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PHENOTYPIC EVALUATION OF TOMATO GERMPLASM FOR THE SOURCE OF RESISTANCE AGAINST TOMATO LEAF CURL VIRUS DISEASE

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ABSTRACT

Tomato leaf curl virus (TLCV) is the most devastating virus of tomato crop. TLCV is transmitted by whitefly (*Bemisia tabaci*) in a persistent and circulative manner. For the management of this problem, twenty seven tomato varieties/lines were screened for the source of resistance against tomato leaf curl virus disease (TLCVD) in field trial because no viricide was available to combat the viral diseases and insect vectors had developed resistance against the insecticides. The observations of TLCVD incidence were recorded on weekly basis**.** Varieties/lines were evaluated on the basis of symptomology. Grafting and whitefly mediated inoculation techniques were adopted to confirm the virus. Out of twenty seven varieties/lines, three were highly susceptible, six were susceptible, four were moderately susceptible, six were moderately resistant and eight cultivars were resistant. No variety/line was highly resistant or immune against TLCVD. Resistant genotypes (varieties/lines) can be used in breeding programs as an eco-friendly management approach.

Key words: TLCV, Resistance, Incidence, Evaluation.

INTRODUCTION

Tomato (*Solanum lycopersicon* L.), belonging to family *solanaceae*, is one of the most popular and widely grown vegetable crop worldwide. This crop has high nutritive value, taste and versatile uses. The yield and quality of tomato fruits are considerably affected by an array of insect pests and diseases at different stages of crop growth. Among the devastating pathogens, tomato leaf curl virus (TLCV) is an important and major constraint in reducing the yield of tomato crop (Singh, 2014).

TLCV is a group of viruses which belongs to the *Geminiviridae* family that contains plant viruses with a circular, single-stranded DNA genome and two incomplete icosahedral geminate particles (Pandey *et al*., 2009). *Geminiviridae* is classified into four genera on the basis of vector type, host range and genome sequences (Fauquet *et al*., 2008). Begomoviruses are the most devastating genera for tomato plant worldwide, especially in tropical and subtropical regions (Seal *et al*., 2006).

TLCV causes tomato leaf curl virus disease (TLCVD), the most widespread among viral diseases and found in several Middle Eastern, African, Asian and Mediterranean countries (Abhary *et al*., 2007). The yield of TLCV infected plants is affected both qualitatively and quantitatively. In case of severe attacks, yield losses reach upto 100% (Sahu *et al*., 2012). TLCVD is differentiated by stunting, chlorosis, upward curling of leaves, crinkling, puckering and yellowing. Infected plants have a bushy appearance with reduced flower and fruit setting (Kumar *et al*., 2012).

TLCV is transmitted by whitefly, *Bemisia tabaci* (Genn.) in a circulative and persistent manner which belongs to order Hemiptera and family *Aleyrodidae* (Boykin *et al*., 2007). *B. tabaci* can acquire the virus from infected source in 5 minutes of acquisition access period (Sohrab *et al*., 2012). A single whitefly can transmit TLCV successfully after 4-8 hours of inoculation access period (Hidayat and Rahmayani, 2007). *B. tabaci* can transmit TLCV horizontally as well as vertically by sexual and transovarial passage respectively (Ghanim *et al*., 2007). The latent period of TLCV in *B. tabaci* lies between 8-24 hours (Lia *et al*., 2010). The virus can also be transmitted through grafting because it involves the union of cambial layers of stock and scion, either of which might be infected with a virus (Mathews, 1970).

Cultivation of susceptible tomato germplasm leads to high whitefly population infestation and virus incidence on host plants (Gilbertson *et al*., 2011). Several pesticides/chemicals applied against the insects failed to control the *B. tabaci* and attributed to the emergence of insecticidal resistance (Hameed *et al.*, 2010). The non-judicious use of pesticides causes environmental pollution and hazards thus increasing the cost of crop production (Aktar *et al*., 2009). Varietal resistance is the best option for the TLCV disease management. The objective of this study was to identify resistant genotypes through phenotypic screening and confirmation of the results through whitefly and graft inoculation. This would be very useful to find cheap, efficient, eco-friendly and viable options as no viricide or other chemical has been

available for the suitable management of TLCVD problem.

