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The Impact of Interference Between Tomato Yellow Leaf Curl and Tomato Mosaic Viruses on Tomato Plants

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Abstract: The interference between *tomato yellow leaf curl* geminivirus (TYLCV) and *tomato mosaic* tobamovirus (ToMV), has great effect on concentration and infectivity of both viruses in infected plants as well as on tomato yield. Using polymerase chain reaction (PCR), nucleic acid hybridization (NASH), enzyme-linked immunosorbant assay (ELISA) and biological assay, it was found that TYLCV or ToMV had high concentration in inoculated tomato plants. While, it had low concentration in TYLCV inoculated tomato plants when inoculated at the first, followed by ToMV inoculation, 15 days later or when the two viruses were inoculated at once. Also, low virus concentration was obtained when tomato plants inoculated with ToMV at first followed by TYLCV inoculation 15 days later. Concerning the external symptoms, it was found that the reduction of TYLCV external symptoms were in ascending order when ToMV, TYLCV or both viruses were inoculated at first. In addition, it was observed that, ToMV was suppressed in all treatments followed by increase with plant age. In contrary to that of TYLCV, ToMV symptoms increased in descending order respectively. The distribution of TYLCV was existent in all parts of inoculated plants. The height and yield of tomato plants also affected by the interference between the two viruses in which this affect depended on which virus was inoculated at first. In general, the effect of two viruses on decreasing tomato yield was more than when the two viruses were found individually.

Key words: Interference, tomato plants, TYLCV, ToMV, PCR and ELISA and NASH.

INTRODUCTION

Many investigators reported that, there is interference (antagonistic effect) among the strains of one virus as well as among different viruses[13,15,10]. Intereference between related strains can also be demonstrated by mixing the two viruses in the same inoculum and inoculating it to a host that gives distinctive lesions for one or both of the two viruses or strains[9].

Cohen and Marco[6] and Eid *et al*.[7] have reported interference between TYLCV and ToMV. Zaher and Eid[16] found that the average heights of tomato plants differed depending on which virus was inoculated first. They added that the external symptoms of TYLCV dominated on the new leaves when it was inoculated first followed by ToMV inoculation and the number of local lesions on *Nicotiana glutinosa* were very much reduced when ToMV was inoculated containing TYLCV. They reported that interference between TYLCV and ToMV was significant when TYLCV was inoculated one week earlier than ToMV or when ToMV inoculum contained TYLCV.

Therefore, this study aimed to minimize TYLCV disease on tomato plants via inoculation with ToMV first. In addition, the interaction between them, their effects on TYLCV concentration and distribution and tomato yield were studied.

MATERIALS AND METHODS

Interaction between TYLCV and ToMV was studied in nursery and open field.

Source of the viruses: TYLCV and ToMV were kindly obtained from Virology Lab., Dep. of Microbiology, Fac. Agric., Ain Shams Univ. TYLCV was maintained in tomato plants (*Lycopersicun esculentum* var. Castle rock). ToMV was maintained in *Nicotiana tabacum* var. Samson. All these plants were grown under greenhouse conditions.

Nursery experiment: Healthy tomato seedlings (30 days old) were cultivated in clay pots (20 cm Ø) and kept in an insect proof greenhouse. Old uniform healthy tomato

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seedlings were divided into six groups each one contain 50 seedlings. The first group was inoculated with ToMV; second group inoculated with TYLCV; third group inoculated with ToMV then TYLCV at once; fourth group inoculated with ToMV followed by TYLCV after 15 days; fifth group inoculated with TYLCV followed by ToMV after 15 days and sixth group remain without inoculation (healthy control). The inoculated tomato plants were potted under observation.

Field experiment: Seedlings under greenhouse experiment (50 days old) were planted in open field under gauze tunnels until the end of trial. Fertilization, irrigation and fungal control were applied as recommended by Egyptian Ministry of Agriculture.

Virus inoculation: Tomato seedlings were mechanically inoculated with ToMV infected sap. While TYLCV was inoculated by syringe injection using 0.1 M phosphate buffer pH 7.2 and infected sap (1:1 W/V) as recorded by Allam *et al*.[2].

