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DEVELOPMENT OF TOMATO HYBRIDS WITH MULTIPLE DISEASE TOLERANCE

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Abstract

Four lines and six testers were crossed to produce 24 F_1 hybrids following Line x Tester technique to screen the material tolerant to blight and cucumber mosaic virus using integrated protocols of whole plant assay, mechanical inoculation and breeding. Check hybrid T-1359 was highly susceptible to late blight (LB) and early blight (EB) to the mark of 94 and 74% respectively and cucumber mosaic virus (CMV) with a 2.8 severity index. One hybrid Nagina x LB5 was scored tolerant to late blight with 35% infection, while four genotypes viz. LB3, LB2, LB4, LB7 and one hybrid Naqeeb x LB5 were tolerant to early blight with 28-30% infection and tolerant to cucumber mosaic virus with 2.1 severity index. Non-additive gene action was pre-dominant in genetic control of blights, viral diseases, yield and all yield related traits. Genotype Roma, LB5 and LB6 were found to be good general combiner for late blight while Roma, Nagina and LB2 for early blight. A high yielding hybrid Naqeeb x LB6 was the best one with 37.55% tolerance to late blight and 33.33% high yielder than T-1359. Most of the hybrids showed better tolerance to CMV as compared to T-1359. Identified good combiners can be used in heterosis and recombination breeding to develop high yielding and disease tolerant tomato genotypes.

Key words: *Solanum lycopersicum* L., Combining ability, Cucumber mosaic virus, *Phytophthora infestans*, *Alternairia solani*, Line x tester analysis.

Introduction

Tomato is susceptible to biotic and abiotic stresses (Akhtar *et al*., 2012; Shamim *et al*., 2014; Khan *et al*., 2015). According to an estimate, early blight (EB) and late blight (LB) in epidemic form cause 49 to 91% yield losses in Pakistan (Azam & Shah, 2003). Cool, humid and rainy conditions favor LB incidence. The LB pathogen '*Phytophthora infestans'* attacks on leaves, stems, fruits and seeds of tomato (Robin & Choen, 2004). The *Alternaria solani* can attack fruits when they approach to maturity at the stem end where the symptoms may be small and sunken or may enlarge to cover most of the fruit (Rotem, 1994; Agrios, 1997; Chaerani & Voorrips, 2007). Low temperature and high humidity (November to January) while high temperature and low humidity (March to June) favors the incidence of LB and EB, respectively, in Pakistan. Farmers rely mainly on frequent applications of fungicides for the control of EB and LB which is quite expensive and not eco-friendly. Shoestring disease caused by CMV is one of the serious viral threats to tomato productivity. Under field conditions, management of CMV mainly depends on vector (aphid: *Myzus persicae*) control via insecticide spray. CMV is endemic in open field grown tomatoes in Pakistan transmitted through seed, sap and non-persistently by aphids. *M. persicae* is efficient and the most studied vector to transmit CMV in tomatoes (Garcia-Arenal & Palukaitis, 2008). Tomato has been found to be infected with all three subgroups while CMV subgroup IA is responsible for infecting tomatoes in Pakistan (Akhtar *et al.,* 2008; Akhtar *et al.,* 2010). Transfer of resistance into elite tomato lines and hybrids is the principal way of developing EB, LB and CMV resistance in tomato. Most of the known and useful disease resistance traits in tomato and other crop species are conferred by single dominant gene, which are appropriate for the development of hybrid cultivars. Pace of research work for development of high yielding and disease resistant hybrids/cultivars of tomato has been extremely slow mainly due to lack of resistant/tolerant genetic resources in cultivated back ground to LB, EB and CMV and poor combining ability (Foolad, 2007; Goncalves *et al.,* 2008).

To overcome this situation, the current research and development work was undertaken to develop primarily high yielding and disease resistant/tolerant hybrids of tomato. Such hybrids could later be released as commercial hybrid. Research out comes will help to improve socio-economic conditions of the country.

Materials and Methods

The breeding material consists of 4 lines (Riogrande, Roma, Nagina and Naqeeb) and 6 testers (LB2, LB3, LB4, LB5, LB6 and LB7). Lines hereafter were designated as female (♀) and tester as male (♂) genotypes. Lines and Testers were crossed within each experiment according to Line x Tester technique (Kempthrone, 1957) to generate 24 F_1 hybrids.

