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Resistance of *Solanum* species to *Cucumber mosaic virus* subgroup IA and its vector *Myzus persicae*

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Abstract Sixty-nine tomato genotypes representing nine *Solanum* species were evaluated for resistance to *Cucumber mosaic virus* (CMV) subgroup IA and its aphid vector *Myzus persicae*. Resistance was assessed by visual scoring of symptoms in the field under natural conditions, and in the greenhouse by artificial inoculations through aphid *M. persicae* and mechanical transmissions in the year 2007 and 2009. Considerable variation in responses was observed among the evaluation methods used. Field evaluations were found liable to errors as different levels were observed for the same genotypes in the different years, however mechanical inoculation was found to be the most useful in identifying CMV subgroup IA resistance, in contrast aphid transmission was most useful in identifying insect transmission resistance. All genotypes observed as highly resistant to CMV subgroup IA in the field or through vector transmission became systemically infected through mechanical inoculations. Using mechanical inoculation, six genotypes (TMS-1 of *S. lycopersicum*, LA1963 and L06049 of *S. chilense*, LA1353, L06145 and L06223 of *S. habrochaites*) were found resistant and another six (L06188 and L06238 of *S. neorickii*, L06219 of *S. habrochaites*, L05763, L05776 and L06240 of *S. pennellii*) were found tolerant showing mild symptoms with severity index (SI) ranging 1-2 and with delayed disease development after a latent period (LP) of 18–30 days. However, these genotypes were found to be resistant to highly resistant in the field and through inoculation by *M. persicae*; and they also supported low population levels of *M. persicae* except TMS-1. Another nine genotypes (LA2184 of S. *pimpinellifolium* L., LA2727 of *S. neorickii*, LA0111, L06221, L06127 and L06231 of S. *peruvianum* L., LA1306, L06057 and L06208 of *S. chmielewskii*) showing a susceptible response after mechanical inoculation were highly resistant, resistant and tolerant after *M. persicae* transmission. The resistant genotypes, identified in the present study can be exploited in the breeding programmes aimed at developing tomato varieties resistant to CMV subgroup IA and broadening the genetic base of CMV-resistant germplasm. The differences observed between mechanical and aphid transmission suggests that one should consider both evaluation methods for tomato germplasm screening against CMV subgroup IA.

Key words *Cucumber mosaic virus* subgroup IA · *Cucumovirus* · Mechanical inoculation · *Myzus persicae* · Resistance · *Solanum* species

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Introduction

Tomato (*Solanum esculentum* L.) is the second most important vegetable grown commercially worldwide; however, its production is under the constant threat of diseases. About 200 diseases are known to infect tomatoes worldwide (Jones et al. 1997). Viruses are the most damaging threat and more than 100 viruses are known to infect tomatoes naturally or experimentally, inflicting heavy yield losses including *Cucumber mosaic virus* (CMV), which caused severe epidemics worldwide in the tomato crop (Tomlinson 1987). CMV is a type species of the genus *Cucumovirus*, in the family Bromoviridae. It is one of the most widespread plant viruses in the world. It is non-persistently transmitted by at least 80 aphid species, and has an extremely broad host range, infecting over 1,300 plant species in more than 500 genera of over 100 families including cereals, fruits, legumes, vegetables, ornamentals, weeds, etc. (Kaper and Waterworth 1981; Edwardson and Christie 1991; Palukaitis et al. 1992; Aramburu et al. 2007; García-Arenal and Palukaitis 2008).

CMV is one of the best characterized tripartite positive-sense single stranded RNA viruses (Takahashi 2008). Proteins 1a, 2a and 3a are expressed from genomic RNAs 1, 2 and 3 respectively, whereas the coat protein (CP) and 2b proteins are expressed from subgenomic RNA4 and RNA 4A, respectively (Palukaitis et al. 1992; Ding et al. 1994; Sulistyowati et al. 2004). The 1a and 2a proteins are apart of viral replicase complex (Hayes and Buck 1990), while cell to cell movement through the plant requires the 3a movement protein (MP) and coat protein (CP) (Suzuki et al. 1991; Taliansky and Garcia-Arenal 1995; Kaplan et al. 1995; Gal-On et al. 1998). CMV has been classified into two major subgroups designated as I and II according to serological relationships and nucleic acid identities (Palukaitis et al. 1992). Recent analysis based on the nucleotide sequence of the 5' non-translated region of the RNA3 group I was further divided into two subgroups namely; IA and IB (Roossinck 2002). Tomato is found to be infected with all three subgroups of CMV (Sudhakar et al. 2006; Akhtar et al. 2008; Pratap et al. 2008). CMV infected plants show a broad spectrum of symptoms from mild to severe mosaic, stunting, filiformity, chlorosis and necrosis depending on viral strain and host (Carrere et al. 1999; Sulistyowati et al. 2004; García-Arenal and Palukaitis 2008). In tomato, some subgroup I strains can cause fern-shaped filiform leaves and stunting (Hellwald et al. 2000; Stamova and Chetelat 2000; Akhtar et al. 2008; Pratap et al. 2008) whereas subgroup II strains lead to severe mosaic, leaf puckering and stunting (Sudhakar et al. 2006).

