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Identification of SSR Markers Linked to Chilli Leaf Curl Virus (ChLCV) Resistance in Chilli (*Capsicum annuum* L.)

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Abstract

This study was conducted during April to September, 2022 at ICAR-IIHR, Bengaluru, Karnataka, India to identify molecular markers linked to Chilli leaf curl virus (ChLCV) resistance in *Capsicum* spp. Simple sequence repeat (SSR) markers were utilised to screen the contrasting parental lines and their F_2 segregating population. A total of 120 SSR primers were screened among the parents, of which 22 exhibited clear polymorphism, resulting in an 18.33% polymorphism rate. However, no polymorphism was observed in the bulks, indicating that the selected markers might not be closely linked to the resistance gene. This suggested that the resistance gene might be located in genomic regions not covered by the chosen SSR markers or that resistance involves more complex genetic interactions, such as quantitative trait loci (QTLs) or minor effect genes. The findings highlighted the need for alternative strategies, including expanding the marker set, utilizing high-throughput genotyping methods such as SNP arrays or whole-genome sequencing, and conducting QTL mapping or genome-wide association studies (GWAS).

Keywords: Chilli, Begomovirus, SSR, leaf curl virus

1. Introduction

Chilli (*Capsicum annuum* L.), is one of the major crops of the Solanaceae family, originated in Central and South America, with *C. annuum var. glabriusculum* identified as its wild progenitor (Aguilar-Melendez et al., 2009, Verma et al., 2022, Timmarao et al., 2023). The genus *Capsicum* comprises 35 species (Carrizo Garcia et al., 2016), including five domesticated species i.e., *C. annuum*, *C. chinense*, *C. frutescens* (*C. annuum var. glabriusculum*; ITIS, 2022), *C. pubescens*, and *C. baccatum*. An additional domesticated species, *C. assamicum*, has been reported from northeastern India (Purkayastha et al., 2012). Chilli is highly nutritious, rich in ascorbic acid (vitamin C) for immunity, carotenoids (pro-vitamin A) for vision and skin health, and tocopherols (vitamin E) for antioxidant protection. It also contains flavonoids with anti-inflammatory properties and capsaicinoids, which offer metabolic and therapeutic benefits. These bioactive compounds make capsicum valuable for both nutrition and medicinal applications (Bal et al., 2022, Bal et al., 2024). Its fruit biochemical compounds (capsaicinoids, capsinoids and carotenoids) have wider applications in the food, pharmaceutical, phytofeed, and cosmeceutical industries (Barik et al., 2022).

In India, green and dry chillies have substantial annual production with green chillies yielding 4,496,000 metric tons (mt) over 413,000 hectares, and dry chillies producing 2,782,000 mt across 852,000 hectares (Anonymous, 2024). Notably, dry chillies constitute 40% of global production and 32.9% of India's total spice exports, valued at ₹ 10,445.92 crore (Anonymous, 2023). Despite its economic significance, chilli cultivation faces severe yield losses due to biotic stresses, particularly Chilli leaf curl virus disease (ChLCVD), caused by begomoviruses and transmitted by the whitefly *Bemisia tabaci*. In tropical and subtropical regions, chilli faces

severe losses, up to 100%, due to chilli leaf curl virus disease (Chattopadhyay et al., 2008; Varma et al., 2011; Senanayake et al., 2012; Mandal et al., 2017; Srivastava et al., 2017; Kumar, 2019). and is characterized by leaf curling, puckering, vein swelling, stunting, and reduced fruiting (Senanayake et al., 2007; Kumar et al., 2011a and 2011b). Recent outbreaks of Chilli leaf curl disease (ChiLCD) have posed a significant threat to chilli cultivation in various regions of Maharashtra (Thakur et al., 2018). The rise of new biotypes of *Bemisia tabaci* and the increasing demand in the international market have contributed to the global spread of Chilli leaf curl disease (ChiLCD) across various countries (Ramos et al., 2019). Additionally, the introduction of novel crops into existing or new agricultural regions may facilitate the spread of begomoviruses, potentially leading to the emergence of new species or isolates. This process could be driven by recombination and mutation (Juarez et al., 2019; Shingote et al., 2022).

