Using Molecular and Biological Tools for Assessment of TYLCV Resistant Tomato Cultivars Commercially Grown in Southern Palestine

استخدام الأدوات الجزيئية والبيولوجية لتقييم أصناف البندورة المقاومة لفيروسTYLCV والمستعملة تجاريا في جنوب فلسطين

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Received: (10/2/2013), Accepted: (26/1/2014)

Abstract

Tomato, considered one of most important and popular crop, was infected by *Tomato Yellow Leaf Curl Virus* (TYLCV) which causes significant yields loss. Biological and molecular tools were used to evaluate the TYLCV incidence on tomatoes grown under commercial conditions. A total number of 72 tomato plants from each eight different tomato cultivars commercially planted in Palestine were tested for their virus infection which occurred naturally. The virus incidence was reported biologically based on visual inspections for the disease symptoms and molecularly by PCR tests, in two growing season periods: Summer (2006) and spring (2007). As a result, no cultivars were found "immune" to virus infection. However, fundamental differences in symptoms development and severity had been discovered. This study, which was carried out for the first time in Palestine, showed that some cultivars such as "3060" could be targeted as promising virus-tolerant ones. The sensitivity of molecular methods over bioassays was evaluated, and combined methods were suggested for any cultivars resistance assessments. Besides, research results revealed that the experimental

conditions in Spring were not in favor of the virus spread, as the main virus vector (*Bemisia tabaci)* population number would be lower in the Summer season; thus the Spring season is seen as the best one for farmers to escape from TYLCV infections.

Keywords: Bioassays, Tolerance, Molecular, Tomato, TYLCV, Palestine.

ملخص

تعتبر البندورة واحدة من المحاصيل المهمة والشعبية، ولكنها تصاب بفيروس تجعد واصفرار اوراق البندورة (TYLCV) مما يسبب خسارة كبيرة في الانتاج. تم استخدام الأدوات البيولوجية والجزيئية لتقييم الإصابة على البندورة والتي نمت في الظروف التجارية. تم اختبار ٧٢ من نبات الطماطم لكل صنف من الأصناف الثمانية للطماطم المختلفة والمستعملة تجاريا في فلسطين، وذلك بفحص اصابتها بالفيروس المنتقل اليها بشكل طبيعي. حيث تم ثبوت وقوع الاصابة الفيروسية بيولوجيا عن طريق عمليات التفتيش البصرية لأعراض المرض، وجزيئيا عن طريق اختبارات تضاعف الحمض النووي (PCR)، وذلك في موسمين: صيف (٢٠٠٦) وربيع (٢٠٠٧). وكانت النتيجة لذلك انه لا توجد "مناعة" لعدوى الفيروس لأي من الأصناف المختبرة. ومع ذلك، فقد كشفت الدراسة، والتي تجرى للمرة الأولى في فلسطين، اختلافات جوهرية في تطور المرض وشدة الاصابة. حيث أظهرت النتائج انه يمكن استهداف بعض الأصناف الواعدة مثل "٣٠٦٠" لكونها الاكثر تحملا للفيروس. تم كذلك تقييم حساسية الاختبارات الجزيئية مقارنة بمثيلاتها البيولوجية، واقتراح استعمال الاختبارين معا في تقييم أي أصناف مقاومة. اضافة الى ذلك، كشفت النتائج أن الظروف التجريبية في الربيع لم تكن في صالح انتشار الفيروس، حيث أن تعداد ناقلات الفيروس الرئيسة (الذبابة البيضاء) تكون أقل من موسم الصيف، مشيرة الى ان هذا الموسم هو الأفضل للمزارعين للهروب من الاصابة بالفيروس (TYLCV).