CMV is endemic in open field grown tomatoes in Pakistan. It is transmitted through seed, sap and non-persistently by aphids. *M. persicae* is efficient and the most studied vector to transmit CMV in tomatoes (García-Arenal and Palukaitis 2008). *M. persicae* is a polyphagous pest and damages the tomato crop by sucking cell sap. This damage weakens plants, which bear less fruit. It excretes honey dew on leaves on which sooty mould develops that affects photosynthesis. Under field conditions, management of CMV mainly depends on vector control. But CMV control is thought to be complicated due to a broad host range of both virus and its vector, since many crops and non-crops can serve as virus reservoirs (Stamova and Chetelat 2000). Secondly, the vector *M. persicae* is becoming resistant to many of the pesticides used in the tomato industry; equally, application of excessive quantities of insecticides poses great risks to humans and the environment. The most effective and environmentally safe method of control is through breeding cultivars resistant to both CMV and its vector *M. persicae*. Akhtar et al. (2008) found that CMV subgroup IA is responsible for infecting tomatoes in Pakistan. Therefore, the present work was carried out to screen cultivated and wild genotypes of *Solanum* species against CMV subgroup IA and *M. persicae* for the first time in Pakistan.

Materials and methods

Plant material

Sixty-nine genotypes of nine *Solanum* species (44 of *Solanum lycopersicum,* 2 of *S. chilense*, 5 of *S. pimpinellifolium L.*, one of *S. cheesmaniae*, 3 of *S. neorickii*, 4 of *S. habrochaites,* 4 of *S. peruvianum L.*, 3 of *S. chmielewskii* and 3 of *S. pennellii*) were compared for their resistance to CMV subgroup IA and its vector *M. persicae* (Table 1).

Field testing

Field observations were recorded during 2006-07 and 2008-09 cropping season at the Nuclear Institute for

Table 1 Response of *Solanum* spp. to CMV Subgroup IA after field infection, aphid transmission and mechanical inoculation

Solanum spp./ Genotypes	Disease response in field in 2007			Disease response in field in 2009			Aphid transmission				[a]Mechanical inoculation		
	Infection percentage	Severity index	Disease response	Infection percentage	Severity index	Disease response	Infection percentage	Latent period	Severity index	Disease response	Latent period	Severity index	Disease response
S. lycopersicum													
Pakit	60	1.7	T	42	1.5	T	60	18	3.0	S	12	3.5	HS
Peelo	80	2.5	S	65	2.1	T	70	18	3.5	HS	12	4.0	HS
CC Haus	70	2.7	S	49	3.3	S	60	20	4.0	HS	12	4.0	HS
88572	80	2.5	S	74	1.3	R	80	17	3.0	S	13	3.3	S
Lyp-1	75	2.9	S	66	2.6	S	70	17	4.0	HS	13	4.0	HS
H-24	83	2.3	T	70	2.4	T	80	18	3.0	S	13	3.3	S
Picdenato	54	1.9	T	80	2.5	S	60	17	3.0	S	12	3.3	S
Tibrido	67	1.8	T	78	1.4	R	70	18	2.6	S	13	3.0	S
Titano	70	2.6	S	75	2.7	S	80	16	3.2	S	12	3.7	HS
T2	83	2.7	S	72	2.2	T	80	16	2.8	S	13	3.0	S
UC-134	82	1.8	T	67	1.2	R	80	16	3.0	S	12	3.3	S
Roma	70	2.9	S	60	2.7	S	70	15	3.0	S	12	3.5	HS
Nagina	83	3.0	S	70	2.9	S	80	18	3.3	S	12	4.0	HS
Money Maker	84	1.9	T	70	2.2	T	70	17	2.8	S	13	3.0	S
Riogrande	79	2.4	T	73	2.6	S	60	16	3.1	S	12	3.5	HS
Rio-J-400	63	2.9	S	78	2.5	S	50	15	3.5	HS	12	4.0	HS
Rio-Mut-400	68	2.8	S	65	2.6	S	60	18	3.5	HS	12	4.0	HS
TMS-1	0	0	HR	5	1.0	R	10	25	1.0	R	18	1.3	R
TMS-2	40	1.8	T	60	1.5	T	70	19	2.8	S	12	3.0	S
TMS-3	59	2.3	T	60	2.5	S	90	17	3.5	HS	12	4.0	HS
LA1226	38	1.7	T	32	1.3	R	50	19	3.5	HS	17	4.0	HS
LA1673	0	0	HR	0	0	HR	30	20	3.5	HS	15	4.0	HS
LA1286	0	0	HR	0	0	HR	20	18	2.8	S	12	3.0	S
L02875	0	0	HR	0	0	HR	10	18	2.9	S	13	3.0	S
L06203	0	0	HR	0	0	HR	10	17	3.5	HS	14	4.0	HS
L06170	0	0	HR	0	0	HR	10	18	2.8	S	13	3.0	S
B-21	80	2.6	S	60	2.2	T	80	18	3.0	S	12	3.3	S
B-22	83	2.3	T	56	1.4	R	70	19	3.5	HS	12	4.0	HS
B-23	58	1.6	T	27	1.0	R	80	18	2.8	S	12	3.0	S

Table 1 (continued)

