava,K.M. and Singh,B.P., 1985, Isolation and partial characterisation of a tobamovirus from cornflower (Centaurea cyanus L.) Indian J.Plant Pathology, **3** : 203-207.

Asselin,A. and Zaitlin,M., 1978, An anamalous form of tobacco mosaic virus RNA observed upon polyacrylamide gel electrophoresis. Virology **88** : 191-193.

Asselin,A., Seirgny,P. and Hogue,R., 1984, A note on the preparation of electrophoretically purified isolates of TMV. Phytoprotection, **65** : 84-88.

Avgelis,A.D., 1986, Viruses of tomato in plastic houses in Crete. Netherlands J.Pl.Path., **92** : 147-152.

Bald,J.G. and Suzuki.T., 1974, A simple method for purifying some plant viruses. Plant Dis. Reptr., **58** : 126-127.

Ball,M.E. and Brakke, M.K., 1968, Leaf dip serology for electron microscopic identification of plant viruses. Virology, **36** : 152-155.

Bem,F. and Murant,A.F., 1979, Comparison of particle properties of Heracleum latent and apple chlorotic leaf spot viruses. J.gen.Virol., **44** : 817-826.

Bennett,C.W., 1969, Seed transmission of plant viruses. Adv. Virus Res., **14** : 221-261.

Benoit,M.A., 1977, Does tobacco seed transmit TMV. Planter, **53** : 197-201.

Benoit,M.A. and Maury,Y., 1976, Location of TMV in tobacco seed. Planter, **52** : 392-396.

Bewley,W.F. and Corbett,W., 1930, The control of cucumber and tomato mosaic diseases in glasshouses by the use of clean seed. Ann. appl. Biol., **17** : 260-265.

Bhalla,R.B. and Sehgal,O.P., 1973, Host range and purification of the nucleic acid of a defective mutant of tobacco mosaic virus. Phytopathology, **63** : 906-909.

Boedkter,H., 1960, Configurational properties of tobacco mosaic virus ribonucleic acid. J.Mol. Biol., **2** : 171-188.

Bos,L., 1975, The application of TMV particles as an internal magnification standard for determining virus particle sizes with the electron microscope. Neth. J.Pl.Path., **81** : 168-175.

Bos,L., 1977, Seed borne viruses. In: Plant health and quarantine in international transfer of genetic resources. ed, W.B.Heiwett and L.Charappa, pp39-69., CRC Press, Cleveland, Ohio, USA, 390pp.

Brandes, J. and Chessin, M., 1965, An Electron microscope study on the size of Sammon's opuntia virus. Virology, **25** : 673.

Brisson,L., Trudel,M.J. and Asselin,A., 1983, Note on presence of ToMV on commercial crops of glasshouse tomato in Quebec. Phytoprotection, **64** : 83-87.

Broadbent,L., 1963, The epidemiology of tomato mosaic III. Cleaning virus from hands and tools. Ann. Appl. Biol., **52** : 225-232.

Broadbent,L., 1964, The epidemiology of tomato mosaic VII. The effect of TMV on tomato fruit yield and quality under glasshouse conditions. Ann. Appl. Biol., **54** : 209-224.

Broadbent,L., 1965a, The epidemiology of tomato mosaic. XI. Seed transmission of TMV. Ann. Appl. Biol., **56** : 177-205.

Broadbent,L., 1965b, The epidemiology of tomato mosaic. VIII. Virus infection through tomato roots. Ann. Appl. Biol., **55** : 57-65.

Broadbent,L. and Fletcher, J.T., 1963, The epidemiology of tomato mosaic .IV.Persistence of virus on clothing and glass house structures. Ann. Appl. Biol., **52** : 233-241.

Broadbent,L., Read, W.H. and Last, F.T., 1965, The epidemiology of tomato mosaic. X.Persistence of TMV infected debris in soil and the effects of soil partial sterilization.Ann.Appl.Biol., **55** : 471-483.

