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Screening of tomato genotypes against tomato leaf curl virus and their morphological and biochemical categorization

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Abstract
Field screening studies were conducted with 34 tomato genotypes against Tomato leaf curl virus disease under experimental fields of Seed Research and Technology Centre, Hyderabad, India. All the tomato genotypes were evaluated for their genetic diversity with respect to morphological and biochemical characters. A wild accession, EC-251672 exhibited highly resistant reaction (0%) to *ToLCV* with no visual symptoms of disease incidence when screened under field conditions. Nine private tomato hybrids viz., Akash-918, NS-539, NS-515, Siri-9005, STH-803, STH-807, To-1827, US-1196, US-2175 and one tomato variety Vybhav showed resistant reaction (1-20% incidence) while all Arka varieties under study from IIHR, Pusa hybrid-1 and susceptible check Punjab chuhara (100%) were found be highly susceptible to ToLCV. The wild genotype EC-251672 possessed narrow *hirsutum* type of leaves, glabrous plant surface and high phenol content was found to be resistant to whitefly vector and *ToLCV* disease. The phenol content of tomato genotypes showed significant negative correlation with the *ToLCV* disease incidence. Hence this wild tomato source can be used in the development of future molecular breeding programmes against *ToLCV* in tomato.

Keywords: Tomato leaf curl virus, wild tomato, phenol, trichomes, host plant resistance, whitefly, genetic diversity

Introduction
Tomato (*Solanum lycopersicon*), is the widely grown vegetable crop in different temperate and tropical climatic conditions of the world and very commonly used colorful fruit in Indian curries. Tomato leaf curl virus (*ToLCV*), a begomovirus transmitted by whitefly, *Bemisia tabaci* causing severe yield losses in tomato especially in summer months. This viral disease is characterized by severe curling, cupping of leaves, thick rubbery shrunken leaves and stunted plant growth with majority of flower (up to 90%) drops down after infection, therefore only few fruits are produced. This Tomato leaf curl virus is so devastating epidemic disease for tomato cultivation in India which causes up to 99–100% yield losses and became a big problem for tomato farmers (Singh *et al.*, 2008) [1].

Morphological or genetic diversity study is an essential technique for the selection of preferred genotypes for plant breeding and development of varieties with known traits of resistance to biotic and abiotic stresses. Collection of germplasm, characterization of resistant traits and evaluation of genotypes are key steps towards the direct use of resistant entries in hybridization programmes. Morphological traits have been identified as equally important as yield components in breaking yield barriers as no crop can perform higher than its genetic potential even under a very conducive environment (Baye *et al.*, 2001) [2].

Management of insect transmitted viral diseases through vector control is not always successful when there is a rapid turn-over rate of vector population for disease transmission in the crop. A more effective solution for the management of vector transmitted viral diseases is by breeding cultivars resistant to ToLCV (Nateshan *et al.*, 1995, Lapidot *et al.*, 1998) [3, 4]. During the past 20 years, not much research was carried out to evolve completely resistant varieties to ToLCV. Therefore exotic wild *Lycopersicon* accessions have been screened for virus resistance in India (Nariani and vasudeva, 1963, Muniyappa *et al.*, 1991, Nateshan *et al.*, 1995) [5, 6, 3]. Nevertheless progress in breeding for ToLCV resistance has been slow (Banerjee and Kalloo, 1987[7], Lapidot *et al.*, 1998) [4] because of the complex genetics of resistance. The most important aim of plant breeding is made to develop high yielding varieties that are resistant to biotic stress for tomato leaf curl virus.

Hence, there is a dearth of resistant and stable tolerant tomato genotypes against tomato leaf curl virus, field screening study was carried out to identify resistant sources for utilization in developing line /variety resistant against tomato leaf curl virus.