Fungal culture for early blight (EB): A wild type isolate of EB (*Alternaria solani*) was obtained from naturally infected tomato plants at NIAB, Faisalabad by transferring the EB-infected tissues onto V8 agar medium (17.7% V8 juice, 0.3% $CaCO_3$, 2% agar). To induce sporulation, mycelial plugs were cut from the isolation medium and were sub-cultured onto freshly prepared medium. The fungus was grown at 24±1°C with a 12 h photoperiod consisting of a combination of UV-C (15 watt germicidal; General Electric, Cleve land) and

florescent light sources. After 8 days of growth under UV-C light, the fungus was sporulated as evidenced by the production of several concentric rings of dark conidia. Conidia were harvested by applying 2 mL of sterilized de-ionized water to the culture plates and gently scraped with a glass microscope-slide. The conidial suspension was filtered through a 0.5-mm 2-pore strainer to remove mycelia debris. The resulting spore suspension was adjusted to 40, 000 conidia per mL using a haemocytometer (Foolad *et al.,* 2000).

Whole Plant Assay for EB: Four week old nursery seedlings of elite lines and check hybrid T-1359 were transplanted in the glass house following Completely Randomized Design (CRD) in pots with three repeats. Each genotype had six plants in each replication. After six weeks, plants were inoculated with *Alternaria solani*. Inoculated plants were maintained in dark for 24 h at relative humidity (RH) of >95%. After that RH was reduced to ~85% and plants were maintained under a 12 h photoperiod. Six days after inoculation the plants were evaluated individually by the proportion of leaf and plant blighted using scale to calculate disease index percentage (Table 1).

Fungal culture for late blight (LB): A wild type isolate of LB (*Phytophthora infestans*) was obtained from naturally infected tomato plants at NIAB, Faisalabad, Pakistan. The culture was obtained by transferring the LB infected tissues onto PARP medium (pimaricin, ampicillin, rifampicin and pentachloronitrobenzene agar). For zoospore production and multiplication, older leaves from the middle of the six-week-old plants of the susceptible genotype Nagina were put onto moistened filter paper in 140 mm Petri plates. The adaxial surfaces of these leaves were injured at the center using a sterile 10 µl micropipette tip and a 5 µl sporangial suspension, collected from PARP medium was placed on the wound of each leaf for 24 h at 18°C in dark. Then 15 mL of sterilized distilled water was added to the plates and they were further incubated for 2-3 days at 18°C in dark. The suspension was then filtered through four layers of sterile muslin cloth to remove other fragments. The zoospore suspension was adjusted in sterilized distilled water to a concentration of 5000 zoospores per mL using a haemocytometer (Akhtar *et al*., 2012).

Whole Plant Assay for late blight (LB): Five to six-week-old greenhouse grown plants of test genotypes and hybrids were sprayed to runoff with a hand sprayer using *Phytophthora infestans* zoospore suspension. Inoculated plants were covered with a plastic tunnel to increase humidity and kept at 18-20°C with a 16 h photoperiod for 7-15 days. There were three replications for each genotype such that each replication had 3 plants. Data regarding the proportion of leaf and plant blighted were visually estimated by using scale to calculate percent disease index percentage (Table 1).

Mechanical Inoculation (MI) for cucumber mosaic virus (CMV): The inoculum of CMV for the mechanical inoculation 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) with a pestle and mortar and squeezed through a very fine muslin cloth. Five plants of each test hybrids, parents and check hybrid were grown in glass house following CRD with three replications. 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 surplus inoculation and were kept under insect free cages for symptoms development. The presence or absence of CMV in the test genotypes i.e. hybrids, parents and check hybrid variety was assayed by double antibody sandwich procedure (DAS-ELISA) as described by Clark & Adams (1977) with commercial polyclonal antibodies to CMV. Data were 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.

Evaluation of the material in field conditions: Healthy seed of test genotypes [lines, testers, hybrids and standard control T-1359] were grown on raised beds during October, 2013. Four to six inches long nursery seedlings of all genotypes were transplanted at tomato breeding field of NIAB, Faisalabad in November, 2013 following randomized complete block design (RCBD) 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 in each replication. Crop remained in field till June, 2014 to record data on number of fruits per plant (NoF), fruit weight (FW) and fruit yield (FY) according to descriptors for tomato (Anon., 1996) recommended by IPGRI, Italy. Gene action and combining ability on blight response, yield and yield components were worked out as per line x tester analysis (Kempthrone, 1957; Arunachalam, 1974). Commercial heterosis was estimated according to standard procedures (Nadaranjan & Gunasekaran, 2005). Negative value of combining ability and commercial heterosis over the standard in blight response and vice versa in yield and yield components were taken desirable to select blight resistant and high yielding hybrids. Non-significant combining ability effects were regarded as average type.

Table 1. Disease rating scale for early and late blight.