In India, four begomoviruses Chilli leaf curl virus (ChLCV), Tomato leaf curl New Delhi virus (ToLCNDV), Tomato leaf curl Joydebpur virus (ToLCJV), and Chilli leaf curl Palampur virus (ChiLCPV) are associated with ChLCVD (Kumar et al., 2011a, b). This highlights the need for identifying molecular markers linked to resistance, which are crucial for marker-assisted breeding to enhance chilli productivity and combat viral diseases. Conventional breeding for ChLCV resistance is challenging due to the quantitative nature of resistance and environmental influences on phenotypic expression. Molecular markers, such as SSRs, provide a powerful tool for identifying genomic regions associated with resistance, enabling precise and efficient breeding strategies. Availability of reference genome *C. annuum* cv. CM334 (Kim et al., 2014) has increased genetic research for improving traits. Traditional backcrossing for gene transfer takes a long time, requiring 6–7 generations (Wang and Bosland, 2006). Therefore, this study aimed to identify SSR markers linked to ChLCV resistance by screening contrasting parental lines and segregating populations.

2. Materials and Methods

The present study was carried out during April-September, 2022 at ICAR-Indian Institute of Horticultural research, Bengaluru, India

2.1. Plant materials

Two parental lines were selected: one resistant to ChLCV (IHR4615) and one susceptible (IHR2451). The F_2 population derived from these contrasting parental lines was used for genetic analysis of obtained polymorphic markers between the parents.

2.2. Phenotyping for ChLCV Resistance

Seedlings of 2-4 leaf stage were evaluated under artificial conditions using whitefly mediated mass inoculation in the screening house. Viruliferous whitefly were released onto the portrays containing seedlings and the intermediate row was sown with susceptible check (Arka Mohini) after 24-48 hrs of feeding the trays were removed from the screening chamber and sprayed with insecticide to kill the whitefly. The trays were then kept in the net house and the disease severity was scored based on a 0-4 scale (Yadav et al., 2022) at weekly interval up to 8 weeks. Resistance and susceptibility were categorized based on disease scores.

2.3. DNA extraction and SSR marker screening

Genomic DNA was extracted from parental lines and F_2 individuals using the CTAB method (Doyle and Doyle, 1990). A total of 120 publicly available SSR markers (Supplementary Table 1) were selected from the linkage map (Minamiyama et al*.,* 2006) which were distributed across the chilli genome were screened for polymorphism between the parental lines. The PCR reaction was set for a total volume of 15 µl and consisted of 4.5 µl nuclease-free water, 2 µl of genomic DNA (30 ng μl^{-1}), 2 µl of each of dNTPs (1 mM), forward and reverse primers (5 pM each), 2.0 µl of 10X polymerase chain reaction (PCR) buffer with $MgCl_2$ and 0.5 µl *Thermus aquaticus* (*Taq*) DNA polymerase (1 unit μl^{-1}; Genei, Bangalore, India).

The DNA fragments were amplified using a touchdown PCR program, which included initial denaturation at 94°C for 3 minutes, 10 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C (the annealing temperature reduced by 1°C per cycle) for 1 minute and extension at 72°C for 1 minute, and then 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes. The amplified DNA fragments were resolved on a 6% polyacrylamide gel (HiMedia Labs. Pvt. Ltd, Mumbai, India) and visualized under UVI-Tec Gel documentation system.

2.4. Parental polymorphism and bulked segregant analysis (BSA)

The 120 markers were screened in the contrasting parents and the obtained polymorphic markers were further screened in the resistant and susceptible DNA bulks which were prepared by pooling equal amounts of DNA from 10 resistant and 10 susceptible F_2 plants and the obtained parental polymorphic SSR markers were screened in the DNA bulks to identify markers associated with resistance.