Cai,W.O., 1985, Rapid detection of virus free gladiolus plantlets by indirect ELISA. Acta Microbiologica Sinica, **25** : 329-333.

Capoor,S.P., 1961, Cassia tora : Local lesion host of tobacco mosaic virus. Curr. Sci., **30** : 353.

Capoor,S.P. and Sharma,D.C., 1965, Studies on Cape Goosberry mosaic disease. Indian Phytopath., **18** : 58-65.

Chang,Y.S., Pei,M.Y., Jen,M.H., Chein,Y.J., Tsao,T.C. and Cheo,C.C., 1964, The size, shape and structure of chinese rape (Brassica campestris L.) - Youcai mosaic virus, YMV and some chemical characteristics of its protein subunits. Scientia Sin., **13** : 1421.

Clark,M.F. and Adams,A.N., 1977 Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. J.Gen. Virol., **34** : 475-483.

Clark,M.F. and Bar Joseph, 1984, Enzyme immunosorbent assays in plant virology. Methods in Virology, **7** : 51-95.

Crowley,N.C., 1959, Studies on the time of embryo infection by seed transmitted viruses. Virology, **8** : 116.

Das,C.R. and Raychaudhuri,S.P., 1953, A probable strain of tomato acuba mosaic virus. Indian Phytopath., **6** : 117-120.

Dawson, J.R.O., Rees,M.W. and Short,M.N., 1975, Protein composition of unusual tobacco mosaic virus strains. Ann.Appl.Biol., **79** : 189.

De,M.L., 1951, Electron microscope determination of size and shape of a new plant virus (Sunnhemp mosaic). Indian J.Physiology, **25** : 525-529.

Dhanju,K.S. and Varma,J.P., 1986, Natural occurence of mixed infection of tomato leaf curl virus and tobacco mosaic virus in tomato. Indian J.Virology, **2** : 108-110.

Fernandez,A.I., Lopez,M., Fernandes,R. and Fernandez,T., 1988, Early detection of TMV in tobacco plants by means of ELISA. Revista Biologia, **11** : 19-24.

Fletcher,J.T. and Harris,P.A., 1979, Survey of diseases of glasshouse tomato crops in England and Wales in 1976. Plant Pathology, **28** : 111-118.

Fletcher,J.T., Harris,P.A., and Bant,J.H. and Collins,B., 1977, Survey of diseases of glasshouse tomato crops in Yorkshire and Lancashire in 1974 and 1975. Plant Pathology, **26** : 49-57.

Francki,R.I.B., Milne,R.G. and Hatta,T., 1985, Atlas of plant viruses, Vol II, CRC Press, Florida, 284pp.

Gardner,M.W. and Kendrick,J.B., 1992, Overwintering of tobacco mosaic. Botan. Gaz., **73** : 469-485.

Gibbs,A.J., 1975, Tobamovirus group. CMI/AAB descriptions of plant viruses. No.184.

Gierer,A. and Schramm, 1956, Infectivity of ribonucleic acid from tobacco mosaic virus. Nature, **177** : 702-703.

Giri,B.K. and Mishra,M.D., 1990, Effect of tomato mosaic virus on pollen viability and yield of tomatoes. Indian Phytopath., **43** : 487-490.

Gooding,G.V.Jr., 1975, Inactivation of TMV on tomato seed with Tsp and NaOCl. Plant Dis. Reptr., **59** : 770-772.

Gooding,G.V.Jr. and Hebert,T.T., 1967, A simple technique for purification of tobacco mosaic virus in large quantities. Phytopathology, **57** : 1285.

Gooding,G.V.Jr. and Suggs,E.G., 1976, Seed borne tobacco mosaic virus in commercial sources of tomato seed. Plant Dis. Reptr., **60** : 441-442.

Goswami,B.K. and Chenulu,V.V., 1974, Interaction of root knot nematode, Meloidogyne incognita and TMV in tomato. Indian J.Nematol., **4** : 69-80.

Habib,H.M., 1986, Infectivity of ToMV in tomato plant parts. Egyptian J.Botany, **29** : 11-19.