MATERIALS AND METHODS

Establishment of disease screening nursery: In order to find out the resistant source against the TLCVD incidence tomato germplasm were evaluated under natural conditions. The experiment was conducted in research area Department of Plant Pathology, University of Agriculture, Faisalabad (Pakistan). Seeds of twenty seven tomato varieties/lines (Roker, Big Beef, 09079, Uovo Roseo, Naqeeb, Caldera, Sitara-TS-101, Pakit, Riogrande, Nuyt-9-11, Nagina, Lyp#1, Roma, Nuyt-25-11, Carmen, BL-1176-Riostone-1-1, Libnan Arif, Nuyt -04-11, Salma, PO-02, 09088, 09080, 10127, 10113, 09091, 10125 and 014276) were taken from Ayub Agricultural Research Institute (AARI) Faisalabad. Row to row and plant to plant distance of 60cm and 30cm was maintained respectively. The experiment was conducted in augmented design. To ensure the presence of virus source in the field, a row of spreader (Fanto) was sown after every three rows of varieties/lines to be tested for resistance. Disease incidence of TLCVD infected plants was recoded weekly in each entry. Disease incidence percentage was calculated as under:

$$\% \text{ Disease incidence} = \frac{\text{No. of infected plants}}{\text{Total No. of plants}} \times 100$$

The resistance against disease was evaluated by modified Ssekyewa, 2006 scale.

Disease rating scale

Grades	%Incidence	Infection Category
0	All plants free of virus symptoms	HR
1	1-20% infection	R
2	21-40% infection	MR
3	41-60% infection	MS
4	61-80% infection	S
5	81-100% infection	HS

HR= Highly resistant, R= Resistant, MR= Moderately Resistant, MS= Moderately Susceptible,
S= Susceptible, HS= Highly susceptible

Area under disease progress curve: Area under disease progress curve (AUDPC) was used to combine multiple observations of the disease progress into a single value (Simko and Piepho, 2012). AUDPC was calculated by the trapezoidal integration of the disease incidence over time for each variety/line, considering the whole period evaluated (Shaner and Finney, 1977), as follows.

$$AUDPC = \sum_{i=1}^{n-1} [(xi+xi+1)/2] (ti+1-ti)$$

Where n is the number of assessment; X, disease incidence (%) and (*ti*+1-*ti*), duration between two consecutive assessments. The disease assessments (TLCVD incidence) over specific periods of time interval (weekly) recorded during the experiment (2012 and 2013) were interpreted according to the above mentioned formula and the AUDPC of TLCVD for each variety/line was calculated during both the years.

Recording of whitefly (*B. tabaci*) population data from disease screening nursery: Whitefly population data was recorded by randomly selecting three diseased plants from each entry and recording the insect population from upper, middle and lower leaves (Perveen *et al*., 2010).

Biological assays for the pathogenecity of virus: The pathogenecity of the virus was confirmed through whitefly and graft inoculation.

(i) Pathogenecity test through whitefly: Ten plants of highly susceptible variety "Salma" was grown in pots and kept in insect free cage. Nine of these plants were inoculated through whitefly transmission technique (Ning *et al*., 2015). One plant was kept as control for the comparison of the results. Almost twenty whiteflies were introduced in the cage containing TLCVD infected tomato plants and given an acquisition access period for two days. Then suspected viruliferous whiteflies were collected from the muslin cage and transferred to the healthy plants at the second leaf stage for a period of two days. Later on, these plants were sprayed with insecticide (Imidacloprid) to kill the whitefly. The symptoms were recorded after seven days by visual observations.

(ii) Pathogenecity test through grafting: Twenty seven varieties/lines were planted in earthen pots placed in green house for the confirmation of TLCV by graft inoculation. There were two to three plants in each pot, with replications. The plants were kept in good condition through recommended agronomic practices. TLCV infected plants was collected from the field for grafting on to healthy plants in the pots. Plants were selected as soon as top leaves show the TLCV symptoms. A slanting cut of 2cm long and 0.2cm deep was made on the stem of infected plant. Wedge grafting was performed as suggested by (Bausher, 2013). The grafted portion was wrapped tightly with parafilm and covered with polythene bags. Non grafted plants were kept as control.

