Occurrence and prevalence of four viruses infecting tomatoes in Northern districts of West Bank, Palestinian Territories

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Abstract – Studying tomato viruses during 2003/2004 using immunosorbent assays revealed that tomato yellow leaf curl virus (TYLCV) was the most dominant disease infecting tomato plants in the northern districts of the West Bank particularly Jenin and Tobas as its incidence ranged from 28-93%. The maximum disease incidence of 90-93% was recorded in Al-Far'a region of Tobas district. Cucumber mosaic virus (CMV) was the second most prevalent virus of tomato in the studied regions as its incidence ranged from 15-51%. Tomato infection with tomato mosaic virus (TMV) and potato virus Y (PVY) was considered insignificant as these viruses were detected in very few tomato samples.

Keywords – Tomato; Viruses; Palestine.

Introduction

Like many other developing countries, Palestine has an important agricultural sector, which has been considered the backbone of its economy. This was particularly true in the years after instituting its autonomy with the establishment of the National Palestinian Authority in 1994. Since then the agriculture became the largest contributor to the country's gross product and provided employment to the economically active population (Palestinian Central Bureau of Statistics (PCBS) 2008).

Vegetables are the most important crops grown in the country, occupying 18734.4 hectares, with an annual production of 645157 metric tons. More than 31% of those vegetables are tomatoes, and the remainder include thirty-five other vegetable crops, of which cucumbers are the most important. Tomato is the main greenhouse crop but it is also cultivated in low tunnels and in open fields, comprising a total annual production of 203999 metric tons (PCBS 2008). Several viruses are known worldwide to attack tomatoes causing serious damage and yield reduction. TMV, TYLCV, CMV and PVY are of the ten most viruses affecting the crop elsewhere (Oetting and Yunis, 2004, Agrios, 1997, Pico *et. al.* 1996, Sawalha. (2009a, 2009b, 2010).

Viral diseases of tomatoes occasionally cause serious damages and large economic losses. The amount of loss depends on the viral disease involved, the strain of the virus, the variety of tomato, the age of the plant at infection time, the presence of other diseases, and the extent that viruses have spread during the planting of the crop (Walkey 1985).

Tomato production in Palestine, however, has not yet reached its full potential due to many factors related to climate and poor management, and above all, due to the high level of pests and diseases (PCBS 2008). In regard to diseases, viral diseases may be considered an important factor responsible for the recurrent crop failure in the country forcing many farmers to switch their choice of crop production from tomato to other vegetables (Sawalha 2010, Ministry of Agriculture, personal communication).

As tomato is the most important vegetable crop in the country, this research aims to study and identify the viruses affecting this crop and to shed light on their occurrence and prevalence. Northern districts including Jenin and Tobas were selected for this study because tomato production in those regions is 48250 metric tons and they occupy about 47% of the total tomato production in the West Bank part of Palestine (PCBS 2008).

Materials and Methods

Regions of study

The research was carried out during the summer growing seasons of 2003 and 2004. Tomato fields were selected randomly in the regions with intensive tomato cultivation to represent all possible conditions of tomato production in these districts. The major tomato producing

fields located in Jenin were selected in Al-Zababdeih, Al-Jededeih, and Qabatyya regions. Two fields were selected in Tobas; one of them was in Kashda village and the other was selected in Al-Far'a region a few hundred meters away from the town's midpoint (Fig 1).

Growing season

The experiments were carried out during the summer growing season because this season is considered the main planting one of tomato in the regions of study throughout the year. In this season, seeds are sown during April and May and the seedlings are transplanted from the middle of May until the middle of June.

The pattern of tomato cultivation in the regions of study

Tomato is planted in the studied area in rows with a width of about 1 to 1.2m. The lands are first prepared by hoeing twice and then adding manure. Chemical fertilizers are added in three equal doses; the first when preparing the land, the second after transplanting and the third at fruit set.

Field visits and sample collection

Field visits in the studied regions began in July and continued until the end September. Samples were collected at least one month after the transplanting of tomato seedlings to ensure that tomato had become infected after being exposed for a sufficient period to the viral source. The selected fields were visited weekly in a regular manner and leaf samples were collected randomly from top parts of plants. The samples were labelled and kept frozen for laboratory testing.

