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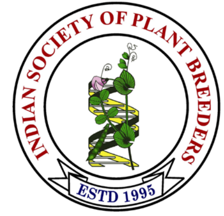

Research Article

Molecular validation of Tomato Yellow Leaf curl Virus (TYLCV) (*Ty-1/3, Ty-2 and Ty-3*), Fusarium wilt (*I-2*) and Root- Knot Nematode (*Mi-1*) resistance in the breeding lines of tomato (*Solanum lycopersicum* L.)

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Abstract
Gene based molecular screening for disease resistance has become a prominent and widely adopted approach in contemporary tomato breeding programs, both in the public and private sectors. The effectiveness of disease-resistant molecular screening relies on the selection of suitable markers that span the entire genomic region for accurate identification of resistant alleles. In this study, various co-dominant and dominant Sequence Characterized Amplified Region (SCAR) markers were employed to screen for resistance genes associated with Tomato yellow leaf curl virus (TyLCV) *Ty*-1, *Ty*-2, *Ty*-3, Fusarium wilt (race-2) *I*-2, and Root-knot Nematode ((*Meloidogyne incognita*) *Mi*-1. The selected markers exhibited high robustness in distinguishing between resistant and susceptible lines. Co-dominant SCAR markers M2 (*Ty*-1 gene) and TG0302 (*Ty*-2 gene) efficiently identify homozygous or heterozygous alleles in Tomato yellow leaf curl virus (TyLCV) screening. The P6-25 marker for *Ty*-3 gene discerns *Ty*-3, *Ty*-3a, and *Ty*-3b alleles, enhancing allele specificity. In Fusarium wilt *I*-2 gene screening, I-2/5 ensures accurate identification, while in Root-knot Nematode *Mi*-1 gene screening, co-dominant marker Mi-23 reliably identify and distinguishes homozygous or heterozygous resistant lines without false positives for *I*-2 and *Mi*-1 genes. This streamlined marker assisted approach optimizes disease resistance screening in tomato breeding, providing valuable insights for disease resistant cultivar development.

Keywords: Tomato, Resistance breeding, TYLCV, Fusarium wilt, Root knot nematode;

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a vital solanaceous vegetable cultivated globally in tropical and sub-tropical regions for fresh market produce (Sushmitha *et al.,* 2018), processing, and nutritional attributes.

(Abbas *et al.,* 2022; Leonardi *et al.,* 2000). Tomato fruits are notable for their nutritional value, offering a wealth of dietary fiber, antioxidants like lycopene, vitamins A, B C (Venkadeswaran *et al*., 2021) minerals like phosphrous, iron, proteins, sugars, carbohydrates, and essential organic acids for a healthy human diet. (Naika *et al*., 2005; Srinivasulu and Singh, 2021). However, tomatoes encounter significant challenges, as over 200 pathogens contribute to substantial yield losses in tomato production (Grey. 1994). Among these, Tomato Yellow Leaf Curl Virus (TYLCV) stands out as a major threat, leading to substantial yield losses upto100 % during summer (Prasanna *et al*., 2015). TYLCV is transmitted by the silverleaf whitefly (*Bemisia tabaci*) notably, seed transmission has also been observed (Kil *et al.,* 2018). Six *Ty* genes, *Ty*-1 through *Ty*-6, have been identified and mapped using DNA markers (Kaushal *et al*., 2020). Whereas, *Ty*-1, originating from *S. chilense*, is on chromosome 6 and codes for an RNA-dependent RNA polymerase, providing resistance to TYLCV. *Ty*-2, from *S. habrochaites*, on chromosome 11, confers high level of resistance to TYLCV-IL strain but not to other TYLCV strains by involving in Leucine-rich repeat gene (NB-LRR) carrying nucleotide binding domain mechanism. While, *Ty*-3, a dominant gene on chromosome 6 from *S. chilense*, is part of efforts to pyramid resistance genes for broad TYLCV resistance. These genes involve RNA-dependent RNA polymerase and play a role in antiviral RNA silencing (Butterbach *et al*., 2014).

