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Variability, genetic relationships and ISSR marker-based characterization of Tomato Genotypes against Tomato Leaf Curl Virus

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Abstract

Tomato leaf curl virus (ToLCV) is one of the most destructive viral diseases limiting tomato production worldwide. Identification of resistant genotypes and assessment of their genetic diversity are essential for the development of durable resistant cultivars. The present investigation was undertaken to evaluate twenty-two tomato genotypes and varieties for natural ToLCV incidence and to assess their genetic diversity using Inter Simple Sequence Repeat (ISSR) markers. Disease incidence varied significantly among the genotypes, indicating substantial variability for resistance and susceptibility under natural field conditions. The resistant variety Arka Rakshak remained free from disease infection, whereas susceptible genotypes exhibited high disease incidence. Genomic DNA isolated from all genotypes was amplified using ISSR markers. Twenty ISSR primers generated a large number of reproducible bands with a high level of polymorphism, demonstrating the effectiveness of ISSR markers in detecting genetic variation among tomato genotypes. Cluster analysis based on Jaccard's similarity coefficient grouped the genotypes into distinct clusters corresponding to their genetic relationships and disease response. Resistant genotypes showed closer genetic association with Arka Rakshak, whereas susceptible genotypes clustered with JT-3 and other highly infected cultivars. The study revealed considerable genetic variability among tomato genotypes and demonstrated the usefulness of ISSR markers for identifying resistant genetic resources and supporting tomato breeding programmes aimed at ToLCV resistance.

Keywords: Tomato (Solanum lycopersicum L.), Tomato Leaf Curl Virus (ToLCV), ISSR markers, genetic diversity, disease resistance, molecular characterization, germplasm evaluation

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops cultivated worldwide owing to its nutritional value, economic importance, and versatile culinary applications. It is a rich source of vitamins, minerals, lycopene, and other antioxidant compounds beneficial to human health (Bergougnoux, 2014; Knapp and Peralta, 2016) [3, 5]. Despite its importance, tomato productivity is severely affected by several biotic stresses, among which Tomato Leaf Curl Virus (ToLCV) is one of the most devastating diseases. Tomato Leaf Curl Virus, a member of the genus Begomovirus, is transmitted by the whitefly (*Bemisia tabaci*) and causes severe yield losses in tropical and subtropical regions. Infected plants exhibit leaf curling, yellowing, stunted growth, and reduced fruit yield. The development of resistant cultivars remains the most economical and environmentally sustainable approach for disease management. Molecular markers have become indispensable tools in plant breeding and genetic diversity studies. Among various marker systems, Inter Simple Sequence Repeat (ISSR) markers are highly informative, reproducible, and cost-effective molecular markers that amplify regions between microsatellite loci without requiring prior genome sequence information. These markers have been extensively used for assessing genetic diversity, cultivar identification, and phylogenetic relationships in crop plants (Zietkiewicz *et al*., 1994; Ng and Tan, 2015) [7, 11]. Evaluation of tomato germplasm for ToLCV resistance combined with molecular characterization can provide valuable information for identifying resistant donor parents and understanding genetic relationships among genotypes. Therefore, the present study was undertaken to assess ToLCV incidence under natural field conditions and to determine the genetic diversity among tomato genotypes using ISSR markers.

Materials and Method

Twenty-two tomato genotypes and varieties were obtained from the Vegetable Research Station, Junagadh Agricultural University, Junagadh, while seed of the resistant variety Arka Rakshak was procured from the Indian Institute of Horticultural Research (IIHR), Bengaluru. The experiment was conducted under natural field conditions. Disease incidence and severity were recorded from five randomly selected plants in each replication. Disease symptoms were scored according to the modified scale of Lapidot (2007) [6]: 0 = no symptoms, 1 = slight yellowing, 2 = yellowing with minor leaf curling, 3 = pronounced yellowing and curling with continued growth, and 4 = severe stunting and curling with arrested growth. Percent disease incidence was calculated and subjected to statistical analysis using a Completely Randomized Design (CRD) Snedecor and Cochran (1967) [10].

Genomic DNA was isolated from young leaf tissues using the modified CTAB method of Doyle and Doyle (1990) [4]. DNA quality and quantity were assessed prior to PCR amplification. A total of fifty ISSR primers were screened, of which twenty primers showing clear and reproducible amplification were selected for diversity analysis. PCR amplification was performed following the procedure described by Ponnuswami (2010) [8] with suitable modifications. Amplified products were resolved through agarose gel electrophoresis and scored for the presence or absence of bands. Disease incidence data were statistically analyzed according to CRD procedures.