3. Results and Discussion

The primary objective of this study was to identify molecular markers associated with resistance to Chilli leaf curl virus-Raichur isolate and determine their location on the *Capsicum* genome. Understanding the genetic basis of resistance is a crucial aspect of molecular mapping. Phenotypic segregation data from the F_2 population confirmed that the resistance trait in IHR4615 follows a 3:1 (resistant: susceptible) ratio, indicating a monogenic dominant inheritance of resistance through Chi square test. Previous studies have also reported that the resistance in this study is controlled by a single

Table 1: Primer pairs selected from linkage map developed by Minamiyama et al. (2006) for the mapping of chilli leaf curl virus disease (ChiLCVD) resistant gene

SSR No.	Marker name	Forward sequence	Reverse sequence	Fragment size	Linkage group
1.	CAMS-015	TCATGTTGATTATGCTTTTGTTCA	CCATGTATTGTATGATACCTGAGAAA	112	2
2.	CAMS-020	CAGCAGTAACAGAGGCAGGTC	CACAAGTGAGTTTATTCATATCACCA	171	5
3.	CAMS-024	TGTTGAGGCTTGGGAAAAAC	CAAGATAATGGGTAGAAAGGCAAC	219	8
4.	CAMS-040	TATAGCCTGTGGGTGCCTTC	TGGGGTGAACAATAGCATGT	248	3
5.	CAMS-051	ACCCAGTTCCCTTTCTTGGT	GAAGGTTAGCGGAATGAACG	151	5
6.	CAMS-056	CATTGTCCAGGCTGATGTTT	AACTCAGGTACACGGGATAAAA	247	1
7.	CAMS-063-2	CCACTCTCCAAAAGCAAACC	TGTTTGCCACTGTATGTGTCTG	199	2
8.	CAMS-065	CCAGTCTCATCCAGCAGACA	CATATGCTGCT CCTGCATTC	213	2
9.	CAMS-070	CCCTGAACTTGTCCTCCAAA	GGGTATGGGGTGTAGGTGTG	248	6
10.	CAMS-072	CCCGCGAAATCAAGGTAAT	AAAGCTATTGCTACTGGGTTCG	153	5
11.	CAMS-075	ACTAATTACACATTCTGCATTTTCTC	AGGCTCGAGTACCACGAAGA	190	5
12.	CAMS-081	GTTGGGGGAGAGTTGGGTAT	TGGGGTGAACACTAGCATGT	238	7
13.	CAMS-199	ACCTCGGCATGTGATA	TTGCATGGGGATATAA	188	5
