

SCREENING OF TOMATO GENOTYPES AGAINST CUCUMBER MOSAIC VIRUS (CMV) CAUSING FERN LEAF DISEASE

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ABSTRACT

Fern leaf disease of tomato is caused by cucumber mosaic virus (CMV) belongs to a genus *Cucumovirus*. A total of 68 diverse tomato genotypes were screened against fern leaf virus under greenhouse conditions by artificial sap inoculation. The genotypes showed different levels of disease reaction. Out of 68 genotypes screened, 15 genotypes showed immune reaction, 9 genotypes showed resistant reaction, 17 genotypes showed moderately resistant reaction, 20 genotypes showed moderately susceptible reaction and remaining genotypes showed susceptible reaction. The resistant reaction of genotypes was reconfirmed by *DAC-ELISA* with positive control Arka vikas. Hence the resistant sources obtained could be utilized as donors in development of resistant variety against tomato fern leaf disease.

INTRODUCTION

Tomato (*Solanum lycopersicon* L.) belongs to family *Solanaceae* and is one of the most popular and important vegetable crop in India. Tomato is popular for its high nutritive value, taste and versatile uses (Passam *et al.*, 2007). The production quality of tomato affected by array of biotic and abiotic stresses. Of these biotic factors especially virus diseases are major constraint in tomato production (Kalloo, 1988). Several viruses are presently known to contribute consistently to yield losses of tomato crop. Some of the important viruses infecting tomato are, *Tomato spotted wilt virus* (TSWV), *Cucumber mosaic virus* (CMV), *Tomato mosaic virus* (ToMV), *Tomato yellow leaf curl virus* (ToLCV) and *Tobacco mosaic virus* (TMV) (Jones *et al.*, 1991; Massumi *et al.*, 2009).

Fern leaf disease of tomato caused by CMV mainly transmitted by aphids (*Aphis gossypii* and *Myzus persicae*) belongs to Bromoviridae family and genus *Cucumovirus* (Maneesha *et al.*, 2005; Chandankar *et al.*, 2013). In India the occurrence of Cucumber mosaic virus on tomato was reported for the first time by Kiranmai *et al.* (1997). The CMV infection on tomato induces yellowing, mottling, twisting of leaves, leaf distortion and fern-like appearance of the leaves (Kiranmai *et al.*, 1997).

Host plant resistance is very effective and economical input of Integrated Pest Management (Singh, 2014). During the past two decades considerable efforts have not been made to develop virus resistant cultivars in tomato except against ToLCV. Tomato cultivars however are not completely resistant to viruses. Identification of resistant genotypes and exploration of resistant sources in tomato germplasm is very much important for the effective and environmentally safer management of CMV. To date, utilization of genotypes as a

resistance source against CMV in tomato has not reported so far in India. In view of the above, the present emphasis on Host Plant Resistance (HPR) could be easily adopted by the farmers. Hence, the present study was carried out to identify the level of resistance/susceptibility in selected and popularly grown tomato varieties/genotypes along with some accessions under green house conditions.

MATERIALS AND METHODS

The plant materials in the present investigation comprised of 68 genotypes of tomato collected from Department of Horticulture, UAS, GKVK and ICAR-Indian Institute of Horticulture Research (IIHR), Bengaluru.

The experiment was laid out under glasshouse conditions at Department of Plant Pathology, UAS, GKVK, Bengaluru. Sixty eight genotypes were screened against CMV causing fern leaf disease. Ten seeds per genotype were raised in pro-trays containing coir pith. Later 20-25 days old seedlings were transferred to 6' x 4' polythene bags. An equal number of Arka Vikas was raised in a similarly as a susceptible check. The genotypes were inoculated by virus inoculum through method described by Mandal *et al.* (2001) and inoculated seedlings were maintained in the insect proof cages for symptom expression.

Forty days after inoculation, disease incidence was scored based on a scale (0-5) given by Bos (1982) (Table 1) and percentage disease index (PDI) was worked out using the formula adopted by Silbernagel and Jafari (1974).

$$PDI = \frac{0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5}{nt(nC - 1)} \times 100$$

Where,

$n_0, n_1, n_2, \dots, n_5$ = No. of plants in score 0, 1, 2, ..., 5, respectively,

nt = total no. of plants,

nC = Total number of categories.