Virus identification and incidence

Virus identification was done serologically using virus-specific antibodies purchased from international companies. Thus, indirect enzyme-linked immunosorbent assay (I-ELISA) adopted by Clark et. al (1986) was used to test the plant samples for CMV, PVY and TMV. With respect to the other case, the triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) was used to test the TYLCV infection. TAS-ELISA was used according to Macintosh et. al. (1992), Muniyappa et. al. (1991), Sawalha et. al. (2000: 339) and Sawalha (2000: 24-89). The antibodies of CMV, PVY and TMV and the goat anti-rabbit conjugate were purchased from Bioreba, Inc. The polyclonal and monoclonal antibodies of TYLCV, along with the rabbit anti-mouse conjugate, were purchased from Adgen Ltd (Scotland, UK.). The

results of the ELISA tests were recorded one hour after the substrate incubation took place using the automated ELISA-Reader. The light absorbance was measured for ELISA wells at 405 nanometer (Sawalha (2009c)

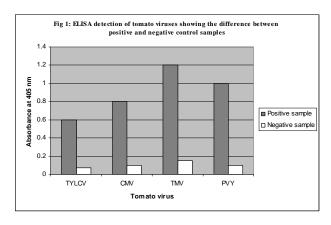
Statistical analysis

Statistical analysis of the data was done using the Two-Sample Tests of Proportions (TSTP) to compare virus occurrence in the studied regions. The results were analysed using a level of significance when $\dot{\alpha} = 0.05$ (Lind *et. al.* 2005).

Results

Virus identification and incidence

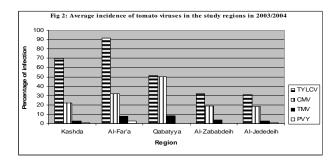
ELISA tests showed that the TYLCV, CMV, TMV and PVY parasitize tomato fields in different percentages. ELISA readings recorded for the virus-infected samples were at least eight times greater than the readings recorded for the virus-free samples (Fig 1).



The four viruses are found in tomato planted in Kashda, Al-Far'a and Al-Jededeih regions. TYLCV is the most dominant virus affecting tomato fields in all regions as its occurrence rate ranges from 28-93% in the tested tomato fields. In addition, the maximum TYLCV infection (93%) is recorded for tomato fields planted in Al-Far'a region. CMV is the second-most dominant tomato virus in all tested fields as it infects tomato fields with an occurrence rate ranging from 15-51%. The maximum occurrence of CMV is found in Qabatyya followed by Al-Far'a and Kashda regions. The minimum percentage of CMV infection is recorded for Al-Zababdeih and Al-Jededeih regions. Both TMV and PVY are present in minute samples collected from the studied regions, while most of the other samples were free from these viruses (Table 1) (Fig 2).

Region	TYLCV				CMV				TMV			PVY				Total samples		
	2003		2004		2003		2004	2004		2003		2004		2003 2004			collected 2003 2004	
	NS	PI	NS	PI	NS	PI	NS	PI	NS	PI	NS	PI	NS	PI	NS	PI		
Kashda	33	70	35	69	11	23	11	21	3	6	0	0	1	2	0	0	47	51
Al-Far'a	43	93	45	90	14	30	17	34	5	10	3	6	2	4	1	2	46	50
Qabatyya	23	51	23	52	23	51	22	50	4	8	4	9	0	0	0	0	45	44
Al-Zababdeih	16	31	16	33	10	20	9	18	2	4	2	4	0	0	0	0	51	49
Al-Jededeih	17	34	15	28	11	22	8	15	1	2	2	3	1	2	0	0	50	54
TOTAL	132	55	134	54	69	29	67	27	15	6	11	4	4	2	1	0.004	239	248