Hansen,N.J., 1976, Electrophoresis of ribonucleic acid on a polyacrylamide gel which contains disulfide cross linkages. Virology, **76** : 37-44.

Hebert,T.T., 1963, Precipitation of plant viruses by polyethylene glycol. Phytopathology, **53** : 362.

Hedrick,J.L. and Smith,A.J., 1968, Size and charge isomer seperation and estimation of molecular weights of proteins by Disc gel electrophoresis. Arch. Biochem. Biophys., **126** : 155-164.

Hibi,T., Yada,K., Takahashi,S. and Shibata,K., 1973, High resolution electron microscopy of TMV J.Electron Microscopy, **22** : 243-253.

Hill,J.H. and Shepherd,R.J., 1972, Molecular weights of plant virus coat proteins by polyacrylamide gel electrophoresis. Virology, **47** : 817-822.

Hobbs, H.A.,Reddy,D.V.R., Rajeshwari,R. and Reddy,A.S., 1987, Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses. Plant Dis., **71** : 747-749.

Hogue, R. and Asselin, A., 1984, Polyacrylamide agarose gel electrophoretic analysis of tobacco mosaic virus disassembly intermediates. Can. J. Bot., **62** : 2336-2339.

Hollings,M. and Huttinga,H., 1976, Tomato mosaic virus.CMI/AAB descriptions of plant viruses.No.156.

Hollings,M., Komuro,Y. and Tochihara,H., 1975, Cucumber green mottle mosaic virus. CMI/AAB descriptions of plant viruses. No.154.

Holmes, F.O., 1929, Local lesions in tobacco mosaic. Bot. Gaz., **39** : 55.

Horne, R.W., Hobart, J.M. and Markkham, R., 1976, Electron microscopy of tobacco mosaic virus prepared with the aid of negative staining - carbon film techniques. J.Gen.Virology., **31** : 265-269.

Ismail, W., Johri, J.K., Zaidi, A.A. and Singh, B.P., 1979, Influence of root knot nematode, tobacco mosaic virus and complex on the growth and carbohydrates of Solanum khasianum. Indian J. Exptl. Biol., **17** : 1266-1267.

John, C.A. and Sova, C., 1955, Incidence of tobacco mosaic virus on tomato seed. Phytopathology, **45** : 636-637.

Kamra, B.S, and Dubey,G.S., 1975, Particle morphology of a TMV strain from Chilli. Indian Phytopath., **28** : 96-98.

Kandaswami,T.K. and Ramakrishnan,K., 1964, Datura ferox L. an additional local lesion host for tobacco mosaic virus. Madras Agric. J., **51** : 254.

Kassanis,B. and Varma,A., 1975, Sunn-hemp mosaic virus CMI/AAB descriptions of plant viruses.No.153.

Khatri,H.L. and Chenulu,V.V., 1966, The occurence of a mosaic disease of phlox in India. Curr. Sci., **35** : 183.

Knight,C.A., Silva,D.M., Dahl,D. and Tsugita,A., 1962, Two distinctive strains of tobacco mosaic virus. Virology, **37** : 236-242.

Koening,R., 1981, Indirect ELISA methods for the broad specificity detection of plant viruses J. Gen. Virology, **55** : 53-62.

Koening,R. and Paul,H.L., 1982, Variants of ELISA in plant virus diagnosis. J. Virological Methods, **5** : 113-125.

Krishna,R., Srivastava,K.M. and Singh,B.P., 1982, Nicotiana plumbaginifolia - an unrecorded carrier of a tobamo and cucumovirus. New Botanist, **7** : 105-109.

Kwaje,S.L. and Young,R.J., 1979, Transmission of TMV by seeds of tomato. Proc. West Virginia Acad. Sci. **51** : 37-42.

Lammelli,U.K., 1970, Clevage of structural proteins during the assembly of the head of bacteriophage T4. Nature, **227** : 680-685.