Introduction

Tomato crop in many countries in the Middle East including Palestine, is infected with several diseases, tomato yellow leaf curl viral disease (TYLCVD) is one of them. Since the 1980s, the disease had become one of the most economically important tomato diseases world-wide, presented in most Mediterranean countries and parts of sub-Saharan Africa, Asia, Japan, Australia, the Caribbean Islands, and recently reported in USA, as in Florida, Georgia and Louisiana (Czosnek *et al.* 1990; Gallitelli *et al*. 1991; Nakhla *et al.* 1994; Polston *et al.* 1994;

Konaté *et al.* 1995; Czosnek and Laterrot 1997; Nakhla and Maxwell 1998; Peterschmitt *et al.* 1999; Accotto *et al.* 2000; Sinisterra *et al*. 2000; Bird *et al.* 2001; Jebbour and Abaha 2002; Urbino and Tassius 2003).

At least nine different virus species had been found associated with TYLCD which altogether are referred to as "*Tomato yellow leaf curl virus*" (TYLCV) (Moriones and Navas- Castillo 2000; Fauquet and Stanley 2005; Stanley *et al*. 2005; Moriones 2007). These viruses belong to the genus *Begomovirus* in the family *Geminiviridae.* The potential of begomoviruses to generate genetic diversity through recombination could be relevant for their ecological fitness, and recombination that forces driving evolution in this group of viruses (Fauquet and Stanley 2005). The family *Geminiviridae* is comprised of four genera: *Mastrevirus***,** *Curtovirus***,** *Topocuvirus* and *Begomovirus* (Van Regenmorte 2000), sharing similarities in genome organization, insect transmission, and host range. The genus *Begomoviruses* consists with monopartite and bipartite genomes, and been transmitted by whiteflies (*Bemisia tabaci* Gennadius (Homoptera: *Aleyrodidae*) in a persistent, circulative, non-propagative manner.

The early report of the disease symptoms caused by *Tomato yellow leaf curl viru*s (TYLCV), on tomato (*Lycopersicon esculentum*) plants describes small, curled and chlorotic leaves, later descriptions of symptoms included stunted plants and loss of fruits because of the premature drop of flowers (Ber *et al.* 1990). Symptoms become visible in tomato in approximately 3-4 weeks after infection.

The domesticated tomato *Solanum esculentum* (formerly *Lycopersicon esculentum*) is the primary host of TYLCV. Most of wild tomato species such as *S. chilense, S. habrochaites* (formerly *L. hirsutum), S. peruvianum*, and *S. pimpinellifolium* were symptomless carriers and could be used in breeding programs for TYLCV resistance (Zakay *et al*. 1991). Beside tomato, other plant species in several botanical families were found potentially host plant species of this virus (Mansour and Al-Musa 1992; Kegler 1994; Cohen and Antignus 1994; Nakhla and Maxwell 1998; Polston *et al*. 2009; Salati *et al.* 2002).

In many cases the disease caused losses in yields that could be reached up to 100% (Polston *et al.* 1999). Tomato plants in commercial fields of most regions of the world were still largely susceptible to various begomoviruses (Polston and Anderson 1997). The management of TYLCV in tomato was difficult and expensive, however, many approaches had been used resistant cultivars to decrease losses of TYLCV. So far, several strategies had been investigated to control TYLCV, most of them were either directed towards insect (vector) control (Hamdan and Abu-Awad 2008) or by breeding crops resistant or tolerant to the virus. Nevertheless those measures could only delay the progress of the virus, but not suppress it (Caciagli *et al.* 1995). Therefore, the potential approaches in reducing the virus incidence still namely resistant crop are in developing (Cohen and Antignus 1994; Morales 2001; Lapidot and Friedmann 2002).

Chemical Treatment Were Inefficient To Limit The TYLCV Spread, Hence, Breeding Resistant Or Tolerant Varieties Of Tomatoes (Lapidot And Friedmann 2002; Morales 2001; Pico *Et Al.* 1996) Was The Only Choice. In Palestine, TYLCV Is Considered One Of The Most Damaging Pathogen On Tomato (Czosnek And Laterrot 1997; Sawalha 2009). Several Tomato Lines Were Imported From Seeds' Companies With Varied Tolerance Levels For The TYLCV, But Data Of Their Susceptibilities Or Resistances To The Virus (TYLCV) Were Not Documented Locally And Thus Were Not Available For Growers. For Such Tale, This Research Studies Were Conducted On Monitoring The Virus Incidence On Tomato Cultivars That Are Mostly Planted In Palestine Under Commercial Conditions (Open Fields), Using Biological And Molecular Tools. Both Methods Were Compared And Analyzed For The First Time On Tomatoes Naturally Infected To *Tomato Yellow Leaf Curl Virus.* Tomato Cultivars Resistance Levels Were Then Estimated To Be Available To Growers, Nurseries As Well As Part For Any Clonal Selections Recommended For Any Future Breading Programs.