Disease rating	Severity symptoms for whole plant assay	Percent disease index (DI)	Disease reaction
0	No visible symptoms apparent	0	Immune
1	A few minute lesions to about 10% of the total leaf area is blighted and usually confined to the 2 bottom leaves	0.01-10	Highly resistant
2	Leaves on about 25% of the total plant area are infected	10.01-25	Resistant
3	Leaves on about 50% of the total plant area are infected	25.01-40	Tolerant
4	Leaves on about 75% of the total plant area are infected	40.01-60	Susceptible
5	Leaves on whole plant are blighted and plant is dead	> 60.01	Highly susceptible

Results

Mean performance of test genotypes for LB, EB, CMV and agronomic traits has been given in Table 2. Commercial hybrid T-1359 was highly susceptible to LB (84%), EB (76%) and to CMV (SI = 2.8). Of test entries, 1 hybrid Nagina x LB5 proved to be tolerant to early blight (DI = 35%), 5 entries viz. LB3, LB2, LB4, Naqeeb x LB5 and LB7 tolerant to late blight (DI = 28% to 37%) and 5 entries viz. Nagina x LB7, LB4, Naqeeb x LB4, Riogrande x LB6 and Nagina x LB3 tolerant to CMV (SI = 2.1 to 2.40). Six promi nent high yielding hybrids were Roma x LB6, Roma x LB3, Riogrande x LB3, Riogrande x LB5, Riogrande x LB6 and Nagina x LB4 with yield range of 3.77 $kg \cdot pl^{-1}$ to 3.14 $kg \cdot pl^{-1}$ compared to 2.19 $kg \cdot pl^{-1}$ of control.

Analysis of variance of treatment mean squares and its partionings (parents, crosses, parent vs., crosses, lines, testers and line x tester interactions) for EB, LB, CMV, yield and yield components were significant in majority of their respective variances that permitted to proceed further for gene action and combining ability analysis (Table 3).

Estimates of genetic components have been shown in Table 4. Considering LB and EB, magnitudes of σ^2SCA and σ^2D were higher than their corresponding general combining ability σ^2GCA and additive σ^2A components. Similarly the comparative ratio (σ^2GCA/σ^2SCA) was less than one (<1) while for σ^2D/σ^2A, was greater than one (>1). Values of σ^2g were considerably low compared to σ^2p. High heritability [h^2 (bs)] and high genetic advance percentage was recorded for LB and EB diseases. Line x Tester interaction contributed 44.62% followed by tester (31.72%) and lines (23.66%) for LB improvement. Line x Tester interaction contributed 40.89% followed by lines (30%) and tester (29.11%) for EB improvement. In case of CMV, estimates of σ^2GCA and σ^2A were almost nil (round figure) except that of σ^2SCA and σ^2D. Genotypic variance σ^2g was less than phenotypic variance σ^2p while both heritability and genetic advanced were moderate. Contribution of lines, testers and line x tester interactions showed higher value of line x tester interaction followed by testers and lines, respectively. Results on yield and yield components revealed higher magnitude of σ^2SCA and σ^2D than those of corresponding σ^2GCA and σ^2A for number of fruits per plant, fruit weight and yield per plant. Similarly the value of ratio σ^2GCA/σ^2SCA was less than unity and value of ratio σ^2D/σ^2A was greater than unity for all the characters. Genotypic variance was less than phenotypic variance for entire traits. High estimates of broad sense heritability and high genetic advance were recorded for number of fruits per plant, fruit weight and yield per plant. Contribution of lines to the total variance was less than tester in all parameters. Line x Tester interaction contributed more than testers in fruit weight.

General combining ability (GCA) effects have been shown in Table 5. Among female, Roma while on male side LB6 and LB5 had significant and desirable negative GCA effects for LB. For EB, line Nagina and tester LB2 possessed highly significant while Roma had average type (non-significant) desirable negative GCA effects. Combining ability of CMV displayed non-significant GCA for the entire parent genotypes except tester LB2. Nevertheless, Riogrande and Nagina among lines and LB3, LB5 and LB7 among testers had average negative GCA effects. GCA effects of Roma, LB6 and LB3 were significantly higher over the rest of the parents for number of fruits per plant. Line Naqeeb and testers; LB5 and LB4 rendered outstanding positive GCA effects for fruit weight. Two lines; Riogrande and Roma and two testers; LB3 and LB6 enumerated highly significant and desirable GCA effects for yield per plant.

Specific combining ability (SCA) effects for different parameters have been given in Table 6. Five hybrids viz. Roma x LB4 followed by Riogrande x LB3, Nagina x LB7, Riogrande x LB5 and Naqeeb x LB3 possessed significant desirable negative SCA effects for LB. Hybrid Nagina x LB7 acquired highly significant negative SCA effects in desirable direction for EB. None of the hybrids could possess significant desirable SCA effects for CMV tolerance though several hybrids showed average negative SCA effects.

