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Characterization of the Virus Causing Fern Leaf Disease on Tomato in Karnataka, India

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Abstract

A mechanically transmissible virus that induced fern leaf disease in tomato was isolated from naturally infected tomato plants in Karnataka. The incidence of the disease in major tomato growing districts of Southern Karnataka, ranged from 0 to 20%. Virus isolated from tomato crops causing fern leaf disease was identified as a strain of cucumber mosaic cucumovirus (CMV) on the basis of host range, physical properties, serology and electron microscopy. Of the 30 plant species belonging to 12 families tested for host range studies by mechanical inoculation 16 plant species viz., *Cucumis sativus, Luffa acutangula, Lagenaria siceraria, Momordica charantia, Citrullus lunatus, Capsicum annum, Nicotiana tabacum, N. glutinosa, N. benthamiana, Vigna ungiculata, Phaseolous aureus, P. mungo, Dolichos lablab, Chenopodium amaranticolor, C. quinoa and Tagetes indica* took infection and expressed symptoms such as vein clearing, mosaic and chlorotic local lesions. Studies on physical properties revealed that the virus has a dilution end point of 10^{-4}, thermal inactivation point of 60 C and longevity *in vitro* of 4 days at room temperature. Inefected samples strongly reacted in DAC-ELISA with CMV antiserum indicating the association of CMV antiserum indicating the association of CMV with the disease. Electron microscopic observation of crude leaf extract placed on carbon coated grid and stained with 2% uranyl acetate also revealed the presence of isometric particles averaged about 30 nm in diameter. Based on the results of the above investigations the causal agent of the tomato fern leaf disease was identified as a strain of CMV.

Key words : Tomato, Characterization, Fern leaf disease, Host range, Physical properties.

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops grown in Karnataka. Tomato is affected by a number of diseases causing substantial losses in yields. Besides fungal, bacterial and phytoplasmal infections, it is also affected by a large number of viral diseases. A virus causing fern leaf disease is gaining importance in recent years. Ferm leaf disease of tomato is severe in Karnataka during 1996. The disease incidence ranged from less one to 20% in different tomato genotypes, cultivars and hybrids (1). Fern leaf or strap leaf or shoestring is the symptoms produced by cucumber mosaic virus on tomato (2). Cucumber mosaic cucumovirus (CMV) infections rank among the most devastating virus diseases in the commercial culture of tomato. Cucumber mosaic virus (CMV) is the type member of cucumovirus genus, in the family Bromoviridae. The genome consist of three distinct single stranded positive sense RNA molecules viz., RNA 1, RNA 2 and RNA 3 it is one of the most important and wide spread virus having wide host range. CMV is most commonly a problem in peppers, cucurbits, tomatoes and bananas (3). The pathology of CMV infections in tomato is quite divers, ranging from asymptomatic to severe stunting with leaf curl, referred as fern leaf syndrome, when associated with satellite Ranks, CMV infections can induce lethal necrosis in tomato (4, 5). CMV exists as variety of isolates that differ in host range and pathogenecity (6, 7). Sympotoms of CMV on several hosts usually consist of mild to strong mosaic, puckering of leaves and stunting with or without leaf deformation. CMV exists as myriad of strains that are difficult to characterize and distinguish (8). Methods that had been used to characterize them include symptoms induced on test plants, serology (9, 8), electrophoretic mobilities of intact virions and RNA's in polyacrylamide gels (9, 10), hybridization with complimentary DNA (11), competition hybridizations (12—14), and peptide and RNA mappings. Though the disease has been known for quite a long period and a good amount of literature is available on various aspects of the disease, information regarding the virus causing fern leaf disease of tomato in India is rather meager or scanty. Thus the

present study was undertaken to characterize the virus associated with the ferm leaf disease of tomato.

Methods

Collection and Maintenance of Virus

Naturally infected of tomato plants showing mosaic, stunting and fern leaf symptoms collected from tomato field in Bangalore district were served as primary virus source for sap inoculation to experimental host plants. The virus isolate was selected by four serial passages from single lesion through *Vigna unguiculata* and subsequently propagated on susceptible tomato cv Arka Vikas and maintained in insect proof glasshouse, and used for further studies.