Materials and Methods

Plant Material and Experimental Design

Different tomato cultivars from mostly cultivated ones were collected according to data brought from local nurseries and based on Palestinian farmers' choice. Most of these cultivars were imported from seeds companies and having commercial names such as: "Teiba, 116, 916, 1684, 3060, 3019, Munna and 56". Some varieties were brought from Zeraim Gedera Seed Company (116; 916 and 1684); Hazera Seed Company (3060; 3019 and 56/56) and local sources as Sharbati nursery (Teiba) and Juzor (Muna). Tomato seedlings were then planted and tested for TYLCV virus infection (*in vivo*) at Hebron University Research Unit in Al-Arroub Agricultural Experimental Station. Seedlings plants were let to grow in open field, in two growing seasons: summer (2006) and spring (2007) as usually done by Palestinian farmers, after ensuring completely randomized block design with four replicates for each cultivar. No insecticides or artificial inoculation were applied. Selected plant seedlings were planted in about 0.4 ha open field at Hebron University Research Unit in Al –Arroub Agricultural Experimental Station to be tested later for their natural infection of TYLCV virus (without human interference or artificial inoculation).

All healthy seeds and/or seedling plants were planted in the field in completely randomized block design with four replicates. The total number of plants for each cultivar was 72, 18 tomato seedlings for each replicate, in the two growing season successively. The distance between plots was 1.5m and within each plant line was 0.5 m. Drip irrigation system was applied and no insecticides had been used. Cultivar 56/56 was only planted in spring.

Bioassays

The infection degree was evaluated biologically based on a scale established for rating TYLCV severity symptoms degree. The scale ranged from 0 (no symptoms) to 3 (severe symptoms). Severity incidences of infection for the TYLCV were recorded after planting and evaluated on weekly basis for each cultivar based on visual inspections

for the virus disease symptoms. Yellowing of apical leaf margins, curling and stunting were the main criteria for evaluation of disease symptom developments, using the following scale for evaluation:

Severity Scale	Description	Details
0	None	no visible symptoms
1	Mild	very slight yellowing of leaflet margins on apical leaf
2	Moderate	few yellowing and minor curling of leaflet ends
3	Severe	intense yellowing and major curling and/ or stunting

The symptom severity rating scale was weekly reported for each cultivar in both growing seasons. Statistical analysis for the degree of infection and symptom severity index were done according to Fisher LSD at $p < 0.05$, which takes the square root of the Residual Mean Square from the ANOVA and considers that to be the pooled SD and computes a standard error of the difference between those two means. Then it computes a t ratio by dividing the difference between means by the standard error of that difference.

Molecular assays

Beside visual inspections, molecular analysis using virus-specific primers in polymerase chain reaction (PCR) had been applied to record incidence of viral infection and to help in tomato cultivars resistance levels assessments. Molecularly, the infection degree was calculated based on PCR amplification product for samples using virus specific primers as recommended by (Navot *et al.* 1991). Since several virus isolates had been sequenced, only two species of TYLCV were recognized in Mediterranean basin: *Tomato yellow leaf curl virus* - Israel (TYLCV-IL) (Navot *et al*. 1991) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV) (Kheyr-Pour 1991); however, TYLCV-IL is currently the most prevalent species in Europe (Gafni 2003).

In this study, primers were designed to amplify 634 bp from the intergenic region (IR) of "TYLCV-Israel" (Navot *et al*. 1991). These primers were TYv2337 (viral sense) [5´-ACGTAGGTCTTGACATCTGTTGAGCTC3'] (anneals at nt 2337-2364) and Tyc138 (viral comp) [5´-AAGTGGGTCCCACATATTGCAAGAC 3'] (anneals at nt 138-125).