Hybrids specifically, Nagina x LB4 and Roma x LB3 had highest positive and desirable SCA effects for number of fruits per plant. Cross Riogrande x LB3, Roma x LB6, Naqeeb x LB4 and Riogrande x LB5 showed desirable positive SCA effects for fruit weight. In case of yield per plant, hybrid Nagina x LB4 had maximum desirable positive SCA effects followed by Naqeeb x LB7, Riogrande x LB2, Roma x LB3 and Roma x LB6.

Table 2. Comparison of mean performance of tomato genotypes.

Hybrids	LB (%)	EB (%)	CMV (SI)	NoF	FW (g)	FY (kg/pl)
Riogrande x LB2	85 a	57 defgh	2.77 abcd	30 efghi	95.00 fghijk	2.86 def
Roma x LB2	45 fgh	68 abcdefg	3.53 abc	32 defg	68.67 mno	2.19 hijkl
Nagina x LB2	50 efg	75 abc	2.60 bcd	25 ghijkl	85.67 ijklm	2.14 hijkl
Naqeeb x LB2	85 a	65 abcdefg	2.70 abcd	14 nop	100.00 efghij	1.40 o
Riogrande x LB3	57 def	55 efghi	2.57 bcd	27 fghijk	126.67 abcd	3.40 abc
Roma x LB3	52 efg	72 abcd	2.60 bcd	40 bc	94.00 fghijk	3.65 ab
Nagina x LB3	47 efgh	69 abcdef	2.40 cd	31 defgh	80.00 jklmn	2.47 efghij
Naqeeb x LB3	53 efg	73 abcd	2.87 abcd	23 hijklm	108.33 defg	2.47 efghij
Riogrande x LB4	77 abc	52 ghij	2.77 abcd	22 ijklm	107.00 defgh	2.39 fghijkl
Roma x LB4	77 abc	57 defgh	2.87 abcd	22 ijklm	86.67 hijklm	1.93 lmn
Nagina x LB4	47 efgh	69 abcdef	2.73 abcd	31 defgh	104.00 efghi	3.14 cd
Naqeeb x LB4	75 abc	70 abcdef	2.33 cd	19 lmnop	133.67 a	2.48 efghij
Riogrande x LB5	78 abc	59 cdefg	2.43 cd	25 ghijklm	132.33 ab	3.18 bcd
Roma x LB5	52 efg	67 abcdefg	2.47 cd	24 hijklm	117.00 abcde	2.73 defg
Nagina x LB5	35 h	57 defgh	2.63 bcd	31 defgh	93.33 fghijk	2.83 def
Naqeeb x LB5	53 efg	37 jk	2.50 bcd	21 klmno	100.00 efghij	2.08 ijklm
Riogrande x LB6	55 efg	63 bcdefg	2.37 cd	41 b	77.33 klmn	3.16 bcd
Roma x LB6	72 bc	70 abcdef	2.77 abcd	37 bcde	103.00 efghi	3.77 a
Nagina x LB6	72 bc	67 abcdefg	2.97 abcd	31 efgh	94.33 fghijk	2.81 defg
Naqeeb x LB6	43 gh	71 abcde	2.70 abcd	33 cdef	89.67 ghijkl	2.92 cde
Riogrande x LB7	77 abc	65 abcdefg	2.60 bcd	22 ijklm	79.00 klmn	1.58 no
Roma x LB7	58 de	72 abcd	2.60 bcd	29 efghij	81.67 jklmn	2.32 ghijkl
Nagina x LB7	53 efg	81 a	2.10 d	19 lmnop	71.67 lmno	1.33 o
Naqeeb x LB7	78 abc	77 ab	2.93 abcd	22 jklmn	109.33 cdefg	2.33 ghijkl
Riogrande	69 cd	78 ab	2.77 abcd	34 bcdef	63.00 nop	2.12 hijklm
Roma	73 abc	77 ab	2.87 abcd	56 a	46.33 p	2.57 efghi
Nagina	80 abc	53 fghij	2.77 abcd	37 bcde	52.33 op	1.93 lmn
Naqeeb	75 abc	78 ab	3.93 a	31 efgh	63.67 nop	1.97 klmn
LB2	77 abc	30 k	2.83 abcd	17 mnop	130.00 abc	2.18 hijkl
LB3	75 abc	28 k	2.73 abcd	20 klmnop	103.00 efghi	2.03 jklmn
LB4	80 abc	31 k	2.27 cd	13 p	126.33 abcd	1.63 mno
LB5	75 abc	42 hijk	2.43 cd	21 klmnop	116.67 abcde	2.43 efghijk
LB6	80 abc	55 efghi	3.77 ab	39 bcd	67.33 mno	2.60 efgh
LB7	82 abc	38 ijk	2.60 bcd	14 op	112.00 bcdef	1.53 no
T-1359	84 ab	76 abc	2.80 abcd	33 cdef	66.67 mnop	2.19 hijkl
LSD 5%	12.88	16.8	1.27	7.96	27.5	0.5
C.V %	11.91	16.8	14.34	17.75	13.5	12.61

Genotypes sharing common letter do not differ significantly at $p>0.05$

Table 3. Mean square for analysis of variance in tomato genotypes.