Solanum spp./ Genotypes	Disease response in field in 2007			Disease response in field in 2009			Aphid transmission				[a]Mechanical inoculation		
	Infection percentage	Severity index	Disease response	Infection percentage	Severity index	Disease response	Infection percentage	Latent period	Severity index	Disease response	Latent period	Severity index	Disease response
B-24	83	2.1	T	58	1.4	R	70	19	2.7	S	12	3.0	S
B-25	73	2.2	T	67	1.7	T	60	18	3.0	S	12	3.5	HS
B-26	81	2.7	S	67	2.5	S	70	18	3.5	HS	12	4.0	HS
B-27	89	2.8	S	77	2.2	T	70	18	2.8	S	12	3.0	S
B-28 (RF)	69	2.9	S	50	2.6	S	70	16	3.5	HS	12	4.0	HS
B-28 (CF)	87	3.5	HS	64	3.3	S	70	13	4.0	HS	8	4.0	HS
B-29	79	2.7	S	68	1.2	R	70	15	3.5	HS	12	4.0	HS
B-30	68	2.1	T	53	1.9	T	60	16	3.6	HS	12	4.0	HS
B-31	73	2.3	T	60	2.0	T	80	18	3.7	HS	12	4.0	HS
B-33	75	1.7	T	59	2.1	T	70	17	3.6	HS	12	4.0	HS
Ch-151	71	2.0	T	58	2.0	T	70	17	3.6	HS	12	4.0	HS
S. lycopersicum													
Walter	6.0	1.0	R	13	1.9	T	50	22	2.8	S	12	3.0	S
PAK140979	57	1.8	T	53	2.1	T	70	17	3.6	HS	12	3.8	HS
PAK10996	64	1.6	T	63	1.8	T	80	17	3.8	HS	12	4.0	HS
PAK10579	67	2.0	T	60	2.1	T	80	18	3.9	HS	12	4.0	HS
S. chilense													
LA1963	0	0	HR	0	0	HR	0	0	0	HR	28	1.0	R
L06049	0	0	HR	0	0	HR	0	0	0	HR	29	1.0	R
S. pimpinellifolium L.													
LA2184	0	0	HR	0	0	HR	10	18	2.0	T	14	2.5	S
LA0722	0	0	HR	0	0	HR	20	16	2.5	S	12	3.0	S
LA1261	0	0	HR	0	0	HR	10	17	3.0	S	13	3.5	HS
L02707	0	0	HR	0	0	HR	10	16	3.0	S	14	3.3	S
L04166	0	0	HR	0	0	HR	10	17	3.0	S	13	4.0	HS
S. cheesmaniae													
LA0317	20	1.2	R	20	1.3	R	50	16	2.5	S	13	3.3	S
S. neorickii													
LA2727	20	1.2	R	25	1.7	T	25	18	1.9	T	15	3.3	S
L06188	0	0	HR	0	0	HR	0	0	0	HR	20	2.0	T

L06238	0	0	HR	0	0	HR	0	0	0	HR	22	2.0	T
S. habrochaites													
LA1353	0	0	HR	0	0	HR	20	0	0	HR	28	1.3	R
L06145	0	0	HR	0	0	HR	0	0	0	HR	30	1.0	R
L06219	0	0	HR	0	0	HR	0	0	0	HR	25	2.0	T
L06223	0	0	HR	0	0	HR	0	0	0	HR	27	1.3	R
S. peruvianum L.													
LA0111	0	0	HR	0	0	HR	10	22	1.3	R	17	3.0	S
L06221	0	0	HR	0	0	HR	10	21	1.8	T	17	2.8	S
L06127	0	0	HR	0	0	HR	10	20	1.9	T	15	3.0	S
L06231	0	0	HR	0	0	HR	0	0	0	HR	14	3.0	S
S. chmielewskii													
LA1306	0	0	HR	0	0	HR	0	0	0	HR	12	3.3	S
L06057	0	0	HR	0	0	HR	0	0	0	HR	13	3.0	S
L06208	0	0	HR	0	0	HR	0	0	0	HR	14	3.0	S
S. pennellii													
L05763	0	0	HR	0	0	HR	0	0	0	HR	28	2.0	T
L05776	0	0	HR	0	0	HR	0	0	0	HR	27	2.0	T
L06240	0	0	HR	0	0	HR	0	0	0	HR	28	2.0	T

HR highly resistant, *R* resistant, *T* tolerant, *S* susceptible, *HS* highly susceptible

[a] Infection percentage was 100 for all the mechanically inoculated genotypes

Agriculture and Biology (NIAB), Faisalabad, Pakistan. Each test genotype was transplanted in triplicate with normal row to row and plant to plant distances during December 2006 and 2008. Conventional agronomic practices were followed to maintain the crop in good condition, however, no plant protection measures were applied against the aphid *M. persicae* to ensure a high inoculum pressure throughout the experiment. Data for CMV symptoms were recorded following a five point (0–4) disease severity index (SI), where 0 = no visible disease symptoms (highly resistant); 1 = mild mosaic or mottling and leaf deformity (resistant); 2 = moderate mosaic or mottling, leaf deformity and filiformity (tolerant); 3 = severe mosaic or mottling or leaf deformity, filiformity, shoestringing, minor to medium stunting with minor flower shedding and minor reduction in fruit setting (susceptible) and 4 = severe mosaic or mottling, leaf deformity, filiformity, shoestringing, stunting with no or few fruit setting (highly susceptible). Individual symptomatic plant ratings for each genotype were added and divided by the number of infected plants to calculate the corresponding SI.

Source and maintenance of CMV subgroup IA

The inoculum of CMV subgroup IA for the mechanical and *M. persicae* transmission study was obtained from naturally infected tomato plants of cultivated tomato variety Nagina and maintained in the glasshouse.

Aphid transmission

Non-viruliferous aphids (*M. persicae*) collected from healthy plants were first starved for 30 min in Petri dishes and then provided with a 2-h acquisition access period (AAP) on tomato leaves infected with CMV subgroup IA. After which they were provided with a 4-h inoculation access period (IAP) on 3-4 week old healthy test plants. A total of 5 potted plants per test genotype were inoculated using 100 viruliferous aphids per plant. After 4-h IAP, plants were sprayed with insecticide to kill aphids. Data was recorded on the percentage of disease transmission, mean latent period, and average disease severity 90 days after inoculation.

Mechanical inoculation

Tomato leaves with typical shoestring disease symptoms were ground in 0.02 M phosphate buffer, pH 7.4; (1 g/ml) with a pestle and mortar and squeezed through a very fine muslin cloth. Young leaves of 4-week old five healthy tomato plants were dusted with 500-mesh carborandum powder and were mechanically inoculated with the freshly extracted sap using cotton pads. Plants were rinsed gently with a stream of water just after inoculation to remove superfluous inoculum and were kept under insect-free cages for symptom development. Data were recorded on the percentage of disease transmission, mean latent period, and average disease severity 90 days after inoculation.