Since viral DNAs were assumed to be accumulated in the uppermost leaf of each plant, total nucleic acids were extracted from tomatoes upper parts from both TYLCV-infected and healthy one's using a modified procedure of Dellaporta heat extraction method as described by Potter *et al.* (2003). Briefly, 5 mg of leaf sample were extracted with 1ml of extraction buffer (100 mM Tris pH 8; 50 mM EDTA; 500 mM NaCl; 10 mM β –Mercaptoethanol and 1.25 % SDS) using a pre-cold mortars and pestles. Samples were allowed to settle at 65 °C for 10 min before adding 1/5 volume of potassium acetate (5 M, pH 8). Incubation on ice for 10 min, followed by 13,000 rpm centrifuge for 20 min at 4 °C. Isopropanol (1:1 volume) was added to supernatant and the mixture was incubated at -20 °C for 10 min, before centrifugation. The pellet was then washed by phenol/Chloroform/Isoamyl alcohol (25:24:1) and DNA was precipitated in absolute alcohol. After washing with 70% ethanol, the pellet was dried and re-suspended in 60 µl of sterile water (HPLC), to be used for PCR reaction mixture (Biolin Ltd, USA) [2.5 µl of DNA sample, 0.25 Mm deoxynucleotide triphosphate (dNTPs, 1x *Taq* DNA polymerase buffer, 0.25 mM $MgCl_2$, 0.5 units *Taq* DNA polymerase], in addition to 2.5 µM of each virus- complementary and sense primers. PCR products were obtained with amplification parameters [94 °C / 5 min; 30X (94 °C / 1 min, 62 °C / 45 sec., 72 °C / 1 min); 94°C / 1 min; 56 °C / 1 min; 72 °C / 10 min] by Thermocycler PTC- 0200 DNA engine (Alpha unit) provided by Biotechnology laboratory at Hebron University. The amplicons were analyzed in 1.2 % agarose gel electrophoresis stained with ethidium bromide.

Results

Symptom-severity rating

TYLCD were found infecting all tomato cultivars planted in both growing seasons but with varied percentage of infection evaluated based on the severity scale abovementioned. The symptoms were observed typically for the virus and they were varied from chlorotic margins, small leaves that are cupped, thick and rubbery to severe stunting, yellowing, leaf cupping and stunting.

The first observed symptoms appeared two weeks after planting in tomato cultivars 116, 916, "1684, and 3019" while in "Munna" cultivar symptoms were produced 5 weeks later. Symptoms were started with strong chlorosis and yellowing of young leaves to be then developed toward upward curling of leaf margins, and culminated in a complete stunting of growth.

In summer growing season, there were fundamental differences in symptoms manifestation time as well as the percentage of infection and symptom severity (Table 1). Although none of the tomato cultivars were immune to the virus infection, was the most promising cultivar showed high level of tolerance in all the parameters used in the experiment as mentioned in Table (1).

Table (1). Biological assays for tomato cultivars infected by TYLCD under natural conditions for summer growing season (2006)*.

Cultivar	Weeks to manifest symptoms	% of infection	Symptom severity scale				Symptom Severity Index
			0	1	2	3	
3019	2	93a	5	0	0	67	2.791
1684	2	71a	21	7	4	40	1.875
116	2	58b	30	10	11	21	1.31
916	2	25c	54	9	4	5	0.444
Teiba	3	21c	57	6	7	2	0.361

…continue table (1)

Cultivar	Weeks to manifest symptoms	% of infection	Symptom severity scale				Symptom Severity Index
			0	1	2	3	
Munna	5	14c	62	7	2	1	0.194
3060	4	7c	67	2	2	1	0.125

*Symptom severity scaled from 0 (no symptoms) to 3 (Severe symptoms). The degree of infection for tomato cultivars infected by TYLCV virus has been calculated for eight weeks after planting. Symptom severity index was calculated using Fisher LSD at $p \leq 0.05$. Each cultivar had 72 plants. Cultivars with same letters had no significant differences

