

## **IDENTIFICATION OF CHILE PEPPER PHYTOPATHOGENIC VIRUSES AT THE GUANAJUATO STATE MEXICO**

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### **SUMMARY**

It was studied the presence of different chile pepper viruses among chile growing fields of six municipalities of Guanajuato, Mexico. For the detection of viruses, it was utilized the immunosorbant enzyme linked, solid phase, double sandwich technique (DAS-ELISA), carried out under 405 and 490 nm wave length, depending on the enzyme used. 42 foliage chile samples with two replicates were analyzed. The viruses more frequently found were: Potato Virus Y (PVVY) (7/42); Tobacco Rattle Virus (TRV) (23/42); and less frequently was found, Cucumber Mosaic Virus (CMV) (1/42). The TRV was found in all the sampled localities, and it was not been previously reported as present among chile plantations in Mexico and therefore, its effect reducing the chile production in this country, is unknown.

**Key words:** Plant viruses, Chile pepper, identification, ELISA

### **INTRODUCTION**

In Mexico 512,000 hectares are devoted to vegetables growing, constituting 3.5% of the Mexican agricultural surface; 15% of that area is devoted to green chile pepper (*Capsicum annuum* L.) and 7% to dry chile pepper (Siller, 2000). The state of Guanajuato has got an exceptional agricultural potential, devoting around 12,000 ha for growing chile pepper, potato and tomato (INEGI, 2003). For chile production, diseases constitute the most important risk factor, and so for, it is important to protect it from the attack of different pathogens. In recent years, the diseases attributed to viruses have carried out important economic losses for chile pepper production in Mexico (Rico, 2002).

These losses are variable from one year to another, and they have been present as a function of the climatic conditions, field management, cultural and chemical control of insects and weeds, becoming to losses of the 100% (Vidales and Alcantar, 1989). These diseases have been increased in almost all the production zones of the country, becoming one of the most important problems for chile cultivation, being "the Bajío region" one of the zones with a great important incidence of these viral diseases (Rico, 2002).

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For the control of any disease, it is important to know which is its causal agent, identifying it through the different laboratory techniques, that allow the implementation of different steps of management and control; the correct diagnosis of the viral agent is prominent because allows in reliable form a successful control through different alternative implemented for that (Gonzalez and Delgadillo, 1989). Nevertheless, it is not enough to only utilize the symptoms recognition to identify the virus responsible of the studied disease, due to the fact that different viruses show similar plant symptoms. Besides that, plants may be infected simultaneously for more than one type or virus variant, for what it is necessary to carry out special diagnosis tests at laboratory and at greenhouse to determine the disease causal agent (Harris, 1994).

Different methodologies have been developed that, by itself, are considered to be useful as a sufficient tool for the identification of a virus, as it is the case of the immunoserology (Cross and Cold, 1997). Among those methodologies, the enzyme linked immunosorbant assay, known commonly as ELISA, has demonstrated to be very specific and useful to detect virus in plants (Cross and Cold, 1997). Based on the previous revision, the present work aimed to identify the causal agents of viral types associates to diseases with viral symptoms, among growing areas of field established chile pepper in the state of Guanajuato, Mexico.

## **MATERIALS AND METHODS**

Collection of chile pepper samples. Several field collection journeys were organized according to the year season, in which the cultivars were established, for collect plant tissue samples showing symptoms of a putative viral disease, such as curling, mosaic, leave deformations, yellowing, blistering, etc., in the municipalities of Cortazar, Irapuato, Romita, Silao, Dolores Hidalgo and San Luis de la Paz, Guanajuato, Mexico. The sampling work was carried out through June 1996 and from July to August of 1997. Once the samples were collected and placed inside polyethylene bags, they were carried to the Plant Pathology lab., and kept in the freezer (-20°C) until the tissue was used for analysis.

Sample processing. This work was carried out as a part in the Laboratory of Plant Pathology of the Agricultural Sciences Institute of the University of Guanajuato (ICA-UG) and as another part in the Plant virology Laboratory of the Irapuato Unit of the Research and Advanced Study Center of the National Polytechnique Institute (CINVESTAV-IPN). For the detection of all the virus it was utilized the inmunoabsorbent enzyme linked, solid phase, double sandwich technique (DAS-ELISA) running through two days (Clarck and Adams, 1977; Cruz and Frías, 1997). The antibodies used for the TbRV (Tobacco Rattle Virus), CMV (Cucumber Mosaic Virus) and PVY (Potato Virus Y); for the TbRV, it was used the enzyme alkaline phosphatase, while for the virus CMV and PVY the enzyme used was the peroxidase.

Antisera origin. The antisera employed for the tests, were obtained from the Agdia company Inc., from US between 1995 and 1996, which were kept at  $4 \pm 2$  °C until the assays were carried out.

Evaluation of results. Evaluation was carried following a diagram with duplication of each sample, obtaining the medium value of each pair of values, and in this form, it was defined which of the samples showed to be positive for the virus in question.