Resistance to aphid *M. persicae*

Tomato genotypes that were evaluated for resistance to CMV subgroup IA were also screened for resistance to *M. persicae* in the field during both years (2007 and 2009). Observations were made in the third week of February, first week of the March, third week of the March and in the first week of the April. The populations of *M. persicae* on three leaves, upper, middle and lower parts of 10 plants per genotypes, were recorded. Genotypes were ranked as highly resistant, resistant and susceptible on the basis of aphid infestation scores following the trinomial sampling scale; 0 = no aphids (highly resistant); 1 = 1–10 aphids per leaf (resistant) and 2 = 11 or more aphids per leaf (susceptible) (Kohler and St. Clair 2005).

CMV detection using ELISA and reverse transcription polymerase chain reaction

The presence or absence of CMV in the test plants was assayed by double antibody sandwich procedure (DAS-ELISA) (Clark and Adams 1977; Palukaitis et al. 1992) with commercial polyclonal antibodies to CMV (BIOREBA AG Switzerland) as recommended by the manufacturers. To confirm the presence of CMV subgroup IA, viral RNA was extracted from the leaves following the method described by Ryu and Park (1995). Reverse transcription polymerase chain reaction (RT-PCR) and restriction fragment length polmorphism (RFLP) were employed to detect and characterize the *Cucumovirus* following the method described by Choi et al. (1999). RT-PCR was carried out using a GeneAmp RNA PCR core kit (Perkin Elmer) according to the manufacturer's instructions.

Results

Field screening of tomato genotypes for CMV subgroup IA

Sixty-nine tomato genotypes of nine *Solanum* species showed a wide range of responses to infection by CMV subgroup IA in the field in 2007 and 2009. Presence or absence of virus was confirmed by typical viral symptoms, ELISA, and RT-PCR using a genus-specific single pair of primers. The infection percentage varied 0–89% during 2007 and 0–80% during 2009 with SIs of 0–3.5 in 2007 and 0–3.3 in 2009 (Table 1). The first disease symptoms appeared on the susceptible genotypes in the last week of February after 7–10 days of the appearance of aphids. Symptoms started with minor mosaic on young emerging leaves, which progressed to severe mosaic or mottling, leaf deformity, filiformity, shoestringing, stunting with no or few fruits most of which were deformed. About 4-5 weeks after infection, when the filiform leaves were well developed, another symptom in the form of excessive numbers of lateral leaflets became apparent. The disease severity increased with time except in April, when the severity decreased due to increased temperature. Plants of susceptible genotypes infected at an early stage of growth expressed severe symptoms while mid and late season infections produced minor symptoms. Severely affected plants produced fewer fruits, some of which were smaller and deformed with delayed maturity, but others were normal in shape but medium in size.

Of the 44 *S. lycopersicum* genotypes evaluated in the field in 2007 on the basis of SI, only 6 genotypes (TMS-I, LA1673, LA1286, L02875, L06203 and L06170) were found highly resistant as they were symptomless and had not accumulated detectable amounts of virus. One genotype (Walter) of *S. lycopersicum* was resistant, 21 genotypes were tolerant, 15 genotypes were susceptible, and one genotype was highly susceptible with SI values of 0, 1, 1.6–2.4, 2.5–3.0 and 3.5 respectively (Table 1). During 2009 ranking of the tested 44 *S. lycopersicum* genotypes was not similar to that observed in 2007. However in 2009, the same five genotypes (LA1673, LA1286, L02875, L06203 and L06170) were highly resistant, 9 genotypes (TMS-I, B-23, UC-134, B-29, 88572, LA1226, Tibrido, B-22 and B-24) were resistant, 17 genotypes were tolerant and 13 genotypes were susceptible with SI values of 0, 1–1.4, 1.5–2.4 and 2.5–3.3 respectively (Table 1).

Most of the genotypes (92%) of the remaining eight wild *Solanum* species exhibited high levels of resistance with similar ranking in both the years (Table 1). Two genotypes of S. *chilense* (LA1963 and L06049), 5 genotypes of *S. pimpinellifolium L.* (LA2184, LA0722, LA1261, L02707 and L04166), 2 of *S. neorickii* (L06188 and L06238), 4 of *S. habrochaites* (LA1353, L06145, L06219 and L06223), 4 of *S. peruvianum L.* (LA0111, L06221, L06127 and L06231), 3 of *S. chmielewskii* (LA1306, L06057 and L06208) and 3 of *S. pennellii* (L05763, L05776 and L06240) were highly resistant. They remained symptomless and were negative for CMV. One genotype LA0317 of *S. cheesmaniae* was resistant with SI of 1.2 and 1.3 during 2007 and 2009 respectively and one genotype LA2727 of *S. neorickii* was resistant in 2007 with SI of 1.2 while it was tolerant in 2009 with SI of 1.7 (Table 1).

