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A Comparison between CAPS and SCAR Markers in the Detection of Resistance Genes in some Tomato Genotypes against *Tomato Yellow Leaf Curl Virus* and Whitefly

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Abstract

The *Tomato yellow leaf curl virus* (TYLCV) is transmitted by the whitefly vector (*Bemisia tabaci*) and can cause big losses in tomato yields in Egypt and worldwide. Several genes linked to resistance to TYLCV (*Ty-1*, *Ty-2* and *Ty-3*) and whitefly (*Mi-1.2*) in wild species and cultivated tomatoes were detected. Cleaved amplified Polymorphic Sequence (CAPS) and Sequence Characterized Amplified Region (SCAR) markers are used as tools in tomato lines carrying different combinations of *Ty-1*, *Ty-2*, *Ty-3* and *Mi-1.2* alleles. In this study, a total of nineteen tomato genotypes have been selected for the existence of resistance alleles, using five CAPS and three SCAR markers. Resistant allele-specific fragments for TYLCV and whitefly have been identified in some of the genotypes used in the current research. Thus, CAPS markers closely linked to *Ty-1* and *Mi-1.2* discriminated homozygous and heterozygous tomato genotypes. In addition, Sequence Characterized Amplified Region (SCAR) markers linked to *Ty-2* and *Ty-3* genes separated both dominant and recessive alleles in tomato plant materials. On the contrary, CAPS (*Ty-2* and *Ty-3*) and SCAR (*Mi-1.2*) markers gave false positive results. These molecular markers are considered advantageous tools for pyramiding resistance genes of several genotypes into a single line, improving the resistance to *begomoviruses*.

Keywords: *Solanum lycopersicon*, Wild tomato, *Ty-1*, *Ty-2*, *Ty-3*, *Mi-1.2*, Disease resistance.

1. Introduction

Tomatoes (*Solanum lycopersicum*) are economically important horticultural crops which belong to the *Solanaceae* family, and are used as food for humans (Peralta *et al*., 2008). Tomato yields are often infected by the *Tomato yellow leaf curl virus* (TYLCV) which causes 100% significant losses in tomato crops in the tropical and subtropical regions around the world (Czosnek, 2007; Mahfouze *et al*., 2015). TYLCV belongs to the genus *Begomovirus* (family *Geminiviridae*) (Belabess *et al*., 2016). The virus is transmitted by the sweetpotato whitefly vector (*Bemisia tabaci*) in a persistent and circulative manner (Mahfouze *et al*., 2017; Geng *et al*., 2018). Although, the use of insecticides for the sake of whitefly insect control can reduce the spread of the virus, epidemics can appear and resistance of whitefly to insecticides has been reported (Feng *et al*., 2010; Schuster *et al*., 2010). The breeding of tomatoes resistant or tolerant to TYLCV is one of the most effective strategies to reduce the yield losses. No resistance has been recorded to date in the *S. lycopersicum* genotype, though some tomato lines have been found to be less susceptible than others. Fortunately, new sources of resistance to TYLCV have been found in several wild tomato species (Ji *et al*., 2007a; Scott, 2007). Several resistance genes to TYLCV have been identified (Ji *et al*., 2007a, 2009; Anbinder *et al*., 2009). The TYLCV resistance gene *Ty-1* originated from *S. chilense* line LA1969 and is located on chromosome 6 of tomato (Zamir *et al*., 1994). The *Ty-2* locus (Hanson *et al*., 2000), was identified in *S. habrochaites* B6013 (Kalloo and Banerjee, 1990), mapped on the long arm of chromosome 11 (Hanson *et al*., 2006). The third TYLCV resistance gene *Ty-3* was identified in resistant tomato genotypes derived from accessions LA2779 and LA1932 of *S. chilense* and is located on the long arm of chromosome 6, 15 cM away from *Ty-1* locus (Ji *et al*., 2007a). The dominant whitefly or root-knot nematode resistance gene *Mi-1* was introgressed from *S. peruvianum* into cultivated tomato (Messeguer *et al*., 1991; Chen *et al*., 2015). The introgressed DNA region carries many genes and pseudogenes, among which only *Mi-1.2* was proven to give resistance to whiteflies (Nombela *et al*., 2003), nematodes (Milligan *et al*., 1998) and aphids (Rossi *et al*., 1998).

A significant advance has been made in the development of molecular markers associated with disease resistance genes (Ji *et al*., 2007b; Pérez de Castro *et al*., 2007; Anbinder *et al*., 2009). The markers associated with resistance genes can be used to select novel resistant sources at early stages without inoculation with the pathogen, hence shortening the length in breeding programs. In addition, molecular markers are powerful

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tools in pyramiding programs, in which different resistance gene alleles are introgressed in a single tomato line to increase the effectiveness and durability of resistance against diseases, also reducing the cost of breeding resistant plants (Vidavski *et al*., 2008; Slater *et al*., 2013).

The objective of this study is to identify and compare two functional molecular markers, namely CAPS and SCAR linked to resistance genes for TYLCV (*Ty-1*, *Ty-2* and *Ty-3*) and whitefly resistance locus (*Mi-1.2*) in wild and cultivated tomato genotypes, which can be used as marker-assisted selection (MAS) in breeding programs.

2. Materials and Methods

2.1. Plant Materials

A total of nineteen tomato genotypes, including commercial cultivars and accessions were used in this research. Fifteen tomato accessions were provided by the Tomato Genetics Resource Center (TGRC), Department of Plant Sciences, University of California, Davis, CA 95616 (http://tgrc.ucdavis.edu) and two accessions supported by the Centre for Genetic Resources (CGN), Netherlands (http://www.wur.nl). In addition, two commercial cultivars were obtained from the Egyptian Company for Seeds, Oils and Chemicals, Egypt to be used in this study (Table 1). Twenty tomato seeds of each genotype were germinated in a greenhouse until true leaves developed at a temperature regime of 27°C:16°C (Light: Dark), a photoperiod of L16:D8 h, and a relative humidity of 68-75%. The seedlings were planted in peatmoss: sand (2:1) in pots irrigated with a nutritive complex N: P: K (20:20:20).

Table 1. Tomato genotypes used in this study.

No.	Genotype	Source	No.	Genotype	Source
1	*Solanum hirsutum* 24036	CGN	11	*S. chilense* 56139	CGN
2	*S. galapagense* 0317	TGRC	12	*S. lycopersicon* cv. Super Marmande	Egypt
3	*S. neoricki* 0247	TGRC	13	*S. lycopersicon* cv. Strain B F1	Egypt
4	*S. arcanum* 1346	TGRC	14	*S. corneliomulleri* 1283	TGRC
5	*S. corneliomulleri* 1274	TGRC	15	*S. habrochaites* 1739	TGRC
6	*S. pennellii* 1733	TGRC	16	*S. pimpinellifolium* 1279	TGRC
7	*S. huaylasense* 1358	TGRC	17	*S. pimpinellifolium* 1332	TGRC
8	*S. pimpinellifolium* 1342	TGRC	18	*S. pennellii* 2963	TGRC
9	*S. peruvianum* 1333	TGRC	19	*S. pennellii* 1942	TGRC
10	*S. habrochaites* 1352	TGRC			

CGN= Centre for Genetic Resources, The Netherlands; TGRC= Tomato Genetics Resource Center (TGRC), Department of Plant Sciences, University of California, Davis.

2.2. Virus Resistance Tests

2.2.1. Source of TYLCV Isolate

The *Tomato yellow leaf curl virus* (TYLCV) isolate was obtained from the Virology Laboratory, Department of Agricultural Microbiology, Faculty of Agriculture, University of Ain Shams, and was previously isolated and identified from systemically infected tomato plants. The isolate was maintained on tomato plants cv. Super Marmande. Systemically, the infected leaves were used as sources of inoculum in all experiments.