Based on the PDI values obtained, the genotypes were classified into five categories *viz.*, Immune (I) where PDI=0; resistant (R) where PDI=1-25%; moderately resistant (MR) where PDI=26-50%; moderately susceptible (MS) where PDI=51-75% and Susceptible (S) where PDI=76-100% (Havey, 1996).

DAC- ELISA was performed as described by Clark and Joseph

(1984) to confirm the presence of CMV in symptomless carriers and symptom bearing plants using CMV specific antiserum (company name) and readings were recorded in ELISA reader.

RESULTS AND DISCUSSION

Results as presented on the table 2 revealed that tomato genotypes exhibited a wide range of disease reaction to the extent of 0 to 93.11% against CMV under glasshouse conditions. Based on PDI, 15 genotypes *viz.*, Mukti, Arka Ahuti, Nandi, Sankranti, US-1035 (F_1), EC-771607, LA-0475, TLB-221, Hisar-N, TLB-129, TLB-192, LA-3923, TLB-133, Navoday and Sarkar were found to be immune without any symptoms. Nine genotypes showed resistant reaction *viz.*, Vybhav, HAT-121, WIR-13706, L-00887, LA-1479, LA-2138B, LA-1478, BWR-5 and S-22 and 17 genotypes showed moderately resistant reaction. Moderately susceptible reaction was found in 20 genotypes. The remaining 7 genotypes namely, Arka Vikas, Local variety, EC-514109, EC-677191, EC-677049, LA-0168 and TL-53(c) showed susceptible reaction (Table 3).

The similar type of varietal screening for host plant resistance were well documented by Hobbs *et al.* (1996) in pepper (*Capsicum annum* L.) lines for CMV resistance. The results

Table 1: Disease Scale Description Category

Scale	Description
0	No symptoms
1	Very light mottling of older leaves and dark green colour in younger leaves
2	Light and dark green areas associated with veins
3	Mosaic, blistering and puckering of leaves
4	Distortion of leaves
5	Stunting of the plants with negligible or no flowering

Table 2: Artificial screening of diverse tomato genotypes and their reaction against CMV causing fern leaf disease

Sl. No.	Genotypes	Percent disease Index (PDI)				Average PDI	Disease and reaction
		10 DAI	20 DAI	30 DAI	40 DAI		
1	Arka Vikas	58.93	79.66	88.56	94.33	80.37	S
2	Local variety	88.12	91.63	94.46	98.26	93.11	S
3	Arka Abha	0.00	24.43	38.23	47.89	27.64	MR
4	Mukti	0.00	0.00	0.00	0.00	0.00	I
5	Arka Ashish	4.60	22.33	36.76	43.65	26.84	MR
6	Arka Ahuti	0.00	0.00	0.00	0.00	0.00	I
7	Arka Alok	23.18	36.63	66.33	93.15	54.82	MS
8	Arka Meghali	45.33	64.9	72.59	96.65	69.87	MS
9	Arka Avinash	0.00	23.64	45.66	73.13	35.61	MR
10	Laxmi	9.13	23.14	32.33	46.73	27.83	MR
11	Nandi	0.00	0.00	0.00	0.00	0.00	I
12	Sankranti	0.00	0.00	0.00	0.00	0.00	I
13	Vybhav	0.00	4.60	29.46	58.34	23.10	R
14	Arka Abhijith (F_1)	19.11	26.13	35.64	56.33	34.30	MR
15	Arka Ananya (F_1)	23.31	34.94	57.70	79.45	48.85	MR
16	Arka Shresta (F_1)	0.00	26.83	48.59	75.48	37.73	MR
17	US-618 (F_1)	0.00	18.94	38.49	52.75	27.55	MR
18	US-1035 (F_1)	0.00	0.00	0.00	0.00	0.00	I
19	Arka Rakshak (F_1)	8.21	38.26	56.33	85.89	47.17	MR
20	Arka Sourabh	23.93	56.66	71.71	84.23	59.13	MS
21	EC-676791	32.66	52.33	69.14	91.57	61.43	MS
22	EC-514109	79.28	87.13	93.42	97.66	89.37	S
23	EC-677123	48.43	62.59	78.29	96.95	71.56	MS
24	EC-677191	58.94	78.38	87.36	94.49	79.79	S
25	EC-109762	14.46	28.35	39.26	56.16	34.56	MR
26	EC-771606	26.37	42.46	71.54	81.31	55.42	MS
27	EC-677049	86.35	89.47	92.83	95.97	91.15	S
28	EC-676730	15.16	43.17	62.53	78.56	49.86	MR
29	EC-771608	21.63	43.92	56.87	86.38	52.20	MS
30	HAT-121	0.00	4.94	23.15	36.23	16.08	R
31	WIR-13706	3.13	18.37	33.16	43.33	24.50	R
32	EC-771607	0.00	0.00	0.00	0.00	0.00	I
33	LA-0168	47.56	74.26	85.47	93.09	75.10	S
34	LA-01468	8.37	26.43	36.13	48.32	29.81	MR

