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Research Article
Screening Of Popular Indian Chili Pepper (*Capsicum annuum* L.) Genotypes Against the *Chili leaf curl virus* Disease

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Abstract

Background and Objective: Chilli pepper is an important spice crop and viral diseases hamper the successful of chilli peppers. The present investigation entitled was carried out to identify the sources for *Chilli leaf curl virus* (ChiLCV) resistance. Screening of genotypes against the ChiLCV is crucial to select the appropriate genotypes to get the successful crop production under the disease pressure. **Materials and Methods:** A collection of 70 popular chilli genotypes across India were evaluated under the open field conditions and using artificial inoculum for the ChiLCV disease. Thereafter, the virus presence and absence was also determined by using the Polymerase Chain Reaction (PCR) using universal primers (AV494/AC1048). **Results:** It determined that 23 genotypes were moderately susceptible, 12 each were susceptible and moderately resistant, 10 were symptomless, 6 were resistant, 5 were highly resistant and 2 were highly susceptible. Further, the 10 symptomless and 5 highly resistant genotypes identified under open field conditions were subjected to artificial screening by using whitefly mediated and graft inoculations. Thereafter, the 6 resistant genotypes identified with artificial inoculation by showed the presence of the virus when confirmed with PCR. However, in the whitefly mediated inoculation, four genotypes viz., Sel-3 (T_1), Sel-4 (T_2), Sel-6 (T_3) and CHIVAR-1 (T_4) did not show any amplification for the presence of the virus. **Conclusion:** Overall, this study provides useful information regarding the behaviour of popular chilli cultivars/genotypes against the ChiLCV disease.

Key words: Chilli pepper, ChiLCV, field screening, artificial inoculation, polymerase chain reaction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chilli is known to be affected by more than 35 viruses. Twenty-four viruses are reported to affect chilli naturally, among them 11 have been reported from India namely *Pepper vein bending virus*, *Pepper veinal mottle virus* and *Chilli leaf curl virus*. Among all these viruses, the *Chilli leaf curl virus* (ChiLCV) is the most destructive in terms of disease incidence and fruit yield loss. In severe conditions, 100% marketable fruits loss have been reported. Although, the begomoviruses infecting a large quantity of economically essential dicot plants worldwide. The genus *Begomovirus* belongs to the family Geminiviridae vectored by the whitefly, *Bemisia tabaci* Gennadius. The *Begomovirus* members characterized by twin icosahedral particles (18×30 nm size) and the genome consist of one or two circular, ssDNA components (2.5-3.0 kb) known as DNA A and DNA B[1]. Chilli leaf curl disease on chilli plant has been reported from India. A strain of *Chilli leaf curl virus*-Pakistan (ChiLCV-PK) was associated with chilli leaf curl disease. The partial DNA-A sequences analysis indicated that this strain was monopartite. Till date genome sequence of four begomoviruses infecting chilli have been characterized from India viz., *Chilli leaf curl virus* (ChiLCV), *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Joydebpur virus* (ToLCJV) and recently *Chilli leaf curl Palampur virus* (ChiLCPV)[2].

The symptoms are broadly of three types: (a) Leaf curling, (b) Vein yellowing and (c) Yellow mosaic. The typical symptoms consist of leaf curling, puckering, rolling, shortening of internodes and petioles, blistering of interveinal areas, thickening and swelling of the veins, older leaves turned out to be leathery and brittle, crowding of leaves and stunting of whole plants[3]. The typical leaf curl symptoms and increase in disease severity in infected plants are due to the presence of cognate beta-satellites associated with the virus. The success of disease resistance breeding depends on the genetic variability and the reliable evaluation tests employed for identification of the resistant sources. It is important to employ most reliable tests of resistance when dealing with destructive diseases like ChiLCV[4]. Various methods have been employed to screen *Capsicum* germplasm for resistance to ChiLCV viz., screening under natural epiphytotic conditions and artificial inoculation (grafting inoculation and whitefly mediated inoculation). Breeding for ChiLCV resistance was started in the late sixties in India and natural field screening was mostly used to identify resistance sources based on disease incidence and severity[5,6].

Polymerase Chain Reaction (PCR) is now widely followed because of smooth application, rapid, sensitivity, specificity for identification and detection of begomoviruses in epidemiological and disease management studies with minimal sample preparation. In all begomoviruses genomes, a region with high homology is present. Universal degenerate primers are designed to anneal to these regions. These universal primers are identical primers with a base change in one or more places. They act as universal degenerate primers which amplify a DNA base in all begomoviruses[7]. Therefore, in order to identify the sources for ChiLCV resistance in a collection of germplasm through natural and artificial screening, it screened a collection of 70 popular chilli genotypes across India were evaluated under the open field conditions and using artificial inoculum. Thereafter, the virus presence and absence in the resistant genotypes was determined using the Polymerase Chain Reaction (PCR) by using universal primers (AV494/AC1048).

MATERIALS AND METHODS

The experimental fields were settled at the Department of Vegetable Science, College of Agriculture, Kerala Agricultural University, Vellayani, India, during 2016-2017. The study aimed at the identification of sources of *leaf curl virus* resistance in the popular chilli cultivars from India. The predominant soil type of the experimental site was red loam to Vellayani series, texturally classified as sandy clay loam. The region appreciates a warm, humid tropical climate.

Plant material and experimental setup: Seventy chilli genotypes had been collected from numerous sources. The list of genotypes and their source of origin is given in Table 1. The randomized complete block design was used with three replications with a spacing of 45×45 cm in a plot size of 3.6×1.8 m. There were 20 plants of each genotype per plot. All other instructions were followed based on the package and practices defined elsewhere[8].

