MOLECULAR SCREENING FOR Ty-1 AND Ty-3 GENES IN SEVENTEEN TOMATO GENOTYPES

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Abstract

Tomato Yellow Leaf Curl Virus (TYLCV) is very devastating threat to tomato production worldwide and considers a major tomato viral disease in tropical and subtropical regions. Many resistance or tolerance genes have been introgressed from tomato relatives for breeding against TYLCV. For instance, some genes confer tolerance by enhancing gene silencing such as Ty-1 and Ty-3 genes which are conditioning resistance to TYLCV disease in tomato. Molecular screening for these genes in sixteen tomato genotypes (GS-12, Ginan, 81, Yara, Toppy, Yassamin, Seren, Zina, Oula, super Mamrande, Rasheda, Nada, B104, Noon, Wigdan and Sheifa) were collected from Iraqi Markets (Al-Sinak, Jamila and Baquba), in the period from December 2018 to January 2019 was done using two molecular DNA-based markers; they were sequence characterized amplified region (SCAR), and cleaved amplified, polymorphic sequence (CAPS) markers. The experiment was conducted at University of Baghdad/ Genetic Engineering and Biotechnology Institute for Postgraduate Studies. Results showed that six tomato genotypes were contain both Ty-1 and Ty-3 (GS-12, Ginan, 81, Yara, Toppy and Yassamin) and two genotypes contain only one gene (Seren contains Ty-3 and Zina contains Ty-1). Also, the results showed that eight genotypes do not contain neither the Ty-1 or Ty-3 (Oula, super Mamrande, Rasheda, Nada, B104, Noon, Wigdan and Sheifa). The markers specific of Ty-1 recorded a PCR product of 320 bp while Ty-3 specific marker showed PCR product of 608 bp.

Keywords: Totamo genotypes, Ty-1 and Ty-3 genes, CAPS, SCAR

Introduction

Tomato (Lycopersicon esculentum Mills.) is one of the most important grown vegetables worldwide due to its valuable content nutrient and it is on top list of every culinary dishes worldwide (Shanmukhi et al., 2018). In 2017 about 5 million hacters were harvested worldwide and the production estimate was about 182 million Mt in 2017 (FAOSTAT). In Iraq tomato is very important crop for local consumption due to high content vitamins, minerals, essential amino acids, sugars and fibers. Differs tomato seed suppliers and tomato seedling nurseries are available in Iraqi market. According to the Iraqi ministry of agriculture / Directorate of Seeds Testing and Certification (D.S.T.C) there are more than one hundred tomato verities has been entered the country. In spite of that not all of these genotypes are registered and approved to be planted in Iraq, many have been found and grown by farmers. Like many other vegetable crops, tomatoes suffer many destructive pathogens of both open field and greenhouse grown conditions. For instance, the tropic diseases and disorders can affect tomatoes during growing season (Peralta et al., 2001). Plant viruses can negatively affect the production of tomato and other vegetables worldwide, including Iraq. Tomato yellow leaf curl viruses (TYLCV) a member of Begomovirus is on the top of destructive tomato pathogens damaging tomato production areas of the world (Glick et al., 2009; Díaz-Pendón et al., 2010). TYLCV can be transmitted by whitefly vector Bemisia tabaci, (Homoptera: Aleyrodidae) which causes direct feeding damage and indirect damage as a TYLCV vector. Lost can be even higher on tomato in developing countries due to lack of knowledge on plant virus disease control (Caciagli, 2009). Bemisia tabaci can spread the virus between many hosts and this way may have an important role in the survival of virus between seasons as source of tomato infection (De Barro et al., 2011). Since it is difficult to control TYLCV’s vector and the absence of resistant gene in tomato’s genome, a major economic control strategy for this pathogen is to determine resistant gene in wild relatives and transfer this gene into tomato’s genome (Scholthof et al., 2011; Hanssen et al., 2010; Glick et al., 2009). Researchers have identified six major resistant genes form wild tomato relatives (Ty-1, Ty-2, Ty-3, Ty-4, Ty-5 and Ty-6) (Hutton and Scott, 2014; Caro et al., 2015; Hanson et al., 2008). Wild tomato relatives such as Lycopersicon chilense, Solanum peruvianum and S. habrochaites contain resistance genes and have been used as a sources of resistance in tomato breeding programs (Vidavsky et al., 2008). Ty-1 was introgressed from S. chilense accession LA1969 and mapped to chromosome 6 of tomato (Zamir et al. 1994; Verlaan et al., 2011). Also, Ty-3, was introgressed from S. chilense accessions, LA1932/LA2779/ LA1938, and also mapped to chromosome 6 (Ji et al. 2007). Both Ty-1 and Ty-3 code for RNA-dependent RNA polymerase (RDR) class v. RDRs can be defined as proteins that synthesize small-interfering RNAs (siRNAs) siRNA-producing dsRNA molecules using a single-stranded RNA (ssRNA) molecule as a template. Also, RDRs have atypical DFDGD motif in the catalytic domain and may be involved in RNA silencing (Verlaan et al., 2013).