RESULTS

Response of tomato germplasm against TLCVD incidence and *B. tabaci* population: Tomato leaf curl disease (TLCD) symptoms appeared on all the varieties/lines evaluated against the TLCV. The earliest symptoms appeared on a highly susceptible variety "Salma". The symptoms started by upward and

downward curling of leaves in infected plants. Infected plants remained stunted and became yellowish in color with less fruit formation. During the year 2012 maximum disease incidence 95.29% and average *B. tabaci* population 8.23 was recorded on Salma, followed by 86.15% (7.22) on 014276 and 82.71% (7.56) on Sitara-TS-101. These cultivars were regarded as highly susceptible in the reaction group 5 (Table. 1). Six varieties/lines showed TLCVD incidence in the range of 61-80% as 74.09% (6.67) on 10125, 71.64% (6.34) on 10127, 69.16% (5.79) on Libnan Arif, 67.39% (5.58) on BL-1-176-Riostone-1-1, 64.87% (5.36) on Big Beef and 63.38% (5.24) on Caldera. These cultivars were rated as susceptible in the reaction group 4.

Four varieties/lines (Uovo Roseo, Nuyt-9-11, PO-02 and 10113) were kept in reaction group 4 and regarded as moderately susceptible. The percent disease incidence and *B. tabaci* population on these varieties/lines was 43.24 (4.09), 47.15 (4.63), 50.73 (4.81), 53.48 (5.17) respectively.

Six varieties/lines (Carmen, Roker, Lyp#1, 09079, Nuyt-25-11 and 09088, were categorized as moderately resistant by showing TLCVD incidence and average *B. tabaci* population 22.45% (3.37), 24.67% (3.51), 27.53% (3.62), 29.42% (3.82), 32.12% (3.95) and 35.27 (4.02) respectively. These varieties/lines were kept in reaction group 2.

The disease incidence and average *B. tabaci* population was minimum 6.26% (2.17) on Naqeeb, 8.34% (2.42) on Pakit, 10.81% (2.67) on Nagina, 13.76% (2.75) on Riogrande, 15.31% (2.98) on 09080, 17.85% (3.05) on Roma, 19.67% (3.35) on 09091 and 18.83% (3.21) on Nuyt-04-11. These eight varieties/lines were graded as resistant in reaction group 1.

Tomato varieties/lines exhibited similar response against TLCVD and *B. tabaci* population during the year 2013. None of the screened varieties/lines was found to be highly resistant against TLCVD and *B. tabaci*. All the varieties/lines were categorized in the same classes as in the year 2012 with more or less TLCVD incidence percentage and *B. tabaci* population (Table. 2). During the year 2013 highly susceptible varieties/lines (Salma, 014276, Sitara-TS-101 showed 94.72% (8.18), 88.06% (7.47) and 83.37% (7.62) disease incidence and *B. tabaci* population respectively. Six susceptible varieties/lines (10125, 10127, Libnan Arif, BL-1176-Riostone-1-1, Big Beef and Caldera) showed 75.31% (6.75), 72.05% (6.38), 68.53% (5.71), 66.48% (5.52), 63.28% (5.24) and 62.44% (5.18) TLCVD incidence and *B. tabaci* population respectively.

TLCVD incidence and *B. tabaci* population on four moderately susceptible varieties/lines (Uovo Roseo, Nuyt-9-11, PO-02 and 10113) recorded as 44.52 (4.16), 46.23 (4.56), 51.58 (4.89) and 54.14 (5.23) respectively.

Six moderately resistant varieties/lines (Carmen, Roker, Lyp#1, 09079, Nuyt-25-11 and 09088) showed TLCVD incidence and average *B. tabaci* population 23.64% (3.46), 25.16% (3.64), 28.31% (3.65), 28.24% (3.68), 32.25% (3.91) and 36.36 (4.07) respectively.

The minimum disease incidence and average *B. tabaci* population was recorded on eight resistant varieties/lines as 5.42% (2.14) Naqeeb, 7.43% (2.34) Pakit, 10.98% (2.63) Nagina, 12.57% (2.69) Riogrande, 14.79% (2.87) 09080, 16.58% (3.02) Roma, 18.06% (3.45) 09091 and 18.35% (3.19) Nuyt-04-11. The area under disease progress curve was directly proportional to the percent disease incidence during two years 2012 and 2013 (Table. 1,2).