Field screening of genotypes for ChiLCV resistance: The field screening was undertaken when the natural ChiLCV pressure was at its peak because of high whitefly population. No plant protection measures were provided. The visual observation on the appearance of ChiLCV symptom was noted at fortnightly periods after transplanting. Chilli genotypes and hybrids were screened for ChiLCV resistance during summer. On each genotype, the severity of symptom was noted on the basis of severity[9,10] scale 0-6. The specific disease reaction was assigned for all the genotypes based on the Coefficient of Infection (CI) and Disease Reaction (DR) as suggested by Kumar *et al.*[11].

Table 1: List of 70 genotypes used for the study

Treatments	Accessions/Genotypes	Source	Treatments	Accessions/Genotypes	Source
T_1	Sel-1	AVRDC, Taiwan	T_{36}	Pant C 1	GBPUAT, Pantnagar
T_2	Sel-3	AVRDC, Taiwan	T_{37}	Punjab Surkh	PAU, Ludhiana
T_3	Sel-4	AVRDC, Taiwan	T_{38}	Kashi Anmol	IIVR, Varanasi
T_4	Sel-5	AVRDC, Taiwan	T_{39}	DCL 524	HRS, Devihosur
T_5	Sel-6	AVRDC, Taiwan	T_{40}	C-31-1	HRS, Devihosur
T_6	Punjab Lal	PAU, Ludhiana	T_{41}	ACC-2-1	HRS, Devihosur
T_7	Punjab Tej	PAU, Ludhiana	T_{42}	I-1	HRS, Devihosur
T_8	Punjab Sindhuri	PAU, Ludhiana	T_{43}	I-2	HRS, Devihosur
T_9	Punjab Guchhader	PAU, Ludhiana	T_{44}	I-3	HRS, Devihosur
T_{10}	Vellayani Athulya	KAU	T_{45}	I-4	HRS, Devihosur
T_{11}	Ujjwala	KAU	T_{46}	CHIVAR-1	IIVR, Varanasi
T_{12}	DCA 268	HRS, Devihosur	T_{47}	CHIHYB-2	IIVR, Varanasi
T_{13}	DCA 167	HRS, Devihosur	T_{48}	CHIVAR-3	IIVR, Varanasi
T_{14}	DCA 157	HRS, Devihosur	T_{49}	CHIHYB-3	IIVR, Varanasi
T_{15}	DCA 142	HRS, Devihosur	T_{50}	CHIVAR-2	IIVR, Varanasi
T_{16}	PS 1	HRS, Devihosur	T_{51}	CHIVAR-4	IIVR, Varanasi
T_{17}	Byadagi Dabbi	HRS, Devihosur	T_{52}	CHIVAR-6	IIVR, Varanasi
T_{18}	Byadagi Kaddi	HRS, Devihosur	T_{53}	CHIVAR-7	IIVR, Varanasi
T_{19}	Jwalasakhi	NBPGR, New Delhi	T_{54}	LCA-334	HRS, Devihosur
T_{20}	EC 354890	NBPGR, New Delhi	T_{55}	KA-2	HRS, Devihosur
T_{21}	EC 599958	NBPGR, New Delhi	T_{56}	CHIVAR-10	IIVR, Varanasi
T_{22}	IC 572483	NBPGR, New Delhi	T_{57}	CHIVAR-8	IIVR, Varanasi
T_{23}	EC 599960	NBPGR, New Delhi	T_{58}	CHIVAR-9	IIVR, Varanasi
T_{24}	IC 572468	NBPGR, New Delhi	T_{59}	CHIVAR-5	IIVR, Varanasi
T_{25}	Naga chilli	N-E region	T_{60}	Japani Longi	PAU, Ludhiana
T_{26}	Arka Lohit	IIHR, Bengaluru	T_{61}	Perennial	PAU, Ludhiana
T_{27}	Anugraha	KAU	T_{62}	VS-7	PAU, Ludhiana
T_{28}	CA-3 (EC-391083)	NBPGR, New Delhi	T_{63}	VS-9	PAU, Ludhiana
T_{29}	CA-5 (EC-596920)	NBPGR, New Delhi	T_{64}	S-217621	PAU, Ludhiana
T_{30}	CA-6 (EC-596940)	NBPGR, New Delhi	T_{65}	Sel. 40	PAU, Ludhiana
T_{31}	CA-8 (EC-599969)	NBPGR, New Delhi	T_{66}	Sel.7-1	PAU, Ludhiana
T_{32}	CA-32 (DWD-2)	NBPGR, New Delhi	T_{67}	Sel. 36-1	PAU, Ludhiana
T_{33}	Jwalamukhi	KAU	T_{68}	PLS-3-1	PAU, Ludhiana
T_{34}	Keerthi	KAU	T_{69}	Sel. 20-1	PAU, Ludhiana
T_{35}	Pusa Jwala	IARI, New Delhi	T_{70}	ms-12	PAU, Ludhiana

Artificial screening for ChiLCV: Ten symptomless and five highly resistant genotypes identified under natural field conditions were subjected to screening under artificial inoculation condition by whitefly mediated inoculation and graft inoculation against *leaf curl virus* isolate. The genotypes used for artificial screening were presented in Table 2. For the maintenance of ChiLCV inoculum, the susceptible chilli plants affected with ChiLCV were selected and replanted in the clay pot and they were kept in an insect-proof cage. Acquisition of virus from ChiLCV infected plant was prepared by using 2 L plastic bottles. The lower end of the bottles were removed and covered with a muslin cloth and the upper ends were closed with the help of cotton plugs. For the acquisition of virus, ChiLCV infected plant branches were inserted inside the bottles which contain non-viruliferous whiteflies. These flies were allowed to feed on the ChiLCV infected branches for 24 h (Acquisition period).

The virus presence and absence was also confirmed based on Polymerase Chain Reaction (PCR) using the universal primers. The ChiLCV symptomatic samples were collected from whitefly and graft inoculated plants[12]. From these samples, the genomic DNA was extracted following the CTAB method. The presence/absence of ChiLCV specific PCR band will be observed based on expected size amplicon (~560 bp).

Statistical analysis: The experimental data from all experiments were analyzed by using computer software PBTools (PBTools-1.4, 2014).

