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TRANSFER OF CUCUMBER MOSAIC VIRUS RESISTANCE INTO HYBRIDS OF TOMATO

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A tomato male sterile line TMS1, tolerant to cucumber mosaic virus (CMV) was crossed with 5 high yielding but CMV susceptible lines namely Naqeeb, Money Maker, B23, B25 and Peto-86 to transfer the resistance into F_1 hybrids. The entire breeding material was subjected to mechanical inoculation followed by double antibody sandwich procedure (DAS-ELISA) to confirm the presence or absence of CMV. Data was recorded on the percentage of disease transmission, mean latent period, average disease severity and yield and yield related traits in field. TMS1 was reconfirmed 'tolerant' through mechanical inoculation but 'resistant' in natural filed conditions. Hybrids did not express tolerance level at par with TMS1 in mechanical evaluation. However, they showed at par response under field evaluation except hybrid TMS1 × B25. Hybrids TMS1 × Naqeeb, TMS1 × MM, TMS1 × B23, TMS1 × B25 and TMS1 × Peto-86 showed better tolerance to CMV over standard hybrids T-1359 and Salar. Line TMS1 proved to be a valuable donor of CMV resistance in cultivated tomato.
Keywords: CMV resistance, male sterile, ELISA, mechanical inoculation, hybrids

INTRODUCTION

Tomato is grown in different parts of Pakistan round the year, particularly during October to June in the Punjab province. During this period, the crop is hit by several biotic stresses mainly responsible for the incidence of fungal and viral diseases. Of 200 infectious diseases of tomato, 100 are viral which cause substantial yield losses of tomato worldwide (Tomlinson, 1987). Symptoms of extreme filiformity (shoestring) caused by subgroup I strains of CMV have been reported for the first time in Pakistan on all cultivated tomato varieties (Akhtar *et al*., 2008). CMV belongs to genus *Cucumovirus* and family *Bromoviridae* which has broad host range of 1,300 plant species of monocot and dicot plants including various cucurbits, tomato, pepper, cereals, fruits, legumes and weeds etc. (Cerkauskas, 2004; Garcia-Arenal and Palukaitis, 2008). This virus is transmitted non-persistently by 80 aphid species; green peach aphid *Myzus persicae* is an important vector of CMV (Cerkauskas, 2004). The large number of aphid vector species and natural host reservoirs accounts for high incidence of CMV in field plants. Tomato is not preferred host for the green peach aphid which normally colonizes various cucurbits and other plants. Most epidemics in tomato occur when the primary virus inoculum exists in reservoirs such as weeds. Current control measures for CMV are focused on management of vector which is not a dynamic solution. Control of CMV is complicated by its broad host range; broad aphid transmissibility and acquiring of gradual resistance of *M. persicae* against pesticides. Besides low productivity of crop, wide use of insecticides is causing environmental pollution in CMV hit farming systems.

The genetic basis of CMV resistance is largely unknown. However, in related nightshade family, *Capsicum annuum*, CMV tolerance was a quantitative character with incomplete dominance; for which several *quantitative trait loci* were responsible (Lapidot *et al*., 1997). Stamova and Chetelat (2000) reported CMV resistance as un-reliable monogenic character, suggesting lack of penetrance, significant environmental effects or the existence of additional resistance factors. It is one of the best characterized tripartite positive-sense single stranded RNA viruses (Takahashi, 2008). Because of these factors, no resistant or tolerant varieties in cultivated background have been developed. Lack of natural genetic resistance in cultivated tomato and/or failure to incorporate resistance from wild relatives of *Solanum* into commercial varieties is the major hindrance in developing CMV resistant cultivars. Therefore, identification/creation and incorporation of CMV resistance within cultivated background seems the most workable breeding strategy. In an attempt to identify CMV resistant genetic resources in tomato, we reported number of accessions of species (*S. lycopersicum*, *S. chilense*, *S. habrochaites*, *S. neorickii* and *S. pennellii*) as resistant and/ tolerant to CMV (Akhtar *et al*., 2010). In the current study reported here, we examined the possibility of transfer of CMV resistance/tolerance from a male sterile line TMS1 (*S. lycopersicum*) into F_1 hybrids via crossing TMS1 with high yielding but CMV susceptible male genotypes (*S. lycopersicum*). The ultimate objective is incorporation of CMV resistance/tolerance into improved cultivars/hybrids.