ISSR banding patterns were scored in binary format. Polymorphism percentage, polymorphic information content (PIC), and ISSR primer index (IPI) were calculated. Genetic similarity coefficients were estimated using Jaccard's coefficient, and cluster analysis was performed using the UPGMA method through NTSYSpc software to generate dendrograms depicting genetic relationships among genotypes.

Results and Discussion

Response of Tomato Genotypes to ToLCV Infection

Significant variation in ToLCV incidence was observed among the evaluated tomato genotypes, indicating substantial genetic variability for disease resistance (Table 1). Arka Rakshak remained completely free from infection and exhibited the highest level of resistance under natural field conditions. In contrast, genotype JTL-15-71 recorded the highest disease incidence (91.66%), followed by JT-3 (86.66%) and JTL-15-05 (83.33%). Intermediate levels of disease incidence were observed in Punjab Chhuhara, ATL-15-13, Selection-22, GT-1, BSS-488, and Arka Vikas. Lower disease incidence was recorded in AT-3, JTL-12-11, H-24, and Bhagya, indicating relatively better tolerance to ToLCV infection (Table 1). Similar variability in ToLCV incidence among tomato genotypes and the susceptibility of cultivars such as Arka Vikas under field conditions have also been reported by Singh (2014) [9]. The observed variability among genotypes provides opportunities for selecting resistant donor parents in breeding programmes.

Table 1: Percent Tomato Leaf curl virus Disease severity in natural field condition

Sr. No.	Name of Tomato Genotypes and varieties	% Disease incidence	
		Original Value	Transformed value (Arc Sin transformation)
1	Arka rakshak	0.00	0.00
2	Punjab chhuhara	76.66	61.69
3	H-24	35.00	36.22
4	Bhagya	38.33	38.22
5	AT-3	28.33	32.12
6	ATL-15-13	76.66	61.43
7	JTL-8-18	55.00	47.89
8	ATL-11-05	50.00	44.98
9	NTL-14-71	41.66	40.15
10	Selection 22	75.00	60.05
11	NTL-12-07	61.66	51.75
12	NTL-12-06	78.33	62.45
13	NTL-12-01	48.33	44.02
14	JTL-12-02	45.00	42.10
15	JTL-12-11	30.00	33.14
16	JTL-15-02	70.00	57.21
17	JTL-15-05	83.33	67.00
18	JTL-15-71	91.66	73.37
19	GT-1	73.33	59.68
20	BSS-488	75.00	59.97
21	Arka vikas	73.33	58.90
22	JT-3	86.66	68.63
	S. Em±	-	2.972
	C.D. @ 5%	-	8.500
	C.V.%	-	10.287

ISSR Marker Polymorphism

Twenty ISSR primers generated a total of 221 amplified bands, of which 200 were polymorphic, indicating a high level of genetic diversity among the evaluated genotypes. The average polymorphism across primers was 86.00%. UBC-857 and UBC-885 produced the highest number of bands, whereas UBC-849 and UBC-859 generated the fewest fragments (Table 2). The percentage polymorphism ranged from 22.22% to 100%, demonstrating the discriminatory power of ISSR markers. PIC values ranged from 0.68 to 0.93, indicating high informativeness of the selected primers. The high level of polymorphism observed in the present investigation agrees with the findings of Aghili *et al.* (2012) [1], who reported the effectiveness of ISSR markers for assessing genetic diversity in tomato germplasm.

Table 2: Number of Amplified Bands, Percent Polymorphism and PIC Obtained by ISSR Primers

Sr. No.	ISSR Primer	Band Size (bp)	Total No. of Amplicons (A)	Polymorphic Bands (B)			Mono-Mor Phic Band	% Poly-Mor Phism (B/A)	PIC*	IPI
				S	U	T				
1	UBC-809	217-1469	14	11	2	13	1	92.85	0.89	12.55
2	UBC-812	186-1602	12	12	0	12	0	100	0.90	10.87
3	UBC-817	433-2508	8	8	0	8	0	100	0.86	6.94
4	UBC-818	196-959	11	8	3	11	0	100	0.82	9.09
5	UBC-823	263-1568	9	6	1	7	2	77.77	0.87	7.88
6	UBC-829	450-1397	10	7	1	8	2	80.00	0.86	8.68
7	UBC-834	141-862	9	9	0	9	0	100	0.84	7.63

