بسم الله الرحمن الرحيم

Hebron university
College of Graduate Studies

Biological and molecular studies on TYLCV severity, and incidence on tomato cultivars in Al-Arroub Agricultural Experimental Station

BY

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بسم الله الرحمن الرحيم

جامعة الخليل
كلية الدراسات العليا

دراسات بيولوجية وجزيئية لمعرفة شدة ونسبة الاصابة بفيروس اصفرار وتجعد القمة النامية الذي يصيب أصناف البندورة في محطة العروب للأبحاث الزراعية - فلسطين

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M.Sc. Program in Plant Protection

Biological and Molecular Studies on Severity and Incidence of TYLCV Infecting Tomato Cultivars in the Southern Highlands of West Bank, Palestine

By:

Sahar Ahmad Sulyman Amro

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DEDICATION

I Would Like To Dedicate This Thesis To My Parents And My Family

III

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AFLP	**amplified fragment length polymorphism**
C	**complementary sense strand**
CP	**coat protein**
IR	**intergenic region**
MP	**movement protein**
ORF	**open reading frame**
PTGS	**post- transcriptional gene silencing**
RAPD -DNA	**randomly amplified polymorphic DNA**
Ren	**replication enhancer protein**
Rep	**replication associated protein,,**
RFLP	**restriction fragment length polymorphism**
SiRNA	**small interferring RNAs**
TBA	**Tris borate EDTA**
TrAP	**transcriptional activator protein**

الخلاصة بالعربية

دراسات بيولوجية وجزيئية لمعرفة شدة ونسبة الإصابة بفيروس اصفرار وتجعد القمة النامية الذي يصيب أصناف البندورة في محطة العروب للأبحاث الزراعية - فلسطين

فيروس اصفرار وتجعد القمة النامية يسبب خسارة كبيرة في محصول البندورة في عدة دول في الشرق الأوسط بما فيها فلسطين . تظهر الأعراض غلى شكل اصفرار وتجعد ونقصان مساحة الأوراق مع تقزم للنبتة وتساقط للأزهار .

في هذه الدراسة تم إجراء بحوث بيولوجية عن طريق مراقبة الإصابة ورصد نسبتها في بعض أصناف البندورة التجارية في فلسطين. بالإضافة للبحوث البيولوجية تم الكشف عن وجود الفيروس عن طريق الاسترداد التلقائي للحمض النووي (PCR) فقد تمت مضاعفة الحمض النووي للفيروس باستخدام إنزيم التضاعف والكشف عنه وهذه التقنية تستخدم للمرة الأولى في الضفة الغربية وقد تم استخدامها في مختبر التقنيات الحيوية في جامعة الخليل لأنها دقيقة وحساسة في الكشف عن المرض مقارنة بالبحوث البيولوجية .

تشير البحوث لنتائج تجربة الصيف انه لا يوجد صنف لديه مناعة ضد المرض ولكن هناك اختلاف واضح بينها من حيث تطور الأعراض وشدتها فبعضها ظهرت عليه أعراض خطيرة مثل صنف3019و1984 وبعضها قليل أو متوسط مثل صنف 3060, منى, طيبة و916.

تشير النتائج التي حصلنا عليها بالطريقتين أن معرفة شدة أعراض المرض التي تصيب بعض الأصناف ضرورية لمعرفة مدى مقاومتها للمرض حيث أن شدة الأعراض تختلف حسب نوع الصنف .

لقد ظهرت أقل نسبة للإصابة باستخدام طريقة الاسترداد التلقائي (PCR) في الصنف 3060 وهي 1% و منى 6% بينما أعلى نسبة كانت في الأصناف 3019 ,116, 911حيث وصلت تقريبا إلى 50%.

تشير هذه النتائج أن هناك توافق كبير مابين النتائج التي تم الحصول عليها بالطريقتين حيث أن الأصناف التي كانت نسبة الإصابة بها عالية بالبحوث البيولوجية هي أيضا نسبتها عالية بالبحوث الجزيئية مثل صنف 3019و1684 والعكس صحيح مثل صنف 3060 وهناك أصناف كانت فيها الطريقة الثانية أدق من الطريقة الأولى حيث سجلت نسبه أعلى وذلك لان الطريقة الثانية أدق وهذا

ينطبق على صنف 916وطيبة , ومع ذلك هناك أصناف كانت الطريقة الأولى نسبتها أعلى وهذا سببه وجود أنواع لنفس الفيروس(strains) وتسبب ظهور نفس الأعراض وهي و TYLCV-Mld و TYLCSV وللكشف عنها يلزم استخدام (primers) كواشف أخرى لم يتم استخدامها في هذه التجربة.

تشير نتائج البحوث البيولوجية وبحوث الاسترداد التلقائي للحمض النووي (PCR) لتجربة الربيع أن نسبة الإصابة أقل بكثير من تجربة الصيف و ذلك بسبب الأعداد الكبيرة للذبابة البيضاء والتي تنقل الفيروس (*Bemesia tabaci*) في الصيف .

لقد تم إجراء مسح لمعرفة نسبة الإصابة في الجزء الجنوبي من فلسطين خلال شهر آب سنة 2008 في مدينة بيت لحم والخليل في الحقول المكشوفة والبيوت البلاستيكية وقد تبين أن نسبة الإصابة في الحقول المكشوفة هي 12% وفي البيوت البلاستيكية 28, % وقد تم تسجيل أعلى نسبة للإصابة في بيت لحم في منطقة مرح رباح حيث بلغت 100% وفي منطقة تقوع حيث بلغت 59 %. تشير النتائج أن نسبة الإصابة في بيت لحم 15% وهي أعلى من نسبة الإصابة في الخليل 3,5 %.

تشير نتائج الدراسات أن الأصناف التي ظهرت عليها نسبة الإصابة قليلة هي تلك الأصناف التي يمكن اختيارها للأبحاث الجينية للحصول على أصناف مقاومة.

ABSTRACT

Biological and molecular studies on TYLCV severity, and incidence on tomato cultivars in Al-Arroub Agricultural Experimental Station

Tomato yellow leaf curl virus (TYLCV, monopartite begomovirus) causing yield losses in tomato crops in many countries in the Middle East including Palestine. Visual disease symptoms consist of curling, yellowing and reduction of the leaflets area together with stunting and flower abortion. In this research study, the virus infection in tomato crops was monitored in southern highlands of West Bank and its incidence was reported. Beside biological assay, PCR methodology was used in detection of the virus for the first time in West Bank. In this tale and as a part of transferring biotechnology to our universities, this technique was checked and optimized at Hebron University Biotechnology Lab for its sensitivity in detecting the virus compared to Bioassay.

Results showed that none of the tomato cultivars planted in the summer experiment was "immune" to the TYLCV infection; however, there were fundamental differences in symptom development and severity. The symptom severity was varied from highly severe symptoms in cultivar (3019) to low or mild symptoms in cultivars (3060, Munna, Teiba, and 916). Combined methods of bioassay and PCR tests revealed that the incidences of TYLCV and severity results are necessary to evaluate this resistance. It seems that the cultivars which showed delay in expressing viral symptoms are those with low viral infection. The low incidence of virus infection detected by PCR had been found in 3060 (1%) and Munna

(6%) cultivars while the highest one was in cultivars 3019,116 & 916 reached approximately to 50%.

Bioassay results were in agreement with molecular detection method in sense that cultivars which had high degree of infection also had high PCR incidence. The sensitivity of molecular methods over bioassays is quite known, and it was ascertained in the degree of infection by bioassays in cultivars 916 and Teiba, however in cultivars such as 116,1684 and 3019 the degree of infection recorded by bioassays was higher than PCR incidence. This due to the existence of different TYLCV isolates such as TYLCSV –ES[1], TYLCV-ES [2] and TYLCV-Mld infecting the plant and could not be detected by the used primers due to their specificity.

Biological and molecular incidence for spring experiment is much lower than summer experiment. This due to the high population of the whitefly vector *Bemesia tabaci* Gennadius (Homoptera : Aleyrodidae) .

Survey for TYLCV in the southern region of West Bank, Palestine during July 2008 (Hebron and Bethlehem) in open fields and in plastic houses showed that the incidence in open fields was 12% and in plastic houses was 0.28 %. The maximum incidence had been recorded in Marah Rabah in Bethlehem which reach 100% and Tkooh 59%. The incidence in Hebron was less than the incidence in Bethlehem which was 3.5%, 15% respectively.

The less infected tomato cultivars resulted from this study could be our genetically choice for tolerance once.

INTRODUCTION

Tomato yellow leaf curl virus (TYLCV) belongs to the *Begomovirus* genus within the *Geminiviridae* family. Begomoviruses are exclusively transmitted by *Bemesia tabaci* Gennadius (Homoptera : Aleyrodidae). Tomato yellow leaf curl diseases (TYLCD) are associated to a complex of viral species, including TYLCV, and all including rather similar symptoms on tomato (*L. esculentum*) plants .

Tomato yellow leaf curl virus was first reported in Palestine in 1939-40 associated with outbreaks of *Bemisia tabaci*. The causal agent was described in 1964 and named *tomato yellow leaf curl virus* (TYLCV) (Cohen and Harpaz, 1964). The virus was isolated in 1988 (Czosnek *et .al.*, 1988) and its genome sequenced in 1991 (Navot *et al.*, 1991).

The early report of the disease describes small, curled and chlorotic leaves. Later descriptions of symptoms included stunted plants and loss of fruits because of the premature drop of flowers.

Since the 1960s, the disease has become one of the most economically important tomato diseases world-wide; it is present in most Mediterranean countries and parts of sub-Saharan Africa, Asia, Japan, Australia, the Caribbean Islands, and was recently reported in the USA, in Florida, Georgia and Louisiana (Czosnek *et al.*, 1990; Nakhla and Maxwell, 1998; Polston *et al.*, 1999). In many cases the disease causes yield losses of the order of 90%.

In general, Geminiviruses are a large and diverse family of plant infecting viruses that share a unique particle structure of fused icosahedra

that forms a twinned (geminate) capsule, and they have genomes comprising covalently closed circular single-stranded DNA approximately 2.7 kb in size (Gafni and Epel, 2002; Gutierrez, 1999; Hanley-Bowdoin *et al*., 2000).

The management of TYLCV in tomato is difficult and expensive both in protected and open field production. Often management techniques are not sufficient and economic losses are incurred. Many approaches have been used to try to decrease losses due to TYLCV although only a few are frequently effective and some cannot be used in all climates and locations. In general, no single approach is effective to manage TYLCV. Combinations of chemical and cultural techniques are employed to reduce the number and movement of the whitefly vector, and to minimize or eliminate inoculum sources of TYLCV. TYLCV is managed primarily through the use of resistant cultivars, pesticides, cultural practices, and exclusion through the use of 50 mesh screens, and regular or UV-absorbing plastics in the case of protected production.

Since few viruliferous whiteflies may transmit the virus to a large number of plants, chemical controls as well as IPM strategies employed for controlling the vector proved unsuccessful to decrease the TYLCV incidence on cultivated tomatoes (Reynaud *et al.*, 2003). Cultivation of tomatoes with resistance to the virus and/or the vector is a more effective solution for a sustained control of TYLCV. No resistance to TYLCV was found in cultivated *L. esculentum* (Laterrot, 1989; Pico *et al.*, 1999b; Pilowsky and Cohen, 2000) and, during last decades, considerable efforts has been done to develop TYLCV resistant cultivars by transferring resistance from wild types of *Lycopersicon* in to cultivated tomatoes. Nevertheless, progress in breeding for TYLCV resistance has been slow because of the complex genetics of resistance, which probably explain why

the cultivars and breeding lines most often are not as resistant as wild species .

To characterize this resistance in different tomato cultivars visual scoring (incidence and severity) and viral presence using PCR method were carried out in Al- Arroub Agricultural Experimental station.

OBJECTIVES

The main scope of the study could be summarized as follows:

1. To optimize at what concentration *Taq* DNA polymerase should be used to detect TYLCV virus.
2. To monitor the disease incidence and severity in summer and spring experiments for tomato cultivars planted in Al-Arroub Agricultural Experimental station by using biological and molecular methods and characterize their resistance level .
3. Compare resistance levels to *Tomato yellow leaf curl virus* among commercial cultivars under natural infection.
4. To monitor the biological incidence of TYLCV in the southern part of the West Bank (Hebron and Bethlehem).
5. Beside biological assay, PCR methodology will be used in detection of the virus for the first time in the West Bank. In this direction and as apart of transferring biotechnology to our universities, this technique will be checked and optimized at Hebron University Biotechnology Laboratory for its sensitivity in detecting the virus compared with bioassays.

CHAPTER ONE: LITERATURE REVIEW

1.1. Geminiviruses.

1.1.1. Morphology

Virions are geminate (about 18 x 30 nm), apparently consisting of two incomplete icosahedra (T=1) with a total of 22 pentameric capsomers (Fig 1.1).

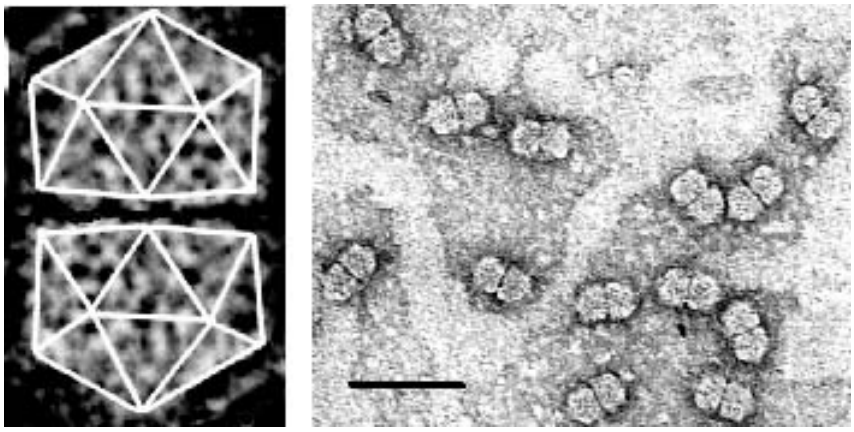

Figure 1.1: Geminiviruses morphology.Left: organization of capsomeres. Right: geminiviruses subunits . Bar = 100 nm.

Right, typical geminiviruses consist of two joined quasi-isometric subunits, with a characteristic "waist" constriction, and pointed ends: purified particles of the species Maize streak virus stained with uranyl acetate. The bar represents 100 nm. (Left), capsids are constructed as shown: triangles superimposed on negatively-stained virion indicate organization of capsomers.

1.1.2. Taxonomy of geminiviruses

Geminiviruses are plant viruses that belong to the family Geminiviridae, first described by Goodman in 1977 (Goodman, 1977a, 1977b). Geminiviruses are characterized by the unique Gemini shape of a fused icosahedral viral particle. The geminate virions consists of a circular single-stranded DNA (ssDNA) genome. The family Geminiviridae is comprised of four genera : *Mastrevirus***,** *Curtovirus***,** *Topocuvirus* and *Begomovirus* (Van Regenmortel *et al*., 1999), all of which share similarities in genome organization, insect transmission, and host range.

The genus *Mastrevirus* consists of geminiviruses with a monopartite genome, and the Mastreviruses are transmitted by leafhoppers, in most cases by a single species in a persistent, circulative, non-propagative manner. They are most often found on monocotyledonous plants, especially on maize. The genus *Curtovirus* includes viruses with monopartite genomes, transmitted by leafhoppers or treehoppers in a persistent, circulative, non-propagative manner. They infect only dicotyledonous plants, especially sugar beet, tomato and melon. The genus *Begomovirus* includes viruses with monopartite and bipartite genomes. Begomoviruses are transmitted by whiteflies in a persistent, circulative, non-propagative manner, and infect dicotyledonous plants. *Bean golden mosaic virus* (BGMV) is the type species. The genus *Topocuvirus* was approved by ICTV in 1999. They infect dicotyledonous plants and are transmitted by leafhoppers.

1.2. Tomato Yellow Leaf Curl Disease

1.2.1. Taxonomy and classification

At least nine different virus species more or less related phylogenetically, and strains of them, have been associated with TYLCD , among others, *Tomato yellow leaf curl virus*, TYLCV (Moriones and Navas- Castillo, 2000; Fauquet and Stanley, 2005; Stanley *et al*., 2005).

