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Characterization of Tomato MAGIC-RIL's for Resistance to Tomato Spotted Wilt Virus (TSWV) Disease

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Abstract

Tospoviruses are one of the economically important disease-causing viruses of tomato (*Solanum lycopersicum*) and other Solanaceous species. Tospoviruses are transmitted predominantly by the thrips (*Franklinnella oxidentallis*) in a persistent manner. As all cultivated tomato varieties and hybrids are susceptible to the virus, the objective of this work was to evaluate the responses of RILs (Recombinant Inbred Lines) of tomato to infection of tospoviruses and their tolerance to the disease. The resistance to tospovirus infection was evaluated in field conditions as well as in contained conditions by mechanical inoculation of tomato seedlings with plant sap-phosphate buffer mixture from tospovirus-infected tomato plants. Among 225 RILs, 203 lines were grouped as immune, two lines were resistant, one line was moderately resistant, one line was moderately susceptible, seven were susceptible and 11 were grouped as highly susceptible to TSWV disease in field condition. In the case of the contained condition, the magnitude of variation for per cent disease incidence of TSWV for RILs ranged from 60.00 to 90.00 with a grand mean of 7.60 at 21 days after inoculation. Out of 225 RILs, about 81 RILs did not exhibit any symptoms of TSWV disease and were immune in both field and contained conditions. Identification of these lines resistant to TSWV disease in both field and contained conditions might be useful for tomato breeding programme.

Keywords : TSWV, Tospoviruses, Tomato, Viral diseases, RILs, Mechanical inoculation

The tomato spotted wilt virus (TSWV) clustered under the genus *Orthotospovirus,* is a genus of negative-strand RNA viruses, in the family Tospoviridae of the order Bunyavirales. *Tospovirus* and family *Bunyaviridae,* infecting over 1000 plant species and causes significant economic damage to many agricultural and horticultural crops (Stevens *et al.,* 1992). Tomato spotted wilt virus disease may affect both the quantity and quality of the crop. Additionally, the movement of infected plant material may result in TSWV being introduced into a new area. Development of control measures for TSWV has been a tedious process due to the wide host range of viruses and the incapability of pesticides to control vectors, and thrips (Cho *et al.,* 1989). Genetic resistance to Tospoviruses has been reported in many wild relatives of *Lycopersicon* spp.

TSWV infects over 1000 plant species and causes significant economic damage to many agricultural and horticultural crops. In some areas, the virus has been found to be ubiquitous in the environment as it can infect many weeds, landscape plants and native plants. Symptoms of tomato spotted wilt differ among hosts and can be variable in a single host species. Young leaves of slightly infected transplants turn

bronze (purplish-brown) and later develop numerous small, dark spots (Soler *et al.,* 2003). The bronzing of foliage may extend to large areas of the leaf surface. The bronzed areas may roll inward and the tissue often dies. Heavily infected transplants remain stunted. Shiny, dark brown streaks appear on stems and petioles. The growing tips of plants may die back. Affected fruit has spots about one cm in diameter with slightly raised, circular markings. Ripe fruit can be distorted and can have alternate red and yellow bands. Sometimes infected plants are killed by severe necrosis (Goldberg and French, 2016).

In *Lycopersicon* species, tospovirus resistance has been found to be genetic. It has been demonstrated that creating genetically resistant cultivars is an effective strategy for managing viral infections in a variety of crops (Fraser, 1990). However, the majority of the reported sources of resistance is specific to certain TSWV isolates (Stevens *et al.,* 1992). 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. 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 this study, potential sources of resistance to TSWV disease were identified among tomato RILs under both field and contained conditions.

Material and Methods

Plant Material

The RILs of tomato derived from MAGIC (Multi-parent Advanced Generation Inter-Cross) were used as a source for the evaluation and screening for resistance to TSWV disease. A total of 225 tomato MAGIC-RILs were raised in Augmented Block Design for field screening during March, 2021, when the vector population for both viral diseases is reported to be high. All recommended package of practices were followed except for the management of TSWV disease on the crop.

The scale of disease severity index given by Hanson (1996) was used for screening of TSWV disease resistance in both field and contained conditions;

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 covering all plants
F	25.1 and above	Highly Susceptible (HS)	Severe necrosis and wilting

The resistance to TSWV disease was screened in field conditions at 45, 60, 75 and 90 days after transplanting.

Mechanical Inoculation of TSWV on Tomato Culture

The procedure recommended by Mandal and Kundu (2008) was followed for screening by mechanical inoculation 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. In order to rule out mixed infection by tomato mosaic virus (ToMV), initially the culture was inoculated to *Nicotina glutinosa* and checked local lesions if any. The virus inoculum was prepared by using freshly harvested tospovirus infected tomato from the field. 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. 1).