Additionally, tomatoes are susceptible to Fusarium wilt caused by *Fusarium oxysporum f. sp. lycopersici*, a soil-borne fungus specifically targets tomatoes, leading to wilting by clogging xylem vessels causing 30- 40 % yield losses (Gordon, 2017). The pathogenic strain exhibits three races, namely race 1, race 2, and race 3 (Bournival *et al.,* 1990). Wild Solanum accessions exhibit resistance to all races, integrated into cultivated tomatoes to develop resistant cultivars. The *I*-2 gene, the first among the I genes, provides resistance to race 2, mapped to chromosome 11 and encoding a coiled-coil NB-LRR protein confer resistant to race 2 of fusarium wilt (Simons *et al*., 1998). The Root-knot nematode *Meloidogyne incognita* belonging to the *Meloidogyne* genus (Trudgill *et al*., 2001), poses a significant threat in tomato production causing substantial quantitative and qualitative yield losses ranging from 25 to 100% (Seid *et al*., 2015). The Mi-1 gene, identified as a single dominant gene (Barham and Winstead, 1957), was initially discovered in wild relatives of *Solanum peruvianum* and subsequently transferred into cultivated tomatoes to create resistance. *Mi*-1 gene imparts active resistance against various RKN species (Smith, 1944), as the *Mi*-1 involves nucleotide-binding site and leucine-rich repeat (NBS-LRR) mechanisms, featuring a potential coiled-coil (CC) domain preceding the NBS. The utilization of gene based molecular markers to screen for the presence of disease resistance alleles is a crucial aspect in tomato breeding programs. Considerable progress has been achieved in the creation of molecular markers linked to disease resistance genes (Anbinder *et al.,* 2009). These markers, correlated with resistance genes, enable the identification of novel resistant sources in the early stages of breeding programs without the need for pathogen inoculation, thereby expediting the breeding process. Furthermore, molecular markers serve as potent tools in pyramiding programs, where diverse alleles of resistance genes are introduced into a single tomato line. This enhances the efficacy and longevity of resistance against diseases while concurrently lowering the cost associated with breeding resistant plants (Slater *et al*., 2013). The present study employs a series of gene-specific molecular markers to screen the presence of resistance genes *Ty*-1, *Ty*-2, and *Ty*-3 against Tomato Yellow Leaf Curl Virus (TYLCV), *I*-2 gene against Fusarium wilt, and *Mi*-1 gene against Root-Knot Nematode in the breeding line of tomato. Subsequently, these resistance genes are utilized for the development of varieties/hybrids with combined resistance to TYLCV, Fusarium wilt, and Root-Knot Nematode. The study also aims to evaluate the effectiveness and robustness of the existing gene-specific molecular markers utilized for the identification of resistance to TYLCV, Fusarium wilt, and Root-Knot Nematode in tomato plants, thereby contributing to an effective breeding program.

MATERIALS AND METHODS

Plant materials: The plant materials consist of 15 advance generation breeding lines developed from pedigree breeding methods, were collected from The World Vegetable Center, Asian Vegetable Research and Development Center (AVRDC)- Taiwan. These lines were screening for the presence of resistant genes against Tomato leaf curl virus (TyLCV)- *Ty*-1, *Ty*-2 and *Ty*-3, Fusarium wilt (race -2) *I*-2 and Root-knot Nematode *Mi*-1. The details of the advance generation breeding lines and their resistant genes against Tomato yellow leaf curl virus (TyLCV), Fusarium wilt (race -2) and Root-knot Nematode (*Meloidogyne incognita*) are given in **Table 1**.

Plant genomic DNA isolation: Genomic DNA extraction from young tomato leaves was performed using the CTAB (cetyl-triethyl-ammonium-bromide) method, as outlined by Doyle (1990). Approximately 0.2g of young leaves were homogenized with 800 µl of extraction buffer containing CTAB [(2 % w/v), 20 mM EDTA, 100 mM Tris–HCl (pH 8.0), 1.4 M NaCl, 1 % PVP, 0.1 % sodium bisulfide, and 0.2 % (v/v) 2-mercaptoethanol] using a mortar and pestle. The resulting homogenate was transferred to a 2.5 ml Eppendorf microcentrifuge tube and subjected to a water bath at 55-50 ˚C for 30 minutes with intermittent shaking. Following incubation, an equal volume of chloroform: isoamyl alcohol (24:1, v/v) was added to the microcentrifuge tube, and the mixture was centrifuged at 13,000 rpm for 15 minutes. The clear aqueous phase was carefully transferred to another

Table 1. The details of the advance generation breeding lines and their resistant genes used for screening against Tomato yellow leaf curl virus (TyLCV), Fusarium wilt (race -2) and Root-knot Nematode

S. No.	Lines	Parentage	Resistant Gene		
			TyLCV	FW	RKN
1	CLN2498D	CLN2114A x (CLN2026C x CLN1466I)	*Ty*-2	-	-
2	CLN3241H-27	CLN3149 x (NC03220 X-20-21-1-18-30)	*Ty* -1/*Ty*-3, *Ty*-2	*I*-2	-
3	CLN3938K-8	CLN3641F1-8-11-14-4-25 x T.Star-29-7-4-16	*Ty*-1/*Ty*-3	-	-
4	CLN4032C-8	CLN3682F1-10-3-4-27-3-8 x T.Star-1-28-21-5-27	*Ty*-1/*Ty*-3	-	*Mi*-1
5	CLN4018G	CLN3641F1-8-11-14-4-25-19 x T.Star-1-28-21-5-20	*Ty*-2	-	-
6	CLN3764D-21	CLN3241F1-34-18-6-26-8 x Savior F1	*Ty*-1/*Ty*-3	*I*-2	-
7	FMTT1733D	CLN3022F2-154-45-8-18-21-6 x CLN2866-237-5-17-20-22	*Ty*-3, *Ty*-2	*I*-2	-
8	FMTT1733E	CLN3022F2-154-45-8-18-21-6 x CLN2866-237-5-17-20-22	*Ty*-3, *Ty*-2	*I*-2	-
9	CLN2026D	(CL5915-93D4 x NC82162) x (CRA84-58-1 x UC204A)	-	I-2	-
10	CLN3682C	CLN3125F2-21-15-13-29-25 x CLN3230F1-20-5-19	*Ty*-1/*Ty*-3, Ty-2	-	*Mi*-1
11	CLN4251A	(CLN3682C x UC204A) x CLN3961F1-6-43-1-14-29	*Ty*-1/*Ty*-3, *Ty*-2	*I*-2	-
12	CLN4251C	(CLN3682C x UC204A) x CLN3961F1-6-43-1-14-29	*Ty*-1/*Ty*-3, *Ty*-2	-	*Mi*-1
13	CLN4066F	CLN3682C x CLN3552B	*Ty*-1/Ty-3, *Ty*-2	*I*-2	*Mi*-1
14	CLN4078A	CLN3682C x CLN4020F1	*Ty*-1/*Ty*-3, *Ty*-2	*I*-2	*Mi*-1
15	CLN3961D	CLN3670F1-10-21-14-13-8 x CLN3552F2-1-19-17-27-28-87	*Ty*-1/*Ty*-3, *Ty*-2	-	-

