DOI : 10.5958/0974-0112.2016.00019.0

Indian J. Hort. 73(1), March 2016: 60-65

Response of some wild species of tomato against *Peanut bud necrosis virus* under open-field conditions

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ABSTRACT

Thrips-borne *Tospovirus* pathogens adversely affect many globally important crops. Among 16 distinct virus species in the *Tospovirus* genus, four species including *Peanut bud necrosis virus* (PBNV) causing necrosis disease in tomato have been reported in India. Identification of stable sources and further utilization of wild relatives as gene sources to increase levels and diversify the bases of resistance may offer good management for the disease. A total of 13 wild species of tomato (*Solanum peruvianum*), two *S. pimpinellifolium*, one *S. chilense*, one *S. pennellii* and three check cultivars (*S. lycopersicum*) along with two cultivars (*S. lycopersicum*) having the *Sw-5* and *Sw-7* genes were evaluated under field conditions during three consecutive seasons (June to October 2008, July to December 2009, August 2010 to February 2011). Among all, a high degree of field resistance (>80%) was detected in seven lines of *S. peruvianum* (L00735, L00671, L00887, L06138), *S. chilense* (TL02226) and *S. pimpinellifolium* (L03708, TL02213) lines. The field data was also supported by negative reaction against a polyclonal antiserum of the nucleocapsid protein (N) of PBNV in direct antigen coating-enzyme linked immunosorbent assay (DAC-ELISA). The cultivars with *Sw-5* and *Sw-7* genes were highly susceptible to PBNV.

Key words: *Solanum lycopersicum*, *S. peruvianum*, *peanut bud necrosis virus.*

INTRODUCTION

Thrips are extremely difficult to find on plants, making control problematic. Tomato necrosis disease caused by *Peanut bud necrosis virus* (PBNV) in India is a distinct *Tospovirus* belonging to serogroup IV (Jain *et al.*, 8; Soler *et al.*, 19; Akram *et al.*, 2). The disease is a serious constraint to production of several crops including tomato in various locations of the subcontinent. PBNV and *Tomato leaf curl virus* are considered to be among the most destructive diseases of tomato in India, causing yield losses ranging from 27 to 90% in summer (Singh and Tripathi, 15). PBNV not only reduces yield up to 90%, but also diminishes the quality of fruit harvested from infected plants (Sain and Chadha, 14). Integrated disease management (IDM) strategies like use of cultural practices, plastic mulches, fine-mesh netting at nursery stage, and resistant or tolerant varieties are effective and ecologically friendly method for reducing the impact in tomato, pepper and peanut (Greenough *et al.*, 7). Cultivar choice is an additional method available to control plant virus disease. Virus-resistant cultivars are one of the most cost-effective IDM components as the resistant cultivars have low environmental impact and have proven to be the most consistent way to minimize losses from *Tospovirus* (Soler *et al.*, 19; Zaccardelli *et al.*, 20). Finding the source of resistance genes and utilizing them in breeding for resistance is an important process for safe and effective *Tospovirus* control.

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MATERIALS AND METHODS

A germplasm collection of 13 wild species of tomato (*Solanum peruvianum* (L.) Mill), two *S. pimpinellifolium,* one *S. chilense*, one *S. pennellii*, two cultivated tomato (*S. lycopersicum*) BL1022 and CK 12 having *Sw-5* and *Sw-7* genes, and three susceptible cultivars TLB 182, K555, TLCV15 were chosen for evaluation for resistance to *Peanut bud necrosis* (PBNV) disease. These entries were evaluated under field conditions using the natural inoculum with infector row method in three consecutive years (2008, 2009, and August 2010-February 2011) (Table 1). Susceptible line *S. lycopersicum* (CNL-2498 E) was selected to be planted as the infector row. The germplasm/line material was procured from AVRDC-The World Vegetable Center, Taiwan.

The field experiments were laid out following a randomized complete block design (RCBD) with three replications for two consecutive years (June-November 2008, July-December 2009, and August 2010-February 2011) in Hyderabad, India to evaluate the resistance of wild tomato germplasm against PBNV infection.

Table 1. PBNV incidence in tomato entries evaluated during 2008, 2009 and 2010-11.

