Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
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http://ansinet.com

Plant Pathology Journal 5 (1): 11-14, 2006
ISSN 1812-5387

Identification of Pepper Viruses by Das-elisa Assays in Gaziantep-Turkey

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Abstract: Pepper growing are negatively affected due to the diseases caused by pepper viruses in Gaziantep. In order to avoid the damage of pepper viruses, survey was performed in Nurdağı and Islahiye districts of Gaziantep province located in southeastern Anatolia. The symptoms observed on pepper plants in yards were consisted of mosaic and curling, vein clearing, chlorotic and necrotic spot, fruit and leaf deformations. In October 2001, samples were collected and performed biological and serological assays. TMV and CMV were determined as responsible agent of these symptoms. GAP (Güney-Doğu Anadolu Projesi = Southeastern Anatolia Project) is one of the most important agricultural recovery projects of Turkey. One of GAP inclusion is Gaziantep become a leading producer of pepper in Turkey. Although damage by viruses is yet very below threshold level, an effective control strategies should be now argued to avoid from possible virus problems in longer term.

Key words: Pepper, virus diseases, DAS-ELISA, identification

INTRODUCTION

Due to favourable soil and climatic conditions, pepper plant has a traditional importance in Gaziantep region. Moreover, the increasing of processing techniques (i.e., paste, powdered, spice, shocking etc), pepper production has an increased importance of being a cash crop. Pepper, is one of the most important export agricultural commodities, is source of vitamin C and β-carotene which is precursor compound of vitamin A. Associations with β-carotene of vitamin C in red pepper have activity as an antioxidant and reduce degenerative potentials of free radicals, highly reactive molecules that react and damage cells in the body. Moreover, several studies have shown that challenging against free radicalls of antioxidants helps reduce risk of certain cancers and of heart attack. Evidences also suggest that vitamin C and β-carotene supplementation have performance enhancing effects on immune system.

While the average of pepper yield of the world is approximately 12.000.000 ton/year, as many as of 23, 10 and 9% of the yielding is from Chine, Turkey and Nigeria, respectively. Turkey 's average pepper harvest is estimated as 1.200.000 ton/year. It includes 340.000 ton of dolma pepper, 810.000 ton of bell pepper and 50.000 ton of charleston pepper.

There is many of factors that decrease quantity and quality of pepper producing as well as in other crops. The most important restrictive factors include plant disease and pests, but plant virus diseases are economically important since yet there are no efficient chemical treatments that protect plants from virus infection. Besides viruses, are cellular pathogens, have the ability for attacking to cause infection on the all living (biotic) organisms which are used as vector for transmitting.

Sepulveda *et al.*[1] reported that approximately 30% of peppers in North Central Chile were infected with two or more viruses that these viruses detected by DAS-ELISA test was CMV, TSWV, AMV, PVY, INSV, ToMV and TMV.

So far, it has been reported that TMV[2-4], CMV[3,4], PVX[3], TEV[5], PMMV[5], PVY pathotype[6-8] have caused infection of pepper plants in Turkey. Although symptoms induced by these viruses vary depending on the host, environmental conditions and individual virus infecting plant, the most common symptoms exhibited of this viruses are necrotic spots, streaking, ring spots, stunting, wilting, etc. Symptoms of virus-infected plants may resemble fungal and bacterial diseases or environmental stresses hence they are confused with plants affected by herbicide or air pollution damage, mineral deficiencies and other plant diseases. So, disease of viruses often is misleading, if it is diagnosed on the basis of physical symptoms alone. Positive identification of viruses requires reliable methodologies. The enzyme-linked immunosorbent assay (ELISA) has been very popular for detection of viruses in plant materials since it was introduced to plant virology by Clark and Adams[6]. In

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Japan, Ikegashira *et al.*[9] for the extraction of viruses from soil and optimized for the detection of pepper mild mottle virus (PMMoV) in soil taken from green pepper (Capsicum annuum) fields has applied DAS-ELLISA due to its reliable method. Moreover four field potyvirus isolates naturally infected sweet pepper in Brasilia was characterized using DAS-ELISA test by Cunha *et al.*[7]. Due to its adaptability, sensitivity and time- and labor-saving and reasonable in cost of reagents, ELISA is used in a wide range of diagnosis of viruses, especially to assay a lot of samples in a relatively short period of time. From indirect ELISA procedures, DAS-ELISA, in practice, is highly strain specific and requires each detecting antibody to be conjugated to an enzyme. Up to now, no yet virus diseases of pepper have been investigated in Gaziantep province, dwelled on southeastern Anatolia and is one of the leading producer of pepper in Turkey. In this dissertation, we have studied that diseases of pepper damaged by viral agents is determined using biological and serological methodologies.