* All the advance generation breeding lines developed from pedigree method of breeding, TyLCV- Tomato yellow leaf curl virus, FW- Fusarium wilt (race -2), RKN- Root-knot Nematode

microcentrifuge tube, and an equal volume of ice-cold isopropanol (3:2, v/v) was added. This solution was then incubated overnight at -20 ˚C to facilitate high-quality DNA precipitation. The sample was subsequently centrifuged at 13,000 rpm for 15 minutes to form a DNA pellet. The resulting DNA pellet was washed with 70% ethanol, air-dried for 5 minutes, and then resuspended in 100 µl of molecular biology-grade (DEPC treated), nuclease, and protease-free water. The DNA concentration and purity were determined using an Eppendorf Bio-spectrometer® Basic, measuring DNA quantity (DNA/µg/mL) and DNA quality (A260/A280). Based on these readings, the DNA was diluted to a concentration of 10 ng/µl for subsequent PCR amplification.

PCR Amplification for the presence of resistant alleles TyLCV**-** *Ty*-**1,** *Ty*-**2,** *Ty*-**3,** Fusarium wilt **(race -2)** *I*-**2** and Root-knot Nematode *Mi*-1: PCR amplification was conducted in a total volume of 25 µl, comprising 10× PCR buffer, 0.25 mM dNTPs, 0.2 U Taq DNA polymerase, 10 pmol of each gene-specific primer, and 20 ng of genomic DNA. The PCR thermal cycling was carried out using the Eppendorf 6331 Mastercycler® Nexus Gradient, employing the following thermal profile: an initial denaturation step at 94 °C for 4 minutes, followed by 35 cycles of denaturation at 94 °C for 40 seconds, annealing at the gene-specific temperature and time, initial extension at 72 °C for 60 seconds, and a final extension step at 72 °C for 10 minutes, followed by a 4 °C hold. The details of SCAR markers, including gene-specific primers and their respective annealing temperatures and times, are given in **Table 2.**

The PCR products were separated by electrophoresis in a 1.5% (w/v) agarose gel stained with ethidium bromide, utilizing a TAE buffer system. Gel documentation was performed using a Bio RAD Gel Doc XR+ System with Image Lab software. The size of the gene-specific PCR products was determined by comparing them with a 100 bp DNA ladder. To ensure the accuracy of the results, PCR amplification and gel electrophoresis were repeated at least twice for each marker.

RESULTS AND DISCUSSION

The effectiveness of disease resistant molecular screening depends up on the selection so suitable markers, as these markers have to span the entire genomic region in accurate identification and differentiate the resistant line carrying gene with that of the susceptible lines that lack in gene of interest. The current investigation demonstrated that all selected co-dominant and dominant Sequence Characterized Amplified Region (SCAR) markers, selected for screening resistant alleles associated with TyLCV- *Ty*-1, *Ty*-2, *Ty*-3, Fusarium wilt (race-2) *I*-2, and Root-knot Nematode *Mi*-1, exhibited high robustness in identifying the resistant gene harbouring line with that the susceptible. Moreover, the selected co-dominant markers demonstrated the capability to discern allele zygosity, distinguishing between homozygous and heterozygous states.

Table 2. The details of SCAR markers with gene-specific primers, annealing temperatures for TyLCV, Fusarium wilt and Root-knot Nematode

