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STUDIES ON A VIRUS CAUSING SEVERE MOSAIC OF TOMATO (*Lycopersicon esculentum* Mill.)

BY

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A DISSERTATION

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IN

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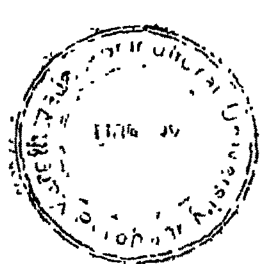

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This is to certify that Shri Gosavi Sudhir Bhagwanrao has satisfactorily prosecuted his course of research for a period of not less than five semester and that the dissertation entitled " STUDIES ON A VIRUS CAUSING SEVERE MOSAIC OF TOMATO " submitted by him is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the dissertation or part thereof has hot been previously submitted by him for a degree of any University.

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This is to certify that the dissertation entitled "STUDIES ON A VIRUS CAUSING SEVERE MOSAIC OF TOMATO" submitted by Shri SUDHIR BHAGWANRAO GOSAVI to the Marathwada Agricultural University in partial fulfilment of the requirement for degree of MASTER OF SCIENCE (Agriculture) in the subject of PLANT PATHOLOGY has been approved by the student's advisory committee after oral examination in colleboration with the external examiner.

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Chapter-1

INTRODUCTION

Chapter-I

INTRODUCTION

Tomato (Lycopersicon esculentum Mill.) is one of the most important Solanaceous crop which rank among the world's largest fruit vegetable crop. Tomato is originated from Brazil from where it has spread to other parts of the world. Tomato have an high vitamin content, the most important being ascorbic acid or vitamin C followed by Vitamin A, thiamine or vitamin B and riboflavin and vitamin B_2(Premnath, 1976).

This crop is grown all over India, the total acerage under tomato in the world is 1.875 million hectares with a total fruit production of about 38.83 million metric tonnes (Anonymous, 1976). Tomato is a well known popular fruit vegetable grown throughout India. Tomatoes always find a place in a kitchen garden whether small or big. The main tomato growing season is winter although it can be grown round the year, if irrigation facilities are available.

The total area under tomato cultivation in India is 40,600 hectares with 4.64 lakh metric tonnes of fruits production. In Maharashtra it is grown over an area of about 15,000 hectares but of which 5,800 hectares under kharif, 7,800 hectares for winter and 1,400 hectares under summer cultivation (Anonymous, 1979). In Marathwada it is grown on an area of 500 hectares out of which 200 hectares is under kharif and 300 hectares is under winter (Anonymous, 1979). However, area under this crop is

increasing since last 4-5 years, as a result of increasing irrigation facilities.

Fruits of tomato not only adorn the table in the form of soups, salads, preserver, pickles and many other tomato product, but also enrich the health of man.

There are several diseases infecting tomato caused by bacteria, fungi, mycoplasma, nematodes and viruses. Tomato crop is often found to be infected by viruses of which some of the viruses cause disease of economic importance. The well characterized viruses which naturally infect tomato include members of nepo, tomato spotted wilt, tombus, potex, poty, tobamo, cucumo and other virus groups (Matthews, 1979).

I   Nepovirus group :

1. Tomato black ring virus (Smith, 1946).
2. Tomato ringspot virus (Price, 1936).

II   Tomato spotted wilt virus group :

3. Tomato spotted wilt virus (Samuel et al., 1930).

III   Tombusvirus group :

4. Tomato bushy stunt virus (Smith, 1935; Ainsworth, 1936; Bawden et al., 1938).

IV   Potex virus group :

5. Potato X virus (Robert's, 1948; Wilkinson and Blodgett, 1948; Takens, 1979).

V   Potyvirus group :

6. Peru tomato virus (Raymer et al., 1972).

VI **Tobamovirus group :**

7. Tobacco mosaic virus (Palm, 1924; Poonam and Verma, 1977).
8. Tomato mosaic virus (Westerdijak, 1910; Allard, 1916; Broadbent, 1976).

VII **Cucumovirus group :**

9. Cucumber mosaic virus (Whipple and Walker, 1941; Hagedorn, 1950; Quantz, 1957).
10. Tomato aspermy virus (Blencowe *et al.*, 1949).

VIII **Other viruses :**

11. Tomato bunchy top virus (Pandey *et al.*, 1981).
12. Tomato leaf curl virus (Verma *et al.*, 1978; Verma *et al.*, 1980; Reddy *et al.*, 1979; 1981).
13. Tomato streak virus (Tsupkora, [illegible] 1980; Eskarous, 1979).
14. Tomato vein clearing virus (Dagar *et al.*, 1979).
15. Tomato yellow leaf curl virus (Mazyad *et al.*, 1979; Makkouk et al., 1979; Al-Musa, 1982).
16. Tomato yellow mosaic (Lastra *et al.*, 1981).
17. Tomato yellow top virus (Riberiro *et al.*, 1981).
18. Tomato yellow vein mosaic virus (Wilson *et al.*, 1981).

During field survey in the kharif season 1984-85 the tomato plants were observed to be infected by virus. The diseased plants were found to be showing severe mosaic,

leaf distortion and leaf elongation with shoe-string like symptoms. Therefore present investigation was undertaken to identify the causal virus.

The studies with following objectives were undertaken for partial characterization of virus causing severe mosaic disease of tomato and the results are incarporated in this dissertation.

1. Transmission
   - a) Mechanical transmission
   - b) Insect (aphid) transmission
   - c) Seed transmission
2. Physical properties in crude sap
   - a) Thermal inactivation point (TIP)
   - b) Dilution end point (DEP)
   - c) Longevity <u>in virto</u> (LIV)
3. Host range
4. Serology
5. Electron microscopy
6. Screening of tomato varieties for resistance against the virus in question.

Chapter-2

REVIEW OF LITERATURE

Chapter-II

REVIEW OF LITERATURE

Tomato are known to be infected spontaneously by many viruses of which some cause disease of economic importance. Viruses causing disease of tomato include the members of nepo, tomato spotted wilt, tombus, potex, poty, tobamo, cucumo and other virus groups (Harrison *et al.*, 1971; Matthews, 1979). Of which few important viruses are reviewed in this chapter.

2.1 Tomato mosaic virus (ToMV) :

Westerdijk (1910) was the first to report that tomato mosaic was caused by a virus. Subsequently Allard (1916) report tomato mosaic from U.S.A. Bewley (1923), distinguished five main types of symptoms. The first consisted of a simple mottling of the foliage without any distortion, the second type resembled the first, but the spots were indistinct in the outline and deep yellow in colour(probably similar to aucuba mosaic of potatoes). The third type was characterized by a distortion of leaf margin with no mottlings; in the fourth type the leaf surface was blistered and the margins distored; and in the fifth type the lamina was severly reduced, resulting in the formation of tendril-like leaves. The evidance available at present show that all these types are different manifestation of the same disease. Besides the mottling or blistering of

the leaves, cohesion and twisting of the various parts of the flowers and sterility of the anthers are common symptoms of mosaic disease, but mottling of the fruit is uncommon in Great Britain.

ToMV is mechanically, insect as well as seed transmissible. Tomato mosaic virus has been transmitted mechanically between different hosts (Miller, 1953). Ainsworth (1935) reported that the disease was found to be readily transmissible to healthy plants by mechanical transmission. Ainsworth (1932) reported that inoculation of tomato with sap from stripe plants often caused mosaic only. Høggan (1934) reported a very low level of transmission which, however, has not been substained (Orlob, 1963; Pirone, 1969). Some progress on this problem was made when Teakale and Sylvester (1962) showed that TMV co[illegible] be inoculated by aphids placed on virus-covered leaves. More recently Lokej and Orlob (1969) demonstrated that aphids can transmit TMV from a virus covered leaf to healthy leaf and they described experiments in which the green peach aphid, Myzus persicae Sulz. transmitted TMV from tomato and double infected tobacco. Cleveland (1931) reported that the Myzus persicae was believed to be mainly responsible for the insect transmission of tomato mosaic from tomato to tomato. Recently Singh and Shastry (1980) reported that the transmission of TMV by aphids were unsuccessful. Caldwell (1934), Gardner and Kendrick (1922),

Jones and Burnett (1935) and Nitzany (1960) have reported no seed transmission of TMV, in thousands of seedlings grown from seed produced by infected plants. Wildman (1959) also reported no knowledge of any confirmed report that TMV is transmitted through seeds. Two of the reports of Doolitte and Beecher (1937) and Raychaudhari (1952) indicated that TMV was transmitted in freshly extracted seed, but not in stored seeds. Berkeley and Madden (1932, 1933) presented evidence that TMV is present in embryos of seed produced by infected plants. The effect of different times and methods of storage (Doolitte and Beecher, 1937; John and Sova, 1955; Raychaudhari, 1952) and seed treatments (Alexander, 1960; Chamberlain and Fry; 1950; Nitzany, 1960) on the reduction and elimination of TMV in tomato seed. The conflicting reports on seed transmission may be due to differences in tomato varieties as suggested by Milbrath (1937). Alexander (1960) suggested that, TMV was recovered from tomato seeds after 3 years storage. Howles (1961) suggested that the tomato seeds infected by TMV in an incubator for 22 days at 72°C, inactivated must of the virus. Tomato mosaic virus transmitted through seeds (Ragozzing and Angelaccio, 1981; Macias, 1981).

The thermal inactivation point (TIP) reported for TMV include 80°C (Ho and Li, 1930); 80°C to 90°C (Howles, 1948); 85°C to 90°C (Smith, 1928; Verma and Kumar, 1982); 88°C (Brever et al., 1980); 90°C to 95°C (Doolitte and

Beecher, 1942; Fernandez-Northcote *et al.*, 1976); 92°C to 93°C (Mazyad *et al.*, 1969); 92°C to 94°C (Mamula *et al.*, 1974); 95°C (Khatri and Singh, 1977); 98°C (Das and Raychaudhari, 1953).

The dilution end point (DEP) reported for TMV include 10^{-6} (Smith, 1928); 1 : 1000,000 (Doolitte and Beecher, 1942); 1 : 10^{-6} (Mazyad *et al.*, 1969); 10^{-6} to 10^{-7} (Fernandez - Northcoate *et al.*, 1976); 10^{-7} (Verma and Kumar, 1982); 10^{-8} (Khatri and Singh, 1977); 1 :100,000 (Das and Raychaudhari, 1953).

The longevity *in-vitro* (LIV) reported for TMV at room temperature include 45 days (Verma and Kumar, 1982); 60 days (Ho and Li, 1936); 6 months (Fernandez-Northcoate *et al.*, 1976); 9 months (Khatri and Singh, 1977); 12 months (Smith, 1928); 6-20 months (Brewer *et al.*, 1930). 36 months at 21°-27°C and 7 years at room temperature (Doolitte and Beecher, 1942); 72 months (Mamula *et al.*, 1974) and 78 months at room temperature (Mazyad *et al.*, 1969).

The virus have very wide host range along with the Atlantic coast region of Morocco. Lockhart and Fischer (1973) reported that the systemic hosts of TMV were *Lycopersicon esculentum* cv. Bonnie best; *Capsicum annuum* cv. Yolowendes; *Nicotiana tabacum* cv. Turkish; *N. glauca*; *Physalis floridana*. Local hosts included *N. glutinosa*, *N. tabacum* cv. Xanthi-nc, *Petunia hybrida*, *Datura stramonium* and non hosts included *Vigna sinensis* cv.

