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Short Communication

Validation of known ToLCV markers associated with ToLCV resistance in tomato through Bulked Segregant Analysis

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Abstract

After phenotyping of the segregating F_2 population derived from a highly resistant ToLCV line of wild tomato IIHR 2101 (*S. habrochaites*) and a susceptible cultivated line AT-3 (*S. lycopersicum*), the ratio of resistant to susceptible tomato plants was found to be approximately 3:1. In the correlation analysis phenol showed a significant and negative correlation (r = -0.8166) with the disease incidence. Total 235 plants from F_2 segregating generation were subjected to molecular marker analysis employing a Bulked Segregant Analysis (BSA) approach. Out of 146 primers surveyed for parental screening, only two were found able to discriminate both the parents (highly resistant and highly susceptible) sufficiently. Two markers (Sp and C2_At5g51110) were identified as linked to ToLCV resistance gene through BSA.

Key words: Tomato, ToLCV, BSA, distant hybridization, mapping population

The tomato (*Solanum lycopersicum* Mill., 2n=2x=24) is an important and most widely grown vegetable crop of the world. India ranks second in the area (0.88 m ha) and second in production (1.88 mt) in the world (FAO 2016). Tomato production is hampered due to abiotic and biotic stresses. Tomato leaf curl disease (ToLCD) caused by Tomato leaf curl virus (TLCV) is major devastating disease among various biotic stresses. The disease may cause up to 75% or more reduction in fruit yield and due to its devastating nature, it has become a national problem (Sastry and Singh 1973; Saikia and Muniyappa 1989). Resistance breeding for ToLCV appears to be a propitious and ecological approach to manage the disease. Resistance to ToLCV have been reported in certain accessions of wild relatives of *Solanum lycopersicum* but the genetic bases of the resistances change from a single dominant gene to a multigenic recessive pattern (Laterrot 1992).

In the era of molecular techniques especially, DNA markers for genetic analysis leads to a great addition in knowledge of genetic nature, behavior and structure of various plant genomes. Bulk Segregant Analysis (BSA) is a swift method for identify marker-trait association. It includes screening of DNA pools developed from a F_2 population which was originated from a cross between two diverse parents. Two pooled DNA samples are contrasting for a targeted phenotype. Those pooled DNA are utilized to identify molecular markers whichdetect polymorphism between them.

AT-3 (susceptible source) and IIHR-2101 (resistant source) were taken as a female and male parent, respectively to develop F_2 mapping population. A total of 250 plants including 235 F_2, 5 plants of IIHR 2101, 5 plants of AT-3 and 5 F_1 tomato plants grown in field under natural condition with zero plant protection. The populations were subjected to natural whitefly attack at Anand in Gujarat. The disease was scored on 0–5 scale at 30 days (vegetative growth), 60 days (flowering stage) and 90 days (matured stage) after transplanting and scaling procedure were followed as per Banerjee and Kalloo (1987a).

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The morphological observations like plant height and number of primary branches were taken while moisture was determined using method described by A.O.A.C. (1980); total phenol total phenol was determined using the Folin-Ciocalteu method described by Magalhaes et al. (2006); total chlorophyll was extracted using amphiphilic DMSO solvent (Hiscox and Israelstam 1979). The genomic DNA was extracted from both the parental lines (IIHR-2101 and AT-3), its hybrid and each plant of the F_2 generation (Doyle and Doyle 1990). BSA was performed according to method explained by Michelmore et al. (1991).

The mean performance, descriptive statistics and correlation of studied traits in the F_2 population with both parental genotypes are given in Tables 1 and 2.

Table 1. Correlation analysis of different characters with disease incidence

	PH	NPB	CHL	PHE	MOIS	DS
PH	1					
NPB	0.52699	1				
CHL	-0.0146	-0.0436	1			
PHE	0.01945	0.01160	0.18543	1		
MOI	-0.0612	-0.0519	0.00058	0.03246	1	
DS	-0.0067	-0.0382	-0.2166	-0.8166**	-0.1178	1

PH= Plant height, NPB= No. of primary branches, CHL= Chlorophyll content (mg/g), PHE= Phenol content (g/100g), MOI= Moisture (%), DS= Disease score

