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CMV, AMV, PVY: DYNAMICS OF VIRUS ANTIGEN ACCUMULATION IN SINGLE AND MIXED INFECTIONS

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Abstract

Pepper plants cultivated in open fields are highly susceptible to plant viruses. That is why, apart from single, viruses appear and in mixed infections. The aim of this study was to examine the eventual interactions in the mixed infections between the three most common viruses on pepper plants in R. Macedonia, such as *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV) and *Potato virus Y* (PVY). Virus antigen accumulation was measured with the use of DAS-ELISA method. The dynamics of the antigen accumulation was measured three times during the vegetation in a three-year trial. Single infections were observed to be more spread than mixed infections during the tested period. Mixed infections appeared in 2 – 12% from the inspected plants. Most of the mixed infections included CMV, being the most spread virus in the tested period. During this trial, a significant interaction between the virus antigen accumulations of the tested viruses in the mixed infections could not be observed, leaving space for further and more profound examinations.

Keywords: DAS-ELISA, viruses, pepper production.

Introduction

Pepper (*Capsicum annuum* L.) is one of the most important cultivated crops in the Republic of Macedonia (Jankulovski, 1997). Considering pepper production, R. Macedonia is amongst the top ten countries in Europe (FAO, 2015). Pepper cultivated in open fields is more susceptible to virus infections, than pepper cultivated in green houses (Bogatzevska et al., 2007). Since most of the pepper plants in R. Macedonia are cultivated in open fields (Tudzarov, 2011), plant viruses represent a major problem and limiting factor in pepper production (Jovanchev et al., 1996; Rusevski and Bandzo, 1998). The most common pepper viruses are: *Cucumber mosaic virus* - CMV, *Alfalfa mosaic virus* - AMV, *Tobacco mosaic virus* - TMV, *Tomato spotted wilt virus* - TSWV, *X* and *Y viruses of potato* - PVX and PVY etc. (Jovanchev et al., 1996; Choi et al., 2005; Ormeño et al., 2006; Kim et al., 2010; Milošević, 2013). These pathogens can cause damage in pepper production up to 100% (Šutić, 1995; Jovanchev et al., 1996). Previous findings on occurrence and distribution of viruses on pepper cultivated in open fields showed that in R. Macedonia the most widespread virus infections were by CMV, followed by AMV and PVY (Rusevski et al., 2009; 2010; 2011; 2013). The damage which these viruses cause to the plants is even more enhanced during mixed infections. In mixed infections, CMV expresses more severe symptoms and causes more extreme growth inhibition (Procházková, 1970; Murphy and Bowen, 2006; Kim et al., 2010), as well as increasing of the virus titer in the infected tissues (Wang et al., 2002; Murphy and Bowen, 2006). PVY is usually found in mixed infections, causing more severe damage (Šutić, 1995). Symptom manifestation of virus infections depends on various factors, amongst which are mixed infections caused by two or more viruses (Kim et al., 2010) and may vary from typical, to masked and atypical symptoms (Nair et al., 2009). Because of that, visual detection has only a preliminary role, while for a final diagnosis and virus determination, especially in mixed infections, laboratory tests such as DAS-ELISA are performed. That is why, during determination of virus occurrence of the most widespread pepper viruses on the territory of R. Macedonia, special attention was given to the mixed infections in the plants and their possible interactions.

Material and methods

Collection of plant samples

The study was conducted during 2012, 2013 and 2014 on pepper plants cultivated in open fields in R. Macedonia. Eight important pepper production regions were included in the survey: areas around Skopje (1 locality), Kumanovo (1 locality), Sveti Nikole (2 localities in 2012 and 1 locality in 2013 and 2014), Kochani (2 localities), Strumica (2 localities), Radovish (2 localities), Prilep (1 locality) and Bitola (2 localities). In each locality, field inspection was conducted three times during the vegetation: end of June, after planting of seedling material in the field; middle of August, during flowering and end of September, while harvesting. Sample collection was performed from seven randomly chosen plants. In order to perform serological testing, young pepper leaves were collected from the upper parts of the plants.

Serological analysis

The presence of the inspected viruses and the dynamics of the antigen accumulation were determined on collected leaf samples tested by Double Antibody Sandwich – Enzyme Linked Immunosorbent Assay (DAS-ELISA), as described by Clark and Adams (1977) and modified as proposed by Bioreba AG (Wernli, 1999), using commercial polyclonal antisera. Plant tissue samples were homogenized in extraction buffer (1:10 w/v). Commercial positive and negative controls produced from the same manufacturer were included on each plate. The tested samples were considered to be positive if the average optical density (OD) value after incubation of one hour at room temperature in the dark was higher at least twice than the average OD of the negative control, measured with an ELISA microplate reader MULTISCAN ASCENT at absorbance of 405 nm (Boonham et al., 2003; Vučurović et al., 2012).

