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Characterization of *Solanum chilense* Accessions for Resistance to Tomato Spotted Wilt Virus (TSWV) Disease

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ABSTRACT: Domesticated tomato (*Solanum lycopersicum* L.) is known to be susceptible to many viral diseases. However, resistance subsists in wild tomato species. The wild relative of tomato, *Solanum chilense* is reported be source of resistant to major diseases of tomato including important viral disease caused by tomato spotted wilt virus (TSWV) infection. In the present study, 17 accessions of *S. chilense* were evaluated for resistance against TSWV disease in field and contained conditions. The contained condition screening involved mechanical inoculation of the TSWV infected leaf sap with Phosphate buffer on leaves of the *S. chilense* plants. The magnitude of variation of TSWV disease incidence ranged from 40.00 with a grand mean disease incidence of 14.12 in field condition to 50.00 with a grand mean of 7.06 in contained condition across the accessions. Among 17 accessions of *S. chilense* screened, 11 accessions did not exhibit any symptoms of TSWV disease and were immune in both field and contained conditions, which indicates these accessions may be a potential source of resistance to incorporate in tomato breeding programs for resistance against TSWV disease and can be used in developing TSWV disease resistant lines of tomato.

Keywords: TSWV, Tospovirus, Wild tomato, *Solanum chilense*, Viral diseases, Tomato.

INTRODUCTION

Tomato originated in South America has been spread to all over world across mountains, deserts and coastal plains resulting in its domestication from its wild relative species over the time. The genome of tomato has undergone genetic bottlenecking during this event resulting its genetic diversity to reduce to less than 5 per cent of the diversity found in its wild relatives (Sim *et al.,* 2011). Since these species can be out crossed with cultivated ones, breeders have introgressed wild genomes into cultivated varieties since 1917, a practice that continues today (Foolad, 2007). Most of the genes governing disease resistance have been introgressed from wild species. Tomato accessions devoid of TSWV disease symptom upon infection have been reported in a number of species, such as *S. arcanum, S. cheesmaniae, S. chilense, S. chmielewskii, S. corneliomulleri, S. galapagense, S. habrochaites, S. neorickii, S. pennellii, S. peruvianum,* and *S. pimpinellifolium* (Ji *et al.,* 2007; Vidavski *et al.,* 2008; Ezin *et al.,* 2010; Pereira Carvalho *et al.,* 2010; Tomás *et al.,* 2011).

As its name suggests, *S. chilense* lives primarily in Chile, including in the Atacama Desert. It is highly resistant to drought. The green fruits of *S. chilense* also have a distinctive darker stripe when mature, like several other wild tomato species. The species is reported be highly resistant to pests and diseases, harboring genes, which provide resistance against important viral diseases and are introgressed into commercial cultivars of Tomato (Padmanabhan *et al.,* 2019).

In *Lycopersicon* species, the resistance to Tospoviruseshas been found to be genetic. The principal work in identifying resistance source for TSWV in tomato resulted in discovering a single dominant resistance gene locus, *Sw5*, originating from the wild species of tomato, *Solanum peruvianum*, which has been introgressed into cultivated tomato plants. The genes, *Sw5-a* and *Sw5-b*, encode proteins of 1245 and 1246 amino acids, respectively, and are members of the coiled-coil (CC), nucleotide-binding-ARC, Leucine rich repeat (*CC-NBS-LRR*) group of resistance candidate genes. Over the years, seven TSWV resistance loci have been identified and designated as allelic (*Sw-1a* and *Sw-1b*), dominant (*Sw-5, Sw-6,* and *Sw-7)* and recessive genes (*sw-2, sw-3,* and *sw-4*) in tomato (Padmanabhan *et al.*, 2019). In comparison to *Sw-5*, the *Sw-6* resistance locus gives only limited resistance to thrips inoculation and is only effective against considerably fewer TSWV isolates (Canady *et al.*, 2001). Instead, *Sw-7* is said to display field resistance to a variety of TSWV isolates, including those that outcompete *Sw 5*. The molecular mechanism underlying the locus *Sw-7*, which was introgressed from *S. chilense* accession LA 1938 and is often mapped to chromosome 12, is yet unknown (Padmanabhan *et al.*, 2019). The objective of the present study was to evaluate *S. chilense* accessions for resistance against TSWV disease in field and contained conditions and to identify potential resistant lines for both the diseases to introgress them in further tomato breeding programs.

MATERIAL AND METHODS

Screening for resistance to TSWV disease in field conditions. The field evaluation of 17 wild accessions (name given as WT series) of *Solanum chilense* was carried out for screening of disease resistance to TSWV disease during March, 2021, when vector population for both viral diseases is reported to be high. All recommended package of practices was followed except for management of TSWV disease on the crop (Fig. 1). The observations were recorded for TSWV disease incidence by using the scale given by Hanson (1996).