Infection severity incidence was found varied according to cultivars, i.e cultivars "3019 and 1684" had high symptom severity and incidence reached (93%, 71%) respectively, while cultivars "3060 and Teiba" showed lower incidence in symptom severity (7% and 14%) respectively in summer growing season. The observations indicated also that these cultivars have fundamental difference in the degree of infection every week after planting. It was noticed that the percentage of infections increased rapidly every week with cultivars "3019, 1684" but not for cultivar "3060". That was also correct for the severity index. Cultivar s "3019 and 1684" had the highest symptom severities (2.79 and 1.8) respectively compared with "3060" cultivar. Although cultivar "3060" represents the least degree of infection, it was clustering with cultivars "Munna, Teiba and 916" (Table 1)

Results from spring growing season (2007) were obtained in twelve weeks. Generally the % of infection recorded at this season was relatively low (max. 8.3%) compared to summer 2006 one (Table 2). The tomato cultivars "56/56 and 1684" were firstly recorded to show symptoms which appeared after four weeks while "Teiba, 916 and 3060" cultivars did not appear any symptoms during that growth period.

Table (2): Biological assays results from spring growing season (2007) for tomato cultivars infected with TYLCD naturally*.

Cultivar	Weeks to manifest symptoms	% of infection	Symptom severity scale				Symptom Severity Index
			0	1	2	3	
56/56	4	13.8	58	11	1	2	0.194
1684	4	8.3	64	6	0	2	0.138
Munna	9	2.7	69	3	0	0	0.014
116	9	2.7	70	2	0	0	0.027
3019	10	1	71	1	0	0	0.013
Teiba	0	0	72	0	0	0	0
916	0	0	72	0	0	0	0
3060	0	0	72	0	0	0	0

*Symptom severity were scaled from 0 (no symptoms) to 3 (Severe symptoms). Symptom severity index was calculated using Fisher LSD at $p < 0.05$.

Surprisingly, the highest infection recorded in summer 2006 for "3019" cultivar, was the last one expressed symptoms in spring (10 weeks later), in spite of "1684" cultivar remained on the top of list for highest degree of infection. Meanwhile cultivars "3060, Teiba and 916" remained with lowest (if not) infection in spring time.

Screening Molecular incidence of TYLCV

The presence of TYLCV in the tomato cultivars was detected by using PCR method. Total DNA from healthy and infected tomato cultivars was extracted using modified procedure of Dellaporta heat extraction method (Potter *et al.* 2003). *Taq* DNA polymerase sensitivity in detection of the virus DNA had been tested and optimum results were used for detection protocol. PCR gave a single product of the expected size at (634 bp) at 0.4 units *Taq* DNA polymerase for all positive samples (Fig. 1). Primers used in this study were able to amplify the (IR) of TYLCV formally known as "TYLCV-Israel".

PCR incidences using primers designed o the (IR) of "TYLCV-Israel" showed that cultivar 3060 and Teiba had the lowest incidence (1%, 5%) respectively (Table 2).

Regarding to molecular detection of TYLCV in tomatoes cultivar planted in spring 2007; PCR incidences for each cultivar were shown in Table 3.

PCR results here shown that cultivar 56/56 had the highest incidence while cultivars 3060, 3019 and Teiba had no positive results. Comparing molecular results for both growing seasons revealed differences in virus incidence for some cultivars (Table 4).

Fundamental differences had been obtained when results between TYLCD infected tomato cultivars were compared during summer and spring season. These differences were observed in all used parameters (days to produce symptoms, symptom severity, and degree of infection). In summer, symptoms were observed after two weeks of planting, while in spring, symptoms (if any) were four weeks later. The early infection could be due to nursery infection, while late ones might be due to open field infection. In addition to that, severe symptoms were not observed in spring except for cultivar "56/56" in which two plants had only severe symptoms, while in summer severity symptoms were devastated (Fig. 2 & 3).