MATERIALS AND METHODS

Tomato male sterile line TMS1 already proved resistant to CMV (Akhtar *et al*., 2010) was crossed with high yielding but CMV susceptible lines viz. Naqeeb, Money Maker (MM), B23, B25 and Peto-86 to create 5 crosses. Male sterile line was used as female/line while other lines as male/tester parent. Crossed seed (F_1) was collected at fruit ripening. The growth type of TMS1 and all testers was determinate except MM which was indeterminate. Therefore, commercial hybrid T-1359 (determinate type) and Salar (indeterminate type) were included to assess the performance of determinate and indeterminate genotypes/hybrids. For the mechanical inoculation, CMV was obtained from naturally infected tomato plants of cultivated tomato variety Nagina and maintained in the glasshouse. Tomato leaves of susceptible check genotype with typical shoestring disease symptoms were ground in 0.02 M phosphate buffer, pH 7.4; (1g ml^{-1}) with a mortar pestle and squeezed through a very fine muslin cloth. Five plants of each test hybrids, parents and check hybrid were grown in glass house following completely randomized design with three repeats. Young leaves of four week old plants were dusted with 500-mesh carborandum powder and were mechanically inoculated with freshly extracted sap using cotton pads. Plants were rinsed gently with a stream of water just after inoculation to remove superfluous inoculation and were kept under insect free cages for symptom development. The presence or absence of CMV in the test genotypes i.e. hybrids, parent lines and check hybrid variety was assayed by double antibody sandwich procedure (DAS-ELISA) as described by Clark and Adams (1977) with commercial polyclonal antibodies to CMV. Data was recorded on the percentage of disease transmission, mean latent period and average disease severity 90 days after inoculation following five points (0-4) disease severity index (SI), where 0 = no visible disease symptom (highly resistant, SI 0); 1 = mild mosaic or mottling and leaf deformity (resistant, SI 0.01-1.4); 2 = moderate mosaic or mottling, leaf deformity and filiformity (tolerant, SI 1.5-2.4); 3 = severe mosaic or mottling or leaf deformity, filiformity, shoestring, minor to medium with minor flower shading and minor reduction in fruit setting (susceptible, SI 2.5-3.4) and 4 = severe mosaic or mottling, leaf deformity, filiformity, shoestring, stunting with no or few fruit setting (highly susceptible, SI 3.5-4.0). Individual symptomatic plant ratings for each genotype were added and divided by the number of infected plants to calculate the corresponding SI (Akhtar *et al*., 2010).

Four to six inch long nursery seedlings of 13 genotypes [6 lines + 5 hybrids + 2 checks] were transplanted at tomato breeding field of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad in November, 2012 following randomized complete block design with three replications. The experiment was set up keeping plant to plant distance 0.5 m and bed to bed distance 1.5 m. Each genotype had seven plants per replication. Crop remained in field till June, 2013 and data was on growth habit, growth type, fruit shape, plant height (cm), days to maturity, number of fruits per plant, fruit length (cm), fruit diameter (cm), average fruit weight (g), 1000-seed weight (g) and fruit yield (kg $plant^{-1}$) in addition to CMV rating as per scale given earlier. All morphological features like growth habit, growth type and fruit shape were recorded in accordance with descriptors for tomato (Anonymous, 1996) recommended by International Plant Genetic Resources Institute (IPGRI), Italy.