SOV	df	LB	EB	CMV	NoF	FW	FY
Replication	2	219.72*	146.36	0.24	14.90	204.03	0.49**
Treatment	33	623.84**	664.50**	0.43**	249.32**	1589.24**	1.13**
Parents	9	43.84	1258.89**	0.87**	555.33**	3169.54**	0.39**
Crosses	23	664.89**	275.73**	0.23	139.73**	959.23**	1.27**
P vs C	1	4899.77**	4256.67**	1.20**	15.80	1856.80**	4.63**
Lines	3	1205.89**	634.26**	0.21	237.87**	1412.13**	1.04**
Testers	5	970.12**	369.22**	0.23	276.17**	1423.71**	2.89**
L x T	15	454.94**	172.86	0.24	74.62**	713.83**	0.78**
Error	66	63.85	109.22	0.16	24.33	161.93	0.10
Total	101	249.90	291.38		97.66	629.11	0.44

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

Table 4. Estimate of genetic components in tomato genotypes.

SOV	LB	EB	CMV	NoF	FW	FY
σ^2GCA	5.29	2.59	0.00	1.64	6.19	0.01
σ^2SCA	130.37	21.21	0.03	16.76	183.97	0.23
σ^2GCA/σ^2SCA	0.04	0.12	0.00	0.10	0.03	0.05
σ^2A	10.59	5.19	0.00	3.28	12.38	0.02
σ^2D	130.37	21.21	0.03	16.76	183.97	0.23
σ^2D/ σ^2A	12.31	4.09	0.00	5.10	14.86	9.14
σ^2g	186.66	185.09	0.09	75.00	475.77	0.35
σ^2p	250.51	294.31	0.25	99.33	637.70	0.44
σ^2e	63.85	109.22	0.16	24.33	161.93	0.10
h^2(bs)	0.75*	0.63*	0.37	0.76*	0.75*	0.78*
S.E h^2(bs)	0.01	0.01	0.23	0.01	0.01	0.20
G.A (% of mean)	36.89	36.37	13.87	56.63	40.99	44.21
Contribution of Lines (%)	23.66	30.00	11.82	22.20	19.20	10.65
Contribution of Testers (%)	31.72	29.11	21.54	42.97	32.27	49.45
Contribution of L x T (%)	44.62	40.89	66.64	34.83	48.53	39.90

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

Table 5. General Combining ability effects of parents for different parameters in tomato genotypes.

Parents	LB	EB	CMV	NoF	FW	FY
			Lines			
Riogrande	2.89	0.61	-0.07	0.85	5.46	0.20**
Roma	-11.67**	-0.44	0.15	3.51**	-5.60	0.20**
Nagina	1.39	-7.33**	-0.09	0.74	-9.26**	-0.11
Naqeeb	7.39**	7.17**	0.01	-5.10**	9.40**	-0.29**
SE of lines	1.88	2.46	0.09	1.16	3.00	0.07
			Testers			
LB2	12.36**	-8.19**	0.24*	-1.74	-10.10**	-0.42**
LB3	-3.47	1.97	-0.05	3.15*	4.82	0.43**
LB4	3.44	-2.11	0.02	-3.51*	10.40**	-0.08
LB5	-6.06*	2.56	-0.15	-2.09	13.24**	0.14
LB6	-12.39**	8.14**	0.04	8.29*	-6.35	0.60**
LB7	6.11*	-2.36	-0.10	-4.09*	-12.01**	-0.67**
SE of testers	2.31	3.02	0.11	1.42	3.67	0.09

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

Heterosis: Estimates of commercial heterosis for LB, EB, CMV, yield and yield components compared to T-1359 has been given in Table 7. Thirteen hybrids showed highly significant reduction (-32.80% to -58.50%) in LB incidence over control T-1359. These hybrids were Roma x LB4 (-58.50%), followed by Riogrande x LB5 (-49.01%), Riogrande x LB3 (-46.64%), Roma x LB6 (-44.66), Nagina x LB6 (-44.66%), Riogrande x LB6 (-40.71%), Roma x LB3 (-38.73%), Nagina x LB5 (-38.73%), Naqeeb x LB6 (-37.55), Roma x LB7 (-36.76%), Nagina x LB7 (-36.76%), Roma x LB5 (-34.78%) and Roma x LB2 (-32.80%). Significant reduction in EB was recorded which ranged from -25.44% to -51.75% in this context, Nagina x LB7 had maximum EB reduction with value of -51.75% followed by Nagina x LB2 (-32.02%), Roma x LB2 (-27.63%), Riogrande x LB2 (-25.44%), Nagina x LB3 (-25.44%) and Roma x LB4 (-25.44%). Regarding CMV, hybrid Nagina x LB7 had maximum desirable commercial heterosis (-25%) over control variety T-1359. Eighteen crosses owned negative but non-significant heterosis (-16.67% to -1.19%) over control T-1359.