Table 2: Cont.....

Sl. No.	Genotypes	Percent Disease Index (PDI)				Average PDI	Disease and reaction
		10 DAI	20 DAI	30 DAI	40 DAI		
35	L-00887	0.00	0.00	0.00	0.00	0.00	I
36	LA-0369	3.26	27.16	42.15	57.27	32.46	MR
37	LA-2138B	0.00	13.33	36.16	48.74	24.56	R
38	LA-0475	0.00	0.00	0.00	0.00	0.00	I
39	LA-0369	19.42	32.35	54.36	72.33	44.62	MS
40	LA-1479	3.10	12.53	25.16	36.78	19.39	R
41	LA-1478	0.00	8.94	21.13	33.33	15.85	R
42	TLB-205	17.25	36.47	49.39	69.64	43.19	MR
43	TLB-221	0.00	0.00	0.00	0.00	0.00	I
44	Hisar-N	0.00	0.00	0.00	0.00	0.00	I
45	TLB-129	0.00	0.00	0.00	0.00	0.00	I
46	CLN-2585D	27.34	43.12	56.37	64.68	47.88	MR
47	PKM-1	47.18	63.25	74.47	89.26	68.54	MS
48	TLB-196	36.24	57.17	76.65	87.93	64.50	MS
49	BWR-5	0.00	14.71	29.49	37.33	20.38	R
50	TLB-192	0.00	0.00	0.00	0.00	0.00	I
51	LA-3923	0.00	0.00	0.00	0.00	0.00	I
52	LCR-9	37.04	47.84	74.28	93.18	63.09	MS
53	L-04360	26.63	39.46	69.47	83.33	54.72	MS
54	L-02846	33.16	46.23	72.59	94.26	61.56	MS
55	Hawai-3996	39.43	64.39	76.36	88.17	67.09	MS
56	TLB-133	0.00	0.00	0.00	0.00	0.00	I
57	TL-53(c)	79.36	86.74	94.23	98.17	89.62	S
58	BL-1199	46.33	57.18	73.33	92.27	67.28	MS
59	L-02831	37.45	59.46	75.29	86.56	64.69	MS
60	BL-1200	47.93	63.13	77.17	95.96	71.05	MS
61	LA-3913	16.24	35.49	59.52	78.63	47.47	MR
62	LA-3914	11.23	28.92	39.46	64.36	35.99	MR
63	CLN-2679F	43.68	61.93	82.33	93.17	70.28	MS
64	CLN-2679E	12.37	39.53	74.20	89.37	53.87	MS
65	TLB-226	18.74	45.51	61.48	82.43	52.04	MS
66	S-22	0.00	3.59	29.35	37.23	17.54	R
67	Navoday	0.00	0.00	0.00	0.00	0.00	I
68	Sarkar (F ₁)	0.00	0.00	0.00	0.00	0.00	I

DAI: Days After Inoculation; I: Immune, R: Resistant, MR: Moderately resistant; MS: Moderately susceptible, S: Susceptible