Results and discussion

Virus occurrence in single and in mixed infections

During the whole three-year trial, CMV was observed to be the most prevalent virus of pepper plants cultivated in open fields in R. Macedonia (51% in 2012, 34% in 2013 and 61% in 2014) (Table 1). In 2012, AMV was detected as second (15%), while in 2013 and 2014 it was PVY (7% and 8%, respectively). Plant viruses regularly occur on pepper plants throughout the vegetation in R. Macedonia (Jovanchev et al., 1996; Rusevski et al., 2011; 2013) and other countries (Choi et al., 2005; Kim et al., 2010; Milošević, 2013), causing economic losses and representing a major threat for pepper production. The frequency of CMV on pepper was confirmed and in other studies (Choi et al., 2005; Ormeño et al., 2006; Kim et al., 2010). During the tested period, viruses appeared more in single, than in mixed infections. Mixed infections were primarily observed in 2012 (12%). Their frequency declined during the examined years, so in 2013 it was 5% and in 2014 mixed infections were determined only in 2% of the tested samples. Further investigation to why this frequency declining occurred during the years, should be performed. Mixed infections with CMV, AMV and PVY were also detected in other studies (Avilla et al., 1997; Kim et al., 2010; Milošević, 2013).

Table 1. Incidence of *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV) and *Potato virus Y* (PVY) in single and mixed infections on pepper plants in R. Macedonia during 2012-2014

Year	Number of tested samples	Single infections			Mixed infections			
		CMV	AMV	PVY	CMV+AMV	CMV+PVY	AMV+PVY	CMV+AMV + PVY
2012	91	37 (41%)	4 (4%)	1 (1%)	7 (8%)	1 (1%)	2 (2%)	1 (1%)
2013	84	24 (29%)	1 (1%)	2 (2%)	0	3 (4%)	0	1 (1%)
2014	84	49 (59%)	2 (2%)	5 (6%)	0	2 (2%)	0	0

The occurrence of mixed infections was considered to be a common event (Murphy and Bowen, 2006). In our study, CMV, being the most widespread pepper virus, was detected in almost all of the mixed infections. Because of its distribution, CMV was observed as part of many mixed infections with other viruses by various authors (Fraile et al., 1997; Kim et al., 2010; Chen et al., 2011; Vučurović et al., 2010; Rusevski et al., 2013). In the studies of Avilla et al. (1997) and Milošević (2013), the most common mixed infection was observed between CMV and PVY, which corresponded to our findings from 2013 and 2014. In 2012 and 2013, a triple mixed infection was observed in the area around Kumanovo. Many other authors have observed mixed infections with more than two viruses (Kim et al., 2010; Vučurović et al., 2010; Rusevski et al., 2013).

Table 2. Incidence of *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV) and *Potato virus Y* (PVY) in single and mixed infections on pepper plants per regions in R. Macedonia during 2012-2014

Tested regions	Year	Single infections			Mixed infections			
		CMV	AMV	PVY	CMV + AMV	CMV + PVY	AMV + PVY	CMV + AMV + PVY
Kochani[2]	2012	0	1 (7%)	1 (7%)	0	0	2 (14%)	0
	2013	5 (36%)	0	0	0	1 (7%)	0	0
	2014	12 (86%)	0	0	0	0	0	0
Prilep[1]	2012	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0
	2014	2 (29%)	0	1 (14%)	0	0	0	0
Bitola[2]	2012	7 (50%)	2 (14%)	0	1 (7%)	0	0	0
	2013	0	1 (7%)	0	0	1 (7%)	0	0
	2014	7 (50%)	2 (14%)	0	0	0	0	0
Skopje[1]	2012	5 (71%)	0	0	2 (29%)	0	0	0
	2013	3 (43%)	0	0	0	0	0	0
	2014	5 (71%)	0	1 (14%)	0	0	0	0
Kumanovo[1]	2012	4 (57%)	0	0	0	1 (14%)	0	1 (14%)
	2013	2 (29%)	0	2 (29%)	0	0	0	1 (14%)
	2014	3 (43%)	0	1 (14%)	0	0	0	0
Strumica[2]	2012	9 (65%)	1 (7%)	0	2 (14%)	0	0	0
	2013	5 (36%)	0	0	0	1 (7%)	0	0
	2014	4 (29%)	0	2 (14%)	0	1 (7%)	0	0
Radovish[2]	2012	0	0	0	0	0	0	0
	2013	5 (36%)	0	0	0	0	0	0
	2014	10 (72%)	0	0	0	1 (7%)	0	0
Sveti Nikole	2012[2]	12 (86%)	0	0	2 (14%)	0	0	0
	2013[1]	4 (57%)	0	0	0	0	0	0
	2014[1]	6 (86%)	0	0	0	0	0	0