Existence of TYLCV and ToMV in tomato plants: TYLCV and ToMV were detected in different parts of tomato plant (petioles of old and new blades, middle veins, stems, roots, flowers without calyx, calyx and fruits) using biological assay, ELISA, PCR and NASH as follows:

1. Biological assay: In the course of detection, the activity of ToMV by sap mechanical transmission from treated tomato plants to healthy indicator plants *Datura stramonium*, *Nicotiana tabacum var. white burly* and *chenopodium amaranticolor*). To study the activity of TYLCV, non-viruliferous whiteflies were transferred to infected plants (30 insects per plant) and left for 60 min for acquisition feeding period. Viruliferous whiteflies were transferred to ten healthy indicator plants for each treatment and left for 24 hours as latent period and inoculation feeding period before killing them by insecticide (acetellic). The inoculated plants were kept in an insect proof greenhouse at temperature about 24°C and symptoms appeared were recorded daily.

2. Serological assay: Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used for rapid detection of ToMV as described by Clark and Adams[5]. ELISA kits were supplied via SANOFL, Sante Animal, Paris, France.

3. Polymerase chain reaction (PCR): TYLCV was detected using PCR according to Navot *et al*.[14] and Aref *et al*.[4]. This assay was carried out at Molecular Biology Lab., Virus and Phytoplasma Dep., Plant Pathology Research Institute Agricultural Research Center. The oligonucleotide geminivirus specific primers for all Eastern Hemisphere whitefly- transmitted geminivirus (OWTGs) were:

1. Forward: 5`-CAGTCCGTTGAGGAAACTTAC-3`.
2. Reverse : 5`-CCCACCAATAACTGTAGC-3`.

4. Nucleic acid spot hybridization: Nucleic acid spot hybridization (NASH) was carried out for the detection and determination of TYLCV concentration in tested tomato plants according to Loebenstein and Akad[11].

Growth parameters and yield: The effects of interaction between TYLCV and ToMV on tomato plants were determined as plant height, number of branches, fresh and dry weight, number of flowers and number of fruits.

RESULTS AND DISCUSSIONS

Interaction between TYLCV and ToMV on tomato plants was studied under nursery and tunnel experiments. The virus symptomatology, distribution and concentration were determined. In addition, effect of the interaction on tomato plant as plants growth, external symptoms and yield was estimated.

Existence of TYLCV: It was found that TYLCV existed in petiole, middle vein leaf, calyx, stem, root and fruits, since gave a positive PCR reaction (Fig. 1a). The TYLCV concentration differed in different parts of infected plants. It was found with high concentration in top blade, middle vein, calyx and stem, since gave a positive NASH results while low concentration in low blade, flower, fruit and root since gave positive NASH results with week spots (Fig. 1b) of PCR product or NASH.

Effect of interference between TYLCV and ToMV on tomato plants:

Virus symptomatology: Detection of TYLCV and ToMV biologically was achieved by mechanical inoculation on indicator plants using syringe injection and common method of inoculation. It was found that the reduction of TYLCV external symptoms were in ascending order when ToMV, TYLCV or both viruses were inoculated first. ToMV was found to be suppressed in all treatments followed by increase with plant age. In contrary to that of TYLCV, ToMV symptoms increased in descending order respectively (Table 1). Cleared that, inoculated indicator plants with ToMV only or with TYLCV reacted with characteristic symptoms due to decrease of ToMV infectivity, followed by inoculation of

Table 1: Effect of interaction between TYLCV and ToMV on viral symptoms on indicator plants.

Indicator plants treatments	Tomato var. Castle rock	*D.stramonium*	*N. tabacum* var. white burly	*Ch. amaranticolor*
Inoculated with ToMV	Se M	Large L.L	Se, M, De	Large L: Ch. L
Inoculated with TYLCV	Cu, Cl, St	Cu, Cl; V.b	-	-
Inoculated with ToMV then TYLCV immediately	mM, Cu, Cl	Little L.K.; Cl	mM	Little L Ch. L
Inoculated with ToMV then TYLCV at 15 days later	Cu, Cl	Very little L.L Cl.	mM	Little L Ch. L
Inoculated with TYLCV then ToMV at 15 days later	Cu, Cl	Very little L.L Cl, Cu	mM	Large L.L

- Abreviations Cl = Chlorosis, Cu=Curling; De = deformation, L.Ch.L. Local chlorotic lesion, L.L = local lesion, mM= mild mosaic, M = mosaic, SeM = Severe mosaic, V.b = Vein banding.
- Symptoms were recorded after 40 days from inoculation.