Entry	Species	Percent disease incidence[a]									
		Year 2008			Year 2009			Year 2010-11			Pooled mean
		49 DAT[b]	89 DAT	AUDPC[c]	50 DAT[b]	75 DAT	AUDPC[c]	90 DAT[b]	120 DAT	AUDPC[c]	
L 00671	*S. peruvianum*	04.2 (06.90)	17.5 (24.61)	16.26	13.9 (21.9)	17.8 (24.9)	18.39	2.38 (8.9)	14.29 (22.2)	16.67	16.53 (23.99)
L 00673	*S. peruvianum*	16.7 (19.93)	26.7 (30.29)	19.62	19.0 (25.9)	30.2 (33.3)	22.96	19.05 (25.9)	52.38 (46.4)	10.72	36.43 (37.13)
L 00678	*S. peruvianum*	06.7 (08.85)	30.0 (32.21)	21.04	-	-	-	-	-	-	30.0 (33.21)
L 00687	*S. peruvianum*	36.7 (36.14)	43.3 (41.15)	16.02	-	-	-	-	-	-	43.3 (41.15)
L 00688	*S. peruvianum*	21.4 (22.41)	40.5 (39.42)	25.62	-	-	-	-	-	-	40.5 (39.53)
L 00689	*S. peruvianum*	24.1 (24.36)	31.5 (33.74)	29.61	-	-	-	-	-	-	31.5 (34.14)
L 00737	*S. peruvianum*	20.7 (21.49)	47.8 (43.76)	33.12	-	-	-	-	-	-	47.8 (43.74)
L 00735	*S. peruvianum* var. *humifusum*	-	-		09.5 (18.0)	09.5 (18.0)	14.25	2.38 (8.9)	9.52 (18.0)	19.05	9.51 (17.96)
L 00738	*S. peruvianum*	20.0 (21.93)	33.3 (34.93)	25.51	-	-	-	-	-	-	33.3 (35.25)
L 00882	*S. peruvianum*	18.3 (21.07)	36.9 (37.15)	28.93	30.0 (33.2)	37.8 (37.9)	32.11	4.76 (12.6)	16.7 (24.1)	14.29	30.47 (30.50)
L 00887	*S. peruvianum*	04.2 (06.90)	35.6 (31.34)	31.41	05.6 (13.6)	05.6 (13.6)	10.09	4.76 (12.6)	16.7 (24.1)	14.29	16.53 (23.99)
L 00890	*S. peruvianum*	20.0 (21.93)	23.3 (28.07)	26.32	-	-	-	-	-	-	23.3 (28.86)
L 06138	*S. peruvianum*	10.7 (15.52)	10.7 (15.52)	39.15	16.7 (24.1)	22.2 (28.1)	20.88	14.29 (22.2)	28.57 (32.3)	3.57	20.49 (26.92)
BL 1022 (*Sw-5*)	*S. lycopersicum*	76.7 (66.15)	100 (90.00)	63.65	84.1 (66.5)	94.4 (76.4)	75.83	59.52 (50.5)	85.71 (67.8)	47.62	93.37 (75.08)
CK-12 (*Sw-7*)	*S. lycopersicum*	56.7 (48.93)	92.9 (77.37)	78.58	-	-	-	-	-	-	92.9 (74.55)
TLB-182 (SC)	*S. lycopersicum*	92.9 (77.37)	100 (90.00)	84.19	80.2 (63.6)	90.5 (72.0)	72.36	54.76 (47.7)	64.29 (53.3)	34.52	84.93 (67.16)
L05789	*S. pennellii*	-	-	-	-	-	-	47.62 (43.6)	47.62 (43.6)	22.62	47.62 (43.6)
L03708	*S. pimpinellifolium*	-	-	-	-	-	-	4.76 (12.6)	11.90 (20.2)	16.67	11.90 (20.2)
TL02213	*S. pimpinellifolium*	-	-	-	-	-	-	7.14 (15.5)	16.67 (24.1)	13.09	16.67 (24.1)
TL02226	*S. chilense*	-	-	-	-	-	-	21.43 (27.6)	21.43 (27.6)	3.57	21.43 (27.6)
TLCV15 (SC)	*S. lycopersicum*	-	-	-	-	-	-	33.33 (35.3)	88.09 (69.8)	35.71	88.09 (69.8)
K555 (SC)	*S. lycopersicum*	-	-	-	-	-	-	50 (45.0)	71.43 (57.7)	35.71	71.43 (57.7)
CD at ($p \leq 0.05$)		26.93	21.77		26.62	27.54		17.09	18.64		20.18