NS: Number of infected samples, PI: Percentage of infection



Statistical analysis revealed that the proportions of TYLCV in the studied regions were greater than CMV, TMV and PVY. The maximum computed Z values ($\alpha =$ 0.05) for the TYLCV compared with the other viruses were recorded in Al-Far'a region followed by Kashda, Qabatyya, Al-Zababdeih and Al-Jededeih. No significant difference was recorded between TYLCV and CMV infecting tomato fields in Qabatyya region as the computed Z value was much less than the critical one (Table 2)

Table 2: Statistical analyses and the Z value of the TSTP. The Z table = 1.65

Region	Virus combination	Computed Z value	Decision
Kashda	TYLCV/CMV	6.74	S
	TYLCV/TMV	9.80	S
	TYLCV/PVY	10.16	S
Al-Far'a	TYLCV/CMV	8.66	S
	TYLCV/TMV	11.81	S
	TYLCV/PVY	12.54	S
Qabatyya	TYLCV/CMV	0.14	NS
	TYLCV/TMV	6.63	S
	TYLCV/PVY	8.30	S
Al-Zababdeih	TYLCV/CMV	2.10	S
	TYLCV/TMV	5.15	S
	TYLCV/PVY	6.17	S
Al-Jededeih	TYLCV/CMV	2.20	S
	TYLCV/TMV	5.55	S
	TYLCV/PVY	5.83	S

S: Significant, NS: Non significant

Discussion

Tomato viruses were identified serologically using I-ELISA for CMV, and TMV, PVY, and TAS-ELISA for TYLCV. TYLCV is the most prevalent virus threatening tomato fields in the studied regions. The higher incidence of TYLCV (31-93%) is attributed to the abundance of whiteflies (Bemisia tabaci) and the overlapping that occurs between tomato growing seasons in the regions (Sawalha 2010). The 93% incidence of the virus in Al-Far'a may be attributed also to the warm climate which helps the whiteflies to appear earlier and to reproduce more compared with the other regions (PCBS 2005, Sawalha 2010). In this regards, Nava-Camberos et. al. (2001) reported that the warm climate is the best condition for the whitefly development, fecundity and survival. Similar results were recorded in Jordan where Al-Musa and Mansour (1983) reported that TYLCV was the predominant virus affecting tomatoes in the Jordan Valley. CMV was detected to be the second-most prevalent virus affecting tomato fields in the studied regions. This infection with CMV may be attributed to allate aphid species which are usually ample in the region, especially the green peach aphid (Myzus persicae Sulzer) and the melon aphid (*Aphis gossypii* Glover). The virus has a wide host range (800 species) from which it can be acquired by aphids and transmitted in a nonpersistent manner and can also be mechanically transmitted (Oetting and Yunis, 2004, Trigiano et. al. 2004). Another reason for the wide spread occurrence of CMV in the studied regions may be attributed to the intensive and annual culturing of cucurbits including cucumber, squash and melon in the region. According to PCBS (2008), these crops are planted in a wide area with an annual production of 15376 and 9250 metric tons in Jenin and Tobas districts, respectively. These crops are the most suitable hosts for CMV, so their presence in the tomato growing sites makes them the viral source from which the allate aphids transmit the disease to tomato (Oetting and Yunis 2004, Sacristian et. al. 2004). The high rate of tomato infection with CMV in Qabatyya region may be attributed to the extensive culturing of tobacco in the village Plains, which are situated very close to tomato growing sites. The plains are the major culturing sites of tobacco in Jenin district. The rare infection of tomato plants with TMV and PVY may be attributed to the scarcity of the viral source in the region at that time.

As the results above indicate, it can be concluded that TYLCV was the key virus affecting tomato fields in the region. Therefore, efforts must be directed towards the control of the disease. Furthermore, because CMV infects tomato fields in much lower percentages, it is of the utmost importance that efforts towards the prevention of further outbreaks must take place. TMV and PVY, on the other hand, play a very insignificant role in tomato production as their occurrence was very low in tomato fields.