2.2.2. TYLCV Inoculation

One-month age tomato genotypes (15 plants/genotype) grown in the greenhouse were inoculated by syringes using TYLCV infected tomato sap according to Allam *et al*., (1994). Inoculated plants were observed for the development of TYLCV symptoms for eight weeks post-inoculation. All of the TYLCV inoculated tomato plants were tested for the presence of viral DNA by PCR using the TYLCV specific primer sets. These materials were evaluated in two seasons during 2015/16 and 2016/17 in the greenhouse.

2.2.3. Extraction of DNA

DNA was extracted from healthy and TYLCV-inoculated fresh tomato leaves, after two months of inoculation. Around 30 mg of tissue was ground in liquid nitrogen and extracted with the DNA purification Kit (Bio Basic, Inc., Markham, Canada) following the manufacturer's instructions. DNA quality and quantity were determined by agarose gel electrophoresis and Spectrophotometer. DNA concentrations were adjusted to 50 ng/µL and the extracts were frozen at -20^{o}C.

2.2.4. Detection of TYLCV by PCR

TYLCV-specific primer sets were designed based on the sequence of TYLCV isolate (KP725055.1) from the GenBank database using the primer3 software program (Rozen and Skaletsky, 2000). The primers set TYLCVF/ TYLCVR were designed to amplify the *AV1* gene (encodes a coat protein) of the TYLCV isolate (manufactured by Bio Basic, Canada) (Table 2). PCR reaction mixtures of 25 µL contained: 2.5 µL of 2.5 mM dNTPs; 2.5 µL 10X PCR buffer; 2.5 µL 25 mM $MgCl_2$; 1 µL 2 U *Taq* DNA polymerase; 2.5 µL each forward and reverse-sense primer at 10 µM; 1 µL sample DNA and 11.5 µL dsH_2O. The PCR was carried out in Thermocycler ((Biometra, biomedizinische Analytik, Germany GmbH). The PCR was carried out by denaturing DNA template at 94°C for four minutes, followed by thirty-five cycles of denaturing at 94°C for one minute, primer annealing at 55°C for one minute and DNA extension at 72°C for one minute. Then, it was followed by a final DNA extension at 72°C for seven minute.

2.3. PCR Amplification of Alleles Resistant to TYLCV (Ty-1, Ty-2 and Ty-3) and Whitefly (Mi-1.2)

2.3.1. Cleaved Amplified Polymorphic Sequence (CAPS) Markers

Five markers TY-1CAPS-TG178, JB1, TY-2CAPS-TG105A, TY-3CAPS-FER-G8 and MI-REXCAPS (manufactured by Bio Basic, Canada) and the sequence of primers are shown in Table 2. PCRs were performed in 25 µL reaction volumes containing 1 µL of 50 ng/µL genomic DNA, 1X buffer, 0.5 µM of each primer, 0.6 mM dNTPs, 1.4 mM $MgCl_2$ and 1 unit of *Taq* polymerase (Promega, USA). The following conditions were used: an initial denaturation step at 95°C for two minutes, followed by thirty-five cycles of 95°C for forty seconds, annealing temperature (Table 2) for forty seconds, 72°C for sixty seconds and a final extension at 72°C for five minutes.

Table 2. Primer sequences used in this study.

Primer and markers	*R*-gene*	Chromosome No	Single nucleotide sequence (5'-3')	Annealing temperature (AT)°C	Restriction enzyme	Molecular size of band (bp)	Molecular size after digestion with *Taq*I (bp)	References
TYLCV AV1 F	-	-	TGACAAAGACATGCGGACCA	55	-	335	-	Present study
TYLCV AV1R	-	-	TGGGCTGTCGAAGTTGAGAC					
TY-1CAPS-TG178F	*Ty1*	6	GGTACTCCTGGAAGGGTTAAGG	56	*Taq*I	1000	>200 200	Barbieri *et al.*, (2010)
TY-1CAPS-TG178R			CACGCTGGTTCTGTTGTATCTC				160	
JB1CAPSF	*Ty1*	6	AACCATTATCCGGTTCACTC	53	*Taq*I	950	450	Pérez *et al.*, (2007)
JB1CAPSR			TTTCCATTCCTTGTTTCTCTG				500	
TY-2CAPS-TG105A F	*Ty2*	11	CTTCAGAATTCCTGTTTTAGTCAGTTGAACC	62	*Taq*I	500	330	Maxwell *et al.*, (2006)
TY-2CAPS-TG105AR			ATGTCACATTTGTTGCTTGGACCATCC				220	
TY-3CAPS-FER-G8F	*Ty3*	6	CATCCC GTGCATCATCCAAAGTGAC	55	*Taq*I	500	50 200 250	Jensen *et al.*, (2007)
TY-3CAPS-FER-G8R			CTAAGGGTGTACCCCAAGGGAAC				300 500	
MI -REX CAPS F	*Mi1.2*	6	TCGGAGCCTTGGTCTGAATT	55	*Taq*I	750	180	Michelson *et al.*, (1994)
MI-REX CAPSR			GCCAGAGATGATTCGTGAGA				570 750	
Ty-2SCAR T0302F	*Ty2*	11	TGGCTCATCCTGAAGCTGATAGCGC	55	-	900	-	Yang *et al.*, (2014)
Ty-2SCAR T0302R			AGTGTACATCCTTGCCATTGACT			800		
Ty-3SCAR P6-25F	*Ty3*	6	GGTAGTGGAAATGATGCTGCTC	53	-	320 450	-	Ji *et al.*, (2008)
Ty-3SCAR P6-25R			GCTCTGCCTATTGTCCCATATATAACC			660		
Mi-1.2SCARMi23F	*Mi1.2*	6	TGGAAAAATGTTGAATTTCTTTTG	57	-	380	-	Seah *et al.*, (2007)
Mi-1.2SCARMi23R			GCATACTATATGGCTTGTTTACCC			430		

**Tomato yellow leaf curl virus* (TYLCV) resistance genes.

2.3.1.1. Restriction Digestion and Analysis

CAPS-based markers were digested by the restriction enzymes *TaqI* (Table 2). 20 µL reaction mixture containing 16.3 µL dsH_2O, 2 µL restriction enzyme 10X buffer, 0.2 µL BSA (Bovine serum albumin), 0.5 µL *Taq*I restriction enzyme 10 U/µL (Promega Corp.) and 1 µL DNA. The reaction mixture was placed in a 65°C water bath for about two hours according to the manufacturer's instructions.

2.3.2. Sequence Characterized Amplified Region (SCAR) Markers

Three markers Ty-2SCART0302, Ty-3SCARP6-25 and Mi-1.2SCARMi23 (manufactured by Bio Basic, Canada) and the sequence of primers are shown in Table 2. PCR parameters were for 25 µL reactions containing 2.5 µL 2.5 mM dNTPs, 5 µL 5X buffer, 2.5 µL 2.5 mM $MgCl_2$, 0.1 µL (0.5 units) *Taq* DNA polymerase (Promega Corp., Madison, WI), 2.5 µL each forward and reverse primers at 10 µM, 1 µL of DNA extract and 8.9 µL dsH_2O. PCR cycles were 94°C for four minutes, thirty-five cycles of 94°C for thirty seconds, annealing temperature (Table 2) for one minute and 72°C for 1.5 minute. These cycles were followed by 72°C for ten minutes and then the reaction was held at 4°C. PCR reactions were performed in the Thermocycler.

All of the PCR and restriction-digested products were analyzed with 1.5% agarose gel electrophoresis in 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3), and were stained with ethidium bromide, and visualized with UV light.

3. Results

3.1. Evaluation of TYLCV Resistance in the Tomato Genotypes

TYLCV inoculation tests were performed on nineteen tomato genotypes which showed different responses to the TYLCV infection (Table 3). Resistant tomato line *S. corneliomulleri* 1274 has not shown TYLCV symptoms eight weeks after inoculation. However, ten tomato genotypes were tolerant to TYLCV which showed mild to no-symptoms including leaf cup shape and necrosis. On the contrary, eight susceptible tomato accessions displayed moderate to severe symptoms involved leaf curling, crinkle, epinasty, vein clearing, small leaf size, yellowing, veinal necrosis and deformation (Figure 1 and Table 3).