RESULTS

In mechanical inoculation, none of tested genotypes was found resistant to CMV however; male sterile line TMS1 responded as tolerant (Table 1). All genotypes were systemically infected with virus. Disease transmission was 100% for all the test genotypes since none of the inoculated

Table 1. Grouping of genotypes based on CMV infection.

Severity index	No. of genotypes	Disease reaction	Genotypes
Mechanical Inoculation			
0	0	HR	0
0.01-1.4	0	R	0
1.5-2.4	1	T	TMS1
2.5-3.4	10	S	TMS1 × Naqeeb, TMS1 × MM, TMS1 × B23, TMS1 × B25, TMS1 × Peto86, Naqeeb, MM, B23, B25, Peto86
3.5-4.0	2	HS	T-1359, Salar
Field conditions			
0	0	HR	0
0.01-1.4	1	R	TMS1
1.5-2.4	4	T	TMS1 × Naqeeb, TMS1 × MM, TMS1 × B23, TMS1 × Peto-86
2.5-3.4	6	S	TMS1 × B25, Naqeeb, MM, B23, B25 and Peto86
3.5-4.0	2	HS	T-1359 and Salar

plant was symptomless and had detectable amount of virus through ELISA. First disease symptom showing mild mosaic was observed in all genotypes/hybrids 12 days post-inoculation which progressed into severe mosaic or mottling, leaf deformity and shoe-stringing. No reduction in disease severity was recorded till the end of the experiment. In field conditions all genotypes showed a wide range of responses to CMV infection. Presence or absence of virus was confirmed by their typical symptom and through ELISA. Male sterile line TMS1 was found as resistant, hybrids TMS1×Naqeeb, TMS1 × MM, TMS1 × B23 and TMS1 × Peto-86 as tolerant while TMS1 × B25 and parent genotypes viz. Naqeeb, MM, B23, B25 and Peto-86 were susceptible. Both control hybrids (T-1359 & Salar) were highly susceptible. Plants of susceptible genotypes infected in field at an early stage of growth, expressed severe symptoms while mid-season or late infections showed minor symptoms. Severe symptoms started with minor mosaic on young emerging leaves, which resulted into severe mosaic or mottling, leaf deformity, shoe stringing and stunting (Table 1).

Comparison of mean performance indicated valuable genetic variability among test genotypes and control (T-1359 & Salar) in mechanical inoculation, field conditions, yield and its related traits (Table 2). All hybrids showed equal scores (3.0) of susceptibility in mechanical inoculation test. However, four hybrids viz. TMS1 x Naqeeb, TMS1 x B23, TMS1 × Peto-86 and TMS1 × MM appeared tolerant (2.33) in natural field conditions. The value of susceptibility to CMV in each control was considerably high in both evaluations. TMS1 came up as tolerant (2.33) through mechanical inoculation but resistant (2.0) in natural filed conditions. Parental material viz. TMS1, Naqeeb, B23, B25, Peto-86 and hybrid TMS1 × Naqeeb, TMS1 × B23, TMS1 × B25, TMS1 × Peto-86 and T-1359 expressed determinate growth habit and bushy plant type while MM, TMS1 × MM and Salar showed indeterminate growth habit with vine plant type. Fruit shape was almost round in the determinate genotypes however; Salar had cylindrical fruit shape. Considerable variations in yield and yield components within genotypes and controls were also registered.