RESULTS

Field screening of chilli genotypes for ChiLCV resistance: The field screening was undertaken to evaluate 70 chilli germplasm against chilli leaf curl disease. The genotypes/accessions were assessed based on severity scale 0-6. The symptom severity on an individual plant basis was noted to calculate Disease Severity Index (DSI). The DSI was multiplied by Disease Incidence (DI) and divided by 100 to get the Coefficient of Infection (CI). The responses of 70 chilli genotypes to ChiLCV under natural field conditions are presented in Table 2.

Table 2: Screening of 70 chilli genotypes against ChiLCV disease under field conditions

		Mean number of infected plants in six classes (symptom severity grade 0-6)										
Treatments	Genotypes	0	1	2	3	4	5	6	Mean PDI (%)	Mean DI (%)	Mean CI (%)	Disease reaction
T_1	Sel-1	3.33	9	3.67	4	0	0	0	23.61	83.33	19.68	MR
T_2	Sel-3	20	0	0	0	0	0	0	0	0	0	SL
T_3	Sel-4	20	0	0	0	0	0	0	0	0	0	SL
T_4	Sel-5	6.33	9.67	4	0	0	0	0	14.72	68.33	10.07	R
T_5	Sel-6	20	0	0	0	0	0	0	0	0	0	SL
T_6	Punjab Lal	7.33	6.33	6.33	0	0	0	0	15.83	63.33	9.97	R
T_7	Punjab Tej	1	2.67	5.67	5	5.67	0	0	43.06	95	40.9	MS
T_8	Punjab Sindhuri	3.67	8.67	3	4.67	0	0	0	23.89	81.67	19.47	MR
T_9	Punjab Guchhader	1	5.33	2.67	5	6	0	0	41.39	95	39.35	MS
T_{10}	Vellayani Athulya	1.67	3.67	2.33	5.67	6.67	0	0	43.33	91.67	39.75	MS
T_{11}	Ujwala	1.67	2.33	5.67	4.33	6	0	0	42.22	91.67	38.71	MS
T_{12}	DCA 268	0	0	1.67	5.67	3.67	9	0	66.67	100	66.67	S
T_{13}	DCA 167	3	0.33	3.67	4	9	0	0	46.39	85	39.43	MS
T_{14}	DCA 157	0	0	0	6.33	5.33	8.33	0	68.33	100	68.33	S
T_{15}	DCA 142	0	0	0.67	3.33	6.33	9.67	0	70.83	100	70.83	S
T_{16}	PS 1	2.67	2.33	1.33	4	9.67	0	0	46.39	86.67	40.22	MS
T_{17}	Byadagi Dabbi	0	1	0	4.33	3.67	11	0	69.72	100	69.72	S
T_{18}	Byadagi Kaddi	0	0.67	0.33	3.33	5.67	10	0	70	100	70	S
T_{19}	Jwalasakhi	2.33	0.67	4.67	6.33	6	0	0	44.17	88.33	39.01	MS
T_{20}	EC 354890	0	0	1	4.67	6.67	7.67	0	67.5	100	67.5	S
T_{21}	EC 599969	5.67	4	3.33	5.33	1.67	0	0	27.78	71.67	19.83	MR
T_{22}	IC 572483	2	1.33	5	5.33	6.33	0	0	43.89	90	39.47	MS
T_{23}	EC 599960	8.33	2.67	9	0	0	0	0	17.22	58.33	10.04	R
T_{24}	IC 572468	1	2	8	4.33	4.67	0	0	41.39	95	39.36	MS
T_{25}	Naga chilli	4.33	6.67	4	5	0	0	0	24.72	78.33	19.33	MR
T_{26}	Arka Lohit	0.67	2	8.67	4.33	4.33	0	0	41.39	96.67	40	MS
T_{27}	Anugraha	2	1	5.67	4.67	6.67	0	0	44.17	90	39.75	MS
T_{28}	CA-3 (EC-391083)	6.67	9	4.33	0	0	0	0	14.72	66.67	9.82	R
T_{29}	CA-5 (EC-596920)	4	8	3.33	4.67	0	0	0	23.89	80	19.1	MR
T_{30}	CA-6 (EC-596940)	1	1.33	9	4.67	4	0	0	41.11	95	39.06	MS
T_{31}	CA-8 (EC-599969)	3.67	7.33	5.33	3.67	0	0	0	24.17	81.67	19.72	MR
T_{32}	CA-32 (DWD-2)	2.33	12	2.67	3	0	0	0	21.94	88.33	19.39	MR
T_{33}	Jwalamukhi	0.67	5	5	3.33	6	0	0	40.83	96.67	39.43	MS
T_{34}	Keerthi	1.67	2.33	5	5	6	0	0	42.78	91.67	39.21	MS
T_{35}	Pusa Jwala	0	0	0	0.33	5	4.33	10.33	87.22	100	87.22	HS
T_{36}	Pant C 1	0	0	1.33	4.33	4	10.33	0	69.44	100	69.44	S
T_{37}	Punjab Surkh	0.33	4.67	5.33	5.33	4.33	0	0	40.56	98.33	39.86	MS
T_{38}	Kashi Anmol	0	0	0	0	0	6.67	13.33	94.44	100	94.44	HS
T_{39}	DCL 524	0	0.33	1.67	3.33	3.33	11.33	0	69.72	100	69.72	S
T_{40}	C-31-1	2	1	5.33	5	6.67	0	0	44.44	90	39.96	MS
T_{41}	ACC-2-1	2.33	1.33	2.33	7.67	6.33	0	0	45.28	88.33	39.99	MS
T_{42}	I-1	5.33	3.33	5.33	6	0	0	0	26.67	73.33	19.57	MR
T_{43}	I-2	2	1.67	3.33	6	7	0	0	45.28	90	40.64	MS
T_{44}	I-3	0	1	0.67	2.67	4.67	11	0	70	100	70	S
T_{45}	I-4	0	0	0	6.67	3.67	9.67	0	69.17	100	69.17	S
T_{46}	CHIVAR-1	0	0	0	0	0	0	0	0	0	0	SL
T_{47}	CHIHYB-2	0.67	4.67	3.67	7	4	0	0	40.83	96.67	39.43	MS
T_{48}	CHIVAR-3	5	4.33	4.33	6.33	0	0	0	26.67	75	19.94	MR
T_{49}	CHIHYB-3	0.33	4.67	5.33	5.33	4.33	0	0	40.56	98.33	39.85	MS
T_{50}	CHIVAR-2	0	0	0	0	0	0	0	0	0	0	SL
T_{51}	CHIVAR-4	9.33	10.67	0	0	0	0	0	8.89	53.33	4.75	HR
T_{52}	CHIVAR-6	1	3.67	4.33	6	5	0	0	41.94	95	39.76	MS
T_{53}	CHIVAR-7	1.33	4	3	6.33	5.33	0	0	41.94	93.33	39.11	MS
T_{54}	LCA-334	0	0	2.33	3.67	5.33	8.67	0	66.94	100	66.94	S