Aphid transmission studies

The transmission of CMV subgroup IA by *M. persicae* was tested on 69 tomato genotypes of 9 *Solanum* species (Table 1). The infection percentage was not significantly different from the field screening response for most of the *S. lycopersicum* genotypes but there were significant differences in SI values. Of the 44 *S. lycopersicum* genotypes evaluated using aphids, all the genotypes which were resistant under field conditions turned out to be susceptible with high SI values, except for one genotype (TMS-I). A wide range of infection percentages (10–90%), LPs (15–25) and SIs (1–4) was observed between the genotypes. TMS-I showed minimum infection percentage (10%), SI value (1) and a marked delay in disease development (25 DPI). Twenty-two genotypes were susceptible and 21 were highly susceptible with SI values of 2.6–3.3 and 3.5–4.0 respectively. The first disease symptom as minor mosaic was observed on the susceptible genotype B-28 (CF) after 13 DPI, which was followed by other susceptible genotypes having LP from 15–20 DPI, while disease symptoms on the resistant genotype TMS-I started after 25 days. Severe disease symptoms were observed on all 43 *S. lycopersicum* genotypes within 10 days after the first symptom appearance

while remaining as mild on TMS-I throughout the experiment. Five genotypes namely LA1673, LA1286, L02875, L06203 and L06170 showing highly resistant response under field conditions in both years became susceptible to highly susceptible through aphid transmission with high SI values ranging from 2.8–3.5 and infection percentages from10–30%. These genotypes showed higher SI values but lower infection percentages as compared to other susceptible genotypes. None of the tested genotypes was found to be free of infection or displaying 100% infection.

There was a wide variation in SI values (0–3.0) and infection percentages (0–50%) in the remaining 25 genotypes belonging to eight wild *Solanum* species (Table 1). The results when comparing field and contained aphid transmission experiments were similar to some extent as majority of the test genotypes (60%) was resistant with mild symptoms after aphid inoculation of the virus (Table 1). Two genotypes of *S. chilense* (LA1963, L06049), 2 of *S. neorickii* (L06188, L06238), 4 of *S. habrochaites* (LA1353, L06145, L06219, L06223), one of *S. peruvianum L.* (L06231), 3 of *S. chmielewskii* (LA1306, L06057, L06208) and 3 of *S. pennellii* (L05763, L05776, L06240) were highly resistant as these were free from the disease and had not accumulated detectable amount of virus. One genotype, LA0111 of *S. peruvianum L,.* was resistant with an SI of 1.3, LP of 22 days and 10% infection; while one genotype of *S. pimpinellifolium L.* (LA2184), one of *S. neorickii* (LA2727) and 2 of *S. peruvianum L.* (L06221, L06127) were tolerant with SI values of 2.5, 1.9, 1.8, 1.9 and percent infection of 10, 25, 10, 10 respectively. The remaining 5 genotypes of *S. pimpinellifolium L.* and *S. cheesmaniae* were susceptible with SIs of 2.5–3.0, LPs of 16-17 days and 10–50% infection (Table 1). Disease symptoms in infected plants persisted throughout the experiment and symptomless plants remained symptomless and uninfected till the end of the experiment *i.e.*, 90 days after inoculation.

Mechanical inoculation studies

Mechanical inoculation was 100% successful in transmitting CMV subgroup IA to all the genotypes of 9 *Solanum* species, but there was a wide variation in SI and LP values within the genotypes. None of the inoculated plants was symptomless and all the treated plants had detectable amounts of virus. The infection percentage and SI values by mechanical inoculation were however different when comparing field and contained aphid inoculations for the 44 *S. lycopersicum* genotypes (Table 1). Notably, only one male sterile genotype TMS-I was resistant with an SI of 1.3, which showed a marked delay in disease development with 25 days of LP. Among the remaining genotypes, 16 were susceptible with SI values of 3.0–3.3, and 27 were highly susceptible with SI values of 3.5–4.0. The first disease symptom showing mild mosaic was observed in highly susceptible genotypes after 12DPI, and this progressed into severe mosaic or mottling, leaf deformity, filiformity, production of excessive number of lateral leaflets, shoestringing (Fig. 1), stunting with no or few deformed fruits (Fig. 2). No reduction in disease severity was recorded untill the end of the experiment.

A low correlation was found between results from field/aphid inoculation and mechanical inoculation for 25 wild genotypes. A number of genotypes from eight wild *Solanum* species, found highly resistant to CMV subgroup IA under field or through aphid transmission, turned out to be susceptible by mechanical inoculation, indicating the reliability of this sap transmission technique. Two genotypes of *S. chilense* (LA1963, L06049) and 3 of *S. habrochaites* (LA1353, L06145, L06223) responded as resistant with SI values of 1.0–1.3 and delayed LP of 27–30 days (Table 1). Two genotypes of *S. neorickii* (L06188, L06238), one of *S. habrochaites* (L06219) and 3 of *S. pennellii* (L05763, L05776, L06240) were

Fig. 1 Mild mosaic & leaf deformity, moderate mosaic & leaf deformity, severe leaf deformity & filiformity, production of excessive number of lateral leaflets, and severe shoestringing (from left to right)

Fig. 2 Deformed fruits on left and healthy on right

found tolerant with SI values of 2.0 and LP of 20–28 days. The remaining 12 genotypes were susceptible with SI values of 2.5–3.0 and two genotypes were highly susceptible with SI values of 3.5–4.0. The first symptom in all the inoculated wild genotypes started as a mild mosaic except for *S. habrochaites* genotypes, which initiated disease as severe blistering on upper leaf surface. Genotypes responding as resistant showed minor symptoms like slight mosaic or mottling while tolerant ones showed moderate mosaic or mottling and leaf deformity followed by minor shoestringing or fern leaves till the end of experiment.