14.	CAMS-090	TCGCTCAAAGCACAT CAAAG	CTTGATTGTTCTTCCACTGCTG	243	8
15.	CAMS-095	CGCTAGCATGACACTCAAGG	AAACGGCAAGGCTACACATC	228	5
16.	CAMS-101	TCAGCAATTAACATGCCAAAA	T GGATTGGGAGAAGAT CGAC	217	6
17.	CAMS-177	ATTCTCTACCCCTGCCTGTG	CTCAGGAGATGTCCCACGAT	229	2
18.	CAMS-122	CATCCACGTGCATAGTCAGG	TTCTAAGTGTTGAAATTATGGGATTT	178	5
19.	CAMS-142	GAGCGCTTAAGTGGTCATAGG	CTACAACGCCCCAAAACAAT	241	7
20.	CAMS-153	TGCACAAATAT GAATCCCAAGA	AAGTCAGCAAACACATCTGACAA	243	6
21.	CAMS-156	CCCTATGCTTTCACAACTCCT	GACGTGGTTATGACGAT AGGC	181	10
22.	CAMS-162	GGACCGTTCAGGAGGTT ACA	GCCATCATTCAAAACCGAAT	210	1
23.	CAMS-163	TCCATATAGCCCGTGTGTGA	GCGTGGGAATACAATGCTAGA	250	5
24.	CAMS-173	CAACCGCCAGTAGACAGGTT	GTGCGTGTGCGTGTGT GTAT	169	4
25.	CAMS-117	TTGTGGAGGAAACAAGCAAA	CCTCAGCCCAGGAGACATAA	223	11
26.	CAMS-190	TTTCTGCAGTGTTACCAATATTTCA	CCCATGGGT CCTACCT CAG	212	5
27.	CAMS-191	CCCGAATCCAAGTCATTGAG	TAAATCCGGTTCCCTTTCCT	224	3
28.	CAMS-194	TCATGGAAAATTAACAACGCATA	GGGGGTTGGAGAAGAAAGTT	245	1
29.	CAMS-089	AACAGCGCTGATCCTTTACC	CAACATCACAGTGGCAGAAGA	221	11
30.	CAMS-201	GGTTATTGGTTTGGTTCATTTTT	TTGGACTAAATGTTGATACGAGAAA	201	9
31.	CAMS-207-2	CTCACGAGCCACTTGAACAC	GCCTTGTTTCCTATCCCAAC	243	5
32.	CAMS-215	CGTGGGTGGTCTAGGATGAT	GCTGGCAAGTCACTCTGGAT	220	7
33.	CAMS-224-2	TCTCTGCTCCAAAATGGTGA	GTGCGAACCCACTTTGAAAT	201	1
34.	CAMS-227	TTTGTCCTTTAATTCACCTTTTGA	GCATCAAAATAAGGATAAAGTTATGG	296	5
35.	CAMS-234	TATAGCCCATGGGTGCCTTT	AAAACCCAATATTAACCATATGCAA	157	6
36.	CAMS-236	TTGTAGTTTGCGTACCATTTGA	ATGAATCCAGGGTTCCACAA	191	2
37.	CAMS-237	CCTTCCCTTGAAATTGATATTTTAC	TCCCCTATCACTCACATCTGC	221	2
38.	CAMS-301	CTGT CCATGCTT GTGATGCT	TGATTTGTGCCT CGTTTGAG	180	8
39.	CAMS-309	GAAAATCGACCCGTTTTGAA	T CAATT CGGACAAAATT AGCAA	235	9