Table 2: Continue

Treatments	Genotypes	Mean number of infected plants in six classes (symptom severity grade 0-6) 0	1	2	3	4	5	6	Mean PDI (%)	Mean DI (%)	Mean CI (%)	Disease reaction
T_{55}	KA-2	0	0	1	4	5	10	0	70	100	70	S
T_{56}	CHIVAR-10	1.33	4	3.33	6	5.33	0	0	41.67	93.33	38.86	MS
T_{57}	CHIVAR-8	0	0	0	0	0	0	0	0	0	0	SL
T_{58}	CHIVAR-9	7.33	6.67	6	0	0	0	0	15.56	63.33	9.85	R
T_{59}	CHIVAR-5	6	2	3.67	8.33	0	0	0	28.61	70	20.03	MR
T_{60}	Japani Longi	10.33	9.67	0	0	0	0	0	8.06	48.33	3.96	HR
T_{61}	Perennial	9.67	10.33	0	0	0	0	0	8.61	51.67	4.51	HR
T_{62}	VS-7	6	3.33	2.67	8	0	0	0	27.22	70	19.07	MR
T_{63}	VS-9	0	0	0	0	0	0	0	0	0	0	SL
T_{64}	S-217621	7.67	6.33	6	0	0	0	0	15.28	61.67	9.46	R
T_{65}	Sel. 40	0	0	0	0	0	0	0	0	0	0	SL
T_{66}	Sel.7-1	0	0	0	0	0	0	0	0	0	0	SL
T_{67}	Sel. 36-1	0	0	0	0	0	0	0	0	0	0	SL
T_{68}	PLS-3-1	10.33	9.67	0	0	0	0	0	8.06	48.33	3.93	HR
T_{69}	Sel. 20-1	9.33	10.33	0	0	0	0	0	8.61	51.67	4.57	HR
T_{70}	Ms-12	5	4.67	4	6.33	0	0	0	26.39	75	19.76	MR
Mean	3.44	3.28	2.86	3.41	2.85	1.82	0.34	34.8	72.79	31.96		
	CD 5%								2.68	5.78	2.72	
	CD 1%								3.52	7.6	3.58	

PDI: Percent disease index, DI: Disease incidence, CI: Coefficient of Infection, SL: Symptom less, HR: Highly resistant, R: Resistant, MR: Moderately resistant, MS: Moderately susceptible, S: Susceptible, HS: Highly susceptible

Field screening against ChiLCV disease: Out of 70 genotypes screened, 10 genotypes were found to be completely free (symptomless) from ChiLCV infection and were, therefore regarded as symptomless genotypes. The genotype which showed a symptomless reaction to ChiLCV included T_2, T_3, T_5, T_{46}, T_{50}, T_{57}, T_{63}, T_{65}, T_{66} and T_{67} (Table 2). Out of the remaining 60 genotypes, five genotypes showed highly resistant reaction and they were T_{51}, T_{60}, T_{61}, T_{68} and T_{69}. The first disease symptom appearance was delayed up to 45 Days After Transplanting (DAT) in genotype T_{51}, whereas, in genotypes T_{60}, T_{61}, T_{68} and T_{69} it was delayed up to 60 DAT (Table 3). Out of the remaining 55 genotypes, six genotypes showed resistant reaction with CI ranging from 5 to 10. The genotypes which showed a resistant reaction to ChiLCV included T_4, T_6, T_{23}, T_{28}, T_{58} and T_{64} (Table 2).

Among six genotypes, T_6 had early disease appearance (within 15 DAT). Remaining five genotypes expressed delayed symptom development and first symptoms were visible 30 DAT in T_{23}; 45 DAT in T_4, T_{28} and T_{58} and 60 DAT in T_{64} (Table 3). Twelve genotypes were moderately resistant with CI ranged from 10 to 20. The genotypes which showed the moderate resistant reaction to ChiLCV included T_1, T_8, T_{21}, T_{25}, T_{29}, T_{31}, T_{32}, T_{42}, T_{48}, T_{59}, T_{62} and T_{70}. Four genotypes (T_8, T_{21}, T_{42} and T_{70}) showed disease infection within 15 DAT, T_1 and T_{25} in 30 DAT, T_{29}, T_{31}, T_{32}, T_{48} and T_{62} in 45 DAT and T_{59} in 60 DAT.