Disease	Gene	Primer	Marker type	Primer sequence (5´-3´)	Annealing temperature (°C)	Fragment Size (bp)	Reference
TyLCV	*Ty*-1/3	M2	Co-Dominant	F: GATCCGTTGATTGAAGAAAT	56˚C- 1 min	264 (R)	Chen *et al.* (2015)
				R: AGGAAGAGGAGAGACAATCC		252 (S)	
	Ty-2	P1-16	Dominant	F: CACACATATCCTCTATCCTATTAGCTG	58˚C- 1 min	300 (R)	Yang *et al.* (2014)
				R: CGGAGCTGAATTGTATAAACACG		600 (S)	
		TG0302	Co-Dominant	F: TGGCTCATCCTGAAGCTGATAGCGC	55˚C- 1 min	900 (R)	Garcia *et al.* (2007)
				R: AGTGTACATCCTTGCCATTGACT		800 (S)	
	Ty-3	SCAR 1	Co-Dominant	F: GCTCAGCATCACCTGAGACA	58˚C- 20 Sec	519 (R)	Dong *et al.* (2016)
				R: TGCAGGAACAGAATGATAGAAAA		269 (S)	
		P6-25	Co-Dominant	F: GGTAGTGGAAATGATGCTGCTC	53˚C- 1 min	Ty3 – 600 (R) Ty3a – 630 (R) Ty3b – 450 (R)	(Ji *et al.,* 2007; Salus *et al.,* 2007; Nevame *et al.,* 2018
				R: GCTCTGCCTATTGTCCCATATATAACC		320 (S)	
FW	I-2	I-2/5	Co-Dominant	F: CAAGGAACTGCGTCTGTCTG	65˚C- 30 sec	633 (R)	Korzh and Dubina, 2022
				R: ATGAGCAATTTGTGGCCAGT		693 or in combined with 760 (S)	
		Z1063	Dominant	F: ATTTGAAAGCGTGGTATTGC	54˚C- 1 min	~ 940 (R) No band (S)	Arens *et al.* 2010
				R: CTTAAACTCACCATTAAATC			
RKN	Mi-1.2	Mi-23	Co-Dominant	F: TGGAAAAATGTTGAATTTCTTTTG	56˚C- 30 sec	430 (S) 380 (R)	El Mehrach *et al* (2005)
				R: GCATACTATATGGCTTGTTTACCC			
		PM3Fb	Dominant	F: CACACATGAGGTATGTTCGTATTATGG	55˚C- 1 min	500 (R)	El Mehrach *et al* (2005)
				R: TCACAGCCTAGCTTTTGAATCAGTACC			

TyLCV – Tomato yellow leaf curl virus, RKN – Root-knot nematode (*Meloidogyne incognita*), FW- Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici* -Race 2

Molecular screening for TyLCV *Ty*-1 gene**:** A co-dominant M2 SCAR marker was utilized to screen the *Ty*-1 resistant allele and those with the susceptibility allele. The marker was designed to span the entire *Ty*-1 genomic locus, selectively amplifying a target DNA fragment linked to a 12 bp deletion within the susceptibility allele at the *Ty*-1 locus. This targeted deletion within the gene serves as a discriminatory factor, enabling the clear identification of lines harbouring the *Ty*-1 resistance allele from those carrying the susceptibility allele. The M2 on PCR amplication gave a single amplified fragment of 264 bp with the ten resistant lines such as, CLN3241H-27, CLN3938K-8, CLN4032C-8, CLN3764D-21, CLN3682C, CLN4251A, CLN4251C, CLN4066F, CLN4078A and CLN3961D carrying *Ty*-1 gene in homozygous condition their genomic allele. Whereas, the five lines, CLN2498D, CLN4018G, FMTT1733D, FMTT1733E and CLN2026D exhibited homozygous susceptible for *Ty*-1 gene and gave a single amplicon at 252 bp, signifying the lack *Ty*-1 gene in their genome **(Fig. 1).** Chen *et al.* (2015) found comparable outcomes from their screening for *Ty*-1 gene using F2 generation and wild accessions *S. chilense* tomato genotypes

Molecular screening for **TyLCV *Ty*-2** gene**:** The two markers, P1-16 dominant and TG0302 co-dominant provide the similar results in identifying the resistant lines carrying *Ty*-2 gene with that of the susceptible lines, which lack in *Ty*-2 gene. Moreover, TG0302 primer effectively distinguished between homozygous and heterozygous condition of *Ty*-2 gene, due to it co-dominant nature. The primer pair of P1-16 produce a single band with the band length of 300 bp for resistant lines such as, CLN2498D, CLN3241H-27, CLN4018G, FMTT1733D, FMTT1733E, CLN3682C, CLN4251A, CLN4251C, CLN4066F, CLN4078A and CLN3961D harbouring Ty-2 gene in their genome. The reaming four lines CLN3938K-8, CLN4032C-8, CLN3764D-21 and CLN2026D amplified at 600 bp indication susceptible for *Ty*-2 gene **(Fig. 2).** Where the TG03032 primers leaves a single band at 900 bp with the homozygous resistant *Ty*-2 gene carrying lines CLN2498D, CLN3241H-27, CLN4018G, FMTT1733D, FMTT1733E, CLN3682C, CLN4251A, CLN4251C, CLN4066F, CLN4078A and CLN3961D. Meanwhile, the lines CLN3938K-8, CLN4032C-8, CLN3764D-21 and CLN2026D amplified at 800 bp exhibited homozygous susceptibility for *Ty*-2 gene **(Fig. 3).** The results