Leberman,R., 1966, The isolation of plant viruses by means of "simple" coacervates. Virology, **30** : 341-347.

Loening,U.E., 1967, The fractionation of high molecular weight ribonucleic acid by polyacrylamide gel electrophoresis. Biochem. J., **102** : 251.

Loening,U.E., 1969, The determination of the molecular weight of RNA by PAGE. The effects of changes in confirmation. Biochemical J., **113** : 131-138.

Lommel,S.A., Mc Cain,A.H., Morris,T.J., 1982, Evaluation of indirect enzyme linked immunosorbent assay for the detection of plant viruses. Phytopathology, **72** : 1018-1022.

Mali,V.R. and Vyanjane,N.T., 1980, Occurence of tobacco mosaic virus on passion flower (Passiflora caerulea). Indian J.Mycol. Pl. Pathol., **10** : 112-114.

Mali,V.R., Diwakar,M.P. and Patil,F.S., 1979, Effect of tobacco mosaic virus infection on growth, nodulation and nitrogen fixation in cowpea. Indian J.Mycol. Pl. Pathol., **9** : 126-127.

Mariappan,V., Govindaswamy,C.V. and Ramakrishnan,K., 1970, Studies on the role of weed hosts in the spread of TMV. Role of Vernonia cinerea. Madras Agric. J., **57** : 680-686.

Mayee,C.D., Kanwar,J.S. and Nandpuri,K.S. 1974a, The comparitive performance of different genotypes of tomato to leaf curl and mosaic. J.Res. PAU., **11** : 362-364.

Mayee,C.D., Kanwar,J.S. and Nandpuri,K.S., 1974b, Field screening of some tomato varieties and hybrids against leaf curl and mosaic viruses. Punjab Veg. Grower., **9** : 71-72.

Mayee,C.D., Kanwar,J.S. and Nandpuri,K.S., 1977, Reaction of some tomato lines to the tobacco mosaic virus. J.Res. PAU., **14** : 59-60.

Mc.Guire,J.M., Wickizer,S.L., Goode,M.J., 1979, Association of tomato mosaic virus with tomato seed. Arkansas Farm Res., **28** : 11.

Miller,P,M., 1953, An apparently new virus of tomatoes in Illinois. Phytopathology, **43** : 480-482.

Miller,P.M. and Thornberry,H.H., 1958, A new viral disease of tomato and pepper. Phytopathology, **48** : 665-670.

Mitra,D.K. and Majumdar,M., 1980, Absorption of ^{32}P by brinjal and tobacco leaf tissues infected with little leaf and TMV. Indian Phytopath., **33** : 619-620.

Murant,A.F. and Taylor,M., 1978, Estimates of molecular weights of nepovirus RNA species by polyacrylamide gel electrophoresis under denaturing conditions. J.Gen.Virology, **41** : 53-61.

Murant,A.F., Mayo,M.A., Harrison,B.D. and Goald,R.A., 1972, Properties of virus and RNA components of raspberry ringspot virus. J.Gen.Virology. **16** : 327-338.

Murant,A.F., Taylor,M., Duncan,G.H. and Raschke,J.H., 1981, Improved estimates of molecular weight of plant virus RNA by agarose gel electrophoresis and electron microscopy after denaturation with glyoxal. J.Gen. Virology. **53** : 321-332.

Murthy,N.S., 1982, Additional list of plant species possessing tobacco mosaic virus inhibitors. Indian J.Agril. Res., **16** : 233-238.

Nagaich,B.B. and Vashisth,K.S., 1963, A virus causing a typical mosaic disease of beans. Indian J.Microbiol., **3** : 113-116.

Nagarajan,S. and Summanvar,S.A., 1973, Ocimum sanctum - A new local lesion host for tobacco mosaic virus. Indian Phytopath., **26** : 342-343.

Nariani,T.K., Kartha,K.K. and Nam Prakash, 1970, Purification of the southern sunn-hemp mosaic virus using butanol and differential centrifugation.Curr.Sci., **39** : 539-541.