The researchers have designed AV1 primer specific of the TYLCV coat protein gene (*AV1*), in order to detect TYLCV in the nineteen tomato genotypes. The results showed that all tested tomato lines recorded of one amplicon of 335 bp except *S. corneliomulleri* 1274 have not recorded any product, which displayed resistance to the TYLCV isolate.

Table 3. Tomato genotypes used to evaluate gene-based markers for resistances to TYLCV.

No.	Genotype	*Tomato yellow leaf curl virus* (TYLCV) resistance genes and DNA markers								Result of infection test	Detection of TYLCV by PCR
		Ty-1		*Ty-2*		*Ty-3*		*Mi-1.2*			
		JB1-CAPS	CAPS-TG178	CAPS-TG105	SCAR T0302	CAPS-FER-G8	SCAR- P6-25	MI-REX CAPS	SCAR-Mi23		
1	*Solanum hirsutum* 24036	H(*Ty1*/ ty_1)	S(ty_1/ty_1)	R(*Ty2*/*Ty2*)	H(*Ty2*/ ty_2)	H(*Ty3*/*ty3*)	R(*Ty3*/*Ty3*)	*Mi*/*mi*	-	LC, Y, C (Susceptible)	+
2	*S. galapagense* 0317	S(ty_1/ty_1)	S(ty_1/ty_1)	H(*Ty2*/ ty_2)	S(ty_2/ty_2)	H(*Ty3a*/*ty3*)	S(ty_3/ty_3)	*mi*/*mi*	*mi*/*mi*	LC, C (Susceptible)	+
3	*S. neoricki* 0247	S(ty_1/ty_1)	S(ty_1/ty_1)	R(*Ty2*/*Ty2*)	S(ty_2/ty_2)	S(ty_3/ty_3)	S(ty_3/ty_3)	*mi*/*mi*	-	LC, Y, C (Susceptible)	+
4	*S. arcanum* 1346	S(ty_1/ty_1)	H(*Ty1*/ ty_1)	R(*Ty2*/*Ty2*)	S(ty_2/ty_2)	H(*Ty3a*/*ty3*)	H(*Ty3b*/ ty_3)	*Mi*/*mi*	*Mi*/*mi*	LCS (Tolerant)	+
5	*S. corneliomulleri* 1274	S(ty_1/ty_1)	H(*Ty1*/ ty_1)	H(*Ty2*/ ty_2)	H(*Ty2*/ ty_2)	H(*Ty3a*/*ty3*)	R(*Ty3*/*Ty3*)	*Mi*/*mi*	-	NS (Resistant)	-
6	*S. pennellii* 1733	S(ty_1/ty_1)	S(ty_1/ty_1)	H(*Ty2*/ ty_2)	R(*Ty2*/*Ty2*)	H(*Ty3*/*ty3*)	R(*Ty3*/*Ty3*)	*Mi*/*mi*	*Mi*/*mi*	SL, D (Susceptible)	+
7	*S. huaylasense* 1358	S(ty_1/ty_1)	R(*Ty1*/ *Ty*1)	H(*Ty2*/ ty_2)	R(*Ty2*/*Ty2*)	H(*Ty3a*/*ty3*)	H(*Ty3*/ ty_3)	*Mi*/*mi*	-	LCS (Tolerant)	+
8	*S. pimpinellifolium* 1342	S(ty_1/ty_1)	S(ty_1/ty_1)	R(*Ty2*/*Ty2*)	S(ty_2/ty_2)	H(*Ty3a*/*ty3*)	S(ty_3/ty_3)	*mi*/*mi*	*mi*/*mi*	LC, SL, D (Susceptible)	+
9	*S. peruvianum* 1333	H(*Ty1*/ ty_1)	R(*Ty1*/ *Ty*1)	H(*Ty2*/ ty_2)	S(ty_2/ty_2)	H(*Ty3a*/*ty3*)	H(*Ty3b*/*ty3*)	*Mi*/*mi*	*mi*/*mi*	NS (Tolerant)	+
10	*S. habrochaites* 1352	H(*Ty1*/ ty_1)	S(ty_1/ty_1)	R(*Ty2*/*Ty2*)	R(*Ty2*/*Ty2*)	H(*Ty3a*/*ty3*)	R(*Ty3*/*Ty3*)	*Mi*/*mi*	*mi*/*mi*	NS (Tolerant)	+
11	*S. chilense* 56139	H(*Ty1*/ ty_1)	S(ty_1/ty_1)	H(*Ty2*/ ty_2)	S(ty_2/ty_2)	H(*Ty3a*/*ty3*)	H(*Ty3*/ ty_3)	*Mi*/*mi*	-	NS (Tolerant)	+
12	*S. lycopersicon* cv. Super Marmande	H(*Ty1*/ ty_1)	S(ty_1/ty_1)	H(*Ty2*/ ty_2)	S(ty_2/ty_2)	H(*Ty3a*/*ty3*)	S(ty_3/ty_3)	*mi*/*mi*	*mi*/*mi*	LC, C, SL, VN (Susceptible)	+
13	*S. lycopersicon* cv. Strain B F1	H(*Ty1*/ ty_1)	S(ty_1/ty_1)	R(*Ty2*/*Ty2*)	S(ty_2/ty_2)	H(*Ty3a*/*ty3*)	S(ty_3/ty_3)	*Mi*/*mi*	*Mi*/*mi*	LC, C (Susceptible)	+
14	*S. corneliomulleri* 1283	H(*Ty1*/ ty_1)	R(*Ty1*/ *Ty*1)	H(*Ty2*/ ty_2)	R(*Ty2*/*Ty2*)	H(*Ty3a*/*ty3*)	R(*Ty3*/*Ty3*)	*Mi*/*mi*	-	LCS, N (Tolerant)	+
15	*S. habrochaites* 1739	S(ty_1/ty_1)	S(ty_1/ty_1)	H(*Ty2*/ ty_2)	R(*Ty2*/*Ty2*)	H(*Ty3a*/*ty3*)	H(*Ty3*/ ty_3)	*Mi*/*mi*	-	NS (Tolerant)	+
16	*S. pimpinellifolium* 1279	S(ty_1/ty_1)	S(ty_1/ty_1)	R(*Ty2*/*Ty2*)	S(ty_2/ty_2)	H(*Ty3a*/*ty3*)	S(ty_3/ty_3)	*mi*/*mi*	*mi*/*mi*	E,VC (Susceptible)	+
17	*S. pimpinellifolium* 1332	S(ty_1/ty_1)	S(ty_1/ty_1)	R(*Ty2*/*Ty2*)	S(ty_2/ty_2)	H(*Ty3a*/*ty3*)	S(ty_3/ty_3)	*mi*/*mi*	*mi*/*mi*	NS (Tolerant)	+
18	*S. pennellii* 2963	S(ty_1/ty_1)	S(ty_1/ty_1)	H(*Ty2*/ ty_2)	H(*Ty2*/ ty_2)	H(*Ty3a*/*ty3*)	R(*Ty3*/*Ty3*)	*mi*/*mi*	*Mi*/*mi*	NS (Tolerant)	+
19	*S. pennellii* 1942	S(ty_1/ty_1)	H(*Ty1*/ ty_1)	H(*Ty2*/ ty_2)	H(*Ty2*/ ty_2)	H(*Ty3a*/*ty3*)	R(*Ty3*/*Ty3*)	*mi*/*mi*	*Mi*/*mi*	NS (Tolerant)	+

R: Resistance allele, homozygote (*Ty*/*Ty*), S: Susceptibility allele, homozygote (*ty*/*ty*), H: Heterozygote (*Ty*/*ty*), PCR= Polymerase chain reaction, CAPS=Cleaved amplified polymorphic sequence, SCAR= Sequence characterized amplified region, (+)= Positive result, (-)= Negative result, C= Crinkle, D= deformation, E= Epinasty, LC=Leaf curl, LCS=Leaf cup shape, NS= No symptoms, SL= Small leaf size, Y= Yellowing, VC= Vein clearing, VN= Veinal necrosis.