SSR No.	Marker name	Forward sequence	Reverse sequence	Fragment size	Linkage group
40.	CAMS-311	GGTGCGCTAGAGATGGAGAG	TTTGAGTGTTCGGGACTGGT	234	6
41.	CAMS-321	CTGCAGGTGGTTCTCTCCAT	TGGCACTCGAACCAGTATGA	235	3
42.	CAMS-324	AACTTGATCCAACGGCTGAG	TCGAAGGAGAGAAACGGTTG	172	7
43.	CAMS-327	GCATCTAAGTCTACGCCCTTG	AAAGCCTTTGGCAATGAACA	243	13
44.	CAMS-330	GGCTACCGCCTTCTGACTTA	TTCGTATCTGGGGT GTCAAA	208	4
45.	CAMS-336	GGTGGAAACTTGCTTGGAGA	CCCAGAACCATCCACCTACT	157	3
46.	CAMS-340	TTTATGCCCATTCACAAAATAA	GGACGAATTCACCGAGTGC	250	10
47.	CAMS-348	CTGAAGTCGGCTAGATGCCTA	TCAAAGCTATGGAGGAAAAGGA	218	3
48.	CAMS-351	CGCATGAAGCAAATGT ACCA	ACCTGCAGTTTGTTGTTGGA	206	4
49.	CAMS-352-2	GCTCCTTATGTGGTGGAGGA	CACCTTCTGACTTTGGCTGA	240	4
50.	CAMS-358	GACCCTTCTCCCCTTTTTCTT	CACATGGACGGATCCTTTTT	218	6
51.	CAMS-360	ACGGTCGATTCCTGTATTGC	GCATGCTAAACCCAATTTTCTC	209	1
52.	CAMS-361	TTGGTGTGGTTAGGGGAGAG	GGCGTTCGAACTTGTGAAAT	207	4
53.	CAMS-373	GGTTGATGGTCCATGTTCAA	CCTCCTACCCTATCCCCAAG	230	12
54.	CAMS-378	GAAATCGACGCGTTTCTAGC	TGTGGGGAGAGAGAGGAAGA	168	1
55.	CAMS-376	GGTGCTGGCATAGATGAACA	TATGTCTGGCTTGGTGCTGA	230	3
56.	CAMS-398	ATGGTCCATGGTCAGCAGAT	GGGCAGAACAGTGGAT GATT	165	7
57.	CAMS-405	TTCTTGGGTCCCACACTTTC	AGGTTGAAAGGAGGGCAATA	241	11
58.	CAMS-417	CCCAAAGACGATTTGCTGA	ATTGCCTGTGAGTGCAACAA	240	10
59.	CAMS-420	CAGCGTTCTATCGTCTCAAATG	TTGACAAACCAGAAATTGATCG	200	5
60.	CAMS-424	TCCACAGCCCACAGTGTCTA	GCTTGTGGTTCCGTGATTTT	167	6
61.	CAMS-450	CCTTCTTCTTTGCCACCTTC	T AGCAGCAGCTGATGGAGAA	220	5
62.	CAMS-451	TGCATTGGTGGGCTAACATA	GCTCTTGACACAACCCCAAT	233	11
63.	CAMS-454	GAGCCTCTTAATGTATCTGAAAACA	AATTTTGGTGAATCGCACCT	243	9
64.	CAMS-456	ATGGAGCTGGGGCTAAAAAT	GCTCAGCAAATTGAGGAGAAG	153	1
65.	CAMS-460	CCTTTCACTTCAGCCCACAT	ACCATCCGCTAAGACGAGAA	215	7
66.	CAMS-478	GAGTGCCATGCTGATTAAGGA	CACGACTGTCTTGCCTGAAC	248	3
67.	CAMS-489	TAAGTTTGTGTCGGGCATGT	ATGGAGCTTGAACTGGGATG	240	1
68.	CAMS-492	GTTCAAACACTTCCCCCTCA	T GT CAT CGTTGGTCGTTACC	250	12
69.	CAMS-494	GGTAGGTGAGGACCCACAGA	AACTATACCCCCGCTGCTCT	247	4
70.	CAMS-606	GACTAGTCCCCGTTCAACCA	TTTGCGAGAAGATGCTTCAG	208	7
71.	CAMS-610	TTGGGACATGACAATTCTGC	AAACGTACATTAGGTAGATCCGGTA	207	1
72.	CAMS-619	TCCTGCTCTCCTCAGAAAGTAAA	GAAACAGGAGGAAGAGGAAGAA	154	9
73.	CAMS 630-2	CCCTCATTATGGAGCAACCA	TTGAGGGAGGGACATAAGACA	172	1
74.	CAMS 635	GAGATTGGTCGGATGGAAAT	AAAATGTCAAAGTCGATCAAACA	154	2
75.	CAMS-644	CGCATGAAGCAAATGT ACCA	ACCTGCAGTTTGTTGTTGGA	206	4
76.	CAMS-647	CGGATTCGGTTGAGTCGATA	GTGCTTTGGTTCGGTCTTTC	221	3
77.	CAMS-655-2	CCTTGCTGCTACCTATTGAAGA	TCCAATCGTCGATATGTTTGTT	224	4
78.	CAMS 668-2	ATCGTCCATTTGTGGGAAAA	TGTGGTCGGAAATAGGAAGAA	158	10
79.	CAMS-679	TTTGCATGTTTTACCCATTCC	ATGT GAAACACAT AGGTAGCACTGA	200	1