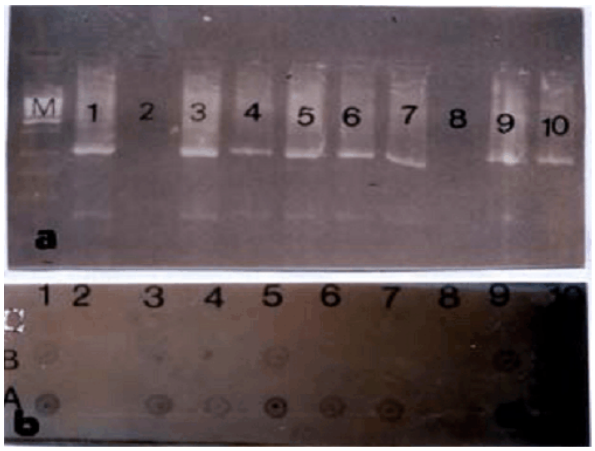

Fig. 1: Agarose minigel electrophoresis (a) and Dot blot hybridization (b) showed the distribution and concentration of TYLCV in Tomato plants whereas:
1. Infected tomato plants (positive control).
2. Healthy tomato plants (negative control).
3,4,5,6,7,8,9 and 10 parts of TYLCV inoculated tomato plants, Top blade; low blade, leaf middle vein, calyx, flower (without calyx), stem and root respectively. A = PCR product and B-DNA extract.

ToMV and TYLCV at once or ToMV and then TYLCV after 15 days. The result of decreasing infectivity of the two viruses is shown in table (1) and figure (2).

Virus infectivity: It was found that ToMV decreased the TYLCV activity in tomato plants. Since inoculated plants with TYLCV then ToMV after 15 days later had a high concentration of TYLCV, where as gave a clear spots of NASH results with PCR products or DNA extracts Fig. (3,a). While inoculated plants with ToMV then TYLCV immediately and ToMV then TYLCV after 15 days later gave not clear spots (Fig. 3,a). As well, healthy plants gave negative NASH results.

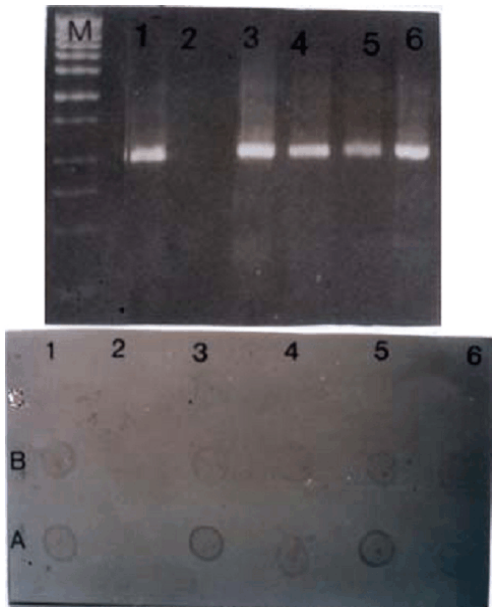

Fig. 2: Agarose minigel electrophoresis (a) and Dot blot hybridization (b) showed the effect of ToMV on TYLCV concentration where as:
1. Infected tomato plants (positive control).
2. Healthy tomato plants (negative control).
3. Inoculated tomato plants with TYLCV.
4. Inoculated tomato plants with ToMV and TYLCV immediately.
5. Inoculated tomato plants with ToMV then TYLCV at 15 days later.
6. Inoculated tomato plants with TYLCV then To MV at 15 days later. (A – PCR product. B = DNA extract).