[1] 1 location per area was tested, 7 marked plants
[2] 2 locations per area were tested, 7 marked plants

Dynamics of virus antigen accumulation in mixed infections

During this study, three times during the vegetation virus antigen accumulation in the infected pepper plants was measured using the DAS-ELISA test. One of the aims of this study was to investigate if and how the viruses included in the mixed infections influence each other's dynamics and virus accumulation. The only mixed infection which did not include CMV was between AMV and PVY in the area around Kochani in 2012 on two infected pepper plants. Dynamics of the virus antigen accumulation of these two viruses is shown in Table 3.

In the mixed infections with AMV+PVY detected on the marked pepper plants included in this study, any higher OD values or significant virus accumulation fluctuations were not observed. Dynamics of virus antigen accumulation of the other types of mixed infections is given in Tables 4, 5 and 6.

Table 3. Dynamics of virus antigen accumulation of AMV and PVY (according to OD absorbance) measured in marked pepper plants with AMV+PVY mixed infection during the tested period 2012-2014

Tested regions	Year	Plant	Tested viruses and number of repetitions					
			AMV			PVY		
			I	II	III	I	II	III
Kochani	2012	1	-	-	0.261	-	-	0.368
		2	-	-	0.226	-	0.267	0.379

I, II, III – different testing periods (I – beginning of vegetation, II – middle of vegetation, III – end of vegetation)
[-] the sample is virus free from the inspected virus (OD absorbance bellow 0.100 is measured)

Table 4. Dynamics of virus antigen accumulation of AMV and CMV (according OD absorbance) measured in marked pepper plants with AMV+CMV mixed infection during the tested period 2012-2014

Tested regions	Year	Plant	Tested viruses and number of repetitions					
			AMV			CMV		
			I	II	III	I	II	III
Bitola	2012	1	-	-	0.273	-	0.777	0.555
Skopje	2012	1	-	-	0.249	-	0.684	1.457
		2	-	-	0.260	-	0.756	0.583
Strumica	2012	1	-	-	0.311	-	0.612	0.554
		2	-	0.192	0.362	-	-	0.727
Sveti Nikole	2012	1	-	-	0.241	-	0.695	0.255
		2	-	-	0.204	-	0.758	0.420

I, II, III – different testing periods (I – beginning of vegetation, II – middle of vegetation, III – end of vegetation)
[-] the sample is virus free from the inspected virus (OD absorbance bellow 0.100 is measured)

In most of the cases of mixed infections with CMV+AMV, after the inoculation with AMV on already infected plants with CMV, declining of CMV virus accumulation was observed. Most evident examples were on the infected plants in the area around Sveti Nikole. In contrary, in the area around Skopje after the AMV infection, the OD absorbance measured during the second control grew up during the third. Based only on the number of these tested plants, a conclusion can't be drawn, but a pattern may be sensed, which draws further investigations in the interactions between AMV and CMV in mixed infections.

Table 5. Dynamics of virus antigen accumulation of PVY and CMV (according OD absorbance) measured in marked pepper plants with CMV+PVY mixed infection during the tested period 2012-2014

Tested regions	Year	Plant	Tested viruses and number of repetitions					
			CMV			PVY		
			I	II	III	I	II	III
Kochani	2013	1	-	0.471	1.896	0.377	0.310	4.322
Bitola	2013	1	-	0.319	0.204	0.237	0.244	0.260
Kumanovo	2012	1	-	-	0.714	-	-	3.420
Strumica	2013	1	0.255	3.008	1.337	0.318	0.251	0.258
	2014	1	-	-	0.772	-	-	0.217
Radovish	2014	1	-	-	0.796	-	-	0.209

I, II, III – different testing periods (I – beginning of vegetation, II – middle of vegetation, III – end of vegetation)
[-] the sample is virus free from the inspected virus (OD absorbance bellow 0.100 is measured)

During the three-year trial, unlike the other types of mixed infections, the mixed infections with CMV+PVY were detected during the whole tested period (Table 5). It was determined that in some cases CMV virus accumulation declined in the CMV+PVY infections (example from the area around Strumica). In other cases, the virus antigen accumulation of both viruses increased (in the area around Kochani. During these mixed infections, very high OD values for both of the tested viruses were measured, giving the assumption of synergistic interaction between these viruses. Choi et al. (2002) have established that in mixed infections between CMV and some *Potyvirus* on zucchini plants, an increase of the CMV virus antigen accumulation occurred.