Determination of detection limits. The value of healthy control was obtained carrying out averaging the 10 lowest average readings of absorbance obtained from each virus with different sample analysis. As a criterion to determine the detection limit, the value of the healthy control was doubled and then was used. Every value above that detection limit was considered positive.

## **RESULTS AND DISCUSSION**

There were analyzed 42 foliage samples of chile plants from the municipalities of Cortazar, Irapuato, Romita, Silao, Dolores Hidalgo and San Luis de la Paz of the state of Guanajuato, confirming more frequently the presence of the virus PVY, TbRV and a minor frequency the presence of CMV. The virus more frequently detected, considering the positive number of samples from the total of 42 processed, was the virus TbRV with 23 positive samples (Table 1), with a minor frequency the virus PVY with 5 positive samples and the CMV with only 1 positive sample.

From Cortazar samples was not detected the virus CMV, the virus PVY was found in 1 sample from 12 collected and the TbRV in 2 samples; from Irapuato samples it was not detected the virus CMV neither the virus PVY, but virus TbRV was found in 4 out of 8 samples analyzed; from Romita samples it was not detected the CMV, the PVY was found in 1 out of 2 samples processed and the TbRV was also in 1 out of 2 samples analyzed; from Silao samples, it was detected the virus CMV in 1 out of 5 samples; particularly, in the locality of San José del Durazno, it was not detected the PVY, but was detected the TbRV in 1 out of 5 samples; from Dolores Hidalgo, it was not found the Virus CMV, but it was detected PVY in 5 out of 11 samples and TbRV in 10 out of 11 samples, advertising markedly the presence of the TbRV and of the PVY in those localities; from San Luis de la Paz samples, it was not detected CMV neither PVY, but it was detected TbRV in 3 of the analyzed samples.

The CMV was detected in low proportion, while in other Mexican regions it is considered as one of the most important virus with a major distribution in chile cultivars (Vega *et al.*, 1989). The virus PVY was detected in several localities sampled, although with a low presence; and has been reported affecting chile cultivars in Mexico (Vega *et al.* 1989). The TbRV was found in all the sampled localities, its presence had not been reported among the chile plantations in Mexico, and therefore its effect reducing the chile production is unknown.

In Mexico up to starting the 80's it was considered that chile pepper virus were: the Cucumber Mosaic Virus (CMV), the Tobacco Mosaic Virus (TMV), and the Etch Tobacco Virus (ETV), the three viruses with an RNA genome. Advancing the 80's, the Geminiviruses

start to play an important role giving place to yield losses; these viruses have a DNA genome (Torres *et al.*, 1994).

Based on the results found in the present work it is important to know several aspects related to the TbRV epidemiology at the chile pepper growing areas such as those of the state of Guanajuato, studying their distribution, their dissemination pattern, their host range, their transmission by seeds, their cultural managing, and to know their effect with different types chile varieties, to be able to establish the control actions needed.

## CONCLUSIONS

The viruses detected in chile pepper cultivated in the state of Guanajuato were PVY, TbRV and the CMV. The virus TbRV was not been reported for Guanajuato, Mexico.

**Table 1. Virus detection in 42 chile pepper samples (*Capsicum annum*)**

Municipality Location	CMV	VIRUS PVY	TbRV	Collect sample date
Victoria de Cortazar	0/2 <sup>2</sup>	0/2	1/2	July 11, 1996
El Charro	0/2	0/2	0/2	July 11, 1996
El Colorado	0/8	1/8	1/8	July 11, 1996
Total	0/12	1/12	2/12	
Irapuato, Gto.				
R. Nuevo de Dolores	0/2	0/2	1/2	July 12, 1996
El Bosque	0/2	0/2	1/2	June 24, 1997
Arandas	0/4	0/4	2/4	June 29, 1997
Total	0/8	0/8	4/8	
Mezquite Gordo	0/1	1/1	1/1	July 29, 1997
Colonia Juárez	0/2	0/2	0/2	July 29, 1997
Total	0/3	1/3	1/3	
Silao, Gto.				
Tupatarito	0/2	0/2	0/2	July 29, 1997
San José del Durazno	1/3	0/3	1/3	July 29, 1997
Total	1/5	0/5	1/5	
Dolores Hgo, Gto.				
Cerrito de Guadalupe	0/6	4/6	5/6	August 10, 1997
Delicias	0/2	1/2	2/2	August 10, 1997
El Llano de Abajo	0/3	0/3	3/3	August 10, 1997
Total	0/11	5/11	10/11	
La Trinidad	0/2	0/2	2/2	August 10, 1997
El Rosario	0/1	0/1	1/1	August 10, 1997
Total	0/3	0/3	3/3	
Great Total	1/42	7/42	23/42	

<sup>2</sup>It indicates the presence of the virus in analyzed 0 samples of 2.