These viruses belong to the genus *Begomovirus* which includes geminiviruses (family *Geminiviridae*) that are transmitted in a circulative persistent manner by the whitefly *Bemisia tabaci* Gennadiu*a*:(*Homoptera: Aleyrodida*). Different virus species have been associated with TYLCD in many countries across the globe: *Tomato yellow leaf curl virus* (TYLCV), *Tomato yellow leaf curl Mali virus (*TYLCMLV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV), *Tomato yellow leaf curl Malaga virus* (TYLCMalV), and *Tomato yellow leaf curl Axarquia virus* (TYLCAxV) (Abhary *et al*., 2007). Hence, TYLCD-associated virus isolates belonging to two or more different species, and sometimes recombinants, have been found in the same country (Monci *et al*., 2002). Two viruses infecting tomato crops in the Middle East, *Tomato yellow leaf curl virus*-Israel (TYLCV-IL) and TYLCV-Mld, were cloned and sequenced in the 1990s. Recently, a third virus strain, *Tomato yellow leaf curl Sardinia virus*-Spain (TYLCSVES), has been identified in Jordan (Anfoka *et al*., 2005)

1.2.2. Geographical distribution of TYLCD

TYLCD was first observed in Palestine in 1939-1940 and the appearance of the disease coincided with an increase in the whitefly population. The causal begomovirus was identified in the 90's as TYLCV (Cohen and Antignus, 1994; Pico *et al.*, 1996). In Africa, TYLCD was first described in Sudan (Yassin and Nour, 1965; Yassin, 1975), Then Nigeria, but the causal agent was identified as TYLCV only in 1997 (Czosnek and Laterrot, 1997). Since, TYLCD has also been reported from many other African countries: in Cameroon (Czosnek and Laterrot, 1997), Burkina Faso (Konaté *et al.*, 1995), Mali and Senegal (D'Hondt and Russo, 1985), and Egypt (Czosnek *et al.*, 1990). The occurrence of TYLCD was reported from the south of Casablanca in 1996-97 and by 1998 it had spread to all

the tomato growing areas of Morocco (Peterschmitt *et al.*, 1999; Jebbour and Abaha, 2002).

In the late 1980s, the first reports were made on the occurrence of TYLCD in the Americas and Europe (Accotto *et al.*, 2000). The disease has also spread to the Caribbean islands (MacGlashan *et al.*, 1994; Nakhla *et al.*, 1994; Polston and Anderson, 1997; Ramos *et al.*, 1996; Sinisterra and Patte, 2000; Bird *et al.*, 2001) reaching the French West Indies in 2001 (Urbino *et al.*, 2003). In 1997 the virus has emerged in Florida and one year later (April 1998) up to 100% incidence was recorded in tomato fields (Polston *et al.*, 1999).

1.2.3. Recombination as source of genetic diversity in TYLCD-associated virus populations

During mixed infections, viruses can exchange genetic material through recombination or re assortment of segments (when the parental genomes are multipartite) if present in the same cell context of the host plant. Hybrid progeny viruses might then arise, some of them well adapted in the population that can cause new emerging diseases. A natural recombination has been observed with the *Tomato yellow leaf curl Sardina virus* (TYLCSV) and TYLCV in Spain, the resulting recombinant appearing better fit than the two original isolates (Monci *et al.*, 2002). Specific aspects about occurrence of recombination among viruses of the TYLCD complex have been reviewed recently by Moriones *et al.* (2007). It was found that recombinant genotypes quickly emerged and spread in the population after the novel introduction of TYLCV virus strains into Spanish epidemics. During field surveys two types of recombinants were found, TYLCMalV and TYLCAxV (Monci *et al.*, 2002; García-Andrés *et al.*, 2006). Interestingly, for these two recombinants, novel pathogenic properties were demonstrated that suggested enhanced ecological

adaptation to the invaded area. Thus, in addition to be readily transmissible by *B. tabaci*, they exhibited a host range wider than either parental virus , which is consistent with selection for a better natural fit. Based on the singular genetic and biological properties of these two recombinant viruses, and following species demarcation criteria proposed for begomoviruses (Fauquet *and* Stanly, 2003), the *Geminiviridae* Study Group of the International Committee on Taxonomy of Viruses considered them as two new virus species in the *Begomovirus* genus (Fauquet and Stanley, 2005).Therefore, the potential of begomoviruses to generate genetic diversity through recombination can be relevant for their ecological fitness, and recombination should be taken into account among forces driving evolution in this group of viruses.

1.3. *Tomato yellow leaf curl virus*

1.3.1. Disease symptoms:

Symptoms become visible in tomato in approximately 2–3 weeks after infection. Leaf symptoms include chlorotic margins, small leaves that are cupped, thick and rubbery (Fig. 1.2). Leaves show yellowing on the edges accompanied by upward curling. The majority (up to 90%) of flowers abscise after infection, and therefore few fruits are produced.

Fig. 1.2. Tomato yellow leaf curl symptoms on tomato plant.

1.3.2. Physical properties

TYLCV, like all members of Geminiviridae , has geminate (twinned) particles, 18–20 nm in diameter, 30 nm long, apparently consisting of two incomplete T = 1 icosahedra joined together in a structure with 22 pentameric capsomers and 110 identical protein subunits (Fig. 1.3) .

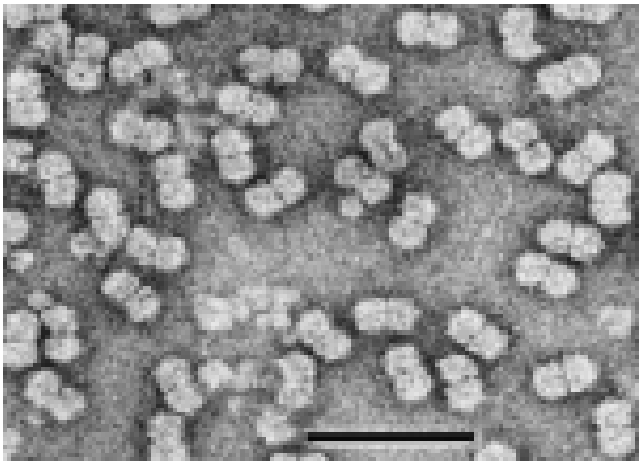

Fig. 1.3. Eectron micrograph of purified, negatively stained TYLCV particles. Bar = 100 nm.

1.3.3. Genome organization of TYLCV virus

TYLCV encodes six partially overlapping open reading frames (ORFs) that are organized bidirectionally (Fig. 1.4) ; two of these ORFs (V1 and V2) are in the virion sense orientation, and four (C1–C4) in the complementary orientation. Between the two transcription units resides an intergenic region of about 300 nucleotides which contains key elements for the replication and transcription of the viral genome; it is organized in a typical iterative structure (Arguello-Astorga *et al*., 1994). The virion-sense ORF V1 encodes the 30 kDa viral coat protein, while the 13.3 kDa V2 transcription products has recently been hypothesized to be involved, together with the 11 kDa protein product of C4, in cell-to-cell movement of viral DNA (Rojas *et al*., 2001). The C1 on the complementary strand encodes for a 41 kDa protein, the only protein known to be involved in viral replication (Desbiez *et al*., 1995). The C2 gene encodes for a 15.7 kDa protein found to be localized in nuclei, and contributes to viral pathogenicity (Van Wezel *et al*., 2001). Recently, it was suggested to be a suppressor of post-transcriptional gene silencing (PTGS) (Van Wezel *et al*., 2002 b). TYLCV C3 has been hypothesized to function as a replication enhancer protein (Hanley-Bowdoin *et al*., 2000).

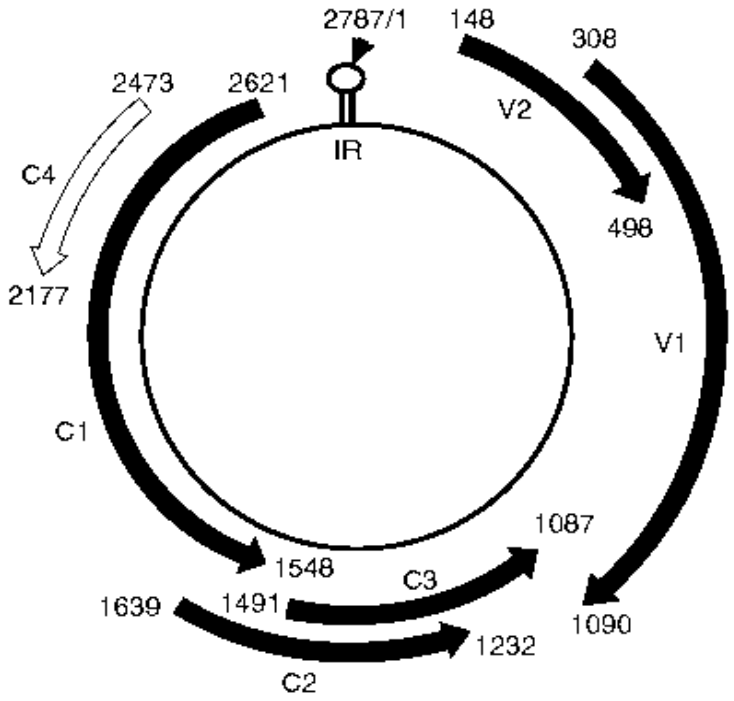

Fig. 1.4 Genome organization of *Tomato yellow leaf curl virus*.

The single-stranded virion DNA comprises 2787 nt. Open reading frames (ORFs) of virion-sense and complementary-sense strand polarity are designated (V) and (C), respectively. ORFs are represented by an arrow; numbers indicate first and last nucleotide of each ORF. V1 encodes the capsid protein (CP), V2 a movement protein, C1 the replication initiator protein (Rep), C2 a transcriptional activator protein (TrAP), C3 a replication enhancer protein (REn), and C4 a symptom and movement determinant. IR: intergenic region. The conserved inverted repeat flanking the conserved nanonucleotide sequence TAATATTAC is symbolized by a stem–loop; an arrow head indicates the cleaving position of Rep in the TAATATT/AC loop; A at the cutting site (/) is nucleotide number one, by definition.

1.3.4. Replication of TYLCV.

Geminivirus genomes encode only a few proteins and thus are dependent on host cell factors for replication such as DNA polymerase - and probably repair polymerases - in order to amplify their genomes, as well as transcription factors. Geminiviruses replicate via a rolling circle mechanism like bacteriophages such as M13, and many plasmids. Replication occurs within the nucleus of an infected plant cell. First the single-stranded circular DNA is converted to a double-stranded circular intermediate. This step involves the use of cellular DNA repair enzymes to produce a complementary negative-sense strand, using the viral genomic or plus-sense DNA strand as a template. The next step is the rolling circle phase, where the viral strand is cleaved at a specific site situated within the origin of replication by the viral Rep protein in order to initiate replication.

This process in an eukaryotic nucleus can give rise to concatemeric double-stranded forms of replicative intermediate genomes, although double-stranded unit circles can be isolated from infected plants and cells. New single-stranded DNA forms of the virus genome (plus-sense) are probably formed by interaction of the coat protein with replicating DNA intermediates, as genomes lacking a CP gene do not form ssDNA. The ssDNA is packaged into geminate particles in the nucleus. It is not clear if these particles can then leave the nucleus and be transmitted to surrounding cells as virions, or whether ssDNA associated with coat protein and a movement protein is the form of the genome that gets trafficked from cell to cell via the plasmodesmata (Gutierrez, 2002). These viruses tend to be introduced into and initially infect differentiated plant cells, via the piercing mouthparts of the insect vector; these cells however, generally lack the host enzymes necessary for DNA replication, making it difficult for the virus to replicate.

1.3.5. Transmission by Vectors

The whitefly *B. tabaci* is the only known natural vector. Adults and crawlers (1st instar) are the only stages where the insect is able to acquire and transmit TYLCV (Cohen and Nitzany, 1966; Mehta *et al*., 1994). Single insects are able to acquire TYLCV and transmit it to tomato plants. Minimum effective acquisition access and inoculation access periods are approximately 10-20 min. The rate of transmission increases with longer acquisition and inoculation access periods. A minimum of 8 h (latent period) from the time acquisition started is required for *B. tabaci* to be able to infect tomato test plants.

In a one insect/one plant inoculation test, females *B. tabaci* were more efficient than male insects (Martin, 1987). Viral DNA can be detected in single insects by PCR after 5 min of access feeding, and in tomato plants as early as 5 min after inoculation feeding (Atzmon *et al*., 1998). A GroEL homologue produced by the insect coccoid endosymbionts is involved in circulative transmission of the virus (Morin *et al*., 1999).

TYLCV is associated with the insect vector for its entire adult life. Insects that emerged during a 24-h period and were reared on a non-host plant after a 24 h-acquisition period retained TYLCV for their entire 35-40 day life (Rubinstein and Czosnek, 1997). During this period, transmission rates decreased from 100% to 15%. The viral DNA was detected during the entire life of the insect whereas the capsid protein was undetectable after 12 days. The long-term association of TYLCV with the insect led to a reduction of ~20% in their life expectancy and of ~50% in the number of eggs laid (Rubinstein and Czosnek, 1997). Until 1998 TYLCV was not supposed to be transmissible to the progeny, only adults or larvae could acquire the virus. However in 1998 TYLCV-MLD was claimed to be transmitted through the egg for at least two generations (Ghanim *et al*., 1998). It was reported (Ghanim and Czosnek, 2000) that TYLCV-MLD

could be sexually transmitted among whiteflies. In another report (Bosco *et al*., 2004) it was shown for the Israeli strain of TYLCV that , neither viral DNA nor infectivity were associated with the progeny of viruleferous whiteflies. Whiteflies feed on phloem so the virus infection increase rapidly in all the plant parts (Caciagli *et al*., 1995) .

1.3.6. Breeding for natural resistance to TYLCV

All commercial tomato cultivars have been found to be completely susceptible to TYLCV, urging breeders to screen wild tomato accessions for potential resistance traits (Pilowsky & Cohen, 2000). Until now, only one major resistance gene to TYLCV had been identified: TY-1 (Zamir *et al.*, 1994) on chromosome 6 of *L. chilense*. Two more resistance modifier genes were mapped to chromosome 3 and 7 (Zamir *et al.*, 1994) of *L. Chilense*. Another TYLCV-resistance gene, originating from *L. pimpinellifolium* had been mapped using RAPD PCR-based markers to chromosome 6, but to a different locus from TY-1 (Chagué *et al.*, 1997).

Different breeders' teams used different wild type genetic background to build lines with high levels of resistance, such as: *L. peruvianum* (Lapidot *et al.*, 1997; Friedmann *et al*, 1998; Vidavsky & Czosnek, 1998), *L.chilense* (Zamir *et al.*, 1994; Scott *et al.*, 1996), *L. pimpinellifolium* (Vidavsky *et al.*, 1998), and *L. hirsutum* (Vidavsky & Czosnek, 1998; Hanson *et al.*, 2000). The first TYLCV-resistant commercial cultivar resulting from breeding programmes is TY20, which carries a resistance derived from *L. peruvianum*, showing a delay in symptom development and viral accumulation (Pilowsky and Cohen, 1990; Rom *et al.*, 1993).

In most cases the sources of TYLCV resistance appeared to be controlled by multiple genes (Pico *et al.*, 1996; Pico *et al.*, 1999a). Examples of the different resistant lines studied are given in the review of Lapidot and Friedmann (2002). Nevertheless, after 20 years of breeding

programs, very few commercial genotypes with increased levels of TYLCV resistance are on the market. Besides, the direct genetic resistance to begomoviruses, resistance to the whitefly vector has been reported in some wild *Lycopersicon* species, such as *L. hirsutum* and *L. peruvianum* (Morales, 2001). It has been associated with the large amounts of sticky substances that plants of these species exudate, entrapping the whiteflies and significantly reducing the transmission of begomoviruses (Channarayappa and Shivashankar, 1992; Morales, 2001).

With such a broad range of tolerance and resistance in nature, only a few breeding lines and varieties have been produced (Rom *et al*., 1993; Lapidot *et al*., 1997; Mason *et al*., 2000). However, in commercial fields of most regions of the world, tomato plants are still largely susceptible to various begomoviruses (Polston and Anderson, 1997; Mason *et al*., 2000; Diaz-Plaza *et al*., 2001). In addition, it is a concern that some asymptomatic, tolerant cultivars support the replication of the virus, and can act as sources of begomovirus for susceptible crops (Lapidot *et al*., 2001).

Liu and Stansly (2000) have tested several surfactants and oils against whitefly nymphs on tomato plants. Although there were good levels of insect mortality in some cases, phytotoxicity was observed in many instances. Their effects on yields were not reported. Nevertheless, the breeding of tomatoes resistant to TYLCV has been slow because of the complicated inheritance of the resistance /tolerance trait .

1.3.7. Genetic engineering for TYLCV

Research on transgenic, TYLCV- resistant tomato began in the early 1990s. A range of strategies have been applied, including the use of antisense RNA, CP genes, an intact replication- association protein gen (*Rep*) and truncated versions of the latter gene .Several viral sequences

have been used in attempts to obtain plants resistant to tomato-infecting begomoviruses, with results that vary from immunity to complete susceptibility.

