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MOLECULAR IDENTIFICATION OF TOMATO GERMPLASM FOR RESISTANCE TO TOMATO YELLOW LEAF CURL VIRUS IN EGYPT

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ABSTRACT

The present investigation was carried out to study molecular marker are known to confer resistance to tomato yellow leaf curl virus (TYLCV) in some local and hybrid Fourteen tomato accessions namely, Jubilee, Peto86,Super Strain B, Advantag, Edkawi, super Bader, Tempo, Azteca, G.5, G.S, Nada, 9062, 9065, 9064. The plants were grown in under greenhouse conditions, open field and test for reactions to TYLCV.

The resistance to TYLCV disease in the tomato accession linked to Ty-2 gene. All the plants in the studs one band only 800 bp were susceptible to the disease; expat 9064 two bands 800bp and 900 bp were resistant.

***Conclusively,** the use of methods of molecular markers to identify resistant varieties and sensitive to viruses. Moreover, the use of modern molecular methods to identify the local plant varieties with jam and before inclusion in breeding programs to save time and effort and money.*

Key words: Molecular, Resistance, Tomato Yellow Leaf Churl Virus.

INTRODUCTION

Tomato, *Lycopersicon esculentum*, is one of most important vegetable crops in the world, with an annual production of more than 160 million tons and it is currently the most highly consumed vegetable in the world. Tomatoes and tomato-based products are considered as healthy foods for

several reasons (Suarez *et al*., 2008). Tomato is one of the most important vegetable crops grown in Egypt for fresh consumption and processing.

Tomatoes are grown all year seasons round in Egypt (515000 Feddan / 8 575000 Ton) (FAO.2013); the tomato is grown both in open cultivation and in green houses for commercial and home use. Virus diseases occasionally cause serious damage and large economic losses. The amount of loss can vary from 5% to 90% depending on the virus disease involved, the strain of the virus, the variety of tomato, the age of the plant at infection time, the temperature during disease development, the presence of other diseases, and the extent that viruses have spread in the planting (Averre and Gooding, 2000).

Tomato yellow leaf curl disease is one of the most devastating tomato diseases in the Mediterranean area. It can be caused by a complex of closely related viruses. They constitute a group of small monopartite single stranded DNA begomoviruses belonging to the Geminiviruses family (Stanley, 1985; Navot *et. al*., 1992). They are transmitted by the whitefly Bemisia tabaci in a circulative manner (Cohen and Nitzany, 1966). Symptoms of the disease consist of a more or less prominent upward curling of leaflet margins, reduction of leaflet area and yellowing of young leaves, together with stunting and flower abortion (Moriones and Castillo, 2000). Since 1980s molecular markers are being widely used as a major tool for molecular breeding of tomato (Barone, 2008). More than 40 genes (including many single genes and quantitative trait loci, Q.T.L) that confer resistance to all major classes of pathogens have been mapped on the tomato molecular map (Grube *et. al*., 2000). The H24 resistance factor, named *Ty-2* was reported to be associated with an introgression from TG36 (84 cM) to TG26 (92 cM) (Hanson *et. al*., 2006). This polymorphic marker was chosen to screen the segregating populations that were screened at AVRDC with a biological assay for resistance to TYLCV (Ji *et.al*., 2007 and Mohmed and Palchamy 2012).

Therefore, this study aimed to molecular screening for *Ty-2* gene using a T0302 marker linked to *Ty-2*, and analyzing the genetic of resistance to TYLCV in tomato genotypes.

MATERIALS AND METHODS

Plant material

Fourteen tomato accessions were used in this study, as summarized in Table 1. The experiment was conducted at Genetic Engineering and

Table 1. Genotypes, Accessions and Sources of tomato plants used in this study.