Materials and Methods

34 tomato genotypes consisting of twenty two commercial tomato hybrids, nine varieties and three exotic germplasm collections were screened at Seed Research and Technology Centre, Prof. Jayashankar Telagana State Agricultural University, Rajendranagar, Hyderabad. The seedlings were grown in plastic containers at controlled conditions in National Bureau of Plant Genetic Resources (NBPGR) glass house, Hyderabad. Twenty five days old seedlings of each genotype were transplanted in the field with 2.5 x 2 feet spacing by following augmented block design with two replications along with national susceptible check Punjab Chuhara and resistant check Vybhav. The data was recorded at weekly intervals on reaction of these genotypes for the development of *ToLCV* symptoms and the vector, *B. tabaci* incidence was initiated after two weeks of transplantation up to 10 weeks after transplantation. The crop was grown under unprotected conditions with all recommended agronomic practices.

The visual morphological characters like, plant growth habit, leaf type, leaf pubescence, petiole pubescence, stem pubescence, etc., were recorded at full foliage stage (30- 35 days). At the time of peak harvest, tomatoes were randomly selected for observing biochemical parameters. The juice was extracted by crushing the fruits in muslin cloth and the % TSS were measured by using Erma hand refractometer at room temperature. Folin - Ciocalteau reagent method was used to estimate total phenols in leaves and expressed as mg / 100 g of leaf sample.

The vector, *B. tabaci* population counts were taken from five randomly selected plants in each entry by observing two upper, two middle and two bottom leaves of each plant. The incidence of *ToLCV* infection was recorded at weekly intervals from 2 to 10 weeks after transplantation. The number of virus infected and healthy plants in each replication were recorded and computed by using the following formula.

$$\textit{ToLCV}\ \text{incidence}\ (\%) = \frac{\text{Total number of plants infected with } \textit{ToLCV}}{\text{Total number of plants observed}} \times 100$$

The categorization of the disease reaction was scored as defined below

Highly resistant (HR)	:	0%.
Resistant (R)	:	1-20%
Moderately resistant (MR)	:	21-50%
Moderately susceptible (MS)	:	51- 75%
Susceptible (S)	:	75 – 100%

Tomato yield was determined by pooling the yield obtained from multiple pickings. The data was subjected to suitable statistical analysis using SPSS package.

Results and Discussion

Screening of tomato genotypes against *ToLCV* disease

Among the 34 tomato genotypes screened against *ToLCV*, only one wild accession, EC- 251672 expressed immune reaction (0%) to ToLCV infection throughout the crop growth period under field conditions (Plate 2). Among the other two wild accessions, EC-395457 and EC-357842 exhibited resistant to moderately resistant reaction with 17.00% and 28.60% *ToLCV* disease incidence respectively. Of the twenty two commercial private hybrids, 16 hybrids *viz.*, NS-539, STH-803, BSS-3000, NS-524, NS-55, Siri - 9005, STH-807, STH-816, US-1196, US-2175, Akash-918, NS-515, NS-582, NS-526, To-1827 and NS- 585 recorded *ToLCV* incidence ranging from 5.0 to 16.7% after 10 weeks of transplantation and were proved to be resistant under field conditions. Three commercial hybrids *viz.*, STH-801, Gem and Lyco exhibiting moderately resistant reaction against *ToLCV* with 27.1%, 30.0% and 35.7% disease incidence respectively. Two commercial private hybrids, STH- 808 and Lakshmi exhibited moderately susceptible reaction to *ToLCV* with an incidence of 51.4% and 55.0% respectively as depicted in Figure 1 and Table 1.

Out of nine tomato varieties, Three varieties *viz.*, Sankranthi (7.1%), Vybhav (10%) and TLBR-1 (16.7%) recorded resistant reaction whereas Nandi (25.7%) exhibited moderately resistant reaction and the other 4 varieties *viz.*, Pkm-1, Marutham, Arka meghali and Pusaruby exhibited susceptible reaction (55 to 100%) to *ToLCV* with 100% disease incidence in Punjab chuhara after ten weeks of transplantation (Table1 and Fig.1)