Mechanical Inoculation

The virus inoculum was prepared by grinding virus infected leaves in 0.01M phosphate buffer, pH 7.0 at the rate of 2 ml/g of infected tissue. A pinch of celite (600 meshes) was added to the inoculum (0.25 mg/ml of standard inoculum) before inoculation as an abrasive. A small piece of sterilized absorbent cotton soaked in the inoculum was rubbed over the upper surface of leaves gently and unidirectionally. Inoculated leaves were washed with jet of water to remove excess inoculum and abrasive. The plants were labeled and kept for observation in insect proof glasshouse for symptoms expression.

Survey to Assess the Status of Fern Leaf Disease

Commercial fields of tomato in major tomato growing districts of Southern Karnataka viz., Kolar, Mandya, Mysore and Bangalore were surveyed to assess the status of fern leaf disease during summer 2005. Depending upon the field size, four to eight random sites of 5 m^2 each were selected and per cent incidence of fern leaf disease was recorded by counting the number of infected plants out of total number of plants, meanwhile age of the crop and variety cultivated was also recorded. Aphid species present on tomato plants were observed and collected by using camel hairbrush and placed in petri plates covered with black cloth and brought to the laboratory for further identification.

Host Range

Thirty plant species belonging to 12 families were used ion host range studies. The virus inoculum was prepared by grinding virus infected leaves of tomato cv Arka Vikas in 0.01M phosphate buffer, pH 7.0 at the rate of 2 ml per gram of infected tissue. A pinch of celite was added to be inoculum. The inoculum was soaked in small pieces of absorbent cotton and rubbed on test plants. Legume plants were inoculated on cotyledonary leaves before emergences of primary leaves; other test plants were inoculated on second and fourth fully expanded leaves. Inoculated plants were kept in insect proof glasshouse and observed for development of symptoms. Inoculated and subsequently developed leaves were back inoculated to *Vigna unguiculata* and Chenopodium amaranticolorto confirm the virus infection.

Virus Stability in Buffered Sap

Physical properties of the virus viz., dilution end point (DEP), thermal inactivation point (TIP) and longevity *in vitro* (LIV) were carried out (15) using *Vigna unguiculata* and *Chenopodium amranticolor* as local lesion assay hosts.

Enzyme Linked Immunosorbent Assay (ELISA)

Serological detection of the virus in infected plant samples was done by direct antigen coating-ELISA (DAC-ELISA) using CMV specific antiserum. ELISA plates were directly coated with antigen samples diluted in carbonate buffer, pH 9.6 (1 g/9 ml). Crude antiserum at 1 : 500 dilutions in antibody buffer was subsequently added to the plate. Goat antirabbit antibodies labeled with alkaline phosphatase at 1 : 1000 dilutions in antibody buffer were added to the plate to detect antigen antibody reaction. The plates were inoculated with P-nitrophenyle phosphate (5 mg/5 ml) for 30 minutes at room temperature. Absorbance OD value was recorded at 405 nm using ELISA reader.

Electron Microscopy

For detection of virus particles in leaf material, one centimeter square infected leaf tissue of tomato cv Arka Vikas was taken and crushed on clean glass

Table 1. Host range of the virus causing tomato fern leaf disease. DC = Downward cupping, M = Mosaic, DGB = Dark green blisters, CLL = Chloratic local lesion, PLT = Pointing of leaf tip, RLL = Reduction in leaf lamina, NLL = Nocrotic local lesion, VC = Vein clearing, LG = Leaf curl.