Early Ranshorn; Phaseolus vulgaris cv. Pinto; Cucumis sativus cv. National Pickling, Vicia faba.

The virus particles were reported as straight rigid rod shaped i.e. 300 nm (Gibbs, 1977; Holling and Huttinga, 1976).

2.2 Tobacco Mosaic virus :

Palm et al., (1924) reported that some species are tabulated as disease carrier for tabacco mosaic for the first time. Poonam and Verma (1977) reported two viruses isolated from field grown tomatoes in Hariyana shows some slig difference from the common or wild strain of tobacco mosaic virus. Savino and Castellano (1978) reported a severe disease of tomato cv. Molese characterized by dwarfing, loss of yield, malformed leaves and enation is reported. The pathogen was a strain of tobacco mosaic virus serologically distinguishable from other strain isolated from tomato with ordinary mosaic symptoms. Symptoms were reported on Solanum marrano on inoculation with infected sap of the purified virus. Vlasov et al., (1978) observed stem mottle disease of tomato in Crimea region a specific strain of tobacco mosaic virus causing stem mottling and bright yellow white mosaic on the leaves. Similar symptoms appeared on fruit. The symptoms were reproduced in inoculation test on tomato and Gomphrena globosa. Andrade et al., (1981)reported severe outbreaks varying in incidence from 5-42 per cent occured, in commercial

planting. The causal agent was identified as ToMV (tobacco mosaic virus, tomato str.) on biological, physical, morphological and serological properties. Davino *et al.*, (1983) reported that tobacco mosaic virus mainly affected tomato. He observed symptoms, inoculation of indicator plants and serological tests and showed various degree of infection by tobacco mosaic.

The efficiency of mechanical transmission have been reported to increase by the use of phosphate buffer (Yarwood, 1952) and abrasive (Celite and Carborandum). A rapid method for indexing for TMV was by rubbing tomato leaves (Murakishi, 1960).

It is well known that many plant viruses can be transmitted by aphids, but the highly infectious tobacco mosaic virus (TMV) seems to have no aphid vector. Only Hoggan (1934) reported a very low level of transmission which, however, has not been substantiated. Recently Singh and Sastry (1980) reported that the transmission of TMV by aphids was unsuccessful. Dubey *et al.*,(1981) from Himachal Pradesh in India reported the virus isolated was transmitted by *Myzus persicae*, *Brevicoryne brassicae* and *Aphis fabae*.

The virus is also transmitted through seed. Karimov (1928) reported seeds are the primary source of infection by TMV. Those with necrotic symptoms are marked smaller than healthy seed and sometimes deformed and

undeveloped. Germination was 10 per cent lower. The seed germination was 10 per cent lower. The coat and endosperm carry TMV in high concs. Dubey, et al., (1981) reported the virus isolated was seed borne and infected many hosts. Kwaje and Young (1979) reported transmission was not systemic in seeds from diseased fruit but TMV was carried on the seed coats. Alekseen et al.,(1974) reported in the Astrakhan region the incidence of TMV on tomato reaches 100 per cent at the end of the growing period. Seeds was the main source of infection. The virus was found on the seed surface in the seed coat and in shoots. Shmyglya et al., (1984) reported seed transmission in Turkish tobacco.

Vlasov et al.,(1978) reported the disease observed in Crimea region caused by a specified strain of tobacco mosaic virus having thermal inactivation point of the new TMV strain was $> 98^{\circ}$ and dilution end point 10^{-10}. Poonam and Verma (1977) reported two isolates of TMV. The dilution end point of isolate 1 was 10^{-4}-10^{-5} and that of isolate 2, 10^{-3}-10^{-4}. The respective thermal inactivation point were 95 and 90°C respectively. Isolate 1 and 2 withstood ageing upto 9 and 13 days at room temperature and upto 15 and 39 days at -20°C respectively. Dubey et al., (1981) reported the thermal inactivation point was 94-95°C and dilution end point 1 : 75,000 - 1 : 80,000 and longevity *in-vitro* 49 days at room temperature and 14 weeks at 7°C. In dried leaves

the infectivity can remain for 18 weeks. Erkan *et al.*, (1983) reported the dilution end point was 10^{-7}-10^{-8}, thermal inactivation point 90°-95°C and longevity *in-vitro* more than 60 days at 20°± 2°C.

Palm (1923) reported that mosaic disease was transmissible to peppers (*Capsicum annuum* and *C. frutescens*), *Solanum torvum*, *S. ferox*, egg plant (*S. melongena*), tomato (*S. lycopersicon*), *Physalis angulata* and *Cucurbita* sp. Palm *et al.*, (1924) reported some hosts as disease carriers for tobacco mosaic were *S. verbascifolium*, *S. torvum*, *Citrullus vulgaris*, *Scoparia dolcis* and *Physalis minima*. Erkan and Yorgana (1983) reported that wilt, stunting, stem necrosis, defoliation, mosaic and leaf and fruit deformation were noted on infected *Capsicum annuum*. The virus isolated failed to infect *Nicotiana glauca*, induced latent infection on tomato chlorotic local lesions on *Gomphrena globosa*, necrotic local lesions on some *Phaseolus vulgaris* cultivars and local and systemic lesions on *Petunia hybrida*.

Van Koot (1940) reported the following methods of purification were applied to tobacco mosaic and single virus streak of tomato at the Wageningen Mycological Laboratory. 1) Stanley's filtration through celite and seperation of the virus by repeated precipitation with ammonium sulphate; 2) Bawden and Pirie's heating to 70°C and seperation of the virus after chemical treatment and 3) Ryiyoff's and Gromyko's elimination of the pigment free juice consists

in filtration through celite, virus purification with 40 per cent ammonium sulphate and only one treatment with 1 or 2 per cent charcoal, all at pH 7. The tomato single virus streak are slightly shorted (15µ) than those of tobacco mosaic (20 to 25µ). The virulence of the tomato virus, as measured by local lesions on N. glutinosa, remained intact during a protracted process of chemical purification where that of tobacco mosaic declined appreciably. Lima and Chagas (1974) reported a purified extract of TMV from systematically infected tobacco leaves was highly infective to tobacco, tomato and Chenopodium amaranticolor. Antiserum obtained by rabbit immunization reacted specifically with the purified virus and with crude sap from infected tomato and tobacco plants.

Dubey et al., (1981) reported rod shaped particles measured 300 x 18 nm. Yilmaz (1981) reported rod shaped particles, C. 300 nm in length. Erken et al., (1983) reported rod shaped particles with 300 x 15 nm in length were noted in infected spp.

Tewari et al., (1973) presented data on varietal reaction of tomatoes to TMV. Few lines had resistance comparable to that of L. peruvianium, 4 others were highly resistant in winter but somewhat susceptible in summer and one variety proved immune. Of the four wild spp., L. peruvianum was the most resistant followed by L. hirsutum.

Mayee *et al.*, (1977) reported of 100 lines screened against the tomato strain of TMV prevalent in the Punjab, 10 showed high resistant. Gracia *et al.*, (1979)reported of 100 plants examined 89 were infected by 1 virus and 11 by combination of 2. Incidence was tomato spotted wilt virus 49 per cent, tobacco mosaic virus 42 per cent, potato virusY16 per cent and cucumber mosaic virus 4 per cent.

Khotimirs and Pogoris (1977) reported that young plants of tomato var. Ukrains'Kii teplichnii with symptoms of TMV were used as initial material. Diagnostic serum was specific reaching only with homologous antigen. The titre of the serum after reimmunisation of rabbits was 1: 512.

2.3 Cucumber mosaic virus (CMV):

One of the earliest reference made by Smith (1949) in England on virus diseases of tomato. They are cucumber mosaic virus and tobacco mosaic virus. It's strain causing aucuba mosaic stripe and enation tomato spotted wilt, tomato black ring and tomato bushy stunt also double virus streak (tobacco mosaic virus and potato virus X), neither of which occur frequently in this country. Kobatake (1974) reported cucumber mosaic virus. He described its symptoms on tomato in Nara Prefecture. Waterworth and Povish (1975) reported a strain of CMV isolated from flowers of Ixora with mosaic and imported from the Philippines was serologically distinct from strain D, Q and S, caused different symptoms on several hosts including cucumber, tomato and Datura. Ignash (1978)

described 3 strains of CMV, CMV strain1isolated from cucumber and turip, strain 2 from tomato and strain 3 of tomato aspermy virus were investigated. Gracia *et al*. (1979) reported virus deceases of tomato in Mendoza provine, of 100 plants examined 89 were infected by 1 virus and 11 by combination of 2. In which incidence of cucumber mosaic virus was 4 per cent. Andrade *et al*., (1981) reported tomato mosaic virus associated with shoe-string symptoms in Chilean tomatoes. In which cucumber mosaic virus was not found in any of 5 field samples assayed. Davino *et al*.(1983) reported incidence of some viruses on tomato and capsicum crops in Sicily. Cucumber mosaic virus was predominanted on *Capsicum annuum*. Fann *et al*., (1982) identified the causal viruses of tomato mosaic in Guangdong provine. CMV, TMV (Common strain) and TMV (tomato strain) were identified in 16, 8 and 4 of the 10 major tomato growing localities respectively and 34.4, 12.6 and 13.9 per cent of the 223 mosaic isolates. Doolittle (1921) reported the influence of temperature on the development of mosaic disease of cucumber. Higher soil and air temperature were found to favour the development of cucumber mosaic.

Omar *et al*., (1982) described the 3 strains of CMV studied needed at least 48 h to multiply in inoculated leaves after which they could be detected in all leaves of the plant showing that virus particles more with

assimilates. No virus is detected in uninoculated leaves were detected 24 h after inoculation, CMV was detected in all components of cucumber fruits, including seeds in exocarp, mesocarp and endocarp of tomato fruits.

Chin (1983) reported transmission of CMV strain O was obtained with *Myzus persicae* (most efficient), *Aphis citricola* and *Toxoptera aurantii*, the last being previously unreported as a vector *A. citricola* and *Hysteroneura setariae*, less efficient, were reported as vectors of the virus for the first time. CMV is also transmitted through dodder spp.

Quiot *et al*., (1979) reported the epidemiology of shoe-string disease and necrosis of tomato caused by CMV in South-East France. Observations over 4 year showed that tomato is less susceptible to CMV than melon. Doolittle (1921) reported that mosaic was transmitted from cucumber to *Asclepias ryrica*, *Capsicum annuum* and *Martynia lonisiania* and from these plant to cucumber. The wild cucumber (*Micrampelis labata*) is however, most important in the over wintering of cucumber mosaic. The disease is transmitted through seeds of the wild cucumber. Migliori *et al*.,(1978) described 21 hosts of the virus including 7 new ones, are listed. The principle weed host was *Commelina diffusa*. The prevalent strain was the leguminous type, systemic on cowpea and *Phaseolus vulgaris*. *C. diffusa* was infected by the systemic Vigna strain as well as by a

local v. strain, singly or in combination. P. vulgaris and several Papilionaceae, particularly V. hoesi, were infected only by systemic v. strain. The local v. strain was predominant on tomato, cucumber and Capsicum annuum var. Gossum.

Hatta and Francki (1981) described electron microscopy of leaf cells infected with the virus revealed membrane-bound vesicles 50-90 nm diameter, some having membrane continuous with the tonoplast.