No.	Genotypes	Accessions	Sources
1	***L. esculentum***	Jubilee	ARC, Egypt
2		Peto 86	
3		Super Strain B	
4		Advanteg	
5		Edkawi	
6		Super Bader	
7		Tempo (LA 2050)	NGB, Sweden
8		Azteca	Commercial hybrid
9		G.5	
10		G.S	
11		Nada	
12		9062	
13		9065	
14		9064	

ARC: Agricultural Research Center, Horticulture Research Institute, El- Dokki, Giza, Egypt. **NGB:** Nordic Gene Bank, Sweden.

Biotechnology Research Institute (GEBRI) Molecular Biology Department, University of Sadat City, Egypt.

***DNA extraction* :**

DNA isolation and purification was carried out using CTAB (Cetyl-tetramethyl ammonium bromide) method, according to Murray and Thompson (1980). Fresh Leaf tissues (200mg) were ground to fine powder in liquid nitrogen using sterile, pre-cooled mortars and pestles. The powder was transferred to a 1.5ml microcentrifuge tube containing 0.6ml of pre-warmed (65°C) DNA extraction buffers. Suspension was incubated at 65°C for 30 min with intermittent swirling after which equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by gentle inversion for 5 min. The content was then spun at 15,000 rpm for 10 min. The aqueous phase was transferred to a fresh 1.5 ml microcentrifuge tube. Then, 0.6ml of chilled isopropanol followed by quick and gentle inversion and incubated at - 20°C for 30 min was added. The precipitated DNA was pooled out using wide bore tips, and further centrifugation was done for the supernatant at 10,000 rpm

for 10 min. The DNA pellet was washed twice with 70% ethanol, air dried and dissolved in 100 µl TE buffer.

***SCAR marker linked to the TYLCV resistant gene, TY-2,in tomato*:**

All the procedures for PCR and for the separation of amplified products were carried out as described **Mohamed, S. M., palchamy, K.(2012).** T0302F and T0302R primer was used to assays locus, TY-2,in tomato Sequence of marker used in this study in Table 2.

Table 2. Sequence of marker.

Marker	Primer sequence
T0302	F:5̀ TGGCTCATCCTGAAGCTGATAGCGC R:5̀ AGTGTACATCCTTGCCATTGACT

PCR amplification

The PCR reaction was carried out in a total volume of 25 µl containing: 10x buffer recommended by suppliers, 2.5 mM MgCl2, 0.5 µM of each primer, 0.4 mM dNTPs, 1 U of Taq DNA polymerase and 40 ng of template DNA. The amplification was carried out in PCR thermocycler machines from Biometra (T-Gradient Thermoblock). PCR cycles were 94°c for 4 min, 35 cycles of 94° C for 30s, 55° C for 1min and 72° C for 1 min, followed by an extension step of 10 min at 72° C.

Gel electrophoresis

Agarose (1.5%) was used for resolving the PCR products and 1 kb DNA marker as a standard DNA were used in the present study. The run was performed for 1 hour at 50 volt in SDE-PLAS submarine (10 cm x 10 cm). Bands were detected on UV-transilluminator, photographed by Gel Documentation System and were analyzed using the Phortix Program.

RESULTS AND DISCUSSION

T0302 screening of *Ty-2* was carried out using genomic DNA from tomato accessions. Electrophoretic patterns of the fragments amplified by PCR from genomic DNA using the primer T0302 shown in (Figures 1). T0302 marker fragments were amplified from the genomic DNA of Fourteen accessions of tomato. The nucleotide sequences of the primer pairs used for amplifying the fragments are shown in (Table 2).