The *CP* gene of TYLCV was used by Kunik *et al.* (1994) to transform tomato by using the full-length of the *CP* gene. The resulting plants showed resistance to challenge by TYLCV which was associated with high levels of expressed CP. However, this resistance was expressed as delay in symptoms rather than total immunity.

Another gene often used for obtaining transgenic resistance to tomato-infecting begomoviruses is the replication-associated (*Rep*) gene. Noris *et al.* 1996, were the first to demonstrate that the expression of a truncated TYLCSV *Rep*, encoding the first 210 amino acids of the Rep protein and potentially co-expressing the C4 protein, could confer high levels of resistance in *Nicotiana benthamiana* plants. However, resistance was overcome with time. This truncated gene was also used to transform tomato plants (Brunetti *et al.*, 1997). Transformed plants that expressed high levels of the truncated TYLCSV Rep protein were resistant to TYLCSV infection, whereas those tomato lines in which the protein was not expressed (lines containing the antisense *Rep* or both sense and antisense *Rep* gene) were susceptible to TYLCSV. Bendahmane and Gronenborn 1997 demonstrated that the use of the full-length antisense *Rep* conferred moderate resistance to TYLCSV in *Nicotiana benthamiana*, and this resistance was inherited in the R 2 generation as well. Interestingly, in both cases the level of homology between the antisense RNA and the challenge virus sequence specified the level of resistance obtained.

Recently, Franco *et al.* (2001) have shown resistance of *Nicotiana benthamiana* to TYLCSV by a double mechanism involving antisense RNA of TYLCSV *Rep* gene and extra-chromosomal molecules; however,

the plants were not protected against TYLCV , which is a more severe virus . As it can be clearly seen, most studies on transgenic plants expressing the *Rep* gene or its antisense RNA were done on *Nicotiana benthamiana*, a known permissive host. Only a short time ago, a construct consisting of 2/ 5 of the TYLCV *Rep* gene was demonstrated to confer high levels of resistance and often immunity to TYLCV in both tobacco and tomato, probably through the mechanism of PTGS (Freitas-Astúa *et al.*, 2001; Polston *et al.*, 2001). The relevance of these studies is based on the fact that several lines of transformed tomato and tobacco plants were immune to TYLCV in the R 1 and R 2 generations, and that similar responses were observed in two different hosts, in independent transformations. These results suggest that the 2/5 TYLCV *Rep* construct is a strong gene silencing inducer. However, since numerous viruses can infect tomatoes, often in mixed infections, it is
imperative that in some regions of the world the resistant plants exhibit broad-spectrum resistance. For that reason, new strategies are leading towards gene pyramiding or crossing of material already resistant to one virus with lines resistant to other viruses, or the use of negative dominant mutants that can confer good levels of resistance not only to the virus from which the sequence was derived, but also to related viruses (such as recombinants and variants or even other begomovirus species).

Further studies demonstrated that *Nicotiana benthamiana* plants expressing the truncated *Rep* of TYLCSV were resistant to the homologous virus, but susceptible to the related TYLCV Murcia strain (TYLCV -ES[1]). According to the authors, the truncated *Rep* acts as a trans-dominant-negative mutant inhibiting transcription and replication of TYLCSV, but not of TYLCV -ES[1] (Brunetti *et al.*, 2001). Finally, although no begomovirus-resistant transgenic tomato plants are yet

available to growers, some of these lines are very promising and might in the near future be cultivated or used in breeding programs.

1.3.8. TYLCV host range

The domesticated tomato *Solanum esculentum* (formerly *Lycopersicon esculentum*) is the primary host of TYLCV. Most of the wild tomato species such as *S. chilense, S. habrochaites* (formerly *L. hirsutum), S. peruvianum*, and *S. pimpinellifolium* include accessions that are symptomless carriers and are used as genitors in breeding programs for TYLCV resistance (Zakay *et al*., 1991).

Laboratory inoculation by viruliferous whiteflies and field sampling surveys have indicated a potentially wide host range of this virus, spanning 13 plant species in 9 botanical families. Host plant families include: *Asclepiadaceae, Asteraceae, Fabaceae, Malvaceae, Solanaceae, Gentianaceae, Cleomaceae, Cucurbitaceae* and *Apiaceae*

(Mansour and Al-Musa, 1992; Cohen and Antignus, 1994 Kegler; 1994; Nakhla Maxwell, 1998). Several cultivated plants including bean (*Phaseolus vulgaris*), petunia (*Petunia hybrida*), and lisianthus (*Eustoma grandiflorum*) are hosts of TYLCV and present severe symptoms upon whitefly mediated inoculation. (Salati *et al.*, 2002).

Weeds, such as *Datura stramonium* and *Cynanchum acutum,* present distinct symptoms, whereas others, such as *Malva parviflora*, are symptomless carriers. Plants used for rearing whiteflies, such as cotton (*Gossypium hirsutum*) and eggplant (*Solanum melongena*), are immune to the virus (Czosnek *et al*., 1993). Additional plant species have been shown to be susceptible to TYLCV but do not exhibit any disease

symptoms, such as the weeds species *Cleome viscose (Caparidaceae)* and *Croton lobatus (Euphorbiaceae)* (Salati *et al.*, 2002*).*

1.3.9. Management of TYLCV

Up to now, almost no efficient measures for the control of TYLCV have become identified . Several strategies have been investigated and mostly are either directed towards insect (vector) control or by breeding crops resistant or tolerant to the virus. Potential insect control measures can be divided into 3 main categories: chemical, biologic and physical measures. Cultural practices for managing *B. tabaci* populations have also been used.

Over the years, chemical or pesticide control measures have only been partially effective, since whitefly populations are polyphagous, can reach very high numbers and because the chemicals were improperly used. As a consequence, massive doses have been used in attempts to eradicate the vector. Resistance to several insecticides have been detected within the invasive B biotype (Costa *et al.*, 1993a; Pico *et al.*, 1996). Another drawback of the use of insecticides turned out to be the killing of natural enemies (Gill, 1992). Natural enemies of whiteflies are numerous, such as the the *Encarsia* and *Eretmocerus* species which are the most commonly used to bio-control *B. tabaci* populations, even in IPM programs (Gerling, 1990; Gerling and Mayer, 1996; Ellsworth and Martinez , 2001). Nevertheless, those measures can only regulate the direct feeding damage or delay the progress of the virus disease, but canot suppress it, as the threshold of only one or two insects per plant needed for TYLCV transmission to occur (Caciagli *et al.*, 1995).

Physical barriers as fine-mesh screens have been used in the Mediterranean basin to protect crops (Cohen and Antignus, 1994). More recently, UV-absorbing plastic screens have been shown to inhibit

penetration of whiteflies into greenhouses (Antignus *et al.*, 1996; Antignus *et al.*, 2001). However the use of physical barriers is not the best solution as it is costly and creates problems of shading, overheating and poor ventilation (Lapidot and Friedmann, 2002).

Cultural practices such as crop-free periods, altering dates of planting , crop rotation, and weed and crop residue disposal, high planting densities, floating row cover, mulches, trap crop, or living barriers performed well. But growers may be reluctant to adopt such cultural practices that require significant changes in their conventional practices (Hilje *et al.*, 2001) and might be time consuming. All these control strategies have shown their drawbacks, and turned out to be not completely efficient in the case of whitefly and hence hence begomovirus control. Therefore, the potentially best way to reduce geminivirus incidence still is by breeding crops resistant to the virus (Cohen and Antignus, 1994; Morales and Anderson, 2001; Lapidot and Friedmann, 2002).

1.4. The vector: *Bemisia tabaci* (Homoptera: Aleyrodidae)

1.4.1. Introduction

The absence of historical records relating to indigenous faunae in some countries prevents a systematic study of the introgression of whiteflies into various (continental) areas. The first description of *Bemisia tabaci* or sweet potato whitefly was in 1889 in Greece (Gennadius, 1889). It was classified in the order Homoptera, family *Aleyrodidae* and subfamily *Aleyrodinae*, and is one of the 1160 aleyrodid species (Gill, 1992). Females can regulate the sex of their progeny by selective egg fertilization. Fertilized females lay diploid and haploid eggs up to 300 per individual, this amount varying greatly, depending on the biotype. The former gives rise to females but the

later to males. The unfertilized females only lay haploid eggs. The progeny sex ratio is affected by insect age. Young females lay more female producing eggs than older females (Berlinger, 1986).

1.4.2. *Bemisia tabaci* Life cycle stages

Bemisia tabaci eggs are oval in shape and somewhat tapered towards the distal end. The egg is pearly white when first laid but darkens over time. At 25 ºC, the eggs will hatch in six to seven days (Byrne and Bellows, 1991).

The first larval instar is capable of limited movement and is called the crawler. The dorsal surface of the crawler is convex while the ventral surface, appraised to the leaf surface, is flat. The crawlers usually move only a few centimeters in search of a feeding site but can move to another leaf on the same plant. After they have begun feeding, they will molt to the second larval instar, usually two to three days after leaving the egg. The second, third and fourth larval instars are immobile with atrophied legs and antennae, and small eyes. The nymphs secrete a waxy material at the margins of their body that helps adhere them to the leaf surface. The second and third larval instars, each last about two to three days. The red-eyed larval stage is sometimes called the "pupa stage".

There is no molt between the fourth larval instar and the red-eyed larval stage but there are morphological differences. The fourth and red-eyed larval stages combined lasts, for five to six days. The stage gets its name from the prominent red eyes that are much larger than the eyes of earlier larval instars (Byrne and Bellows, 1991).

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Adult females insert their eggs into the foliage of host plants and the newly-hatched nymphs settle for larval life with little movement on the plant chosen by the parent. Winged adults fly about, however, and move

between crops ((Byrne and Bellows, 1991). Adults live for a week or more (Byrne and Bellows 1991) and much of the egg production depends on the food ingested during adulthood.

1.4.3. Damage

Damage is caused not only by direct feeding, but also through transmission of viruses. *Begomoviruses* are the most numerous of the *B. tabaci* transmitted viruses The European and Mediterranean Plant Protection Organization ("EPPO") (2004) states that, "Since the early 1980s, *B. tabaci* has caused escalating problems to both and can cause crop yield losses of between 20% and 100% (Brown and Bird, 1992). field and protected agricultural crops and ornamental plants. Heavy infestations of *B. tabaci* and *B. argentifolii* may reduce host vigor and growth, cause chlorosis and uneven ripening, and induce physiological disorders.

The larvae produce honeydew on which sooty moulds grow, reducing the photosynthetic capabilities of the plant, resulting in defoliation and stunting. Ellsworth and Martinez-Carrillo (2001) state that, "*B. tabaci*'s small size belies its ability to move relatively large distances locally, placing many hosts within communities at risk of infestation.This ability to disperse is made worse by its extensive movement through commerce of plant products around the globe. The small size and rapid reproductive potential are other characteristics that result in explosive population growth. The damage potential of this pest as a direct plant stressor, virus vector, and quality reducer (e.g., by contamination with excreta) is substantial. These attributes, among others, render this species a shared pest within agricultural communities."

Cassava mosaic disease (CMD) and cassava mosaic *geminiviruses* (CMGs) are transmitted by the whitefly (Colvin *et al.* 2004) destroying cassava crops. Agriculture in tropical and subtropical regions are most

threatened, with crops such as beans, cucurbits, peppers, cassavas and tomatoes particularly being affected (Brown, 1994). Tomato yellow leaf curl virus (TYLCV) limits tomato production in several geographic regions, including the Middle East and the Far East (Zeidan *et al.* 1998).

1.4.4. Host range

B. tabaci is one of the only six whitefly species that have a very broad host range of herbaceous plants (Mound and Halsey, 1978). Indeed *B. tabaci* has an extremely wide host range, despite the substantial differences in the host range of the different biotypes of *B. tabaci* (Cock, 1986; Price *et al*, 1986).

B. tabaci can feed on more than 900 plant species (Cock, 1986; Greathead, 1986) belonging to 74 botanical families. A more precise study (Servin *et al.*, 1999) has indicated that 73% of the cultivated plants and 66% of the weeds are host of the biotype B. For the other biotypes less information is available.

Host plant families reported in the literature often include the following: *Leguminosae, Malvaceae, Solanaceae, Euphorbiaceae, Convolvulaceae, Cucurbitaceae, Labiacae, Verbenaceae, Cruciferae, Amaranthaceae, Rosaceae, Asteraceae* and *Moraceae*. New additions to this list have been reported (Simmons *et al.*, 2000; Oliveira *et al.*, 2001), such as *Cleomaceae, Sterculicaceae, Rubiaceae, Valieranaceae*, and *Hypericacae*. With all these host plants described in the literature and despite the polyphagous nature of *B. tabaci*, outbreaks of *B. tabaci* in various parts of the world during the last decade and apparent differences in host range at different localities suggest a broad range of genetic variability within and between populations (Basu, 1995). This is quite coherent with the numerous new biotypes with different host range found worldwide (Perring, 2001).

1.4.5. Geographical distribution

Of the many whitefly species in the world, *B. tabaci* is one of the most viruliferous and it has now become globally distributed over all continents except the Antarctics (Martin *and* Rybick, 2000). The present-day distribution of *B. tabaci* is presumably related to its close association with agricultural monocrops (Brown *et al.*, 1995). The European and Mediterranean Plant Protection Organization ("EPPO") (2004) reports that *B. tabaci* may have originated in India, but the evidence is not conclusive. The insects are self-propelled over local distances.

1.4.6. Biotypes

Since the 1980's, a marked increase of *B. tabaci* populations was observed, being also found on ornamental plants and plant species previously described as non-hosts. This emergence has led to intensified studies on the systematic of *B. tabaci.* In the United States, a certain strain was found to be more polyphagous, producing silver leaf symptoms (Maynard, 1989; Yokomi *et al.*, 1990) on the genus *Cucurbita* and differed from biological parameters that characterized the initial biotype (denoted biotype A).

It was also demonstrated that this newly described strain produces more honeydew, has a higher survival rate on more hosts and lays more eggs than the biotype A (Byrne and Miller, 1990). Analysis of the honeydew showed no chemical differences between the new strain and the A biotype. The conclusions of this study proposed that this strain had access to more phloem sap and that this might be the major difference between the biotype A and the new strain. Then, numerous authors recognized even more differences between the two *B. tabaci* strains and even proposed to separate them as two species (Gill, 1992; Bartlett and Gawel, 1993; Costa *et al.*, 1993b; Perring *et al.*, 1993a; Bellows *et al.*, 1994), i.e. *B. tabaci* versus *Bemisia argentifolii*.

This classification was again controverted by others (Campbell *et al.*, 1993) and as a result this strain remained at the biotype level, being denoted biotype B. More recently, studies have been performed using molecular techniques to differentiate *B. tabaci* populations, such as isoelectric focusing electrophoresis (Perring, 1992; Brown, 2000), AFLP (Cervera *et al.*, 2000), mitochondrial marker analysis (Brown and Frohlich, 1995; Frohlich *et al.*, 1999), RAPD-PCR (Gawel and Bartlett, 1993; Perring *et al.*, 1993b; De Barro and Driver, 1997; Guirao *et al.*, 1997; Kirk *et al.*, 2000) and most recently microsatellite studies (De Barro *et al.*, 2003; Tsagkarakou and Roditakis, 2003).

Theses analyses plus additional biological tests allowed not only the distinction of just 2 separate biotypes (A and B), but even more, and usually considered as indigenous to a certain part of the world (Bedford *et al.*, 1994b; Frohlich *et al.*, 1999; De Barro and Hart, 2000; Kirk *et al.*, 2000). In a review on *B. tabaci* (Perring, 2001) all reported biotypes have been clustered into 7 groups as follows; group 1: new world biotypes A, C, N, R*;* group 2: cosmopolitan biotypes B, B2*;* group 3: Benin (biotype E) and Spain (biotype S)*;* group 4: India, biotype H*;* group 5: Sudan (biotype L), Egypt, Spain (biotype Q), Nigeria (J)*;* group 6: Turkey (biotype M), Hainan, Korea; and group 7: Australia (biotype AN). Up to date 24 distinct populations of *B. tabaci* have been given a biotype designation.

CHAPTER TWO: MATERIALS AND METHODS

This chapter describes the materials, chemicals, equipment, and methodology of experiments conducted during this research

2.1. Experimental site

The study took place in Al –Arroub Agricultural Experimental Station .The open field used was with an area of about one dunum planted with different tomato cultivars in two seasons.

