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 were 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 (Messegue 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 whitelies (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
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 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.

Table 1. Tomato genotypes used in this study.

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

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 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. RNA 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°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 Sagalski, 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 MgCl2; 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 dH2O. The PCR was carried out in Thermocycler (Biomateria, 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 minutes.

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. PCR reactions 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 MgCl2 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.
2.3.1. Restriction Digestion and Analysis

CAPS-based markers were digested by the restriction enzymes Taq I (Table 2). 20 µL reaction mixture containing 16.3 µL dsH2O, 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-2SCAR T0302, Ty-3SCAR P6-25 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 MgCl2, 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 dsH2O. 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.

Table 2. Primer sequences used in this study.

<table>
<thead>
<tr>
<th>Primer and markers</th>
<th>R-gene*</th>
<th>Chromosome No</th>
<th>Single nucleotide sequence (5’-3’)</th>
<th>Annealing temperature (AT)°C</th>
<th>Restriction enzyme</th>
<th>Molecular size of band (bp)</th>
<th>Molecular size after digestion with TaqI (bp)</th>
<th>References</th>
</tr>
</thead>
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<tr>
<td>TYLCV AV1 F</td>
<td>-</td>
<td>-</td>
<td>TGACAAAGACATGCGGACCA</td>
<td>55</td>
<td>TaqI</td>
<td>335</td>
<td>-</td>
<td>Present study</td>
</tr>
<tr>
<td>TYLCV AV1R</td>
<td>-</td>
<td>-</td>
<td>TGGGCTGTCGAAGTTGAGAC</td>
<td>56</td>
<td>TaqI</td>
<td>1000</td>
<td>200</td>
<td>Barbieri et al., (2010)</td>
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<tr>
<td>TY-1CAPS-TG178F</td>
<td>Ty1</td>
<td>6</td>
<td>GTACTCTCCGGAAAGGTTAAAGG</td>
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<td>TaqI</td>
<td>950</td>
<td>450</td>
<td>Pérez et al., (2007)</td>
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<tr>
<td>JB1CAPSR</td>
<td>Ty1</td>
<td>6</td>
<td>TTTCCATTCTCGTTCCTG</td>
<td>55</td>
<td>TaqI</td>
<td>750</td>
<td>250</td>
<td>Jensen et al., (2007)</td>
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<tr>
<td>TY-3CAPS-TG165F</td>
<td>Ty2</td>
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<td>ATGTACATTTGTGGTTGAACATCC</td>
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<td>TaqI</td>
<td>900</td>
<td>800</td>
<td>Yang et al., (2014)</td>
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<td>AGGTGACATCTCCTGGAATTCGTAGACT</td>
<td>55</td>
<td>TaqI</td>
<td>800</td>
<td>750</td>
<td>Michelson et al., (1994)</td>
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<td>TY-3CAPS-FER-G8F</td>
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<td>TGGAAAAATGTGAATTTCTTTTG</td>
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<td>TaqI</td>
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<td>Ty-2SCAR T0302F</td>
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<td>TGGCTCACTGTAAGCTAGACTGC</td>
<td>55</td>
<td>TaqI</td>
<td>900</td>
<td>800</td>
<td>Yang et al., (2014)</td>
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<tr>
<td>Ty-2SCAR T0302R</td>
<td>Ty2</td>
<td>11</td>
<td>AGGTGACATCTCCTGGAATTCGTAGACT</td>
<td>55</td>
<td>TaqI</td>
<td>800</td>
<td>750</td>
<td>Michelson et al., (1994)</td>
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<tr>
