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Screening for genetic divergence in tomato genotypes against tomato leaf curl virus

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Abstract

During summer 2005 out of 50 genotypes screened for tomato leaf curl virus under field conditions, none of the lines tested were resistant, however, six genotypes showed mild infection and nine genotypes showed moderate infection. In the second season, *i.e.*, 2006 only Nandi and Vybhav showed moderate resistant reaction, along with the new commercial hybrids Hy-558, Hy-530, NS-563 and NS-719. The variety Vybhav was found superior over other varieties against the disease. The presence of virus in the symptomatic hosts was confirmed by ELISA and PCR. The plant height of the genotypes contributes to maximum extent (52.21 %) to the divergence followed by yield per plant and per cent disease incidence (10.86 % each), but the vector population contributed least (0.97 %). As a result of D^2 clustering, the commercial hybrids possessing lot of diversity fall in to four different clusters, cluster II had got six entries, cluster III 3 entries, cluster IV 6 entries and cluster V only one entry wheras cluster I had 50 entries.

Key words: Tomato leaf curl, ELISA, PCR and screening

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important and most widely grown vegetable crop in India and ranks second in importance among vegetables. It is grown for its edible fruit, which can be consumed, either raw or cooked or in the form of various processed products.

The tomato leaf curl virus disease (ToLCVD) is caused by a range of circular single ssDNA virus (more than 20 species) species in the genus Begomovirus (Geminiviridae: subgroup – III) (Polston and Anderson, 1997; Faquet and Stanely, 2003) which are transmitted by the whitefly *Bemisia tabaci* Genn. and is the most important and destructive viral pathogen in many parts of India (Vasudeva and Sam Raj, 1948: Sastry and Singh, 1973; Saikia and Muniyappa, 1989; Harrison *et al.*, 1991). The symptoms of ToLCVD includes leaf curling, leaf chlorosis, vein clearing and stunting. If the infection occurs at the early stage it may lead to sterility.

The incidence of ToLCV in tomato growing areas of Karnataka ranged from 17-100 per cent in different seasons. The per cent yield loss observed ranged from 50-70 per cent in tomato cv. Pusa Ruby grown in February-May (Saikia and Muniyappa, 1989). Diversity in tomato leaf curl begomoviruses (TLCBs) in southern India has been apparent since the early 1980s when Reddy *et al.* (1981) reported that in a single tomato variety, TLCB isolates gave rise to five distinct symptom types. Variability was subsequently also found in the epitope profiles of TLCBs collected from Karnataka (Muniyappa *et al.,* 1991b), with groupings suggesting that the tomato crop and some neighboring weed species were hosts to the same TLCB strains/species.

The severity and rate of spread of ToLCV has become a major limiting factor for cultivation and challenging to farmers and scientific community. The existence of variability among the virus isolates and vector is the main reason for the break down of resistance in the leading varieties. Therefore, the screening and identification of resistance source is an important practice in the management of the disease.

Materials and methods

Tomato varieties, cultivars, commercial hybrids and breeding lines, were sown in nursery beds and 24-25 days old seedlings were transplanted in the main field. Each variety/ line, was planted in two rows of 6 meter length during summer of 2005 and the lines which showed mild and moderate infection were screened during second season (summer 2006) along with some commercial hybrids. An artificial inoculation was carried out for lines showed mild and moderate rection in summer 2005, along with commercial hybrids using standard procedures (Muniyappa, 2000).

The presence of virus in the symptomatic plants was confirmed by ELISA and PCR using coat protein specific primers (Deng, 1994). Observations were recorded on appearance of first symptom and incidence of both diseased plants and vector population upto 12 weeks after planting.

The following scale was employed for scoring the disease reaction, suggested by Muniyappa *et al.* (1991a).

Resistant (R)	: No symptom.
Mild infection (M)	: Light yellowing along the margins but no curling and very few plants are infected.
Moderate infection (Mo)	: Slight yellowing along the margins, slight curling, puckering and stunting.
Susceptible	: Very severe curling, puckering, stunting, reduction in leaf size, and reduced fruit formation.