References

- [1]. A. Al-Musa, A. Mansour, Plant Viruses Affecting Tomato in Jordan. Identification and Prevalence, Phytopath. Z, 106, (1983) 186-190.
- [2]. B. Pico, M. Diez, F. Muez, Viral disease causing the largest economic losses to tomato crop. II. The tomato yellow leaf curl virus-a review, Scientia Horticulturae, 67 (1996) 151-196.
- [3]. D. Lind, W. Marchal, S. Wathen, Statistical Techniques in Business & Economics, Twelfth Edition, McGraw-Hill Irwin, New York, 2005, pp. 262-263
- [4]. D. Walkey, Applied Plant Virology, Heinemann; London, 1985, pp. 6-92.
- [5]. G. Agrios, Plant Pathology, Fourth Edition, Academic Press; London, 1997, pp. 479-554.
- [6]. H. Sawalha, A. Mansour, M. El-Khateeb, Serological and PCR detection of tomato yellow leaf curl virus from infected plant tissues and whiteflies, Seventh Arab Congress of Plant Protection, Amman-Jordan, 22-26 October, (2000).
- [7]. H. Sawalha, Whitefly population and incidence of tomato yellow leaf curl virus in tomato fields grown in the northern regions of the West Bank, Al-Aqsa University Journal (Natural Sciences Series), 13 (2010) 7-24.
- [8]. H. Sawalha, Occurrence of tomato yellow leaf curl virus on volunteer tomato, jimsonweed and tobacco in North West Bank: Distribution of the virus natural reservoirs in summer season, An Najah University for Research, 23 (2009b) 73-91.
- [9]. H. Sawalha, Palestinian isolate of tomato yellow leaf curl virus: capsid and nucleic acid retention in *Bemisia*

- *tabaci*, transmission, and field study of virus association with the vector and non-vector nsects, An Najah University for Research, 23 (2009a) 93-115.
- [10]. H. Sawalha, Purification, Antiserum Production, Biological and Molecular Studies of Tomato Yellow Leaf Curl Virus, PhD. dissertation, University of Jordan, Amman, Jordan, 2000, pp. 24-89
- [11]. H. Sawalha, The use of PCR, IC-PCR, TAS-ELISA, TBIA, and biological methods to determine the time needed to detect TYLCV in inoculated jimsonweeds, The First Conference on Biotechnology Research and Application in Palestine, Furno Hall, Bethlehem University, Bethlehem, Palestine, 3-4 April, 2009c, pp. 28
- [12]. M. Clark, R. Lister, M. Bar-Joseph, ELISA Techniques, Methods in Enzymology, 115 (1986) 771-773
- [13]. Palestinian Central Bureau of Statistics, Agricultural Statistics, Ramallah; Palestine, 2008, pp. 77-110.
- [14]. Palestinian Central Bureau of Statistics, Meteorological Conditions in the Palestinian Territories Annual Report, Ramallah; Palestine, 2005, pp. 39-49.
- [15]. R. Oetting, H. Yunis, Field Guide to Common Insects, Mites, and Diseases of Greenhouse Grown Sweet Peppers, Cucumbers and Tomatoes, Hakohav Press; Kfar Qari, 2004, pp. 58-79.
- [16]. R. Trigiano, M. Windham, A. Windham Plant Pathology, Concepts and Laboratory Exercises, CRC Press; London, 2004, pp. 28-29.
- [17]. S. Macintosh, D. Robinson, B. Harrison, Detection of three whitefly-transmitted gemini viruses occurring in Europe by testing with heterologous monoclonal antibodies, Annals of Applied Biology, (1992) 279-303.
- [18]. U. Nava-Camberos, D. Riley, M. Harris, Camberos, U., Riley, D., and Harris, M. (2001). "Temperature and host plant effects on development, survival, and fecundity of *Bemisia tabaci* (Homoptera: Aleyrodidae), Environmental Entomology, 30 (2001) 55-63.
- [19]. V. Muniyappa, M. Swanson, G. Duncan, B. Harrison, Particle purification, properties and epitope variability of Indian tomato yellow leaf curl gemini virus, Annual of Applied Biology, 118 (1991) 595-604.

Vitae

Dr. Sawalha was born in Palestine. He obtained a Ph.D. degree in Horticulture and Plant Protection from the University of Jordan in 2000.

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