Table 6. Dynamics of virus antigen accumulation of AMV, PVY and CMV (according to OD absorbance) measured in marked pepper plants with AMV+PVY+CMV mixed infection during the tested period 2012-2014

Tested regions	Year	Plant	Tested viruses and number of repetitions								
			AMV			CMV			PVY		
			I	II	III	I	II	III	I	II	III
Kumanovo	2012	1	-	-	0.280	-	0.561	0.405	-	-	4.016
	2013	1	2.575	-	0.703	-	0.263	0.733	1.699	4.866	3.999

I, II, III – different testing periods (I – beginning of vegetation, II – middle of vegetation, III – end of vegetation)
[-] the sample is virus free from the inspected virus (OD absorbance bellow 0.100 is measured)

Triple mixed infections with all of the tested viruses was detected in 2012 and 2013 in the area around Kumanovo on two occasions (Table 6). In 2012, a decrease in the CMV virus antigen accumulation was observed, after the pepper plant was infected with AMV and PVY. Unlike the previous year, in 2013 an increase in the virus antigen accumulation of CMV was observed. Also, an increase of AMV and decrease of PVY accumulation in the triple mixed infection was detected. Overall, after observing the fluctuations in the virus antigen accumulations of the inspected viruses (AMV, CMV and PVY) in the mixed infections on the marked pepper plants, several correlations could be pointed out: antagonistic effect in AMV+CMV and synergistic interaction in CMV+PVY. Because of the small number of tested samples, no significant correlation could be determined. Other authors also came to different conclusions regarding this matter. Kim et al. (2010), which were inspecting mixed infections with CMV on pepper, could not find any significant correlation between the interactions of the different viruses present in mixed infections. Unlike these results, Choi et al. (2002) have established that in mixed infections between CMV and some *Potyvirus* on zucchini plants, an increase of the CMV virus antigen accumulation occurred.

Conclusions

During the tested period on the pepper plants cultivated in the open fields in R. Macedonia, it was determined that single infections were more dominant and widespread, than mixed infections of AMV, CMV and PVY. The occurrence of the mixed infections declined from 12% in 2012, 5% in 2013, to only 2% in 2014. After analyzing the dynamics of the virus antigen accumulation of the inspected viruses in the mixed infections, a door has opened for further investigations in this field, leaving space for speculation of possible interactions between tested viruses. The dynamics of replication of the virus particles and their distribution in the systemically infected host plants needs to be understood and studied further, in order to better understand the interactive influence of various external and internal factors that impact virus fluctuations in mixed infections.

References

1. Avilla, C., Collar, J. L., Duque, M. and Fereres, A. (1997). Yield of bell pepper (*Capsicum annuum*) inoculated with CMV and/or PVY at different time intervals, zeitschrift fur pflanzenkrankheiten und pflanzenschutz. Journal of plant diseases and protection, 104: 1-8.
2. Bogatzevska, N., Stoimenova, E. and Mitrev, S. (2007). Bacterial and Virus diseases spread in Bulgaria and Macedonia on field and greenhouse pepper. Plant protection, 18: 17-21.