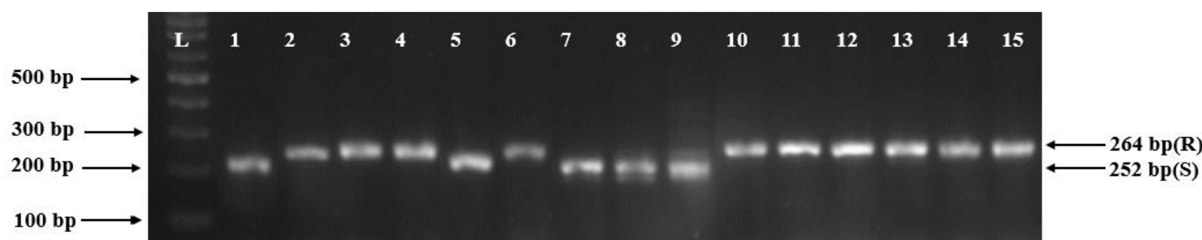

Fig. 1 Molecular validation of TyLCV, *Ty*-1 gene using M2 primer pair

L- indicates lane- 100 bp ladder, 1- CLN2498D, 2- CLN3241H-27, 3- CLN3938K-8, 4- CLN4032C-8, 5- CLN4018G, 6- CLN3764D-21, 7- FMTT1733D, 8- FMTT1733E, 9- CLN2026D, 10- CLN3682C, 11- CLN4251A, 12- CLN4251C, 13- CLN4066F, 14- CLN4078A, 15- CLN3961D, R indicate- Resistant lines amplified at 264 bp, S- indicate- Susceptible line amplified at 252 bp

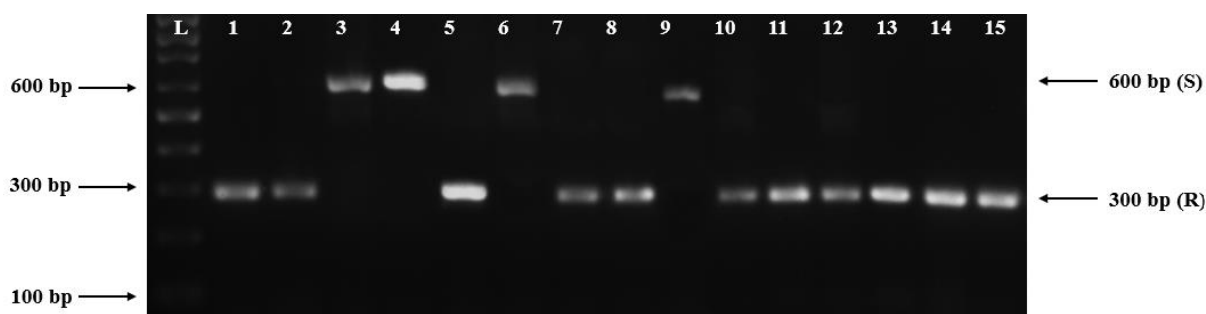

Fig. 2 Molecular validation of TyLCV, *Ty*-2 gene using P1-16 primer pair

L- indicates lane- 100 bp ladder, 1- CLN2498D, 2- CLN3241H-27, 3- CLN3938K-8, 4- CLN4032C-8, 5- CLN4018G, 6- CLN3764D-21, 7- FMTT1733D, 8- FMTT1733E, 9- CLN2026D, 10- CLN3682C, 11- CLN4251A, 12- CLN4251C, 13- CLN4066F, 14- CLN4078A, 15- CLN3961D, R indicate- Resistant lines amplified at 300 bp, S- indicate- Susceptible line amplified at 600 bp

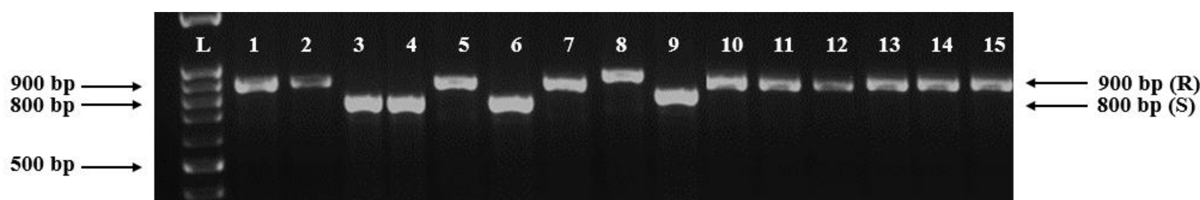

Fig. 3 Molecular validation of TyLCV, *Ty*-2 gene using TG0302 primer pair

L- indicates lane- 100 bp ladder, 1- CLN2498D, 2- CLN3241H-27, 3- CLN3938K-8, 4- CLN4032C-8, 5- CLN4018G, 6- CLN3764D-21, 7- FMTT1733D, 8- FMTT1733E, 9- CLN2026D, 10- CLN3682C, 11- CLN4251A, 12- CLN4251C, 13- CLN4066F, 14- CLN4078A, 15- CLN3961D, R indicate- Resistant lines amplified at 900 bp, S- indicate- Susceptible line amplified at 800 bp

of the current study are in consistent with those of Garcia *et al*. 2007 regarding the detection of the *Ty*-2 locus in tomato germplasm derived from *Solanum habrochaites* using the Co-dominant SCAR marker.