[a]Value in parentheses are Arc sine transformed values; [b]DAT = days after transplanting; [c]AUDPC = area under disease progress curve

Tomato seedlings were raised in trays using sand, soil, and compost mixture @ 1:1:2 under 60-mesh nylon net. Seed of *S. peruvianum* species were sown 10 days earlier (June 23, 2008) than the check lines (CK12, BL1022) and susceptible tomato line (TLB-182), as the wild tomato grows slower than improved varieties. Transplanting of the wild entries was done 10 days after transplanting (DAT) of (August 2) the improved cultivars. Ten seedlings of each entry were transplanted on 5 m long raised ridges in a double-row plot, at 90 cm × 90 cm spacing at M/s JK Seeds Research farm, Hyderabad. Cultural practices were followed according to the recommendation for irrigated tomato in Andhra Pradesh (Anon, 1). One PBNV 'infector' tomato (highly susceptible line CNL-2498 E) row in every fifth plot and susceptible check (TLB-182) in every replication were transplanted 15-20 days before transplanting of the screening material. The lines L00671, L00673, L00882, L00887 and L06138 observed with low disease incidence and negative reaction with DAS-ELISA in the 2008 experiment were selected for field confirmation trials during 2009 and 2010-11. These entries, along with *S. peruvianum* var. *humifusum* (L00735), check line BL1022 having *Sw-5* genes, and a susceptible control were evaluated (7 plants per replication). During the 2010-2011 trial, six *S. peruvianum*, one *S. chilense* and two *S. pimpinellifolium* lines along with BL1022, TLB 182 and two new lines K555 and TLCV15 as checks were evaluated using the infector row method. The nursery was raised on July 16 and transplanted on August 9, 2010 at the RCSA field (14 plants in each replication).

PBNV disease incidence was identified on the basis of field symptoms (Swift, 18). The plants were inspected at seven-day intervals to note the appearance and development of the symptoms of PBNV infection starting from transplantation to last harvest. The tomato plants that remained asymptomatic until last harvest was designated as healthy plants. On the basis of the symptoms caused by the virus, the data on the incidence of PBNV was collected at two stages of the plant growth: at 49 and 89 DAT in 2008. 50 and 75 DAT in 2009, and 80 and 120 DAT in 2010-11 using 0-1 scores, 0 = healthy plant, 1 = systemic symptoms without or with stunting. The percent incidence of PBNV was calculated by counting the plants showing PBNV infection by following the formula: Percent incidence $=(X_1/X) \times 100$, where X_1 = number of infected plants and X = total number of plants. Because the standard deviations for PBNV score were proportional to their corresponding means, disease incidence data were arcsine-transformed to stabilize error variance before analysis of variance (ANOVA) at *P* <0.05% and <0.01% levels of significance (Snedecor and Cochran, 16). The Arc sine formula gives the values being identical to the Arc sine tabular values and also facilitates the calculation in the Microsoft Excel program.

Area under disease progress curve (AUDPC) was also calculated (for assessment of disease incidence) for each genotype using disease incidence (transformed data), which was the proportion (0-1.0) of symptomatic plants in the plot, using the formula: $AUDPC = \sum_{i=1}^{n-1} ([Y_i + Y_i+1]/2) [T_i+1 - T_i]$ where: Y_i+1 = apparent incidence (0-1.0) at the i^{th} observation, T_i = time (days) at the i^{th} observation, n = total number of observations. Where y_i is the disease incidence in percent at i^{th} assessment, t_i is the time of the i^{th} assessment in days from the first assessment date, and is the total number of days the disease was assessed (Campbell and Madden, 3).

Enzyme-linked immunosorbent assay (ELISA) was carried out and PBNV infections were verified by serological identification. Infected *S. lycopersicum* leaves were used as a positive control. Two to 10 fresh leaves samples from symptomatic and non-symptomatic surviving plants of each entry were collected. The samples were subjected to a polyclonal antiserum against a nucleocapsid protein of PBNV in an alkaline phosphate-based direct antigen coating-enzyme linked immunosorbent assay (DAC-ELISA) (Cho *et al.*, 4; Jain *et al.*, 8). Results of finished ELISA plates were measured at 405 nm on Molecular Devices-E-Max plate reader. Samples were considered positive if the absorbance at 405 nm was more than the twice the average buffer of healthy tomato control reading, whichever was higher.