Kuniyasu et al., (1975) reported the MR 9, MR 12, Ohio 690712 and L 253 had the fewest symptoms following inoculation with CMV.

2.4 Potato virus X (PVX):

Experiments on the spread of five strains of potato virus X were made with seven potato varieties and tomato plants both under glass and in the field. The rate of spread was much greater in tomato than in potato plants and virulent strains of the virus, which achieve a high concentration in infected plants, spread more rapidly than avirulent strain (Roberts, 1948). Wilkinson and Blodgett (1948) reported local lesions without, systemic infection, developed when Gomphrena globosa the globe amaranth was inoculated mechanically with potato virus X, provided carborundum was used. Lesions appeared in 2 to 3 days and were encircled by a red ring within a week after inoculation.

Talens (1979) reported a new strain of potato virus X, which identified as a ring spot strain of potato virus X and identified from seed stock used for commercial seed potato production in the Mountain Prov. Davino *et al.*, (1983) reported incidence of potato virus X on tomato crop in Sicily. In India Dubey (1983) reported association of potex virus with a mosaic disease of tomato.

The virus was mechanically transmitted with ease but not by aphid; *Myzus persicae* (Verma and Baranwal 1983). Dubey (1983) reported a virus isolated from field tomato which was found to be sap and seed transmissible. Transmission was reported by grasshoppers, *Melanoplus differentiallis* (Walters, 1951). Virus was also transmitted by fungus, *Synchytridium endobioticum* (Nienhauns and Stille, 1965). The virus [illegible] also infect some dodder species (Schmelzer, 1956). Singh and Mall (1974) reported physiological changes induced by potato virus X on tomato plants.

The virus affected many hosts. Talens (1979) reported that the virus caused ringspot, systemic line pattern and necrotic streaks on tobacco, mosaic on *Capsicum annuum*, *Datura stramonium*, tomato and *Nicotiana rustica*, necrotic local lesions on *Gomphrena globosa* and chlorotic local lesions on *Chenopodium amaranticolor*. Dubey (1983) described a wide host range for potato virus X on tomato. Out of 61 plants species from 14 families, the virus [illegible] infected only a few members of family Solanaceae, Fabaceae,

Brassicaceae and Chenopodiaceae. He described the host range as *Lycopersicon esculentum* L. vars. White and No.8, *Capsicum annuum* L., *C. pendulum* Willd, *Solanum nigrum* L., *Chenopodium amaranticular* Reyn and Coste, *C. album* L., *Datura metel* L., *Calendula officinalis* L., *Glycine max*, *Nicotiana glutinosa* L., *N. tabacum* L. and *Solanum tuberosum* L. Dubey (1983) reported that tomato was attacked by several strains of potato virus X. A comparision of virus, which he described with different strains of potato virus X reported on tomato and other hosts.

The virus in sap lost its infectivity on heating at 80°C for 10 min but not at 75°C. The dilution end point was found to be in between 10^{-5} - 10^{-6} and longevity *in-vitro* 18-21 days at room temperature (24°-34°C), 30-33 days at 7°C and 27 days in desiccated leaves of tomato (Dubey,1983).

In 1979, Talens reported that electron miscroscopic demonstration of flexuous rods 15 x 550nm in partially purified preparation, confirmed the identity of the virus. Yilmaz (1981) reported the particle size is c. 540 nm with filamentous rods. Verma and Baranwal (1983) reported the virus had slightly flexible particles with length ranging between 540-600 nm. Dubey (1983) reported virus particles appeared as flexible rods with a model length of 530x15nm.

2.5 Tomato spotted wilt virus (TSWV):

Spotted wilt on tomato first described by Carne (1924) in Victoria and other eastern parts of Australia.

Samuel and Bald (1933) described in South Australia, tomato spotted wilt though usually present only on isolated tobacco plants is a common disease of this crop. McWhorter (1934) also report this disease from Oregon greenhouse in England. Berkely (1935) reported that since 1931 disorder suspected to be spotted wilt of tomato was present in Ontario but only in 1934 it was possible to undertake experimental studies which showed conclusively that this or a very closely related disease was present in that province and Sascatchewan in Canada. Chamberlain and Taylor (1936) reported a serious tomato disease, known locally as "stripe" or 'brown top' but considered to be identical with spotted wilt was present for some years in the HuttValle, New Zealand. Bonnemaison (1939) discusses the information available on spotted wilt of tomato and its insect vector, Thrips tabaci in France. Norris (1943) has obtained evidence that the tomato spotted wilt virus was a complex of related strains, probably three, one of which (the necrotic) appears are the ringspot and mild strains. No tipblight indicating complete seperation of the necrotic strains has yet been observed in Australia. Best (1961) recommended experiment on earlier work was confirmed by further experiment at the Waite agric. Res. Inst., Univ. Adelaide. Inouye and Inouye (1972) reported the virus was isolated from dahlia var. Komyo showing severe chlorotic and necrotic ringspots, the first record of it in Japan except for an isolate from an imported dahlia in

quarantine. Zafar (1975) reported TSWV from Pakistan for the first time. The virus cause severe infection of tomato plants and fruits at the Agric. Res. Farms at Tarnab, Peshawar and other tomato fields in the area. Todd <u>et al</u>., (1975) reported the first occurrence of TSWV from India in the Nilgiri Hills. The disease caused a severe infection on Marglobe tomatoes in 1964. Kobataka <u>et al</u>. (1976) first reported the virus from Nara pretecture in Japan. McGuire <u>et al</u>., (1979) also reported this disease from Arkansas. Joshi (1980, 1981) reported TSWV from Andhra Pradesh in India. The virus was causing bronzing and necrosis and was identified from symptoms.

Samuel and Bald, (1933) described the symptoms of TSWV on tobacco, the symptoms consisted of necrotic spots on the younger leaves, necrotic areas along the lateral veins, usually on the lower half of the leaf and various combination of these symptoms as well irregular necrotic spot and line patterns.

The virus was sap transmissible (Gardner and Whipple 1934) when rubbed to a large number of hosts. McWhorter (1934) reported that the virus was sap transmissible when inoculation with rubbing were made to the leaves of Petunia plants from tomatoes grown in a greenhouse in Oregon. Chamberlain and Taylor (1936) also report the disease was sap transmissible. Vlasov <u>et al</u>., (1977) reported that in inoculation tests in several indicators a strain of TSWV from tomato failed

to infect tobacco but a strain of the same virus from tobacco infected both tobacco and tomato. The virus was also transmitted by *Thrips tabaci* as reported by several workers (Bald *et al*., 1931; Ainsworth, 1932; Gardner *et al*., 1935; Sakimura, 1961). Samuel and Bald (1933) reported that TSWV was transmitted by *Frankliniella insularis* and *Thrips tabaci*, which was also reported by Berkeley (1935). Paliwal (1976) reported two isolates of TSWV from BC transmitted by *Frankliniella occidentalis* which was common in BC but not in Eastern Canada. He also reported that neither isolate was transmitted by *Thrips tabaci* which was a TSWV vector in some other countries. Iwaki *et al*., (1984) reported silver mottle disease of watermelon caused by TSWV, which was transmitted by thrips. This is the first report of TSWV naturally infecting Cucurbitaceae.

Bald and Samuel (1931) reported spotted wilt was experimentally transferred from tomato to tobacco and 14 other species of *Nicotiana* as well as to 7 species of *Solanum*, *Capsicum annuum*, *Datura stramonium*, *Hyoscyamus niger*, *Lycium ferocissimum*, *Petunia hybrida*, *Physalis peruviana*, *Salpiglossis* sp., *Schizanthus* sp., *Aster* sp., and *Chrysanthemum* sp., Ainsworth (1932) described some hosts, which were tobacco *N. macrophylla*, *N. glutinosa*, *D. stramonium*, *Solanum capsicastrum*, garden petunias, dahlias, cinerarias and *Chrisanthemum* sp. were found naturally infected. Gardner *et al*., (1935) reported some additional hosts for

TSWV which include cauliflower, celery, Nicotiana glauca and species of Amaryllis, Begonia, Brooallia, Campanula, Cheiranthus, Delphinium, Gloxinia, Godetia, Gavillordia Laya, Papaver, Pentstemon, Pumula, Salvia and Verbena.

Berkeley (1935) reported that TSWV from Canada had a thermal inactivation point 45°C and the longevity in-vitro 1/2 to 2 hours. Gardner et al. (1935) reported that the virus has been found to survive 73 hours storage at 0°C. Chester (1934) reported that the virus was unstable in-vitro and usually inactivated by temperature below 55°C. Recently Inouye and Inouye (1972) reported the thermal inactivation point for TSWV was 40-45°C Dilution end point 10^{-3}-10^{-4} and longevity in-vitro 1-2 hours at 20-23°C.

Electron micrographs of the virus particles in negatively stained dip preparation showed spherical particles c. 70-90 nm diameter, but in most causes colapsed and/or variously deformed (Inouye and Inouye 1972).

Rao et al., (1980) reported that incidence of the virus on 23 cvs. in the field in rabi 1978 and kharif 1979 (tabulated) was 1.58-15.50 and 100 per cent respectively. The low incidence in rabi was due to scarcity of thrips vector at that time.

2.6 Tomato bushy stunt virus (TBSV):

Fischer and Lockhart (1977) reported two variants of TBSV were isolated from tomato and Capsicum respectively.

This apparently is the first report of natural infections of Capsicum by TBSV. Börges *et al.*, (1979) reported TBSV from Portugal on glasshouse tomato, was identified on the basis of symptoms, on various hosts inoculated by sap inoculation, gel diffusion serology and immuno electron microscopy with TBSV serum strain B-3. Novak *et al.*, (1980) reported the first record of TBSV on plants of the Plumbaginaceae. Novak *et al.*, (1981) reported that by bioassays and serological tests TBSV was detected for the first time in Czechoslovakia on lettuce plants with leaf curl mosaic and small necrotic spots on main and secondary leaf veins. Novak and Lanzova (1982) gives details about the incidence of TBSV in plum trees with shorkalice fruit symptoms and in cherry with detrimental canker.

Novak *et al.*, (1980) reported that the virus was sap transmissible. Campbell (1968) reported that at the A.R.C. virus Res. Unit. Cambridge, England, attempts to transmit TBSV to tomato and *Datura stramonium* by using a naturally occurring tomato associated isolate of *O. brassicae* as a vector were unsuccessful, although all the test plants became infected by the *Olpidium*. The virus was also transmitted through dodder sp.

Novak *et al.*, (1980) reported *Gomphrena tartaricum* and *Solanum sinuata* being new natural hosts for TBSV. Novak and Lanzova (1982) reported some new natural hosts, including *Fragaria ananassa*, *Fraxinus excesior*, *Lonicera henryi*,

Robinia psendoacacia, Cotinus cogqvria, Daphue mezereum skimmia japanica, Limonium tartaricum and Rumex crispus.

The thermal inactivation point of TBSV was at 84-85°C, dilution end point was 10^{-6} and stability in sap at 22°C upto 45 days (Novak et al.,1981).

Bawden and Pirie (1943) reported a new method for the purification of TBSV. Borges et al., (1979) reported the isometric virions by the disp method. Yilmaz (1981) reported that the particle length was about 30 nm with isometric rods.