Analysis of variance for CMV response, yield and yield components has been presented in Table 3. Magnitude of genotypic variance (σ^2g) for mechanical inoculation and field conditions were less than phenotypic variance (σ^2p). High heritability ($h^2b.s$) and high genetic advance (G.A) were recorded for mechanical inoculation while moderate heritability and moderate genetic advance under field conditions. Coefficient of the variation (C.V) in mechanical inoculation was less than that of field conditions. Genotypic mean square for yield and its related traits were highly significant. The genotypic variances were at lower side across all the traits studied. High heritability and high genetic advance was recorded for plant height, number of fruits per plant, fruit length, fruit weight, 1000-seed weight and yield per plant. However, for days to maturity and fruit diameter, high heritability with moderate genetic advance was recorded. Coefficient of variation appeared in limits for all the characters. In perusal of Table 4, all hybrids showed enhanced commercial heterosis to CMV tolerance. Determinate hybrids viz. TMS1 × Naqeeb, TMS1 × B23 and TMS1 × Peto-86 surpassed significantly by -22% while TMS1 × B25 by -11.11% over standard T-1359. Indeterminate hybrid TMS1 × MM also excelled

Table 2. Mean comparisons of tomato genotypes for CMV response, yield and yield components.

Genotypes	CMV response		Plant		Fruit shape	Plant height (cm)	Days to mat. (days)	Number of fruits plant^{-1}	Fruit length (cm)	Fruit dia. (cm)	Avg. fruit weight (g)	1000 seed wt. (g)	Fruit yield (kg plant^{-1})
	MI	Field	type	growth									
TMS1 x Naqeeb	3.00b	2.33bc	B	D	R	123.67de	135.00cd	60.31def	4.20de	4.60ad	55.33abc	3.42a	3.33d
TMS1 x B23	3.00b	2.33bc	B	D	R	118.47e	129.33d	41.37f	4.47d	4.87a	61.00a	2.61c	2.47ef
TMS1 x B25	3.00b	2.67ab	B	D	R	135.40d	140.67bc	48.51ef	4.33de	4.47cde	49.00b-e	3.12b	2.38f
TMS1 x Peto86	3.00b	2.33bc	B	D	R	112.67e	136.00cd	45.30f	4.07e	4.53bcd	44.33de	3.38a	1.96f
TMS1	2.33c	2.00c	B	D	R	-	-	-	-	-	-	-	-
Naqeeb	3.67a	3.00a	B	D	R	59.00h	148.00b	60.77def	5.47b	4.33def	54.00a-d	2.70c	3.50d
B23	4.00a	3.00a	B	D	R	73.72gh	161.33a	45.00f	4.97c	4.20efg	33.00fg	2.43de	3.28d
B25	4.00a	3.00a	B	D	R	90.67f	148.00b	75.14bcd	6.70a	4.00g	57.33ab	2.30e	6.00a
Peto-86	4.00a	3.00a	B	D	R	64.33h	165.67a	89.67b	5.43b	4.63abc	61.33a	2.61c	4.31bc
T-1359 (control)	4.00a	3.00a	B	D	HR	255.93a	148.00b	82.68bc	4.90c	4.13fg	56.67abc	3.09b	4.63b
TMS1 x MM	3.00b	2.33bc	Vine	ID	R	236.20b	130.00d	60.31def	4.37de	4.77ab	46.67cde	2.56cd	3.11de
MM	4.00a	3.00a	Vine	ID	R	184.86c	162.67a	89.67b	4.27de	4.63abc	29.09g	2.28e	2.31f
Salar (control)	3.67a	3.00a	Vine	ID	R/C	79.67fg	138.33c	90.44b	5.27bc	4.03g	41.00ef	2.67c	3.71cd
LSD 5%	0.48	0.61				15.24	8.05	20.12	0.39	0.27	10.64	0.15	0.66
LSD 1%	0.65	0.83				20.71	10.95	27.35	0.53	0.37	14.46	0.20	0.89

Genotypes sharing similar letters do not differ significantly at $P \leq 0.05$.

MI= Mechanical inoculation; B= Bushy; D= Determinate; ID= Indeterminate; R= Round; HR= Highly round and R/C= Round/cylindrical

Table 3. ANOVA for CMV, yield and yield components.