Both the parents exhibited significant ($p < 0.05$) difference for all the studied traits. Male parent IIHR 2101 was found significantly superior for all the studied traits. A substantial variation among F_2s was also observed for studied characters. The values of GCV, PCV, h^2 and genetic advance for studied traits are given in Table 3. A high estimate of PCV compared to GCV was documented for phenol and chlorophyll. The PCV were consistently greater than its corresponding GCV demonstrating that the obvious variability in characters may not only because of genotypes but climate as well as soil condition were also significant in expressing studied traits. Correlation analysis was carried out to establish an association between ToLCV incidence and component characters (Table 1). In the present study phenol showed significant and negative correlation ($r = -0.8164$) with the disease incidence (Table 1). The results indicated that phenol was found to be increased with decreased incidence of ToLCV disease. Other characters like chlorophyll, moisture, plant height and number of primary branches did not show significant correlation with disease scoring (-0.2166, -0.1178, -0.006 and -0.038, respectively). The present finding are in accordance of the results reported by Saraswathi and Shivashankar (1998).

There were 99 SSR, 47 SCAR and CAPS primers used. BSA indicated that among all the primers Sp and C2_AT5g51110 markers distinguished the resistant and susceptible bulks and found putatively associated with ToLCV resistance (Fig. 1A and 1B).

Table 2. Trait wise performance of population

Trait	Range of F_2	Mean of P1	Mean of P2	Mean of F1	P1 VS P2
Phenol	0.307-2.731	0.42±0.148	1.2±0.418	2.05±0.170	4.532**
Moisture	26.11-87.80	42.33±1.784	81.42±1.69	82.64±2.08	38.38**
Height	60-519	117.75±9.29	174.56±10.49	218.83±10.13	9.17**
Chlorophyll	0.16-18.30	6.30±1.69	10.51±1.602	8.89±1.24	3.79**

Table 3. Variance and genetic component of traits

Trait	VAR of P_1	VAR of P_2	VAR of F_1	GCV	PCV	Heritability	Genetic advance	Genetic advance ean
Phenol	0.02	0.18	0.03	39.18	44.75	76.66	0.81	70.66
Moisture	3.18	2.86	4.33	6.32	6.69	89.35	9.79	12.31
Height	98.60	110.20	102.65	31.80	32.05	98.50	169.28	65.02
Chlorophyll	2.88	2.58	1.54	34.16	38.94	76.94	5.45	61.72

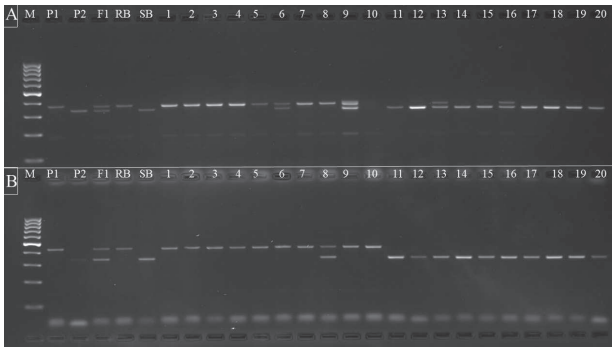

Fig. 1. (A) BSA of Sp (B) BSA of C2_At5g51110; Lane 1: M-Marker; Lane 2: P1-AT-3; Lane 3: P2- IIHR-2101; Lane 4: F1-F_1 of AT-3×IIHR-2101; Lane 5: RB- Resistance bulk; Lane 6: SB- Susceptible bulk; Lane 7-16:1-10 Plant samples used to make Resistance bulk; Lane17-26 :11-20 Plant samples used to make susceptible bulk

Sp and C2_At5g51110 were established to be associated with the ToLCV resistance.

Conclusively, the F_2 population segregated in a 3:1 ratio for ToLCV resistance: susceptibility as well as for indeterminate: determinate plant height. The F_2 population showed a dominant mode of inheritance for both resistance to ToLCV disease and height associated traits. *S. habrochaites* L. (IIHR-2101) may be further used as a parent to develop high yielding, ToLCV disease resistant varieties of tomato. The CAPS markers, SP and C2_AT5g51110 can be used for mapping QTLs as well as for Marker-Aid Breeding for developing ToLCV-resistant lines of tomato.

Authors' contribution

Conceptualization of research (AP); Designing of the experiments (AP); Contribution of experimental materials (AP); Execution of field/lab experiments and data collection (RP); Analysis of data and interpretation (RP, APM, MAM); Preparation of manuscript (RP, KK).

Declaration

The authors declare no conflict of interest.

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