	Host plant	Family	Number of plants inoculated	Number of plant infected	Pre cent infection	Incubation period (days)	Types of symptom
1	*Cucumis sativus*	Cucurbitaceae	10	8	80	7	DC, M, DGB
2	*Cucurbita pepo*	„	10	-	-	-	-
3	*Luffa accutangula*	„	10	7	70	8	M
4	*Momordica charantia*	„	10	6	60	8	CLL
5	*Lagenaria siceraria*	„	10	4	40	15	M, DGB
6	*Citrullus lanatus*	„	10	4	40	20	M
7	*Capsicum annuum*	Solanaceae	10	10	100	20	M, PLT, RLL
8	*Solanum melongena*	„	10	-	-	-	-
9	*Nicotiana glutinosa*	„	10	10	100	10	M, PL, RLL
10	*Nicotiana tabacum* var samsun	„	10	9	90	12	VC, LC
11	*Nicotiana benthamiana*	„	10	10	100	15	M, RLL
12	*Datura stramonium*	„	10	-	-	-	-
13	*Vigna umguiculata*	Leguminaceae	10	10	100	7	NLL
14	*Phaseolus aureus*	„	10	10	100	8	NLL
15	*Phaseolus mungo*	„	10	10	100	8	NLL
16	*Dolichos lablab*	„	10	7	70	15	VC, M
17	*Phaseolus vulgaris*	„	10	-	-	-	-
18	*Arachis hypogea*	„	10	-	-	-	-
19	*Chenopodium amaranticolor*	Chenopodiaceae	10	10	100	6	CLL
20	*C. quinoa*	„	10	10	100	6	CLL
21	*Celosia argentea*	Amaranthaceae	10	-	-	-	-
22	*Raphanus sativus*	Cruciferae	10	-	-	-	-
23	*Carica papaya*	Cariaceae	10	-	-	-	-
24	*Tagetes indicus*	Compositae	10	7	70	20	M
25	*Parthenium hysterophorus*	Astraceae	10	-	-	-	-
26	*Euphorbia geniculata*	Euphorbiaceae	10	-	-	-	-
27	*Coleus aromaticus*	Lamiaceae	10	-	0	-	-
28	*Coleus forskohlii*	„	10	-	-	-	-
29	*Coleus aromaticus varigatus*	„	10	-	-	-	-
30	*Musa paradigiaca*	Musaceae	10	-	-	-	-

slide by using 0.01M phosphate buffer (pH 7.0). A drop of extract was placed on the carbon coated grid of electron microscope and allows to stand for 2 minutes. Then the grid was washed with 10 drops of distilled water and stained with 2% uranyl acetate. The excess stain was removed by touching the edge of the grid with a piece of filter paper and examined in JEOL-100S electron microscope.

Results and Discussion

Symptomatology

The inoculated plants of tomato cv Arka Vikas developed symptoms like light and dark green mosaic 2 to 10 days after inoculation and later they developed symptoms like leaf distortion, shoestring or filiform leaf due to reduction in leaf lamina which gives “fern leaf” like appearance after 15—20 days after inoculation. Such of the infected plants are severely stunted and produce less number of fruits with reduced size and weight.

Distribution and Incidence of the Disease

Incidence of the virus causing tomato fern leaf disease in South Karnataka during summer 2005, ranged from 0 to 20%. Maximum incidence of 20% was recorded in Mandya district and no incidence was recorded in many parts of Bangalore district. All the varieties and hybrids grown in the surveyed area were found to be susceptible. *Aphis craccivora* and

Table 2. Dilution end point (DEP) of the virus causing tomato fern leaf disease. Assay host : *Chenopodium amaranticolor*. NL = No lesion.

	Dilution	Number of leaves inoculated	Total lesions produced	Average number of lesions/ leaf
1	10^{-1}	5	700.0	140
2	10^{-2}	5	257.0	51.4
3	10^{-3}	5	172.0	34.4
4	10^{-4}	5	95.00	19.0
5	10^{-5}	5	NL	NL
6	10^{-6}	5	NL	NL
7	10^{-7}	5	NL	NL
8	10^{-8}	5	NL	NL
9	10^{-9}	5	NL	NL
10	10^{-10}	5	NL	NL
11	Control (no dilution)	5	850.0	170

Table 3. Thermal inactivation point (TIP) of virus causing tomato fern leaf disease. Assay host : *Chenopodium amaranticolor*. NL = No lesion.

	Temperature (C)	Number of leaves inoculated	Total lesions produced	Average number of lesions/ leaf
1	30	5	515.0	103.0
2	35	5	460.0	92.0
3	40	5	355.0	71.0
4	45	5	270.0	54.0
5	50	5	162.0	32.4
6	55	5	82.50	16.5
7	60	5	25.00	05.0
8	65	5	NL	NL
9	70	5	NL	NL
10	75	5	NL	NL
11	80	5	NL	NL
12	85	5	NL	NL
13	90	5	NL	NL
14	95	5	NL	NL
15	Control	5	550	110

Myzus persicae were the aphid species found on tomato plants in surveyed fields, which act as vectors of the virus.