Table 3: Grouping of tomato genotypes against CMV based on different levels of disease reaction

PDI(%)	Reaction	Genotypes
0	Immune(15)	Mukti, Arka Ahuti, Nandi, Sankranti, US-1035 (F ₁), EC-771607, LA-0475, TLB-221, Hisar-N, TLB-129, TLB-192, LA-3923, TLB-133, Navoday, Sarkar (F ₁)
1-25	Resistant(9)	Vybhav, HAT-121, WIR-13706, L-00887, LA-1479, LA-2138B, LA 1478, BWR-5, S-22
26-50	Moderately resistant(17)	Arka Abha, Arka Ashish, Arka Avinash, Laxmi, Arka Abhijith (F ₁), Arka Ananya (F ₁), Arka Shresta (F ₁), US-618 (F ₁), Arka Rakshak (F ₁), EC 109762, EC-676730, LA-01468, LA-0369, TLB-205, CLN-2585D, LA-3913, LA-3914
51-75	Moderately susceptible(20)	Arka Alok, Arka Meghali, Arka Sourabh, EC-676791, EC-677123, EC-771606, EC-771608, LA-0369, PKM-1, TLB-196, LCR-9, L-04360, L-02846, Hawai-3996, BL-1199, L-02831, BL-1200, CLN-2679F, CLN-2679E, TLB-226
76-100	Susceptible(7)	Arka Vikas, Local variety, EC-514109, EC-677191, EC-677049, LA-0168, TL-53(c)

PDI: Per cent disease index

also Lend further support of earlier worker, Frangioni *et al.* (2003) observed the immune reaction of pepper line, AF-97A to CMV and tolerance in AF-188, AF-1178, AF-98 A, AF-99 A and AF-136 A.

In order to confirm the absence of virus in the symptomless (Immune) plants DAC-ELISA was carried out and Arka Vikas used as positive control. The ELISA readings indicated that, the absorbance value was two times higher in the infected plants (0.725) than healthy plants (0.130 to 0.251) and results

are presented in the Table 4.

Our results supported by Herison *et al.* (2003) who evaluated, 69 hot pepper (*Capsicum annum*) lines for resistance to cucumber mosaic virus (CMV-02). Lines C1024, KA-2, PBC495 and C1042 showed 0% disease intensity (DI) consistently, whereas lines viz., LV2323, Tit Paris and PBC068 recorded 10% DI. Rashid *et al.* (2007) screened the pepper lines viz., C-4, C-8 and local check showed, positive reaction and the remaining lines showed negative reaction in ELISA test. The

Table 4: ELISA absorbance values of susceptible check (positive control) and immune genotypes

Sl. No.	Genotypes/lines	OD value*	Reaction
1	Healthy tomato	0.138	-ve
2	Arka Vikas	0.725	+ve
3	Mukti	0.219	-ve
4	Arka Ahuti	0.251	-ve
5	Nandi	0.153	-ve
6	Sankranti	0.198	-ve
7	US-1035 (F ₁)	0.176	-ve
8	EC-771607	0.159	-ve
9	LA-0475	0.237	-ve
10	TLB-221	0.152	-ve
11	Hisar-N	0.166	-ve
12	TLB-129	0.147	-ve
13	TLB-192	0.184	-ve
14	LA-3923	0.204	-ve
15	TLB-133	0.227	-ve
16	Navoday	0.173	-ve
17	Sarkar (F ₁)	0.192	-ve

*Absorbance value: Average of 3 reaction

results also in accordance with Ashfaq *et al.* (2014), who evaluated forty Chilli pepper genotypes both local and imported, for source of resistance to CMV chilli isolate. On the basis of 0-5 disease rating scale and ELISA, nine genotypes viz., C-2, CV-2, CV-5, BSS-269, PGRI, M-2001, CM-2001, M-97 and CP-328 were remained free of infection and categorized as highly resistant.

In present study genotypes grouped under immune and resistant category would be utilized as donors to develop CMV resistant lines. Hence, this study may serve as a basic guideline for researchers aiming at the development of resistance to CMV in tomato or in other hosts and genotypes utilized here serve as source to identify resistance to other viruses.

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