2.1.1. Plant material

Tomato cultivars used in the experiments were purchased from Almojahed nursery, Hebron, Palestine. The cultivars were 116, 916, 1684, 3060, 3019, Teiba, Munna and 5656. The cultivar 5656 was used in the spring experiment only and was not used in the summer experiment.

Different tomato cultivars have been planted in Al-Arroub experimental station to be tested for TYLCV infection. Two experiments were done:

Summer experiment (started on 5/8/2006 and terminated in 30/9/2006).

Spring experiment (started on 26/4/2007 and terminated in 22/7/2007).

2.1.2. Field study

Twenty five- day- old seedlings were planted in the field in summer (2006) Fig (2.1) and spring (2007) seasons Fig (2.2). Each cultivar was replicated four times in a randomized complete block design. Each experimental plot had 18 plants. The distance between plots was 1.5 m and the distance between plants within each plot was 0.5 m; a drip irrigation system was used for irrigation .

2.1.2.1 Biological studies for TYLCV incidence for summer experiment (2006)

Twenty five –day –old seedlings of tomato cultivars were planted in an open field of Al Arroub Agricultural Station during summer 2006. Seven cultivars were used in this experiment replicated four times in a randomized complete block design; each replicate had 18 tomato seedlings. The distance between each experimental plots was 1.5 m and the distance between plants in each plot was 0.5m .

Tomato cultivars 116, 916 and 1684 were planted on 5/8/2006 while tomato cultivars 3060, 3019, Teiba and Munna were planted on 11/8/2006. Plants were irrigated by drip irrigation system and no insecticides had been used. The experimental layout are shown in the figure (2.1) .

116	916	Munna	1684	Teiba	3060	3019
3019	Teiba	116	1684	3060	916	Munna
3019	1684	Munna	116	3060	916	Teiba
Munna	3019	1684	116	916	3060	Teiba

Figure (2.1) Layout of open field planted with different tomato cultivars used for the summer experiment in Al- Arroub Agricultural Experimental Station (2006).

TYLCV symptom severity rating was evaluated according to the visibility of disease symptoms regarding to the yellowing of leaflet margins on apical leaf, curling and stunting. Symptom development evaluation scale used in this experiment was similar to the one used by Maruthi et al (Maruthi *et al*., 2003):

0=No visible symptoms,

1= Very slight yellowing of leaflet margins on apical leaf,

2= Some yellowing and minor curling of leaflet ends,

3= Severe symptoms, yellowing, curling, severe stunting.

TYLCV incidence and severity on each cultivar, in addition to the number of days required for symptoms appearance after planting were evaluated on weekly basis and started on 11/8/2006 and terminated on 30/9/2006. Statistical Analysis of disease incidence and severity data was done according to Fisher LSD at $p \leq 0.05$.

2.1.2.2. Biological studies for TYLCV incidence for spring experiment (2007)

Twenty five –day –old seedlings of tomato cultivars were planted in an open field of Al Arroub Agricultural Station during spring 2007. Eight cultivars were used in this experiment replicated four times in a randomized complete block design . Each replicate have 18 tomato seedlings. The distance between experimental plots was 1.5m and the distance between plants in each plot was 0.5m.

Tomato cultivars 56, 116, 916 and 1684, 3060, 3019, Teiba and Munna were planted on 26/4/2007. These cultivars were the same cultivars used in summer experiment except for cultivar 56 which was used in spring experiment only. The experimental layout are shown in the Figure (2.2). Disease severity and disease incidence for each cultivar and number of days to observe early symptoms were evaluated every week started from 8/5/2007 and terminated on 30/7/2007. The symptom severity rating scale was mentioned in section (2.1.2.1).

Comparison between the resistance level according to TYLCV incidence of infection and disease severity for tomato cultivars was done, Comparison between spring results and summer results was then reported.

1684	Munna	116	56	916	3060	3019	Teiba
916	116	1684	56	Munna	3019	3060	Teiba
916	3060	56	3019	116	Teiba	1684	Munna
116	56	916	3019	Munna	1684	3060	Teiba

Fig. (2.2) Layout of open field planted with different tomato cultivars in Al-Arroub Agricultural Experemental Station for spring experiment (2007).

2.2. Chemicals and equipment

2.2.1. Primers

Primers used in this study were used to amplify the intergenic region (IR) of TYLCV. These primers were able to amplify 634 bp from their region of the TYLCV formally known as "TYLCV-Israel" (Navot et al., 1991).. Primer pairs TYv2337/Tyc 138 were used in this study as previously described by Anfoka *et al.*(2005). These primers were obtained from Biolin ltd., USA.

TYv2337 (viral sense) [5´-ACGTAGGTCTTGACATCTGTTGAGCTC3']
and Tyc138 (viral comp) [5´-AAGTGGGTCCCACATATTGCAAGAC
3'].

2.2.2. Chemicals used for PCR reaction mixture preparation

The chemicals used to prepare PCR reaction mixture: 0.25mM deoxynucleotide triphosphate (dNTPs). 1x *Taq* DNA polymerase buffer, 0.25mM $MgCl_2$, 0.5 units *Taq* DNA polymerase, 2.5µM of each

complementary and virus –sense primer and 2.5µl of DNA sample . All components of the PCR reaction were obtained from Biolin ltd., USA.

2.2.3. Chemicals used for DNA extraction

10mM B –Mercaptoethanol , 100Mm Tris , 50mM EDTA pH 8, 500mM NaCl, 1.25% SDS, potasium acetate, Isopropanol, phenol, Chloroform , Isoamyl alchohol, absolute ethanol , 70% ethanol, molecular grade water (HPLC).

2.2.4 . Chemicals used for analysing PCR results

For PCR analysis: Agarose gel, Ethedium bromide , 1x TBE, DNA ladder (1kbp) .

2.2.5. Laboratory equipment and tools

The equipments and tools that was needed for our experiments are provided from Hebron University (Biotechnology laboratory and plant protection research lab . Thermocycler (model PTC- 200, MJ research Inc.,Water town, MA, USA). Ultra-low temperature freezer (model MDF- U 3086S, Sanyo, USA). Centrifuge (model Universal 16 R, A. Hettich,D-78532 Tuttilingen) . Gel electrophoresis apparatus for PCR analysis. UV trans-illumination apparatus (model Image Master VD, Ge health company) .

2.3. Methodology of the research

2.3.1. Optimization of *Taq* DNA polymerase

Taq DNA polymerase at the concentration of 5 units/µl was diluted to get a concentration range from 0.5-2.5 units /µl. Since commercial *Taq* DNA polymerase was very expensive, an experiment had been set to figure out the minimum of concentration of *Taq* DNA polymerase needed to detect this virus . For this purpose, different concentrations of *Taq* DNA polymerase were tested : 0.5µl (2.5 units), 0.4µl (2.0 units), 0.3µl (1.5 units), 0.2µl (1unit) and 0.1µl (0.5 unit). Each concentration was applied on two positive, two negative, two water and two unknown tomato leave samples.

2.3.2. Molecular studies for TYLCV incidence for summer experiment (2006)

2.3.2.1. Samples collection

Viral DNA concentrated in the uppermost leaf of each plant was assessed by using polymerase chain reaction method. In this method, leaves were collected from each plant in 30/9/2006 using dissecting blades and kept at -80ºC for later PCR detection.

2.3.2.2. Nucleic acid extraction

Total nucleic acids were extracted from both TYLCV-infected and healthy tomato cultivars using a modified procedure of Dellaporta heat extraction method as described by (Potter *et al*,. 2003). Preparation of the extraction buffer was done as shown in table (2.1).

Table (2.1) Extraction buffer used for detection of TYLCV virus

Chemicals	To make 100ml
	58 ml H_2O
100mM Tris pH 8	10 ml 1M Tris ,PH 8
50mM EDTA pH 8	10 ml 0.5M EDTA
500mM NaCl	10 ml 5 M Nacl
10mM B –Mercaptoethanol	70 µl B-Mercaptoethanol
1.25 % SDS	12 ml 10 % SDS

Five mg of leaf sample were extracted with 1ml of extracting buffer (Table 2.1), using a pre-cooled mortars and pestles. After vortexing, samples were allowed to settle at 65 °C for 10 min before adding 1/5 volume of potassium acetate (5M, pH 8). Samples were incubated on ice for 10 min, and then centrifuge at 13,000 rpm for 20 min at 4◦C. 500 µl of supernatant were taken and added to equal volume of Isopropanol. Samples were incubated for 10 min at -20 ◦C, and then centrifuged for 10 min at 13,000 rpm. The pellet was then treated with phenol /Chloroform /Isoamyl alchohol (25: 24 :1) in order to remove RNase, before centrifuging samples again at 13,000 rpm for 10 min; the aqueous phase was then collected. Aqueous phase(150µl) with three volumes of absolute ethanol were mixed and stored for 30 min at -20. After centrifuging for 10 min at 13,000rpm, the pellet was washed by 30 µl of 70% ethanol, dried and re-suspended in 60 µl of molecular grade water (HPLC), to be stored laty at -20ºC.

2.3.2.3. Polymerase chain reaction (PCR)

Amplification of the intergenic region of TYLCV was done using the primer TYv2337 which is the primer sense and had the sequence (5´-ACGTAGGTCTTGACATCTGTTGAGCTC-3') (anneals at nucleotide position 2337-2364).The primer Tyc138 was the complementary to the positive strand and anneals at nucleotide position 138-125) and had the sequence (5´AAGTGGGTCCCACATATTGCAAGAC 3'). These primers were able to amplify 634 bp from their region of the TYLCV formally known as "TYLCV-Israel" (Navot *et al.*, 1991). After DNA extraction, preparation for the PCR reaction took place. The components of the reaction were [0.25Mm deoxynucleotide triphosphate (dNTPs), 1x *Taq* DNA polymerase buffer, 0.25mM MgCl, 0.5 units *Taq* DNA polymerase, 2.5µM of each complementary and virus –sense primer and 2.5µl of DNA sample. The parameters for the PCR reaction were optimized for 25µl. All components of the PCR reaction were obtained from Biolin ltd., USA. The reaction components were placed in the thermocycler with amplification program as [94°C/5min; 30X (94°C/1 min, 62°C/45 sec., 72°C/1 min.); 1X (94°C/1 min; 56°C/1 min; 72°C/10 min**).**

2.3.2.4. Gel Electrophoresis

The resulting PCR fragments were analyzed by 1.2 % agarose gel electrophoresis after staining with ethidium bromide. The main steps used for analyzing PCR products were as follows:

1. Agarose gel (1.2%) was prepared by dissolving 1.2 gm in 1x TBE
2. After cooling the molten agarose, ethidium bromide was added at 70°C.
3. Loading buffer (5 µl) was added to tubes which have the amplified PCR reaction mixture .

4. Loading the wells carefully by adding 15 µl of amplified PCR reaction mixture and 10 µl of DNA marker.
5. Run the gel at 130V for 20 min.
6. Visualize the amplified fragments by UV trans-illumination. Verify results against DNA marker and positive control.
7. Photograph the gel to provide a permanent record.

2.3.3. Molecular studies for TYLCV incidence for spring experiment (2007)

Viral DNA that concentrated in the uppermost leaf of each plant was assessed by using polymerase chain reaction method. In this method, leaves are collected from each plant in 30/7/2007 by using dissecting blades and kept at -80ºC for later PCR detection. The methods used in this experiment are the same methods used in summer experiment which is mentioned in section (2.3.2). Then comparisons between resistance levels for tomato cultivars according to molecular incidence were done.

2.3.4. Survey of *Tomato Yellow Leaf Curl virus* in the Southern part of the West Bank, Palestine .

To determine the incidence of *Tomato Yellow Leaf Curl Virus* (TYLCV) in the southern part of West Bank, a survey took place between 1/7/2008 and 1/8/2008 in open fields and greenhouses.

The survey was based on symptoms observation (leaf curl, yellowing, abnormal reduction in plant size, stunting and poor fruit set). The tomato planted in the spring season by the farmers were treated with insecticides. In Autumn, the farmers usually do not cultivate tomato because of the high population of the white fly *Bemesia tabacci*.

Survey of *Tomato Yellow Leaf Curl Virus* conducted mainly in two districts: Bethlehem and Hebron. In Bethlehem district seven areas were surveyed (Marah Rabah, Almenyah, Wady Fokin, Beteer, Al walajah, Beit Fajar, and Tkooh) Table (2.2) . In Hebron thirteen areas were surveyed (Dweer Ban, Tafoh, Beit Kahel, Tarkomiah, Beit Ola, Shyokh Alarrub, Albweereh, Saeer, Beit Ommar, Halhool, Dora and Yatta Table 2.3. In each district cultivar type, number of tomato plants, number of infected plants and date of planting had been recorded Table (2.2) and Table (2.3).

Table 2.2. Survey for TYLCV incidence in different regions of Bethlehem

Area name	Type	Cultivar type	Number of plants	Date of planting
Marah Rabah	Open field	144	150	1/6/2008
Almenyah	Open field	773	3500	01/07/2008
Wady Fokin	Open field	Manar	1440	28/04/2008
Beteer	Plastic house	Unknown	3000	Unknown
Al walajah	Open field	Sarah	500	18/05/2008
Al walajah	Open field	Sarah	200	28/05/2008
Al walajah	Open field	56	1000	04/07/2008
Beit Fajar	Open field	259	600	20/05/2008
Marah Rabah	Plastic house	Manar	1000	25/03/2008
TKOOH	Open field	56	1000	28/05/2008
TKOOH	Open field	593	1500	20/06/2008
TKOOH	Plastic house	Unknown	700	10/03/2008

Table 2.3. Survey for TYLCV incidence in different regions of Hebron

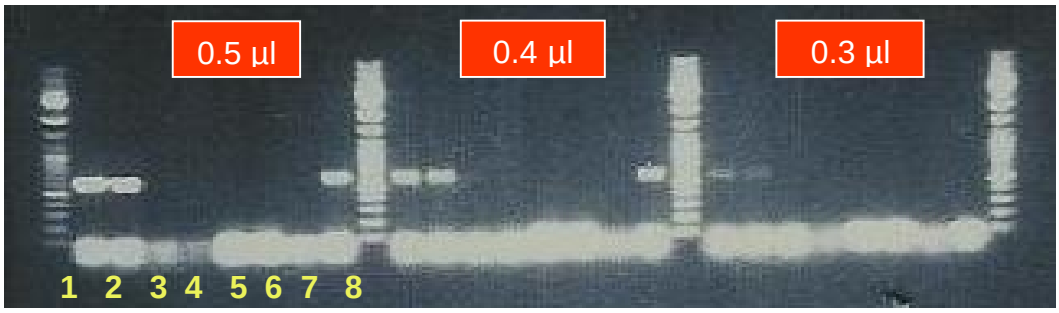
Area name	Type	Cultivar type	Number of plants	Date of planting
Dweer Ban	Open field	Unknown	2800	Unknown
Tafoh	Plastic house	Unknown	5000	Unknown
Tafoh	Open field	16/84	50	18/05/2008
Beit Kahel	Open field	Unknown	1500	18/05/2008
Tarkomiah	Plastic house	30/10	1700	13/03/2008
Tarkomiah	Open field	56	900	15/04/2008
Beit Ola	Open field	144	3000	25/05/2008
Shiokh El Arrub	Open field	Sarah	3500	15/05/2008
Albweereh	Open field	30/19	2000	01/06/2008
Albweereh	Open field	773	2000	01/06/2008
Saear	Open field	Sarah	2000	20/05/2008
Halhool	Open field	56	1000	14/05/2008
Beit Ommar	Open field	773	600	14/05/2008
Beit Ommar	Open field	unknown	1500	01/07/2008
Dora (Wadi Abu Al kamra)	Open field	30/19	2000	28/05/2008
Dora (Wadi Abu Al kamra)	Open field	Unknown	1500	02/06/2008
Dora (Wadi Abu Al kamra)	Plastic house	Manar	1000	20/03/2008
Dora (Wadi Abu Al kamra)	Plastic house	Manar	1500	20/03/2008
Dora (Wadi Abu Al kamra	Plastic house	Manar	1000	17/03/2008
Yatta (Rakaa)	Open field	16/84	1500	01/05/2008

Yatta (Rakaa)	Open field	Sarah	500	20/05/2008
Yatta (Rakaa)	Plastic house	Manar	1000	25/04/2008
Yatta (Rakaa)	Plastic house	Manar	1200	25/04/2008

CHAPTER THREE: RESULTS

3.1. Optimization of *Taq* DNA polymerase

Taq DNA polymerase sensitivity in detection of the virus DNA had been tested. The results are shown in figure (3.1) .