Twenty-three genotypes were found to be moderately susceptible with CI ranging from 20 to 40. The genotypes which showed moderate susceptible reaction were T_7, T_9, T_{10}, T_{11}, T_{13}, T_{16}, T_{19}, T_{22}, T_{24}, T_{26}, T_{27}, T_{30}, T_{33}, T_{34}, T_{37}, T_{40}, T_{41}, T_{43}, T_{47}, T_{49}, T_{52}, T_{53} and T_{56} (Table 3). In the genotype, T_9 the first disease symptom appeared 30 DAT. Five genotypes (T_{30}, T_{47}, T_{49}, T_{52} and T_{53}) were free from infection upto 45 DAT (Table 3). Twelve genotypes viz., T_{12}, T_{14}, T_{15}, T_{17}, T_{18}, T_{20}, T_{36}, T_{39}, T_{44}, T_{45}, T_{54} and T_{55} showed a susceptible reaction. Two genotypes T_{35} and T_{38} showed a highly susceptible response (Table 2). Based on the Coefficient of Infection (CI) and disease reaction under field conditions (Table 2), it was found that greater number of genotypes were moderately susceptible (MS) (23), followed by moderately resistant (MR) (12), susceptible (S) (12), symptomless (SL) (10), resistant (R) (6), highly resistant (HR) (5) and highly susceptible (HS) (2).

Artificial screening for ChiLCV resistance: Selfed progenies of 10 symptomless (SL) genotypes (T_2, T_3, T_5, T_{46}, T_{50}, T_{57}, T_{63}, T_{65}, T_{66} and T_{67}) and five Highly Resistant (HR) genotypes (T_{51}, T_{60}, T_{61}, T_{68} and T_{69}) under field conditions were raised under insect-proof cage.

Whitefly mediated inoculation under insect-proof cage: Out of 10 symptomless genotypes, six genotypes viz., T_2, T_3, T_5,

Table 3: Days taken to first ChiLCV symptom expression in 70 genotypes under natural field conditions

Treatments	Genotypes	Severity grade Days After Transplanting (DAT)					Final severity grade	Coefficient of infection	Disease reaction
		15 DAT	30 DAT	45 DAT	60 DAT	75 DAT			
T_1	Sel-1	0	0.66	2.3	3.33	3.33	3.33	19.68	MR
T_2	Sel-3	0	0	0	0	0	0	0	SL
T_3	Sel-4	0	0	0	0	0	0	0	SL
T_4	Sel-5	0	0	0.66	1	2	2	10.07	R
T_5	Sel-6	0	0	0	0	0	0	0	SL
T_6	Punjab Lal	0.66	0.66	1.66	1.66	1.66	2	9.97	R
T_7	Punjab Tej	1	1.66	1.66	4	4	4	40.9	MS
T_8	Punjab Sindhuri	0.66	1	2	3	3	3	19.47	MR
T_9	Punjab Guchhader	0	1	2.33	4	4	4	39.35	MS
T_{10}	Vellayani Athulya	1	1.66	3.33	3.33	3.66	4	39.75	MS
T_{11}	Ujwala	0.66	1.66	1.66	2.66	3.66	4	38.71	MS
T_{12}	DCA 268	1	2.33	3.33	4	5	5	66.67	S
T_{13}	DCA 167	1.66	2.33	3	4	4	4	39.43	MS
T_{14}	DCA 157	1	2	2.33	4.33	5	5	68.33	S
T_{15}	DCA 142	0.66	0.66	2.66	4.66	5	5	70.83	S
T_{16}	PS 1	1.66	2	2	4.33	4.33	4	40.22	MS
T_{17}	Byadagi Dabbi	2.66	3.33	3.66	3.66	5	5	69.72	S
T_{18}	Byadagi Kaddi	2.66	3	4	4	5.33	5	70	S
T_{19}	Jwalasakhi	1.66	1.66	2.66	2.66	4	4	39.01	MS
T_{20}	EC 354890	1	1.66	3.66	4.66	5.33	5	67.5	S
T_{21}	EC 599969	1.66	2	2	2	3	3	19.83	MR
T_{22}	IC 572483	1.66	1.66	3	3.66	3.66	4	39.47	MS
T_{23}	EC 599960	0	1.33	2.33	2.33	2.33	2	10.04	R
T_{24}	IC 572468	1.33	2.66	2.66	3.66	3.66	4	39.36	MS
T_{25}	Naga chilli	0	1	2	2	3.33	3	19.33	MR
T_{26}	Arka Lohit	1.33	1.66	1.66	4	4	4	40	MS
T_{27}	Anugraha	1.66	2.33	3.33	3.33	4.33	4	39.75	MS
T_{28}	CA-3 (EC-391083)	0	0	0.66	2	2	2	9.82	R
T_{29}	CA-5 (EC-596920)	0	0	0.66	2	3	3	19.1	MR
T_{30}	CA-6 (EC-596940)	0	0	1	3	4	4	39.06	MS
T_{31}	CA-8 (EC-599969)	0	0	0.66	2	3	3	19.72	MR
T_{32}	CA-32 (DWD-2)	0	0	1	2	3	3	19.39	MR
T_{33}	Jwalamukhi	1.66	2	3.33	3.33	4	4	39.43	MS
T_{34}	Keerthi	1	2.33	2.33	4	4	4	39.21	MS
T_{35}	Pusa Jwala	3.66	4.33	4.33	6	6	6	87.22	HS
T_{36}	Pant C 1	3	3.33	3.66	5	5	5	69.44	S
T_{37}	Punjab Surkh	1.66	2.33	2.66	3.66	3.66	4	39.86	MS
T_{38}	Kashi Anmol	3.66	3.66	4.66	6	6	6	94.44	HS
T_{39}	DCL 524	1.66	1.66	3	4	4	5	69.72	S
T_{40}	C-31-1	0.66	2	3	34.33	4.33	4	39.96	MS
T_{41}	ACC-2-1	0.66	1.66	1.66	3	4	4	39.99	MS
T_{42}	I-1	0.66	2	2	3	3	3	19.57	MR
T_{43}	I-2	1	1.66	1.66	2.66	3.66	4	40.64	MS
T_{44}	I-3	0.66	2	2	4	5	5	70	S
T_{45}	I-4	1.66	2.33	4.33	5	5	5	69.17	S
T_{46}	CHIVAR-1	0	0	0	0	0	0	0	SL
T_{47}	CHIHYB-2	0	0	2.66	3.66	3.66	4	39.43	MS
T_{48}	CHIVAR-3	0	0	2	3	3	3	19.94	MR
T_{49}	CHIHYB-3	0	0	2.66	3.66	4.33	4	39.85	MS
T_{50}	CHIVAR-2	0	0	0	0	0	0	0	SL
T_{51}	CHIVAR-4	0	0	1	1	1	1	4.75	HR
T_{52}	CHIVAR-6	0	0	1	2.66	3.66	4	39.76	MS
T_{53}	CHIVAR-7	0	0	2.66	3.66	4.33	4	39.11	MS
T_{54}	LCA-334	2.33	4.33	4.33	5	5	5	66.94	S
T_{55}	KA-2	0.66	1.66	2.33	4.33	5.33	5	70	S