Grade	Disease incidence (%)	Reaction category	Symptoms
A	0.0	Immune (I)	No symptoms
B	0.1-5.0	Resistant (R)	Initial symptoms on young leaflets
C	5.0-10.00	Moderately Resistant (MR)	Symptoms extended up to petioles
D	10.1-15.0	Moderately Susceptible (MS)	Necrosis of growing buds including buds
E	15.1-25.00	Susceptible (S)	Necrosis extended up to stem
F	25.1 and above	Highly Susceptible (HS)	Severe necrosis and wilting

The number of diseased and healthy plants was recorded by counting leaf-curled plants per plot and the per cent disease incidence was calculated. The accessions were screened for TSWV disease resistance at 45, 60, 75 and 90 days after transplanting in field conditions.

Screening for resistance to TSWV diseases in contained conditions. The accessions of *S. chilense* (Bioversity Int.) were used as a source for the evaluation and screening for the resistance to TSWV disease. The procedure recommended by Mandal and Kundu (2008) was used in the present study. Infected tomato leaves were collected from infected plants in the field. The virus infection was identified primarily on the basis of rings symptoms on fruits, leaf and stem necrosis of infected plants. When local lesions were found, it was inoculated to cowpea (cv. C-152) and identified chlorotic spots. The virus was maintained on *Nicotinana benthamina* seedlings (Fig. 2).

Inoculation on tomato seedlings. The sap was prepared using Potasium phosphate buffer (0.01 M) pH 7.0 along with 0.2 % sodium sulphite, 2-mercaptoethanol (0.01 %) / thioglycerol (0.1 %) and Carborundum (0.01 %) inoculum concentration: 1:10 wt /vol. Buffer prepared was chilled by storing at 4°C. Tissue was grinded in the above buffer and was kept on ice. The sap was inoculated to seedlings at mature stage *i. e.,* 16-18 days old seedlings (Seedlings raised in growth chamber having temperature: 20 ± 10 C, Light: 12 hr. dark and 12 hr. light with 7000-8000 lux and RH (%): 85 ± 2 %) (If in polyhouse, Shade- 50 % Temperature: 28 ± 2°C). Inoculation was carried during evening hours. The inoculated plants were washed with tap water before drying the leaves and kept. Seedlings were maintained for symptoms at temperature: 20 ± 10 C, Light: 12 hr. dark and 12 hr. light with 7000-8000 lux and RH (%): 85 ± 2 %). The observations were recorded at 7, 14 and 21 days after inoculation.

RESULTS AND DISCUSSION

The disease incidence of TSWV for 17 accessions of *S. chilense* in field condition were recorded after 75 DAT, as there was no TSWV symptoms observed. The mean performance of selected quantitative traits for 17 accessions of *S. chilense*in field condition is given in Table 1. The per cent disease incidence for TSWV recorded for 17 accessions of *S. chilense* at 60 DAT, 75 DAT and 90 DAT in field conditions is listed in Table 2. At 60 DAT, all 17 accessions of *S. chilense* were grouped as immune based on disease severity scale. The magnitude of variation in disease incidence increased to 20.00 with a grand mean of 1.76 at 90 DAT. Among 17 accessions, 15 were immune, one line (WT-9) was resistant and accession, WT-10 was grouped as moderately resistant to TSWV disease incidence at field condition (Fig. 3). The assessment of disease incidence of TSWV for 17 accessions of *S. chilense* in contained conditions was conducted by mechanical inoculation of TSWV sap mixture from infected host plants. The per cent disease incidence of TSWV among accessions recorded at 7 DAI, 14 DAI and 21 DAI in contained conditions is presented in Table 3. The magnitude of variation for per cent disease incidence of TSWV for accessions ranged from 0.00 to 20.00 with a grand mean of 1.76 at 7 DAI. The disease incidence increased to 30.00 per cent at 14 DAI through a mean of 2.94. At 21 DAI, the per cent disease incidence was severe ranging from 0.00 to 50.00 with a grand mean of 7.06 (Figure 4).