Inoculation on Tomato Seedlings

The sap was prepared using Potassium phosphate buffer (0.01 M)-pH 7.0 along with 0.2 per cent sodium sulphite, 2-mercaptoethanol (0.01%) / thioglycerol (0.1%) and Carborundum (0.01%) inoculum concentration: 1:10 wt/vol. The 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 ± 10^0C, Light: 12 hr. dark and 12 hr. light with 7000-8000 lux and RH (%): 85 ± 2%) (If in polyhouse, Shade- 50% Temperature: 28 ± 2^0C). Inoculation was carried out 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 ± 10^0C, Light: 12 hr. dark and 12 hr.

Fig. 1 : Artificial inoculation of tospovirus by the mechanical method in contained condition on a) & b) cowpea, c) & d) *Nicotiana benthamiana*, e) RILs and wild accessions in contained condition

light with 7000-8000 lux and RH (%): 85 ± 2 %). Symptoms were observed at 10-12 dpi. The degree of disease severity was determined on the basis of visual observations by using a scale given by Hanson (1996). The TSWV disease resistance in contained conditions was screened at 7, 14 and 21 days after inoculation.

Results and Discussion

The Estimates of descriptive statistics for selected quantitative traits among MAGIC-RILs of tomato are presented in Table 1. The disease incidence of TSWV for RILs in field conditions was recorded after 60 DAT, as there were no TSWV symptoms observed among the plants till that period. The distribution of Tomato MAGIC-RILs based on the TSWV disease incidence scale given by Hanson (1996) are listed in Table 2. The per cent disease incidence for TSWV recorded for 225 RILs at 60 DAT, 75 DAT and 90 DAT is presented in Table 3. At 45 DAT, all 225 RILs were grouped as immune based on the disease severity scale. The magnitude of variation for TSWV disease incidence ranged from 0.00 to 20.00 with a mean disease incidence of 1.02 at 60 DAT. The disease incidence at 75 DAT increased to 30.00 with a grand mean of 1.96. The grand mean of disease incidence at 90 DAT was 3.73 ranging from 0.00 to 60.00. Out of 225 RILs, 203 were grouped as immune, two lines were resistant, one line was moderately resistant, one line was moderately susceptible, seven were susceptible and 11 were grouped as highly susceptible to TSWV disease in field conditions (Fig. 2). The per cent disease incidence of TSWV for 225 RILs in contained conditions by mechanical inoculation of TSWV sap mixture from infected host plants recorded at 7 DAI, 14 DAI and 21 DAI is presented in Table 4. The magnitude of variation for per cent disease incidence of TSWV for RILs ranged from 0.00 to 60.00 with a grand mean of 3.82 at 7 DAI. The disease incidence increased to 80.00 per cent at 14 DAI with a mean of 5.51. At 21 DAI, the per cent disease incidence was severe ranging from 0.00 to 90.00 with a grand mean of 7.60 (Fig. 3).

Table 1

Estimates of descriptive statistics for selected quantitative traits among MAGIC-RILs of tomato

Traits	Min	Max	Range	Mean	Std. Error	Std. Deviation
Days to 50 % flowering	23.3	39.75	16.45	32.32	0.24	3.61
Plant height (cm)	36.65	181.55	144.9	113.95	1.55	23.72
Number of branches per plant	5.80	39.26	33.46	20.74	0.48	7.25
Number of clusters per plant	18.42	37.92	19.5	27.52	0.27	4.15
Number of fruits per cluster	2.83	7.57	4.74	5. 23	0.06	0.94
Number of fruits per plant	59.86	295.01	235.15	161.28	3.67	56.07
Average fruit weight (g)	2.94	44.63	41.69	22.62	0.61	9.36
Number of locules per fruit	1.76	5.87	4.11	3.68	0.06	0.84
Fruit length (cm)	1.77	6.13	4.36	4.15	0.06	0.88
Fruit diameter (cm)	2.78	6.17	3.39	4.82	0.03	0.53
Total yield per plant (kg)	2.96	10.11	7.15	6.25	0.11	1.61
Trichome density (t/cm^2)	102.34	473.58	371.24	279.92	5.83	89.06
Chlorophyll content (SPAD)	36.96	47.05	10.09	42.48	0.15	2.28
Total Phenol content (mg/g)	4.25	22.29	18.04	13.86	0.27	4.14
Reducing Sugar content (g/100g)	4.00	6.75	2.75	5.49	0.04	0.60
Tannins content (g/100g)	2.06	9.67	7.61	6.34	0.09	1.34
Terpenoid content (mg/100g)	281.79	951.35	669.56	606.33	10.65	162.55