Cherif and Spire (1983) reported that of tomato varieties tested 5 were resistant and 10 tolerant. No local variety of Capsicum annuum was resistant.

2.7 Tomato aspermy virus (TAS):

Tomato aspermy virus was first isolated by Ainsworth (1939) from chrysanthemum. Brierly et al., (1955) induced symptoms in chrysnathemum on inoculation with the virus of mosaic. Procter (1975) reported that TAV was found in Chrysanthemum in N_2 for the first time, in commercial gardensin Christechurch, Wanganui and Auckland in New Zealand. Ignash and Ikounietse (1976) reported that the tobacco cv. Samson is recommended for multiplication of the virus. When sap of N. glutinosa infected 10 days earlier, was used as inoculum 100 per cent infections were possible during the summer. In the glasshouse the plants should be grown at air temperature 18-25°C. Pop (1979) reported that from 1972, tomato crops in the Bucharest district showed symptoms of dwarfing and

marked tip deformation, as a result of the absence, reduction or deformation of leaflets. Tests and serological reactions indicated that the disease is caused by a severe strain of tomato aspermy virus. Kuti and Moline (1984) also reported the virus from Maryland.

Yassin (1975) reported evidence suggesting that tomato condensed top virus on tomato in Sudan is possibly a strain of tomato aspermy virus. Both viruses can produce necrotic local lesions and almost complete cessation of growth at the apex of infected tomato plants, fruits are often seedless and have aborative seeds.

The virus was sap, seed, insect and dodder transmissible reported by several scientists. Procter (1975) reported that the virus was transmitted mechanically among different hosts. He also reported that the virus was transmitted by *Myzus persicae*. Yassin (1975) reported that the physical properties of the virus were similar and both were transmitted by *Myzus persicae*. The viruses were serologically related and evoke similar response in cucumber, (top virus of tomato, and tomato aspermy virus). Basky (1983) reported that the aphid activity was greater in June than in July.

Isolate from *Campanula rapunculoides* was seed borne in *Stellaria media* (Noordam *et al*., (1965). The virus was not seed borne in *N. glutinosa*, *N. tabacum* and *Callistephus chinensis* (Brierley *et al*., 1955). The virus was poorly transmitted by *Cuscuta subinclusa* and *C. europea*; not

transmitted by three other species (Schmelzer, 1957).

Procter (1975) reported that the virus was transferred experimentally to Calendula officinalis, Tropaeolum majus, aster, capcisum, chrysanthemum, lettuce, tomato, Nicotiana glutinosa, Chenopodium amaranticolar, tobacco, Solanum nitrum, Tetragonia expansa and Zinnia. Bounty spinach was a suitable propogative host, yielding 1-5 mg virus per 100 gm leaf tissue 12-14 days after inoculation. C. quinoa was a reliable assay host, producing necrotic local lesions 6-9 days after inoculation (Kuti and Moline 1984).

The longevity of the virus was 7-11 days and the thermal inactivation point 65-70°C. The dilution end point between 1-50,000 and 1-100,000 (Procter, 1975). Longevity in-vitro of TAVM by Kuti and Moline (1984) was 30-48 at 25°C, thermal inactivation point 60-65°C and the dilution end point 10^{-4}-10^{-5} in Bounty Spinach.

Raos et al., (1982) reported that distant antigenic relationship between Australian strains of the virus were investigated by three serological methods radial double immunodiffusion in agar, Elisa and immuno electron microscopy (IEM) using the antibody detoration technique. Results indicated that the LISA method was especially suitable for studying antogenic relationships among viruses. Kuti and Moline (1984) reported the particle size of TAV was 30 nm in diameter with isometric particles.

2.8 Tomato ringspot virus (TRSV):

First detected in India in 1930, the disease has since been found affecting upto 90 per cent (Samson and Imle 1942); Smith (1932) reported an account on its further investigation of the ringspot virus of Solanum capsicastrum.

The tomato ringspot disease was transmitted mechanically by the carborundum method to tomato, potato and Emilia sonchifolia from tomato and E. ronchifolia and by grafting from tomato to tomato and potato. The incubation period in tomato was 10 to 16 days, depending on vigour of growth, the more vigrous growth after inoculation the more rapidly the symptoms appear. The virus quick lost its infectivity in extracted juice, and after one week was recovered from diseased plants only with difficulty. It was not seed transmitted.

Smith (1932) reported the transmissibility of this disease by Thrips tabaci. It also tends to throw doubt on the author's previous experiments indicating Myzus persicae as on occasional transmitter. Iwaki and Komuro (1974) reported that soil of narcissus in Chiba containing Xiphinema americanum transmitted TRSV to Petunia, air drying of the soil at 22-30°C, for 1-3 weeks removed infectivity.

Smith (1932) reported TSRV's identification with spotted wilt of the tomato. It is highly probable that spotted wilt has been at times recorded on tomato mosaic, streak or stripe.

A study of the host range of the virus showed that all the species of Solanaceae tested, including potato, tobacco, chilli pepper, egg plant and petunia were susceptible to it, and it was not transmissible to lupins, dahlias, asters, zinnaias and plantain. Samson and Imde (1942) from India reported the host range for TRSV. The virus from tomato and *Datura stramonium* was successfully inoculated into other tomato varieties and 19 members of the Solanaceae, including potato, Turkish tobacco, *Nicotiana attinis*, eggplant, Ruby King pepper, *Hyoseyamus niger*, *Solanum pseudocapsicum*, *S. curolinense*, and *Nicandra physaloides* (the two last named and *D. stramonium* being natural hosts), two species of Amaranthaceae (*A. tricolor* and *A. retroflexus* and *Martynia louisiana* : from all these except pepper, *H. niger*, *A. retraflexus*, *S. pseudo-capsicum* and *M. louisiana* the virus was recovered by return inoculation to *D. stramonium* and tomato. The symptoms of the ringspot closely resembled these of spotted wilt (though bronzing is not a characteristic symptom) but the thermal death point of the farmer was higher (56 to 58°C compared with 42°C) and its host range narrower. Moreover plants suffering from ringspot were not isolated from infection by suspected spotted wilt virus. The virus survived ageing *in-vitro* for 21 hours compared with 6 for the tomato spotted wilt virus. Salazar *et al.*, (1975) reported that he obtained a nepovirus from potato in Peru which was destinctly

serologically related to tobacco ringspot virus. The virus was inactivated in 10 minute at 57-60°C. when diluted 10^{-3}-10^{-4} or stored at room temperature for 9-10 days. Tomato was readily infected and the virus was always systemic in _Chenopodium amaranticolor_ and _C. quinoa_

2.9 Tomato black ring virus (TBRV):

The virus was first reported from India by Sastry (1966) causing necrotic disease of tomato seedlings at I.A.R.I. New Delhi in 1962. Iwaki and Komura (1973) reported the virus from Trumpet narcissus with symptoms of mosaic and brown spot, was identified by transmission, tests, host range, physical properties, perticle size and serological reactions as the beet ringspot strain of tomato black ring virus, not previously reported from Japan.

Chernoivanova (1973) also reported some dangerous virus diseases of potato and tomato. These include map top potato virus and tomato black ring virus. Buturovic _et al._, (1979) reported this virus from Yugoslovia, the isolate which previously found on potato and grape vine, were collected from sugarbeet, Capsicum, tobacco and potato. All were serologically identical.

Kaminaska (1983) reported tomato blackring and cucumber mosaic were isolated from Forsythia in Poland. Among 96 plants examined, 45 per cent of the older ones and 34 per cent of the younger ones are infected. TBRV was the most

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frequent isolate. The virus was sap, seed and nematode transmitted.

Iwaki and Komuro (1973) reported that TBRV was seed transmitted in chickweed (Steliaria) and soybean. Whereas it was also transmitted by infected seed material and through soil (Chernoivanova, 1973). Harrison et al., 1975 worked on nepoviruses included the seed transmission TBRV which they found that this si may be determined by RNA-1. Migliori et al., (1984) from France demonstrated soil transmission and the nematode Longidorus attennatus present in the rhizosphere of infected plants, transmitted the virus to artichoke, tobacco and Physalis floridana. Forer et al., (1975) reported that oxymyl applied to field soil prevented L. elongatus from acquiring and transmitting TBRV for at least 6 weeks, although number of nematodes were not greatly decreased compared with the untreated control. Glasshouse and lab tests showed that oxamyl (1 ppm) in soil water largely prevented L. elongatus transmitting virus to bait plants over a period of 1 month Xiphinema diversicaudatum was equally affected by smaller cones. 0.1 ppm being sufficient to inhibit virus transmission in one test. Harrison et al., (1961) reported that at Rothamsted and the Scot. Hort. Res. Inst., Invergowrie, Dudde, L. elongatus was always found in soil where patches of plants in raspberry and straw-berry plantation were infected by the beet ringspot (Scottish)

strain of TBRV, but was seldum discovered, eleswhere in the same plantation.

Host range was wide and infect many species of wild and cultivated monocotyledonous and dicotyledonous plants. An isolate from Robinia pseudoacacia infected 76 species in 29 dicotyledonous families after mechanical inoculation (Schelzer, 1963) Chenopodium amaranticolor, C. quinoa, Nicotiana rustica, N. tabacum, Phaseolus vulgaris, Petunia hybrida and N. clevelendii.

In tobacco sap, the virus usually losses infectivity after 10 minute at 60-65°C, storage at room temperature for 2-3 weeks or dilution to 10^{-3}-10^{-4}. The lesion number decreases with dilution by more than the dilution factor (Harrison, 1957). Buturovic et al., (1983) reported that TBRV was inactivated at 60-68°C and the dilution end point was 10^{-5} to 10^{-6}. The virus particle [illegible] was isometric, about 30 mm in diameter with a 5- or 6- sided angular outline (Harrison et al., 1960).

2.10 Peru tomato virus (PTA):

Raymer et al., (1972) described for the first time a new virus of tomato causing leaf distortion, stunting and severe yield reduction was isolated from tomato plants near Trujillo, Peru. The virus was designated as peru tomato virus (PTV). The virus was classified in the potyvirus group on the basis of particle morphology, aphid transmissibility,

induction of cylindrical inclusions in infected cells and serological relationship to some poty viruses (Fernandez-Northcote *et al.*, 1979).

The virus was transmitted by vector *Myzus persicae* after acquisition access period of 30 second inoculation access times of 15 minutes (Raymer *et al.*, 1972) seed transmission was not detected. Seedlings derived from infected plants of tomato, pepper and *Nicotiana physalodes* were virus-free. Natural infection was detected in tomato, pepper, *Physalis pernviana*, *Solanum nigrum* and *Nicandra phyralodes*. Except for a few Chenopodiaceous species, which reacted with local lesions, the host range was restricted to the family Solanaceae. No infection was obtained by sap inoculation of 84 species and 21 cultivar in 17 families (Fernandez-Northcote, 1978; Fernandez-Northcote *et al.*, 1980a; Fribourg, 1979).

Raymer *et al.* (1980) reported some host plants for PTV were, *Lycopersicon pimpinellifolium*, *Nicotiana debneyi*, *N. occidentalis*. He also reported that virus was transmitted by *Myzus persicae* in a non persistent manner. *Datura stramonium* was immune to PTV.