In recent years, about seven TSWV resistance loci have been recognized and designated as allelic (*Sw-1a* and *Sw-1b*), dominant (*Sw-5*, *Sw-6*, and *Sw-7*) and recessive genes (*sw-2*, *sw-3*, and *sw-4*). The locus *Sw-5*, formerly introgressed in the cultivar 'Stevens', is presently the primary source of TSWV resistance in commercial tomato varieties across the globe. *Sw-5* gives resistance to tospoviruses that are closely related to TSWV isolates, such as tomato chlorotic spot tospovirus (TCSV) and groundnut ring spot tospovirus, in addition to imparting a broad spectrum resistance to these tospoviruses (GRSV) (Soler *et al.*, 2003). Padmanabhan *et al.* (2019) studied the possible contribution of PR-5 (Pathogen Related protein) in the resistance showcased by Sw-7. Remarkably, PR-5 overexpressed plants exhibited enhanced resistance, delaying the accumulation of the virus and as well as expression of symptoms. Similar outcomes were reported by Reddy *et al.* (2008) by screening tomato cultivars for TSWV disease resistance and observed that cultivars, Alcobasa-V and PKM-1 were resistant to GBNV/tomato tospovirus in field conditions.

The disease incidence of TSWV for 17 accessions of *S. chilense*in field conditions were recorded after 75 DAT, as there was no TSWV symptoms observed among the plants till that period. About 90 per cent of wild accession were immune in field condition, which indicates the high resistance of *S. chilense* against TSWV due to the presence of *Sw* genes (*Sw-5* and *Sw-7*), which play a major role in resistance against TSWV infection (Canady *et al.*, 2001; Padmanabhan *et al.*, 2019). The assessment of disease incidence of TSWV for 17 accessions of *S. chilense* in contained condition revealed a comparatively lower per cent of disease incidence than it did for RILs, indicating the strong genetic resistance governed by specific resistant genes in *S. chilense* accessions. The locus *Sw-7*, identified in *S. chilense* and introgressed in commercial cultivars is reported as an alternative locus harboring resistance to a broad range of TSWV strains and poses as a potential source of resistance to incorporate in tomato breeding programs for resistance against TSWV disease.

Table 1: Mean performance of selected quantitative traits for 17 accessions of *S. chilense* in field condition.

RILs	DT50F	PH	BPP	FCPP	FPC	FPP	AFWT	LPP	FL	FD	TYPP	TRDSTY	CSPAD	PHL	SUG	TAN	TERP
WT-1	27.03	119.32	8.91	21.35	6.35	101.92	12.87	3.18	2.18	2.42	6.53	734.37	42.17	18.54	5.48	9.85	754.32
WT-2	26.16	122.19	9.03	22.15	7.81	104.33	14.28	2.64	2.18	2.13	3.03	728.95	43.28	20.64	4.32	8.22	683.20
WT-3	26.59	123.4	14.27	20.97	6.93	98.73	13.01	3.27	1.93	2.39	4.25	734.65	42.1	20.30	5.17	9.20	627.58
WT-4	25.68	129.03	18.93	23.46	8.05	111.1	11.28	3.65	2.26	1.97	2.94	762.01	43.28	19.70	5.43	10.03	842.18
WT-5	26.43	116.09	23.1	24.35	6.92	112.73	12.33	3.92	2.73	2.08	3.09	734.2	42.87	18.67	4.32	10.32	654.37
WT-6	27.94	123.33	26.43	21.83	5.17	121.26	13.19	3.74	1.86	1.96	5.33	695.98	44.63	21.36	5.48	8.27	716.54
WT-7	26.55	125.76	18.72	20.94	7.93	109.43	11.86	3.36	2.65	1.50	4.25	732.2	41.86	24.61	4.03	9.64	876.54
WT-8	26.92	118.76	13.28	23.15	5.06	104.29	14.47	2.61	2.37	1.59	3.63	756.23	46.27	20.57	5.33	10.24	532.19
WT-9	27.64	121.29	10.46	22.16	6.92	118.35	13.21	3.86	2.43	2.14	4.36	763.2	45.98	14.60	5.16	10.65	766.95
WT-10	25.03	114.34	9.71	19.83	7.81	100.67	11.45	4.17	2.17	2.26	4.92	733.2	43.28	21.42	5.33	8.92	873.29
WT-11	23.58	122.04	11.27	26.48	7.37	124.35	11.27	3.28	2.41	1.68	3.26	760.34	46.92	19.69	5.4	9.20	764.39
WT-12	26.77	124.3	8.38	22.31	6.83	104.18	13.54	3.77	1.68	1.96	3.04	762.1	43.29	20.46	5.84	9.39	885.32
WT-13	28.1	119.84	11.24	20.84	8.19	115.46	12.65	2.83	2.03	2.12	3.27	765.94	42.18	18.72	6.45	10.83	734.17
WT-14	27.93	117.38	22.63	25.63	7.64	202.37	13.85	4.36	2.19	2.31	4.36	762.1	41.28	21.36	5.92	9.33	568.36
WT-15	25.79	120.14	21.29	22.31	8.91	209.92	12.47	3.27	2.26	1.63	3.03	733.2	45.38	21.57	6.45	10.44	543.94
WT-16	24.05	126.86	29.63	23.49	6.45	113.51	11.25	2.18	2.31	2.26	4.51	670.23	46.39	19.53	5.1	10.26	843.29
WT-17	26.71	128.94	21.54	25.73	7.32	101.36	13.19	3.89	2.16	2.36	2.93	683.2	44.37	18.70	6.45	10.93	764.32