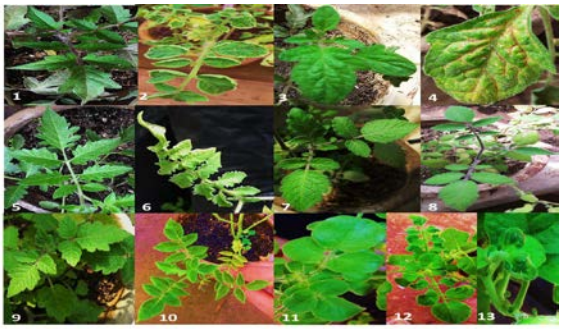

Figure 1. TYLCV symptoms in different tomato genotypes compared with the healthy control.

1-The healthy control of *S. lycopersicon* cv. Super Marmande; 2- *S. lycopersicon* cv. Super Marmande infected with TYLCV, showing leaf curl and small leaf size; 3-The healthy control of *S. lycopersicon* cv. Super Marmande; 4- *S. lycopersicon* cv. Super Marmande diseased with TYLCV, showing veinal necrosis; 5- The healthy control of *S. corneliomulleri* 1283; 6- *S. corneliomulleri* 1283 inoculated with TYLCV, showing leaf cup shape; 7- The healthy control of *S. habrochaites* 1739; 8- *S. habrochaites* 1739 inoculated with TYLCV, showing no symptoms; 9- The healthy control of *S. hirsutum* 24036; 10- *S. hirsutum* 24036 diseased with TYLCV, showing leaf curl, crinkle and yellowing; 11- The healthy control of *S. Pennellii* 1733; 12- *S. Pennellii* 1733 infected with TYLCV showing small leaf size; 13- *S. Pennellii* 1733 infected with TYLCV, showing deformation.

3.2. Screening Markers Linked to Ty-1, Ty-2, Ty-3 and Mi-1.2 Resistance Loci

Validation of PCR-based markers is linked to the three TYLCV resistance loci (*Ty-1*, *Ty-2* and *Ty-3*) and one whitefly resistance gene (*Mi-1.2*) in nineteen tomato genotypes (Table 3).

3.2.1. Ty-1 Locus

PCR amplification of DNA from the tomato genotypes and subsequent digestion by *Taq*I were performed using JB-1 CAPS marker, and scored a unique band of 950 bp with nineteen lines tested before restriction (Figure 2 and Table 3). After restriction with *Taq*I, twelve tomato lines revealed a susceptible allele (*ty-1*) size of 450 bp, which confirms the gene absence *Ty-1* such as *S. galapagense* 0317, *S. neoricki* 0247, *S. arcanum* 1346, *S. corneliomulleri* 1274, *S. huaylasense* 1358, *S. pimpinellifolium* 1342, 1279 and 1332, *S. habrochaites* 1739 and *S. pennellii* 1733, 2963 and 1942. On the other hand, seven accessions exhibited heterozygous alleles (*Ty1*/*ty1*) of 450 and 500 bp which confer the presence of the *Ty-1* gene i.e., *S. hirsutum* 24036, *S. peruvianum* 1333, *S. habrochaites* 1352, *S. chilense* 56139, Super Marmande, Strain B F1 and *S. corneliomulleri* 1283 (Figure 2 and Table 3). None of the tomato lines recorded dominant homozygous for *Ty*1 (*Ty1*/*Ty1*). JB-1 CAPS has not discriminated between resistant and susceptible genotypes.

The TY-1CAPS-TG178 primer pair gave one amplicon of 1000 bp with all of the tomato genotypes (Figure 3). Its nucleotide sequence was 500 bp longer than the sequence deduced from unigene because of the presence of four intron sequences in the genomic DNA. PCR products were distinguishable after digestion with restriction enzyme *Taq*I. Three lines which were homozygous plants for *Ty*1 (*Ty1*/*Ty1*) exhibited two alleles (200 and band slightly larger than 200 bp) viz., *S. huaylasense* 1358, *S. peruvianum* 1333 and *S. corneliomulleri* 1283. Three tomato genotypes had three different alleles of 160, 200 and band slightly larger than 200 bp which were heterozygous (*Ty1*/*ty1*) e.g., *S. arcanum* 1346, *S. corneliomulleri* 1274 and *S. pennellii* 1942. Besides, the other thirteen genotypes were homozygous plants for *ty-1* (*ty1*/*ty1*) showed two alleles with molecular sizes 200 and 160 bp (Figure 3 and Table 3).

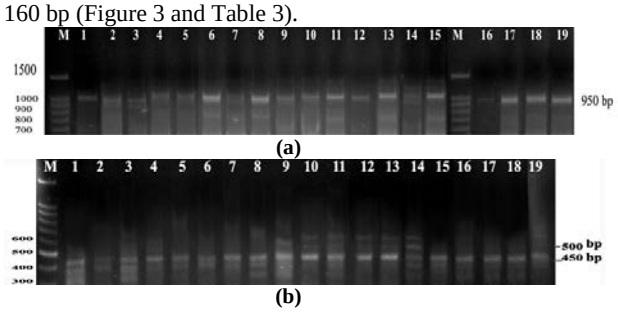

Figure 2. **(a)** PCR profiles of *Ty-1* locus amplified by JB-1CAPS primer from 19 tomato genotypes, lane M= 100 bp DNA ladder. **(b)** *Taq*I digestion of PCR products amplified by JB1CAPS primer. Lane 1: *Solanum hirsutum* 24036 (resistant, heterozygous); lane 2: *S. galapagense* 0317 (susceptible, homozygous); lane 3: *S. neoricki* 0247 (susceptible, homozygous); lane 4: *S. arcanum* 1346 (susceptible, homozygous); lane 5: *S. corneliomulleri* 1274 (susceptible, homozygous); lane 6: *S. pennellii* 1733 (susceptible, homozygous); lane 7: *S. huaylasense* 1358 (susceptible, homozygous) ; lane 8: *S. pimpinellifolium* 1342 (susceptible, homozygous); lane 9: *S. peruvianum* 1333(resistant, heterozygous); lane 10: *S. habrochaites* 1352 (resistant, heterozygous); lane 11: *S. chilense* 56139 (resistant, heterozygous); lane 12: *S. lycopersicon* cv. Super Marmande (resistant, heterozygous); lane 13: *S. lycopersicon* cv. Strain B F1 (resistant, heterozygous); lane 14: *S. corneliomulleri* 1283(resistant, heterozygous); lane 15: *S. habrochaites* 1739 (susceptible, homozygous); lane 16: *S. pimpinellifolium* 1279 (susceptible, homozygous); lane 17: *S. pimpinellifolium* 1332 (susceptible, homozygous); lane 18: S. *pennellii* 2963 (susceptible, homozygous) and lane 19: S. *pennellii* 1942 (susceptible, homozygous).