On the other hand, plants inoculated with TYLCV only or TYLCV then ToMV immediately, TYLCV then ToMV after 15 days later and ToMV then TYLCV after 15 days later had a high concentration of TYLCV as well, infected tomato plants, since the agarose mini gel electrophoresis of PCR anplification of TYLCV for them

Table 2: Effect of TYLCV inoculation on ToMV concentration in tomato plants.

Treatments	* Absorbance at 405 nm. One hour inocubation	Two hours inocubation
- ToMV inoculation	0.625	0.704
- ToMV and TYLCV inoculation at once	0.301	0.340
- ToMV and TYLCV inoculation at 15 days later	0.390	0.434
- TYLCV and ToMV inoculation at 15 days later	0.182	0.199
- Negative ToMV	0.113	0.174
- Positive ToMV	0.718	0.782

* The mean of four replicates

Fig. 3: Effect of ToMV on severity of symptoms on TYLCV inoculated tomato plants.

gave a positive results amplifing 1.1 Kbp by using primers pair IRC. 21. C 1046 (Fig. 3, b).

Infectivity of ToMV was determined using ELISA. The inoculated tomato plants with ToMV only gave the highest ToMV concentration (0.625 and 0.704 after one and two hour incubation respectively). While inoculated plants with ToMV then TYLCV (15 days later) gave a moderate concentration (0.390, 0.434) followed by ToMV then TYLCV at once (0.301, 0.340) (Table 2).

The opposite trend was obtained when inoculated tomato plant with TYLCV then ToMV after 15 days later gave lowest concentration of ToMV (0.182; 0.199). All results were compared with negative and positive ToMV control (0.113, 0.114) and (0.718, 0.782) respectively.

Tomato plant height: The inoculated tomato plants with TYLCV only lead to the highest decrease (34.3%). While ToMV-inoculated tomato plants lead to the lowest decrease (10.1%). Followed by ToMV then TYLCV, immediately (18.1); ToMV then TYLCV at 15 days later, (10.1) and TYLCV then ToMV at 15 days latter (18.2%) compared with non-inoculated tomato plants.

Tomato plant yield: The effect of interaction between ToMV and TYLCV on plant yield under tunnel was recorded in the end of season. The yield of TYLCV inoculated plant gave 0.24 kg; ToMV inoculated plants gave 0.85 kg; ToMV then TYLCV immediately; ToMV then TYLCV or TYLCV then ToMV at 15 days latter gave 0.44; 0.58 and 0.34 kg respectively compared with non-inoculated tomato plants 1.492 kg.

Earlier studies have demonstrated that TYLCV has great effect on tomato leaves (yellowing, curling and reduction of area) much more than on stems (stunting with increased main lateral branches),[2,3].

In the course of studying the existence of TYLCV in different parts of tomato plant, it was found that highest concentration of TYLCV was in stem and middle vein as determined by using NASH. This result may be due to that, curly top viruses exist in close association with the phloem tissue of their hosts[2].

In the current study, we found that interaction or interference between ToMV and TYLCV on tomato plants lead to reduction of TYLCV infection, severity of symptoms and increasing of yield compared with the inoculated tomato plants with only TYLCV. It can be said that, the previous results differed according to which virus is inoculated first consequently due to occupation of the sites of the virus reaction in plant cells. When ToMV was inoculated first, it decreased the effect of TYLCV on tomato plant heights. Our results were in agreement with that obtained by[13,8,7]. On the other hand, domination of TYLCV after eight weeks, suppression of ToMV symptoms and existence of TYLCV in different tomato plants as well as increasing the highest concentration of TYLCV in stem and middle (viens obtained using NASH). It may be due to the existence of TYLCV in close association with host phloem tissue and thus

possible spread with speed[2]. Present results indicated that, TYLCV affected tomato leaves (yellowing, curling and reduction leaf area) much more than that of tomato stem (stunting with increased main lateral branches). Our results are in agreement with that obtained by Allam *et al*.[2] and Aref and El-Dougdoug[3].

The difference between the results obtained by that of PCR and NASH may be attributed to the method sensibility of detection or determination.

