J. Hortic. Sci.
Vol. 19(2), 2024

https://doi.org/10.24154/jhs.v19i2.3475

Original Research Paper

Genetic analysis and validation of molecular markers associated with begomovirus resistance in chilli (*Capsicum annuum* L.)

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ABSTRACT

Chilli is a crop of significant economic value, used as a vegetable cum spice. It has wide applications in the cosmeceutical, pharmaceutical, food and phyto feed industries. Chilli leaf curl disease (ChLCD) has become a major threat to crop production. Exploring host resistance is a sustainable approach to combating the viruses. In the present investigation, we studied the genetic inheritance of Chilli leaf curl virus in two resistant sources *i.e.* IHR4615 and IHR4597. The segregation ratios in the F_2 populations indicated monogenic dominant gene governing the resistance. Further, the publicly available markers associated with begomoviruses resistance were screened in parents. *pepy1* and *Pepy2* genes resistance specific markers didn't show any polymorphism among parents. PAU-LC-343-1 marker associated with *Tomato leaf curl Joydebpur virus* showed parental polymorphism, and its evaluation in the F_2 population of IHR4615 × IHR2451 revealed that the marker is 40.16cM away from the resistance QTL governing *ChLCV-Raichur isolate* resistance, indicating both these loci are different. This marker can be explored for pyramiding resistant loci to develop broad spectrum resistance against begomoviruses in chilli.

Keywords: Chilli leaf curl virus, chilli, inheritance, resistance

INTRODUCTION

Chilli is a commercially important spice cum vegetable crop cultivated globally. Fruits are rich in biochemical compounds for pungency and colours, which have extensive applications in various industries (Timmarao et al., 2025 & Barik et al., 2022). India is the world's highest exporter of dry chillies, accounting for 33.80% of India's total spice exports, valued at 12492.48 crore rupees (Spice Board, 2024). Annually, India produces 4,463,000 MT of green chillies over 419,000 hectares and 2,596,000 MT of dry chillies across 871,000 hectares (Anonymous, 2022-23).

The crop productivity of chilli in India remains low due to various stresses (biotic and abiotic) and varied climatic aberrations. Among biotic stresses, viral diseases are particularly detrimental, causing significant yield losses. Chilli leaf curl disease (ChiLCD) is a major problem in major chilli-growing regions. The etiology of ChiLCD involves over 16 mono and bi-partite begomoviruses, extensively documented within the Indian subcontinent (Senanayake et al., 2007; Shih et al., 2007; Kumar et al., 2012; Kumar et al., 2015; Mishra et al., 2020). These viruses are transmitted by the whitefly species complex in a circulative persistent manner. Typical symptoms include curling of leaves, blistering of interveinal areas, vein swelling, reduced fruit-bearing, and plant stunting. Several factors, including dry spells, elevated temperatures, relative humidity, a wide host range, improper agricultural practices, and the new viral strains, have contributed to the proliferation of whiteflies and the spread of begomoviruses, resulting in severe yield losses. Moreover, indiscriminate pesticide use fosters resistance in insect vectors and poses environmental and pesticide residues in the produce. Resistance breeding offers a sustainable and durable approach to managing virus-induced diseases.

In southern states of India, it is found that *Chilli leaf curl virus- Raichur* isolate (Gen bank Accession No. MK161454 for DNA-A and MK172892 for betasatellite) is a major dominant virus causing chilli leaf curl disease (Yadav et al., 2022). In northern zones of India, *Tomato leaf curl Joydebpur virus* (Srivastava et al., 2017; Thakur et al., 2019) is the predominant virus causing chilli leaf curl disease. Through natural epiphytic screening of germplasm, PBC 142, DLS-Sel10 and WBCSel5 were identified as resistant donors against chilli leaf curl disease resistance at ICAR-IARI, New Delhi (Srivastava et al., 2017). Through natural and artificial screening, an accession S-343 having resistance to ChiLCD, mainly caused by *Tomato leaf curl Joydebpur virus* was reported, and genetics showed a monogenic dominant gene conferring resistance (Thakur et al., 2019). Using this accession, genic male sterile-based F_1 hybrid CH-27 (Bullet type fruits segment) has been developed, having good resistance against ChiLCVD (Dhaliwal et al., 2015). Further, resistance was mapped on chromosome 6, and SSR markers (PAU-LC-343-1 and CA 516044) were reported as markers linked with resistance, which were at 8.9 and 6.8 cM genetic distance, respectively, from the resistant gene (Thakur et al., 2020).