Plant breeders have combined many resistance alleles found in wild tomato species to create resistant cultivars. Ty-1 and Ty-3 have been widely used in hybrid tomato breeding due to the high level of resistance to TYLCV and it is found dominant (Hutton et al., 2017). Breeders have been applied molecular DNA-based markers; such as sequence characterized amplified region (SCAR), and cleaved amplified polymorphic sequence (CAPS) in commercial plant breeding programs since the early 1990s. They have been used for more than 40 genes (including many single genes and quantitative trait loci, QTL) that confer resistance to all major classes of pathogens. They have proven helpful for the rapid and efficient transfer of useful traits into
agronomical desirable genotypes and hybrids. These markers linked to disease resistance loci can now be used for marker-assisted selection (MAS) programs and useful for cloning and sequencing the genes. Furthermore, they can be used for accumulation of several resistance genes in a single genotype (“pyramiding” resistance genes). In addition, markers linked to resistance genes may be also useful for cloning and sequencing the genes. Hence, DNA based markers can allow identification of desirable genotypes in the laboratory instead of the field, which can enhance plant breeding efficiency and saving time and efforts.

The purpose of this study is to test the presence of Ty-1 and Ty-3 genes in sixteen tomato genotypes available in Iraqi markets.

**Materials and Methods**

Experiment was conducted at University of Baghdad/Genetic Engineering and Biotechnology Institute for Postgraduate Studies in 2019.

Sixteen tomato genotypes (Table 1) were grown in March 2009 in a growth chamber.

**Table 1 :** Tomato genotypes used for genotyping for SCAR and CAPS markers to identify Ty-1 and Ty-3 genes.

<table>
<thead>
<tr>
<th>No.</th>
<th>Genotypes</th>
<th>Product</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81</td>
<td>Green reef</td>
<td>Turkey</td>
</tr>
<tr>
<td>2</td>
<td>B104</td>
<td>Syngenta</td>
<td>Thailand</td>
</tr>
<tr>
<td>3</td>
<td>Ginan</td>
<td>Seminis</td>
<td>Thailand</td>
</tr>
<tr>
<td>4</td>
<td>GS-12</td>
<td>Syngenta</td>
<td>Thailand</td>
</tr>
<tr>
<td>5</td>
<td>Nada</td>
<td>Elite</td>
<td>Holland</td>
</tr>
<tr>
<td>6</td>
<td>Noon</td>
<td>Diamond</td>
<td>USA</td>
</tr>
<tr>
<td>7</td>
<td>Oula</td>
<td>Seminis</td>
<td>Germany</td>
</tr>
<tr>
<td>8</td>
<td>Rachida</td>
<td>Paracid</td>
<td>Holland</td>
</tr>
<tr>
<td>9</td>
<td>Sereen</td>
<td>Syngenta</td>
<td>Holland</td>
</tr>
<tr>
<td>10</td>
<td>Shefa</td>
<td>Diamond</td>
<td>Spain</td>
</tr>
</tbody>
</table>