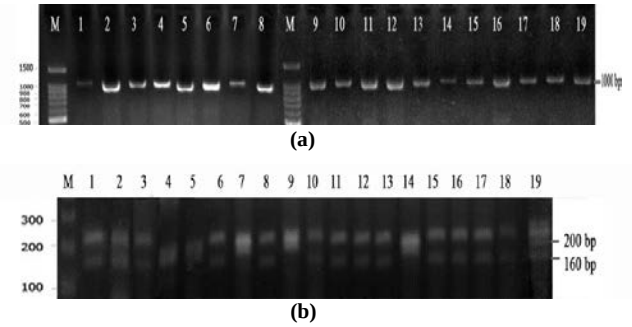

Figure 3. **(a)** PCR profiles of *Ty-1* locus amplified by TY-1CAPS-TG178 primer from 19 tomato genotypes, lane M= 100 bp DNA ladder. **(b)** *Taq*I digestion of PCR products amplified by TY-1CAPS-TG178 primer. Lane 1: *Solanum hirsutum* 24036 (susceptible, homozygous); lane 2: *S. galapagense* 0317 (susceptible, homozygous); lane 3: *S. neoricki* 0247 (susceptible, homozygous); lane 4: *S. arcanum* 1346 (resistant, heterozygous); lane 5: *S. corneliomulleri* 1274 (resistant, heterozygous); lane 6: *S. pennellii* 1733 (susceptible, homozygous); lane 7: *S. huaylasense* 1358 (resistant, homozygous); lane 8: *S. pimpinellifolium* 1342 (susceptible, homozygous); lane 9: *S. peruvianum* 1333(resistant, homozygous); lane 10: *S. habrochaites* 1352 (susceptible, homozygous); lane 11: *S. chilense* 56139 (susceptible, homozygous); lane 12: *S. lycopersicon* cv. Super Marmande (susceptible, homozygous); lane 13: *S. lycopersicon* cv. Strain B F1 (susceptible, homozygous); lane 14: *S. corneliomulleri* 1283(resistant, homozygous); lane 15: *S. habrochaites* 1739 (susceptible, homozygous); lane 16: *S. pimpinellifolium* 1279 (susceptible, homozygous); lane 17: *S. pimpinellifolium* 1332 (susceptible, homozygous); lane 18: S. *pennellii* 2963 (susceptible, homozygous) and lane 19: S. *pennellii* 1942 (resistant, heterozygous).

3.2.2. Ty-2 Locus

TY-2CAPS-TG105A marker was used to select the tomato lines carrying the *Ty-2* gene. One amplicon of 500 bp was obtained from all of the tested tomato genotypes. The PCR products were distinguishable after the cleavage with restriction enzyme *Taq*I (Figure 4). Two different alleles appeared in tomato plant materials; resistant allele 1 consisted of a fragment of 330 bp and appeared in homozygous eight tomato genotypes for *Ty2* (*Ty2*/*Ty2*) i.e., *S. hirsutum* 24036, *S. neoricki* 0247, *S. arcanum* 1346, *S. pimpinellifolium* 1342, *S. habrochaites* 1352, Strain B F1, and *S. pimpinellifolium* 1279 and 1332. Both alleles 330 and 200 bp appeared in heterozygous 11 tomato lines (*Ty2/ty2*) as shown in Table (3). On the other hand, there is not any homozygous susceptible accessions carry allele 2 of 200 bp (*ty2*/*ty2*) (Figure 4 and Table 3). This marker did not discriminate between homozygous and heterozygous accessions from each other.

The two co-dominant TY-2CAPS-TG105A marker scored false positive results for the presence of the *Ty-2* locus. This marker did not separate resistant and susceptible alleles. On the contrary, the PCR products of Ty-2SCAR T0302 marker were well separated from each susceptible or resistance allele at each locus. The PCR results successfully amplified DNA fragments for the *Ty-2* locus from all homozygous and heterozygous tomato lines. The five resistant lines (*Ty2*/*Ty2*) viz., *S. pennellii* 1733, *S. huaylasense* 1358, *S. habrochaites* 1352 and 1739 and *S. corneliomulleri* 1283 scored one allele of 900 bp (Figure 5). Moreover, ten susceptible accessions (*ty2/ty2*) gave PCR fragments of 800 bp e.g., *S. galapagense* 0317, *S. neoricki* 0247, *S. arcanum* 1346, *S. peruvianum* 1333, *S. chilense* 56139, Super Marmande, Strain B F1 and *S. pimpinellifolium* 1342, 1279 and 1332. The four heterozygous tomato genotypes scored two alleles 800 and 900 bp, for example, *Solanum hirsutum* 24036, *S. corneliomulleri* 1274, and *S. pennellii* 2963 and 1942 (Figure 5 and Table 3).

3.2.3. Ty-3 Locus

PCR amplification of DNA from the tomato genotypes using primer TY-3CAPS-FER-G8, gave one fragment with a molecular size of 500 bp in all of the homozygous and heterozygous tomato genotypes as shown in Figure (6). A subsequent digestion, when possible, was performed using *Taq*I restriction enzyme. Two accessions *S. hirsutum* 24036 and *S. pennellii* 1733 which were heterozygous for *Ty-3* showed three different alleles of 50, 250 and 300 bp in the presence of two *Taq*I sites. On the other hand, *S. neoricki* 0247 which was homozygous of *ty-3* had one fragment of 500 bp without being digested due to lack of a *Taq*I site in this fragment. However, the rest of the sixteen tomato lines which were heterozygous for *Ty-3*a exhibited two different bands with molecular sizes 200 and 300 bp, in the result of these accessions having one *Taq*I site (Figure 6 and Table 3). TY-3CAPS-FER-G8 marker gave false positive results for the presence of the *Ty-3* locus. In addition, it has not distinguished between *Ty-3*, *Ty-3a*, *Ty3-b* and *ty-3* alleles of resistant and susceptible tomato lines.

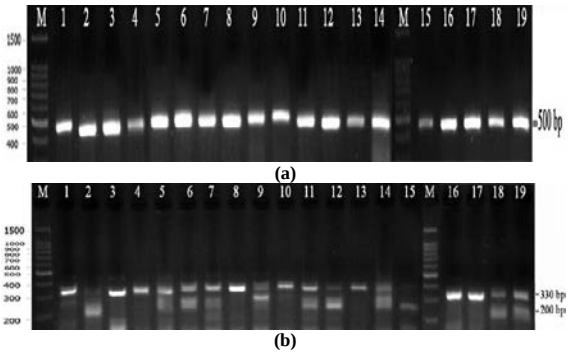

Figure 4. (a) PCR products of *Ty-2* locus amplified by TY-2CAPS-TG105A primer from 19 tomato genotypes, lane M= 100 bp DNA ladder. **(b)** *Taq*I digestion of PCR products amplified by TY-2CAPS-TG105A primer. Lane 1: *Solanum hirsutum* 24036 (resistant, homozygous); lane 2: *S. galapagense* 0317 (resistant, heterozygous); lane 3: *S. neoricki* 0247 (resistant, homozygous); lane 4: *S. arcanum* 1346 (resistant, homozygous); lane 5: *S. corneliomulleri* 1274 (resistant, heterozygous); lane 6: *S. pennellii* 1733 (resistant, heterozygous); lane 7: *S. huaylasense* 1358 (resistant, heterozygous); lane 8: *S. pimpinellifolium* 1342 (resistant, homozygous); lane 9: *S. peruvianum* 1333 (resistant, heterozygous); lane 10: *S. habrochaites* 1352 (resistant, homozygous); lane 11: *S. chilense* 56139 (resistant, heterozygous); lane 12: *S. lycopersicon* cv. Super Marmande (resistant, heterozygous); lane 13: *S. lycopersicon* cv. Strain B F1 (resistant, homozygous); lane 14: *S. corneliomulleri* 1283 (resistant, heterozygous); lane 15: *S. habrochaites* 1739 (resistant, heterozygous); lane 16: *S. pimpinellifolium* 1279 (resistant, homozygous); lane 17: *S. pimpinellifolium* 1332 (resistant, homozygous); lane 18: S. *pennellii* 2963 (resistant, heterozygous) and lane 19: S. *pennellii* 1942 (resistant, heterozygous).