One hybrid Riogrande x LB6 had significantly higher standard heterosis (24.48%) for number of fruits per plant. Sixteen hybrids showed outstanding heterosis (34.43% to 100.40%) for fruit weight. Hybrid Naqeeb x LB4 was at the top (100.40%) followed by Riogrande x LB5 (98.40%), Riogrande x LB3 (89.91%), Roma x LB5 (75.41%), Naqeeb x LB7 (63.92%), Naqeeb x LB3 (62.42%), Riogrande x LB4 (60.42%), Nagina x LB4 (55.92%), Roma x LB6 (54.42%), Naqeeb x LB2 (49.93%), Naqeeb x LB5 (49.93%), Riogrande x LB2 (42.43%), Nagina x LB5 (39.93%), Roma x LB3 (40.93%), Nagina x LB6 (41.43%) and Naqeeb x LB6 (34.43%). Eleven hybrids excelled in yield than that of T-1359 giving a range of 24.66% to 72.30%. Maximum commercial heterosis was exhibited by Roma x LB6 (72.30%) followed by Roma x LB3 (66.82%), Riogrande x LB3 (55.40%), Riogrande x LB5 (45.05%), Riogrande x LB6 (44.44%), Nagina x LB4 (45.53%), Naqeeb x LB6 (33.33%), Riogrande x LB2 (30.59%), Nagina x LB5 (29.38%), Nagina x LB6 (28.46%) and Roma x LB5 (24.66%).

Table 6. Specific Combining ability effects for different parameters in tomato genotypes.

Hybrids	LB	EB	CMV	NoF	FW	FY
Riogrande x LB2	8.36	-1.03	-0.06	4.11	2.21	0.52**
Roma x LB2	-5.42	-1.64	0.49*	3.03	-13.07	-0.16
Nagina x LB2	1.53	1.92	-0.21	-0.95	7.60	0.11
Naqeeb x LB2	-4.47	0.75	-0.21	-6.19*	3.26	-0.46*
Riogrande x LB3	-15.81**	0.47	0.03	-4.21	18.96*	0.21
Roma x LB3	5.42	5.19	-0.16	6.33*	-2.65	0.45*
Nagina x LB3	17.36**	-3.25	-0.12	0.00	-12.99	-0.42*
Naqeeb x LB3	-6.97**	-2.42	0.24	-2.11	-3.32	-0.24
Riogrande x LB4	9.94*	-4.44	0.17	-2.06	-6.29	-0.29
Roma x LB4	-18.17**	-6.06	0.04	-4.70	-15.57*	-0.75**
Nagina x LB4	5.44	10.83	0.14	6.51*	5.43	0.77**
Naqeeb x LB4	2.78	-0.33	-0.36	0.25	16.43*	0.28
Riogrande x LB5	-15.22**	2.89	0.00	-1.36	16.21*	0.27
Roma x LB5	11.33*	-4.06	-0.19	-5.05	11.93	-0.18
Nagina x LB5	-5.06	6.17	0.21	5.17	-8.07	0.24
Naqeeb x LB5	8.94	-5.00	-0.02	1.24	-20.07**	-0.34
Riogrande x LB6	-1.89	0.64	-0.26	4.99	-19.21*	-0.20
Roma x LB6	9.33*	-4.31	-0.08	-2.31	17.51*	0.41*
Nagina x LB6	-3.72	3.25	0.35	-5.56	12.51	-0.24
Naqeeb x LB6	-3.72	0.42	-0.01	2.87	-10.82	0.04
Riogrande x LB7	14.61**	1.47	0.12	-1.46	-11.88	-0.51**
Roma x LB7	-2.50	10.86	-0.11	2.70	1.85	0.23
Nagina x LB7	-15.56**	-18.92**	-0.37	-5.18	-4.49	-0.45*
Naqeeb x LB7	3.44	6.58	0.36	3.94	14.51	0.72**
SE of SCA	4.61	6.03	0.23	2.85	7.35	0.18

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

Table 7. Estimate of commercial heterosis (%) in tomato hybrids.