MATERIAL AND METHODS

Infected plant samples showing symptoms of leaf deformation, mosaic, wilting, leaf curling, vein banding, dwarfing, local necrotic lesions, fruit deformation were collected from yards in 13 locations of Nurdağı and in 6 locations of Islahiye districts at Gaziantep province, Turkey, in 2001-2002 spring. The samples put into plastic bags were labeled and brought to the laboratory by placing on ice bucket then kept -20°C freezer until using. The samples were grounded in the pestle and mortar by Na-Phosphate buffer pH 7.4. After filtering of samples by double layer gauze, liquid part of sediment was used for experiments.

Growing of test plants: The test plants were *C. amaranthicolor, C. quinoa, C. murale, D. stramonium, D. innoxa, C. sativus, N. glutinosa, N. benthamiana, N. clevelandii, N. rustica, N. tabaccum L.* ''Samsun'', *N. tabaccum L.* ''Samsun NN'', *N. tabaccum L* ''Xanthi'', *N. tabaccum L* ''Xanthi NC''. Seeds of these test plants were sown in potting mix composed of soil: sand: fertilizer (1:1:1) to plastic containers (25×18×8 cm) and subsequently transferred in the glasshouse (21-26°C). After germination of seeds when seedlings were at 2-4 leaves stage they were transplanted to the plastic cups containing soil. Test plants were sprayed with Benlate 0.6 g L^{-1} and DDVP 1.5 mL L^{-1} to prevent fungal agents and insect injuries.

Transmitting of viruses to test plants by mechanic inoculation: Leaves surfaces of test plants consisted of *C. amaranthicolor, C. quinoa, C. murale, D. stramonium, D. innoxa, C. sativus, N. glutinosa, N. benthamiana, N. clevelandii, N. rustica, N. tabaccum L.* ''Samsun'', *N. tabaccum L.* ''Samsun NN'', *N. tabaccum L* ''Xanthi'', *N. tabaccum L* ''Xanthi NC'' were sprinkled carborandum powder and smeared with extracts of infected plant then incubated in climate room for 2-4 weeks. Extraction buffer was consisted of 20 mM Phosphate pH 7.0+0.1 % of 2-mercaptoethanol. Symptoms were evaluated in 2-4 week after incubation.

Detection of viruses by DAS-ELISA: Antisera of the suspected samples tested using DAS-ELISA method Clark and Adams[6] were purchased from SIGMA Company. Antisera was consisted of TMV, CMV, TSWV, PMMV. Related chemicals of ELISA was provided from Molecular Biology Laboratory of Biology Department of Gaziantep University.

The leaves of infected young plants were grounded in the extraction buffer (phosphate buffered saline (PBS) pH 7.0). Each samples (1 g in 10 mL extraction buffer) were filtered by double layer gauze then tested by DAS-ELISA in duplicate. Absorbance values were measured at 405 nm on ELISA reader (EL_X 800 bioelisa Reader biokit). While negative controls were included healthy samples, twice the mean value of healthy specimen were estimated as positive control.

RESULTS AND DISCUSSION

As shown Table 1, TMV and CMV produced symptoms on the leaves of indicator plants at varying degrees depending on varieties of the test plant. Elapsed time to occurrence of visible symptoms from inoculation changed according to test plant species between 3-4 days and 3 weeks. The wide range of symptoms were observed in pepper plantations in this region are, leaf yellowing, witch's brom, dense mosaic, vein banding, vein clearings, choloritc local lesions, shoostrerring. While symptoms of TMV were characterized by chlorotic local lesions, necrotic local lesions, wilting, seldom systemic mosaic, white mosaic and deformations, also CMV generally caused to chlorotic local lesions and systemic mosaic symptoms (Fig. 1). TSWV and PMMV did not produce any symptom. TSWV can cause disease in a wide variety of plants including pepper, tomato and lettuce. The virus is common in both temperate and subtropical areas of the world. Thrips transmit the virus, so use of insecticides to