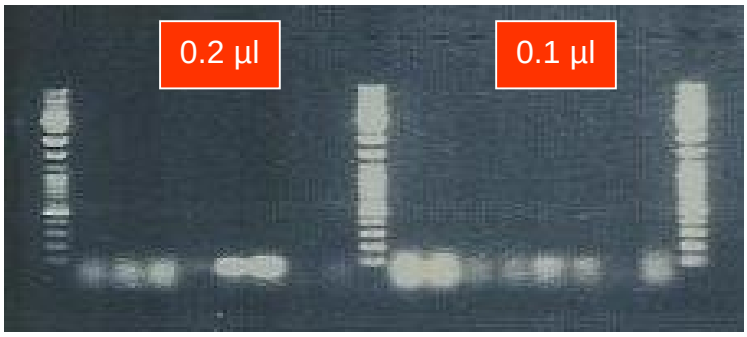


Fig 3.1. *Taq* DNA polymerase sensitivity in detection of TYLCV at different concentration .

1.2% agarose gel electrophresis of DNA intergenic region amplicons. Samples 1 and 2 are positive control; 3 and 4 are negative control samples; 5 and 6 are water control samples, and 7and 8 are randomly selected tomato samples**.**

PCR gave a single product of the expected size at (634bp) reflecting the ability of the primers to anneal to either viral genome. It was found that 0.4µl (2 units) of *Taq* DNA polymerase were enough to detect clearly all positive samples.

3.2. Biological studies for TYLCV incidence for summer experiment (2006)

3.2.1. TYLCV incidence for tomato cultivars for summer experiment (2006)

Results presented in table 3.1 shows that there were fundamental differences in disease incidence for tomato cultivars. The results showed that some of these cultivars had high disease incidence noticed on cultivar 3019 (93%), 1648 (71%) and 116 (58%), while other cultivars had low disease incidence such as cultivar 3060 (7%), Teiba (21%) and Munna (14%).

Statistical analysis for TYLCV incidence after 55 days for tomato cultivars presented in table 3.1 showed that cultivars 3060, Munna, Teiba, and 916 are not significantly differ from each other but significantly different from other cultivars . Cultivars 116 and 1684 are not significantly differ from each other but significantly different from other cultivars, while cultivar 3019 was significantly different from the other cultivars.

The weekly observations for TYLCV incidence for eight weeks are presented in table 3.2 showed that TYLCV incidence increase rapidly for cultivars 3019, 1684 and 116 and slowly for the other cultivars . Results showed that TYLCV incidence increase very slowly for Cultivar 3060 which was the most promising one . TYLCV incidence measurement chart presented in Fig 3.2

Table 3.1. TYLCV incidence and statistical analysis for tomato cultivars after eight weeks of planting for summer experiment (2006).

Cultivar	TYLCV incidence				Total	Mean* ±SE
	Block 1	Block 2	Block 3	Block 4		
116	72.2	55.5	55.5	50	233.29	58.3 b ± 4.8
916	33.3	27.7	22.2	16.6	99.8	24.95 c ± 3.58
1684	94.4	100	50	38.8	283.2	70.8 b ± 15.45
3060	11.1	5.5	11.1	0	27.7	6.9 c ± 2.65
3019	77.7	100	100	94.4	372,1	93 a ± 5.3
Teiba	16.6	27.7	22.2	16.6	83.1	20.77 c ± 2.65
Munna	22.2	11.1	16.6	5.5	55.4	13.85 c ± 3.59

*:Values within the colum followed by the same letter are not significantly different according to Fisher LSD at $p \leq 0.05$

Table 3.2.The weekly observations for TYLCV incidence for tomato cultivars for eight weeks for summer experiment (2006).

Weeks	116	916	3019	1684	3060	Teiba	Munna
1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0
2	1.4±1.4	1.4±1.4	1.4±1.4	1.4±1.4	0±0	0±0	0
3	5.5 ±2.3	2.8±1.6	1.4±1.4	2.7±1.6	1.4±1.4	1.4±1.4	0
4	25±3.5	11±3.9	25±8.3	13.8±3.6	1.4±1.4	4.1±2.6	0
5	30.5±5.8	15.2±2.7	43±13.7	19.4±1.6	1.4±1.4	6.9±1.4	4.12±1.4
6	31.9±5.7	18±2.7	75±8.3	55.5±11.3	1.4±1.4	8.3±2.8	9.7±4.1
7	50±8.8	22±3.9	93±5.3	62.5±15.4	5.5±2.2	13.8±5.3	11.1±5.1
8*	58b±4.8	25c±3.6	93a±5.3	70.8b±15.4	6.9**c**±2.6	20.8c±2.6	13.8c±3.6

*:Values within the raw followed by the same letter are not significantly different according to Fisher LSD at $p \leq 0.05$

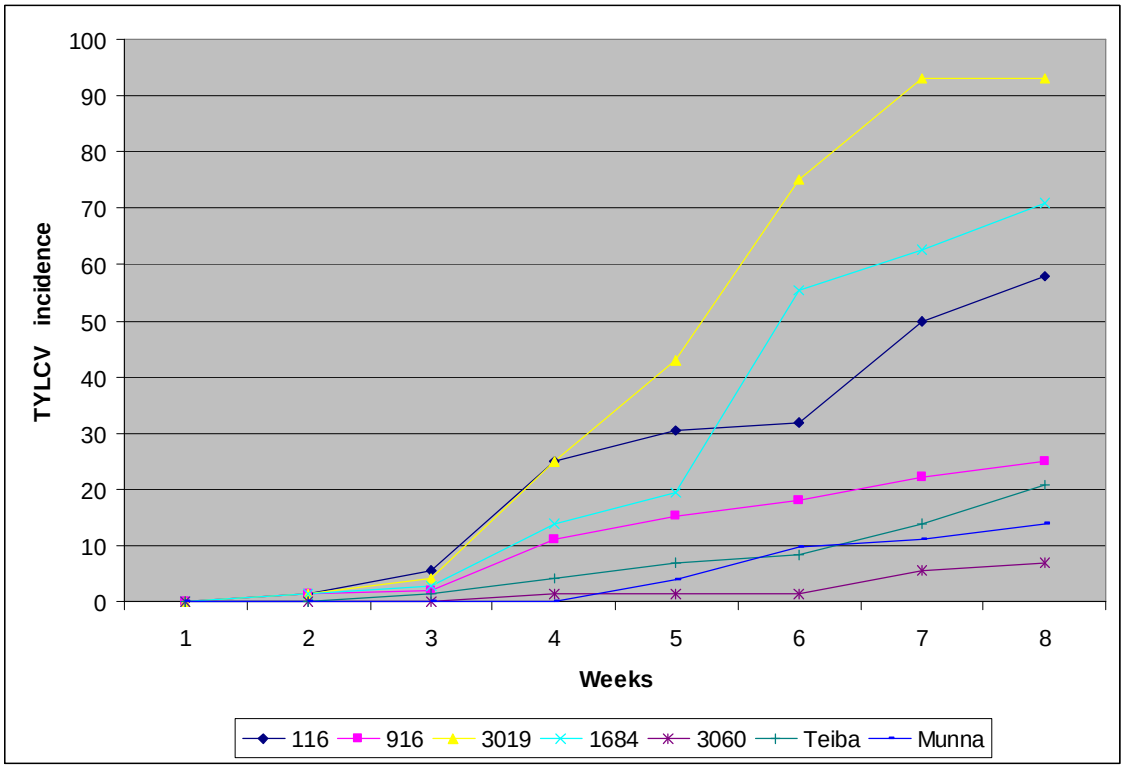


Fig. 3.2. TYLCV incidence % measurement chart for tomato cultivars for summer experiment (2006) in Alarrub Agricultural Experimental station.

3.2.2. Symptom assessment for tomato cultivars infected by TYLCV for summer experiment (2006)

The plants of the different tomato cultivars were screened for symptom development and disease severity . There were fundamental differences both in the number of days to observe early symptoms and the degree of symptom development among the different cultivars . These results are presented in table 3.3. Results showed that the first observation of symptoms appeared after two weeks in tomato cultivars 116, 916, 1684,

and 3019. In the cultivars Munna, 3060, and Teiba, symptoms were produced after 34, 27 and 20 days, respectively.

Table 3.3. Symptom assessment and statistical analysis in different tomato cultivars infected by TYLCV for eight weeks for summer experiment (2006).

Cultivar	Days to observe early symptoms	Disease severity[a]				Total	(Mean*±SE)
		Block 1	Block 2	Block 3	Block 4		
116	13	1.444	1.333	1.277	1.222	5.276	1.319 b ± 0.04
916	13	0.5	0.611	0.388	0.277	1.776	0.444 c ± 0.07
1684	13	2.555	2.833	1.222	0.888	7.498	1.875 b ± 0.48
3060	27	0.333	0.111	0.055	0	0.499	0.125 c ± 0.07
3019	13	2.333	3	3	2.833	11.166	2.79 a ± 0.158
Teiba	20	0.222	0.5	0.388	0.222	1.332	0.333c ± 0.06
Munna	34	0.388	0.111	0.222	0.055	0.776	0.194 c ± 0.07

a: Severity: 0 : no symptoms, 1 : very slight yellowing of leaflet margins on apical leaf , 2 : some yellowing and minor curling of leaflet ends , 3: severe symptoms, yellowing, curling, severe stunting.

*Values within the colum followed by the same letter are not significantly different according to Fisher LSD at $p \leq 0.05$

These symptoms began with strong chlorosis and yellowing of the young leaves progressed to an upward curling of leaf margins, and culminated in a complete stunting of growth. Symptoms are shown in Fig (3.3) and (3.4).

Fig 3.3. Symptoms of *Tomato yellow leaf curl virus.*.

The results showed that tomato cultivars had different symptom severities every week after planting and cultivars 3019, 1684 and 116 had the highest symptom severities which were 2.79 and 1.8 and 1.31, respectively. Cultivar 3060 had the lowest symptom severity which was 0.125 only.

Fig 3.4. Comparison between an infected plant with TYLCV and a healthy plant. Left : infected plant with severe stunting, yellowing, leaf cuping and stunting. Right: healthy plant

Statistical analysis for disease severity after 55 days for tomato cultivars presented in table 3.3 showed that cultivars 3060, Munna, Teiba, and 916 are not significantly differ from each other but significantly different from other cultivars . Cultivars 116 and 1684 are not significantly differ from each other but significantly different from other cultivars, while cultivar 3019 was significantly different from the other cultivars.

The symptom severity for these tomato cultivars had been recorded for eight weeks after planting and represented in Table 3.4.

Table 3.4. The symptom severity for tomato cultivars infected by TYLCV for eight weeks for summer experiment (2006). (Mean*±SE)

Weeks	116	916	1684	3060	3019	Teiba	Munna
1	0	0	0	0	0	0	0
2	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0	0.01 ±0.01	0	0
3	0.09 ±0.04	0.055 ±0.03	0.04 ±0.02	0	0.041 ±0.03	0.01 ±0.01	0
4	0.34 ±0.09	0.208 ±0.106	0.291 ±0.07	0.01 ±0.01	0.416 ±0.211	0.041 ±0.01	0
5	0.66 ±0.46	0.291 ±0.08	0.472 ±0.06	0.01 ±0.01	1.083 ±0.389	0.069 ±0.01	0.04 ±0.01
6	0.77 ±0.15	0.347 ±0.07	1.38 ±0.22	0.01 ±0.01	1.66 ±0.212	0.111 ±0.05	0.138 ±0.06
7	1.05 ±0.14	0.388 ±0.09	1.68 ±0.48	0.097 ±0.07	2.548 ±0.19	0.208 ±0.01	0.166 ±0.08
8*	1.32b ±0.04	0.44c ±0.07	1.87b ±0.48	0.13c ±0.07	2.79c ±0.158	0.33c ±0.06	0.19c ±0.07

Symptom severity scale:0 = No symptoms,1= Very slight yellowing of leaflet margins on apical leaf, 2= Some yellowing and minor curling of leaflet ends, 3= Severe symptoms, yellowing, curling and severe stunting. *Values within the colum followed by the same letter are not significantly different according to Fisher LSD at $p \leq 0.05$

The results showed that the symptom severity increased rapidly in cultivar 3019 and 1684 which had high disease incidence while cultivar 3060 the symptom severity increased very slowly. The symptom severity measurement chart for tomato cultivars are shown in Fig (3.5).

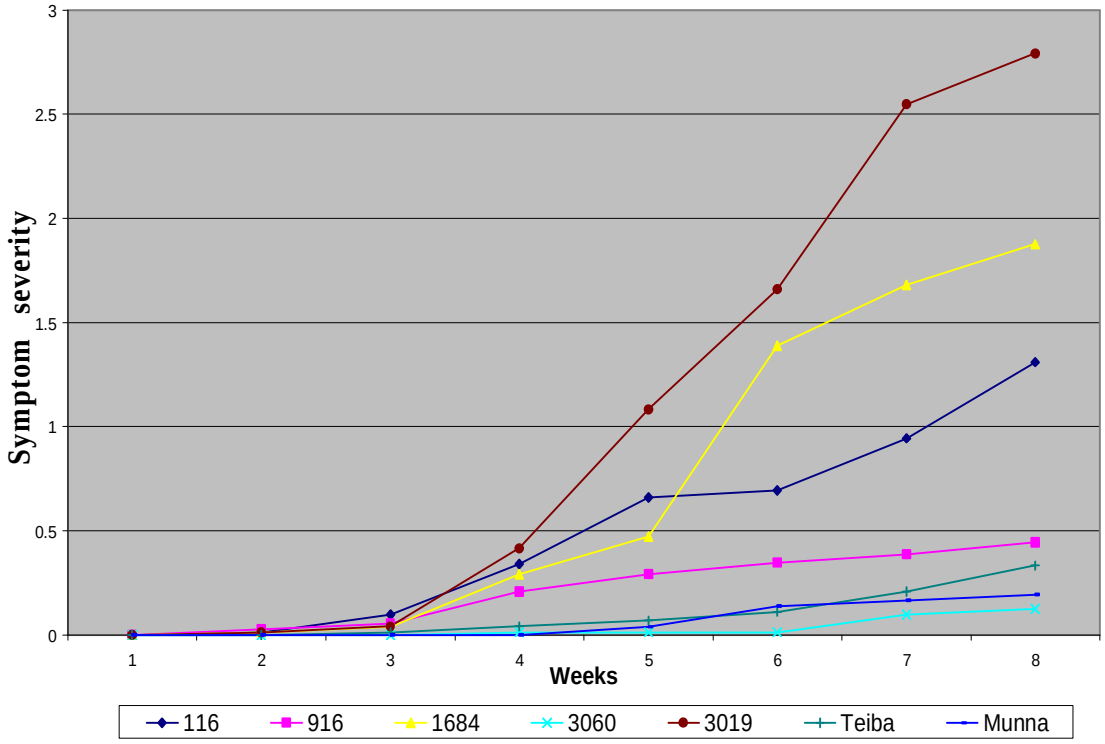


Fig 3.5 .The disease severity measurement chart for TYLCV for summer experiment (2006) in Al-Arroub Agricultural Experimental station.

3.3. Molecular studies for TYLCV incidence for summer experiment (2006)

The presence for TYLCV in the tomato cultivars was detected using the PCR method. Total DNA from healthy and infected tomato cultivars was extracted by using a modified procedure of Dellaporta heat extraction method as described by (potter *et al*,. 2003). Primers used in this study was able to amplify the intergenic region of TYLCV. These primers were able to amplify 634 bp from their region of the TYLCV formally known as "TYLCV-Israel". The PCR fragments are detected by 1.2 % agarose gel electrophoresis after staining with ethidium bromide. PCR incidence in the tomato cultivars was monitored to study the relationship between the

resistance level of tomato cultivars, as determined by symptom severity and disease incidence. TYLCV had been detected in infected tomato cultivars by using PCR method. PCR gave a single product of the expected size at (634bp) reflecting the ability of the primers to anneal to either viral genome Fig (3.6).