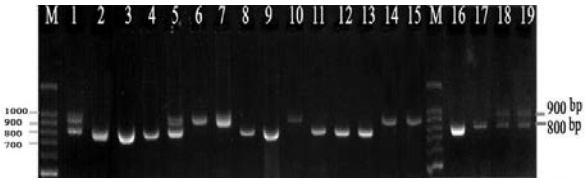

Figure 5. PCR amplicons of *Ty-2* locus amplified by primer Ty-2SCAR T0302 from 19 tomato genotypes, lane M= 100 bp DNA ladder. Lane 1: *Solanum hirsutum* 24036 (resistant, heterozygous); lane 2: *S. galapagense* 0317(susceptible, homozygous); lane 3: *S. neoricki* 0247 (susceptible, homozygous); lane 4: *S. arcanum* 1346 (susceptible, homozygous); lane 5: *S. corneliomulleri* 1274 (resistant, heterozygous); lane 6: *S. pennellii* 1733 (resistant, homozygous); lane 7: *S. huaylasense* 1358 (resistant, homozygous); lane 8: *S. pimpinellifolium* 1342 (susceptible, homozygous); lane 9: *S. peruvianum* 1333 (susceptible, homozygous); lane 10: *S. habrochaites* 1352 (resistant, homozygous); lane 11: *S. chilense* 56139 (susceptible, homozygous); lane 12: *S. lycopersicon* cv. Super Marmande (susceptible, homozygous); lane 13: *S. lycopersicon* cv. Strain B F1 (susceptible, homozygous); lane 14: *S. corneliomulleri* 1283 (resistant, homozygous); lane 15: *S. habrochaites* 1739 (resistant, homozygous); lane 16: *S. pimpinellifolium* 1279 (susceptible, homozygous); lane 17: *S. pimpinellifolium* 1332 (susceptible, homozygous); lane 18: S. *pennellii* 2963 (resistant, heterozygous) and lane 19: S. *pennellii* 1942 (resistant, heterozygous).

Ty3SCAR P6-25 gave one amplified fragment with a molecular size of 450 bp in seven resistant tomato genotypes which were homozygous (*Ty3*/*Ty3*) e.g., *S. hirsutum* 24036, *S. corneliomulleri* 1274 and 1283, *S. pennellii* 1733, 2963 and 1942 and *S. habrochaites* 1352. Besides, *S. peruvianum* 1333 and *S. arcanum* 1346 which were heterozygous (*Ty3*b/*ty3*) recorded two amplicons of 320 and 660 bp (Figure 7 and Table 3). On the other hand, three heterozygous tomato lines (*Ty3*/*ty3*) scored two alleles 320 and 450 bp such as *S. huaylasense* 1358, *S. chilense* 56139 and *S. habrochaites* 1739. The other seven susceptible accessions which were recessive homozygous

(*ty3/ty3*) gave PCR fragments of 320 bp (Figure 7 and Table 3). Furthermore, there are no tomato genotypes that have the *Ty*3a locus.

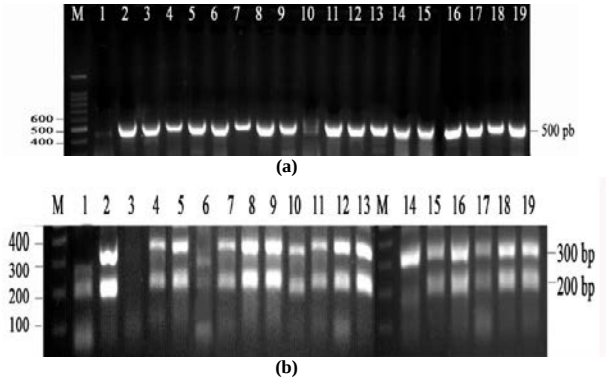

Figure 6. (a) PCR products of *Ty-3* locus amplified by TY-3CAPS-FER-G8 primer from 19 tomato genotypes, lane M= 100 bp DNA ladder. **(b)** *Taq*I digestion of PCR products amplified by TY-3CAPS-FER-G8 primer. Lane 1: *Solanum hirsutum* 24036 (resistant, heterozygous); lane 2: *S. galapagense* 0317 (resistant, heterozygous); lane 3: *S. neoricki* 0247(susceptible, homozygous); lane 4: *S. arcanum* 1346 (resistant, heterozygous); lane 5: *S. corneliomulleri* 1274 (resistant, heterozygous); lane 6: *S. pennellii* 1733 (resistant, heterozygous); lane 7: *S. huaylasense* 1358 (resistant, heterozygous); lane 8: *S. pimpinellifolium* 1342 (resistant, heterozygous); lane 9: *S. peruvianum* 1333 (resistant, heterozygous); lane 10: *S. habrochaites* 1352 (resistant, heterozygous); lane 11: *S. chilense* 56139 (resistant, heterozygous); lane 12: *S. lycopersicon* cv. Super Marmande (resistant, heterozygous); lane 13: *S. lycopersicon* cv. Strain B F1 (resistant, heterozygous); lane 14: *S. corneliomulleri* 1283 (resistant, heterozygous); lane 15: *S. habrochaites* 1739 (resistant, heterozygous); lane 16: *S. pimpinellifolium* 1279 (resistant, heterozygous); lane 17: *S. pimpinellifolium* 1332 (resistant, heterozygous); lane 18: S. *pennellii* 2963 (resistant, heterozygous) and lane 19: S. *pennellii* 1942 (resistant, heterozygous).

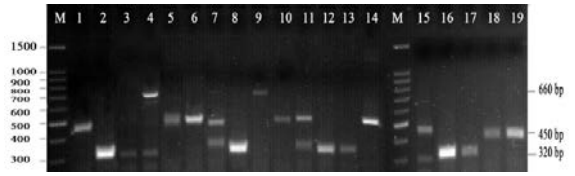

Figure 7. PCR amplicons of *Ty-3* locus amplified with primer Ty3SCAR P6-25 from 19 tomato genotypes, lane M= 100 bp DNA ladder. Lane 1: *Solanum hirsutum* 24036 (resistant, homozygous); lane 2: *S. galapagense* 0317 (susceptible, homozygous); lane 3: *S. neoricki* 0247(susceptible, homozygous); lane 4: *S. arcanum* 1346 (resistant, heterozygous); lane 5: *S. corneliomulleri* 1274 (resistant, homozygous); lane 6: *S. pennellii* 1733 (resistant, homozygous); lane 7: *S. huaylasense* 1358 (resistant, heterozygous); lane 8: *S. pimpinellifolium* 1342 (susceptible, homozygous); lane 9: *S. peruvianum* 1333 (resistant, heterozygous); lane 10: *S. habrochaites* 1352 (resistant, homozygous); lane 11: *S. chilense* 56139 (resistant, heterozygous); lane 12: *S. lycopersicon* cv. Super Marmande (susceptible, homozygous); lane 13: *S. lycopersicon* cv. Strain B F1 (susceptible, homozygous); lane 14: *S. corneliomulleri* 1283 (resistant, homozygous); lane 15: *S. habrochaites* 1739 (resistant, heterozygous); lane 16: *S. pimpinellifolium* 1279 (susceptible, homozygous); lane 17: *S. pimpinellifolium* 1332 (susceptible, homozygous); Lane 18: S. *pennellii* 2963 (resistant, homozygous) and lane 19: S. *pennellii* 1942 (resistant, homozygous).

3.2.4. Mi-1.2 Locus

The PCR using MI-REXCAPS primer pairs yielded a 750 bp DNA fragment in all of the studied genotypes, before digestion with *Taq*I (Figure 8). After digestion, a single 750 bp fragment for recessive homozygous eight genotypes (*mi*/*mi*) was observed. For instance, *S. galapagense* 0317, *S. neoricki* 0247, *S. pimpinellifolium* 1342, 1279 and 1332, Super Marmande and *S. pennellii* 2963 and 1942. However, the remaining eleven genotypes had three loci of 750, 570 and 180 bp which were heterozygous (*Mi*/*mi*) such as *S. hirsutum* 24036, *S. arcanum* 1346, *S. corneliomulleri* 1274 and 1283, *S. pennellii* 1733, *S. huaylasense* 1358, *peruvianum* 1333, *S. habrochaites* 1352 and 1739, *S. chilense* 56139, and Strain B F1 (Figure 8 and Table 3). The MI-REXCAPS marker distinguished between susceptibility and resistance alleles at *Mi-1.2* locus of the homozygous and heterozygous genotypes.