Source of variation	Mean squares for CMV response			Mean squares for yield and yield components							
	d.f.	CMV (MI)	CMV (field)	d.f.	Plant height	Days to maturity	Number of fruits per plant	Fruit length	Fruit diameter	Average fruit weight	1000 seed weight	Fruit yield
Replication	2	0.03	0.08	2	451.73**	75.25**	165.33**	0.01	0.02	28.2**	0.01	0.14
Treatment	12	0.97**	0.41**	11	12871.97**	476.43**	1577.46**	1.73**	0.25**	336.24**	0.46**	3.96**
Error	24	0.08	0.13	22	80.99	22.61	141.21	0.05	0.03	39.45	0.01	0.15
σ^2g		0.29	0.09		4263.66	151.27	478.75	0.56	0.08	98.93	0.15	1.27
σ^2p		0.38	0.23		4344.65	173.89	619.96	0.61	0.10	138.38	0.16	1.42
σ^2e		0.08	0.13		80.99	22.61	141.21	0.05	0.03	39.45	0.01	0.15
h^2(b.s)		0.78	0.42		0.98	0.87	0.77	0.92	0.75	0.71	0.95	0.89
G.A at 5%		28.83	15.12		104.20	16.27	57.74	30.29	11.04	35.31	28.34	64.29
C.V %		8.3	13.52		7.03	3.27	17.32	4.68	3.58	12.8	3.2	11.33

*, ** = Significant at 0.05 and 0.01 levels of probability, respectively *** = TMS1 being a male sterile line was not included in analysis of variance for yield and yield components

Table 4. Commercial heterosis for CMV response, yield and yield components in tomato genotypes.

Hybrids	CMV field	CMV (MI)	Plant height (cm)	Days to maturity (days)	Number of fruits per plant	Fruit length (cm)	Fruit diameter (cm)	Average fruit weight (g)	1000 seed weight (g)	Fruit yield (kg plant^{-1})
TMS1 × Naqeeb	-22.22*	-25.00**	55.22**	-8.78**	-27.04*	-14.29**	11.38**	-2.34	10.57**	-23.03**
TMS1 × B23	-22.22*	-25.00**	48.70**	-12.61**	-49.96**	-8.84*	17.84**	7.66	-15.64**	-43.01**
TMS1 × B25	-11.11	-25.00**	69.95**	-4.95	-41.32**	-11.56**	8.15*	-13.52	0.97	-45.15**
TMS1 × Peto86	-22.22*	-25.00**	41.42**	-8.11**	-45.19**	-17.01**	9.77**	-21.76*	9.49**	-54.84**
T-1359 (control)	3.00	4.00	255.93	148.00	82.68	4.90	4.13	56.67	3.09	4.63
TMS1 × MM	-22.22*	-18.26**	-7.73*	-6.02*	-24.98*	-16.51**	18.28**	-23.5*	-3.63	-15.91
Salar (control)	3.00	3.67	79.67	138.33	90.44	5.27	4.03	41.00	2.67	3.71
SE	0.30	0.23	7.35	3.88	9.70	0.19	0.13	5.13	0.07	0.32

*, ** = Significant at 0.05 and 0.01 levels of probability, respectively

Table 5. Heterobeltiosis estimates for CMV response, yield and yield components in tomato genotypes.

Hybrids	CMV field	CMV (MI)	Plant height (cm)	Days to maturity (days)	Number of fruits per plant	Fruit length (cm)	Fruit diameter (cm)	Average fruit weight (g)	1000 seed weight (g)	Fruit yield (kg plant^{-1})
TMS1×Naqeeb	16.67	28.76**	109.60**	-8.78**	-0.75	-23.17**	6.15	2.47	26.39**	-4.70
TMS1×B23	16.67	28.76**	60.71**	-19.83**	-8.08	-10.07*	15.87**	84.85**	7.27*	-24.69*
TMS1×B25	16.67*	28.76**	49.34**	-4.95	-35.44*	-35.32**	11.67**	-14.53	33.85**	-60.41**
TMS1×Peto86	33.33	28.76**	75.13**	-17.91**	-49.47**	-25.15**	-2.16	-27.72**	29.46**	-54.62**
TMS1×MM	16.67	28.76**	27.77**	-20.08**	-41.37**	2.34	2.88	60.4**	12.26**	34.88*