Genotypes	LB	EB	CMV	NoF	FW	FY
Riogrande x LB2	0.79	-25.44*	-1.19	-8.47	42.43**	30.59**
Roma x LB2	-32.80**	-27.63*	26.19*	-3.69	2.95	-0.15
Nagina x LB2	-9.09	-32.02**	-7.14	-24.06	28.44	-2.28
Naqeeb x LB2	-9.09	-14.47	-3.57	-57.50**	49.93**	-36.23**
Riogrande x LB3	-46.64**	-10.09	-8.33	-18.82	89.91**	55.40**
Roma x LB3	-38.73**	-5.26	-7.14	21.01	40.93*	66.82**
Nagina x LB3	-9.09	-25.44*	-14.29	-6.46	19.94	12.79
Naqeeb x LB3	-30.83**	-5.26	2.38	-30.45*	62.42**	12.94
Riogrande x LB4	-7.90	-21.93	-1.19	-32.41**	60.42**	9.28
Roma x LB4	-58.50**	-25.44*	2.38	-32.36**	29.94	-11.72
Nagina x LB4	-15.02	-12.28	-2.38	-6.89	55.92**	43.53**
Naqeeb x LB4	-11.06	-7.89	-16.67	-43.42**	100.40**	13.09
Riogrande x LB5	-49.01**	-6.14	-13.10	-26.00*	98.40**	45.05**
Roma x LB5	-34.78**	-16.67	-11.90	-29.09*	75.41**	24.66*
Nagina x LB5	-38.73**	-12.28	-5.95	-6.63	39.93*	29.38*
Naqeeb x LB5	-15.02	-7.89	-10.71	-36.13**	49.93**	-4.87
Riogrande x LB6	-40.71**	-1.75	-15.48	24.48*	15.94	44.44**
Roma x LB6	-44.66**	-9.65	-1.19	10.48	54.42**	72.30**
Nagina x LB6	-44.66**	-8.77	5.95	-7.71	41.43**	28.46*
Naqeeb x LB6	-37.55**	6.58	-3.57	0.11	34.43*	33.33**
Riogrande x LB7	0.79	-14.47	-7.14	-32.36**	18.44	-27.70*
Roma x LB7	-36.76**	-3.51	-7.14	-11.79	22.44	6.09
Nagina x LB7	-36.76**	-51.75**	-25.00*	-43.92**	7.45	-39.12**
Naqeeb x LB7	-7.11	0.88	4.76	-34.02**	63.92**	6.39

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

Discussion

Hybrid Nagina x LB5 seemed to possess new allelic combination tolerant to LB irrespective to their susceptible parents; possibly tolerance to LB might emerged from crossing of *susceptible* x *susceptible* alleles of parents. However, an opposite situation was seen in EB wherein new allelic combinations originating from *susceptible* x *susceptible* or *susceptible* x *tolerant* parents did not show decreased level of tolerance to EB in all hybrids except Naqeeb x LB5. Earlier it has been reported that EB being a 3-phase disease is more complex than LB due to number of fungal strains and host plant interactions influenced by environmental conditions (Foolad *et al*., 2008). Male genotypes LB3, LB2, LB4 and LB7 had better tolerance to existing EB strain and even better than T-1359. Variable response of CMV tolerance in the breeding material could be due to CMV inoculums pressure and abiotic factors (temperature, light, humidity etc.) affecting plant growth. Little is known about the extent of transmission of CMV resistance in literature. Success in the development of CMV resistant genotypes of tomato had been hampered due to narrow genetic base of the cultivated tomato and complexity of the virus RNA. The present study elucidated 5 genotypes (Nagina x LB3, Naqeeb x LB4, Riogrande x LB6, Nagina x LB7 and LB4) tolerant to CMV visa vie T-1359 which may be exploited for the development of virus tolerant superior genotypes.

Analysis of variance (Table 3) of breeding material proved to be appropriate on account of having desirable variations among genotypes for LB, EB, CMV, yield and its contributing traits. Such genetic differences fulfilled one of the major pre-requisites needed to transfer disease resistance and high productive genetic contents into hybrids, likely to come out after hybridization. Considerable differences between hybrids and their parents highlighted the role of hybrid vigor. The results were in close harmony to earlier workers (Dhaliwal *et al.,* 2003; Saleem *et al.,* 2011; Saleem *et al*., 2013a). Analysis of variance for lines, testers and line x tester interaction were not significant across all characters. Significance of lines and testers shows expression of additive gene action being homozygous inbred genotypes while that of line x tester interaction portrays non-additive gene action (Panwar, 2005).