The Mi-1.2SCARMi23 gave different results from the MI-REXCAPS primer in the genotypes carrying *Mi-1.2* or not carrying *Mi-1.2*. The PCR with Mi-1.2SCARMi23 primer pairs yielded 430 band with homozygous seven susceptible lines (*mi*/*mi*) such as *S. galapagense* 0317, *S. pimpinellifolium* 1342, 1279 and 1332, *S. peruvianum* 1333, *S. habrochaites* 1352 and Super Marmande. Heteroduplex five genotypes (*Mi*/*mi*) exhibited two amplified fragments of 430 and 380 bp viz., *S. arcanum* 1346, Strain B F1 and *S. pennellii* 1733, 2963 and 1942. Furthermore, the other seven accessions have not scored any bands (Figure 9 and Table 3).

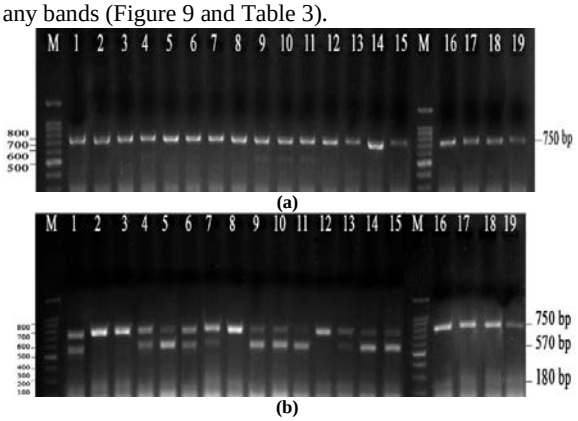

Figure 8. (a) PCR products of *Mi-1.2* locus amplified by MI-REX CAPS primer from 19 tomato genotypes, lane M= 100 bp DNA ladder. **(b)** *Taq*I digestion of PCR products amplified by MI-REX CAPS primer. Lane 1: *Solanum hirsutum* 24036 (resistant, heterozygous); lane 2: *S. galapagense* 0317 (susceptible, homozygous); lane 3: *S. neoricki* 0247 (susceptible, homozygous); lane 4: *S. arcanum* 1346 (resistant, heterozygous); lane 5: *S. corneliomulleri* 1274 (resistant, heterozygous); lane 6: *S. pennellii* 1733 (resistant, heterozygous); lane 7: *S. huaylasense* 1358 (resistant, heterozygous); lane 8: *S. pimpinellifolium* 1342 (susceptible, homozygous); lane 9: *S. peruvianum* 1333 (resistant, heterozygous); lane 10: *S. habrochaites* 1352 (resistant, heterozygous); lane 11: *S. chilense* 56139 (resistant, heterozygous); lane 12: *S. lycopersicon* cv. Super Marmande (susceptible, homozygous); lane 13: *S. lycopersicon* cv. Strain B F1 (resistant, heterozygous); lane 14: *S. corneliomulleri* 1283 (resistant, heterozygous); lane 15: *S. habrochaites* 1739 (resistant, heterozygous); lane 16: *S. pimpinellifolium* 1279 (susceptible, homozygous); lane 17: *S. pimpinellifolium* 1332 (susceptible, homozygous); lane 18: S. *pennellii* 2963 (susceptible, homozygous) and lane 19: S. *pennellii* 1942 (susceptible, homozygous).

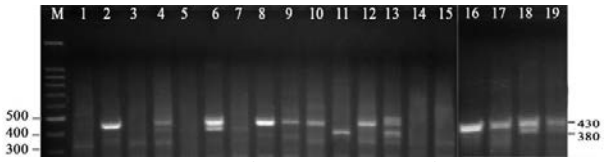

Figure 9. PCR amplicons of *Mi-1,2* locus amplified with primer Mi-1.2SCARMi23 from 19 tomato genotypes, lane M= 100 bp DNA ladder. Lane 1: *Solanum hirsutum* 24036 (no alleles); lane 2: *S. galapagense* 0317 (susceptible, homozygous); lane 3: *S. neoricki* 0247 (no alleles); lane 4: *S. arcanum* 1346 (resistant, heterozygous); lane 5: *S. corneliomuelleri* 1274 (no alleles); lane 6: *S. pennellii* 1733 (resistant, heterozygous); lane 7: *S. huaylasense* 1358 (no alleles); lane 8: *S. pimpinellifolium* 1342 (susceptible, homozygous); lane 9: *S. peruvianum* 1333 (susceptible, homozygous); lane 10: *S. habrochaites* 1352 (susceptible, homozygous); lane 11: *S. chilense* 56139 (no alleles); lane 12: *S. lycopersicon* cv. Super Marmande (susceptible, homozygous); lane 13: *S. lycopersicon* cv. Strain B F1 (resistant, heterozygous); lane 14: *S. corneliomulleri* 1283 (no alleles); lane 15: *S. habrochaites* 1739 (no alleles); lane 16: *S. pimpinellifolium* 1279 (susceptible, homozygous); Lane 17: *S. pimpinellifolium* 1332 (susceptible, homozygous); lane 18: S. *pennellii* 2963 (resistant, heterozygous) and lane 19: S. *pennellii* 1942 (resistant, heterozygous).

4. Discussion

Molecular markers have several applications in plant breeding programs. The availability of molecular markers associated with genes which suggest desirable traits allows the shortening of the breeding programs. Many resistance genes discovered in different wild tomato species have been introgressed in the cultivated tomato. Some of them are mapped to chromosome 6, and are genetically very close. Identifying the loci of a marker linked specifically with one of these resistance genes can be complex. Some tomato genotypes integrating many genes from different wild species often share the same alleles for a marker. This can give false positive results (Slater *et al*., 2013).

In the present study, one wild accession *S. corneliomulleri* 1274 was resistant to TYLCV which has not recorded any symptoms. Also, it was observed in this study that most the genotypes viz., *S. arcanum* 1346, *S. huaylasense* 1358, *S. peruvianum* 1333, *S. habrochaites* 1352 and 1739, *S. chilense* 56139, *S. corneliomulleri* 1283, *S. pimpinellifolium* 1332, and *S. pennellii* 2963 and 1942 were tolerant to the TYLCV infection. In contrast, eight tomato lines, including *S. hirsutum* 24036, *S. galapagense* 0317, *S. neoricki* 0247, *S. pennellii* 1733, *S. pimpinellifolium* 1342 and 1279, Super Marmande, and Strain B F1 were susceptible to TYLCV which showed the typical symptoms to TYLCV. These results were confirmed by amplification of 335 bp TYLCV DNA band in all of the studied tomato genotypes except *S. corneliomulleri* 1274. Similar studies were made by Chomdej *et al*., (2007), Abdulbaset *et al*., (2008) and Seo *et al*., (2018) found that all genotypes of *S. lycopersicum* had shown various degrees of disease symptoms. Thus, six lines of *S. peruvianum* were resistant and remained symptomless.

In this study, five CAPS and three SCAR molecular markers tightly linked to *Ty-1*, *Ty-2*, *Ty-3* and *Mi-1.2* genes have been employed for nineteen tomato genotypes. Such data can assist plant breeders in fast screening against TYLCV resistance at the seedling stage and in the development of stable resistant lines by gene pyramiding through MAS. PCR amplification of DNA from nineteen tomato lines and subsequent digestion by *Taq*I were performed using both JB-1 CAPS and TY-1CAPS-TG178 markers. For the JB-1CAPS marker, after restriction with *Taq*I, twelve tomato lines had a susceptible allele (*ty-1*) size of 450 bp such as *S. galapagense* 0317, *S. neoricki* 0247, *S. arcanum* 1346, *S. corneliomulleri* 1274, *S. huaylasense* 1358, *S. pimpinellifolium* 1342, 1279 and 1332, *S. habrochaites* 1739, and *S. pennellii* 1733, 2963 and 1942. Moreover, seven accessions exhibited heterozygous alleles (*Ty1*/*ty1*) of 450 and 500 bp including *S. hirsutum* 24036, *S. peruvianum* 1333, *S. habrochaites* 1352, *S. chilense* 56139, Super Marmande, Strain B F1 and *S. corneliomulleri* 1283. JB-1 CAPS have not discriminated between resistant and susceptible genotypes. These findings were in agreement with Prasanna *et al*., (2015) who observed that the JB1 marker linked to TYLCV resistance *Ty-1* showed inconsistent amplification, and hence was not considered for further evaluation.