AVGFT: Average fruit weight (g);	SPAD: Chlorophyll SPAD;	FD: Fruit diameter;	FL: Fruit length;	FPC: Fruits per cluster;
DT50: Days to 50 % flowering;	NBPP: Number of branches per plant;	PLHT; Plant height;	TAN: Tannins content;	PHL: Total Phenols content;
NCPP: Number of clusters per plant;	NFPP: Number of fruits per plant;	TERP: Terpenoid content;	TRD: Trichome density;	TYPP: Total yield per plant;
LPF: Number of locules per fruit;	SUG: Reducing sugars content			

Table 2: Mean per cent disease incidence for TSWV disease among *S. chilense* accessions in field condition.

Per cent disease incidence	Mean	Range		Std. Error	Std. Deviation
		Minimum	Maximum		
60 DAT	0.00	0.00	0.00	0.00	0.00
75 DAT	0.59	0.00	10.00	0.59	2.43
90 DAT	1.76	0.00	20.00	1.28	5.29

DAT: Days after transplanting

Table 3: Mean per cent disease incidence for TSWV disease by mechanical inoculation among *S. chilense* accessions in contained condition.

Percent disease incidence	Mean	Range		Std. Error	Std. Deviation
		Minimum	Maximum		
7 DAI	1.76	0.00	20.00	1.28	5.29
14 DAI	2.94	0.00	30.00	1.87	7.72
21 DAI	7.06	0.00	50.00	3.18	13.12

DAI: Days after inoculation

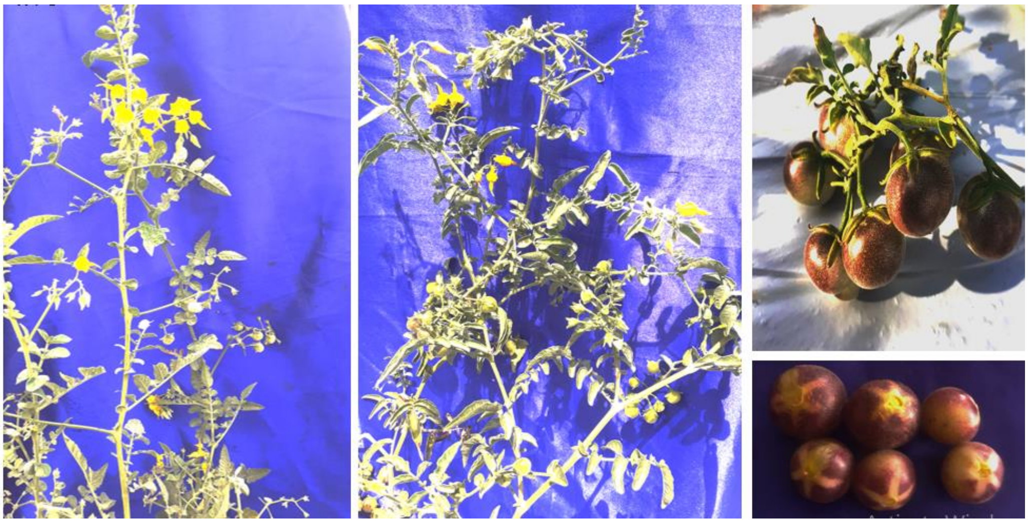

Fig. 1. *S. chilense* accessions screened for TSWV disease resistance in field conditions.

Fig. 2. Artificial inoculation of Tospovirus by mechanical method in contained condition on cow pea, *Nicotiana benthamiana* in contained conditions.

Fig. 3. TSWV disease symptoms observed in accessions of *S. chilense* in field conditions.

Fig. 4. The symptoms of TSWV disease observed in accessions of *S. chilense* in contained condition.

CONCLUSIONS

Among 17 accessions of *S. chilense*, about 11 accessions named WT-1, WT-2, WT-3, WT-4, WT-8, WT-12, WT-13, WT-14, WT-15, WT-16 and WT-17 did not exhibit any symptoms of TSWV disease and were immune in both field and contained conditions. Theoretical research on the advantages of different breeding strategies is supported by empirical genetic models of trait genetic architecture derived from mapping studies.

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Conflict of Interest. None.

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