The time taken for first symptom appearance after transplanting was recorded in all the variety/ lines/genotypes.as following:

Days taken for first appearance of symptoms after transplanting	Characteristics
10-12	Very Early (VE)
21-30	Early (E)
31-40	Moderately Late (ML)
41-50	Late (L)
51 and above	Very Late (VL)

Genetic divergence: The Mahalonobis's D^2 analysis was used for assessing the genetic divergence among the tomato genotypes. The different characteristics like PDI, vector population, plant height, number of branches and number of fruits/plant, fruit weight, yield plant^{-1}, were taken in to consideration. The square of the Mahalonobis's generalized distance between any two populations is given by the formula (Mahalonobis, 1936):

$D^2 = \Sigma \Sigma\ i\ j\ \delta_i\ \delta_j$

Where, D^2 = Square of the generalized distance

i j =Reciprocal of the common dispersal matrix.

$\delta_i = (\mu_{i1} - \mu_{i2})$

$\delta_j = (\mu_{j1} - \mu_{j2})$

μ= Vector of mean values for all the characters.

Clustering of the D^2 values: All the n (n-1)/2 D^2 values were clustered using Toucher's method (Rao, 1952). The following method of clustering D^2 values was used. The two genotypes having the lowest D^2 value between them were selected and a third genotype which had on an average the smallest D^2 values from the first two was added.

Similarly, fourth was chosen which showed, smallest average D^2 from the first three. If at any stage increase in average D^2 values due to addition of a new genotype exceeded the average of those already included, then that genotype was taken out. The genotypes that were already included in that group were considered as the first cluster. The procedure was repeated for other genotypes omitting those that are already included in the former cluster.

The average distance of all the genotypes within the cluster and between any two clusters (inter cluster distance) were calculated.

Results

The results of the study revealed that out of 50 breeding lines (Table 1) screened, none of them was resistant. However, six lines *viz*., Alcobasa, Vybhav, L-32, S-21, Sankranti and Nandi showed mild infection. The per cent infection on these lines was 5, 5, 5, 2, 2 and 8 per cent, respectively and symptom expression was delayed. Twelve lines namely, Alcobasa-V, L-10, L-15, L-17, L-24, L-25, L-26, L-30, L-34, D-4, PKM-1 and V-1 showed moderate infection, while all other lines showed susceptible reaction to ToLCV (Table 2). The time taken for expression of symptoms varied from genotype to genotype. Majority of them showed early (21-30 DAT) to very early (10-20 DAT) infection symptoms.

In the second season, 15 breeding lines which showed mild and moderate infection of ToLCV during summer 2005 were used along with seventeen commercial hybrids. Results of the study showed that none of the lines or hybrids tested were

Table 1. Response of tomato genotypes against Tomato leaf curl virus under field conditions during summer 2005