The genetic components (GCA, SCA, σ^2D, σ^2A) presented non-additive gene action for LB, EB yield and yield components (Table 4). Our findings were in close harmony to earlier reports (Saleem *et al.,* 2011; Saleem *et al.,* 2013a). Zero expression of GCA and σ^2A and lower values of SCA and σ^2D pointed deviations of genetic control to somewhat non-additive gene effects for CMV. The prospectus to select CMV tolerant genotypes convoluted due to moderate heritability and moderate genetic advance which means restricted flow of CMV transmission into offspring after hybridization. Broad sense heritability alone, does not predict the transformation of characters from parents to offspring unless cited with genetic advance. High heritability and high genetic advance for EB, LB, number of fruits per plant and fruit weight could ensure selection of superior genotypes tolerant to existing strain of EB and LB. Paternal lines appeared more important than maternal for late blight, number of fruits per plant, fruit weight and yield per plant due to their relative contribution. The phenomenon would be of course decisive in the choice of seed and pollen parent. Hybrids assumed vital importance with appreciable share (44.62% for LB; 40.89% for EB and 39.90% for yield). Saleem *et al*., (2009, 2011, 2013b) had reported similar work in tomato.

The average performance and nature of GCA effects (Table 2 *vs.* Table 5) of lines and testers could not be seen in linear order; indeed GCA effects depended upon the inherent genetic makeup of the female or male parent irrespective to their individual *per se performance* as reported earlier (Saleem *et al.,* 2009; Saleem *et al.,* 2011) in tomato. Statistically significant combining ability effects carry high weightage than average or low effects in selection of parent or hybrid genotypes fit for recombination and or heterosis breeding (Nadarajan & Gunasekaran, 2005). However, this is not equally applicable to all cases. Breeders prefer average effects in primary targeted traits (if they are in desirable direction) in selection process where improvement in more than one trait is required simultaneously. To make better mates, Roma as female and LB5 and LB6 as male appeared ideal mates for the development of LB tolerant and high yielding genotypes through recombination breeding. However, for EB, the situation became complex due to opposite (+ive or –ive) GCA effects (EB and yield) among and within eventual lines and testers. However, the females (Roma and Nagina) and male LB2 which carried either significant or average GCA effects for EB and yield should be crossed with additional genotypes as per line x tester fashion to elucidate the better high yielding and EB tolerant couples. According to Singh & Narayanam (2009), the GCA variance is primarily due to fixable additive genetic variance or additive × additive epistasis interaction and reflect high breeding value therefore, a multiple crossing program involving aforementioned LB, EB tolerant and high yielding genotypes would result in generation of superior genotypes following pedigree method of selection. In tomato this strategy has been exercised (Saleem *et al.,* 2009; Saleem *et al.,* 2011; Saleem *et al*., 2013a). Parent genotypes possessed average GCA effects except tester LB2 for CMV resistance. The prospectus for the isolation of CMV donor and recipients mates is narrow. A multiple reciprocal crossing program involving LB2 and additional tomato genotypes might be rewarding to find better male and female mates.

Promising specific combinations did not appear best for all studied characters. The similar situation was realized in heterosis *visa vie* specific combining ability. Consequently it necessitated to consider multifactor approach at a time to isolate the most favorite hybrids. Based on mean performance (Table 2), specific combining ability effect (Table 6) and heterosis (Table 7), hybrid Naqeeb x LB6 was the best one with 37.55% better in tolerance to LB and 33.33% higher yielder than T-1359. Hybrid Naqeeb x LB6 retained negative SCA effects for blight and positive effects for yield. It was derivative of low x high GCA combination of parents for LB and yield, respectively. Other hybrids like Roma x LB2 and Nagina x LB5 tolerant to LB and Naqeeb x LB 5 tolerant to EB could be rated good one nevertheless, due to negative SCA

effects of yield might be deferred. According to Singh & Narayanam (2009), SCA is due to dominance genetic variance and all three types of epistasis (additive × additive, additive × dominance and dominance × dominance) and is best suited for commercial hybrids seed development.

Although several hybrids showed better tolerance to CMV against T-1359, yet none of them had a linear trend among *per se performance* (CMV severity index and yield), combining ability effects and commercial heterosis; possibly due to negative relation between CMV tolerance and productivity parameters as reported elsewhere (Saleem *et al*., 2013). However, because of low CMV severity index recorded for LB4 and good GCA effects for LB2, LB3, LB5 and LB7, a multiple crossing program involving those and additional genotypes could be devised which would bring superior genotypes resistant or tolerant to CMV via heterosis or recombination breeding.

Conclusion

Several genotypes tolerant to blight and CMV were isolated and developed. The good general combiners for LB were Roma as ♀ and LB5 and LB6 as ♂. In case of early blight, Roma and Nagina as ♀ and LB2 as ♂ was the good combiner. One high yielding and blight tolerant hybrids Naqeeb x LB6 and several hybrids tolerant to CMV have been isolated for further evaluation in multi-location adaptability trials.

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