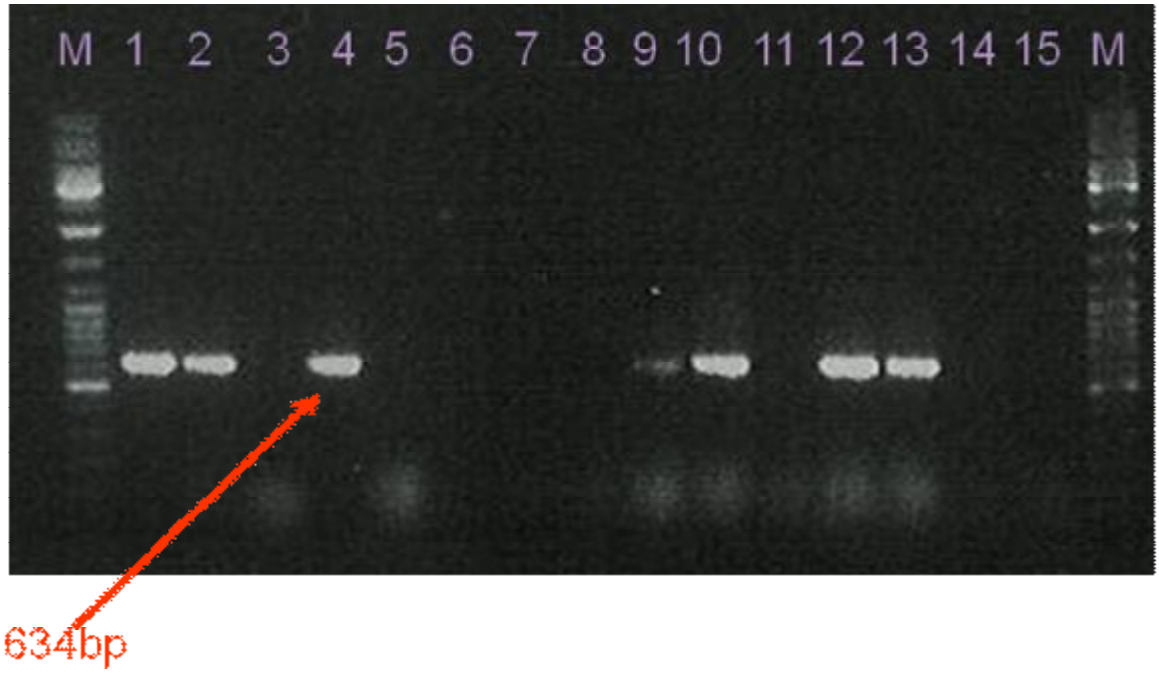


Fig 3.6. Detection of TYLCV in tomato cultivars by using PCR method.

1.2% agarose gel electrophresies of DNA intergenic region amplicons using degenerate primers. A sample 1 is positive control. Samples (2, 4, 9,10,12&13 are virus positive; 14&15 are healthy ones. "M" refers to the DNA ladder that used as a marker (1000bp).

The percentage of samples in which TYLCV was detected for each cultivar had been calculated based on the total number of samples collected . These results are summarized in Table (3.5).

Table 3.5. PCR analysis for TYLCV on tomato cultivars collected in summer (2006) (Mean*±SE).

Cultivar	positive % for TYLCV				Total	(Mean*±SE)
	Block 1	Block 2	Block 3	Block 4		
3060	0	0	0	5.55	5.55	1.38 d ± 1.38
Munna	16.6	0	0	10	26.66	6.66 d ± 4.06
Teiba	33.3	23.5	27.7	27.7	112.2	28 c ± 2.01
916	61.1	44.4	55.5	44.4	205.4	51.3 a ± 4.17
1684	43.7	42.8	41.6	38.8	166.9	41.7 b ± 1.06
3019	50	52.9	43.7	53.3	199.9	49.9 a ± 2.21
116	50	50	58.8	47	205.8	51.4 a ± 2.5

* Mean Percentage of samples in which TYLCV was detected by using PCR technique (based on the total number of samples collected). Values within the colum followed by the same letter are not significantly different according to Fisher LSD at $p \leq 0.05$

Results showed that the cultivar 3060, Teiba and Munna had the lowest percentage which was 1%, 5% and 28%, respectively .The highest percentage was in cultivars 3019, 116 and 916 reached approximately to 50% then cultivar 1684 which had 42%. Statistical analysis for the percentage of samples in which TYLCV was detected had been done . Results presented in table (3.5) . Results showed that cultivar 3060 and Munna were not statistically differ from each other but they are statistically different from the other cultivars. Cultivars 916, 3019, and 116 were not statistically differ from each other but they are statistically different from the other cultivars ,while cultivars 1684 and Teiba was statistically different from the other cultivars. Fig (3.7) show PCR analysis for TYLCV on tomato cultivars .

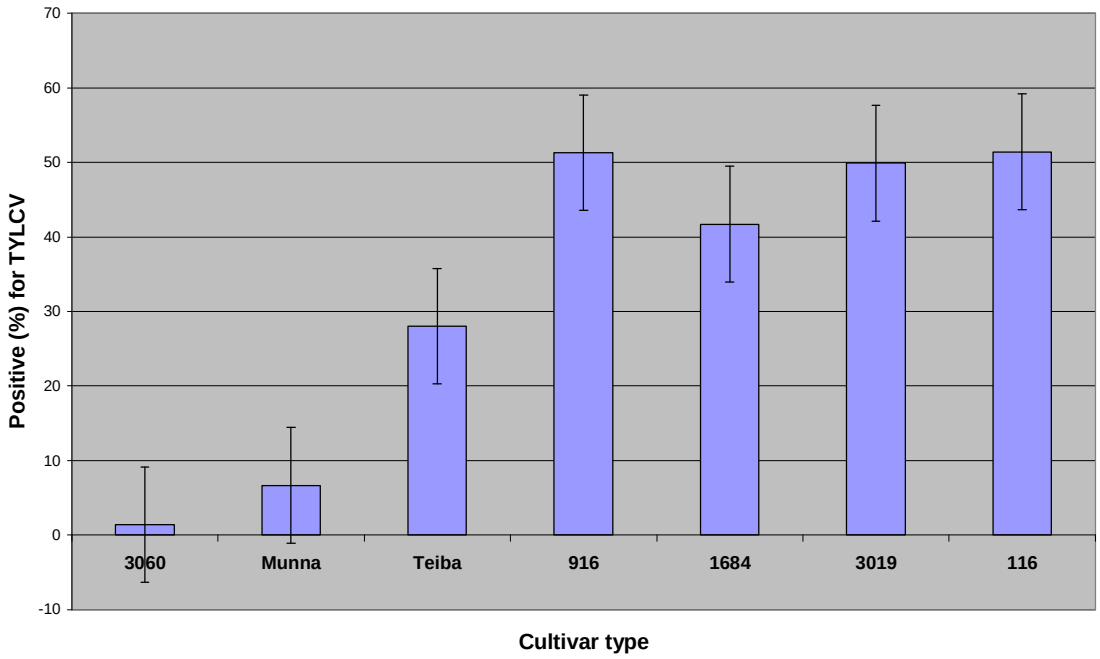


Fig 3.7. PCR analysis for TYLCV on tomato cultivars for the summer experiment 2006 in Alarrub Experimental Station.(Fisher Lsd =7.75, p≤ 0.05)

PCR analysis in a highly susceptible cultivar which was cultivar 3019 and a highly tolerant cultivar which was 3060 had shown in fig (3.8) and fig (3.9).

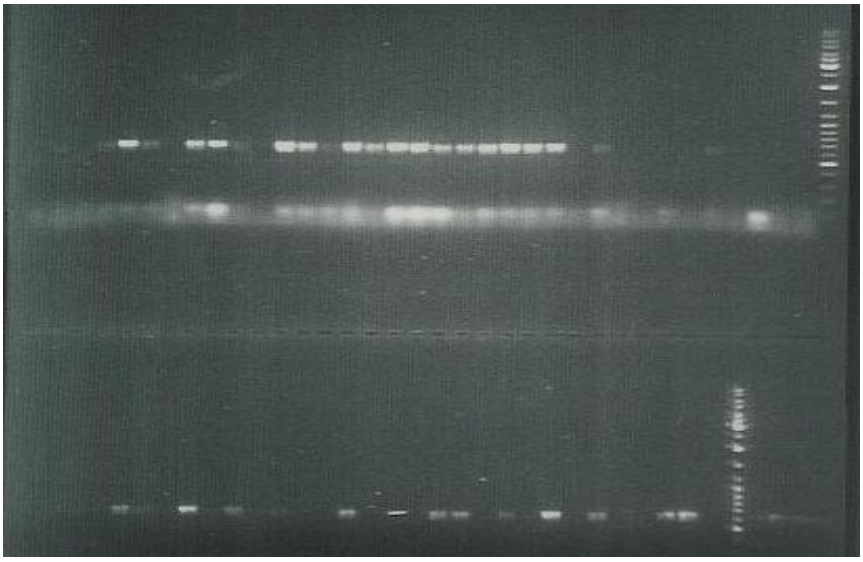


Fig 3.8. PCR analysis in a highly susceptible tomato cultivar infected by TYLCV.

1.2% gel electrophresies of DNA intergenic region amplicons using degenerate primers. PCR results for cultivar 3019 .

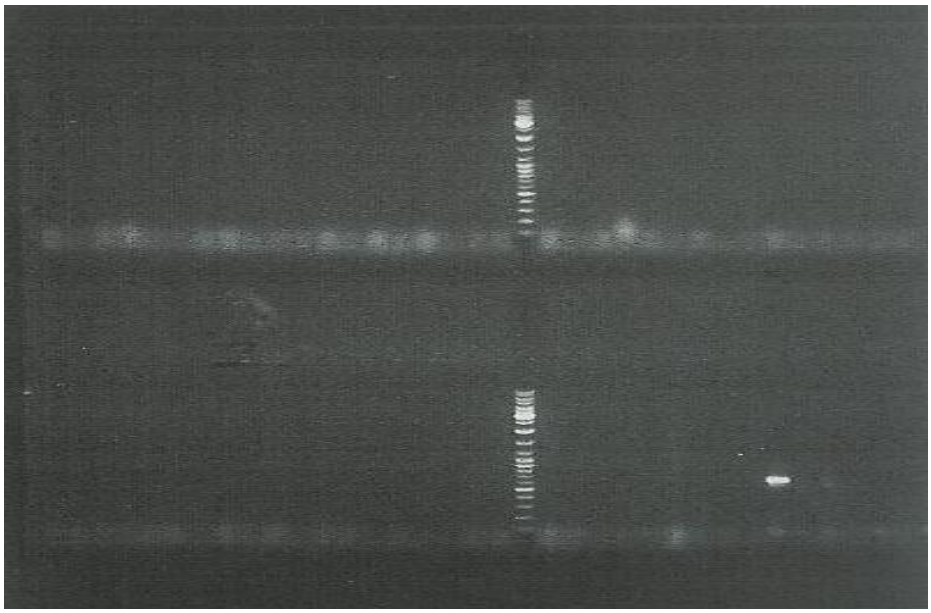


Fig 3.9. PCR analysis in a highly tolerant tomato cultivar infected by TYLCV.

1.2% agarose gel electrophresies of DNA intergenic region amplicons using degenerate primer.

A comparison between PCR analysis (positive % for TYLCV) and disease incidence after eight weeks had been done . Results presented in Table (3.6). The observations showed that cultivar 3060 which had low disease incidence (7%) also had low PCR incidence (1%) while Cultivar 3019 which had high disease incidence (93%) also had high PCR incidence (50%). This means that molecular assay (PCR) determined by biological assay (disease incidence) indicated similar trend in that cultivars which had high disease incidence also had high positive (%) for TYLCV and cultivars which had low disease incidence also had low positive (%) for TYLCV. However, to some extent the correlation was less applicable for cultivar 916 in which the disease incidence 25% is lower than PCR analysis 51%. This is because molecular assay is more sensitive than biological assay .

Table 3.6 . Comparison between field incidence and PCR analysis for tomato cultivars infected by TYLCV for summer experiment 2006 in Al-Arroub Agricultural Experimental Station. (Mean*±SE).

Cultivar type	*Disease incidence %	*Positive (%) for TYLCV
116	58.3 b ± 4.8	51a ± 2.5
916	25 c ±3.58	51a ± 4.17
1684	70.8 b ±15.45	42 b ±1.06
3060	7 c ± 2.65	1d ± 1.38
3019	93 a ± 5.3	50 a ± 2.21
Teiba	20c ± 2.65	28 c ± 2.01
Munna	14 c ± 3.59	6.6d ± 4.06

* Mean : Values within the colum followed by the same letter are not significantly different according to Fisher LSD at $p \leq 0.05$

The correlation between PCR analysis and disease incidence presented in Fig (3.10).

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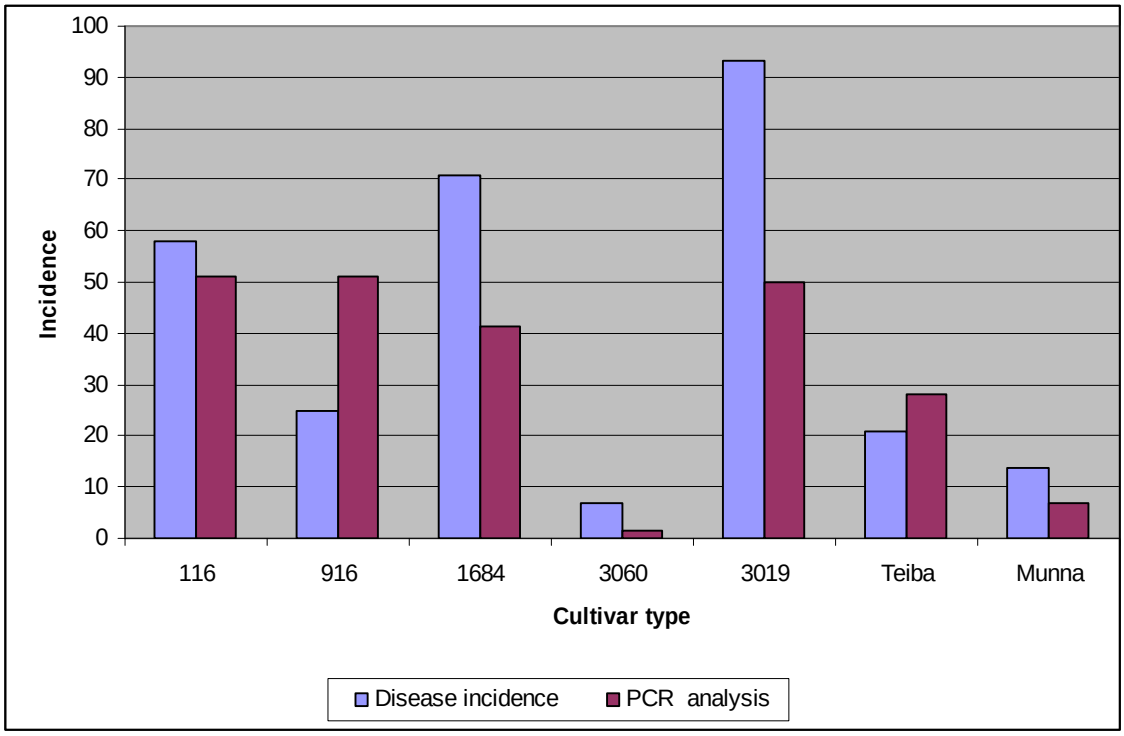

Fig 3.10. Comparison between disease incidence and PCR analysis for tomato cultivars infected by TYLCV for summer experiment 2006 in Al-Arroub Agricultural Experimental Station .

3.4 Biological studies for TYLCV incidence for spring experiment (2007)

3.4.1. TYLCV incidence for tomato cultivars for spring experiment (2007)

TYLCV incidence was screened during the growth period in spring for twelve weeks for each cultivar . Results presented in table (3.7) showed that the highest incidence was for cultivar 56 (13.8%) and cultivar 1684 (8.3%) . Cultivars Munna, 116 and 3019 had (4.1%), (2.7%), (1%), respectively , while cultivars 3060, Teiba and 916 did not infected by TYLCV during spring.

Table 3.7. TYLCV incidence for tomato cultivars after twelve weeks of planting for spring experiment (2007).

Cultivar	TYLCV incidence				Total	(Mean*±SE)
	Block 1	Block 2	Block 3	Block 4		
3060	0	0	0	0	0	0 c ± 0
Munna	0	5.5	0	11.1	16.6	4.12 b±2.6
Teiba	0	0	0	0	0	0 c ± 0
916	0	0	0	0	0	0 c ± 0
1684	11.1	5.5	11.1	0	27.7	8.25b ± 1.58
3019	5.5	0	0	0	5.5	1.37c ± 1.37
116	5.5	0	5.5	0	11	2.75 c± 1.58
56*	11.1	5.5	22.2	16.6	55.4	13.8 a ± 3.59

*:Values within the raw followed by the same letter are not significantly different according to Fisher LSD at $p \leq 0.05$

The weekly observations for disease incidence for twelve weeks had been calculated and presented in table (3.8). Results showed that disease incidence increase rapidly for tomato cultivars 56 and 1684 in which symptoms was observed early and slowly in the other cultivars in which symptoms was observed lately . The disease incidence measurement chart for these tomato cultivars had been done and presented in Fig 3.11

Table 3.8. The weekly observations for TYLCV incidence for tomato cultivars after 12 weeks for spring experiment 2007.