Resistance of *Solanum* species to aphid *M. persicae*

Table 2 indicates that none of the test genotypes was found free from aphids under field conditions. The population levels of *M. persicae* were different in both years. However, similar resistance levels were observed for the same genotypes in both years. Aphid infestations started in the second week of February on susceptible genotypes and persisted in the field till the second week of April in both years. Of the 44 genotypes of *S. lycopersicum* evaluated, only three namely LA1226, Walter and LA1286 were resistant, with an average of 3.3, 4.3 and 7.3 aphids per leaf respectively in 2007 and 3.3, 9.0 and 5.5 aphids per leaf respectively in 2009. The remaining 38 were susceptible with 11.3–55.3 aphids per leaf in 2007 and 10.5–117 aphids per leaf in 2009. Five genotypes viz., LA1673, LA1286, L02875, L06203 and L06170 carried 14, 7.3, 12.3, 13.3 and 13.8 aphids per leaf respectively in 2007 and 10.8, 5.5, 13.4, 10.5 and 12.3 aphids per leaf respectively in 2009. These genotypes were not only highly resistant against CMV subgroup IA in the field but also showed less infection percentage under contained aphid inoculation. The genotype LA1226 showing a minimum number of aphids per leaf in both years was resistant to CMV subgroup IA in 2007 and tolerant in 2009 but highly susceptible after aphid and mechanical inoculation. In 2007, in the third week of February, 16 genotypes of *S. lycopersicum* were resistant and 33 were susceptible; in the first week of March, 4 were resistant and 40 were susceptible; in the third week of March, only 6 were resistant and 38 were susceptible; in the first week of April, 21 were resistant and 24 were susceptible. However during 2009 in the third week of February, 9 genotypes were resistant and 35 were susceptible; in the first week of March, 3 were resistant and 41 were susceptible; in the third week of March, 3 were resistant and 41 were susceptible; in the first week of April, 15 were resistant and 29 were susceptible. Overall the correlation found between aphid densities for the same genotype at different intervals across both years was too low to draw meaningful conclusions.

Of the 25 wild genotypes of *Solanum* species, 22 genotypes carried low to moderate aphid densities (1.3–8.3 and 1.3–9.3 aphids per leaf in 2007 and 2009 respectively) and were rated as resistant (Table 2). The remaining three genotypes viz., LA0317, LA2727 and l06221 were susceptible with 12.8–16.8 and 11–14.5 aphids per leaf in 2007 and 2009 respectively. Most of the aphid-resistant wild genotypes were highly resistant, resistant and tolerant against CMV subgroup IA when plants were evaluated in the field (no symptom, no detectable virus) or inoculated by aphids (with 0–20% infection). There were great variations for infestation levels between time intervals for the same genotype in both years. But maximum infestation levels for resistant genotypes were found between first and third week of April. In 2007, in the third week of February one genotype was highly resistant, 20 were resistant and 4 were susceptible; in the first week of March, 18 were resistant and 7 were susceptible; in the third week of March, only 3 were highly resistant, 20 were resistant and 2 were susceptible; in the first week of April, 13 were highly resistant, 11 were resistant and one was susceptible. However in 2009, in the third week of

Table 2 Aphid *M. persicae* population on *Solanum* spp. in the field

Solanum species/ Genotype	Seed source[a]	Growth habit[b]	Aphid population in 2007						Aphid population in 2009					
			Third week of February	First week of March	Third week of March	First week of April	Mean	Response	Third week of February	First week of March	Third week of March	First week of April	Mean	Response
S. lycopersicum														
Pakit	A	ID	1	24	78	6	27.3	S	35	33	25	6	24.8	S
Peelo	A	D	14	22	39	6	20.3	S	10	23	20	18	17.8	S
CC Haus	A	D	11	18	153	24	51.5	S	25	15	9	10	14.8	S
88572	A	D	5	33	26	9	18.3	S	20	12	12	3	11.8	S
Lyp-1	A	D	21	31	131	11	48.5	S	50	35	40	15	35.0	S
H-24	A	D	17	25	124	29	48.8	S	46	58	37	5	36.5	S
Picdenato	A	D	8	49	52	40	37.3	S	30	40	46	39	38.8	S
Tibrido	A	D	4	56	123	24	51.8	S	21	18	15	6	15.0	S
Titano	A	D	7	24	53	23	26.8	S	8	13	17	13	12.8	S
T2	A	D	12	56	79	39	46.5	S	97	48	26	21	48.0	S
UC-134	A	D	30	132	40	19	55.3	S	24	31	46	22	30.8	S
Roma	A	D	24	37	46	14	30.3	S	78	60	62	49	62.3	S
Nagina	A	D	10	30	65	15	30.0	S	51	79	54	32	54.0	S
Money Maker	A	ID	6	57	80	23	41.5	S	44	28	18	21	27.8	S
Riogrande	A	D	52	44	48	10	38.5	S	27	23	30	12	23.0	S
Rio-J-400	B	D	27	36	15	10	22.0	S	99	64	55	37	63.8	S
Rio-Mut-400	B	D	31	38	17	8	23.5	S	63	60	45	12	45.0	S
TMS-1	B	D	7	75	45	30	39.3	S	104	117	154	93	117.0	S
TMS-2	B	ID	50	52	66	25	36.5	S	49	135	54	79	79.3	S
TMS-3	B	ID	60	51	45	31	46.8	S	60	39	35	22	39.0	S
LA1226	D	ID	4	4	2	3	3.3	R	3	3	4	3	3.3	R
LA1673	D	D	1	24	22	9	14.0	S	2	18	19	4	10.8	S
LA1286	D	ID	4	4	15	6	7.3	R	3	7	9	3	5.5	R
Lo2875	E	SD	10	19	8	12	12.3	S	5	19	20	9	13.4	S
Lo6203	E	D	5	8	31	8	13.0	S	2	16	20	4	10.5	S
Lo6170	E	D	9	13	16	7	13.8	S	8	18	17	6	12.3	S
B-21	B	ID	14	29	1	1	11.3	S	20	32	31	28	27.8	S
B-22	B	D	10	30	13	7	15.0	S	14	20	22	19	18.8	S
B-23	B	D	63	45	10	9	13.8	S	50	35	33	16	33.5	S

