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Studies on Transmission of Cucumber Mosaic Virus (CMV) Through Seed in Tomato

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ABSTRACT: *Tomato is under the constant threat of diseases and about 200 diseases are known to infect tomatoes worldwide. Among these, Cucumber mosaic virus (CMV) is most devastating disease, as it can completely destroy the crop. Thirty tomato genotypes/cultivars along with susceptible check Arka Vikas were screened for resistance to CMV by sap/mechanical inoculation with purified CMV inoculum under greenhouse conditions. After development of prominent symptoms on the inoculated plants, samples were collected from each test entry and confirmed by DAC-ELISA. Seeds from the CMV inoculated tomato plants were collected 60 days after inoculation and sown in earthen pots for the confirmation of transmission of CMV through seeds in tomato. None of the genotypes/cultivars shown development of symptoms/ transmission of CMV through seeds after germination when tested through ELISA.*

Keywords: *Cucumber mosaic virus, ELISA, Sap inoculation, Symptoms, Tomato.*

INTRODUCTION

Tomato [*Solanum lycopersicum* (Mill.) Wettsd] is one of the most popular and widely grown vegetables in the world, ranking second in importance to potato in many countries. India stands fourth in production of tomatoes next to China, U.S.A. and Turkey. Tomato is under the constant threat of diseases and about 200 diseases are known to infect tomatoes worldwide (Jones *et al.*, 1997). Among these, *Cucumber mosaic virus* (CMV) is most devastating disease, as it can completely destroy the crop (Galliteli *et al.*, 1991). The characteristic field symptoms of CMV disease include stunting, yellowing, mottling of leaves, extreme filiformity or shoe stringing of leaf blades, depending on virus strain and the host (Carrere *et al.*, 1999 and Emy Sulistyowati *et al.*, 2004). CMV also impairs the fruit yield and quality of tomato fruits as CMV infected plants often produce small and misshapen fruits, besides delaying the fruit maturity. CMV occurs worldwide and is considered as a very important disease in temperate, tropical and subtropical regions of the world. In tomato, subgroup I strains cause fern shaped filiform leaves and stunting (Hellwald *et al.*, 2000; Stamova and Chetelat, 2000; Akhtar *et al.*, 2008 and Pratap *et al.*, 2012), whereas subgroup II strains lead to severe mosaic, leaf puckering and stunting (Sudhakar *et al.*, 2006).

Transmission efficiency varies with the aphid species, virus strain, host plant species, environmental conditions, and crop season. The virus is not seed borne in tomato (Zitter, 1984 and ACES, 2011).

MATERIALS AND METHODS

Thirty tomato genotypes, obtained from National Bureau of Plant Genetic Resources Regional Station, Hyderabad were used in the study (Table 1), using cv. Arka Vikas as susceptible check. Seeds of Arka Vikas were obtained from Agricultural Research Institute, Rajendranagar, Hyderabad. The accessions consist of cultivated *Solanum lycopersicum* (23 accessions), *Solanum lycopersicum* var. *cerasiforme* (2), *Solanum peruvianum* (1), and *Solanum pimpinellifolium* (4). Tomato seeds of all the genotypes and the check were sown in earthen pots (60 cm dia) containing soil compost mixture (3:1 sandy loam and FYM). It was ensured that at the time of virus inoculation, three healthy seedlings were maintained per pot (replication) and three replications were maintained for each genotype.

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Young leaves showing typical CMV symptoms were collected. The collected samples were ground in a chilled mortar and pestle using inoculation buffer/phosphate buffer. The Inoculation/Phosphate buffer (0.05 M; pH 7.0) is prepared by using Potassium di-hydrogen phosphate (KH_2PO_4) 2.40 g; di-potassium hydrogen phosphate (K_2HPO_4) 5.40 g; mercaptoethanol 1.56 ml and distilled water1000 ml. Twenty days old seedlings at 4 leaves stage were inoculated with freshly prepared standard extract of virus inoculum @ 1:10 dilution. Uninoculated plants served as control. All plants were kept under observation up to six weeks for development of symptom under insect proof conditions. Second inoculation was done 14 days after first inoculation. Tomato cultivars/genotypes screened were further tested serologically by drawing representative leaf samples to confirm the presence of CMV using Direct Antigen Coating-Enzyme Linked Immunosorbent Assay (DAC-ELISA) (Hobbs *et al.,* 1987).