<td>Ty-3SCAR P6-25F</td>
<td>Ty3</td>
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<td>GGTAGTGAAATGATGCTGCT</td>
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<td>TaqI</td>
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<td>660</td>
<td>Is et al., (2008)</td>
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<tr>
<td>Ty-3SCAR P6-25R</td>
<td>Ty3</td>
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<td>GCTCTGCTATATGCCTCCATATAACC</td>
<td>53</td>
<td>TaqI</td>
<td>450</td>
<td>660</td>
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<tr>
<td>Mi-1.2SCARMi23F</td>
<td>M1.2</td>
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<td>TGGAAAAATGTGAATTTCTTTTG</td>
<td>57</td>
<td>TaqI</td>
<td>380</td>
<td>430</td>
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<tr>
<td>Mi-1.2SCARMi23R</td>
<td>M1.2</td>
<td>6</td>
<td>GCATACTATATGGCTTGGTTACC</td>
<td>57</td>
<td>TaqI</td>
<td>430</td>
<td>430</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Genotype</th>
<th>Tomato yellow leaf curl virus (TYLCV) resistance genes and DNA markers</th>
<th>Result of infection test</th>
<th>Detection of TYLCV by PCR</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Solanum hirsutum 24036</td>
<td>H(Ty1/ Ty1) R(Ty2/Ty2) H(Ty3/Ty3) M/mi -</td>
<td>LC, Y, C (Susceptible)</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>S. galapagense 0317</td>
<td>S(Ty1/ Ty1) S(Ty3/Ty3)</td>
<td>LC, C (Susceptible)</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>S. neoricki 0247</td>
<td>S(Ty1/ Ty1) S(Ty2/Ty2)</td>
<td>LC, Y, C (Susceptible)</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>S. arcunum 1346</td>
<td>S(Ty1/ Ty1) H(Ty2/ Ty2)</td>
<td>LCS (Tolerant)</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>S. cornelioiuller 1274</td>
<td>S(Ty1/ Ty1) H(Ty2/ Ty2) H(Ty3a/ Ty3) R(Ty3/Ty3) M/mi -</td>
<td>NS (Resistant)</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>S. pennellii 1733</td>
<td>S(Ty1/ Ty1) R(Ty2/Ty2)</td>
<td>SL, D (Susceptible)</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>S. huaylasense 1358</td>
<td>S(Ty1/ Ty1) R(Ty1/ Ty1)</td>
<td>LCS (Tolerant)</td>
<td>+</td>
</tr>
<tr>
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<td>S. pimpinellifolium 1342</td>
<td>S(Ty1/ Ty1) R(Ty2/Ty2) S(Ty3a/ Ty3) S(Ty3a/ Ty3) M/mi/mi</td>
<td>LC, SL, D (Susceptible)</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>S. pereianum 1333</td>
<td>S(Ty1/ Ty1) R(Ty1/ Ty1)</td>
<td>NS (Tolerant)</td>
<td>+</td>
</tr>
<tr>
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<td>S. habrochaites 1352</td>
<td>S(Ty1/ Ty1) R(Ty2/Ty2)</td>
<td>NS (Tolerant)</td>
<td>+</td>
</tr>
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<td>S. chilense 5639</td>
<td>S(Ty1/ Ty1) H(Ty2/ Ty2) S(Ty3a/ Ty3)</td>
<td>NS (Tolerant)</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>S. lycopersicon cv. Super Marenae</td>
<td>S(Ty1/ Ty1) R(Ty2/Ty2) S(Ty3a/ Ty3)</td>
<td>LC, C, SL, VN (Susceptible)</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>S. lycopersicon cv. Strain B F 1</td>
<td>S(Ty1/ Ty1) R(Ty2/Ty2) S(Ty3a/ Ty3)</td>
<td>LC, C (Susceptible)</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>S. cornelioiuller 1283</td>
<td>H(Ty1/ Ty1) R(Ty1/ Ty1)</td>
<td>LCS, N (Tolerant)</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>S. habrochaites 1739</td>
<td>S(Ty1/ Ty1) R(Ty2/Ty2)</td>
<td>NS (Tolerant)</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>S. pimpinellifolium 1279</td>
<td>S(Ty1/ Ty1) R(Ty2/Ty2) S(Ty3a/ Ty3)</td>
<td>E,VC (Susceptible)</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>S. pimpinellifolium 1332</td>
<td>S(Ty1/ Ty1) R(Ty2/Ty2) S(Ty3a/ Ty3)</td>
<td>NS (Tolerant)</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>S. pennellii 2966</td>
<td>S(Ty1/ Ty1) R(Ty2/Ty2)</td>
<td>NS (Tolerant)</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>S. pennellii 1942</td>
<td>S(Ty1/ Ty1) R(Ty2/Ty2)</td>
<td>NS (Tolerant)</td>
<td>+</td>
</tr>
</tbody>
</table>