Several hypotheses have been proposed to explain interference between viruses[15,12]. In which their hypothesis was, attributed to (1) Depletion of host metabolites and structures in which the initial virus could sequester the ribosomes, leaving the challenge virus nucleic acid unbound and susceptible to degradation. Another suggestion is that the host-coded domain of the viral replicase has been depleted by the protecting strains. These theories assume that different viruses utilize different host components. (2) Capture of viral nucleic acid. In which the (-) strand of the challenge virus is captured by excess (+) RNA of protecting strain. (3) Involvement of coat protein in which coat protein of the protecting strain prevent uncoating of the challenge virus or block attachment sites on the replicase. Difference in results obtained with PCR and NASH may be attributed to the method sensibility of detection or reduction of TYLCV concentration in samples as well as to the difference in inoculation methods. In addition, Loebenstein *et al*.[10] reported that, use of cross protection or virus interference for disease control in practice is limited. They added that, mild strains of ToMV have been used to protect glasshouse-grown tomatoes from severe isolates causing tomato mosaic and citrus tristeza virus are widely used to protect sweet orange trees.

REFERENCES

1. Aguilar, F.S. and F. Penz, 2000. Different forms of interference between two tobamoviruses in two different hosts. Plant Pathology, Vol. 49 Page 659,
2. Allam, E.K., M.A. Abo El-Nasr, B.A. Othman and S.D. Thabet, 1994. A new method for mechanical transmission of tomato yellow leaf curl virus. Egyptian Phytopathological Society, 7th Congress of Phytopathology, Cairo, pp: 77-91.
3. Aref, N.M. and K.A. El-Dougdoug, 1996. Biological and molecular diagnosis of three different symptoms of TYLC-disease in open field. Annals of Agric. Science, 41:1, 173-185.
4. Aref, N.M., N.A. Abdallah, E.K. Allam and M.A. Madkour, 1994. Use of polymerase chain reaction and radiolabeled specific probe to identify tomato yellow leaf curl virus DNA from infected plants. Egyptian Phytopathological Society. The 7th Congress of Phytopatho-logy, Cairo, pp: 93-109.
5. Clalrk, M.F. and A.N. Adams, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34: 475-483.
6. Cohen, S. and S. Marco, 1970. Periodic occurrence of an anti-TMV factor in the body of whiteflies carrying tomato yellow leaf curl virus. Virology, 40: 363-368.
7. Eid, S.A., M.A. Tolba and A.M. Sabek, 1979. A preliminary investigation on interference of tomato leaf curl virus with multiplication of tomato mosaic virus in tomato. 3rd Egypt Phytopathol Congress, pp: 158-166.
8. Knight, C.A., 1975. Chemistry of viruses. Springer-Verlag Wein, New York, pp: 325.
9. Hull, R., 2004. Matthew's Plant Virology, 4th edition. Elsevier Academic Press, pp: 447.
10. Loebenstein, G., R.H. Lawson and A.A. Brunt, 1995. Resistance and interference, In Virus and Virus like Disease of Bulb and Flower Crops. (John Wiley and Sons, New York, pp: 191-202.
11. Loebenstein, G. and F. Akad, 1997. Improved detection of potato leaf roll luteovirus in leaves and tubers with a digoxigenin labeled cRNA probe. Plant Dis. 81: 489-91.
12. Matthews, R.E.F., 1991. Plant virology. San Diego, USA, Academic Press.
13. Matthews, R.E.F., 1970. Plant Virology. Academic Press, New York, pp: 477.
14. Navot, N., M. Zerdan, E. Pichersky, D. Zamier and H. Gasneck, 1992. Use of polymerase chain reaction to amplify tomato yellow leaf curl virus DNA from infected plants and viruliferous whiteflies. Phytopathology, 82: 1199-1202.
15. Urban, L.A., J.L. Sherwood, J.A.M. Rezende and U. Melcher, 1990. Examination of mechanisms of cross protection with non-transgenic plants. In Recognition and Response in plant-virus Interactions R.S.S. Fraser (ed), Springer-Verlag, Berlin, pp: 415-426.
16. Zaher, A. Nahed and S.A. Eid, 1984. Antagonistic effect of leaf curl virus on mosaic virus in tomato. Agric. Res. Rev., 65: 309-314.