**Table 2 :** Sequences and amplification conditions of PCR primers used in this study.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>5' Sequences 3'</th>
<th>Tm</th>
<th>Length</th>
<th>Product size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deng A</td>
<td>TAATATTACCKGKWGVCSC</td>
<td>53</td>
<td>20</td>
<td>520</td>
<td>Deng et al.1994</td>
</tr>
<tr>
<td>Deng B</td>
<td>TGGACYTTTRCAWGGBCCTTCACA</td>
<td>53</td>
<td>20</td>
<td>520</td>
<td>Deng et al.1994</td>
</tr>
<tr>
<td>(CAPS)-F</td>
<td>ATGAGAGACAAAAAATGCTTTC</td>
<td>52.27</td>
<td>20</td>
<td>608</td>
<td>Ji et al. 2007</td>
</tr>
<tr>
<td>(CAPS)-R</td>
<td>TCAGGGTTTCACTTCTATGAAT</td>
<td>54.80</td>
<td>22</td>
<td>320</td>
<td>Ji et al. 2007</td>
</tr>
<tr>
<td>(SCAR)-F</td>
<td>GGTAGTGGAAAATGATGCTGC</td>
<td>59.38</td>
<td>22</td>
<td>320</td>
<td>Ji et al. 2007</td>
</tr>
<tr>
<td>(SCAR)-R</td>
<td>GCTCTGCCTATTGTCCCATATAACC</td>
<td>61.12</td>
<td>27</td>
<td>320</td>
<td>Ji et al. 2007</td>
</tr>
</tbody>
</table>

**Agarose Gel Electrophoresis**

After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria.

**Solutions**

1. X TAE buffer, loading dye, DNA ladder marker, Ethidium bromide (10mg/ml).

**Preparation of agarose**

- 100 ml of 1X TAE was taken in a beaker.
- 1 gm (for 1%) agarose was added to the buffer.
- The solution was heated to boiling (using Micro Wave) until all the gel particles were dissolved.
- 1μl of Ethidium Bromide (10mg/ml) was added to the agarose.

**Casting of the horizontal agarose gel**

The agarose solution was poured into the gel tray after both the edges were sealed with cellophane tapes and the agarose was allowed to solidify at room temperature for 30 minutes. The comb was carefully removed and the gel was placed in the gel tray. The tray was filled with 1X TAE-electrophoresis buffer until the buffer reached 3-5 mm over the surface of the gel.

**DNA loading**

PCR products were loaded directly. For PCR product, 5μl was directly loaded to well. Electrical power was turned on at 100v/m Amp for 75min. DNA moves from Cathode to
plus Anode poles. The Ethidium bromide stained bands in gel were visualized using Gel imaging system.

Results and Discussion

Results showed that six tomato genotypes were contain both TY-1 and TY-3 (GS-12, Ginan, 81, Yara, Toppy and Yassamin) and two genotypes contain only one gene (Seren contains TY-3 and Zina contains TY-1). Also, the results showed that eight genotypes do not contain neither the TY-1 or TY-3 (Oula, super Marmande, Rasheda, Nada, B104, Noon, Wigdan and Sheifa). The SCAR marker specific of TY-1 recorded a PCR product of 320 bp (figure 1) while TY-3 CAPS specific marker showed PCR product of 608 bp (figure 2).

Fig. 1: Detection of Ty-3 gene by Conventional PCR using SCAR marker for 10 tomato genotypes using 100 bp Ladder on 1% agarose gel at 100 volts for 90 minutes.

Fig. 2: Detection of Ty-1 gene by Conventional PCR using CAPS marker for 10 tomato genotypes using 100 bp Ladder on 1% agarose gel at 100 volts for 90 minutes.

References