Sl. No.	Genotype	Symptom Expression (DAT)	Disease incidence (%)	Whitefly population (Number/ plant)	Yield/ plant (g)	Disease reaction
1	L-01	VE	40	2.8	1125	S
2	L-02	E	40	3.2	1100	S
3	L-03	VE	60	4.0	400	S
4	L-04	VE	85	2.0	552	S
5	L-05	VE	30	2.4	870	S
6	L-06	ML	80	3.6	900	S
7	L-07	E	60	2.0	720	S
8	L-08	VE	55	3.0	375	S
9	L-09	E	30	2.0	500	S
10	L-10	E	20	1.0	240	MR
11	L-11	VE	45	3.0	1160	S
12	L-13	VE	35	2.0	572	S
13	L-14	VE	40	2.6	400	S
14	L-15	VE	20	2.4	750	MR
15	L-16	VE	40	2.0	37.5	S
16	L-17	VE	15	1.0	385	MR
17	L-19	VE	35	2.8	350	S
18	L-21	VE	20	2.2	360	S
19	L-23	VE	15	0.4	700	MR
20	L-24	E	20	2.0	216	MR
21	L-26	VE	15	0.2	520	MR
22	L-30	VE	15	1.0	225	MR
23	L-31	VE	25	2.2	150	S
24	L-32	E	5	0.4	1800	M
25	L-33	VE	20	1.8	360	S
26	L-34	E	20	0.4	150	MR
27	L-35	VE	55	2.0	200	S
28	L-35-1	VE	40	2.0	1705	S
29	L-36	VE	35	3.2	240	S
30	L-38	VE	30	2.2	300	S
31	L-39	VE	35	3.2	780	S
32	L-43	VE	40	3.0	350	S
33	L-44	VE	50	2.8	72	S
34	L-49	VE	30	2.0	336	S
35	L-58	E	45	2.2	450	S
36	L-86	VE	45	3.6	230	S
37	Alcobasa- B	VL	5	0.2	1350	M
38	Alkobasa-V	E	10	1.2	560	MR
39	Arka Vikas	VE	55	1.0	1350	S
40	CA-1	VE	30	2.0	660	S
41	D-4	VE	10	0.4	840	MR
42	D-25	VE	40	2.2	1500	S
43	Sankranti	VL	2.0	1.0	656	M
44	Nandi	VL	8	1.2	620	M
45	Vybhav	VL	5	0.2	1116	M
46	PKM-1	VL	10	0.2	765	MR
47	S-21	VL	2.0	0.4	720	M
48	UC-204B	VE	40	2.0	600	S
49	V-1	VE	20	2.0	1100	MR
50	Megha	VE	100	2.0	780	S

Reaction types: R- Resistant, M-Mild Reaction, MR-Moderate Reaction, S-Susceptible.

Table 2. Grouping of genotypes screened during summer 2005 in to different reaction types against tomato leaf curl virus disease

Sl. No	Reaction types	Genotypes
1	Resistant	-
2	Mild Reaction	Alcobasa-B, Vybhav, L-32, S-21, Sankranti and Nandi.
3	Moderate Reaction	Alcobasa-V, L-10,L-15, L-17, L-23, L-24, L-26, L-30, L-34, D-4, PKM-1 and V-1.
4	Susceptible	L-1, L-2, L-3, L-4, L-5, L-6, L-7, L-8, L-9, L-11, L-13, L-14, L-16, L-19, L-21, L-31, L-33, L-35, L-35-1, L-36, L-38, L-39, L-43, L-44, L-49, L-58, L-86, UC 204 B, D-25, Arka Vikas, CA-1 and Megha

found resistant, but few lines such as Nandi, Vybhav, Hy 558, HY 530, NS-503 and NS-719 showed mild reaction of ToLCV and Sankranti, PKM-1 and Utsav showed moderate reaction. All other lines were found susceptible. It was interesting to note that majority of the lines, which showed mild, and moderate reactions during summer 2005 became susceptible during, 2006. The cultivars like Nandi (8.33 PDI), Vybhav (6.89 PDI) were found better performed both from the point of view of resistance and yield. Among the hybrids, HY 558, HY 530 (Sungro hybrids) NS-719 and NS-563 (Namdhari hybrids) showed good response both for yield and for disease resistance (Table 3 and 4).

The results of the ELISA showed that lines which showed mild and moderate reactions had less virus titer compared to susceptible lines. The results of artificially inoculated plants showed that under high disease pressure Nandi and PKM-1 were also susceptible.

Genetic divergence: Plant height of the genotypes contributed maximum extent (52.21 %) to the divergence followed by yield per plant and per cent disease incidence (10.86 % each) but the vector population contributed least (0.97 %) to the divergence (Table 5).