Nariani,T.K., Vishwanath,S.M., Raychaudhuri,S.P. and Mahavir,A.V., 1977, Studies on a mosaic disease of musk melon. (Cucumis melo L.). Curr. Sci., **46** : 47-48.

Niazi,F.R., Jagadishchandra,K. and Nam Prakash, 1973, A new strain of tobacco mosaic virus infecting sunnhemp. Indian Phytopath., **26** : 115-121.

Noordam,D., 1973, Identification of plant viruses, Methods and Experiments. Oxford and IBH publishing Co., New Delhi.

Oshima,N. and Harrison,B.D., 1975, Ribgrass mosaic virus.CMI/AAB descriptions of plant viruses.No.152.

Paul,H.L., 1975, Odontoglossum ringspot virus.CMI/AAB descriptions of plant viruses.No.155.

Pelham,J., 1966, Resistance in tomato to tobacco mosaic virus. Euphytica, **15** : 258-260.

Pelham,J., 1972, Strain genotype interaction of tobacco mosaic virus in tomato. Ann. Appl. Biol., **71** : 219-228.

Phatak,H.C., 1974, Seed borne plant viruses. Identification and diagnosis in seed health testing. Seed Sci. & Technol., **2** : 130-155.

Phatak,H.C. and Verma,V.S., 1970, Systemic lesions of tobacco mosaic virus in Datura. Acta. Phytopath., **5** : 191-195.

Poonam and Varma,J.P., 1976, Resistance to tobacco mosaic virus in tomato. Proc. Natl. Acad. Sci. India B, **46** : 226-228.

Ramakrishnan,K., Kandaswamy,T.K. and Thangamani,G., 1964a, Mosaic disease of tomato. Madras Agric. J., **51** : 94.

Ramakrishnan,K., Rangaraju,R., Thangamani,G., Sarojini,A.P. and Kandaswamy,T.K., 1964b, Studies on new mosaic disease of Phaseolus aureus. Madras Agric. J., **51** : 378-380.

Ramakrishnan,K., Kandaswamy,T.K. and Thangamani,G., 1965, Reaction of Chilli (Capsicum spp.) to tobacco mosaic virus. Madras Agric. J., **52** : 490-491.

Rao,M.H.P. and Reddy.D.V.R., 1971, A new strain of TMV on tomato. Indian Phytopath., **24** : 672-678.

U. A. S.
University Library
DHARWAD.
Acc. No. TH 6366

Raychaudhuri,S.P., Nariani,T.K. and Das,C.R., 1962, Cyamopsis tetragonaloba (L.) - a local lesion host of sunnhemp mosaic virus. Indian Phytopath., **15** : 79-80.

Rejinders,L., Sloof,P., Sival,J. and Borst,P., 1973, Gel electrophoresis of RNA under denaturing conditions. Biochim Biophys. Acta., **324** : 320-333.

Rejinders,L., Aalbers,A.M.J. and Van Kammen,A., 1974, Molecular weights of plant viral RNAs determined by gel electrophoresis under denaturing conditions. Virology, **69** : 515-521.

Reddy,D.V.R., Hari Prasada Rao,M., Padma,M.C. and Gopal Rao,V., 1969, Haemagglutination test to detect tobacco mosaic virus antigens. Indian J.Exptl. Biol., **7** : 162-165,

Sachidananda,J., 1966, A note on a mosaic disease of Nicotiana sylvestris. Indian Phytopath., **19** : 321-322.

Saikia,A.K. and Muniyappa,V., 1989, Epidemiology and control of tomato leaf curl virus in southern India.Trop.Agric., **66** : 350-354.

Sammons, I.M. and Chessin,M., 1961, Cactus virus in the United States. Nature, **191** : 517.

Sandhu,K.S. and Chohan,J.S., 1978, Identification and serological characterization of tobacco mosaic virus from chilli (Capsicum annum). Indian J.Mycol. Pl. Pathol., **8** : 214-215.