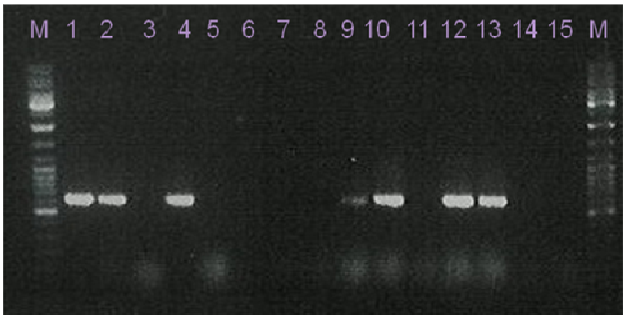

Figure (1): TYLCV intergenic region in tomato cultivars were successfully detected by PCR. Amplicons of 634bp were analyzed in 1.2% agarose gel electrophresies and found in samples (1, 2, 4, 9 and 10). Samples 12 &13 are virus positive control while 14&15 are healthy ones. "M" refers to the DNA ladder that used as a marker (1000bp).

Table (2): TYLCV incidence for tomato cultivars infected during summer growing season 2006 in Alarrub Experimental Station using PCR.

Cultivar	Total samples	PCR positive	% of PCR incidence
116	68	35	51
916	72	37	51
3019	64	32	50
1684	60	25	42
Teiba	71	20	28
Munna	45	3	5
3060	72	1	1
Total	**452**	**153**	**34**

Table (3): TYLCV incidence for tomato cultivars infected during spring growing season 2007.

Cultivar	Total samples	PCR positive	% of PCR incidence
56/56	72	6	8
116	68	4	6
1684	72	3	4
Munna	72	3	4
916	72	2	3
3060	72	0	0
3019	70	0	0
Teiba	67	0	0
Total	**565**	**18**	**3**

Table (4): Comparison between PCR results for spring and summer growing seasons.

Growing Season	Cultivars							
	56/56	116	916	1684	3060	3019	Teiba	Munna
Summer 2006	NA	51%	51%	42%	1%	50%	28%	5%
Spring 2007	8%	6%	3%	4%	0	0	0	4%

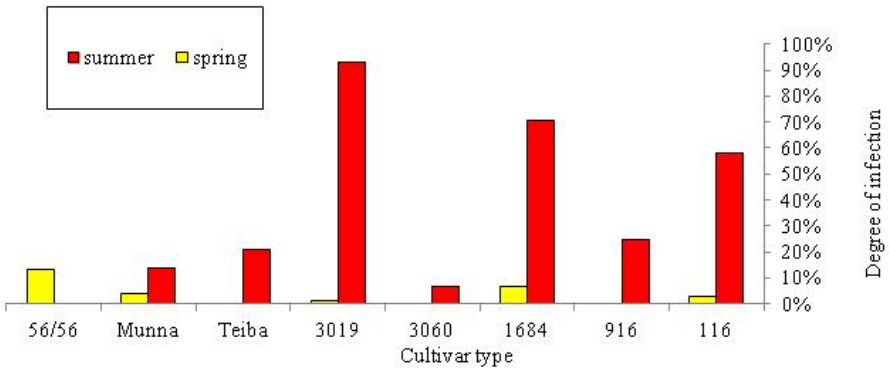

Figure (2): Comparison of TYLCD degree of infection on tomato cultivars for summer and spring growing seasons.