RESULTS AND DISCUSSION

Most of the tomato species evaluated (Table 1) showed the prevalence of PBNV incidence with the highest percent (85-100%) in susceptible checks (TLB-182- BL1022, TLCV15, CK-12). Symptom expression varied among the accessions ranging from sudden wilt to severe necrosis of leaves, stem, meristem, buds, pods, and fruits. Necrosis and wilting were the most common symptoms observed at 40 and 60 DAT. Leaves became necrotic but remained attached to the stem. Stems showed necrosis and irregular brownish-black patches resulting in death of the plants.

Percent disease incidence at 89 DAT during 2008 ranged between 17.5-47.8% in *S. peruvianum* species, and 90-100% *S. lycopersicum* species. In 2009 it was 9.5-94.4%, and during 2010-2011

the percentage of disease incidence ranged from 9.5 to 88.1% in *S. peruvianum*, *S. chilense*, *S. pennellii*, *S. pimpinellifolium* and *S. lycopersicum* (Table 1). Overall, *S. lycopersicum* lines were more susceptible than the other lines evaluated. However, the level of prevalence varied significantly among accessions. Based on the visual symptom scores and their pooled mean, the lowest PBNV incidence was recorded in L06138, L03708, L00887, L00671 followed by TL02213, TL02226 and L00890. Similar results on TSWV prevalence on different tomato lines/varieties in the field have been reported earlier by Greenough *et al.* (7) and Swift (18) and also in previous reports on resistance of tomato wild species against *Tospovirus* (Paterson *et al.*, 11; Krishna Kumar *et al.*, 9; Stevens *et al.*, 17). Of the 68 fresh leaf samples from symptomatic and nonsymptomatic plants of 15 lines, all samples from 7 tomato lines (L00735, L00887, L00882, L00889, L0673, L00671, L00688) I and 11 from 13 samples of L06138 showed negative reaction against a polyclonal antiserum of the nucleocapsid protein (N) of peanut bud necrosis in DAC-ELISA.

Thus results of our study are strongly in contrast to the earlier findings, suggesting that the wild tomato species, especially *S. peruvianum*, *S. pimpinellifolium* and *S. chilense*, are resistant or immune against TWSV with no systemic infection under field conditions (Paterson *et al.*, 11; Krishna Kumar *et al.*, 9; Stevens *et al.*, 17; Rosello *et al.*, 13) and laboratory conditions (Cho *et al.*, 4; Paterson *et al.*, 11). However, the resistance conferred by the *Sw-5* gene, which relies on the development of a hypersensitive response (Soler *et al.*, 19), no longer constitutes a durable resistance system against the disease. Cho *et al.* (4) has reported that certain TSWV isolates overcome this resistance. Our results support the finding of Gordillo *et al.* (6) who have reported a varied range of resistance against TSWV6 isolate from Hawaii and An_{wa}-1 from Western Australia in *S. lycopersicum* accessions ranging from 33-68%. They have reported the highest percentage of resistance against TWSV6 isolate in L00689, L00887 and L00673.

In our study, cultivated tomato lines CK-12 and BL01022 having genes *Sw-7* and *Sw-5*, respectively were found to be highly susceptible to PBNV. This data contrasts with the previous reports suggesting that *Sw-5* confirms dominant resistance against TSWV. In our study *Sw-5* and /or *Sw-7* did not impart PBNV resistance as previously reported, which suggests that *Sw-5* and *Sw-7* genes are isolate-dependent or that resistance breakdown, has occurred. Various researchers are of the opinion that TSWV is found in nature as a heterogenous population of isolates (de Avila *et al.*, 5) with genetic potential for adoption to a wide range of hosts (Qui *et al.*, 12). Resistance-breaking occurs when new, more virulent isolates arise through mutation, selection, or introduction from other countries (Qui and Moyer, 12). Furthermore, through multiplication or prolonged contact of TSWV isolates with the resistance gene, carrier plants can lead to the development of new, more virulent isolates that overcome the resistance (Qui and Moyer, 12).