In Burley 21 tobacco sap, the virus was inactivated by heating to 55-60°C for 10 minute or by dilution beyong 10^{-5}. It retained infectivity after storage for 10-21 days

at 20-22°C. Infectivity survived for 8 years in tobacco leaves dried over anhydrous $CaCl_2$. Raymer *et al*., (1980) reported that dilution end point of the virus in sap was 1:1000-1:10,000 and the thermal inactivation point was 60-70°C.

Raymer *et al*., (1980) reported electron micrographs of leaf dip from infected tissue contained flexous rods 750 nm in length and particle width is about 12 nm.

Chapter-3

MATERIALS AND METHODS

Chapter-3

MATERIALS AND METHODS

3.1 Source and isolation of virus :

The initial source of the virus inoculum was from field grown cultivars of tomato showing severe mosaic, leaf distortion and leaf elongation symptoms. For isolation of virus, the stock culture of the isolate was maintained seperately in glasshouse on *Nicotiana glutinosa* for further detailed studies.

3.2 Transmission studies:

3.2.1 Mechanical transmission :

For mechanical transmission, inoculation were made by conventional leaf rub method. Inoculum was prepared by triturating symptomatic leaves of *Nicotiana glutinosa* with a mortor and pestal in a cold, 0.1 M phosphate + merchaptoethenol buffer (pH 7.0) and applied with cotton swab to the leaves of text plants previously dusted with 800 mesh carborandum. The test plants were inoculated by conventional leaf rub method with a cotton swab. Carborandum (800 mesh) was used as an abrasive. Immediately after virus inoculation, leaves of the test plant were rinsed with tap water. The plants used for mechanical inoculation were raised from healthy seeds in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture. Test plant were maintained in an insect free glasshouse for 4-6 weeks and observations were recorded with respect to the sequence of symptom

development.

3.2.2 Aphid transmission :

For aphid transmission, cotton aphids (*Aphis gossypii* Glov.) raised from single aphid colony were used. For raising an aphid colony, the healthy leaves of cotton were placed in different petridishes, on a slightly wet filter paper and an apterous form of single aphid was transferred with small camel hair brush to the test leaves. Petridish was closed for eight hours and the newly borne aphids were transferred to healthy seedlings of cotton. The healthy seedling of these plants were kept in muslin cloth cages and apterous forms of aphids were transferred to clean petridishes for one hours of pre-acquisition fasting. This was followed by an acquisition feeding of 40-60 second on detached virus infected leaves of *Nicotiana glutinosa*, each aphid during the virus acquisition probe was watched with a stereo-microscope and timed with a watch. Aphids were allowed to make only brief probes of 40-60 seconds duration. Aphids still in probing position at 40 seconds were picked off with small camel hair brush and transferred in batches of 2,5,10,15,20,25 and 30 to eight healthy *Nicotiana glutinosa* plants for inoculation feeding of one hours. Later aphids were killed by spraying with 0.02 per cent dimethoate insecticide and plants were

maintained in an insect free glasshouse for 4-6 weeks and the observations were recorded for the symptoms on test plants. The healthy seedlings receiving non viruliferous aphids served as control. Test plants used were Nicotiana glutinosa and tomato.

3.2.3 Seed transmission :

For seed transmission, the seeds were collected from virus infected tomato plants. Seeds were sown in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture and maintained in an insect free glasshouse. The observations were recorded for per cent seed germination and the level of seed transmission was calculated by counting diseased plants from total population.

3.3 Physical properties :

The studies on physical properties viz. thermal inactivation point (TIP) , dilutation and point (DEP) and longivity in-vitro(LIV) were carried out by following procedures.outlined by Bos et al. (1960).

3.3.1 Thermal inactivation point (TIP):

The virus infected Nicotiana glutinosa leaves were used as source for crude sap for determination of thermal inactivation point. Aliquotes of 2 ml sap was pipetted in each of 10 test tubes and subjected separately for 10 minutes exposure to heat treatments at 40,45,50,55,60,70, 75,80,85 and 90 in a thermostatic water bath and

immediately after the heat treatment, cold water was run over outsides the tubes to cool the contents. Each heat treatment was inoculated on ten uniform sized leaves of _Chenopodium amaranticolor_ assay host. The leaves of an assay host similarly inoculated with untreated standard extract served as control. All inoculated plants were maintained in an insect free glasshouse. Observations were recorded for lesions of an assay hosts.

3.3.2 Dilution end point (DEP):

An experiment was conducted to find out upto what extent sap could be infective after dilutions, the infective extract was diluted in ten fold (x^{10}) series from undiluted 10^{-1} to 10^{-10}each dilution treatment was inoculated on ten uniform sized leaves of _Chenopodium amaranticolor_. Inoculated plants were maintained in an insect free glasshouse. Observation were recorded for lesion counts of an assay host. The sums were plotted on logarithmic scale against the dilution on an arbitrary linear scale.

3.3.3 Longivity in-vitro (LIV):

In order to know the longivity of virus in crude sap, an experiment of aging _in-vitro_ was carried out. Standard extract was kept at room temperature (25-35°C) in a rubber stoppered tubes. Sap stored in this condition was inoculated at an interval of 15 days on healthy

seedling of *Chenopodium amaranticolor* assay host. Each treatment was inoculated on five uniform sized leaves of *Chenopodium amaranticolor*, assay host. The inoculated plants were maintained in an insect free glasshouse and observation were recorded for lesion counts and sums were plotted on a logrithmic scale against the time on an arbitrary linear scale.

3.4 Host range :

For host range studies, twentythree selected plants species belonging to different families viz. Amaranthaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Leguminaceae and Solanaceae were raised from healthy seeds in earthen pots containing steam sterlized soil, sand and compost (2:1:1) mixture and maintained in an insect free glasshouse.

Five plants of each hosts species were inoculated with the sap extracted from virus infected *Nicotiana glutinosa* systemic leaves by conventional leaf rub method. All the leguminous plants were inoculated on primary leaves before the emergence of first trifoliate. All other plants were inoculated on the first leaf or full expanded leaves. The inoculated host plants were ob-served for 4-6 weeks. The plants that did not show any symptoms after the period of 4-6 weeks were back indexed on *Nicotiana glutinosa* test plants for detection of latent infection if any.

The host range experiment was conducted during September, 1984 to June, 1985 and the temperature ranges were minimum 17.74°C and maximum 45.17°C and humidity range was AM 61.52 and PM 26.02 per cent.

The following plants species were used as test hosts for tomato mosaic virus.

I Amaranthaceae

1. *Gomphrena globosa* L.

II Chenopodiaceae

2. *Chenopodium amaranticolor* coste and Reyn.

3. *C. Quinoa* Willd.

4. *C. album*

5. *C. murale* L.

III Compositae

6. *Zinnia elegans* Jacq.

IV Cucubitaceae

7. *Cucumis sativus* L. cv. kheera.

8. *Cucurbita moschata* L.

V Leguminosae

9. *Archis hypogaea* L. SBXI

10. *Phaseolus vulgaris* L. cv. local.

11. *Vigna unguiculata* (L) Walp cv. C.152.

12. *V. radiata* (L) Wilczek S-1.

13. *Canaralia insiformis* DC.

14. *Limabean Phaseolus lunatus* L.

15. *Pisum sativum* L.

VI Solanaceae

16. *Capsicum annuum* L. cv. Np-46-A

17. *Datura stramonium* L.

18. *Lycopersicon esculentum* Mill cv. Karnataka Hybrid

19. *Nicotiana tabacum* L. cv. White burley.

20. *N. gultinosa* L.

21. *N. rustica* L.

22. *Petunia hybrida* Vilm.

23. *Solanum nigrum* L.

3.5 Purification :

The partial purification of the tomato mosaic virus was carried out by following the methods described by Bald and Suzaki (1974) method and Clark (1968) method. With the following modification.

3.5.1 Bald and Suzuki (1974) method:

100 g tissue of the virus isolate from while burley tobacco showing typical symptoms were homogenized in a blender with 200 ml of distilled water containing 0.3 g sodium sulphate (anhydrous), 0.9 g hentonite and 18 ml of n-butanol (7 per cent acqueous solution). The resultant slurry was vaccum filtered through muslin cloth in a Buchner funnel. The filtrate was then centrifuged at low speed (6000 rpm) for 30 minutes in a Janetzky K-70

refrigerated centrifuge by using super speed attachment rotor. High speed supernatant was discarded and pellet was resuspended in a distiled water, about 1/10 of original volume and pooled sample was further subjected to second cycle of low speed (6000 rpm) centrifugation for 20 minutes. Low speed pellet was discarded and to every 20 ml of supernatant, 1 ml of 7 per cent n-butanol was added. Second super speed centrifugation was run at 17,000 rpm for 90 minutes. Supernatant was discarded and final pellet was resuspended in a 0.1 M phosphate buffer (pH 7.0). The evaluation of purification procedure was done by inoculating test plants with virus preparation at each steps.

3.5.2 Modified Clark (1968) procedure:

The leaves of white burley tobacco plants 20-25 days after virus inoculation were used in this purification procedure also. The tissue (100 g) was homoganized in a waring blender in 0.2 M phosphate buffer. pH 6.5 (1.5 m/g leaf), containing 0.5 per cent 2-mercapthoethanol. The slurry was squeezed through muslin and shaked with 20 per cent (v/v) chloroform, followed immediately by centrifugation at 4,000 rpm (R-30 rotor) in a VAC 601 model of Janetzky refriferated ultracentrifuge for 5 minutes. Excess chloroform was removed by blowing air on to the surface of the continuously stirred liquid. The virus was then sedimented at 18,000 rpm (R-40 rotor) for 90 minutes. The virus containing pellet was suspended in 0.01 M ammonium acetate at pH 6.5 and finally

centrifuge at 4,000 rpm (4500 g) in a K-70 model of Janetzky refrigerated centrifuge for 5 minutes. The supernatant was used as partially purified preperation in infectivity test, electron microscopy and serology.

3.6 Electron microscopy:

Partially purified virus preperations were used in electron microscopy. Partially purified virus preperations was fixed in 2 per cent formaldehyde for 10 minutes. A drop of fixed virus was transferred to 300 mesh coppergrids coated with formver membrane and carbon. After 5 minutes, excess liquid was removed with filter paper, stained with 2 per cent phosphostungstic acid (PTA) pH 7.0 and examined in a Philips model 201C transmission electron microscope at 60 kv. The microscope was caliberated employing germanium shadowed carbon replica having 21,600 lines/cm. The actual magnification was used for computing the size of the virus particles.

3.7 Serology:

For establishing the serological relationship of the present virus with the tomato mosaic virus, the serological test viz, drop precipitation test on slide, ring interface precipitation test, in tubes were employed. Antiserum of tomato mosaic virus (TMV) isolate antisera of cucumber mosaic virus, tobacco mosaic & PVX obtained from CPRI Simla were used in serological test. The serological test are described as below.

3.7.1 Drop precipitation test on slides (Bercks et al., 1972):

Drops of test antigens were mixed directly with drops of specific antiserum on mocroscopic slides. These were incubated under high humidity for a few hours at about 20°C and examined under a microscope at about 40x. The diluent used for antigens and antisera was phosphate buffered saline (PBS) : 0.01 M (neutral phosphate buffer +0.15 M NaCl). The antiserum dilution used was 1:4 and antigen 1:10. Healthy sap from white burley tobacco, PBS and normal serum were included as controls.