Table 3: Continue

Treatments	Genotypes	Severity grade Days After Transplanting (DAT) 15 DAT	30 DAT	45 DAT	60 DAT	75 DAT	Final severity grade	Coefficient of infection	Disease reaction
T_{56}	CHIVAR-10	1	1.66	2.33	3.66	4	4	38.86	MS
T_{57}	CHIVAR-8	0	0	0	0	0	0	0	SL
T_{58}	CHIVAR-9	0	0	1.66	1.66	1.66	2	9.85	R
T_{59}	CHIVAR-5	0	0	0	2	3	3	20.03	MR
T_{60}	Japani Longi	0	0	0	1	1	1	3.96	HR
T_{61}	Perennial	0	0	0	1	1	1	4.51	HR
T_{62}	VS-7	0	0	2	2	3	3	19.07	MR
T_{63}	VS-9	0	0	0	0	0	0	0	SL
T_{64}	S-217621	0	0	0	1	1	1	9.46	R
T_{65}	Sel. 40	0	0	0	0	0	0	0	SL
T_{66}	Sel.7-1	0	0	0	0	0	0	0	SL
T_{67}	Sel. 36-1	0	0	0	0	0	0	0	SL
T_{68}	PLS-3-1	0	0	0	1	1	1	3.93	HR
T_{69}	Sel. 20-1	0	0	0	1	1	1	4.57	HR
T_{70}	ms-12	0	1	1	2.33	3.33	3	19.76	MR

SL: Symptom less, HR: Highly resistant, R: Resistant, MR: Moderately resistant, MS: Moderately susceptible, S: Susceptible, HS: Highly susceptible

Table 4: Reaction of symptomless and highly resistant genotypes (under field conditions) against ChiLCV under whitefly mediated inoculation

Treatments	Genotypes	Reaction under field conditions	Appearance of symptom after inoculation (days)	Mean PDI (%)	Mean DI (%)	Mean CI (%)	Disease reaction	Virus presence by PCR
T_{2}	Sel-3	Symptomless	0	0	0	0	SL	-
T_{3}	Sel-4	Symptomless	0	0	0	0	SL	-
T_{5}	Sel-6	Symptomless	0	0	0	0	SL	-
T_{46}	CHIVAR-1	Symptomless	0	0	0	0	SL	-
T_{50}	CHIVAR-2	Symptomless	0	0	0	0	SL	+
T_{57}	CHIVAR-8	Symptomless	0	0	0	0	SL	+
T_{63}	VS-9	Symptomless	23.67	15.56	60	9.33	R	+
T_{65}	Sel-40	Symptomless	26.67	6.67	40	2.67	HR	+
T_{66}	Sel-7-1	Symptomless	27.67	7.78	46.67	3.78	HR	+
T_{67}	Sel-36-1	Symptomless	22.33	14.44	60	8.67	R	+
T_{51}	CHIVAR-4	Highly resistant	20.00	23.33	86.67	20.22	MR	Not tested
T_{60}	Japani Longi	Highly resistant	22.33	15.56	60	9.33	R	Not tested
T_{61}	Perennial	Highly resistant	22.67	14.44	60	8.67	R	Not tested
T_{68}	PLS-3-1	Highly resistant	21.00	25.56	80	20.44	MR	Not tested
T_{69}	Sel-20-1	Highly resistant	19.33	15.56	60	9.33	R	Not tested
	Mean		13.71	9.26	36.89	6.16		
	CD 5%		0.99	2.20	7.06	1.93		
	SE (m)		0.34	0.76	2.43	0.66		
	SE (d)		0.48	1.075	3.44	0.94		

PDI: Percent disease index, DI: Disease incidence, CI: Coefficient of infection, SL: Symptom less, HR: Highly resistant, R: Resistant, MR: Moderately resistant, MS: Moderately susceptible, S: Susceptible and HS: Highly susceptible, -: Absence, +: Presence of 550 bp viral genome

T_{46}, T_{50} and T_{57} remained symptomless under artificial whitefly mediated conditions (Table 4). Two genotypes, namely T_{63} and T_{67} were found resistant and the first disease symptoms appeared on 23.67 and 22.33 days after inoculation, respectively. The genotype T_{65} and T_{66} were found highly resistant and the first symptom development started 26.67 and 26.33 days after inoculation, respectively. Out of five highly resistant genotypes, T_{60}, T_{61} and T_{69} expressed resistant reaction under whitefly mediated inoculation. The symptom development started from 22.00, 21.67 and 22.67 days after inoculation in genotypes T_{60}, T_{61} and T_{69}, respectively. Two genotypes, namely T_{51} and T_{68} showed moderate resistant reaction and the symptom development started from 22.33 and 21.67 days after inoculation, respectively (Table 5).