Molecular screening for TyLCV *Ty*-**3** gene: The two co-dominant SCAR markers SCAR1 and P6-25 on *Ty*-3 gene screening exhibited the similar results in precise detection of *Ty*-3 gene carrying lines. Meanwhile, the P6-25 marker provides additional genetic insights into *Ty*-3 alleles at the specified locus, specifically discerning between *Ty*-3a and *Ty*-3b alleles in their genome. The SCAR1 co-dominant marker on PCR amplication gave a single molecular band at 519 bp with that of the twelve resistant lines such as, CLN3241H-27, CLN3938K-8, CLN4032C-8, CLN3764D-21, FMTT1733D, FMTT1733E, CLN3682C, CLN4251A, CLN4251C, CLN4066F, CLN4078A and CLN3961D carrying *Ty*-3 resistant allele in homozygous

condition. Whereas, the three lines namely, CLN2498D, CLN4018G and CLN2026D amplified at 269 bp indicating the lack of *Ty*-3 gene and governing for *Ty*-3 gene susceptibility **(Fig. 4).** The current findings regarding the SCAR 1 marker align with the observations made by Dong *et al.* (2016), on screening the segregating populations derived from crosses involving *S. lycopersicum* - A45 (resistant) and *S. lycopersicum* - A39 (susceptible) using the Ty-3 gene- SCAR 1 based marker. The P6-25 markers gave a monomeric band of 630 bp with the three lines namely, CLN3938K-8, CLN4032C-8 and FMTT1733E carrying *Ty*-3a resistant allele. On the other hand, CLN3241H-27, CLN3764D-21, FMTT1733D, CLN3682C, CLN4251A, CLN4251C, CLN4066F, CLN4078A and CLN3961D lines amplified at 450 bp signifying these lines harbouring *Ty*-3b resistant allele in their genome. The reaming three lines namely, CLN2498D, CLN4018G and CLN2026D gave a single band at 320 bp indication this line lack of *Ty*-3 gene of interest **(Fig. 5).** The findings were in accordance with the results of Prasanna *et al.* 2015. When he screened using P6-25 marker for *Ty*-2 and *Ty*-3 gene, in the F_4 plants (double heterozygous) and F_5 progenies (segregating population developed from *Ty*-2 and *Ty*-3).

Molecular screening for fusarium wilt I-2 gene: The co-dominant SCAR markers I-2/5 and dominant Z1063 marker employed in I-2 gene screening demonstrated congruent and precise detection results, underscoring their efficacy in identifying *I*-2 gene carrying lines. The marker I-2/5 yielded a single molecular band at 633bp, consistently observed across eight resistant lines such as, CLN3241H-27, CLN3764D-21, FMTT1733D, FMTT1733E, CLN2026D, CLN4251A, CLN4066F and CLN4078A indicative the homozygous presence of the *I*-2 resistant allele. Conversely, seven lines, CLN2498D, CLN3938K-8, CLN4032C-8, CLN4018G, CLN3682C, CLN4251C and CLN3961D exhibited a 760 bp band, signifying the absence of the *I*-2 gene and susceptibility to *I*-2 **(Fig. 6).** The present results are in line with the finding of Svetlana and Elena. 2022 and Shamshin *et al*. 2019, When they screened the existing tomato hybrids with the I2/5,

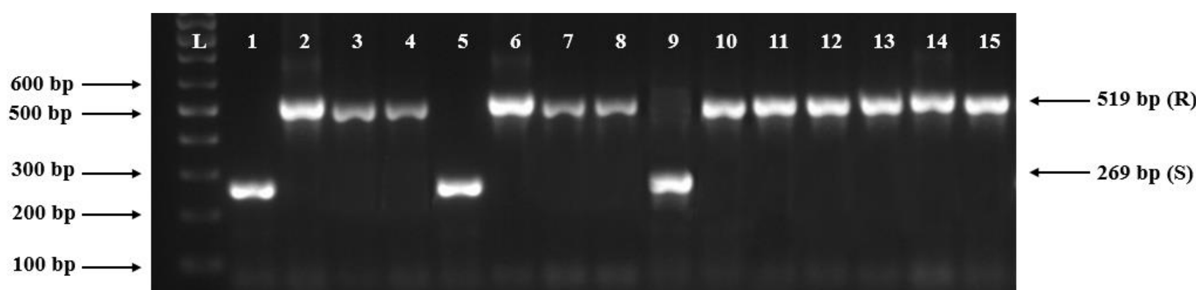

Fig. 4 Molecular validation of TyLCV, *Ty*-3 gene using SCAR 1 primer pair

L- indicates lane- 100 bp ladder, 1- CLN2498D, 2- CLN3241H-27, 3- CLN3938K-8, 4- CLN4032C-8, 5- CLN4018G, 6- CLN3764D-21, 7- FMTT1733D, 8- FMTT1733E, 9- CLN2026D, 10- CLN3682C, 11- CLN4251A, 12- CLN4251C, 13- CLN4066F, 14- CLN4078A, 15- CLN3961D, R indicate- Resistant lines amplified at 519 bp, S- indicate- Susceptible line amplified at 269 bp