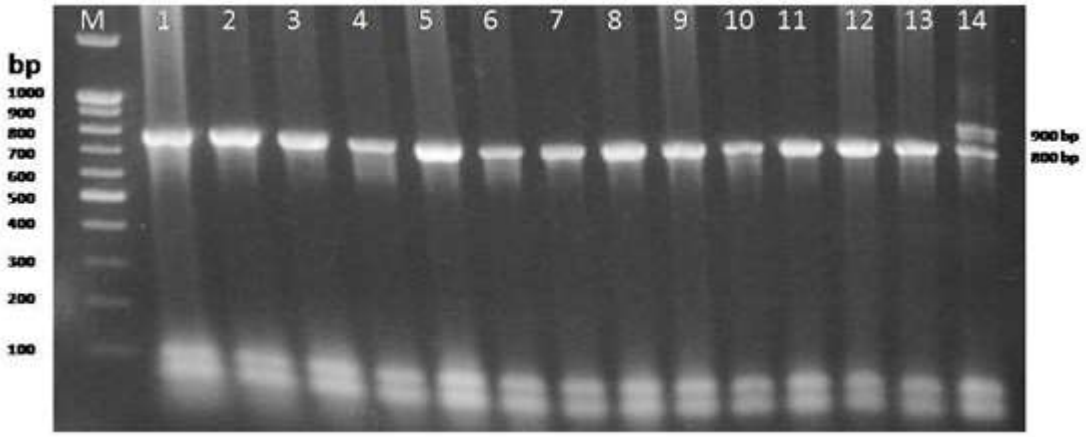

Figure 1. Agarose-gel (1.5%) electrophoresis stained with ethidium bromide shows products amplified with primer T0302from genomic DNA of Fourteen tomato accessions.

1. Jubilee	**6. super Bader**	**11. *Nada***
2 Peto 86	**7. Tempo (LA 2050)**	**12. *9062***
3. super Strain B	**٨. Azteca**	**13. 9065**
4. Advantag	**9. G.5**	**14. 9064**
5. Edkawi	**10. G.S**	
M: 1 kp DNA marker 100 bp – 1000 bp)		

Upon PCR amplification (using annealing temperature of 55°C) two bands was observed in (Figure 1), the amplified product was approximately 800 bp and 900 bp in length as expected according to analysis by Phoretix Program 1D Gel Analysis Software Version 4.01(Table 3). All tomato varieties shown one band expect commercial hybrid 9064 observed two bands 800 bp and 900 bp (Figure 1). T0302 primer was tested with germplasm gave the 900 bp and 800bp fragment (Figure 1). T0302F/T0302R primer effectively detected the two genotypes, *ty2/ty2 800bp* and *Ty2/Ty2 (800bp and 900bp) (*Hanson, 2006; Mohmed and Palchamy, 2012).

Table 3. PCR patterns of fourteen tomato accessions.

M	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1000														
900	0	0	0	0	0	0	0	0	0	0	0	0	0	1
800	1	1	1	1	1	1	1	1	1	1	1	1	1	1
700														
600														
500														
400														
300														
200														
100														

No.	Accession	Genotypes Ty-2	Reaction to TYLCV
1	Jubilee	-/-	Susceptible
2	Peto 86	-/-	Susceptible
3	Super strain B	-/-	Susceptible
4	Advantag	-/-	Susceptible
5	Edkawi	-/-	Susceptible
6	super Bader	-/-	Susceptible
7	Tempo (LA 2050)	-/-	Susceptible
8	Azteca	-/-	Susceptible
9	G.5	-/-	Susceptible
10	G.S	-/-	Susceptible
11	Nada	-/-	Susceptible
12	*9062*	-/-	Susceptible
13	9065	-/-	Susceptible
14	9064	+/+	Resistant

Conclusively, the use of methods of molecular markers to identify resistant varieties and sensitive to viruses. Moreover, the use of modern molecular methods to identify the local plant varieties with jam and before inclusion in breeding programs to save time and effort and money.

REFERENCES

Averre, C.W. and Gooding, G.V.(2000). Virus diseases of greenhouse tomato and their mangment. http://www.ces.ncsu.edu/depts./pp/notes/oldnites/vg15 .htm

Barone, A.(2008). Molecular markers-assisted selection for resistance to pathogens in tomato. *Marker Assisted Selection*: A fast tract to increase genetic gain in plant and animal breeding, Session1: MAS in plants.