SSR No.	Marker name	Forward sequence	Reverse sequence	Fragment size	Linkage group
80.	CAMS 684	CAGGGTGAGCCAAACCATAG	TCAAGACTACTAGGTCGTGGAATG	232	6
81.	CAMS-806	TGTCACAAGTGT CAAGGTAGGAG	CCCCAAAAATTTTCCCTCAT	227	10
82.	CAMS 808	AATGGCTTGCCTAACACAGG	CGGAATTGATCCCTGACAAA	229	2
83.	CAMS-811	GAAGAAACGAAGGATGAACAAAA	CCTGTTTCCTCTTCCTCAGC	260	9
84.	CAMS-838	CCAGGATGGTGTTAAGGGTTT	GTCGCATCAATGAGCAT AGG	229	6
85.	CAMS-844	GCAAAGAAAAAGAAAAGCCTGA	CTGCAACTGCTGCTTCATTC	223	1
86.	CAMS 846	TCTTTTTCCTCGCCATCTTC	CACTGTTATGGCAAAAGAGGA	189	3
87.	CAMS-855	AAGTGTCAAGGAAGGGGACA	CCTAACCACCCCCAAAAGTT	243	8
88.	CAMS 861	GCATGCAAGCTTAGCCAAC	TGAGATTGAAGCTAGAATTTTGGA	183	2
89.	CAMS-864	CTGTTGTGGAAGAAGAGGACA	GCTTCTTTTTCAACCTCCTCCT	222	7
90.	CAMS 865	AGAAATCGTGGTTGGGTGAG	CACTTTGGCACATTTTGCTG	179	11
91.	CAMS-876	TGTGTCTGAAGCGGAACAAA	AAGCAGTGAGCGACGAAG	243	9
92.	CAMS 880	GAGCCAAGAAAAAGGTGGAA	CAACTCATCGTTCAACAACACA	237	6
93.	CAMS-885	AACGAAAAACAAACCCAATCA	TTGAAATTGCTGAAACTCTGAA	248	2
94.	CAMS 888	CCTCGGAGTGGTTTGTTGAT	GCTTGTTCACGCCACCTTAT	211	2
95.	CAMS 891	CTCCGAGAAGGATGTCAGGA	ACTGAGCGACTGATGCCTCT	204	6
96.	CAMS-892-2	CGTCGATTGACCATTGAGTG	CCTTCTAGGAGGCATGTTTTT	184	2
97.	CAMS094	TGTAGCTCACATCGTCTCCACT	GCATTGCATTTCACTTGCAT		
98.	CAMS228	GAGGGCTAAGCAAAGCAGAA	TGCATGTTTCCCTTAGTTTCC		
99.	CAMS406	TAAAAATCGCGGAAAGTTGC	GTCGTTCTATGCGGCATTTT		
100.	CAeMS068	ATCAAATCTCAACACATGGTGGCT	GTTTACTGTATCTCCGGCCCTGTCA		
101.	CAMS071	AATGGGATCTGCATGAGACA	TTCCCTAAAAGATGGTGATTCC		
102.	CAMS823	TCCTCCTCCTTCTCGTGTTC	AAAGAAGCAGCAGGTGAAGA		
103.	CAeMS035	AGGTCTATCGGAAACAGCCTTTCT	GTTTGATCACATCCCAGTCGAATCCTA		
104.	CAeMS060	ATCAAGACAACAACATCATGGGGA	GTTTCGCCTATCAACAATGGCAAATACA		
105.	CAeMS138	ACACACACAATTTCCCTCACTCAC	GTTTCTCTCAAATCCCTCCGTTGTTC		
106.	CAMS396	GTCGGCCGTCATTCACTATT	AGCTTGATGCACCTGGTCTT		
107.	CAeMS144	ATAACTTTGATTCCTAGTTCGGCG	GTTTGAACCCCCAATCATCATATCCTCA		
108.	CAMS032	TGCCACATAGGTTGGCTTTC	CAAAGCCAATGCACATAATCA		
109.	CAMS066	AAAAACATGCACCAGTCCTT	CAACCGCCTGAATTTTCTCT		
110.	CAMS493	TCGATGACGAAAAAGTGTGAA	AGGGCAAAAGACCCATTCTT		
111.	CAeMS015	ATGCCTTGGTGGTGGTTAAATCTG	GTTTAGCGGTATGGACTGCGTACATCTT		
112.	CAeMS073	ATGCTTCTAAGAAACCCCACAACA	GTTTCTCATAAAGGGGTTGGGATTGA		
113.	CAMS212	TTCCCTTTCCCAACATGGTA	ACACCCGAAGATGGGTTAGA		
114.	CAMS368	GAGTGGATAAGCAAGGACGTTT	TTTGCTTCCCTTTTTGCTTC		
115.	CAeMS009	ACGCACCAACGAATATCTATCTCA	GTTTCCGTCCAGATCTACTTTTCCTGC		
116.	CAMS091	TGCTAAACTTGGTTCCCTATCC	CGAAGATGGATTAGCGGGTA		
117.	CAMS179	CATGTCATGAAGTTGATAAGACAATG	TGTTCCAGTGAAAGGCTTCTT		
118.	CAMS871	ACAAAGCATCGGCTGAAAAT	GCGACCAAGTACCAACAGGT		
119.	CAMS452	GAAGTCTGGGACCTCTTTTGG	TTCATTTTGATCTTCACGAACG		
120.	CAMS476	TTTTCCCTTTCCAGTTGTTCA	ATGGGTGAAGTGTGAAAAGAA		