*, ** = Significant at 0.05 and 0.01 levels of probability, respectively

significantly by -22% above control Salar. Under field conditions, determinate hybrids were better than T-1359 by an increase of -25% however, indeterminate hybrid TMS1x MM showed an increase of -18.26% over Salar. For plant height only TMS1 × MM was better than Salar by -7.73%. Four hybrids namely TMS1 × B23 followed by TMS1 × Naqeeb, TMS1 × Peto86 and TMS1 × MM showed comparatively early days to maturity from -12.61% to -6.02% over their respective controls. None of the hybrids was vigorous over commercial hybrid for number of fruits per plant, fruit length, fruit weight and yield per plant. Hybrid TMS1 × MM showed an increase of 18.28% in fruit diameter followed by TMS1 × B23 (17.84%), TMS1 × Naqeeb (11.38%), TMS1 × B25 (8.5%) and TMS1 × Peto86 (9.77%) over their respective controls. For 1000-seed weight, two hybrids TMS1 × Naqeeb and TMS1 × Peto86 showed increase of 10.57% and 9.49% against T-1359. None of the hybrids attained higher level of commercial heterosis and heterobeltiosis for yield (Table 4 & 5) and tolerance to CMV against male sterile line TMS1 (Table 2).

DISCUSSION

The results of present study revealed significant variability for yield and yield related traits among all the tested genotypes as described by Saleem *et al*. (2013). Similarly,

breeding material of current study showed different response of resistance in mechanical and field evaluations which might be due to intrinsic genetic make of each line/hybrid (Saleem *et al*., 2009), variable biotic factors (CMV inoculum pressure, aphid (*M. persicae*), plant age etc.) and abiotic factors (temperature, light and humidity etc.) as reported earlier elsewhere (Ceraskauskas, 2004; Akhtar *et al*., 2010). Line MM possessed indeterminate plant growth habit controlled by gene (*sp*+) while all other genotypes had *sp* gene that refers to determinate growth habit. Gene sp^+ behaved as dominant over determinate plant growth controlling gene (*sp*), therefore, the hybrids either from $sp^+ \times sp$ and $sp^+ \times sp^+$ combination were all indeterminate. Similarly all hybrids had round fruit shape because of round fruit of TMS1. Gene controlling round fruit shape exerted dominant effect over long shapes. Similar report on inheritance of growth habit and fruit shape had already been documented by Khan *et al*. (1981).

TMS1 had functional male sterility of *ps-2* gene. Its hybridity with determinate males (Naqeeb, Peto-86, B23 and B25) and indeterminate male (MM) was absolutely marvelous since as seed parent it gave100% birth of offspring/hybrids. The information about the extent of transmission of CMV resistance to F_1 crosses in literature is limited however, the observed tolerance level under mechanical and field conditions over the check hybrids in different crosses showed an encouraging evidence of transfer of CMV resistance, though this level was less than that of TMS1. All CMV tolerant hybrids TMS1 × Naqeeb, TMS1 × MM, TMS1 × B23, TMS1 × B25, TMS1 × Peto-86 and TMS1 × MM did not show significant commercial heterosis in term of yield response possibly due to less number of male counterparts. It therefore, necessitates using TMS1 frequently as female parent in crossing with large number of male lines to compliment traits not acquired by it. It will ultimately lead to identify good combiner/counterpart to develop hybrids with enhanced level of CMV resistance over commercial hybrids via heterosis breeding and secondly to select superior genotypes via recombination breeding among segregating generations of these crosses till a desirable level of CMV resistance and yield is attained. The best strategy to transfer CMV resistance with high yield potential might or could be backcross breeding.

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