B-24	B	D	15	28	23	11	19.3	S	60	61	90	34	61.3	S
B-25	B	D	26	42	27	12	26.8	S	84	55	62	20	55.3	S
B-26	B	ID	21	43	48	10	30.5	S	43	51	47	64	51.3	S
B-27	B	SD	38	84	62	30	53.5	S	74	49	47	25	48.8	S
B-28 (RF)	B	D	41	76	18	7	35.5	S	51	47	38	36	43.0	S
B-28 (CF)	B	D	20	89	21	8	34.5	S	33	30	42	14	29.8	S
B-29	B	D	34	52	87	35	52.0	S	20	46	24	6	24.0	S
B-30	B	D	12	25	43	20	25.0	S	14	39	20	6	19.8	S
B-31	B	D	55	48	19	13	33.8	S	40	48	37	24	37.3	S
B-33	B	D	50	40	30	12	33.0	S	30	50	30	18	32.0	S
Ch-151	C	D	18	63	34	20	33.8	S	32	16	14	3	16.3	S
S. lycopersicum														
Walter	C	ID	5	4	1	7	4.3	R	3	9	17	7	9.0	R
PAK140979	C	D	19	22	14	10	16.3	S	26	35	44	38	35.6	S
PAK10996	C	D	32	20	13	9	18.5	S	26	23	19	8	19.0	S
PAK10579	C	D	44	54	10	9	29.3	S	59	46	46	34	46.3	S
S. chilense														
LA1963	D	D	3	2	3	0	2.0	R	2	2	3	0	1.8	R
L06049	E	D	1	6	1	0	2.0	R	1	3	1	0	1.3	R
S. pimpinellifolium L.														
LA2184	D	D	7	15	7	2	7.8	R	6	11	9	1	6.8	R
LA0722	D	D	1	7	3	1	3.0	R	1	6	5	0	1.3	R
LA1261	D	D	1	4	7	3	3.8	R	1	5	7	2	6.8	R
Lo2707	E	SD	0	15	10	5	7.5	R	0	10	5	3	3.0	R
L04166	E	ID	4	9	5	2	5.0	R	2	4	3	2	3.8	R
L. cheesmani														
LA0317	D	D	4	27	13	7	12.8	S	7	25	20	6	14.5	S
S. neorickii														
LA2727	D	D	31	16	10	10	16.8	S	30	15	5	5	13.8	S
L06188	E	D	12	8	3	3	6.5	R	10	18	3	1	8.0	R
L06238	E	D	2	6	0	0	2.0	R	1	4	1	0	1.5	R
S. habrochaites														
LA1353	D	SD	2	4	0	0	1.5	R	1	3	1	0	1.3	R
L06145	E	SD	1	3	1	0	1.3	R	1	2	2	0	1.3	R
L06219	E	SD	3	28	2	0	8.3	R	7	23	7	0	9.3	R
L06223	E	SD	3	7	1	0	2.3	R	2	6	1	0	2.3	R

Table 2 (continued)

Solanum species/ Genotype	Seed source[a]	Growth habit[b]	Aphid population in 2007						Aphid population in 2009					
			Third week of February	First week of March	Third week of March	First week of April	Mean	Response	Third week of February	First week of March	Third week of March	First week of April	Mean	Response
S. peruvianum L.														
LA0111	D	D	23	7	0	0	7.5	R	12	8	2	0	5.5	R
L06221	E	D	1	17	33	15	16.5	S	1	18	20	5	11.0	S
L06127	E	D	18	14	3	3	9.5	R	13	17	4	1	8.8	R
L06231	E	D	3	6	1	0	2.5	R	2	4	1	0	1.8	R
S. chmielewskii														
LA1306	D	SD	5	9	5	0	4.8	R	3	7	8	0	4.5	R
L06057	E	D	7	10	3	0	5.0	R	5	9	2	0	4.0	R
L06208	E	D	6	6	3	0	3.8	R	3	4	2	0	2.3	R
S. pennellii														
L05763	E	D	2	6	5	1	3.5	R	2	7	5	1	3.8	R
L05776	E	D	2	8	4	2	4.0	R	3	4	6	0	3.3	R
L06240	E	D	1	7	3	0	2.8	R	1	5	3	0	2.3	R

[a] [A = Ayub Agricultural Research Institute, Pakistan; B = Nuclear Institute for Agriculture and Biology, Pakistan; C = National Agricultural Research Council, Pakistan; D = Tomato Genetic Resources Centre (TGRC), USA; E = Asian Vegetable Research and Development Centre (AVRDC), Taiwan]

[b] [D = Determinate type; ID = Indeterminate type; SD = Semi-determinate type]

February, one genotype was highly resistant, 21 were resistant and 3 were susceptible; in the first week of March, 18 were resistant and 7 were susceptible; in the third week of March, 23 were resistant and 2 were susceptible; in the first week of April, 15 were highly resistant and 10 were susceptible. In most cases, aphid densities were enough to cause disease as was observed in the case of *S. lycopersicum* genotypes.

Discussion

Tomato shoestring, caused by CMV, has emerged as a disease of economic importance in Pakistan due to increasing infestation of its vector *M. persicae*, which has developed resistance against a number of insecticides. This has raised the concern that it could cause a serious problem as it has inflicted heavy yield losses in eastern France (Gebre et al. 1990), Bulgaria (Stamova et al. 1990), eastern Spain (Jorda et al. 1992) and southern Italy (Crescenzi et al. 1993). The use of genetic plant resistance against diseases and pests is an environmentally compatible and effective control method (Martin and Fereres 2003).