Host Range

The virus causing tomato fern leaf disease could infect many plant species belonging to different families namely, Cucurbitaceae, Solanaceae, Leguminaceae, Chenopodaceae and Composite (Table 1). The virus induced systemic mosaic symptoms on *C. sativus, Luffa acutangula, Lgenaria siceraria* and *Citrullus lanatus* 7—20 days after inoculation and produce chloratic local lesion on *Momordica charantia* 8 days after inoculation whereas *Cucurbita pepo* has not took infection. In Solanaceae family *Capsicum annuum, Nicotiana benthamiana* and *N. glutinosa* produced symptoms like mosaic, leaf distortion and light and dark green islands 10—20 days after inoculation. *N. tabacum* var Samson produce symptoms like vein clearing and leaf curl 12 days after inoculation. *Solanum melongena* and *Datura stramonium* has not took infection. The virus induces necrotic local lesions on *Vigna unguiculata*. *Phaseolus aureus* and *P. mungo* at 7—8 days after inoculation and vein clearing, systemic mosaic symptoms on *Dolichos lablab* but no symptom on *Arachis hypogea* and *Phaseolus vulgaris.* The virus induces chloratic local lesions on *Chenopodium amaranticolor* and *C. quinoa* 6 days after inoculation. On targets indices the virus produces systemic mosaic. Plant species belonging to Amaranthaceae, Cruciferae, Cariaceae, Astraceae, Euphorbiaceae, Lamiacea and Musaceae were resistant to the virus under study and have not shown andy symptoms.

Virus Stability in Buffered Sap

Investigations on the physical properties of the virus revealed that the virus was infective unto dilution of 10^{-4} in crude sap and no symptoms were produced in 10^{-5} dilution (Tables 2—4). Therefore the virus under study had dilution end point of 10^{-4}. The virus was infective at 60 C when exposed for 10 minutes but not beyond 60 C. Further the virus remained viable unto 96 hours (4 days) at room temperature (27 ± 1C) indicating that it has a LIV of 96 hours.

Enzyme Linked Immunosorbent Sssay (ELISA)

Serologial studies carried out by DAC-ELISA using CMV specific antiserum to detect the virus associated with fern leaf disease indicated that the samples strongly reacted with the antiserum and gave positive reaction indicating that the disease was probably caused by a strain of cucumber mosaic virus.

Table 4. Longevity in vitro (LIV) of virus causing tomato fern keaf disease. Assay host : *Chenopodium amaranticolor.* NL = On lesion.

	Storage period (hours)	Number of leaves inoculated	Total lesions produced	Average lesion of lesions/ leaf
1	0	5	650.0	130.0
2	12	5	605.0	121.0
3	24	5	482.0	96.4
4	36	5	405.0	81.0
5	48	5	335.0	67.0
6	60	5	157.0	31.4
7	72	5	107.0	21.4
6	84	5	60.00	12.0
7	72	5	107.0	21.4
8	84	5	60.00	12.0
9	96	5	27.50	5.5
10	108	5	NL	NL
11	120	5	NL	NL
12	132	5	NL	NL
13	144	5	NL	NL
14	156	5	NL	NL
15	168	5	NL	NL
16	180	5	NL	NL
17	192	5	NL	NL

Electron Microscopy

Tomato leaves infected with the virus causin fern leaf disease were observed under electron microscope by following leaf dip method revealed the presence of isometric virus particles approximately 30 nm in diameter.

This study that the incidence of tomato fern leaf disease in Southern Karnataka ranged from 0 to 20%. All the varieties and hybrids grown were susceptible to the disease (12, 14, 10). *Aphis craccivora* and *Myzus persicae* were present on tomato plant which in spread of the disease (13, 9). The virus was sap transmissible to the test plant and produces various kinds of symptoms like local lesion on *Chenopod amaranticolor* and systemic mosaic on *Cucumis sativus. Capsicum annuum* and other host plants (1, 8, 15—19). Dilution end point (DEP), thermal inactivation point (TIP) and longevity *in vitro* (LIV) were 10^{-4}, 60 C and 96 hours respectively. Physical properties resemble those of CMV on tomato (9, 15, 18—21).

Virus isolated from fern leaf disease infected plant reacted strongly to CMV specific antiserum in DAC-ELISA and gave positive color development indicating the association of CMV with the disease. Similar positive results obtain by many workers (3, 9, 15). Electron microscopic observations by following leaf dip method revealed the presence of isometric virus particles resembling CMV particles. Similar virus particles are obtained from CMV infected tomato by many workers (9).

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