Against *Chilli leaf curl virus*- Raichur isolate, through artificial screening, resistant sources such as IHR4517, IHR4615 and IHR4630 were identified and in IHR4630, it was reported to be governed by single dominant genes (Yadav et al., 2022). DNA markers will help in breeding programs and in literature, there are few markers available associated with begomoviruses resistance in chilli *i.e.* SSR markers linked to Chilli leaf curl disease resistance (Thakur et al., 2020), *pepy1* gene-specific markers associated PepYLCAV resistance (Koeda et al., 2021) and *Pepy2* gene markers associated with PepYLCIV and PepYLCAV resistance (Koeda et al*.,* 2022). *pepy1* and *Pepy2* are begomovirus resistance genes reported so far in *Capsicum*. In the present study, these markers were screened in the resistant sources identified for chilli leaf curl virus. This study mainly emphasized on understanding genetic inheritance for chilli leaf curl virus resistance and further validation of publicly available markers was carried out to check their cross validation, which helps in breeding ChiLCD-resistant cultivars or hybrids.

MATERIALS AND METHODS

Plant material

Two genotypes *viz.*, IHR4615 and IHR4597, which are highly resistant to chilli leaf curl virus-Raichur isolate (Yadav et al., 2022), were crossed with two highly susceptible (HS) genotypes *viz.*, IHR2451 and IHR4392, and the corresponding F_1 were selfed to develop F_2 segregating populations. The F_2 population, along with parents and F_1, were raised in the net house for screening.

Screening for ChLCV-Rai resistance

Whitefly-mediated mass inoculation (Yadav et al., 2022) was carried out in a controlled environment within a 40-mesh insect-proof net house. Chilli Leaf Curl Virus (ChLCV-Rai) was maintained on chilli plants and fresh, non-viruliferous whiteflies were fed on infected plants for 3 to 4 days to make them virulent. Seedlings at the 3-4 true-leaf stage were placed along with a susceptible check cultivar and inoculated with viruliferous whiteflies. The inoculum plants were shaken intermittently for the spread of viruliferous whiteflies. After 2- 3 days, seedlings were moved to another virus-free area and further monitored for symptom development for eight weeks. The disease scoring of individual plants was recorded at seven days interval up to eight weeks (Fig. 1) as per the standard disease score (Yadav et al., 2022). PCR confirmation of the virus was carried out using virus specific markers (Venkataravanappa et al., 2012).

Statistical analysis

The parental genotypes, along with the F_1 and F_2 generations of the respective crosses *i.e.* IHR4615 (HR) × IHR2451 (HS) and IHR4392 (HS) × IHR4597 (HR), were recorded for phenotypic data on resistance. A Chi-square test was carried out with the single gene hypothesis (Snedecor & Cochran, 1967).

Validation of markers associated with begomovirus resistance

The available public domain markers are associated with begomoviruses resistance (Table 1), like SSRs with *Tomato leaf curl Joydebpur* virus (Thakur et al., 2020), *pepy1* gene-specific markers conferring PepYLCIV (Koeda et al., 2021) and *Pepy2* gene-specific markers conferring PepYLCAV isolates resistance (Koeda et al., 2022) were screened among the highly resistant (IHR4615) and highly susceptible (IHR2451) parents and in the segregating F_2 population of IHR4615 × IHR2451.