3.7.2 Ring interface precipitin test (Ball, 1974):

Two fold serial delutions of antigen or antiserum was made in phosphate buffered saline (PBS). 0.01 M neutral phosphate buffered + 0.=5 M NaCl. Antiserum dilutions were made in 30 per cent glycerine. A small volume (about 0.5 ml) of antiserum in glycerine was placed in the botton of the tubes (10x74 mm in diameter). The placed antigen (about 0.5 ml) in PBS was overlayered carefully by holding the tubes at sharp angle and by using pipette to assist the flow of the antigen down the side of the tube so that the content with the antiserum was made very gradually. Then the tubes were placed upright in a rack and incubated at room temperature. The observations were made for presence of precipitate at interface between antigen and antibody.

3.8 Antiserum production :

For production of antiserum, Newzealand white inbred rabbits were given four intramuscular injection in the hind leg at weekly intervals with 1 ml of partially purified virus suspension mixed with a equal volume of Freund's incomplete adjuvant. Seven days after the last intramuscular injection, an intravenous injection (Booster) with 1 m of virus in 0.5 ml phosphate buffer was given in the marginal vein of rabbit ear. Bleeding was done after sevendays after the final intravenous injection. Serum was collected and stored in vials at 0 to 4°C in freeze after having added few drops of sodium azide (0.025 per cent). The titre of the antisurum was determined by the ring interface precipitation test.

3.9 Varietal screening :

Test tomato varieties/lines were grown from healthy seeds in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture and maintained in an insect free glasshouse. First formed true leaves of test varieties/lines were inoculated with infectious sap by leaf rub method. Ten plants per test varieties/lines were inoculated for each sample.

1. Susceptable (S) = Varieties/Lines reacting with either local or only systemic symptoms or not
2. Resistant (R) = Varieties/lines reacting with only local symptom and virus not recoverable by back inoculation on test plant from non-inoculated

3. Tolerant (T) = Varieties/lines free from both local and systemic symptoms but virus recoverable by back inoculation on test plant.

4. Immune (I) = Varieties/lines free from both local and systemic symptoms and virus also not recoverable by back inoculation on test plant.

The following tomato varieties/lines were used in screening experiment.

1. Pusa ruby
2. Selection-1
3. Hybrid-10
4. Punjab zubari
5. Selection-13
6. Selection-19
7. 580-1-1
8. Hawaii-7990
9. Karnataka hybrid

RESULTS

Chapter-4

RESULTS

4.1 Mechanical transmission :

The virus of tomato was readialy transmitted by sap inoculation with 0.1 M K-phosphate buffer + 1 per cent 2-mercaptoethanol (pH 7.0) from tomato to tomato. On tomato plants after inoculation the virus did not produced any local symptoms. Systemic symptoms were induced by the virus after about 22 days of inoculation. These included mosaic, followed by leaf distortion with considerable leaf elongation of the plants. Finally the shoestring like symptoms were shown by the plants. In some varieties of tomato mottling of leaves and blistering and malformation of the leaves were shown (Fig 1a,b).

4.2 Aphid transmission :

The results on aphid transmission of tomato mosaic virus indicated that the cotton aphid i.e. Aphis gossypii failed to transmit the virus from tomato to tomato and other test host i.e. Nicotiana glutinosa in the non-persistent manner.

4.3 Seed Transmission :

The results on seed transmission indicated that out of the 150 seeds grown from infected plants of tomato, one hundred and thirty were germinated. The level of seed transmission was to the extent of 30 per cent in tomato variety Pusa Ruby (Table 1).

Table 1: Seed transmission of a virus causing severe mosaic of tomato.

Sr. No.	Source host	No. of seeds sown.	No. of seeds germinated.	Per cent seed transmission	Reaction*		Virus recovery on** *C. amaranticolor*	
					(L)	(S)	(L)	(S)
1.	Tomato (Pusa Ruby)	150	130	30	-	M, Ld, Le, Sh	+	+

* = (L) = Local
(S) = Systemic
M = Mosaic
Ld = Leaf distortion
Le = Leaf elongation
Sh = Shoe-string

** = + = Virus recovery positive
\- = Virus recovery negative

4.4 Physical properties :

The results on physical properties in crude sap viz. thermal inactivation point (TIP), dilution end point (DEP), and longevity *in-vitro* (LIV) are shown in Table 2 and also presented graphically in Fig 2a, b and c. It is revealed from the table and graphs that the virus was inactivated between 80 and 85°C and between 10^{-7} and 10^{-8} dilutions. All the inoculated leaves of the assay host become infected at temperature to 80°C but not at 85°C. However, local lesions decreased progressively as the temperature were increased. All inoculated leaves of the assay host became infected at dilutions to 10^{-7} but not at 10^{-8}; local lesions decreased progressively as the dilutions were increased. The virus was viable upto 40 days at room temperature (27 to 30°C). All the assay host inoculated leaves became infected upto 40 days. However local lesions decreased progressively as the aging was increased at room temperature.

4.5 Host range :

The virus of tomato had fairly wide host range and infected 12, out of 23 plant species belonging to Amaranthaceae, Chenopodiaceae and Solanaceae families. Of the 12 susceptible hosts, 3 were local hosts, local lesions hosts included *Gomphrena globosa* (Fig 3a), *Chenopodium quinoa* and *C. murale*. The local and systemic hosts included *C. amaranticolor* (Fig 3b) and *Nicotiana rustica*. The only systemic hosts included *Lycopersican esculentum*, *N. glutinosa*

Table 2: Physical properties of a virus causing severe mosaic of tomato.

Sample number	Treatments[a]					
	TIP $0^{o}C$	No. of lesions	DEP	No. of lesions	LIV in days	No. of lesions
1.	Control	50.8[b]	Control	50.8	Control	50.8
2.	40	47.0	10^{-1}	34.4	5	35.0
3.	45	42.8	10^{-2}	31.0	10	39.7
4.	50	36.3	10^{-3}	25.7	15	22.7
5.	55	31.0	10^{-4}	16.6	20	15.3
6.	60	25.6	10^{-5}	10.6	25	12.8
7.	65	18.7	10^{-6}	5.3	30	7.2
8.	70	11.3	10^{-7}	1.7	35	3.2
9.	75	5.2	10^{-8}	0.0	40	1.3
10.	80	2.2	10^{-9}	0.0	45	0.0
11.	85	0.0	10^{-10}	0.0	50	0.0
12.	90	0.0	-	-	-	-

a = TIP = Thermal inactivation point

DEP = Dilution end point

LIV = Longevity *in-vitro*

b = Figures represent average number of lesions on *Chenopodium amaranticolor* assay host based on five leaves.

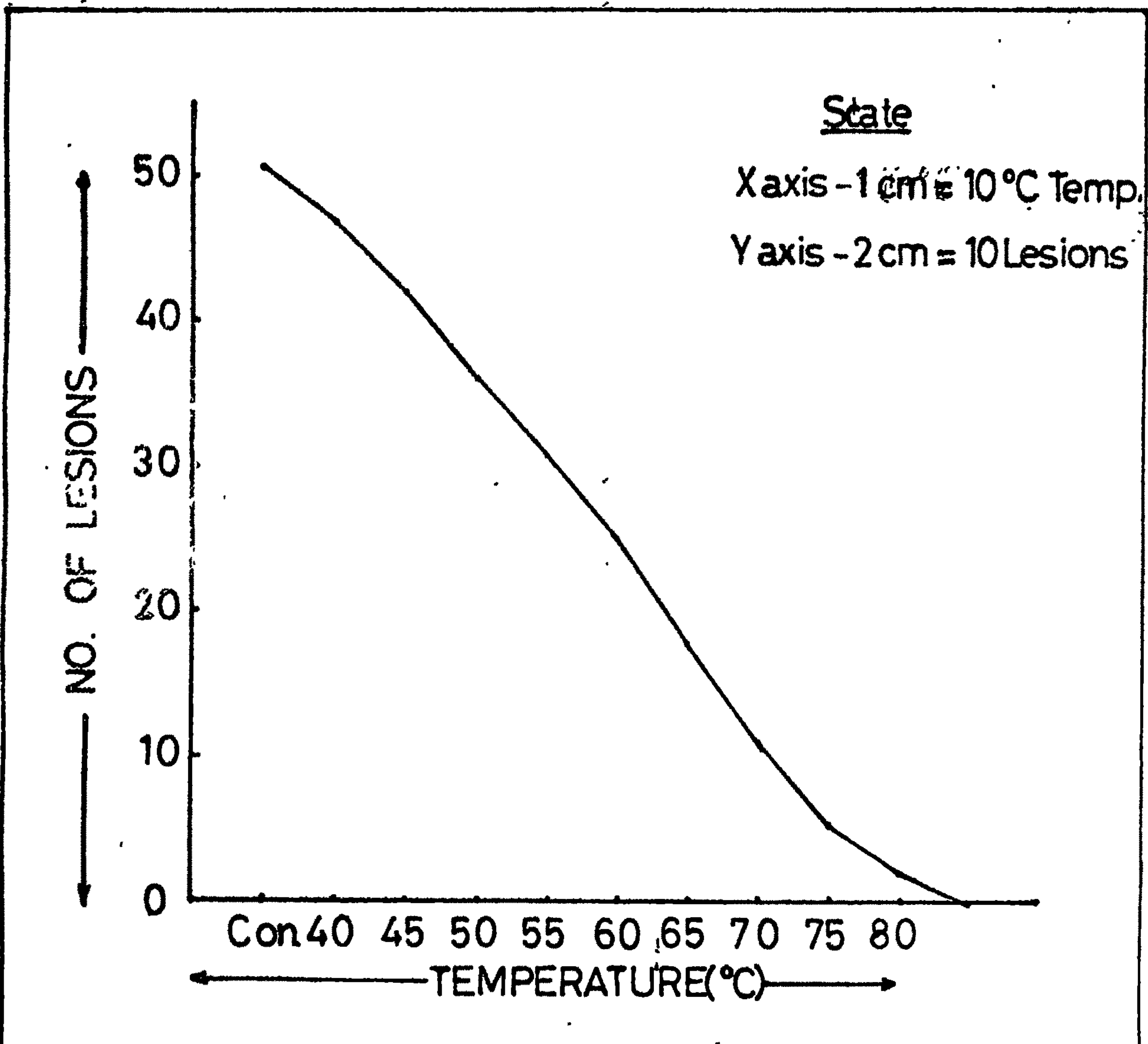

Fig.2a: Thermal inactivation point (TIP) of a virus causing severe mosaic of tomato.