Cohen, S. and Nitzany, F.E.(1966). Transmission and host range of Tomato Yellow Leaf Curl Virus. Phytopathology 56:1127–1131.

FAO(2013). *Food and Agricultural Organization*. Statistics Division, FAOSTAT [Online] Available: http://faostat.fao.org.

Grube, R.C.; Radwanski, E.R. and Jahn, M.(2000). Comparative genetics of disease resistancwithin the *Solanaceae*, *Genetics*, **155:** 873-887.

Hanson, P.; Green, S.K. and Kuo, G.(2006). *Ty2*, a gene on chromosome 11conditioning geminivirus resistance in tomato, *Tomato Genetic Cooperative Report*, **56**:17-18.

Ji, Y.; Hanson, P.; Graham, E. and Maxwell, D.P.(2007). Sources of resistance, inheritance, and location of genetic loci conferring resistance to members of the tomato infection begomoviruses, In: Czosnek H (ed) Tomato yellow leaf curl virus disease: *Management, Molecular biology, Breeding for Resistance.* Kluwer, Dordrecht.

Mohamed, S. M. and Palchamy, K. (2012). Molecular-marker-screening for *Ty-2* gene in two tomato genotypes (*Solanum lycopersicon* L.), *International Journal of Scientific & Technology Research*, **1**: 4:45-47.

Moriones, E. and Castillo, J. N. (2000). Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide, *Virus Research* **71**: 123–134.

Murray, H.G. and Thompson,W.F. (1980). Rapid isolation of high molecular weight DNA, *Nucleic Acids Res*., **8**: 4321–4325.

Navot, N.; Zeidan, M.; Pichersky, E.; Zamir, D. and Czosneck, H.(1992). Use of the polymerase chain reaction to amplify Tomato Yellow Leaf Curl Virus DNA from infected plants and Viruliferous whiteflies. *Phytopathology*, **82**:1199–1202.

Stanley, J.(1985). The molecular biology of geminiviruses. *Adv Virus Res*, **30**:139–177.

Suarez, M.H.; Pallares, J.R.; Mesa, D.R.; Rodriguez, E.R. and Romero, C.D. (2008). Variation of the chemical composition of tomato cultivars (*Lycopersicon esculentum* Mill.) according to resistance against the tomato yellow leaf curl virus (TYLCV), *J. of the Science of Food and Agriculture*, **88**:1882–1891.

التوصيف الجزيئى لمقاومة اصناف الطماطم لفيروس اصفرار و تجعد الاوراق فى مصر

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الملخص

أجريت هذه الدراسة لاختبار الواسمات الجزيئة الخاصة بجينات المقاومة لفيروس اصفرار و تجعد الاوراق فى اربعة عشر صنف من الطماطم المحلية و الهجن و هى Jubilee, Peto 86, Super Strain B, Advanteg , Edkawi , Super Bader, Tempo(LA 2050), Azteca, G.5, G.S, Nada, 9062, 9065 and 9064. و التى تم زراعتها فى الحقل المفتوح و الصوبة وتم عمل فحص لجين *TY2* المقاوم للفيروس.

جميع الاصناف تحت الدراسة اظهرت الحزمه ٨٠٠ قاعدة نتيكلوتيدية و التى تدل على ان هذه الاصناف حساسة للفيروس ، ماعدا الصنف ٩٠٦٤ الذى اظهر الحزمة ٨٠٠ قاعدة و ٩٠٠ قاعدة نيكلوتيدية و اللتان تدل على ان هذا الصنف مقاوم للفيروس.

التوصية:

* استخدام طرق الواسمات الجزيئية للتعرف على الأصناف المقاومة و الحساسة للفيروسات.

* استخدام الطرق الجزيئية الحديثة للتعرف على الأصناف المحلية لدى مربى النبات وذلك قبل إدخالها فى برامج التربية لتوفير الوقت والجهد و المال.