Table 1: Resistance reaction of tomato genotypes to *Tomato leaf curl virus*

S.no	Entry	Reaction	% disease incidence
1	Ec-251672	Highly resistant	0
2	NS-539,, STH -803,Akash-918, Bss-3000, NS-524, NS-55, Siri-9005, STH -807, STH -816, US-1196, US-2175, NS-515, NS-582, NS-526, To -1827, NS-585, Sankranthi, * Vybhav, TLBR -1, Ec-395457	Resistant	20%
3	STH -801, Gem, Lyco,Nandi, Ec-357842	Moderately resistant	21-50%
4	STH -808, Lakshmi,Pkm-1, Marutham	Moderately susceptible	51-75%
5	Arka Meghali, Pusa Ruby,* * Punjab chuhara, Pusa hybrid-1	Susceptible	76- 100%

**Susceptible check *Resistant check

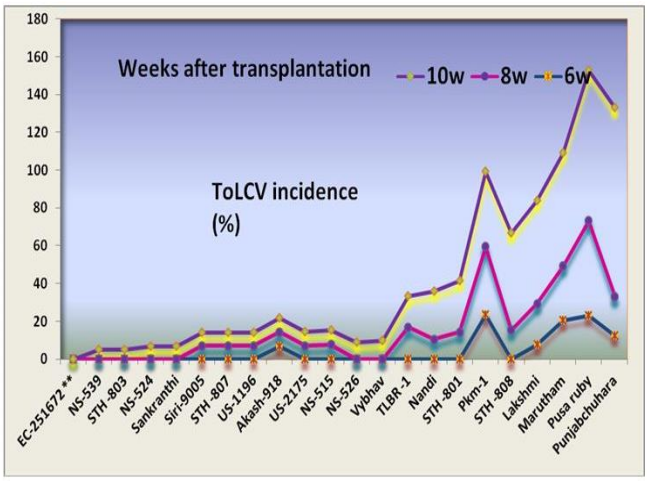


Fig 1: Screening of Tomato genotypes for ToLCV disease incidence

The present findings are in line with the research outcome of Narasegowda *et al.*, 2003) [8] who screened 34 wild and domesticated tomato lines for resistance to TYLCV and ToLCV under glass house conditions. The wild tomato accessions of *Lycopersicon hirsutum*, LA1777 and P1 390659 were found to be good resistant sources to both *ToLCV* and TYLCV. They recorded Arka vikas as susceptible variety. The three commercial hybrids FA9, FA29 and FA38, respectively showed 89, 80% and 93% *ToLCV* incidence. The Vybhav variety which exhibited resistant reaction to ToLCV also shares common platform with the findings of Muniyappa *et al.*, (2002)) [9], Shankarappa (2002)) [10], Who claimed Sankranthi, Nandi and Vybhav varieties as resistant parental lines. In the present study, the sankranthi and Vybhav were found resistant and Nandi showed moderately resistant reaction to ToLCV in the field conditions. There was no significant variation in whitefly population among the different tomato genotypes (1.43 to 2.23 whiteflies/ plant) except on wild accession EC-251672 which is highly resistant to *ToLCV* and recorded 0.15 whiteflies / plant. (Table 1).

Morphological Characters of Tomato genotypes

Growth habit: Out of 34 genotypes (Hybrids /varieties /accessions), 18 varieties/hybrids showed indeterminate growth habit and the remaining 16 genotypes are of semi determinate type (Table2). None of the genotypes showed determinate growth habit.

Leaf type: Wide morphological variability was recorded with respect to leaf type in different genotypes. Only one wild accession, EC-251672 exhibited hirsutum type of leaves (6) and two genotypes *viz.*, EC-395457 and Pusa hybrid-1 recorded potato leaf type (2) and nine genotypes *viz.*, Lyco, Nandi, NS-582, PKM-1, Sankranthi, Siri-9005, STH-801, STH-808 and TLBR-1 are with Peruvianum type of leaves (4). The remaining genotypes showed standard type of leaves. (Table 2).