Sangar,R.B.S., Katwale,T.R., Saraf,R.F. and Parihar,M.S., 1988, Field screening of chilli varieties to viral diseases in Madhya Pradesh. Farm Science J., **3** : 69-71.

Sastry,K.S.M. and Khan,M.A., 1971, Mosaic virus complex of Digitalis lanata. Indian J. Microbiol., **11** : 125-126.

Sastry,K.S.M., Thakur,R.N., Gupta,J.H. and Pandotra,V.R., 1971, Three virus diseases of Eucalyptus citriodora. Indian Phytopath., **24** : 123-126.

Scalla,R., Boudon,E. and Rigaud,J., 1976, Sodium dodecyl sulphate PAGE detection of two high molecular weight proteins associated with TMV infection in tobacco. Virology, **69** : 339-345.

Scott,H.A., 1974, Interpretations of Gel diffusion tests. Phyopathology, **63** : 439.

Sela,I., Reichman,M. and Weissbach,A., 1984, Comparison of dot molecular hybridisation and enzyme linked immunosorbent assay for detecting tobacco mosaic virus in plant tissues and protoplasts. Phytopathology, **74** : 385-389.

Shankar,G., Nariani,T.K. and Nam Prakash., 1971, Purification and serology of bottlegourd (Lagenaria siceraria) mosaic virus. Indian J.Microbiology, **3** : 43-52.

Sharma,O.P., Mahajan,R. and Surjan Singh, 1984, Field reaction of some root knot resistant tomato varieties and hybrids to tobacco mosaic and tobacco leaf curl viruses. Indian J.Mycol. Pl. Pathol., **14** : 148-150.

Shoba cherian and Muniyappa,V., 1995, Indexing of field samples of tomato and determination of seed borne nature of tomato mosaic tobamovirus. Proc. National seminar on Seed Pathology, Manasagangotri, March 3-5 (Abstr.).

Siegel,A., Zaitlin,M. and Duda,C.T., 1973, Replication of Tobacco Mosaic Virus. IV. Further characterization of viral related RNAs. Virology, **53** : 75-83.

Singh,R., 1969, Influence of varying water supply on the susceptibility of Chenopodium amaranticolor to infection with tobacco mosaic virus. Sci. and Cult., **35** : 155-157.

Singh,S.J., 1988, A new mosaic disease of Banana. Curr. Sci., **57** : 1248-1249.

Singh,A.B. and Pandey,P.K., 1979, A new strain of tobacco mosaic virus causing mosaic disease in pigeon pea. Curr. Sci., **48** : 261.

Silva,J.O., Aragao,S.D., Cupertino,F.P., 1986, Evaluation of sources of resistance to ToMV and M.javanica in Lycopersicon. Fitopatologia Brasileira., **11** : 991-1000.

Sigurgeirssioni,T. and Stanley,M.W., 1947, Electron microscope studies on tobacco mosaic virus. Phytopathology, **37** : 26-38.

Sri Ram & Shukla,D.D., 1975-76, Studies on virus disease of chillies (Capsicum annum and C.frutescens) in Rajasthan. Indian J.Mycol. Pl. Pathol., **5** : 29 (Abstr.).

Stanley,W.M., 1935, Isolation of a crystalline protein possessing the properties of tobacco mosaic virus. Science, **81** : 644-645.

Steere,R.L., 1964, Purification p.211-234. In: M.K.Corbett and H.D.Sisler (ed) Plant Virology. Univ. Florida Press, Gainesville. 231 pp.

Sudarshana,M.R. and Reddy,D.V.R., 1989, Penicillinase based enzyme linked immunosorbent assay, for the detection of plant viruses. J.Virological Methods, **26** : 45-52.

Tanigiuchi,T., 1976, Inhibition of TMV infection by detergents. Phytopath.Z., **86** : 246-251.

Taylor,R.H., Grogan,R.C. and Kimble,K.A., 1961, Transmission of tobacco mosaic virus in tomato seed. Phytopathology. **51** : 837-842.