Based on the extent of divergence in the genotypes they were grouped into five clusters (Table 6). The results indicated that majority of them were under cluster 1, (50 genotypes) having less divergence. Commercial hybrids possessed lot of diversity fall in

Table 3. Response of tomato genotypes against Tomato leaf curl virus under field conditions during summer 2006

Sl. No	Natural					Artificially inoculated			
	Genotype/ variety	Symptom expression (DAT)	Disease incidence (%)	Whitefly population (Number/ plant)	Yield plant^{-1} (g)	Disease reaction	Disease incidence (%)	ELISA absorbance values	PCR reaction
1	L-5	E	100.00	2.0	880	S	100.00	1.50	+
2	L-10	E	100.00	0.6	260	S	100.00	1.28	+
3	L-15	E	90.90	2.8	620	S	90.00	1.29	+
4	L-17	E	100.00	0.8	320	S	100.00	1.32	+
5	L-23	E	81.81	0.6	660	S	90.00	1.41	+
6	L-26	E	100.00	2.2	520	S	100.00	1.63	+
7	L-30	E	100.00	3.8	220	S	100.00	1.54	+
8	L-32	E	94.44	2.4	1500	S	90.00	1.46	+
9	Sankranti	ML	13.04	3.6	620	MR	10.00	1.10	+
10	Nandi	ML	08.33	4.2	580	M	20.00	1.04	+
11	Vybhav	ML	06.89	2.2	1260	M	0.00	1.06	+
12	V-1	E	100.00	2.2	400	S	100.00	1.80	+
13	PKM-1	ML	36.36	0.4	650	MR	40.00	1.09	+
14	Alkabasa-V	E	100.00	3.2	350	S	100.00	1.60	+
15	Arka Vikas	E	100.00	0.2	800	S	100.00	1.50	+
16	NS-53	E	100.00	1.2	460	S	100.00	1.40	+
17	NS-563	E	08.69	2.2	1529	M	10.00	1.02	+
18	NS-564	ML	100.00	0.4	272	S	100.00	1.64	+
19	NS-585	L	33.33	0.2	1495	MR	30.00	1.20	+
20	NS-658	E	76.66	0.8	840	S	80.00	1.66	+
21	NS-719	L	06.66	0.4	650	M	10.00	1.06	+
22	NS-816	E	100.00	1.4	256	S	100.00	1.56	+
23	NS-812	E	65.21	1.2	720	S	70.00	1.64	+
24	NS-2530	E	100.00	2.6	258	S	100.00	1.70	+
25	NS-2535	E	86.66	1.2	300	S	80.00	1.81	+
26	Utsav	E	36.00	2.2	1230	M	40.00	1.00	+
27	Indira	ML	77.77	1.4	1254	S	70.00	1.54	+
28	Malini	E	50.00	0.8	864	S	70.00	1.70	+
29	Sonam	L	64.28	0.8	1240	S	80.00	1.60	+
30	HY-530	L	07.60	0.8	1385	M	10.00	1.00	+
31	HY-558	L	09.09	0.6	2448	M	10.00	1.20	+
32	Megha	E	100.00	0.4	780	S	100.00	1.32	+

Table 4. Grouping of genotypes and commercial hybrids screened during summer –2006 in to different categories against tomato leaf curl virus disease

Sl. No	Reaction types	Genotypes
1	Resistant	-
2	Mild Reaction	Nandi, Vybhav, Hy-530, Hy-558, NS-563 and NS-719
3	Moderate Reaction	Sankranti, PKM-1 and Utsav.
4	Susceptible	L-5, L-10, L-15, L-17, L-23, L-26, L-30, L-32, Alkobasa-V, Arkavikas, NS-564, NS-53, NS-658, NS-812, NS-816, NS-2530, NS-2535, V-1, Malini, Sonam, Indira and Megha

Table 5. The per cent contribution of each character towards divergence in tomato

Sl. No.	Character	Contribution
1	Per cent disease incidence	10.86
2	Vector population	00.97
3	Plant height (cm)	52.21
4	Number of branches	08.99
5	Number of fruits/plant	10.06
6	Fruit weight (g)	06.01
7	Yield /plant (g)	10.86
Total		100.00

to four different clusters indicating considerable genetic distance among them. The cluster II had six entries, III had 3 entries, cluster IV had 6 entries and cluster V had only one entry.