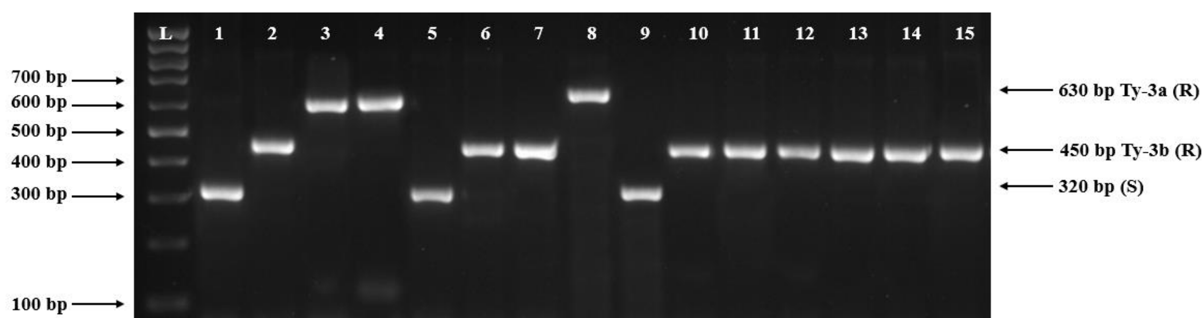

Fig. 5 Molecular validation of TyLCV, *Ty*-3 gene using P6-25 primer pair

L- indicates lane- 100 bp ladder, 1- CLN2498D, 2- CLN3241H-27, 3- CLN3938K-8, 4- CLN4032C-8, 5- CLN4018G, 6- CLN3764D-21, 7- FMTT1733D, 8- FMTT1733E, 9- CLN2026D, 10- CLN3682C, 11- CLN4251A, 12- CLN4251C, 13- CLN4066F, 14- CLN4078A, 15- CLN3961D, R indicate- Resistant lines carrying *Ty*-3a gene amplified at 630 bp and *Ty*-3b gene amplified at 450 bp, S- indicate- Susceptible line amplified at 320 bp

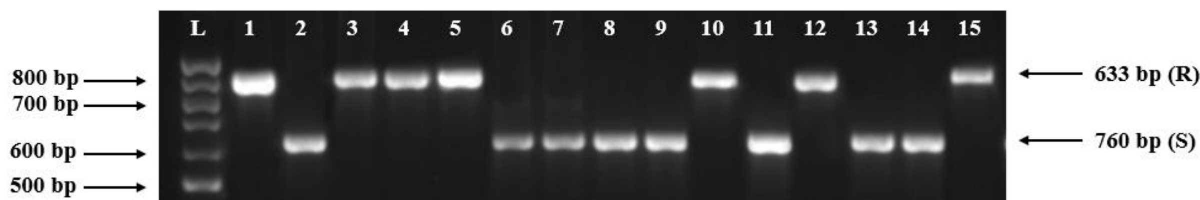

Fig. 6 Molecular validation of Fusarium wilt (Race-2), *I*-2 gene using I-2/5 primer pair

L- indicates lane- 100 bp ladder, 1- CLN2498D, 2- CLN3241H-27, 3- CLN3938K-8, 4- CLN4032C-8, 5- CLN4018G, 6- CLN3764D-21, 7- FMTT1733D, 8- FMTT1733E, 9- CLN2026D, 10- CLN3682C, 11- CLN4251A, 12- CLN4251C, 13- CLN4066F, 14- CLN4078A, 15- CLN3961D, R indicate- Resistant lines amplified at 633 bp, S- indicate- Susceptible line amplified at 760 bp

IOH2 and At-1 markers for detection of race 1 and race 2 of *Fusarium oxysporum f. ssp. Lycopersici*. Whereas. The dominant Z1063 markers gave a monomeric band of 900 bp approximately with the resistant *I*-2 gene carrying lines namely, CLN3241H-27, CLN3764D-21, FMTT1733D, FMTT1733E, CLN2026D, CLN4251A, CLN4066F and CLN4078A. While the markers do not produce a band with the seven susceptible lines CLN2498D, CLN3938K-8, CLN4032C-8, CLN4018G, CLN3682C, CLN4251C and CLN3961D. These no banding pattern signifies that these lines lack in harbouring *I*-2 gene and govern for susceptibility to *I*-2 gene **(Fig. 7).** The current findings align with the study conducted by Popoola et al. (2014), where they utilized Nigerian tomato accessions and two Cleaved Amplified Polymorphic Sequence (CAPS) markers to detect *Fusarium oxysporum f. ssp. lycopersici*, focusing on race 1 and race 2 strains. These markers improved the accuracy of identifying tomato accessions with resistance to Fusarium vascular wilt.