Taylor,G.A., Lewis,G.D. and Rubatzky,V.L., 1969, The influence of time of tobacco mosaic virus inoculation and stage of fruit maturity upon the incidence of tomato internal browning. Phytopathology, **59** : 732-736.

Tewari,V.P., 1982, Progress of research work on breeding for virus resistance in chilli. Madras Agric. J., **69** : 277.

Tewari,R., Choudhury,B. and Raychaudhuri,S.P., 1972, Tobacco mosaic virus resistance in Lycopersicon. Indian Phytopath, **25** : 392-400.

Thakur, R.N. and Sastry, K.M. 1971, Antiviral properties of different plant extracts on petunia distortion strain of TMV. Indian J. Mycol. Plant Pathol., **1** : 126-131.

Torrance,L. and Harrison,B.D., 1981, Properties of Scottish isolates of cocksfoot mild mosaic virus and their comparsion with others. Ann. Appl. Biol. **91** : 283-295.

Torrance,L. and Jones,R.A.C., 1981, Recent developments in serologicals methods suited for use in routine testing for plant viruses. Plant Pathol., **30**: 1-24.

Uyemoto,J.K. and Hummer,D.K., 1973, Uranyl acetate useful as a specimen supporting membrane on EM grids. Phytopathology, **63** : 797-798.

Vandewalle,J.M. and Siegel,A., 1976, A study of nuceotide sequence homology between strains of tobacco mosaic virus. Virology, **73** : 413- 418.

Van Regenmortel,M.H.V., 1965, Plant Virus serology. Advan Virus Res., **12** : 207-271.

Van Regenmortel,M.H.V., 1967, Serological studies on naturally occuring strains and chemically induced mutants of tobacco mosaic virus. Virology, **31** : 467-480.

Van Regenmortel,M.H.V. 1975, Antigenic relationships between strains of tobacco mosaic virus. Virology. **64** : 415-420.

Van Regenmortel, M.H.V. 1981, Tobamoviruses, In: Handbook of plant virus infections and Comparative diagnosis. Krustak. E (ed.) Elsevier, Amsterdam, 541 pp.

Van Regenmortel, M.H.V. and Burckard,J., 1980, Detection of a wide spectrum of tobacco mosaic virus strains by indirect enzyme linked immunosorbent assays (ELISA). Virology, **106** : 327-334.

Varma,A. and Gibbs,A.J., 1978, Frangipani mosaic virus. CMI/AAB descriptions of plant viruses.No.196.

Verma,H.M. and Chowdhury,B., 1984, Occurence of a shoestring mosaic disease on Physalis minima L.Indian J. Mycol. Plant. Pathol., **2** : 95-99.

Verma,G.S., Varma,J.P. and Abidi,S.M.H., 1964, Pathogenicity of strains of tobacco mosaic virus. Indian J. Microbiology, **4** : 21-25.

Verma,V.S. and Singh,S., 1973, Trianthema portulacastrum - a local lesion host for TMV. Zentral bl. Bakt. Parasitk de Abl., **128** : 302-303.

Verma,V.S., Singh,S., Mishra,M.D. and Nam Prakash, 1972, Mosaic disease of a medicinal plant Solanum khasianum from India. Phytopath. Z., **75** : 52-58.

Villareal,L.R., 1980, Tomatoes in the tropics. Westview press, Inc, USA, 674 pp.

Wang, A.L. and Knight, C.A., 1967, Analysis of protein components of tomato strains of tobacco mosaic virus. Virology, **31** : 101-106.

Wetter,C., 1984, Serological identification of four tobamoviruses infecting pepper. Plant Dis., **68** : 597-599.

Wray,W., Boulikas,T., Wray,P.V and Hancock,R., 1981, Silver staining of proteins in polyacrylamide gels. Anal. Biochem., **118** : 197-203.

Zaitlin,M. and Israel,H.W., 1975, Tobacco mosaic virus (type strain). CMI/AAB descriptions of plant viruses.No.151.