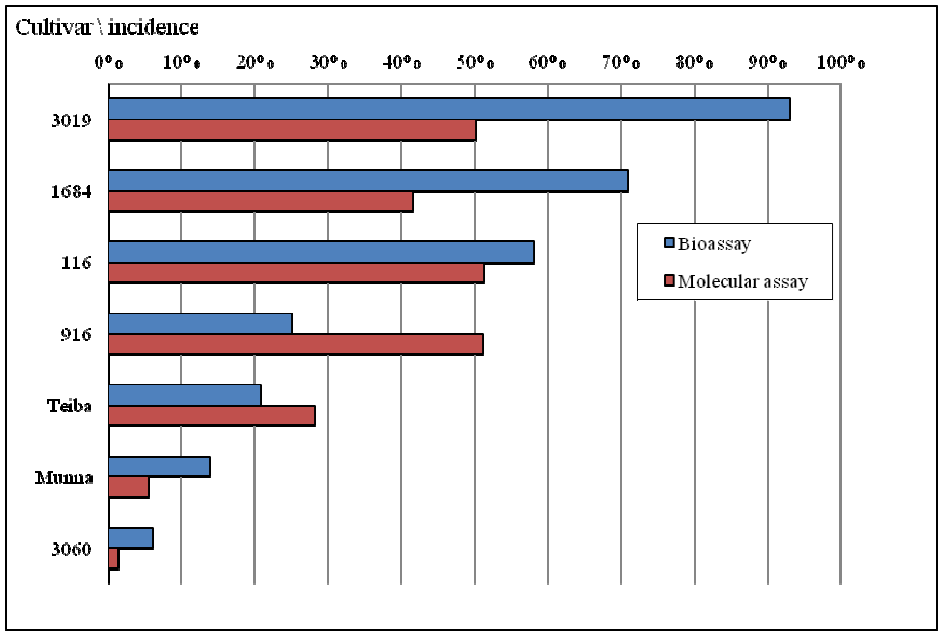

Figure (3): A comparison between PCR incidence (molecular assay) and biological assay (Severity indexing) or summer experiment 2006, showing that molecular assay was in agreement with biological one**.**

Molecular analysis for detection of the virus using specific primers designed on IR region of TYLCV-IL isolate were used on both spring and summer growing seasons. When PCR incidence was tested and compared in tomato cultivars, it showed that cultivar "3060" had the lowest PCR incidence (1%) followed by "Teiba" (5%) and "Munna" (28%) for summer growing season (Table 2). Expectantly, molecular assays were in agreement with bioassays (Fig.3) with lower percentage due to specificity of the technique. In other hand, these discrepancies in results may be due to existence of other TYLCV isolates.

Discussion and Conclusion

It was obvious that TYLCD could be expressed with varying levels of incidence as well as of severity leading for inaccurate detection and quantification of viral DNA (Pico *et al*. 1998; Pico *et al*. 1999). Also the disease discrepancies were observed on host plants due the varying responses against different TYLCV isolates used in each experiments as well as differences in method of inoculation (Pico *et al*. 2001). Infection with Tomato yellow leaf curl disease was coincided with an increase in the whitefly population of *Bemisia tabaci*. Later on, disease causal agent was described in 1964 (Cohen and Harpaz 1964) and the virus genome was isolated then sequenced in early 1990s (Czosnek *et .al.* 1988; Navot *et al*. 1991). "*Tomato yellow leaf curl viruses*" (TYLCV-IL; TYLCSV and TYLCMalV) were isolates found to be associated with TYLCD (Anfoka *et al*. 2005; Gafni 2003).

Selecting a source for resistance to TYLCV might be difficult. Wild tomatoes could be used as source of virus resistance but need efforts in developing screening methods and effective breeding programs. Many TYLCV-resistant tomato varieties are now available for both field and greenhouse production systems, however, these resistant varieties can lose their protection and develop symptoms of tomato yellow leaf curl (Czosnek 2007).

This research was conducted for screening resistant tomato cultivars from local nurseries that considered most popular and commercially planted ones in Palestine, using biological and molecular assays methods.

Results of this study showed that spring season was not suitable for studying resistance level for tomato cultivars due to low temperature and thus, low population of *Bemisia tabaci* (the major virus vector). In summer growing season were the disease incidence was very high due to high level of vector *B. tabaci*, results had shown none of the planted cultivars were immune to the virus infection. Even though, some cultivars (as seen in cultivar "3060") exhibited lower percentage of infection rather than others. In this season the degree of infection for cultivar "3019" is the highest (93%), but this cultivar did not produce any symptoms in spring season. It may be due to weather conditions made it favorable for vector reproduction, hence the virus spread or both. This suggested using summer growing season as an indication for testing levels of resistance for each variety; meanwhile, spring time would be advisable for farmers to grow tomatoes in Palestine in open field (Mansour and Al-Musa 1992).