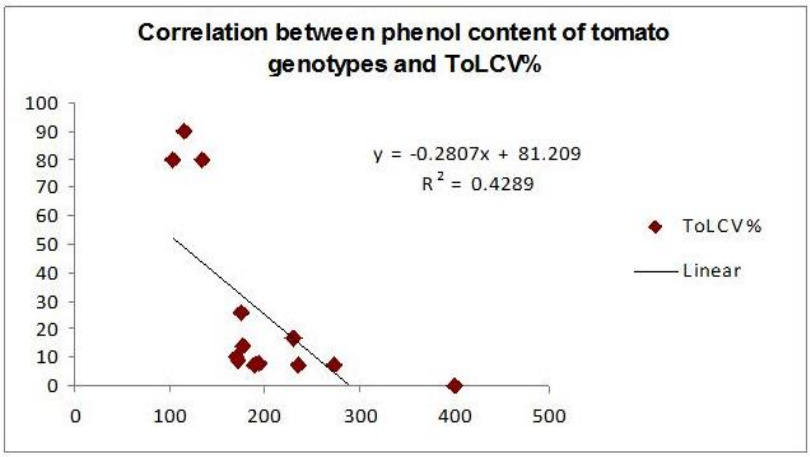
Table 2: Morphological, biochemical characters and yield of tomato genotypes

S. No	Variety / Hybrid	Plant growth Habit 1	Leaf Type 2	Leaf Pubescence 3	Petiole Pubescence 4	Stem Pubescence 5	TSS (%) Brix	Phenols (mg/100g leaf)	Yield t/ha
Wild accessions(NBPGR, Hyderabad)									
1	EC-251672	3.0	6.0	0.0	0.0	0.0	0.0	400.0	10.0
2	Ec-357842	2.0	3.0	3.0	3.0	5.0	3.0	136.8	33.0
3	Ec-395457	2.0	2.0	3.0	3.0	5.0	4.5	142.8	36.0
Hybrids									
4	Pusa Hybrid-1	2.0	2.0	3.0	3.0	5.0	3.8	103.8	31.0
5	Lyco	3.0	4.0	3.0	3.0	5.0	4.8	181.3	33.0
6	STH -816	3.0	3.0	3.0	3.0	5.0	3.0	189.5	33.0
7	TO -1827	2.0	3.0	3.0	7.0	5.0	4.8	176.8	33.0
8	NS-515	2.0	3.0	3.0	3.0	5.0	5.5	194.8	35.0
9	NS-526	2.0	3.0	3.0	3.0	5.0	4.5	171.3	36.0
10	STH -807	3.0	3.0	3.0	3.0	5.0	3.5	121.5	37.0
11	NS-582	3.0	4.0	3.0	3.0	5.0	4.0	127.0	39.0
12	NS-585	3.0	3.0	3.0	3.0	5.0	5.5	98.8	41.0
13	NS-539	3.0	3.0	3.0	3.0	5.0	4.0	103.6	45.0
14	NS-55	2.0	3.0	3.0	3.0	5.0	4.0	156.8	45.0
15	Siri-9005	2.0	4.0	3.0	3.0	5.0	3.0	173.0	45.0
16	Bss-3000	2.0	3.0	3.0	3.0	5.0	3.2	135.3	48.0
17	Gem	3.0	3.0	3.0	3.0	5.0	4.3	108.4	48.0
18	US-2175	3.0	3.0	3.0	3.0	3.0	3.2	69.6	49.0
19	STH -808	3.0	4.0	3.0	3.0	3.0	3.0	172.8	51.0
20	Lakshmi	3.0	3.0	3.0	3.0	5.0	3.0	186.8	52.0
21	NS-524	2.0	3.0	3.0	3.0	5.0	4.0	234.8	52.0
22	US-1196	3.0	3.0	3.0	3.0	7.0	3.5	123.8	55.0
23	STH -803	3.0	3.0	3.0	3.0	5.0	3.5	134.4	56.0
24	Akash-918	2.0	3.0	3.0	3.0	5.0	4.0	190.0	60.0
25	STH -801	3.0	4.0	3.0	3.0	3.0	3.0	119.3	69.0
Varieties									
26	Pkm-1	2.0	4.0	3.0	3.0	5.0	5.5	241.3	27.0
27	Arka Meghali	3.0	3.0	3.0	3.0	5.0	4.0	134.5	30.0
28	Nandi	2.0	4.0	3.0	3.0	3.0	3.0	177.3	32.0
29	Marutham	2.0	3.0	5.0	3.0	5.0	3.2	156.8	35.0
30	Pusa ruby	3.0	3.0	3.0	3.0	5.0	4.5	180.3	37.0
31	Punjab	3.0	3.0	7.0	7.0	7.0	4.0	114.3	39.0

	chuhara**								
32	TLBR -1	2.0	4.0	3.0	3.0	5.0	4.5	230.5	42.0
33	Vybhav*	3.0	3.0	3.0	3.0	5.0	3.5	170.5	46.0
34	Sankranthi	2.0	4.0	3.0	3.0	5.0	4.5	273.5	48.0
	CD at 5%						0.27	0.95	0.01