Table 1
List of tomato accessions evaluated for transmission of CMV through seeds

S.No	*Accession number*	*Species*
1	EC251672	*Solanum lycopersicum* Mill.
2	EC251790	*Solanum peruvianum*
3	EC514006	*Solanum lycopersicum* var. cerasiforme
4	EC514013	*Solanum lycopersicum* var. cerasiforme
5	EC514134	*Solanum lycopersicum*
6	EC615014	*Solanum lycopersicum*
7	EC615018	*Solanum lycopersicum*
8	EC617056	*Solanum lycopersicum*
9	EC617076	*Solanum lycopersicum*
10	EC617080	*Solanum lycopersicum*
11	EC617083	*Solanum lycopersicum*
12	EC617084	*Solanum lycopersicum*
13	EC617088	*Solanum lycopersicum*
14	EC617089	*Solanum lycopersicum*
15	EC620388	*Solanum lycopersicum*
16	EC625642	*Solanum lycopersicum*
17	EC620389	*Solanum lycopersicum*
18	EC631430	*Solanum lycopersicum*
19	EC654284	*Solanum lycopersicum*
20	EC676742	*Solanum lycopersicum*
21	BSBS - 47	*Solanum pimpinellifolium*
22	KARS - 425	*Solanum pimpinellifolium*
23	Punjab Chauhara	*Solanum lycopersicum*
24	Marutham	*Solanum lycopersicum*
25	PSR - 10693	*Solanum pimpinellifolium*
26	Pusa Ruby	*Solanum lycopersicum*
27	SR - 6525	*Solanum pimpinellifolium*
28	STH - 801	*Solanum lycopersicum*
29	STH - 816	*Solanum lycopersicum*
30	US - 1196	*Solanum lycopersicum*
Susceptiblecheck	Arka Vikas	*Solanum lycopersicum*

Sixty days after inoculation the fruits were harvested from the plants and seeds were collected in the polyethylene bags both from inoculated and uninoculated plants. The collected seeds were dried and sown in earthen pots (60 cm dia) containing soil compost mixture (3:1 sandy loam and FYM). After germination the plants were examined for the transmission of CMV through seeds by observing the type of symptoms developed and confirmed by DAC-ELISA. Absorbance values were recorded at 405 nm with Biotek - ELISA micro plate reader. Samples were considered 'positive' when the absorbance value exceeded two times of that of the negative control.

RESULTS AND DISCUSSION

Transmission of CMV through seeds was not observed in thirty tomato genotypes/cultivars along with susceptible check Arka Vikas. All the test entries were found negative to CMV antisera when tested through ELISA. The negative control value in ELISA was recorded as 0.175. None of the genotypes/ cultivars absorbance value shown either greater or

Table 2
DAC-ELISA absorbance values of tomato genotypes/cultivars

S. No.	*Accession number*	*ELISA absorbance values*
1	EC251672	0.061 - 0.070
2	EC251790	0.052 - 0.071
3	EC514006	0.051 - 0.082
4	EC514013	0.056 - 0.072
5	EC514134	0.087 - 0.091
6	EC615014	0.036 - 0.046
7	EC615018	0.067 - 0.074
8	EC617056	0.066 - 0.072
9	EC617076	0.069 - 0.072
10	EC617080	0.070 - 0.074
11	EC617083	0.064 - 0.070
12	EC617084	0.069 - 0.076
13	EC617088	0.073 - 0.080
14	EC617089	0.026 - 0.028
15	EC620388	0.056 - 0.075
16	EC625642	0.057 - 0.062
17	EC620389	0.046 - 0.064
18	EC631430	0.035 - 0.080
19	EC654284	0.045 - 0.061
20	EC676742	0.074 - 0.087
21	BSBS - 47	0.064 - 0.083
22	KARS - 425	0.042 - 0.076
23	Punjab Chauhara	0.072 - 0.081
24	Marutham	0.070 - 0.075
25	PSR - 10693	0.085 - 0.091
26	Pusa Ruby	0.075 - 0.084
27	SR - 6525	0.077 - 0.084
28	STH - 801	0.072 - 0.074
29	STH - 816	0.050 - 0.061
30	US - 1196	0.031 - 0.068
31	Arka Vikas (Susceptible check)	0.063 - 0.042

double the value of negative control, hence all the test entries were considered as negative to CMV. Absorbance values of ELISA were given in the (Table 2). According to Zitter, 1984 and ACES, 2011 CMV is not seed borne in tomato and does not persist in plant debris in the soil. The results are in consonance with the findings of (Zitter, 1984 and ACES, 2011).

CONCLUSION

The results confirmed that the CMV was not transmitted through seed in tomato as the virus cannot persist in soil and plant debris. However, CMV is transmitted through seeds in 19 other crop species (ACES, 2011).

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