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Investigation on Viruses Causing Yellowing Disease in Pepper in Hatay-Turkey

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Abstract

Important viruses mainly caused "pepper yellowing disease" in field-grown and greenhouse-grown peppers, their hosts and vectors were investigated in Hatay province of Turkey. Young shoot, leaf and fruit samples taken from suspected pepper plants and weeds were investigated for presence of main viruses by DAS-ELISA and biological indexing. The mostly observed symptoms were chlorosis and yellowing symptoms in pepper growing areas of Iskenderun and Samandağ districts in Hatay. Among the tested viruses, *Potato virus Y* (PVY) was determined as the most common virus in pepper growing areas in Hatay (13.8%); followed by *Cucumber mosaic virus* (CMV) (5.5%),. Mixed infections caused by double, triple and multiple viruses including CMV, *Beet western yellows virus* (BWYV), *Potato leaf roll virus* (PLRV), *Potato X virus* (PVX), PVY, *Tomato mosaic virus* (ToMV) and *Tomato spotted wilt virus* (TSWV) were identified in pepper plants with low infection rates. *Amaranthus retroflexus, Chenopodium quinoa, Convolvulus arvensis, Malva sylvestris, Physalis angulata, Mercurialis annua, Solanum nigrum, Sonchus oleraceus* and *Xhanthium strumarium* were commonly found in pepper fields. A few pepper plants were found to be infested by dodder (*Cuscuta campestris*) and *Orobanche ramosa*. *Portulaca oleracea, Urtica dioica* and *Plantago major* were observed in pepper growning under greenhouse conditions. *Orobanche ramosa* was also determined in two pepper growing greenhouses*. Amaranthus retroflexus* (28.5%)*, M. sylvestris* (40.0%)*, S. nigrum* (30.0%) and *S. oleraceus* (33.3%) were found to be infected by AMV; *A. retroflexus* (40.0%)*, M. sylvestris* (57.1%)*, P. angulata* (16.,6%) and *S. nigrum* (50%) samples were found to be infected by CMV, PVY was detected in *M. sylvestris* (20.0%)*, M. annua* (41.6%) and *P. angulata* (83.3%) samples; TSWV was detected in *P. major* (25.0%) samples, respectively. Other viruses such as *Beet curly top virus* (BCTV) or begomoviruses and phytoplasmas which were not tested in this study were thought to be also causal agents of "pepper yellowing disease" in pepper growing areas in Hatay-Turkey.

Key words: DAPI, ELISA, greenhouse, pepper, yellows disease, virus, weed

Giriş

Pepper (*Capsicum annuum* L.*, Solanaceae*) is one of the most widely consumed foods in the world. Pepper production has increased in recent years worldwide. The world production of pepper was about 32.3 million tonnes in 2014. The main pepper producer countries are China, Mexico, Turkey, Indonesia, Spain and the United States. Turkey was one of the main pepper producer countries in the world in 2014. Turkey has a big potential of pepper cultivation and it was also one of the most important pepper producer among the countries in the Mediterranean basin. It is produced under protected condition by 25% of the total pepper in Turkey. Therefore, the pepper is an important crop for the Turkish agriculture industry, representing a high share of Turkish exports. The amount of pepper production in Turkey is about 2.4 million tonnes. The Mediterranean region of Turkey where include Hatay province is the main pepper production area by a high prevalence (33%). Although, local pepper

types mainly produced, many varieties of peppers have also been grown in Hatay. Besides outdoor cultivation, greenhouse production has been widely used in many years in the province. Pepper is one of the most important crops and the production in Hatay province was 61 131 tons (FAO, 2014; Anonymous, 2014; Anonymous, 2016).