Plant growth 1. Determinate 2. Semi determinate 3. Indeterminate	2. Leaf Type 1. Small/narrow 2. Potato leaf 3. Standard 4. Peruvianum type 5. Pimpinellifolium type 6. Hirsutum type	3. Leaf pubescence 0-Absent 3-Sparse 5-Medium 7-Dense	4. Petiole Pubescence 0-Absent 3-Sparse 5-Medium 7-Dense	5. Stem Pubescence 0-Absent 3-Sparse 5-Medium 7-Dense

Plate 1: Highly resistant wild Tomato genotype - EC 251672 – Flowers and Fruits



*significant at 5% level

Fig 2: Correlation between Phenol content and (%) ToLCV incidence in Tomato

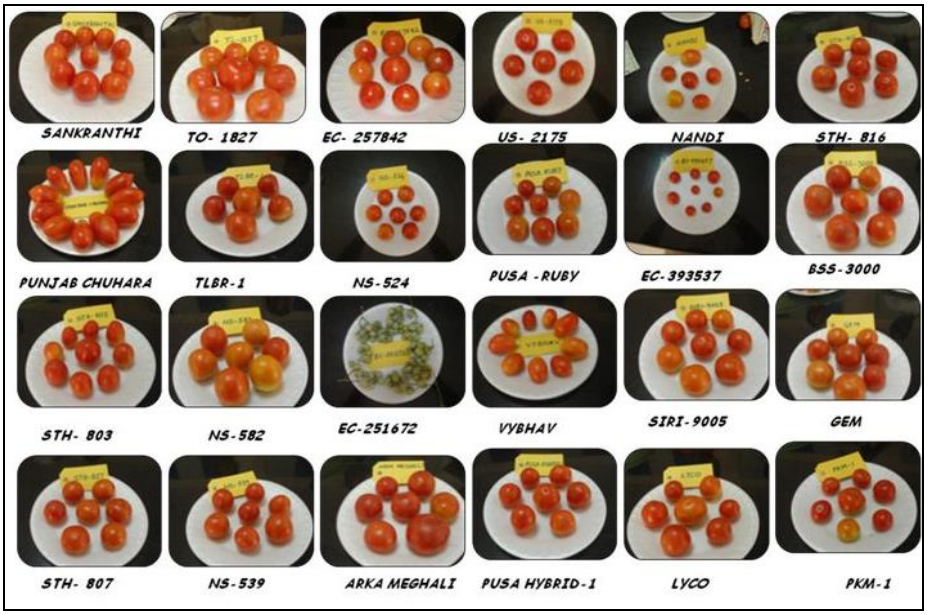


Plate 1: Tomato fruits if different genotypes screened for ToLCV resistance

Leaf pubescence: Only one wild genotype, EC-251672, which is immune to tomato leaf curl virus (0%) was found to be glabrous without any hairs on entire plant surface where as thick dense hairs were observed Punjab chuhara, a highly susceptible check with 100% disease incidence. Marutham recorded medium hairiness on leaves and the other genotypes possessed sparse hairs on surface of leavesb. (Table 2).

Petiole pubescence: Dense hairs were observed on petioles of two genotypes *viz.*, Punjab chuhara and To –1827 and the remaining entries possessed sparse hairs where as the pubescence was not present on the petioles of highly resistant wild genotype EC-251672.

Stem pubescence: Most of the genotypes possessed medium hairs on stem surface whereas Punjab chuhara and US –1196 recorded dense stem hairs while Nandi, STH-801, STH-808 and US-2175 possessed sparse hairs. Glabrous, shiny and wax coated stem was observed in the wild tomato accession EC-251672 (Table 2).