Fig. 1 : Phenotypic scoring scale adopted for chilli leaf curl virus resistance assessment

Table 1 : List of public domain markers validated /screened in the study

Primer Name	Primer sequence (5'–3')	Annealing temperature	Reference
PAU-LC-343-1	F: TGTGTGTGTGTAAATCTCCAA R: ACGGCATGTAAATAAAGTTCA	54.9°C	Thakur et al., 2020
CA 516044	F: ATCTTCTTCTCATTTCTCCCTTC R: TGCTCAGCATTAACGACGTC	52.1°C	
S05_13024889 (*pepy-1*)	F: CATAAGGTAAAATTCTTATTAATCTAGGAG R: TCGGAAACAGCCTTTCTACTTC	60°C	Koeda et al., 2021
CaRDR Indel (*Pepy-2*)	F: CACCACTGTAGAAATKAATGGAMA R: CGAACTTATGTTCTCACAACAATC	60°C	Koeda et al., 2022

Table 2 : Segregation of resistance against chilli leaf curl virus resistance in two sets of segregating populations of chilli

Population	Number of plants in different scales				Total plants		Best fit ratio	χ2 value	P<0.05
	I/HR	R	IR	S	R	S			
				IHR4615 × IHR2451 populations					
IHR 4615 (HR)	20	0	0	0	20	0	-	-	-
IHR 2451 (HS)	0	0	0	20	0	20	-	-	-
F_1	30	0	0	0	30	0	-	-	-
F_2	78	67	36	19	145	55	03:01	0.67	0.41
				IHR4392 × IHR4597 populations					
IHR 4392 (HS)	0	0	0	25	0	25	-	-	-
IHR 4597 (HR)	25	0	0	0	25	0	-	-	-
F_1	20	0	0	0	20	0	-	-	-
F_2	79	62	35	24	141	59	03:01	2.16	0.14

RESULTS AND DISCUSSION

Genetics of resistance

The complete resistance was observed in F_1 plants from both crosses *i.e*. IHR4615 (HR) × IHR2451 (HS) and IHR4392 (HS) × IHR4597 (HR), strongly indicating the dominant nature of the resistance trait. A total of 200 F_2 individual plants of each cross, *i.e*. IHR4615 (HR) × IHR2451 (HS) and IHR4392 (HS) × IHR4597 (HR) were phenotyped for the disease reaction after artificial challenge inoculation (Table 2).

In the cross of IHR4615 (HR) × IHR2451 (HS), out of two hundred F_2 plants, one hundred forty-five plants were found to be in the resistant group and fifty-five plants were found to be in the susceptible group. Similarly, in the cross IHR4392 × IHR4630, out of two hundred F_2 plants screened, one hundred forty-one were found to be resistant, and fifty-nine plants were found susceptible. Resistant and susceptible plants in both populations were segregated in the ratio of three resistant: one susceptible indicating that the resistance is governed by a major single dominant gene (Table 2) and earlier reports of begomoviruses resistance were reported to be conferred by single dominant gene action in S-343 genotype against chilli lead curl disease mainly caused by ToLCJoV (Thakur et al., 2019) and in IHR4615 and IHR4630 against ChLCV-*Raichur* isolate (Yadav et al., 2022). Koeda et al. (2022) reported the single dominant gene action of resistance in PG1-1 (*C. annuum*) against PepYLCAV (*Pepper yellow leaf curl Aceh virus*) and PepYLCIV locus mapped on chromosome 7. However, there are reports of monogenic recessive gene action in Bhut Jolokia against PepLCV (Rai et al., 2014), DLS-Sel-10 against ChLCV (Maurya et al., 2019) and in BaPep-5 against PepYLCAV and PepYLCIV (Koeda et al., 2021). This is mainly attributed to viruses and genetic resources used in the studies. Dominant resistance genes can be more easily incorporated into breeding lines and are typically more stable under varying environmental conditions compared to recessive genes.