In conclusion, our study shows that four out of 13 *S. peruvianum*, two *S. pimpinellifolium,* and one *S. chilense* showed high degree of resistance (>75%) and reacted negatively to PBNV antiserum. These accessions are likely to be useful in developing an IDM strategy to reducing the impact of PBNV. Cultivated tomato lines CK-12 and BL01022 with the genes *Sw-7* and *Sw-5,* respectively, were highly susceptible as well as positive to PBNV antiserum. The difference between previous reports and the susceptibility observed in the wild tomato accessions may be due to the natural isolate (PBNV) variation or due to the use of different accessions in previous screening programmes. The high selection pressure associated with this gene has probably contributed to the emergence of resistance-breaking isolates throughout the world. It is now essential to continue the search for new source of resistance, as well as to confirm the genetic control of the resistance already identified in several tomato accessions.

The highest level of field resistance was observed in four *S. peruvianum* (L00735, L00671, L00887 and L06138), two *S. pimpinellifolium* accessions (L03708, TL02213), and one *S. chilense* accession (TL02226) (Fig. 1) and the results suggest that these accessions may be used as a source of PBNV resistance in tomato breeding programmes for the region. However, the resistance also may be due to the lack of thrips transmission/ infestation on the accessions showing resistance (Krishna-Kumar *et al.*, 10), and some genotypes may be susceptible when the mechanical or viruliferous-thrips inoculation method is used (Rosello *et al.*, 13). Therefore, laboratory screening through mechanical inoculation and thrips transmission as well as molecular studies should be carried out to identify valuable accessions. In addition, to speed up conventional improvement programs, the results of both methods coupled with molecular marker techniques in screening germplasm for resistance to PBNV should be used to ascertain the resistance mechanism. With the availability of more durable resistance genes from wild sources, there is scope to develop PBNV-resistant elite lines and cultivars.

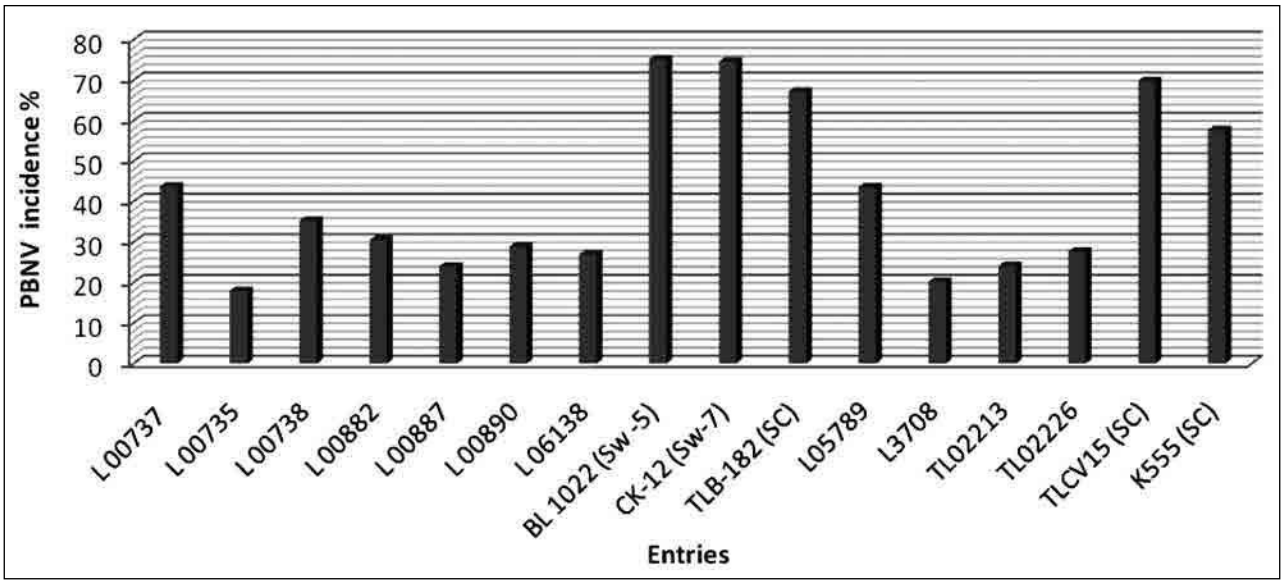

Fig. 1. Pooled mean for PBNV incidence (arcsine transformed) in different entries (CD = 20.18 at *P = 0.05*).

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Received : June, 2016; Revised : November, 2015;
Accepted : January, 2016