Biochemical Characters of Tomato genotypes

The total phenol content was estimated in the leaves of 34 genotypes while total soluble solids (TSS %) was estimated in tomato fruits (Table 2).

Total soluble solids (TSS %): Among the 34 genotypes, maximum TSS content (5.50 ^{0} Brix) was found in PKM-1 and NS-515 and lowest (0 ^{0} Brix) in EC-251672 wild tomato accession. Most of the entries recorded TSS % in the range of 3.0 to 5.0 ^{0} Brix (Table 2).

Total phenols: Significantly highest phenol content of 400 mg/100 gm leaf sample was recorded in EC-251672 and lowest of 69.6 mg/100 gm was recorded in US-2175. High phenol content in the leaves was recorded with most of the *ToLCV* resistant genotypes. (> 150 mg/100 gm) in comparison to Punjab chuhara (114.3 mg / 100 gm) check. (Table 2). The correlation studies computed between disease incidence and biochemical parameters like total phenols, TSS% and *ToLCV* incidence of selected genotypes revealed that the total phenol content was negatively correlated with *ToLCV* incidence in tomato genotypes (r = - 0.65). The tomato genotypes with high phenol content recorded low *Tomato leaf curl virus* incidence. There is no significant correlation between TSS% and *ToLCV* incidence of tomato genotypes. (Figure 2). Earlier more total phenol in tomato leaf curl virus resistant/ tolerant has also been recorded by Hayati (1978) [11]), Banerjee and Kalloo (1989) [12] and Ramesh kumar Singh *et al.* (2010) [13] which is inconsonance with our findings.

Yield (t/ha): Yield potentiality of 34 tomato genotypes ranged from 5 t/ha to 69 t/ha. STH-801 with moderate resistance to *ToLCV* recorded highest yield (69 t/ha) followed by Akash-918 (60 t/ha) which showed resistant reaction to *ToLCV* (Fig 2. and Plate 1). The wild accession EC-251672, which is highly resistant to *ToLCV* recorded significantly lowest yield (5t/ha) with small fruits in bunches which are of no economic value (Table 2 and Plate 2)

The morphological characters and the biochemical constituents observed in tomato genotypes showed that the wild accession EC 251672 with its indeterminate growth habit, *hirsutum* type of narrow leaves and glabrous surface without hairs on entire plant surface was found to be highly resistant to ToLCV and its vector, *B. tabaci* which possessed very high phenol content (400mg/100g) and 0% TSS (Plate 2). This is the first report of the immune reaction of the wild tomato accession, EC 251672 against ToLCV. The present investigation was in line with the findings of Singh *et al.*, 2015 [14], who reported that the wild germplasm accessions EC-520061 (*S. habrochaites*) and EC- 521080 (*S. pimpinellifolium*) were shown to be highly resistant to Tomato leaf curl virus disease. Ten genotypes comprising of 9 hybrids *viz.*, Akash-918, NS-539, NS-515, Siri-9005, STH-803, STH-807, To-1827, US-1196, US-2175 and one variety *i.e* Vybhav showed resistant reaction to *ToLCV*. High phenol content was observed in resistant genotypes while low phenol content was recorded in susceptible varieties. Significant negative correlation was obtained between phenol content of tomato genotypes and *ToLCV* incidence indicating that high phenol content is offering resistance to *ToLCV*. However Total phenol content in the leaves and glabrous plants without hairs on plant surface were emerged as more consistent determinants of resistance against Tomato leaf curl virus so it deserves due attention as a biochemical parameter in future Tomato leaf curl virus resistance breeding programmes.

Conclusion

The resistant Tomato hybrids and varieties can be recommended to the farmers for cultivation in the endemic areas of Tomato leaf curl virus disease especially during summer months. The wild tomato accession EC-251672, which is found to be highly resistant to *ToLCV* and its vector, whitefly with glabrous leaves and high phenol content can be utilized in the future resistance breeding programmes for development of resistant tomato varieties/hybrids against *ToLCV*.

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