Screening of available public domain markers associated with begomoviruses

The available public domain markers PAU-LC-343-1, CA 516044 (SSRs) linked to Chilli leaf curl disease in S-343 (Thakur et al., 2020), S05_13024889 gene-specific marker for recessive *pepy-1* gene associated with resistance to begomovirus such as PepYLCAV and PepYLCIV in BaPep-5 (Koeda et al., 2021) and a *Pepy-2* gene-specific Indel marker (CaRDR Indel) associated with PepYLCAV and PepYLCIV resistance in *C. annuum* accession PG-4 (Koeda et al., 2022) were screened in the parental lines IHR4615 (HR) and IHR2451 (HS) (Fig. 2). Two gene-specific markers (S05_13024889 & CaRDR Indel) associated with *pepy-1* and *Pepy-2* didn't show any parental polymorphism between parents indicating these genes are not present in IHR4615 and IHR4597.

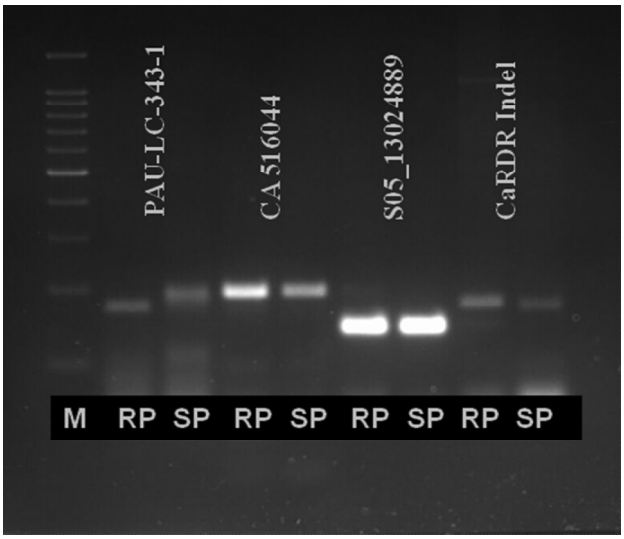

Fig. 2 : Genotyping of parental lines with begomoviruses resistance specific markers (M: 100bp ladder; RP: resistant parent (IHR4615); SP: susceptible parent (IHR2451)

Only the PAU-LC-343-1 marker differentiated between both parents whereas, the CA 516044 marker failed to differentiate between the contrasting parents. Further to determine whether the marker is linked to loci governing the *ChLCV-Rai* isolate resistance the PAU-LC-343-1 marker was then screened in the F_2 segregating individual plants of IHR4615 × IHR2451 cross and the gel images (Fig. 3) were scored and single marker analysis was carried out using the corresponding phenotypic data revealing that the PAU-LC-343-1 marker exhibited a PVE of 4.61% (LOD: 3.93) and was located 40.16cM away from the resistance QTL governing ChLCV-Rai.

These results indicate that the resistance loci in IHR4615 and IHR4597 conferring *Chilli leaf curl virus Rai* isolate are different from those of loci identified on chromosome 6 in S-343 (Thakur et al., 2020) for ChLCD (predominantly by *Tomato leaf curl*