TABLE 2

Distribution of Tomato MAGIC-RILs at different stages of crop growth based on TSWV disease incidence scale given by Hanson (1996)

Disease rating	Reaction	45 DAT	60 DAT	75 DAT	90 DAT
0.0	Immune (I)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 176, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 210, 221, 222, 223, 224, 225	1, 3, 4, 5, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 210, 222, 223, 224, 225	1, 3, 4, 5, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 53, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, , 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 210 , 222, 223, 224, 225	1, 3, 4, 5, 6, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 53, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 154, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 210, 222, 223, 224, 225

Disease rating	Reaction	45 DAT	60 DAT	75 DAT	90 DAT
0.1-5.0	Resistant (R)	-	2, 77, 221	221	155, 156
5.0-10.00	Moderately Resistant (MR) n	-	6, 7, 8, 9, 10, 11, 12, 13,16, 32, 33, 54, 94, 153, 177	2, 77	221
10.01-15.00	Moderately Susceptible (MS)	-	-	51, 52, 54, 94, 153, 177	2, 77
15.01-25.00	Susceptible (S)	-	-	6, 7, 8, 9, 10, 11, 12, 13, 16, 32, 33	51, 52, 54, 77, 94, 153, 177
25.00 & above	Highly Susceptible (HS)	-	-	-	6, 7, 8, 9, 10, 11, 12, 13,16, 32, 33

Fig. 2 : TSWV infected tomato plants in the field of MAGIC-RIP of tomato

TABLE 3

Mean per cent disease incidence for TSWV disease among RILs of tomato in field condition

Per cent disease incidence					
Stages of observation	Mean	Range		Std. Error	Std. Deviation
		Maximum	Minimum		
45 DAT	0.00	0.00	0.00	0.00	0.00
60 DAT	1.02	0.00	20.00	0.23	3.45
75 DAT	1.96	0.00	30.00	0.43	6.46
90 DAT	3.73	0.00	60.00	0.81	12.11

DAT: Days after transplanting

TABLE 4

Mean per cent disease incidence for TSWV disease by mechanical inoculation among RILs in contained condition

Per cent disease incidence					
Stages of observation	Mean	Range		Std. Error	Std. Deviation
		Maximum	Minimum		
7 DAI	3.82	0.00	60.00	0.79	11.90
14 DAI	5.51	0.00	80.00	1.01	15.14
21 DAI	7.60	0.00	90.00	1.28	19.21

DAI: Days after inoculation

Fig. 3 : The symptoms of TSWV infection in artificial screening of MAGIC-RIP of tomato and *S. chilense* accessions in contained conditions

The disease incidence of TSWV for F_6 RILs in field conditions was recorded after 60 DAT, as there were no TSWV symptoms observed among the plants till that period. At 90 DAT, out of 225 RILs, 203 were grouped as immune, two lines were resistant, one line was moderately resistant, one line was moderately susceptible, seven were susceptible and 11 were grouped as highly susceptible for TSWV disease in field conditions. 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 resistance to TSWV disease in both field and contained conditions by some of the lines among RILs might be due to the presence of *Sw* genes, which play a major role in resistance against TSWV disease infection (Canady *et al.,* 2001).

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. 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*) (Padmanabhan *et al.,* 2019). 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 ringspot tospovirus, in addition to imparting a broad spectrum resistance to these Tospoviruses (GRSV) (Soler *et al.,* 2003). The disease incidence of TSWV in contained conditions by mechanical inoculation of TSWV sap mixture from infected host plants of tomato at 7 DAI recorded 60 per cent, indicating high disease severity among RILs. At 21 DAI the magnitude of disease severity was upto 90 per cent revealing a weaker resistance among RILs against TSWV incidence than against ToLCV disease, which could be due to the different sets of genes governing the resistance mechanism against two viral diseases.

Among 225 RILs, about 81 RILs did not exhibit any symptoms of TSWV diseases and were immune in both field and contained conditions. Development of control measures for TSWV has been a tedious process due to the wide host range of viruses and the incapability of pesticides to control vectors. Genetic resistance to Tospoviruses has been reported in many wild relatives of *Lycopersicon* spp. Identification of genes involved in TSWV disease resistance and yield-related QTLs identified, which can be manipulated for high yield in a disease condition. Fine mapping of the picked region of the linkage map for better resolution to facilitate map-based map cloning.

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