For the TY-1CAPS-TG178 marker, three genotypes showed two homozygous bands of 200 and band slightly larger than 200 bp thus conferring the presence of the dominant gene *Ty-1* such as *S. huaylasense* 1358, *S. peruvianum* 1333 and *S. corneliomulleri* 1283. These accessions appeared tolerant or resistant to TYLCV. Moreover, the other genotypes which showed three amplicons of 160, 200 and band slightly larger than 200 bp were heterozygous (*Ty1*/*ty1*) such as *S. arcanum* 1346, *S. corneliomulleri* 1274, and *S. pennellii* 1942. They displayed resistance or tolerance to the TYLCV infection. However, the remaining genotypes which had a recessive gene for *ty-1* showed two alleles with the molecular sizes of 200 and 160 bp. Ji *et al*., (2007b) mentioned that *Ty-1*, which originated from *S. chilense* LA1969 accession and *Ty-1* is almost completely dominant to TYLCV. Milo, (2001) found out that resistance to TYLCV is controlled by *Ty-1* dominant gene, which has been mapped to chromosome 6.

For *Ty-2*, the two markers, TY-2CAPS-TG105A and Ty-2SCAR T0302 were used for the detection of homozygous *Ty-2*/*Ty-2* and *ty-2*/*ty-2* and heterozygous *Ty-2*/*ty-2* in nineteen tomato genotypes. In this study, Ty-2SCAR T0302 primer set separated the homozygous and heterozygous plants better than the TY-2CAPS-TG105 primer. Thus, the latter primer gave false positive results, when nineteen lines were evaluated. Also, this marker has not detected all the lines that have the *Ty-2* gene. In addition, TY-2CAPS-TG105 marker amplified PCR fragments from the recessive homozygous tomato such as Super Marmande, Strain B F1, *S. pimpinellifolium* 1279, 1332 and 1342, *S. peruvianum* 1333, *S. chilense* 56139, *S. galapagense* 0317, *S. neoricki* 0247 and *S. arcanum* 1346. So, the Ty-2SCAR T0302 primer has been a better marker than the TY-2CAPS-TG105 marker. These results concerning this marker are consistent with the results previously observed by Garcia *et al*., (2007) who mentioned that the SCAR T0302 primer is a better marker than the TG105 CAPS marker, as the latter may also discover an introgression that might be linked with the *I2* gene, and this would give false positive results. Kalloo and Banerjee, (1990) found out that *Ty-2* originated from *S. habrochaites* in the resistant tomato line H24 and showed completely dominant TYLCV inheritance (Ji *et al*.,

2007b). *Ty-2* has been mapped to a 19-cM region on the long arm of chromosome 11 (Barbieri *et al*., 2010).

In the current study, the two markers TY-3CAPS-FER-G8 and Ty3SCAR P6-25 have been used for the detection of the *Ty-3* gene. Only, co-dominant Ty3SCAR P6-25 marker distinguished between *Ty-3*, *Ty-3a*, *Ty-3b* and *ty-3* alleles. In contrast, TY-3CAPS-FER-G8 gave false positive results. Thus, all of the tested tomato lines recorded resistance to TYLCV except *S. neoricki* 0247, using TY-3CAPS-FER-G8. On the other hand, one locus of 450 bp (*Ty3*/*Ty3*) revealed using Ty3SCAR P6-25 primer in homozygous seven tomato accessions e.g., *S. hirsutum* 24036, *S. corneliomulleri* 1274, *S. habrochaites* 1352, *S. corneliomulleri* 1283 and *S. pennellii* 1733, 2963 and 1942. The expected 320 bp *ty3* fragment was recorded in the susceptible seven tomato genotypes such as *S. galapagense* 0317, *S. neoricki* 0247, *S. pimpinellifolium* 1342, 1279 and 1332, Super Marmande, and Strain B F1. Two alleles of 660 and 320 bp, were amplified from heterozygous lines (*Ty3b*/*ty3)* such as *S. arcanum* 1346 and *S. peruvianum* 1333. In addition, three heterozygous lines (*Ty3*/*ty3*), namely *S. huaylasense* 1358, *S. chilense* 56139, and *S. habrochaites* 1739 were easily detected by this primer which amplified two alleles of 320 and 450 bp. Moreover, there have not been any tomato lines which have the *Ty*3a gene. These results are in agreement with Neha *et al*., (2016) and Ji *et al*., (2007b) who used a co-dominant SCAR analysis to differentiate between the *S. lycopersicum* recessive allele *ty-3* and the *S. chilense* dominant allele *Ty-3*. Jensen Katie *et al*., (2007) detected a new locus of introgression (*Ty-3b*) in the tomato. Mejia *et al*., (2010) found out that *Ty-2* alone gave no resistance to TYLCV in the tomato, but pyramiding *Ty-2* and *Ty-3* together supplied a higher level of resistance better than *Ty-3* alone.

For the *Mi-1.2* locus, it was observed that MI-REXCAPS scored more accurate results than the Mi-1.2SCARMi23 marker. Some accessions gave results with MI-REXCAPS and have not recorded any results with Mi-1.2SCARMi23 such as *S. hirsutum* 24036, *S. neoricki* 0247, *S. corneliomulleri* 1274 and 1283, *S. huaylasense* 1358, *S. chilense* 56139, and *S. habrochaites* 1739. Furthermore, eleven heterozygous tomato lines (*Mi*/*mi*) displayed three alleles of 180, 570 and 750 bp, using MI-REXCAPS i.e., *S. hirsutum* 24036, *S. arcanum* 1346, *S. corneliomulleri* 1274 and 1283, *S. pennellii* 1733, *S. huaylasense* 1358, *peruvianum* 1333, *S. habrochaites* 1352 and 1739, *S. chilense* 56139, and Strain B F1. In contrast, the other eight genotypes were recessive homozygous have not digestion site by *Taq*I using the MI-REXCAPS marker. Veremis and Roberts, (2000) mentioned that resistance to whitefly or root-knot nematodes originates from wild tomatoes e.g., *S. peruvianum*, *S. arcunum*, *S. corneliomulleri* and *S. huaylasense*. The latter formerly belongs to *S. peruvianum* complex for tomato. Cortada *et al*., (2010) reported that *S. huaylasense* accession LA1358 is a new source of whitefly or root-knot nematode resistance; this resistance can be attributed to the presence of *Mi*-genes. Firdaus *et al*., (2012) discovered different levels of whitefly resistance in tomato wild relatives such as *S. chilense*, *S. pimpinellifolium*, *S. pennellii*, *S. habrochaites*, and *S. habrochaites* f. *glabratum*. In the current study, the MI-REXCAPS marker has been explored alternatively to the SCAR method because CAPS detected new accessions carrying the *Mi-1.2* resistant gene to whitefly or root-knot nematode. These results were in agreement with Williamson *et al*., (1994) who mentioned that The CAPS marker is widely applied to detect the *Mi-1.2* in the tomato, and has been reported relatively reliable. El-Mehrach *et al*., (2005) found out that the REXCAPS marker could not be applied in tomato hybrid lines with introgressions of *S. chilense* and *S. habrochaites.*

5. Conclusion

In this study, CAPS and SCAR markers have been employed to detect resistance genes to TYLCV and whitefly in nineteen tomato accessions. The researchers have identified one line *S. corneliomulleri* 1274 carrying (*Ty-1*, *Ty-2*, *Ty-3*) and (*Mi-1.2*) and showing resistance to TYLCV and whitefly, respectively. In addition, ten genotypes were TYLCV tolerant carrying one or more TYLCV and whitefly resistant alleles. So, molecular markers can be applied in the breeding programs to expedite the procedures of pyramiding these resistance genes of several genotypes into a single line, hence improving the resistance to *begomoviruses*.

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