8	UBC-841	152-1206	13	11	2	13	0	100	0.86	11.21
9	UBC-843	303-932	8	6	2	8	0	100	0.68	5.50
10	UBC-844	296-1818	9	7	2	9	0	100	0.84	7.59
11	UBC-845	117-1699	15	13	2	15	0	100	0.91	13.65
12	UBC-847	183-1641	9	2	0	2	7	22.22	0.88	7.97
13	UBC-849	129-801	7	7	0	7	0	100	0.82	5.78
14	UBC-855	267-1765	13	11	1	12	1	92.30	0.90	11.82
15	UBC-857	180-2885	16	10	1	11	5	68.75	0.92	14.72
16	UBC-858	242-1255	9	5	0	5	4	55.55	0.87	7.86
17	UBC-859	262-951	7	6	1	7	0	100	0.82	5.78
18	UBC-885	201-1679	16	13	1	14	2	87.5	0.92	14.78
19	UBC-887	191-2088	9	6	0	6	3	66.66	0.88	7.93
20	UBC-891	213-1565	17	13	0	13	4	76.47	0.93	15.87
Total			221	171	19	200	31			
Average			-	-	-	10		86.0035	0.86887	9.70958

S = Shared; U = Unique; T = Total Polymorphic Bands; PIC = Polymorphism information content; IPI = ISSR Primer Index = Number of Bands X PIC.

Genetic Relationship among Tomato Genotypes

Genetic similarity coefficients among the twenty-two genotypes ranged from 0.273 to 0.906 (Table 3, Figure 1). The lowest similarity was observed between JTL-12-11 and Punjab Chhuhara, indicating wide genetic divergence. The highest similarity was observed between Bhagya and H-24, suggesting close genetic relatedness. Resistant genotype Arka Rakshak exhibited high similarity with H-24, Bhagya, and AT-3, all of which showed relatively lower disease incidence. Conversely, susceptible genotype JT-3 displayed high similarity with Arka Vikas, JTL-15-71, and GT-1, all characterized by higher disease incidence.

UPGMA cluster analysis grouped the genotypes into two major clusters. Cluster B contained most genotypes and further divided into sub-clusters, while Cluster A contained only Punjab Chhuhara, indicating its distinct genetic background. The clustering pattern generally corresponded with field responses to ToLCV infection. Resistant and moderately resistant genotypes were grouped together, whereas highly susceptible genotypes clustered separately, supporting the association between molecular diversity and disease response. Similar clustering patterns and genetic relationships among tomato genotypes based on ISSR markers were reported by Al-Hammouri *et al.* (2012) [2], who demonstrated the usefulness of UPGMA clustering for distinguishing genetically diverse tomato accessions.

Table 3: Jaccard’s Similarity Coefficient of 22 tomato Genotypes based on ISSR

varieties	Arkar akshak	Punjb chhuhara	H-24	Bhagya	AT-3	ATL-15-13	JTL-8-18	ATL-11-05	NTL-14-71	Selection 22	NTL-12-07	NTL-12-06	NTL-12-01	JTL-12-02	JTL-12-11	JTL-15-02	JTL-15-05	JTL-15-71	GT-1	BSS-488	Arka vikas	JT-3
Arkar akshak	1																					
Punjab chhuhara	0.36	1																				
H-24	0.86	0.31	1																			
Bhagya	0.83	0.31	0.91	1																		
AT-3	0.81	0.35	0.82	0.83	1																	
ATL-15-13	0.70	0.35	0.69	0.71	0.79	1																
JTL-8-18	0.78	0.33	0.82	0.84	0.79	0.72	1															
ATL-11-05	0.77	0.32	0.79	0.83	0.75	0.64	0.82	1														
NTL-14-71	0.78	0.33	0.82	0.83	0.85	0.75	0.84	0.80	1													
Selection22	0.59	0.44	0.57	0.58	0.64	0.67	0.60	0.54	0.64	1												
NTL-12-07	0.73	0.31	0.74	0.79	0.77	0.66	0.79	0.73	0.77	0.57	1											
NTL-12-06	0.72	0.32	0.72	0.76	0.74	0.62	0.78	0.75	0.75	0.55	0.87	1										
NTL-12-01	0.75	0.33	0.76	0.78	0.74	0.65	0.79	0.75	0.76	0.58	0.75	0.76	1									
JTL-12-02	0.75	0.34	0.77	0.81	0.82	0.73	0.77	0.77	0.81	0.59	0.78	0.75	0.79	1								
JTL-12-11	0.69	0.27	0.72	0.75	0.71	0.63	0.78	0.70	0.75	0.55	0.75	0.73	0.74	0.7	1							
JTL-15-02	0.72	0.33	0.71	0.73	0.75	0.74	0.75	0.69	0.77	0.63	0.75	0.73	0.73	0.8	0.78	1						
JTL-15-05	0.76	0.32	0.75	0.79	0.73	0.67	0.78	0.74	0.74	0.59	0.74	0.73	0.71	0.8	0.74	0.74	1					
JTL-15-71	0.73	0.34	0.73	0.76	0.74	0.68	0.77	0.72	0.74	0.60	0.73	0.74	0.74	0.8	0.71	0.75	0.85	1				
GT-1	0.71	0.33	0.73	0.76	0.72	0.66	0.78	0.71	0.74	0.59	0.72	0.74	0.69	0.7	0.74	0.73	0.82	0.83	1			
BSS-488	0.70	0.33	0.72	0.74	0.74	0.71	0.75	0.67	0.76	0.60	0.74	0.72	0.71	0.7	0.71	0.79	0.79	0.79	0.80	1		
Arka vikas	0.73	0.34	0.75	0.75	0.72	0.69	0.78	0.69	0.75	0.62	0.73	0.74	0.74	0.7	0.72	0.79	0.81	0.82	0.84	0.87	1	
JT-3	0.72	0.34	0.71	0.71	0.74	0.65	0.77	0.70	0.73	0.59	0.71	0.71	0.71	0.7	0.72	0.72	0.78	0.79	0.79	0.77	0.80	1