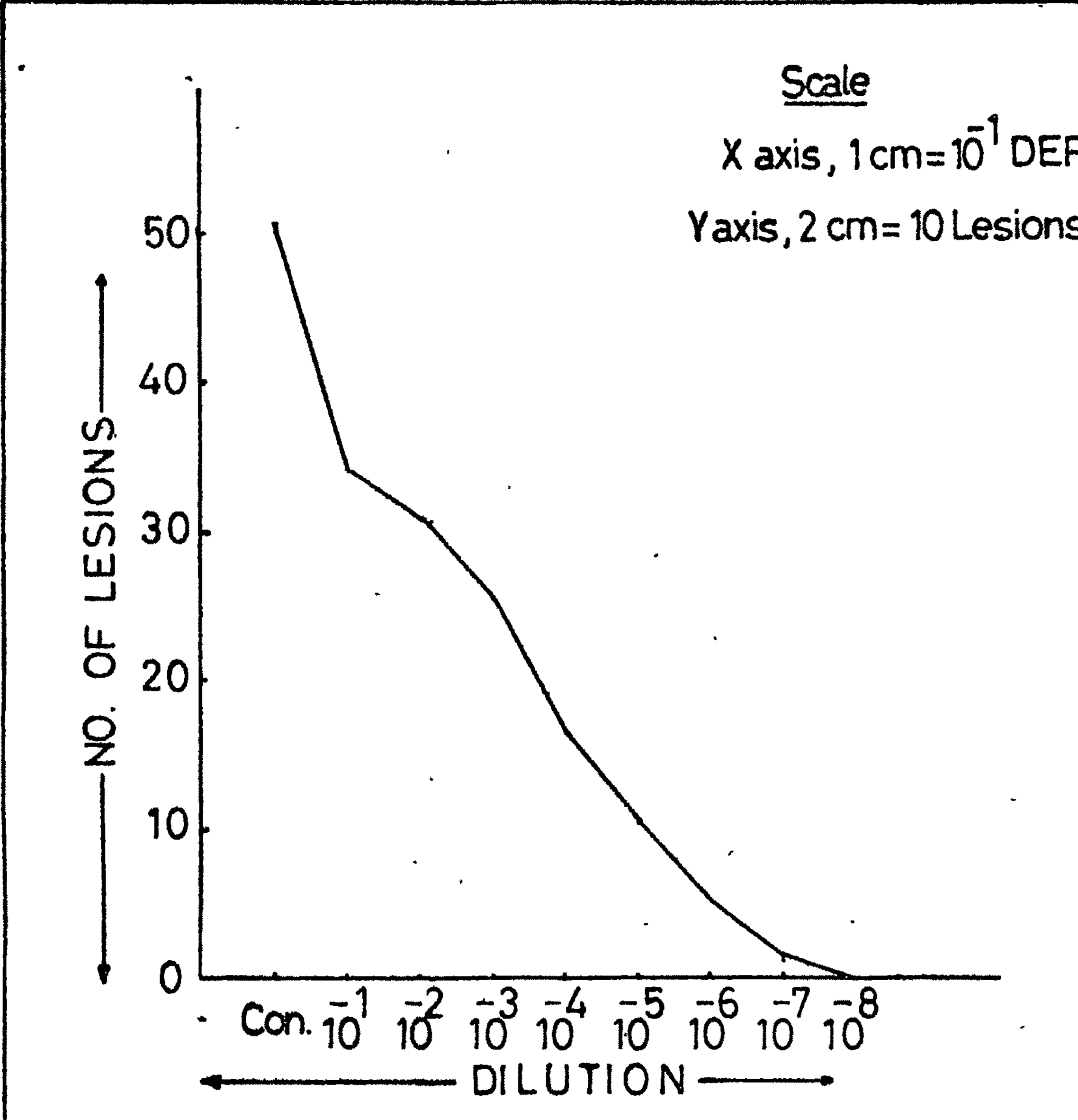

Fig.2b: Dilution end point (DEP) of a virus causing severe mosaic of tomato.

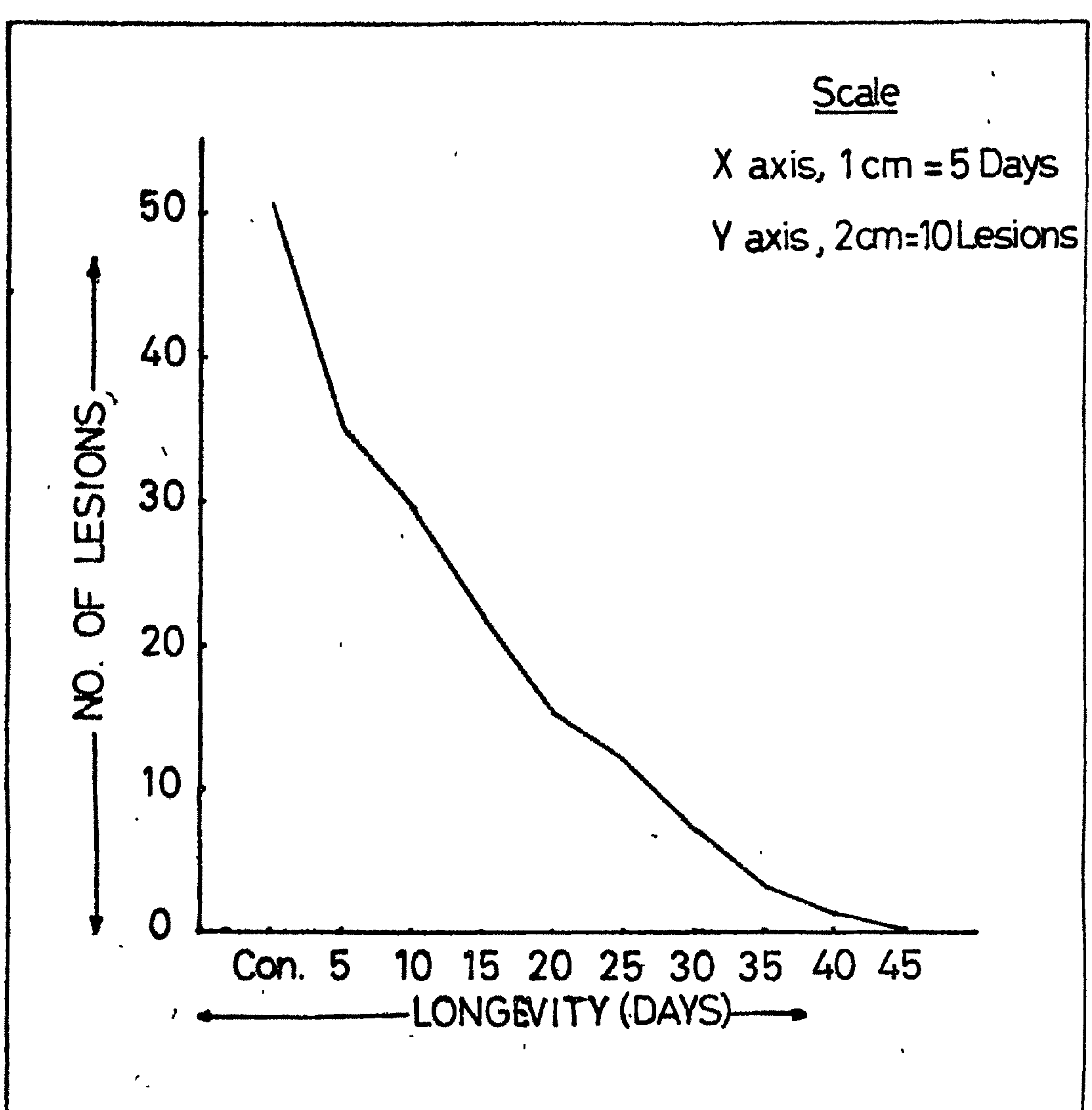

Fig.2c: Longivity in vitro (LIV) of a virus causing severe mosaic of tomato.

(Fig 4a), N. tabacum (Fig 4b), Petunia hybrida, Capsicum annuum, Datura stramonium and Solanum nigrum. The local symptoms produced on [illegible] three hosts were either nectroic local lesions or chlorotic local lesions. The two local and systemic hosts produced symptoms necrotic and chlorotic local lesions and mosaic and leaf distortion on N. rustica. Seven systemic hosts, systemic symptoms produced on these hosts included mosaic, leaf distortion, leaf elongation, mottling and stunting (Table 3). The non hosts of the virus included C. album, Zinnia, cucumber, groundnut, french bean, cowpea, mung bean, swoard bean, limabean and pea.

4.6 Purification :

4.6.1 Bald and Suzaki (1974) method :

The results of infectivity test during different steps of purification are given in Table 4a. It is revealed from the table that the partially purified virus was infectious to C. amaranticolor assay host at 10^{-7} dilutions based on the original weight of the tissue (Table 4b). It is evident from the table that loss of virus was found at all the steps of purification. Therefore, this method was not found to be satisfactory for the partial purification of severe mosaic virus of tomato.

4.6.2 Modified Clark (1968) method :

The result of infectivity test during different steps of purification are given in the Table 5. It is revealed from the table that the partially purified virus was infectious to C. amaranticolor assay host at 10^{-9}

Table 3 : Host range of a virus causing severe mosaic of tomato.

Sr. No.	Host	Reactions*				Virus recovery on *C. amaranticolor*		Remarks
		Local	Incubation	Systemic	Incubation	Local	Systemic	
1.	2.	3.	4.	5.	6.	7.	8.	9.
I	Amaranthaceae							
	1. *Gomphrena globosa*	NLL	9	-	-	+	-	LH
II	Chenopodiaceae							
	2. *Chenopodium album*	-	-	-	-	-	-	NH
	3. *C. amaranticolor*	NLL	2	Chl, mt	8	+	+	LSH
	4. *C. murale*	CLL	5	-	-	+	-	LH
	5. *C. quinoa*	CLL	5	-	-	+	-	LH
III	Compositae							
	6. *Zinia elegans*	-	-	-	-	-	-	NH
IV	Cucurbitaceae							
	7. *Cucumis sativus* cv. Bengalore special	-	-	-	-	-	-	NH
	8. *Cucurbita moschata*	-	-	-	-	-	-	NH
V	Leguminosae							
	9. *Arachis hypogea* cv. SB-XI	-	-	-	-	-	-	NH
	10. *Phaseolus lunatus* cv. Local	-	-	-	-	-	-	NH
	11. *Phaseolus vulgaris* cv. Local	-	-	-	-	-	-	NH
	12. *Pisum sativum* cv. Bonneville	-	-	-	-	-	-	NH
	13. Swerdbean	-	-	-	-	-	-	NH

Continued.

Table 3: Continued.

1.	2.	3.	4.	5.	6.	7.	8.	9.
	14. Vigna radiata cv. S-1	-	-	-	-	-	-	NH
	15. Vigna unguiculata cv. Pusa Vaishaki	-	-	-	-	-	-	NH
VI	Solanaceae							
	16. Capsicum annuum cv. SKV	-	-	M	14	+	-	SH
	17. Datura stramonium	-	-	M	15	+	-	SH
	18. Lycopersicon esculentum	-	-	M, Ld, La	22	+	-	SH
	19. Nicotiona glutinosa	-	-	M, Ld, La	20	+	-	SH
	20. N. rustica	CLL	4	M, Ld	15	+	+	LSH
	21. N. tabacum cv. White Burley	-	-	Mt	17	+	-	SH
	22. Petunia hybrida	-	-	Ld, Stn	15	+	-	SH
	23. Solanum nigrum	-	-	Mt	18	+	-	SH

ChL = Chlorotic local lesions
Ld = Leaf distortion
LH = Local host
M = Mosaic
NH = Non-host
SH = Systemic host
+ = Virus recovery positive

Chl = Chlorotic or chlorosis
Le = Leaf elongation
LSH = Local systemic host
Mt = Mottling
NLL = Necrotic local lesions
Stn = Stunting
- = Virus recovery negative

Table 4 a : Purification of a virus causing severe mosaic of tomato after Bald and Suzuki (1974) method.