dominant gene (Thakur et al., 2019, Yadav et al., 2022). The presence of a dominant resistance gene simplifies its incorporation into elite genotypes compared to a recessive gene, as backcrossing can efficiently transfer the dominant allele without requiring selfing after every two backcrosses.

The effectiveness of molecular markers in breeding programs depends on the level of polymorphism they exhibit. Limited DNA polymorphism within a species can restrict the applicability of markers in genetic studies. However, SSR markers have proven effective in species with low genetic diversity (Minamiyama et al., 2006). In this study, SSR markers displayed a polymorphism rate of 18.33%, likely due to the genetic similarity between the parental lines, as both belonged to the same cultivated species. Previous reports also attribute low SSR polymorphism to genetic relatedness within the *Capsicum annuum* complex (Arjun et al., 2018). Comparable polymorphism rates have been observed in other studies, such as 21% in mapping the male-sterile *ms-10* gene in hot pepper (Aulakh et al., 2016) and 24% in an intraspecific *Capsicum* population (Minamiyama et al., 2006). Given these findings, interspecific crosses are often recommended for developing mapping populations in species with low polymorphism.

3.1. Parental screening and bulk segregant analysis

A total of 120 SSR primer pairs were screened to evaluate genetic variation between the two parental lines. Variations in fluorescence intensity or amplicon size between the DNA samples were used to identify polymorphism. Polyacrylamide gel electrophoresis (PAGE) was chosen over agarose gel electrophoresis because of its superior resolution. Prior research found that PAGE had a substantially higher polymorphism detection rate (21.68%) than agarose gel (6.27%) (Aulakh et al., 2016). The overall polymorphism rate was 18.33%, with 22 (CAMS051, CAMS065, CAMS072, CAMS101, CAMS173, CAMS340, CAMS358, CAMS378, CAMS405, CAMS460, CAMS808, CAMS811, CAMS844, CAMS855, CAMS864, CAMS865, CAMS876, CAMS892-2, CAMS406, CAMS071, CAMS396, and CAMS871) of the 120 SSR primers that were tested showing clear polymorphism across the parental lines. To find markers linked to resistance, these polymorphic primers were then used in bulk segregant analysis (Michelmore et al., 1991). Upon screening these 22 polymorphic markers in the bulks none of the parental polymorphic markers exhibited polymorphism in the bulks. This outcome suggests that the markers may not be closely linked to the gene conferring ChLCV resistance. Alternatively, the resistance gene may be located in a genomic region not covered by the selected SSR markers, or the genetic architecture of resistance might involve more complex interactions, such as quantitative trait loci (QTLs) or minor genes.

The absence of marker polymorphism in the bulks highlights the need for alternative approaches to identify resistance-associated markers. Expanding the marker set to include additional SSR markers or incorporating high-throughput marker technologies such as SNP genotyping arrays or whole-genome sequencing could improve coverage and resolution. Furthermore, conducting QTL mapping or genome-wide association studies (GWAS) may reveal new regions associated with resistance. Fine mapping using larger populations and candidate gene analysis could also provide deeper insights into the genetic basis of ChLCV resistance.

4. Conclusion

This study confirmed monogenic dominant inheritance of ChLCV resistance in IHR4615. Despite low SSR polymorphism, the findings provide insights into resistance genetics. Bulk segregant analysis failed to identify closely linked markers, suggesting the need for expanded marker sets or advanced techniques like SNP genotyping, whole-genome sequencing, and QTL mapping. Future research should refine marker-assisted selection strategies to accelerate breeding for ChLCV-resistant *Capsicum* varieties.

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