Virus diseases have a particular importance in pepper cultivation for causing severe losses in yield and quality (Erkan, 1991; Palloix ve ark., 1994; Ekbiç ve ark., 1997; Gorsane et al., 1999). Approximately, 100 viruses were determined to be able to infect pepper and about 60 viruses have been known to cause natural infections on pepper. Among these *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Chile veinal mottle virus* (CVMV), *Potato virus Y* (PVY), *Tobacco etch virus* (TEV), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Pepper veinal mottle virus* (PVMV), *Potato virus X* (PVX), *Potato virus Y* (PVY), *Tomato spotted wilt virus* (TSWV) are the most important and common ones in pepper production (Zitter and Florini, 1984; Sharma et al., 1989; Edwardson and Christie, 1997; Tameru, 2004). PVY is one of the most important plant viruses and common viruses in solanaceous plants (Scholthof et al., 2011). Pepper production is affected by several important viruses in Turkey *(*Palloix, et al., 1994; Arlı-Sökmen, et al., 2005; Sertkaya, 2008; 2012; Buzkan et al., 2013).

The objective of this study was to investigate the important viruses mainly caused “pepper yellowing disease” in field-grown and greenhouse-grown peppers and their natural hosts in Hatay-Turkey.Common weed species infected with the viruses in pepper growing areas were also determined.

Material and Methods

Surveys were carried out in the main pepper growing areas as Reyhanlı, Samandağ, Altınözü, Antakya and İskenderun districts in Hatay in 2013-2015.

Total of 20 fields and 10 greenhouses were randomly selected and at least 500 plants per field and all plants in each greenhouse were examined during vegetation period. Total of 303 shoot and leaf samples taken from suspected pepper plants and weeds were investigated by symptomatological, Double Antibody Sandwich-Enzym Linked Immunosorbent Assay: DAS-ELISA (Clark and Adams, 1977) and biological indexing (sap inoculation) methods for presence of AMV, BWYV, CMV, PLRV, PVX, PVY, PMMoV, PepMoV, ToMV and TSWV.

Healthy test plants (*Capsicum annuum*, *Chenopodium amaranticolor*, *Lycopersicon esculantum*, *Nicotina benthamiana, N. glutinosa* and *Phaseolus vulgaris*) were obtained by germinating of seeds in peat:tuff mixture (1:1) and used in biological indexing. Sap inoculated plants (25-30 days old) by using 0.1 M phosphate buffer were kept in an insect-proof and controlled climated room at 16-8 (light:dark) photoperiod at a temperature of 26°C day and 22°C night for symptom observation for 6 weeks and then also tested by DAS-ELISA. ELISA results obtained by reading (ELISA Reader: SEAC SIRIO S). Absorbance values (optical densities, 405 nm) of the samples higher than 3 times the average of negative controls were considered positive result (Thomas ve ark., 1986). Suspected plant samples exhibited symptoms related to phytoplasma infections were also examined by DNA staining with DAPI (4’-6-diamidino-2-phenylindole) technique (Sertkaya, 2004).

Results and Discussion

During surveys in pepper growing areas in Hatay , different symptoms as chlorosis, stunting, reduced leaf size, curling upward of leaves without crinkling, leaf deformation with *zig-zagging* of midrib, severe yellowing of leaves, yellow mottling or patterning on leaves or fruits, flower malformations and/or lack of flowers were mainly observed on suspected pepper plants (Figure 1).

Figure 1. Main symptoms as severe stunting, chlorosis and yellowing (A-E), mosaics and *zig-zagging* of midrib (G), and yellow mottling or patterning on leaves (H-L) related virus infections on pepper plants in Hatay-Turkey.

The plants mainly exhibited mosaics and/or leaf curling etc. without general yellowing or chlorosis symptoms were not collected. Chlorosis or yellowing symptoms on pepper plants were mostly observed in pepper growing areas of İskenderun and

Samandağ districts in Hatay. Various symptoms as browning or necrosis in fruit skin and flesh caused by noninfectious disorders or other pathogens were also observed during field studies. Because ripening fruit was not adequately shaded by leaf cover, sunscald damage has commonly occured in Reyhanlı and Altınözü districts. chimera on leaves, cracking and discoloration symptoms on pepper fruits were also observed by the low rates (0.2-1%) in the same areas (Figure 2).