Graft inoculation under greenhouse conditions: Out of 10 symptomless genotypes under field conditions, none were completely free from ChiLCV infection. Four genotypes showed a highly resistant reaction and six showed a

Table 5: Reaction of symptom-less and highly resistant genotypes (under field conditions) against ChiLCV by graft inoculation under greenhouse conditions

Treatments	Genotypes	Reaction under field conditions	Appearance of symptom after grafting (days)	Mean PDI (%)	Mean DI (%)	Mean CI (%)	Disease reaction	Virus presence by PCR
T_2	Sel-3	Symptomless	32.00	8.89	40.00	3.56	HR	+
T_3	Sel-4	Symptomless	34.33	8.89	53.33	4.89	HR	+
T_5	Sel-6	Symptomless	33.33	7.78	40.00	3.11	HR	+
T_{46}	CHIVAR-1	Symptomless	34.33	7.78	40.00	3.11	HR	+
T_{50}	CHIVAR-2	Symptomless	25.67	22.22	73.33	16.44	MR	+
T_{57}	CHIVAR-8	Symptomless	26.00	20.00	80.00	16.00	MR	+
T_{63}	VS-9	Symptomless	26.33	23.33	80.00	18.67	MR	+
T_{65}	Sel-40	Symptomless	26.67	21.11	66.67	14.22	MR	+
T_{66}	Sel-7-1	Symptomless	26.33	20.00	80.00	16.00	MR	+
T_{67}	Sel-36-1	Symptomless	27.33	24.44	80.00	19.56	MR	+
T_{51}	CHIVAR-4	Highly resistant	22.33	35.56	100.00	35.56	MS	Not tested
T_{60}	Japani Longi	Highly resistant	22.00	37.78	100.00	37.78	MS	Not tested
T_{61}	Perennial	Highly resistant	21.67	36.67	100.00	36.67	MS	Not tested
T_{68}	PLS-3-1	Highly resistant	21.67	35.56	106.67	37.78	MS	Not tested
T_{69}	Sel-20-1	Highly resistant	22.67	36.67	106.67	39.11	MS	Not tested
	Mean		26.84	21.67	71.67	18.90		
	CD 5%		1.22	3.90	11.17	4.55		
	SE(m)		0.42	1.34	3.84	1.57		
	SE(d)		0.59	1.90	5.44	2.22		

SL: Symptom less, HR: Highly resistant, R: Resistant, MR: Moderately resistant, MS: Moderately susceptible, S: Susceptible, HS: Highly susceptible, +: Presence of 550 bp viral genome

moderately resistant reaction under graft inoculation. The four highly resistant genotypes include T_2, T_3, T_5 and T_{46} and the first disease symptoms appeared 32.00, 34.33, 33.33 and 34.33 days after graft inoculation, respectively. The genotypes viz., T_{50}, T_{57}, T_{63}, T_{65}, T_{66} and T_{67} showed a moderately resistant reaction. In these genotypes, the days to first appearance of disease ranged from 25.67 in genotype T_{50} to 27.33 in T_{67} (Table 5). The genotypes which showed highly resistant reaction under field conditions were moderately susceptible under artificial graft inoculation. The genotypes which showed moderately susceptible reaction were T_{51}, T_{60}, T_{61}, T_{68} and T_{69} (Table 5). Days to first symptom appearance in these genotypes ranged from 22.00 (T_{60}) to 22.67 (T_{69}).

Molecular detection of ChiLCV by Polymerase Chain Reaction (PCR): In order to confirm the presence of virus from artificially inoculated plants, the DNA from the top young leaves of the artificially inoculated plants were subjected to Polymerase Chain Reaction (PCR) using geminivirus universal primers (AV494/AC1048) for confirmation of ChiLCV. After whitefly inoculation, six genotypes (T_2, T_3, T_5, T_{46}, T_{50} and T_{57}) were symptomless, two (T_{65} and T_{66}) were highly resistant and two (T_{63} and T_{67}) were resistant. Out of six symptomless genotypes, four genotypes, namely T_2, T_3, T_5 and T_{46} did not show virus-specific amplification, which confirmed the absence of viral genome in the inoculated plants (Table 4). However, two symptomless genotypes (T_{50} and T_{57}), two highly resistant (T_{65} and T_{66}) and two resistant genotypes (T_{63} and T_{67}) showed amplification of 560 bp DNA fragment specific to viral genome indicating the presence of viral genomes in the plants. Under graft inoculation, all tested genotypes (4 highly resistant and 6 moderately resistant) showed the presence of virus (Table 5) by amplification of 560 bp DNA fragment specific to the viral genome.

DISCUSSION

Identification of resistance sources is of utmost importance in any resistant breeding program. Identification of true resistance from large population through artificial challenge inoculation becomes difficult and cumbersome. Keeping this in mind, natural field screening seemed best to eliminate the genotypes, which showed obvious susceptible reaction under natural epiphytotic conditions. In the present experiment, 70 genotypes were screened under natural disease conditions. The phenotypic observations suggested that the chilli plants infected at an early stage remained severely stunted. Their terminal and axillary shoots tend to stay erect and their leaflets were reduced in size and abnormally shaped. A wide range of *leaf curl virus* symptoms variability was noticed under natural field conditions. Enations on leaves and vein thickening were pronounced in some plants. Upward curling of leaves, leaf bending and cupping were also observed. Severely affected plants showed bushy

appearance (stunted growth) due to shortened internodes with numerous small and curly leaves in the upper portion of the plants. These plants were also devoid of flowers and fruits. Senanayake *et al.*[13] observed the most notable field symptoms like curling, mottling, puckering and stunting of plants under field conditions.

The susceptible genotypes T_{35} (Pusa Jwala) and T38 (Kashi Anmol) showed very severe disease infection (highly susceptible) with 100% disease incidence and the first symptoms of the disease were observed within 15 days after transplanting of the crop. Development of early and severe symptoms on these genotypes suggested that the disease was in epidemic form and screening under natural field conditions was effective. The differential response of genotypes to ChiLCV incidence and symptom expression could be attributed to the fact that the disease incidence and its spread are influenced by the occurrence and population dynamics of the vector whitefly and the weather conditions in the agro-ecosystem[14,15]. Whiteflies had an affinity for some particular genotypes than others and this resulted in some hybrids being more susceptible to the virus than others under field conditions[16]. The symptom less reaction of genotypes can either be attributed due to non-preference mechanism or merely due to escape of whiteflies[9]. Several resistant or tolerant genotypes identified so far are mainly based on field screening.