Table. 1. Response of tomato germplasm against TLCVD and *B. tabaci* population during 2012

Varieties/lines	Average whitefly population/Leaf	Disease Incidence (%)	Ratings	AUDPC	Level of Resistance or Susceptibility	Graft inoculation 6 plants/ variety
						Infected Plants
Roker	3.51	24.67	2	880.95	MR	2
Big Beef	5.36	64.87	4	2287.95	S	4
09079	3.82	29.42	2	1062.95	MR	2
Uovo Roseo	4.09	43.24	3	1530.9	MS	3
Naqeeb	2.17	6.26	1	236.6	R	0
Roma	3.05	17.85	1	642.25	R	1
Caldera	5.24	63.38	4	2235.8	S	4
Sitara-TS-101	7.56	82.71	5	2912.35	HS	5
Pakit	2.42	8.34	1	309.4	R	1
Riogrande	2.75	13.76	1	499.1	R	1
Nuyt-9-11	4.63	47.15	3	1667.75	MS	3
Nagina	2.67	10.81	1	395.85	R	1
Lyp#1	3.62	27.53	2	981.05	MR	2
Nuyt-25-11	3.95	32.12	2	1141.7	MR	3
Carmen	3.37	22.45	2	803.25	MR	2
BL-1176-	5.58	67.39	4	2376.15	S	4

Riostone-1-1						
Libnan Arif	5.79	69.16	4	2438.1	S	4
Nuyt -04-11	3.21	18.83	1	676.55	R	2
Salma	8.23	95.29	5	3352.65	HS	6
PO-02	4.81	50.73	3	1793.05	MS	3
09088	4.02	35.27	2	1251.95	MR	3
09080	2.98	15.31	1	553.35	R	1
10127	6.34	71.64	4	2524.9	S	4
10113	5.17	53.48	3	1889.3	MS	4
09091	3.35	19.67	1	705.95	R	2
10125	6.67	74.09	4	2610.65	S	5
014276	7.22	86.15	5	3032.75	HS	5

AUDPC: 236.6-705.95 = R, 803.25-1251.95= MR, 1530.9-1889.3= MS, 2235.8-2610.65= S, 2912.35-3352.65= HS
R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, S= Susceptible and HS= Highly susceptible

Table. 2. Response of tomato germplasm against TLCVD and *B. tabaci* population during 2013

Varieties/lines	**Average whitefly population/Leaf**	**Disease Incidence (%)**	**Ratings**	**AUDPC**	**Level of Resistance or Susceptibility**	**Graft inoculation 6 plants/ variety**
						Infected Plants
Roker	3.64	25.16	2	898.1	MR	2
Big Beef	5.24	63.28	4	2232.3	S	5
09079	3.68	28.24	2	1005.9	MR	2
Uovo Roseo	4.16	44.52	3	1575.7	MS	4
Naqeeb	2.14	5.42	1	207.2	R	1
Roma	3.02	16.58	1	597.8	R	2
Caldera	5.18	62.44	4	2202.9	S	5
Sitara-TS-101	7.62	83.37	5	2935.45	HS	6
Pakit	2.34	7.43	1	277.55	R	1
Riogrande	2.69	12.57	1	457.45	R	2
Nuyt-9-11	4.56	46.23	3	1635.55	MS	4
Nagina	2.63	10.98	1	401.8	R	1
Lyp#1	3.65	28.31	2	1008.35	MR	3
Nuyt-25-11	3.91	31.25	2	1111.25	MR	3
Carmen	3.46	23.64	2	844.9	MR	2
BL-1176-Riostone-1-1	5.52	66.48	4	2344.3	S	5
Libnan Arif	5.71	68.53	4	2416.05	S	5
Nuyt -04-11	3.19	18.35	1	659.75	R	2
Salma	8.18	94.72	5	3332.7	HS	6
PO-02	4.89	51.58	3	1822.8	MS	4
09088	4.07	36.36	2	1290.1	MR	3
09080	2.87	14.79	1	535.15	R	2
10127	6.38	72.05	4	2539.25	S	6
10113	5.23	54.14	3	1912.4	MS	4
09091	3.45	18.06	1	649.6	R	2
10125	6.75	75.31	4	2653.35	S	6
014276	7.47	88.06	5	3099.6	HS	6

AUDPC: 207.2-649.6 = R, 844.9-1290.1= MR, 1575.7-1912.4= MS, 2202.9-2653.35= S, 2935.45-3332.7= HS
R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, S= Susceptible and HS= Highly susceptible.