Molecular screening for Root-knot nematode *Mi*-1 gene:The co-dominant SCAR markers Mi-23 and the dominant PM3Fb marker utilized in the screening of the *Mi*-1 gene exhibited a similar and accurate detection of *Mi*-1 gene. Additionally, the Mi-23 primer proved to be effective in discerning between homozygous and heterozygous conditions of the *Ty*-2 gene, attributable to its co-dominant nature. The Mi-23 on amplication gave one amplicon of 380 bp with the respective homozygous resistant lines such as, CLN4032C-8, CLN3682C, CLN4251C, CLN4066F and CLN4078A carrying Mi-23 gene in their locus. While the reaming nine lines namely, CLN2498D, CLN3241H-27, CLN3938K-8, CLN4018G, CLN3764D-21, FMTT1733D, FMTT1733E, CLN2026D, CLN4251A and CLN3961D showed susceptibility to *Mi*-1 gene lacking lines and amplified at 430 bp **(Fig. 8).** On the other hand, the PM3Fb markers only gave a single band of 500 bp with the five resistant lines namely CLN4032C-8, CLN3682C, CLN4251C, CLN4066F and CLN4078A signifying Mi-1 gene. Where the lines such as, CLN2498D, CLN3241H-27, CLN3938K-8, CLN4018G, CLN3764D-21, FMTT1733D, FMTT1733E, CLN2026D, CLN4251A and CLN3961D produce no band up on amplification, this absence of banding patter determine these lines lack in *Mi*-1 gene for governing resistance to root-knot nematode **(Fig. 9).** The current findings align with the research by El Mehrach *et al*. (2005) and Garcia *et al*. (2007), regarding the screening of the *Mi*-1 gene, which confers resistance to root-knot nematodes.

In conclusion, the gene-based molecular screening with SCAR markers, including M2, TG0302, P6-25, I-2/5, and Mi-23 are robust in distinguishing resistant alleles of tomato yellow leaf curl virus (TyLCV), fusarium wilt and root-knot nematode. This approach accelerates resistant breeding, provides genetic insights and aids in developing resilient tomato cultivars.

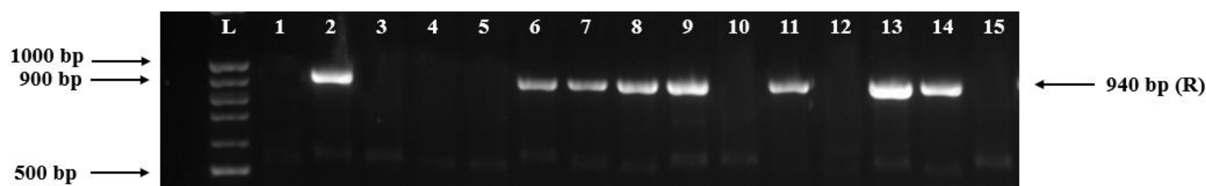

Fig. 7 Molecular validation of Fusarium wilt (Race-2), *I*-2 gene using Z1063 primer pair

L- indicates lane- 100 bp ladder, 1- CLN2498D, 2- CLN3241H-27, 3- CLN3938K-8, 4- CLN4032C-8, 5- CLN4018G, 6- CLN3764D-21, 7- FMTT1733D, 8- FMTT1733E, 9- CLN2026D, 10- CLN3682C, 11- CLN4251A, 12- CLN4251C, 13- CLN4066F, 14- CLN4078A, 15- CLN3961D, R indicate- Resistant lines amplified at 940 bp, Susceptible line has no band formation amplified

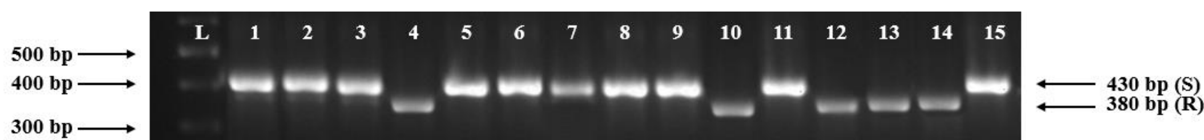

Fig. 8 Molecular validation of Root-knot nematode, *Mi*-1 gene using Mi-23 primer pair

L- indicates lane- 100 bp ladder, 1- CLN2498D, 2- CLN3241H-27, 3- CLN3938K-8, 4- CLN4032C-8, 5- CLN4018G, 6- CLN3764D-21, 7- FMTT1733D, 8- FMTT1733E, 9- CLN2026D, 10- CLN3682C, 11- CLN4251A, 12- CLN4251C, 13- CLN4066F, 14- CLN4078A, 15- CLN3961D, R indicate- Resistant lines amplified at 380 bp, S- indicate- Susceptible line amplified at 430 bp

Fig. 9 Molecular validation of Root-knot nematode, *Mi*-1 gene using PM3Fb primer pair

L- indicates lane- 100 bp ladder, 1- CLN2498D, 2- CLN3241H-27, 3- CLN3938K-8, 4- CLN4032C-8, 5- CLN4018G, 6- CLN3764D-21, 7- FMTT1733D, 8- FMTT1733E, 9- CLN2026D, 10- CLN3682C, 11- CLN4251A, 12- CLN4251C, 13- CLN4066F, 14- CLN4078A, 15- CLN3961D, R indicate- Resistant lines amplified at 500 bp, Susceptible line has no band formation amplified

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