The present study is a comprehensive evaluation of *Solanum* species for resistance against CMV subgroup IA and its vector *M. persicae* under natural infection, and in the greenhouse using *M. persicae* and mechanical inoculation. There were great differences among 69 genotypes for SIs and infection percentages in both the years. All symptomatic genotypes tested positive for CMV while symptomless genotypes tested negative by ELISA. Significant differences in densities of *M. persicae* were observed among *Solanum* species, but none was found to be highly resistant. In the majority of the test genotypes under field evaluation, disease increased with the increase in aphid population but in others it was the reverse. This shows that the insect population was sufficient to result in high level of disease but there were other factors involved. A major difficulty of breeding for disease resistance has been lack of the screening methods. Field screening is the principal method, but it has limitations as the disease might occur with varying degrees of incidence as well as severity (Delatte et al. 2006). These variable levels may be due to the lack of a single or a combination of factors including spatial and temporal variation in inoculum levels, environmental conditions, vector host preference, host resistance to vector, age of plants, soil conditions, etc. (Hoogstraten 1992: Rahman et al. 2005; Akhtar et al. 2009). To overcome these difficulties it was necessary to confirm the field results with artificial transmission by *M. persicae* and mechanical inoculation.

Tomato genotypes belonging to 9 *Solanum* species showed variable responses when inoculated with CMV subgroup IA using *M. persicae*. A significant number were found to be infected with virus. Fifteen genotypes were found highly resistant (no symptoms, no detectable virus), one was resistant and 4 were tolerant. However, all the genotypes were systemically infected with CMV subgroup IA when inoculated mechanically. Interestingly six genotypes (TMS-1, LA1963, L06049, LA1353, L06145, L06223) were found resistant and another six genotypes (L06188, L06238, L06219, L05763, L05776, L06240) were found tolerant when mechanically inoculated with CMV. These genotypes also showed resistance in the field as well as through aphid transmission and supported a low level of *M. persicae* population, except the TMS-1 genotype of *S. lycopersicum* which supported high population levels of *M. persicae* in both years but showed a low level of disease. Previous studies have demonstrated that sources of resistance or tolerance to CMV exist in *S. lycopersicum*, *S. pimpinellifolium L.*, *S. peruvianum L.*, *S. habrochaites*, *S. cheesmaniae*, *S. chilense* and *S. lycopersicodies* (Phills et al. 1977; Gebre et al. 1990; Nitzany 1992; Stoimenova et al. 1992; Stamova 1993; Parella et al. 1997; Abad et al. 2000; Stamova and Chetelat 2000; Ali and Hassan 2002; Cillo et al. 2007). The present study showed that these genotypes resist not only vector transmission but also the virus, and supports the findings of Pioven et al. (1995) who demonstrated that virus resistance may exist at two levels i.e., against pathogen entry and/or at the level of systemic spread.

The majority of plant viruses rely on insect vectors for their transmission and completion of their life cycle (Westwood and Stevens 2010). Identification of resistance through vector transmission is another way of breeding broad resistance to viruses (Boissot et al. 2008; Akhtar et al. 2009). Nine genotypes belonging to *S. pimpinellifolium L.* (LA2184), *S. neorickii* (LA2727), *S. peruvianum L.* (LA0111, L06221, L06127, L06231) and *S. chmielewskii* (LA1306, L06057, L06208) showed tolerant to highly resistant

response to CMV subgroup IA under field conditions and through vector transmission, but were found to be susceptible through mechanical inoculation. This suggests the presence of a resistance mechanism to vector transmission. It could either be due to resistance to the vector or an inability of the vector to introduce sufficient quantity of virus particles into the plants to cause susceptible reaction as earlier reported by Tripathi and Varma (2002) for *Tomato leaf curl virus* and by Akhtar et al. (2009) for *Cotton leaf curl Burewala virus*. The above identified genotypes could be useful in minimizing losses by CMV subgroup IA and other aphid-transmitted viruses as they may constitute a first barrier against the virus by reducing the initial entry of the virus into the plant cell, as previously described by Delatte et al. (2006). Resistance to numerous insect pests and viruses has also been previously documented in several wild species of *Solanum*, particularly in *S. habrochaites*, *S. chmielewskii*, *S. neorickii*, *S. chilense* and *S. pennellii* (Farrer and Kennedy 1991; Kumar and Ullman 1993).

Pico et al. (1998) stated that a successful plant breeding programme for disease resistance depends on the successful identification of sources of resistance and accuracy in resistance assessment techniques. The present results showed that the resistance response to CMV subgroup IA varied according to the virus transmission methods employed. Disease screening by the vector transmission may produce misleading results as vector resistance can be interpreted as resistance to the virus. Mechanical inoculation can overcome this problem, because it is more efficient and standardized. The field screening does not discriminate between these levels but can serve as a first step for further studies by vector or mechanical inoculation. However, vector transmission is necessary because genotypes with vector resistance may be lost during mechanical inoculation.

The present study has shown that sources of resistance against CMV subgroup IA are available in *S. lycopersicum, S. chilense*, *S. neorickii*, *S. habrochaites* and *S. pennellii*,while resistance against vector transmission is available in *S. pimpinellifolium L.*, *S. peruvianum L.*, and *S. chmielewskii*. However, none of the resistant genotypes identified can be released directly for general cultivation because of their unacceptable agronomic characteristics. Incorporation of these resistant sources into commercial genotypes may contribute towards sustainable resistance to CMV subgroup IA. With the rapid spread and emergence of new aphid transmitted viruses in tomato growing areas of the world, a broad-spectrum virus resistance is needed. We hypothesize that the identified resistance in some genotypes against the virus and its vector in the current study could also be effective against other aphid transmitted viruses. Results of the present findings provide clues to breeders on the existence of genetic resistance within nine *Solanum* species with respect to their response to CMV subgroup IA. A breeding programme is therefore envisaged to introgress all of the resistance factors from these genotypes into agronomically suitable genotypes of tomato.

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