	Treatments	Virus isolate
I	Crude sap	32.5[a]
II	Crude sap + buffer	35.0
III	1st cycle	
1	<u>Low speed</u>	
	a. Pellet	30.0
	b. Supernatent	55.0
2	<u>High speed</u>	
	a. Pellet	140.0
	b. Supernatant	70.0
IV	2nd cycle	
1	<u>Low speed</u>	
	a. Pellet	20.0
	b. Supernatant	140.0
	c. Supernatant+7% n-butanol	60.0
2.	<u>High speed</u>	
	a. Pellet	180.0
	b. Supernatant	55.0
3	<u>Low speed</u>	
	a. Pellet	35.0
	b. Supernatant	160.0

a = Average number of lesions on *C. amaranticolor* based on five leaves.

Table 4 b : Dilutions of partially purified virus.

Virus preperation	No. of lesions on *C. amaranticolor*
I Undiluted	160.0
II Diluted	
1. 10^{-1}	70.0
2. 10^{-2}	62.0
3. 10^{-3}	55.0
4. 10^{-4}	37.0
5. 10^{-5}	23.0
6. 10^{-6}	10.0
7. 10^{-7}	2.3
8. 10^{-8}	0.0

Table 5: Infectivity test during purification steps of severe mosaic of tomato following procedure of Clerk (1968).

	Purification steps		Infectivity test
I	Extraction sap		
	Tissue + buffer		40.5*
II	Clarification by low speed centrifugation		
	a) Pellet		8.0
	b) Supernatant		60.0
III	Clarification by high speed centrifugation		
	a) Pellet		205.0
	b) Supernatant		11.0
IV	Clarification of suspended pellet by brief centrifugation		
	a) Pellet		13.0
	b) Supernatant		321.0
V	Partially purified virus preparation		
	Dilutions:	10^{-1}	198.0
		10^{-2}	124.0
		10^{-3}	98.0
		10^{-4}	76.0
		10^{-5}	63.0
		10^{-6}	21.0
		10^{-7}	11.0
		10^{-8}	9.0
		10^{-9}	3.0
		10^{-10}	0.0

* = Average number of lesions based on five leaves of *C. amaranticolor*.

dilutions based on the original weight of the tissue. It is also evident from the table that no loss of virus was found at all the steps of purification. Therefore, this method was found satisfactory for the partial purification of severe mosaic of tomato. Of the two methods, the method, modified Clark (1968) was found comparatively better than the method of Bald and Suzuki (1974) for partial purification of severe mosaic of tomato (Table 6).

4.7 Electron microscopy:

Electron microscopy studies indicated that the partially purified virus preparations contain considerable number of elongaged flexible particles measuring 540 nm in length (Fig 5).

4.8 Serology :

The results on serological tests are shown in Table 7. The field tomato virus eitherin crude, clarified, or in partially purified preperation was serologically related, to potato virus X but not with tomato mosaic, tobacco mosaic and cucumber mosaic viruses.

4.9 Antiserum production :

The particulars about antiserum production of the virus is given in Table 8. The virus was found to be a moderate immunogen. The titre of the virus as determined by ring interface precipitation test was 1:1024. The homologus antiserum gave reaction with sap from healthy leaves of N. glutinosa tobacco plants upto 1: 32 dilutions in ring interface precipitation test but not with PBS

Table 6: Comparative infectivity test used for the purification of severe mosaic of tomato.

Purification test	Purification procedures and infectivity test	
	Bald and Suzuki (1974)	Clark (1968)
Partially purified virus preparation.		
A. Undiluted	160.0	321.0*
B. Diluted		
10^{-1}	70.0	198.0
10^{-2}	62.0	124.0
10^{-3}	55.0	98.0
10^{-4}	37.0	76.0
10^{-5}	23.0	63.0
10^{-6}	10.0	21.0
10^{-7}	2.3	11.0
10^{-8}	0.0	9.0
10^{-9}	0.0	3.0
10^{-10}	0.0	0.0

* = Average number of lesions produced on _C. amaranticolor_ based on five leaves.

Table 7 : Serological relationship of a virus causing severe mosaic of tomato with other viruses.

Sr. No.	Antiserum*	Reactions**
1.	CMV	-
2.	PVX	+
3.	TMV	-
4.	ToMV	-

* = CMV = Cucumber mosaic virus

PVX = Potato virus X.

TMV = Tobacco mosaic virus

ToMV = Tomato mosaic virus

** = + = Serologically related

\- = Serologically not related.

Table 8: Particulars about antiserum production.

1.	Antiserum against	Severe mosaic of tomato, tomato isolate.
2.	Identification number	
3.	Virus purified from	*N. glutinosa*.
4.	Number of injections	4
5.	Types of injection	Intramuscular with Freund's incomplete adjuvant.
6.	Injection intervals	Weekly.
7.	Date of injection	31-1-85 to 20-2-85
8.	Date of booster	27-2-85
9.	Date of bleeding	5-3-85
10.	Titre against virus	1:1024
11.	Date of determination	10-3-85
12.	Method of determining titre	Ring interface precipitation test and drop precipitation test.
13.	Titre against host consituents.	1:32
14.	Date of determination	10-3-85
15.	Method of determining titre	Ring interface precipitation test.
16.	Storage	At $4^{o}C$ in freeze
17.	Suggested method and dilution for serodiagnosis	Ring interface precipitation test at 1:64 dilution.
18.	Preservative added	Na-azide (0.025%) few drops.

Fig 1a: Systemic symptoms of a virus causing severe mosaic of tomato on *Lycopersicon esculentum* (Mosaic symptoms).

Fig 1b: Systemic symptoms of a virus causing severe mosaic of tomato on *Lycopersicon esculentum* (Shoe-string like symptoms)

fig 1a:

Fig. 1a

fig 1b:

Fig 3a: Local symptoms of a virus causing severe mosaic of tomato on _Gomphrena globosa_.

Fig 3b: Local symptoms of a virus causing severe mosaic of tomato on _Chenopodium amaranticolor_.

Fig 3c: Systemic symptoms of a virus causing severe mosaic of tomato on _Chenopodium amaranticolor_.

Fig 3a:

Fig 3b

Fig 3c

Fig 4a: Systemic symptoms of a virus causing severe mosaic of tomato on *Nicotiana glutinosa*.

Fig 4b : Systemic symptoms of a virus causing severe mosaic of tomato on *Nicotiana tabacum* cv. White Burley.

fig 4a:

fig 4b:

Fig 5

and normal serum.

4.10 <u>Varietal screening</u> :

Of the 9 tomato varieties/lines screened, not a single variety was found immune whereas only one found tolerant to severe mosaic of tomato virus (Table 9). The tolerant variety/line include Hawaii-7990. All other varieties/lines screened were susceptible to the virus.

Table 9: Reaction of tomato varieties/lines to a virus causing severe mosaic of tomato.

Sr. No.	Tomato cultivars	Reactions*				Virus recovery on ** *C. amaranticolor.*	Disease*** rating
		Local	Incubation	Systemic	Incubation		
1.	Pusa ruby	-	-	M, Bl, Mt, Ss	20	+	S
2.	Selection-1	-	-	M, Mt, Mf	18	+	S
3.	Selection-13	-	-	M, Bl	18	+	S
4.	Selection-19	-	-	M, Mt, Mf	18	+	S
5.	580-1-1	-	-	M, Mt	19	+	S
6.	Punjab zubari	-	-	M, Mt	19	+	S
7.	Hawaii-7990	-	-	-	-	+	T
8.	Karnataka hybrid	-	-	Mt	16	+	S
9.	Hybrid-10	-	-	M, Mt, Mf	18	+	S

* = Bl = Blistering
M = Mosaic
Mf = Malformation
Mt = Mottling
Ss = Shoe-string

** = + = Virus recovery positive
\- = Virus recovery negative

*** = S = Susceptible
T + Tolerant

Chapter-5

DISCUSSION

Chapter-5

DISCUSSION

The present virus isolate is identified as potato virus X (Potex group, Fribourg, 1975) based on particle morphology and diameter, serology, seed transmission, physical properties and reaction of the selected host.

The result on the mechanical and seed transmission of the present potato virus X were similar to those reported by Reberts (1948), Wilkinson and Bladgett et al.,(1948), Verma and Baranwal (1983) and Dubey (1983). The results on aphid transmission of the present virus are negative and those are similar to reported by Verma and Buranwal et al. (1983).

As for the physical properties of the severe mosaic virus of tomato they fall in the general range of physical properties reported for potex grou (Fribourg,1975). Thermal inactivation point of the present severe tomato mosaic virus was found to be slightly higher than the one reported by Dubey (1983) and Allan et al.(1974). The dilution end point was found to be similar to PVX reported by Fribourg (1975) and slightly higher than the one reported by Dubey (1983). The ageing in-vitro of the present severe tomato mosaic virus was similar to one reported by Allan et al.(1974).

The reaction of 23 selected hosts of the present virus was similar to those reported by Dubey (1983) except *Gomphrena globosa* test plant of PVX. Allan et al.(1974) and Fribourg (1975) reported that *Gomphrena globosa* was good

local lesions host of PVX. Our results are similar as reported by (Wilkinson and Blodgett, 1948; Allan et al., 1974 and Fribourg, 1975). For the partial purification of the severe mosaic of tomato virus, method outlined by Clark (1968) with modification in spead was found satisfactory than the method outlined by Bald and Suzuki (1974). Since these method have not been employed by other workers for the purification of potato virus X infecting tomato, comparision is limited. As for particle morphology and dimentions are concerned the present virus was similar to potato virus X (Dubey, 1983). The present virus is serologically closely related to potato virus X. The present virus isolate is a moderate immunogen and gives high titred antiserum (Ball, 1974). On the basis of particle morphology and dimention, seed transmission, host range, physical properties, serology and electron microscopy the present virus isolate causing severe mosaic of tomato is identified as a potato virus X, tomato strain.

Chapter-6

SUMMARY

Chapter-6

SUMMARY

A virus causing severe mosaic of tomato was readily transmitted by mechanical means and through the seeds of tomato. The virus was not transmitted by aphid vector. The virus was seed borne in tomato cultivars viz., Pusa Ruby (30.00 per cent). The virus was inactivated between 80 to 85°C and between 10^{-7} to 10^{-8} dilutions. The virus was viable upto 40 but not 45 days at room temperature. The virus infected 12, out of 23 plant species belonging to Amaranthaceae, Chenopodiaceae and Solanaceae families.

The hosts of the virus included Gomphrena globosa, Chenopodium quinoa, C. murale, C. amaranticolor, Nicotiana rustica, Lycopersicon esculentum, N. glutinosa, N. tabacum, Petunia hybrida, Capsicum annuum, Datura stramonium and Solanum nigrum. The non hosts of the virus included C. album, Zinnia, Cucumber, groundnut, french bean, cowpea, mungbean, swoard bean, limabean, and pea.

For the partial purification of the virus the method outlined by Clark (1968) was found to be satisfactory than the method outlined by Bald and Suzuki (1974). Partially purified virus preparation contained considerable number of elongated flexible particles measuring 540 nm in length. The virus was found serologically related to potato virus X (PVX) but not with ToMV, TMV and CMV. The virus was

found to be a moderate immunogen since the homologous antiserum titre was 1:512. Based on particle morphology, diameter, host range, aphid transmission and serological properties, the present virus isolate causing severe mosaic of tomato is identified as a member of potex-virus group. Of the 9 tomato varieties/lines screened, none was found to be resistant to severe mosaic of tomato virus (Potex virus).

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