Under natural conditions, resistance exhibited by some lines cannot be inferred as a true resistance because those lines may manage to escape from whitefly (vector) and hence weren't infected. Sometimes it may also due to feeding of other sucking pests that lead to the slight resemblance of leaf curl symptoms. Annual, seasonal and local variations strongly influence the incidence and severity of virus under natural field conditions[17]. So, to identify their nature of resistance, the lines that were screened as high resistance and symptomless under field conditions were subjected to artificial whitefly and graft inoculation.

In whitefly mediated screening, the test plants were inoculated by using viruliferous whiteflies under single plant micro cages. The 10 genotypes which showed symptomless reaction under field conditions expressed a varied level of resistance under artificial whitefly mediated inoculation. Genotypes T_2, T_3, T_5, T_{46}, T_{50} and T_{57} were remained symptomless under artificial whitefly inoculation. The genotype T_{65} and T_{66} showed slight curling and clearing of upper leaves under whitefly mediated inoculation and rated as highly resistant. Genotypes T_{63} and T_{67} showed mild curling and swelling of veins, hence rated as resistant. The five highly resistant genotypes (T_{51}, T_{60}, T_{61}, T_{68} and T_{69}) under field screening were turned out to be resistant (T_{51} and T_{68}) and moderate resistant (T_{60}, T_{61} and T_{69}) under whitefly inoculation conditions. The differential response of genotypes under natural and artificial conditions could be attributed to several reasons. Under artificial conditions, high and uniform inoculum pressure is ensured.

Despite efforts to ensure inoculum under the field conditions, some plants still escape infection and are erroneously regarded as symptomless or resistant. One of the reasons for escape under high disease pressure could be due to host non-preference by the vector, whitefly. Symptoms on moderately resistant or tolerant genotypes grown in the field could be inconspicuous, especially if the plant escapes early infection[18]. Pico *et al.*[19] suggested that artificial cage inoculation is the most efficient, adequate and reliable technique to screen against ToLCV (*Tomato leaf curl virus*) and screening of tomato for ToLCV resistance under natural infestation conditions could be misleading.

After whitefly inoculation, six genotypes were symptomless, two were highly resistant and two were resistant. Out of these six symptomless genotypes, four genotypes namely T_2, T_3, T_5 and T_6 did not show any amplification for the presence of virus whereas, two genotypes (T_{50} and T_{57}) showed the presence of viral genomes in the plants when subjected to PCR amplification by using degenerate primers. After graft inoculation, all ten genotypes showed symptom development. These genotypes were confirmed for the presence of virus by amplification of 560 bp DNA fragment specific to the viral genome. Though the virus is present in all the graft inoculated plants, the apparent symptoms vary with genotypes, i.e., four genotypes (T_2, T_3, T_5 and T_{46}) were highly resistant and six (T_{50}, T_{57}, T_{63}, T_{65}, T_{66}, T_{67}) were moderately resistant. This suggested that there is a better resistance mechanism working in highly resistant genotypes T_2, T_3, T_5 and T_{46} and they could be used as testers in the hybridization programme of the present investigation. To confirm the resistance in the symptomless genotypes viz., GKC-29, BS-35 and EC-49 (after graft inoculation). Kumar *et al.*[11] subjected these plant samples to PCR amplification by using degenerate primers and they confirmed the absence of viral genome from these symptomless plants. Overall, the use of genotypes with a high degree of resistance was recommended to obtain better results under disease pressure. Furthermore, the mechanism of resistance must be evaluated in detail by involving the resistance genotypes in the breeding programs and also by applying the omics-based methods.

CONCLUSION

On the basis of Coefficient of Infection (CI) all the genotypes were assigned specific disease reaction. To facilitate the attack of chilli leaf curl disease in the experiment, plant protection measures were not used for proliferation of the vector whitefly. Out of 70 genotypes screened, ten genotypes were found to be completely free from ChiLCV infection and were regarded as symptomless (SL) genotypes. The genotype which showed symptomless reaction to ChiLCV included Sel-3 (T_2), Sel-4 (T_3), Sel-6 (T_5), CHIVAR-1 (T_{46}), CHIVAR-3 (T_{50}), CHIVAR-8 (T_{57}), VS-9 (T_{63}), Sel-40 (T_{65}), Sel-7-1 (T_{66}) and Sel-36-1 (T_{67}). Five genotypes showed Highly Resistant (HR) reaction included CHIVAR-4 (T_{51}), Japani Longi (T_{60}), Perennial (T_{61}), PLS-3-1 (T_{68}) and Sel-20-1 (T_{69}). In order to establish true resistance, the genotypes that were symptomless and highly resistant under field conditions were subjected to artificial screening. In whitefly mediated inoculation single plant inoculation technique was used, where the individual seedling was inoculated at two true leaves stage by 10 viruliferous whiteflies after acquiring virus from ChiLCV infected chilli source. The genotypes, which showed highly resistant reaction under field conditions were moderately susceptible (T_{51}, T_{60}, T_{61}, T_{68} and T_{69}) under artificial graft inoculation. Moreover, out of six symptomless genotypes after whitefly inoculation, four genotypes namely T_2, T_3, T_5 and T_{46} did not show any amplification for the presence of the virus, confirming the absence of viral genome in the inoculated plants. Since the virus was present in all the graft inoculated plants, but the apparent symptoms varied with genotypes, there was a better resistance mechanism working in the four highly resistant genotypes.

SIGNIFICANCE STATEMENT

Chilli pepper is an important spice crop from a global perspective and there is a continuous rise in the demand for chilli pepper, but, the ChiLCV is a threat to chilli pepper cultivation. Here, it screened the widespread chilli pepper genotypes from India for their potential against the ChiLCV disease. We hope this research will be useful for the breeders and the farmers to determine the chilli pepper genotype for the successful production of the crop even under the presence of ChiLCV disease.

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