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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 Marmande, 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 Marmande, 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 *Begomoviruses* 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*, (Homopetra: 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 ϒ. 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.

No.	Genotypes	Product	Origin
1	81	Green reef	Turkey
2	B104	Syngenta	Thailand
3	Ginan	Seminis	Thailand
4	GS-12	Syngenta	Thailand
5	Nada	Elite	Holland
6	Noon	Diamond	USA
7	Oula	Seminis	Germany
8	Rachida	Paracid	Holland
9	Sereen	Syngenta	Holland
10	Shefa	Diamond	Spain
11	Super Marmande	Holland	Holland
12	Tobby	Syngenta	Holland
13	Wigdan	Seminis	Mexico
14	Yara	Atlas	China
15	Yasamin	Syngenta	Thailand
16	Zina	FAO	Holland

Two DNA based molecular markers SCAR and CAPS which linked to *Ty-1* and *Ty-3*genes respectively were used to detect the presence of these genes in tomato genotypes available in the Iraqi market. DNA was extracted from tomato leaf seedlings according to the protocol of Wizard Genomic DNA Purification Kit, Promega as the following steps:

Forty milligrams of leaf tissue were grinded, 600µl from Nuclei Lysis Solution then added.

- All mixes were Incubate overnight at 65°C, then cooled to room temperature.
- For RNA lysis, 3µl of RNase Solution. Mixed, then incubated at 37°C for 15 minutes.
- For Protein Precipitation, 200µl of Protein Precipitation Solution were add to cell lysate.
- Then mixed well by vortexing. Then incubated deep freeze (-30°C). After that, centrifuge at 13,000 rpm for 5 minutes.
- Diluted DNA transfer to a clean tube containing 600µl of room temperature isopropanol.
- After mixing gently, centrifuge as in "Pellet Cells" above, and supernatant was decanted.
- From room temperature 70% ethanol, 600µl were added then centrifuge for 2 minutes at 13,000 rpm.
- Ethanol then aspirate and air-dried the pellet.
- DNA pellet was rehydrated in 100µl of Rehydration Solution for 1 hour at 65°C.

The polymerase chain reaction (PCR) procedure was carried out following the standard protocol with specific primers (Table 2).

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

Primer name	5' Sequences 3'	Tm	Length	Product size	Reference
Deng A	TAATATTACCKGWKGVCCSC	53	20	520	Deng *et al.*1994
Deng B	TGGACYTTRCAWGGBCCTTCACA		23		
(CAPS)-F	ATGAAGACAAAAACTGCTTC	52.27	20	608	Ji *et a*l. 2007
(CAPS)-R	TCAGGGTTTCACTTCTATGAAT	54.80	22		
(SCAR)-F	GGTAGTGGAAATGATGCTGCTC	59.38	22	320	Ji *et al.* 2007
(SCAR)-R	GCTCTGCCTATTGTCCCATATATAACC	61.12	27		

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.
- The agarose was stirred in order to get mixed and to avoid bubbles.
- The solution was allowed to cool down at 50-60 ºC.

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).

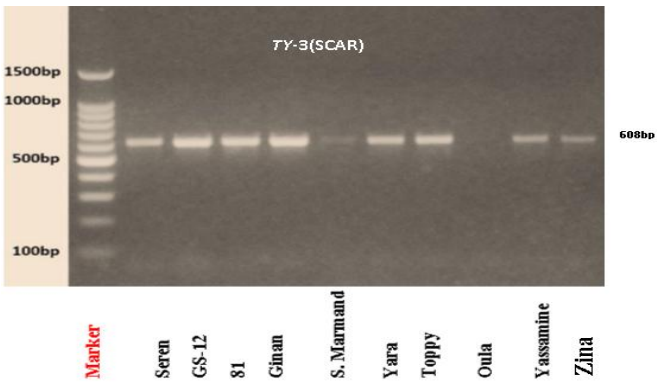

Fig. 1 : Detection of *Ty-3* gene by Conventional PCR using SCAR marker for 10 tomato genotypes using 100 bp Ladder on 1% agarose gel at 100 volts for 90 minutes.

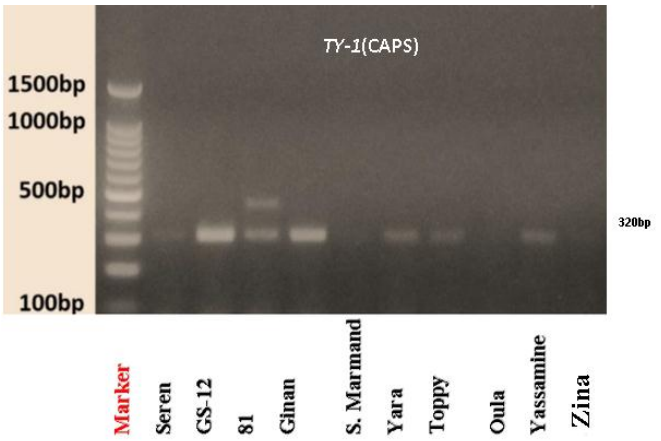

Fig. 2 : Detection of *Ty-1* gene by Conventional PCR using CAPS marker for 10 tomato genotypes using 100 bp Ladder on 1% agarose gel at 100 volts for 90 minutes.

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