Weeks	**116**	**916**	**1684**	**3060**	**3019**	**Teiba**	**Munna**	**56**
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	1.3±1.3	0	0	0	0	1.3±1.3
5	0	0	1.3±1.3	0	0	0	0	1.3±1.3
6	0	0	1.3±1.3	0	0	0	0	1.3±1.3
7	0	0	1.3±1.3	0	0	0	0	1.3±1.3
8	0	0	1.3±1.3	0	0	0	0	1.3±1.3
9	1.3±1.3	0	1.3±1.3	0	0	0	1.37±1.37	1.3±1.3
10	1.3±1.3	0	2.75±1.58	0	1.37±1.37	0	1.37±1.37	6.9±1.3
11	2.7±1.58	0	6.8±2.63	0	1.37±1.37	0	2.7±1.58	8.3±1.58
12*	2.7c±1.6	0c	8.3**b**±1.58	0c	1.37c±1.37	0c	4.1b±2.63	13.8**a**±3.6

*:Values within the raw followed by the same letter are not significantly different according to Fisher LSD at $p \leq 0.05$

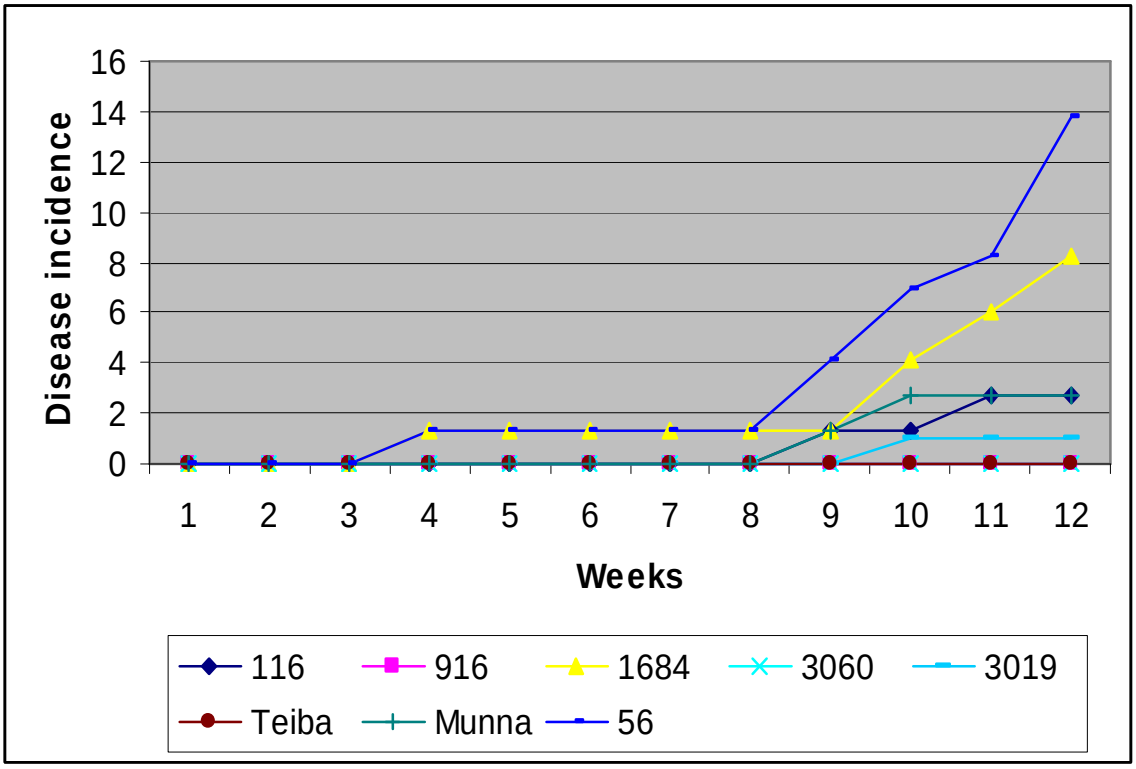

Fig 3.11. Disease incidence measurement chart for tomato cultivars infected by TYLCV for spring experiment 2007.

3.4.2. Symptom assessment for tomato cultivars infected by TYLCV for spring experiment (2007)

TYLCV symptom development and severity was screened during the growth period in spring for twelve weeks . Results presented in table (3.9) showed that the first observation of symptoms appeared after four weeks in tomato cultivars 56 and 1684, while in tomato cultivars Teiba, 916 and

3060 symptoms did not observed during the growth period. The second observation of symptoms appeared after nine weeks in tomato cultivars 116 and Munna then cultivar 3019 in which symptoms was observed after ten weeks . Results showed that cultivars 56 and 1684 had the highest disease severity.

Table 3.9. Symptom assessment in different tomato cultivars infected by TYLCV for twelve weeks for spring experiment (2007) .

(Mean*±SE)

Cultivar	Days to observe early symptoms	Disease severity[a]				Total	(Mean*±SE)
		Block 1	Block 2	Block 3	Block 4		
3060	0	0	0	0	0	0	0 c ± 0
Munna	63	0	0.055	0	0.111	0.166	0.04bc ± 0.026
Teiba	0	0	0	0	0	0	0 c ± 0
916	0	0	0	0	0	0	0 c ± 0
1684	28	0.11	0.166	0.222	0.055	0.553	0.138 ab± 0.04
3019	70	0.055	0	0	0	0.055	0.013 c ± 0.01
116	63	0.055	0	0.055	0	0.11	0.027 c ± 0.01
56	28	0.11	0.166	0.222	0.277	0.775	0.194 a ± 0.035

a: Severity: 0 : no symptoms, 1 : very slight yellowing of leaflet margins on apical leaf , 2 : some yellowing and minor curling of leaflet ends , 3: severe symptoms, yellowing, curling, severe stunting.

* Mean score of symptoms for each genotype. Values within the colum followed by the same letter are not significantly different according to Fisher LSD at $p \leq 0.05$

The weekly observation for disease severity had been calculated for twelve weeks and presented in table (3.10) Results showed that disease severity increase rapidly for tomato cultivars 56 and 1684 in which symptoms was observed early and slowly in the other cultivars in which symptoms was observed lately .

Table 3.10. The weekly observations for symptom severity for tomato cultivars infected by TYLCV for spring experiment 2007.

Weeks	116	916	1684	3060	3019	Teiba	Munna	56
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0.027 ± 0.027	0	0	0	0	0.013 ±0.13
5	0	0	0.027 ±0.027	0	0	0	0	0.027 ±0.027
6	0	0	0.027 ±0.027	0	0	0	0	0.041 ±0.41
7	0	0	0.027 ±0.027	0	0	0	0	0.041 ±041
8	0	0	0.041 ±0.041	0	0	0	0	0.041 ±0.041
9	0	0	0.041 ±0.041	0	0	0	0	0.095 ±0.420
10	0.013 ±0.008	0	0.097 ±0.0.3	0	0.013 ±0.012	0	0.027 ±0.01	0.125 ±0.018
11	0.018 ±0.009	0	0.11 ±0.034	0	0.013 ±0.008	0	0.027 ±0.28	0.138 ±0.015
12*	0.027c ±0.009	0c	0.138ab ± 0.032	0c	0.013c ±0.008	0c	0.04bc ±0.015	0.194**a** ±0.024

* Mean Values within the raw followed by the same letter are not significantly different according to Fisher LSD at $p \leq 0.05$

The disease severity measurement chart for these tomato cultivars had been done and presented in Fig 3.12 .

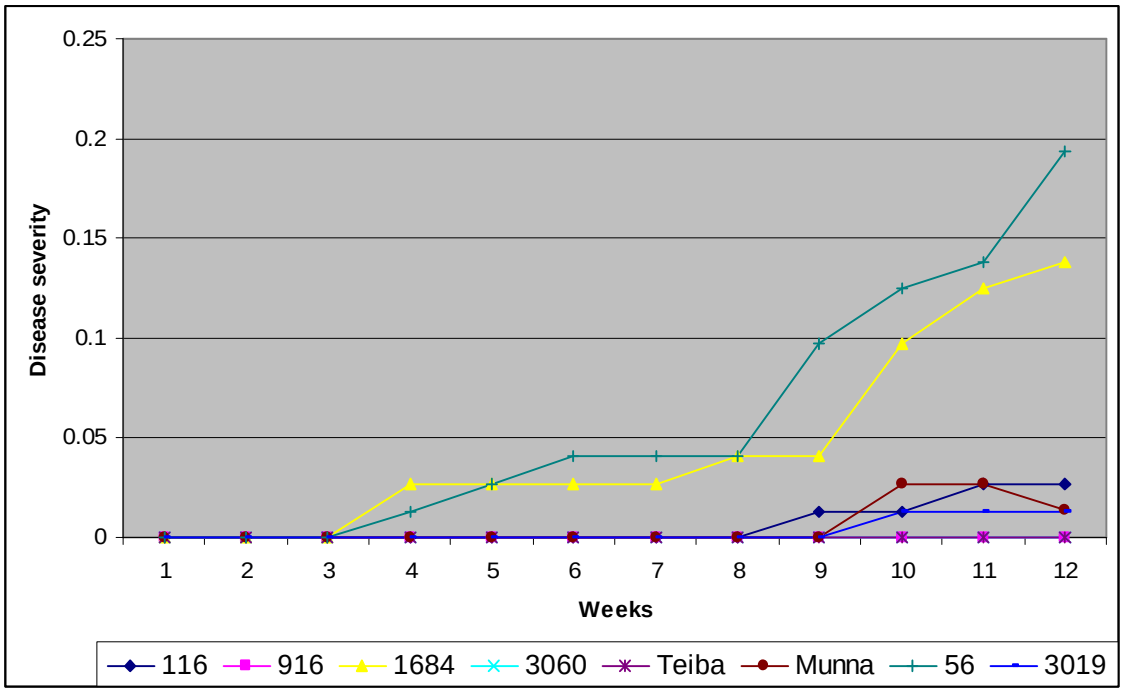

Fig 3.12. The symptom severity measurement chart for tomato cultivars infected by TYLCV for spring experiment 2007.

Comparison between tomato cultivars infected by TYLCV during summer and spring seasons according to disease incidence had been done and shown in Table 3.11.

Table 3.11. Comparison between tomato cultivars infected by TYLCV for summer and spring experiments according to disease incidence.

Cultivar type	116	916	1684	3060	3019	Teiba	Munna	56
Summer experiment (2006)	58	25	70.8	6.9	93	20.8	13.8	not done
Spring experiment (2007)	2.7	0	8.3	0	1.0	0	2.7	13.8

Resulrs showed that there were fundamental differences between tomato cultivars infected by TYLCV during summer and spring season. These differences are observed in all the parameters used (days to observe early symptoms, symptom severity, disease incidence). In summer symptoms was observed after two weeks of planting while in winter symptoms was observed after four weeks and in some cultivars symptoms did not observed at all such as cultivar 3060, 916 and Teiba table (3.3) and table (3.11).

In spring the disease incidence for tomato cultivars is very low when compared with summer results Fig 3.13. In summer the disease incidence for cultivar 3019 is 93% while in spring the disease incidence 1% only.

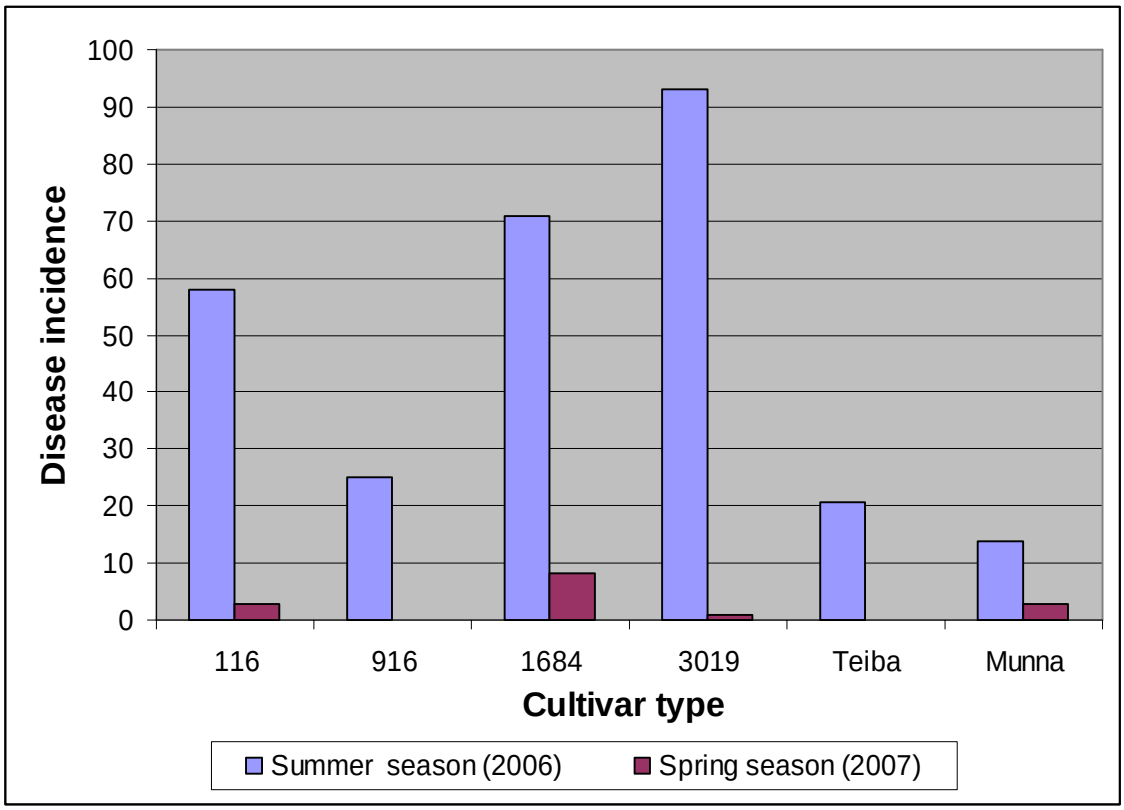

Fig 3.13. Comparison between summer and spring results according to disease incidence for different tomato cultivars.

Also severe symptoms were not observed during spring except for cultivar 56 in which two plants had only severe symptoms while in summer severe symptoms was observed Table 3.3 and Table 3.9 .

These results show that spring season is not suitable for studying the resistance level for tomato cultivars because The experimental conditions were not favorable to the virus , low temperature, Low population of *Bemesia tabaci* and the evaluation was through spring season when the plants did not exhibit severe symptoms. For these reasons tomato are planted in spring season in open fields in Palestine and other countries.

3.5. Molecular studies for TYLCV incidence for spring experiment 2007

The presence for TYLCV in the tomato cultivars was detected using the PCR method . The percentage of samples in which TYLCV was detected for each cultivar had been calculated based on the total number of samples collected . These results are summarized in Table (3.12).

Table 3.12 . PCR analysis for TYLCV on tomato cultivars collected in spring (2007).(Mean*±SE)

Cultivar	Positive (%) for TYLCV				Total	Mean*±SE
	Block 1	Block 2	Block 3	Block 4		
116	6	5	8	6	25	6 b ± 0.63
916	2	4	3	3	12	3 c ± 0.41
1684	4	3	5	4	16	4 c ± 0.41
3060	0	0	0	0	0	0
3019	0	0	0	0	0	0
Teiba	0	0	0	0	0	0
Munna	4	3	5	4	16	4 c ± 0.408
56	7	8	8	9	32	8 a ± 0.41

* Mean Percentage of samples in which TYLCV was detected by using PCR technique (based on the total number of samples collected). Values within the colum followed by the same letter are not significantly different according to Fisher LSD at $p \leq 0.05$

Results show that cultivar 56 and 116 had the highest PCR positive results which was 8% and 6%, respectively while cultivars 3060, 3019 and Teiba had no positive results . Cultivars 1684 and Munna had 4%.

A comparison between PCR analysis (Positive (%) for TYLCV) and biological assay after twelve weeks had been done. Results presented in Table 3.13 and Fig 3.14. Results showed that molecular assay by using PCR determined by biological assay (disease incidence) indicated similar trend in sense that cultivars which had high disease incidence also had high PCR positive results for TYLCV and cultivars which had low disease incidence also had low PCR positive results for TYLCV results. Cultivars in which symptoms did not observed such as cultivar 3060 and Teiba also had no PCR positive results However, to some extent the correlation was less applicable for cultivar 116 and 916 in which the disease incidence is lower than PCR results . this is because molecular assay is more sensitive than biological assay

Table 3.13. Comparison between PCR analysis (Positive (%) for TYLCV) and biological assay for spring experiment 2007.