The intra cluster distance varied from 11.23 in cluster III to 16.05 in IV. This indicates the presence of divergent genotypes within different clusters. The inter cluster D^2 values also ranged widely with minimum value of 19.49 between cluster I and IV to maximum of 31.55 between cluster II and IV indicating only some diversity among the genotypes (Tables 7 and Table 8).

Discussion

In general, majority of the lines tested were found susceptible and only few lines showed moderate reaction. The variety Vybhav, which was found superior over other varieties was on par with some of the better hybrids used in the study. Similar to this, screening of genotypes for managing the disease have been reported by Som and Choudhary (1976), Hassan *et al.* (1984), Banerjee and Kalloo (1987a), Pilowsky and Cohen (1990) and Muniyappa *et al.* (1991a). The cultivars such as Vybhav, HY-530 and HY-558, which were found tolerant could be used in the areas of high disease pressure (Table 3 and 4).

Success in locating resistance to ToLCV breeding is directly related to the availability of diversity in germplasm for resistance either to ToLCV or its vector. The genes for resistance to ToLCV have been reported in wild species like *L. hirsutum*, *L. peruvianum*, *L. pimpenellifolium* (Banerjee and Kalloo, 1987, and Pilowsky and Cohen, 1990). But transfer of these genes to cultivated species was possible in very stray cases. In view of that an attempt was made to screen 65 genotypes/hybrids. The reaction of genotypes to ToLCV under epiphytotic conditions during the summer seasons was assessed. It was found that majority of the genotypes were susceptible to ToLCV under field conditions. However, few were found resistant and they had

Table 6. Clustering pattern of 65 tomato varieties/ genotypes/hybrid following D^2 analysis

Cluster No.	Number of entries	Genotypes
I	50	B-Alcobasa-V, Vybhav, L-32, S-21, Sankranti, Nandi, Alcobasa-V, L-34, L-23, D-4, L-24, L-26, L-30, PKM-1, V-1, L-17, L-15, L-10, L-43, L-39, L-49, L-38, L-36, L-35-1, L-21, L-33, L-31, CA-1, L-58, L-44, UC 204 B, L-86, L-35, D-25, Arkavikas, L-1, L-11, L-16, L-19, L-14, L-13, L-6, L-9, L-8, L-7, L-4, L-2, L-5, L-3 and Megha,
II	6	NS-564, NS-2635, NS-816, NS-812, NS-53 and NS-2530.
III	3	Utsav, Sonam and Indira.
IV	6	Malini, Hy-530, Hy-558, NS-585, NS-719 and NS-563.
V	1	NS-658.

Table 7. The average inter and intra cluster distances for 65 tomato genotypes

Cluster	I	II	III	IV	V
I	15.14	26.94	20.27	19.49	25.16
II		13.53	18.28	31.55	22.56
III			11.23	19.79	18.51
IV				16.05	25.23
V					0.00

Diagonal values indicate intra cluster distance. Above diagonal values indicate intercluster distance.

Table 8. Cluster mean of seven characters in tomato

Cluster	Character						
	1	2	3	4	5	6	7
I	31.65	1.54	654.77	31.20	20.25	8.49	50.12
II	89.26	1.25	422.05	47.72	9.05	3.72	41.16
III	59.78	1.13	1225.77	67.33	19.00	4.44	48.22
IV	18.45	0.64	1305.72	65.22	20.16	5.27	57.00
V	73.55	0.86	860.00	70.00	13.66	4.66	83.66

good fruit set and growth. There was lot of diversity among the genotypes towards ToLCV with the per cent disease incidence varying from 6.89-100 per cent. This was confirmed from the data on D^2 values that distributed genotypes into five clusters. The resistant/tolerant groups of genotypes were congregated in to cluster I and cluster IV. Further it was found that plant height contributed the highest to divergence (52.21 %) followed by per cent disease incidence and fruit yield per plant. It is clear from the results that hybrids have more divergence than the genotypes or advanced breeding lines.

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