Figure 2. Chimera (A), sunscald damage (B) and cracking of fruit (C) symptoms on pepper plant in Hatay.

Total of 303 samples of symptomatic pepper plants were investigated to identify the viruses (AMV, BWYV, CMV, PLRV, PVX, PVY, PMMoV, PepMoV, ToMV and TSWV) which have been mainly detected in pepper in Turkey, whether the causal agent of "yellowing disease" in pepper plants in Hatay.

Based on the DAS-ELISA results, PVY infection was determined as the most common in pepper growing areas in Hatay (13.8%) following by the CMV (5.5%), respectively (Table 1). in pepper plants exhibiting severe yellowing or chlorosis symptoms were mostly foud to be infected by PVY and CMV. PVY is known as a dominant virus in *Solanaceaus* crops including pepper (Sharma et al., 1989). Various symptoms that include mottling and yellowing in pepper were reported to be induced by PVY (Gebre-Selassie et al., 1983).

Although, typical symptoms of AMV and TSWV were severely inspected on pepper plants (Figure 1, photos at the bottom), single infections of AMV, PVX, PLRV and TSWV were not detected in pepper samples. Therefore, mixed infections caused by double, triple and multiple viruses including CMV, BVYC, PLRV, PVX, PVY ToMV and TSWV were identified in pepper plants with low rates (Table 1). The highest infection rates were generally determined in Altınözü and Samandağ districts. Triple infection of CMV+PVY+TSWV was found in pepper fields in Iran (Soleimani et al., 2014). Mixed infection of pepper by PVY and TSWV was also reported in Zimbabwe (Karavina et al., 2016).

During biological indexing, severe symptoms such as chlorosis, pale yellowing of veins or all parts of new shoots on top of the plants developed in test plants (*C. annuum*, *L. esculantum*, *N. benthamiana, N. glutinosa* and *P. vulgaris*) 4-6 weeks after inoculation. AMV, CMV and PVY infections were detected in symptomatic test plants used inoculated with sap samples of severe yellowing pepper plants.

Hand cross sections of two healthy and five suspected pepper leaf petiole and stem samples treated with DAPI were *detected* for the presence of *phytoplasmas* using fluorescent microscope. Typical fluorescence was observed in phloem tissues of three symptomatic plants but not in those of healthy pepper plants.

Table 1. Viruses detected in pepper growing areas in Hatay by DAS-ELISA and their infection rates (%)

Viruses	No. of Collected Samples					No. of Infected Samples	
	An[3]	Ar[3]	Re[3]	Al[3]	Sa[3]	Total	Infection Rate (%)
AMV	0	0	0	0	0	0	0
BWYC	0	0	0	3	3	4^1 +2^2	1.3^1+ 0.6^2 = 1.9
CMV	0	3	4	6	3	8^1 +9^2	2.6^1+2.9^2 = 5.5
PePMV	0	0	0	0	1	1	0.3
PLRV	0	0	0	0	0	0	0
PMMoV	0	0	1	2	0	3	0.9
PVX	0	0	0	0	0	0	0
PVY	2	15	2	11	11	37^1 +5^2	12.2^1+1.6^2 = 13.8
TEV	0	3	0	1	4	7^1 +1^2	2.3^1+ 0.3^2 = 2,6
ToMV	0	0	0	1	7	6^1 +2^2	1.9^1+ 0.6^2 = 2.6
TRV	0	0	0	1	0	1^2	0.3^2
TSWV	0	0	0	0	0	1^2	0.3^2
BWYC+ToMV	0	0	0	1	1	2	0.6
CMV+PLRV	0	0	0	2	1	3	0.9
CMV+PVY	0	2	0	1	0	3	0.9
TRV+TEV	0	0	0	1	0	1	0.3
CMV+PVX+ PLRV	0	0	0	1	0	1	0.3
CMV+PVY+ TSWV	0	0	0	1	0	1	0.3
CMV+PVY+ PVX+ PLRV	0	0	0	1	0	1	0.3