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.
revealed a susceptible allele (Table 3). After restriction with Taq JB-1 CAPS marker, and scored a unique band of 950 bp and subsequent digestion by Taq whitefly resistance gene (Mi-1.2) lycopersicon confirms the gene absence 0317, TYLCV resistance loci (Marmande; 4- and small leaf size; 3-The healthy control of 1-The healthy control of compared with the healthy control.

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 whitely 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 TaqI 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 TaqI, 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. hualasense 1358, S. pimpinellifolium 1342, 1279 and 1332, S. habrochaites 1739 and S. pennellii 1733, 2963 and 1942. On the other hand, seven accesses 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 Ty1 (Ty1/Ty1). JB-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 TaqI. Three lines which were homozygous plants for Ty1 (Ty1/Ty1) exhibited two alleles (200 and band slightly larger than 200 bp) viz., S. hualasense 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 1. TYLCV symptoms in different tomato genotypes compared with the healthy control.

(a) PCR profiles of Ty-1 locus amplified by JB-1CAPS primer from 19 tomato genotypes, lane M= 100 bp DNA ladder. (b) TaqI 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. hualasense 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 1379 (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 2. (a) PCR profiles of Ty-1 locus amplified by JB-1CAPS primer from 19 tomato genotypes, lane M= 100 bp DNA ladder. (b) TaqI 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. hualasense 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 1379 (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) TaqI 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 (susceptible, homozygous); lane 5: S. corneliomulleri 1274 (susceptible, resistant, heterozygous); lane 6: S. pennellii 1733 (susceptible, homozygous); lane 7: S. hualasense 1358 (susceptible, resistant, heterozygous); 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, heterozygous); lane 15: S. habrochaites 1379 (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 TaqI (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. arcuatum 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 Ty2/ty2 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. arcuatum 1346, S. peruvianum 1333, S. chilense 56139, Super Marmande, Strain B F1 and S. pimpinellifolium 1342, 1279 and 1332. The four heterozygous tomato genotype scored two alleles were: 800 bp 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 TaqI restriction enzyme. Two accessions S. hirsutum 24036 and S. pennellii 1733 which were heterozygous for Ty3 showed three different alleles of 50, 250 and 300 bp in the presence of two TaqI sites. On the other hand, S. neoricki 0247 which was homozygous of ty3-1 had one fragment of 500 bp without being digested due to lack of a TaqI site in this fragment. However, the rest of the sixteen tomato lines which were heterozygous for Ty3-3a exhibited two different bands with molecular sizes 200 and 300 bp, in the result of these accessions having one TaqI 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, Ty-3-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) TaqI digestion of PCR products amplified by TY-2CAPS-TG105A primer. Lane 1: Solanum hirsutum 24036 (resistant, homozygous); lane 2: S. galapagense 0317 (resistant, homozygous); lane 3: S. neoricki 0247 (resistant, homozygous); lane 4: S. arcuatum 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, homozygous).

Figure 5. PCR amplicons of Ty-2 locus amplified by primer Ty2SCAR 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. arcuatum 1346 (susceptible, 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 (susceptible, homozygous); lane 13: S. lycopersicon cv. Strain B F1 (susceptible, 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, homozygous).

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. arcuatum 1346 which were heterozygous (Ty3b/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 Ty3a 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) TaqI 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. arcuam 1346 (resistant, heterozygous); lane 5: S. corneliomulleri 1274 (resistant, heterozygous); lane 6: S. pennelli 1733 (susceptible, homozygous); lane 7: S. huylasadense 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 1342 (susceptible, homozygous); lane 17: S. pennelli 2963 (resistant, heterozygous) and lane 18: S. pennelli 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. arcuam 1346 (resistant, heterozygous); lane 5: S. corneliomulleri 1274 (resistant, homozygous); lane 6: S. pennelli 1733 (resistant, homozygous); lane 7: S. huylasadense 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 (susceptible, homozygous); lane 14: S. corneliomulleri 1283 (resistant, homozygous); lane 15: S. habrochaites 1739 (resistant, heterozygous); lane 16: S. pimpinellifolium 1342 (susceptible, homozygous); lane 17: S. pennelli 2963 (resistant, homozygous) and lane 19: S. pennelli 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 TaqI (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. pennelli 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. arcuam 1346, S. corneliomulleri 1274 and 1283, S. pennelli 1733, S. huylasadense 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, peruvianum 1333, S. habrochaites 1352 and Super Marmande. Heteroduplex five genotypes (Mi/mi) exhibited two amplified fragments of 430 and 380 bp viz., S. arcuam 1346, Strain B F1 and S. pennelli 1733, 2963 and 1942. Furthermore, the other seven accessions have not scored any bands (Figure 9 and Table 3).
Figure 9. PCR amplicons of Mi-1.2 locus amplified with primer Mi-1.2SCARM23 from 19 tomato genotypes, lane M= 100 bp DNA ladder. Lane 1: Solanum hisitum 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. corneliomulleri 1274 (no alleles); lane 6: S. pennelli 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. lycopersicum cv. Super Marmande (susceptible, homozygous); lane 13: S. lycopersicum 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. pennelli 2963 (resistant, heterozygous) and lane 19: S. pennelli 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 of 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. pennelli 2963 and 1942 were tolerant to the TYLCV infection. In contrast, eight tomato lines, including S. hisitum 24036, S. galapagense 0317, S. neoricki 0247, S. pennelli 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 Chomdje 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 TaqI were performed using both JB-1 CAPS and TY-1CAPS-TG178 markers. For the JB-1CAPS marker, after restriction with TaqI, 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. pennelli 1733, 2963 and 1942. Moreover, seven accessions exhibited heterogeneous alleles (Ty1/ty1) of 450 and 500 bp including S. hisitum 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. peruviam 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. pennelli 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-2SCARR 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-2SCARR T0302 primer set separated the homozygous and heterozygous plants better than the TY-2CAPS-TG105A 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-2SCARR 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 TGI05 CAPS marker, as the latter may also discover an introgression that might be linked with the J2 gene, and this would give false positive results. Kallou and Banerjee, (1990) found out that Ty-2 originated from S. habrochaites in the resistant tomato line HZ4 and showed completely dominant TYLCV inheritance (H 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. neorickii 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. pennelli 1733, 2963 and 1942. The expected 320 bp Ty-3 fragment was recorded in the susceptible seven tomato genotypes such as S. galapagense 0317, S. neorickii 0247, S. pinnipellifolium 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. arcuatum 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 been not 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. neorickii 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. arcuatum 1346, S. corneliomulleri 1274 and 1283, S. pennelli 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 TaqI using the MI-REXCAPS marker. Veremis and Roberts, (2000) mentioned that resistance to whitefly or root-knot nematode originates from wild tomatoes e.g., S. peruvianum, S. arcuatum, 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. pinnipellifolium, S. pennelli, 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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