Cultivar type	**Disease incidence**	**Symptom severity**	**Days to observe early symptoms**	**Positive (%) for TYLCV**
116	3±	0.027 c ± 0.01	65	6 b ± 0.63
916	0	0 c	0	3 c ± 0.41
1684	8±	0.0138 b ± 0.03	25	4 c ± 0.41
3060	0	0 c	0	0
3019	1±	0.013 c ± 0.01	73	0
Teiba	0	0 c	0	0
Munna	3±	0.027 bc ± 0.025	73	4 c± 0.408
56	14±	0. 194 a ± 0.035	25	8 a 0± 0.41

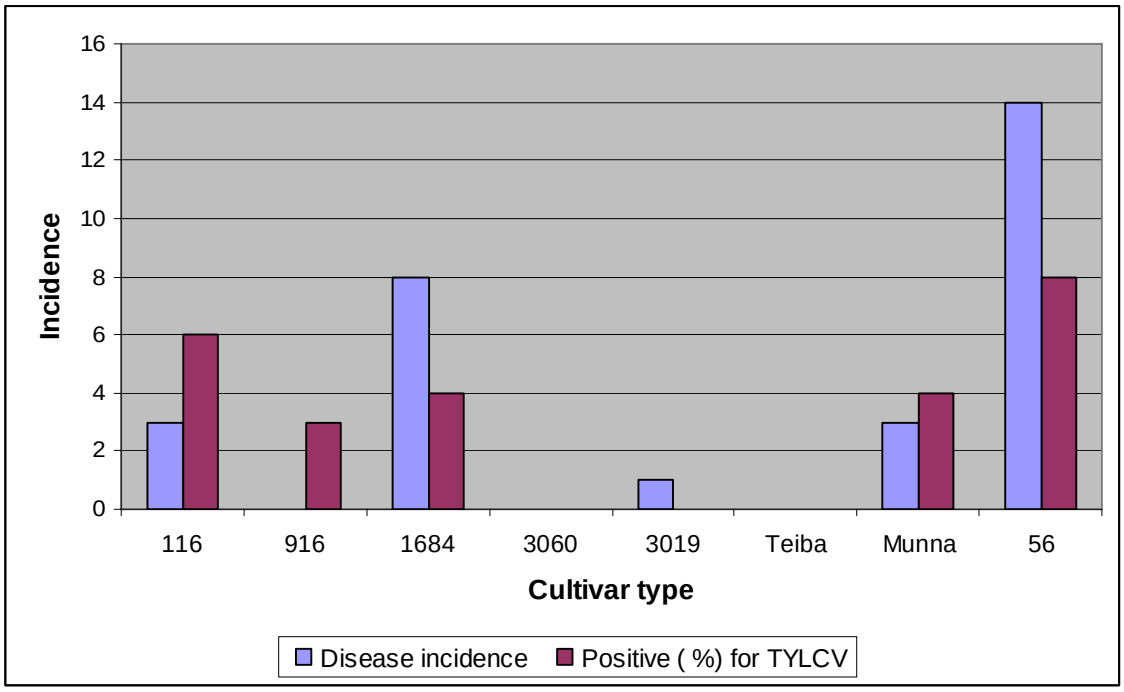

Fig 3.14. Comparison between disease incidence and PCR results for tomato cultivars infected with TYLCV for spring experiment 2007 in Al-Arroub Agricultural Experimental Station .

Fundamental differences had been obtained when we compared PCR results for cultivars that had been planted in spring and those that had been planted in summer Table (3.14) and Fig (3.15). Results show that PCR incidence in spring season is much lower than PCR incidence for summer season. TYLCV appears to be more spread in Summer due to the high population of whitefly *Bemesia tabaci*, the main transmissible agent of the virus.

Table 3.14. Comparison between PCR results for spring experiment and summer experiment.

Experiment	**116**	**916**	**1684**	**3060**	**3019**	**Teiba**	**Munna**	**56**
Summer experiment	51%	51%	42%	1%	50%	28%	7%	not done
Spring experiment	6%	3%	4%	0	0	0	4%	8%

3.6. Survey of *Tomato Yellow Leaf Curl virus* in the Southern part of the West Bank, Palestine .

Survey had been conducted for studying TYLCV incidence in the Southern region of the West Bank of Palestine in open fields and plastic houses for tomato plants which was planted in spring season . The survey was started from 1/7/2008 to 1/8/2008 in different regions in Hebron and Bethlehem based on symptom observation (leaf curl, yellowing, abnormal reduction in plant size, stunting and poor fruit set)

Results presented in Tables (3.15) and (3.16). Results showed that disease incidence was recorded to be 12 % in open fields and 0.28 % in plastic houses. Results showed that in some fields such as Marah Rabah in Bethlehem the incidence was 100% and in Tkooh the incidence was 59 %. The incidence in plastic houses was lower than open fields because the uses of double door system for the plastic houses reduce the damage caused by TYLCV. In Hebron the incidence was 3.5% while in Bethlehem the incidence was 15%. In autumn the population of whitefly is very high so the farmers did not plant tomato in this season so survey had not been conducted in this season.

Table 3.15. Survey for TYLCV incidence in different regions of Bethlehem

Area name	Type	Cultivar type	Number of plants	Number of infected plants	Disease incidence	Date of planting
Marah Rabah	Open field	144	150	150	100	1/6/2008
Almenyah	Open field	773	3500	5	14	01/07/2008
Wady Fokin	Open field	Manar	1440	0	0	28/04/2008
Beteer	Plastic house	Unknown	3000	30	1	Unknown
Al walajah	Open field	Sarah	500	71	14	18/05/2008
Al walajah	Open field	Sarah	200	0	0	28/05/2008
Al walajah	Open field	56	1000	0	0	04/07/2008
Beit Fajar	Open field	259	600	2	0.3	20/05/2008
Marah Rabah	Plastic house	Manar	1000	0	0	25/03/2008
TKOOH	Open field	56	1000	48	5	28/05/2008
TKOOH	Open field	593	1500	885	59	20/06/2008
TKOOH	Plastic house	Unknown	700	7	1	10/03/2008

Table 3.16. Survey for TYLCV incidence in different regions of Hebron

Area name	Type	Cultivar type	Number of plants	Number of infected plants	% of infection	Date of planting
Dweer Ban	Open field	Unknown	2800	37	1.3	Unknown
Tafoh	Plastic house	Unknown	5000	3	0.06	Unknown
Tafoh	Open field	16/84	50	4	8	18/05/2008
Beit Kahel	Open field	Unknown	1500	2	0.13	18/05/2008
Tarkomiah	Plastic house	30/10	1700	0	0	13/03/2008
Tarkomiah	Open field	56	900	117	13	15/04/2008
Beit Ola	Open field	144	3000	435	14.5	25/05/2008
Shiokh Al arroub	Open field	Sarah	3500	175	5	15/05/2008
Albweereh	Open field	30/19	2000	20	1	01/06/2008
Albweereh	Open field	773	2000	22	1	01/06/2008
Saear	Open field	Sarah	2000	100	5	20/05/2008
Halhool	Open field	56	1000	30	3	14/05/2008
Beit Ommar	Open field	773	600	0	0	14/05/2008
Beit Ommar	Open field	unknown	1500	9	0.6	01/07/2008
Dora (Wadi Abu	Open field	30/19	2000	20	2	28/05/2008

Alkamra)						
Dora (Wadi Abu Al kamra)	Open field	Unknown	1500	45	3	02/06/2008
Dora (Wadi Abu Al kamra	Plastic house	Manar	1000	2	0.2	20/03/2008
Dora (Wadi Abu Al kamra	Plastic house	Manar	1500	0	0	20/03/2008
Dora (Wadi Abu Al kamra	Plastic house	Manar	1000	0	0	17/03/2008
Dora (Singer)	Open field	Unknown	500	40	8	Unknown
Yatta (Rakaa)	Open field	16/84	1500	105	7	01/05/2008
Yatta (Rakaa)	Open field	Sarah	500	27	5.3	20/05/2008
Yatta (Rakaa)	Plastic house	Manar	1000	1	0.1	25/04/2008
Yatta (Rakaa)	Plastic house	Manar	1200	0	0	25/04/2008

DISCUSSION

TYLCV is the most damaging pathogen of tomato in Palestine and in many other regions of the world. Proper detection of the pathogen and the development or selection of the resistance cultivars are among the key elements for development of integrated management strategies.

One of the difficulties in selecting a source for resistance to TYLCV stands for the fact that the disease might be expressed with varying disease incidence as well as of severity and of the method of TYLCV detection, which does not always provide accurate quantification of viral DNA (Pico *et al*., 1998 ; Pico *et al*., 1999a). Discrepancies observed between studies also may be due the varying responses against the different TYLCV isolates used in the experiments and differences in method of inoculation (Pico *et al*., 2001). Thus, in our experiment screening for resistant tomato cultivars occurred by using biological methods (days to observe early symptoms, symptom severity, disease incidence) and molecular methods by using PCR.

Symptoms were evaluated by using a scale established for rating TYLCV symptoms. The scale ranged from 0 (no symptoms) to 3 (severe symptoms) this scale was discussed in section (2.1.2.1).

In this study, we tried to identify the resistance level for different tomato cultivars infected by TYLCV in summer and spring seasons under natural conditions by using biological and molecular methods. The experimental conditions in summer were favorable to the virus due to moderate temperature and high population of *Bemesia tabaci.*

Screening tests for summer experiment showed that there was no cultivar resistant to TYLCV infection. Although none of these cultivars are immune

to the virus, results showed fundamental differences between the severity of infection among these cultivars.

Biological assays showed that some of these cultivars had high disease incidence with sever symptoms noticed on cultivars 3019 (93%), 1684 (71%) and 116 (58%), while cultivar 3060 was the most promising one showing low disease incidence (7%) with mild symptom that scored for eight weeks after planting. An increase of virus infection based on symptoms severity were noticed every week for highly susceptible cultivars (3019,1684) and slowly for the resistant cultivars (3060).

Statistical Analysis for TYLCV incidence after 55 days for tomato cultivars showed that cultivars 3060, Munna, Teiba, and 916 are not significantly differ from each other but significantly different from other cultivars . Cultivars 116 and 1684 are not significantly differ from each other but significantly different from other cultivars, while cultivar 3019 was significantly different from the other cultivars Table (3.1).

Then PCR method using a modified procedure of Dellaporta heat extraction was used to verify this resistance between the tomato cultivars. The amplification of DNA occurred by using PCR primers. Before that *Taq* DNA polymerase concentration was optimized to find that 0.4µl (2 units) from *Taq* DNA polymerase were able to detect clearly all positive samples (Fig 3.1).

When the percentage of samples in which TYLCV was detected by using PCR technique(based on the total number of samples collected) had been done and compared between tomato cultivars. Results showed that cultivar 3060 had the lowest PCR results 1% then Munna 7% and Teiba 28% (Table 3.5) while for cultivars 3019, 916, 116 and 1684 were ranging from 40-50%.

A comparison between molecular assay and biological assay had been held Table 3.6 and Fig 3.10) to show that molecular assay was in agreement with biological one. However, to some extent the correlation was less applicable for cultivar 916 in which the disease incidence 25% was lower than PCR incidence 51%. This indicated that PCR method is more sensitive in detection than visual scoring (field incidence , symptom severity) suggesting that the infection was occurred in later stages so the symptoms were not observed. Our results was in accordance with Briddon and Markham, 1994 who suggested that Polymerase chain reaction PCR can be widely acceptable method for the detection of geminiviruses. These results indicated that PCR incidence can serve as indicator for resistance, but it is better if not used as the sole indicator.

In some cultivars, PCR results (percentage of samples in which TYLCV was detected by using PCR technique) was lower than disease incidence evaluated biologically, suggested that this discrepancies in results may be due to existence of other TYLCV isolates. Since all virus isolates are associated with yellow leaf curl symptoms, it is very difficult to correlate a given symptoms with a particular virus strain. In recent years, sequence analysis had shown that considerable sequence diversity exist among members of TYLCV complex. The source for this diversity is recombination. Recombination 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 (Maxwell, 2002; Padidam *et al*, 2002).

Detection and differentiation of the species and recombinant strains of TYLCV complex (Acotto *et al*, 2000) were always based on PCR coupled either with RFLP or sequencing methods. When TYLCV is the only strain infecting tomato and when working under controlled conditions PCR

technique is a very fast and sensitive and simple. But in our experiment primers of other strains like TYLCV-Mld and TYLSCV-ES[2] were not applied due to budget deficit. These strains had been identified in Jordan (Anfoka *et al,*. 2005). Thus we suggest further molecular investigation using the other primers for distinguishing among the other isolates that may be detected in Palestinian's tomatoes.

From the results obtained in summer experiment which is conducted under natural conditions, we conclude that there are differences in the level of resistance among the commercial tomato cultivars. The source of resistance in this cultivars may be due to that the whiteflies have a preference for some tomato cultivars than others because the fitness of the whiteflies affected by the physiological conditions. This was also indicated by Channarayappa and Shivashankar, 1992 as well as Morales and Anderson, 2001. They suggested that resistance to the whitefly vector had been associated with the large amounts of sticky substances that plant of some wild species exudates, entrapping the whiteflies and significantly reducing the transmission of begomoviruses.

Biological incidence and molecular incidence obtained from summer experiment showed that cultivar 3060 was the most promising cultivar. Another experiment had been done for tomato cultivars planted in spring season. Different results have been obtained in all parameters used to characterize the resistance level in summer experiment (disease incidence, severity, days to observe early symptoms and PCR analysis) Tables (3.7), (3.8), (3.9) and (3.12) . In some cultivars symptoms did not observed at all such as 3060, 916 and Teiba. The disease incidence and symptom severity in the infected plants is very low when compared with summer results. In summer the disease incidence for cultivar 3019 was 93% while in spring this cultivar had 1% only . In spring the higher disease incidence

observed in cultivar 56 and reached 13.8% which is extremely low table (3.11). Also severe symptoms were not observed except for cultivar 56 in which two plants had only severe symptoms table (3.9).

PCR analysis had been evaluated for spring experiment and compared with biological assay (Table 3.15 and Fig 3.14). These results were in agreement with biological assay in that the higher the disease incidence the higher the PCR positive results . The correlation was less applicable for cultivar 916, which had higher PCR incidence (5.8%) than the disease incidence (3%) because molecular assay was more sensitive in detection the virus than biological assay. Differences had been observed in PCR incidence for spring experiment with PCR positive results for summer one, as the last one was higher (Table 3.13. Fig 3.14). This suggested that the experiment conditions in spring were not in favor of the virus because the population of its vector (*Bemesia tabaci*) was low compared with the summer one where the population density for *Bemesia tabaci* was high (Mansour and Al-Musa, 1992). It would be quite possible that reduction in TYLCV incidence during spring was due to unfavorable conditions for whiteflies as well as for TYLCV development during these months.

Survey of TYLCV incidence in the southern region of the West Bank had been conducted during July 2008 in open fields and plastic houses .Survey was based on symptom observation (Leaf curl, yellowing, abnormal reduction in size, stunting and poor fruit set). TYLCV incidence was recorded to be 12 % in open fields and 0.28% in plastic houses Table (3.15) and Table(3.16.). Results showed that in some fields such as Marah Rabah area in Bethlehem district was reached 100% and in Tkooh area 59%. In plastic houses it was lower than open fields due to the uses of double door system for the plastic houses that reduced transmissibility of the virus by its vector. As a total, the disease incidence reached in Hebron up to 3.5%

while in Bethlehem it was 15%. In autumn, since the population of whitefly is very high, farmers did not plant tomato in that season, so no survey could be conducted.

In this studies 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 breeding programs that aimed to produce TYLCV resistant cultivars. Selecting plants solely on the basis of presence and absence of symptoms (biological assay) in infected fields leads for a considerable escape because there was a concern that 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*,. 2001) thus the use of molecular tools beside biological assay in different tomato cultivars became important .

It seems that the cultivars which showed delay in viral expressing symptoms are those with low viral infection, thus could be our genetically choice for tolerant cultivars.

The current approaches to detect viral infections based on indicator plants or serological assays. The vast difficulties to obtain sufficient quantities of viral antigens for production of antisera, together with the imprecise evaluation of symptoms, makes molecular procedures an important tool for more accurate detection. Other techniques such as DNA hybridization had also been used for similar purpose; however, they typically yield in consistent results (Polston *et al*., 1990).

Finally, the results of this study should be taken into consideration in any developing of breeding programs for TYLCV resistance tomato cultivars within Palestinian territories, since TYLCV incidence and PCR analysis

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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