[1]The rate of single infection

[2]The infection rates calculated with mixed-multible infections

[3]An: Antakya, Ar: Arsuz, Re: Reyhanlı, Al: Altınözü, Sa: Samandağ

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Hand cross sections of two healthy and five suspected pepper leaf petiole and stem samples treated with DAPI were *detected* for the presence of *phytoplasmas* using fluorescent microscope. Typical fluorescence was observed in phloem tissues of three symptomatic plants but not in those of healthy pepper plants.

Weed species were also inspected and tested for presence of the viruses. *Amaranthus retroflexus, Chenopodium quinoa, Convolvulus arvensis, Malva sylvestris, Physalis angulata, Mercurialis annua, Solanum nigrum, Sonchus oleraceus* and *Xhanthium strumarium* were found to be main common weed species in pepper fields. A few pepper plants were found to be infested by dodder (*Cuscuta campestris*) and *Orobanche ramosa*. *Portulaca oleracea, Urtica dioica* and *Plantago major were observed in pepper grown under greenhouse* conditions. *Orobanche ramosa* was also determined in two pepper greenhouses. Suspected weed samples were detected for presence of the viruses to determine the roles as the inoculum source of the virus infections in pepper crop in Hatay.

Amaranthus retroflexus (28.5%), *M. sylvestris* (40.0%), *S. nigrum* (30.0%) and *S. oleraceus* (33.3%) were found to be infected with AMV; *A. retroflexus* (40.0%), *M. sylvestris* (57.1%), *P. angulata* (16.6%) and *S. nigrum* (50%) samples were found to be infected with CMV, *M. sylvestris* (20.0%), *M. annua* (41.6%) and *P. angulata* (83.3%) samples were found in PVY; TSWV was detected in *P. major* (25.0%) samples (Table 2). It is suggested to be the reason of these results; weeds are important natural hosts for the main viruses in pepper fields in Hatay. Black nightshade (*S. nigrum*) was reported to be an important inoculum source of CMV in pepper crop in Hatay (Sertkaya et al., 2003).

Table 2. Number of virus infected weed species collected from pepper growing areas in Hatay and their infection rates (%)

Weed Species	No. of Virus Infected Plant / No. of Tested Plant			
	AMV	CMV	PVY	TSWV
Amaranthus retroflexus	4/14 (%28.5)	2/5 (%40.0)	-	-
Malva sylvestris	2/5 (%40.0)	8/14 (%57.1)	2/10 (%20.0)	-
Mercurialis annua	-	-	5/12 (%41.6)	-
Physalis angulata	-	1/6 (%16.6)	5/6 (%83.3)	-
Plantago major	-	-	-	2/8(%25.0)
Solanum nigrum	3/10 (%30.0)	3/6 (% 50.0)	-	-
Sonchus oleraceus	3/9 (%33.3)	-	-	-
Total : 17	12/38 (%31.5)	14/31 (%45.1)	12/28 (%42.8)	2/8(%25.0)

According to results of symptom inspections on plants, DAS-ELISA, biological indexing and DAPI assays, "pepper yellowing disease" in Hatay was caused by more than one agent. Due to the collected viruses were not detected in all the symptomatic samples, it is thought those symptoms were also caused by other pathogens. According to results of this study that other viruses such as *Beet curly top virus* (BCTV), begomoviruses, and phytoplasmas were thought to be also causal agents of "yellowing disease" in pepper plants in Hatay. Studies on those potential agents must be carried out to find out the causal agent/s of "yellowing disease" of pepper in Hatay-Turkey.

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