DISCUSSION

Host plant resistance is the best method to control TLCVD but limited sources of resistance are available (Freitas-Astua *et al*., 2002). There is a lack of natural resistance in domesticated varieties of tomato as compared to wild species. Breeding programs would be successful by transferring resistance genes from wild

accessions into cultivated tomato (Bai and Lindhout, 2007). In the current study, none of the varieties or lines evaluated were found to be immune or highly resistant against TLCVD during two years 2012 and 2013. These findings were in accordance with Ragupathi (2000) who evaluated 160 varieties of tomato for resistance against TLCVD in India. Only two wild species *Lycopersicon hirsutum* f. *glabratum* (LA 1223) and *Lycopersicon hirsutum* (LA 1353) were immune to TLCVD incidence. Tomas *et al*., (2011) found the resistant reaction only in wild species. Two wild *Solanum habrochaites* cultivars EELM-388 and EELM-889 were found resistant against TYLCV after a wide germplasm screening through whitefly mediated inoculation or by agroinoculation using an infectious clone, with no symptoms or virus accumulation observed in inoculated plants. The remaining cultivars were moderately susceptible with more than 50% infection. Azizi *et al*., (2008) after screening of 134 domesticated accessions and 6 wild tomato lines under natural conditions, none of the varieties was resistant to TYLCV in domesticated tomato while all six lines of wild species were resistant based on symptom development and DNA amplification. Gomez *et al*., (2004) developed four tomato lines by introgression from the wild tomato *L. chilense* which showed less virus accumulation as compared to the commercial F_1 hybrids ARO 8479 and HA 3108. Rubio *et al*., (2003) showed a positive correlation between symptom intensity and virus titre.

In the present study, eight varieties/lines were found resistant and six were moderately resistant against TLCVD during both years during 2012 and 2013. These results are strengthened by the least number of resistant varieties in domesticated tomato after screening against TLCVD that has been reported in the literature. Singh (2014) screened 32 tomato genotypes against TLCVD under greenhouse out of which three genotypes showed resistant reaction and eight genotypes found moderately resistant. The wild tomato genotypes cannot be easily exploited for cultivation (Selth *et al*., 2004) but their resistant genes can be introduced into the domesticated genotypes for the improvement in resistance (Hulbert *et al*., 2001). Resistant varieties not only help in the economic and eco-friendly management of the disease also result in higher yields (Nawaz *et al*., 2013). In the following experiment, thirteen varieties/lines showed moderately susceptible to highly susceptible reaction against TLCVD. Significant differences in disease incidence in tomato varieties/lines could be attributed to the fact that whitefly has more affinity for some genotypes than others (Osei *et al*., 2012). Rom *et al*., (1993) confirmed the resistant potential of the tomato cultivars through molecular screening apart from phenotypic screening and reported that tolerant lines contained 10-50% less viral DNA as compared to susceptible ones when analyzed by alkaline transfer and dot spot hybridization using cloned viral DNA as a probe. Montasser *et al*., (2012) unveiled the yield potential of the resistant cultivars by performing biochemical analysis and found that the internal structure of the leaves of resistant varieties was less damaged than susceptible ones. Chemical components of tomato leaves especially chlorophyll a and b, total lipids, saturated and unsaturated fatty acids, reducing sugars and proteins, decreased in infected leaves when compared with healthy leaves of test plants. Moshe *et al*., (2012) studied the stress responses of resistant and susceptible tomato plants are different. Susceptible plants were stunted and do not yield, while resistant plants remained symptomless and yielded. Sources of carbon and nitrogen were less affected by TYLCV in resistant than in susceptible plants, which could make resistant plants more balanced and fit to sustain infection.

Conclusion: No highly resistant or immune variety/line was identified against tomato leaf curl virus disease after field screening followed by whitefly and graft mediated inoculation. Variety Naqeeb showed highest level of resistance based upon symptomatology followed by whitefly and graft inoculation.

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