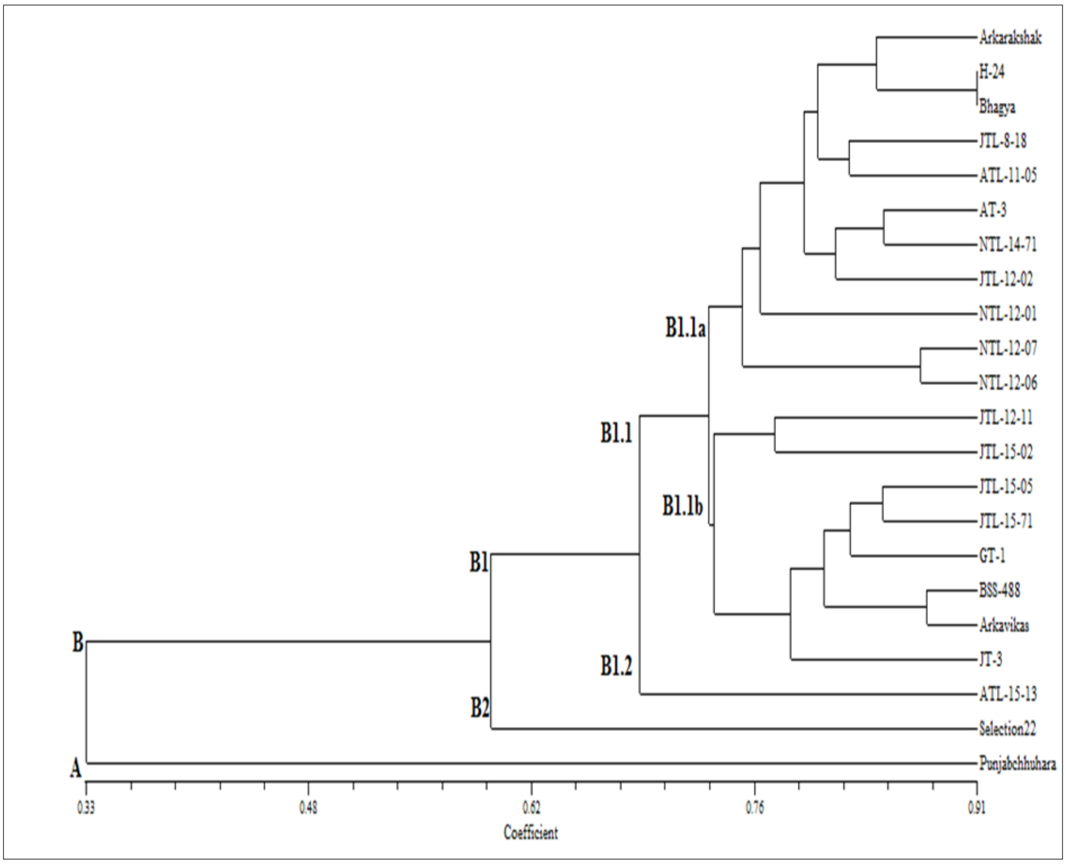

Fig 1: Dendrogram depicting the genetic relationship among 22 tomato genotypes based on the ISSR data from 20 Primers

Conclusion

The study revealed substantial variability among tomato genotypes for Tomato Leaf Curl Virus incidence under natural field conditions. Arka Rakshak exhibited complete resistance and may serve as a valuable donor parent in resistance breeding programmes. ISSR markers proved highly effective in detecting genetic diversity, generating a high proportion of polymorphic bands and clearly differentiating resistant and susceptible genotypes. Cluster analysis successfully grouped genotypes according to their genetic relationships and disease reactions. The identified resistant and genetically diverse genotypes can be utilized in future breeding programmes aimed at developing ToLCV-resistant tomato cultivars.

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