Fig. 1: Symptoms of TMV and CMV on the leaves of pepper in plantation

Table 1: Symptoms produced on indicator plants of TMV and CMV

Indicator plants	Symptoms produced	
	TMV	CMV
C. amaranthicolor	cll	cll
C. quinoa	cll, nll	cll
D. stramonium	nll, cll	-
D. innoxa	nll	-
C. sativus	w, cll	sm
N. glutinosa	nll	sm
N. rustica	cll	sm
N. benthamiana	w	-
N. clevelandii	w	-
N. tabacum L. "Samsun"	sm, wm	-
N. tabacum L. "Samsun nn"	nll, d	sm
N. tabacum L "Xanthi"	nll	-
N. tabacum L "Xanthi-nc"	nll	-

cll: chlorotic local lesions, nll: necrotic local lesions, w: wilting, sm: systemic mosaic, wm: white mosaic, d: deformations

control the vector reduces disease incidence. Elimination of virus reservoirs (weed and ornamental species) near the crop is important but difficult to achieve. PMMV is not known to be spread by insects, but is very easily spread the routine handling of the young plants, especially at transplanting. Although both of these viruses, TSWV and PMMV has not been yet detected in peppers in the region, insect activities have been implicated with virus problems in pepper crop. Unfortunately, it is difficult to control virus infections, once plants are infected, it is too late to do anything except dispose of diseased plants. Thus, it may be time in order to develop an effective control strategies in longer term to avoid from possible virus problems.

Thirty three out of 81 specimen were reacted positively with CMV (16 samples) and TMV (17 samples) antisera. While Nurdağı district was first in distribution of viruses with infecting of 17 out of 45 samples, Islahiye was second with infected 16 out of 36 samples. It was estimated as 25 and 37% of CMV; 19 and 22% of TMV contamination ratio for Islahiye and Nurdağı districts, respectively. Based on the statistical analyses, there were no significant differences between TMV and CMV contaminations observed in the districts. While the diseases caused by CMV and TMV in pepper are apparently present worldwide, likewise Sepulveda *et al*.[1] described the relative importance of viruses encountered in Chile was as follow: Cucumber Mosaic Virus (CMV) 23.3%; Tomato Spotted Wilt Virus (TSWV) 20.8%; Alfalfa Mosaic Virus (AMV) 14.8%; Potato Virus Y (PVY) 14.5%; Impatients Necrotic Spot Virus (INSV) 3.1% (first identification in peppers in Chile); Tomato Mosaic Virus (TMV) 2.2% and Tobacco Mosaic Virus (TMV) 4.9%. Jalapeno peppers plant grown in New York were observed first infection of TMV in 2002 season and it has been noted that TMV caused commercially losses Murphy and Zitter[10].

Tobacco mosaic virus has a wide host range but is especially a concern on solanaceous crops. TMV is not transmitted by insects, TMV is a very stable that is readily spread by contact or human activities when they handle plants or when cutting tools become contaminated. TMV

can persist in dried tobacco leaves, so tobacco products can also be a source of TMV. Cucumber mosaic virus has a wide host range of over 700 species of plants. CMV is primarily spread by aphids that can acquire the virus in as little as 5 to 10 seconds. Aphids then move the virus from plant to plant for a few hours. CMV is also spread mechanically in the plant sap when cuttings are taken from infected stock plants. CMV is also both seed and pollen transmitted in petunia where symptoms develop in very young plants.

Once the virus is detected in the field, the major control method is to immediately eradicate plant materials which indicate symptoms. The viruses are usually introduced into a field by either infected seeds or especially aphid vectors from infected plants on nearby fields. Three percent of pepper crop in Turkey is provided from Gaziantep province, however it is expected an increase of 8-10 times more when constructions and operations of barrages complex that is being built in vicinity of Gaziantep province is completed. GAP (Güney-Doğu Anadolu Projesi = Southeastern Anatolia Project) increased water use in agriculture and thus caused dispersion of various vector insects particularly aphids in the region, as a result, it is considered that viruses will infest in very large areas in the longer term if the spread of insect are not restricted by practically application of insecticides.

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