3. Boonham, N., Walsh, K., Smith, P., Madagan, K., Graham, I. and Barker, I. (2003). Detection of potato viruses using microarray technology: towards a generic method for plant viral disease diagnosis. Journal of Virological Methods, 108: 181-187.
4. Chen, S., Gu, H., Wang, X., Chen, J. and Zhu, W. (2011). Multiplex RT-PCR detection of Cucumber mosaic virus subgroups and Tobamoviruses infecting Tomato using 18S rRNA as an internal control. Acta Biochim Biophys Sin, 43: 465–471.
5. Choi, G.S., Kim, J.H., Lee, H.D., Kim, J.S. and Ryu, K.H. (2005). Occurrence and distribution of viruses infecting pepper in Korea. Plant Pathol. J, 21(3): 258-261.
6. Choi, S. K., Yoon, J. Y., Ryu, K. H., Choi, J. K., Palukaitis, P. and Park, W. M. (2002). Systemic movement of a movementdeficient strain of Cucumber mosaic virus in zucchini squash is facilitated byg a cucurbit-infecting potyvirus. Journal of General Virology, 83: 3173–3178.
7. Clark, M.F. and Adams, A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of the plant viruses. J. Gen. Virology, 34 (3): 457-483.
8. FAO. (2015). http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor
9. Fraile, A., Alonso-Prados, J. L., Aranda, M. A., Bernal, J. J., Malpica, J. M. and Garcia-Arenal, F. (1997). Genetic exchange by recombination or reassortment is infrequent in natural populations of a tripartite RNA plant virus. J Virol, 71: 934–940.
10. Jankulovski, D. (1997). Pepper and tomato. NIP "BAS – TRADE", Skopje: 1-126.
11. Jovanchev, P., Pejchinovski, F., Jankulovski, D., Rusevski, R., Bandzo, S. and Popsimonova, G. (1996). Health status of pepper plants in the Republic Macedonia in 1995. Annual Book of Proceedings of Plant Protection, 7: 159-169.
12. Kim, M.S., Kim, M.J., Hong, J.S., Choi, J.K. and Ryu, K.H. (2010). Patterns in disease progress and the influence of single and multiple viral infections on pepper (*Capsicum annuum* L.) growth. Eur J Plant Pathol, 127: 53–61.
13. Milošević, D. (2013). Diversity and characterization of pepper viruses in Serbia. Ph.D. Thesis. Faculty of Agriculture, University in Belgrade, Belgrade, Serbia.
14. Murphy, J. F. and Bowen, K. L. (2006). Synergistic disease in pepper caused by the mixed infection of *Cucumber mosaic virus* and *Pepper mottle virus*. Phytopathology, 96: 240-247.
15. Nair, R. M., Habili, N. and Randles, J. W. (2009). Infection of *Cullen australasicum* (syn. *Psoralea australasica*) with *Alfalfa mosaic virus.* Australasian Plant Disease Notes, 4: 46–48.
16. Ormeño, J., Sepúlveda, P., Rojas, R. and Araya, J.E. (2006). *Datura* Genus Weeds as an Epidemiological Factor of *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), and *Potato virus Y* (PVY) on Solanaceus Crops. AgriculturaTécnica (Chile), 66 (4): 333-341.
17. Procházková, Z. (1970). Interaction of Cucumber Mosaic Virus and Potato Virus Y with Tobacco Mosaic Virus. Biol. Plant., 12: 297-304.
18. Rusevski, R., Bandzo, K., Kuzmanovska, B. and Gjamovski, V. (2009). Virus status of pepper plants in the area around Kochani in 2008. Plant protection, Skopje, 20: 84-88.
19. Rusevski, R., Bandzo, K., Kuzmanovska, B., Sotirovski, K. and Risteski, M. (2013). Occurrence, distribution and dynamics of virus antigen accumulation in pepper cultivation on open fields in Republic of Macedonia during 2008-2009. African Journal of Agricultural Research, 8 (28): 3836-3841.
20. Rusevski, R., Bandzo, K., Kuzmanovska, B. and Stanoevska, M. (2010). Virus status of pepper plants cultivated on open fields in R. Macedonia in 2009. Plant protection, Skopje, 21: 44-50.
21. Rusevski, R., Bandzo, K., Kuzmanovska, B. and Stanoevska, M. (2011). Virus status of pepper plants cultivated on open fields. In: XVI International meeting on Biotechnology – Book of Proceedings, Čačak, Serbia, 2011: 429-434.
22. Rusevski, R. and Bandzo, S. (1998). Influence of some microclimate characteristics (temperature and humidity) on the aphid population and virus infections on pepper in green houses. Annual Book of Proceedings of Plant Protection, Skopje, 9: 141-159.
23. Tudzarov, T. (2011). Novel plant species and technologies in the vegetable production. Alfa 94, Skopje, R. Macedonia.

24. Vučurović, A., Bulajić, A., Stanković, I., Ristić, D., Berenji, J., Jović, J. and Krstić, B. (2010). Occurrence, molecular detection and identification of Cucumber mosaic virus infecting pumpkin and squash in Serbia. In: 10th Meeting on plant protection, Zlatibor, 2010: 58-59.
25. Vučurović, A., Bulajić, A., Stanković, I., Ristić, D., Berenji, J., Jović, J. and Krstić, B. (2012). Non-persistently aphid-borne viruses infecting pumpkin and squash in Serbia and partial characterization of Zucchini yellow mosaic virus isolates. Eur J Plant Pathol, 133: 935–947.
26. Wang, Y., Gaba, V., Yang, J., Palukaitis, P. and Gal-On, A. (2002). Characterization of synergy between *Cucumber mosaic virus* and potyviruses in cucurbit hosts. Phytopathology, 92: 51-58.
27. Wernli, B. (1999). Bioreba AG
28. Šutić, D. (1995). Plant viruses. Institute for plant protection and environment, Belgrade: 1-394.