Biological assays showed high degree of infection with severe symptoms noticed on cultivars "3019" (93%), "1684" (71%) and "116" (58%), while cultivar "3060" was with low degree of infection (7%) and mild symptom scored in eight weeks after planting, making the last cultivar as most promising for tolerating the viral infection. An increase of virus infection based on symptoms severity were noticed every week for highly susceptible cultivars (3019, 116) and slowly for the tolerant cultivars (3060). Statistical Analysis for the degree of infection after 8 weeks showed that cultivars "3060", "Munna", "Teiba" and "916" are not significantly different among each other but significantly different from others.

Since all curl virus isolates are associated with yellow leaf curl symptoms, it is very difficult to correlate a given symptoms with a particular virus strain. Recently, sequence analysis had shown considerable sequence diversity existed among members of TYLCV complex. The source for this diversity is recombination which is not a rare event among begomoviruses and seems to contribute significantly to increase genetic variability of virus genomes leading to the emergence of virulent or well adapted strain (Padidam *et al.* 1999). It is important to

mention that all cultivars manifested symptoms but were not able to be detected by PCR, suggesting these tomatoes could hold virus variants rather than TYLCV-IL or even other new strains. It is recommended to carry out molecular investigation on these plants to detect the virus isolates that could be new strains or even subspecies of the virus.

However, to some extent the correlation was less applicable for cultivar "916" in which the degree of infection (25%) was lower than PCR incidence (51%). PCR method is known to be more sensitive and specific in detection of the virus compared to visual symptoms scoring. This may reveal the level of tolerance of that cultivar compared to others, suggesting that the infection may occur in later stages so the symptoms were not observed. For that, these results indicated that PCR can serve as indicator for resistance, but it is better if not used as the sole indicator (Briddon and Markham 1994).

Under natural conditions, results obtained during summer season, can conclude that there were differences in the level of resistance among the commercial tomato cultivars that might be due to vector preference for some tomato cultivars than others. It is indicated the reproduction of the whiteflies is affected by physiological and environmental conditions. Some investigations have suggested that resistance to the whitefly had been associated with the large amounts of sticky substances that some wild plant species exudates, entrapping the whiteflies and significantly reducing the transmission of begomoviruses (Channarayappa *et al.* 1992; Morales 2001).

It was revealed that spring season is considered the best one for farmers to escape from viral infections as many cultivars such as"3060", "3019" and "Teiba" did not produce any symptoms at all, meanwhile summer experiment indicated only cultivar "3060" as promising one. These results were confirmed by PCR detection method, suggested that the experiment conditions in spring were not in favor of the virus because the population of its (*Bemisia tabaci*) was low compared with the summer one where the population density for *Bemisia tabaci* was high (Mansour and Al-Musa 1992).

Correlations between resistance levels were confirmed by PCR since those cultivars which had high degree of infection also exhibit high PCR incidence. However, the correlation was less applicable for cultivar "916", which had higher PCR incidence than degree of infection confirming that molecular assay as more sensitive in detection than biological assay, but both should be used in any evaluation of the virus incidence studies.

In this study, we were able to demonstrate that TYLCV was widely dispersed throughout the main tomato growing areas in Palestine. These findings are in accordance with previous reports of TYLCV distribution in Mediterranean basin (Czosnek and Laterrot 1997). Screening procedure for TYLCV resistance is necessary for any breeding programs that aimed to produce TYLCV resistant cultivars. Selecting plants solely on the basis of presence and absence of symptoms (biological assay) could be misleading as some of the virus isolates asymptomatic. Tolerant cultivars support replication of the virus, can act as a source of TYLCV for susceptible crops (Lapidot *et al.* 1997) thus the use of molecular tools beside biological assay in different tomato cultivars became inevitable. It seems that the cultivars which showed delay in viral expressing symptoms are those with low viral infection, hence could be our genetically choice for tolerant cultivars.

Finally, the results of this study should be considered in any breeding programs for TYLCV resistance tomato cultivars in Palestinian territories, since the degree of infection based on visual symptoms and PCR incidence were found to be varied, and some promising cultivar such as cultivar "3060" could be targeted as virus-tolerant tomato cultivar.

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