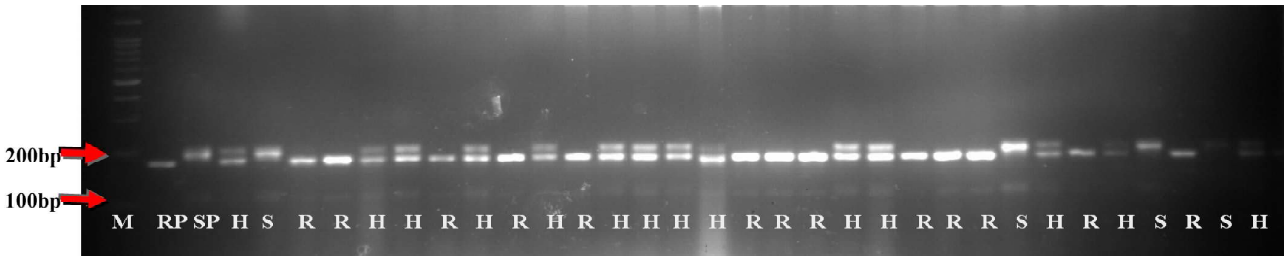

Fig. 3 : Gel profile of PAU-LC-343-1 marker in IHR4615 (HR) × IHR2451 (HS) F_2 segregating population
(M: 100bp ladder; RP: resistant parent (IHR4615); SP: susceptible parent (IHR2451); R: resistant (171bp); S: susceptible (192bp); H: heterozygous

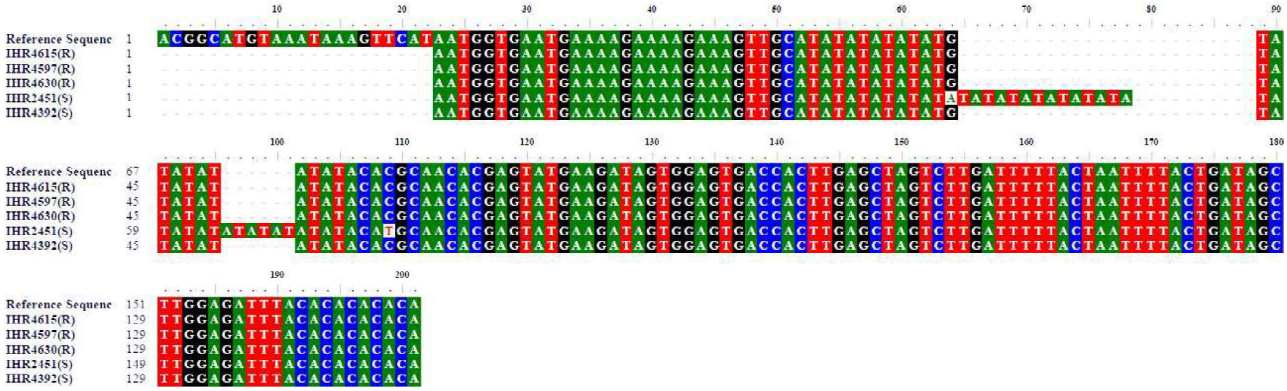

Fig. 4 : Multiple Sequence alignment of the PAU-LC-343-1 primer PCR products of resistant and susceptible genotypes

Joydebpur virus) resistance. Sanger sequencing of the eluted PCR products of both resistant and susceptible parents used in the study revealed that in IHR2451, there is a deletion of 9bp compared to a 29bp deletion (Fig. 4) in highly resistant (IHR4615, IHR4597) parents and a susceptible (IHR4392) parent.

CONCLUSION

Chilli leaf curl disease is a major constraint to chilli crop production. Exploring host plant resistance will enable the development of resistant varieties/hybrids. The segregation for resistance in F_2 populations from highly resistant and highly susceptible accessions (IHR4615 (HR) × IHR2451 (HS) and IHR4392 (HS) × IHR4597 (HR)) demonstrated that ChLCV-Rai resistance is controlled by a monogenic dominant gene that can be easily explored in the development of resistant F_1 hybrids. Further, two genes *pepy1* and *Pepy2* specific markers didn't show any polymorphism in parents. PAU-LC-343-1 marker associated with ChLCD was found to be 40.16cM away from the resistance QTL governing ChLCV-Rai resistance indicating both these loci are different. These markers can be explored for pyramiding resistant loci to develop broad spectrum resistance against begomoviruses in chilli.

ACKNOWLEDGMENT

The senior author acknowledges the Director, ICAR-Indian Institute of Horticultural Research, Bengaluru, India, for providing facilities to carry out this research work & ICAR, New Delhi, for granting Senior Research Fellowship for the Ph.D. program.

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(***Received : 06.9.2024; Revised : 21.12.2024; Accepted : 28.12.2024)***