http://dx.doi.org/10.1590/S1516-8913201400219
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
AN INTERNATIONAL JOURNAL

Serological Identification of Virus in Watermelon Production Fields in the Tocantins State

Raimundo Wagner de Souza Aguiar[1*], Adelmo Rodrigues[1], Augustus Caeser Franke Portella[1], Magnolia de Mendonça Lopes[1], Mirtes Freitas Lima[2], Renato de Oliveira Resende[3] and Tatsuya Nagata[3]

[1]Setor Ciências Exatas e Tecnológicas; Universidade Federal do Tocantins; Gurupi - TO - Brasil. [2]Embrapa-Hortaliça; Brasília - DF - Brasil. [3]Departamento de Biologia Celular; Instituto de Ciências Biológicas; Universidade de Brasília; Brasília - DF - Brasil

ABSTRACT

Watermelon (Citrullus lanatus) cultivated in almost all tropical and subtropical regions of the world, has its largest output in China, and then, according to FAO data, Turkey, Iran and Brazil, being one of the main crops cultivated in State of Tocantins, Brazil. In this work was investigated the occurrence and distribution of the watermelon viruses, totaling 752 samples taken in a stratified experimental design in four representative regions of production: Gurupi (150), Lagoa da Confusao (232), Formoso do Araguaia (265) and Porto Nacional (105). The sampling and collecting the leaves of plants with the presence of symptoms were performed once a week during the entire cultivation cycle. As a result, were observed by Dot-ELISA method, different types of viruses, such as Papaya ringspot W (PRSV-W), Zucchini yellow mosaic virus (ZYMV), Watermelon mosaic virus (WMV) (potyvirus), Cucumber mosaic virus (CMV) (Cucumovirus) and Zucchini lethal chlorosis virus (ZLCV) (Tospovirus). Of these, PRSV-W was predominant (22%), followed by WMV (15%), ZLCV (11%), CMV (5%) and ZYMV (4%). Mixed infections with PRSV-W + WMV and PRSV-W + ZLCV were also observed around 20% frequency (expressed with symptoms differently from a single infection). The results provide important support for the program management viruses.

Key words: Watermelon, *Citrullus lanatus*, potyvirus, cucumovirus, tospovirus, serological assay

INTRODUCTION

Watermelon [*Citrullus lanatus*(Thunb.) Matsum. and Nakai] is a host species for some viruses that have direct effects on fruit production, with significant losses in several producing regions in Brazil. The watermelon crop in Tocantins State occupies an area of approximately 4,000 hectares with production reaching about 90.7 tons, generating an average yield of 25.5 tons/hectare. Plant virus diseases are critical problems in agriculture and occurrence may be excluded if preventive strategies are established (Rodrigues et al. 2009). Although the crop is of great economic and social importance in the state, there are few studies on the occurrence and species of viruses in the main producing regions. Identification of the major viruses present in watermelon crops provides support for further studies to establish integrated management of viruses in producing regions and development of virus-resistant cultivars.

Among the types of viruses infecting watermelon stand out: *Papaya ringspot virus* type: watermelon (PRSV-W) (Rezende and Pacheco 1997; Oliveira et al. 2000; Moura et al. 2001), *Zucchini yellow mosaic virus* (ZYMV) (Oliveira et al. 2000; Moura et al. 2001) and *Watermelon mosaic virus*

*Author for correspondence: rwsa@uft.edu.br

(WMV) (Sá and Kitajima 1991; Oliveira et al. 2000). These are well-known potyviruses in watermelon. Additionally, *Cucumber mosaic virus* (CMV) (cucumovirus) (Eiras et al. 2001) and *Zucchini lethal chlorosis virus* (ZLCV) (tospovirus) (Resende et al. 1996; Giampan et al. 2009) are also recognized as important causal agents in this crop. Transmission of these viruses takes place in a non-persistent manner by aphids, except for ZLCV, which is transmitted by the insect vector thrips (Rezende et al. 1997). Virus infection has a variety of symptoms in the field, perhaps because of the particular virus present in the host and possible mixed infections (Borém and Milach 1998). In Brazil, the occurrence of viruses in cucurbit-producing regions is variable and dispersion is dynamic because of the host species, virus species and migratory capacity of these vectors (Moura et al. 2001).

This study evaluated the prevalence and distribution of PRSV-W, ZYMV, WMV, CMV and ZLCV in watermelon-producing regions in Tocantins State.

MATERIAL AND METHODS

The Collection of watermelon plant leaves with possible symptoms of virus infection were performed according to Lima et al. (2009). The collection period occurred between January 2009 and January 2011, in watermelon culture fields located in the main producing regions of Tocantins State. A total of 752 samples were obtained from four cities: Gurupi (150 samples), Lagoa da Confusão (232 samples), Formoso do Araguaia (265 samples) and Porto Nacional (105 samples). Sampling and collection of plant leaves with symptoms was performed once a week throughout the crop cycle.

Then plant leaves with symptoms located in each area in transversal edges followed by zigzag type were collected and randomly. The plant leaves were packaged in plastic bags, identified, placed in coolers containing ice and, once in the laboratorial, samples were immediately processed. The analyses were performed at the Integrated Pest Management Laboratory, Federal University of Tocantins (Campus Gurupi) with the collaboration of Embrapa Hortaliças (CNPh, Brasilia).

Collection, maintenance and symptoms of isolates

Samples were collected from watermelon plants (cv. Crimson Sweet) with viral symptoms to obtain the inoculum. Preparation of inoculum-containing 1g leaves in suspension was done according to Lima et al. (2009) by the addition of 10 mL of the 0.01 M potassium phosphate buffer (K_2HPO_4) (pH 7.0) with 0.1% sodium sulfite (Na_2SO_3). The symptoms of sample with virus type previously identified were analyzed on *Cucurbita pepo* L., *Nicotiana benthamiana* Domim. and *Datura stramonium* Thunb. The virus inoculum (PRSV-W, WMV, ZYMV, ZLCV and CMV) were sprinkled with abrasive silicon carbide by rubbing the virus suspension against the leaf tissue and kept in watermelon plants until the appearance of symptoms for further analysis and to study expression of symptoms. The symptoms were assessed according to the severity rating system used by Oliveira et al. (2000), as follows: ruffled edges, blistering, spur, leaf narrowing, shoestring, leaf distortion, leaf rolling, necrosis, mosaic, stunting, and no symptoms. All viral symptoms in single and/or multiple infections were evaluated.

Serological identification of isolates WMV, CMV, ZYMV, PRSV-W and ZLCV

The viruses identification was performed by Dot-ELISA with specific antisera (kindly provided by Dr. Mirtes, Embrapa Hortaliças – CNPh) for WMV, CMV, ZYMV, PRSV-W and ZLCV present in samples collected from commercial crop fields. The samples were macerated and prepared according to Banttari and Goodwin (1985) at a ratio of 1g leaf tissue to 10 mL PBS solution (0.08 M $NaH_2PO\ 4H_2O$, 0.02 M K_2HPO_4, 1.4 M NaCl; 0.02 M KCl, pH 7.4). Sample extract (4 µL) was placed on a nitrocellulose membrane (Hybond-C; GE Life Sciences, Rydalmere, Sydney). The membrane was dried at room temperature for 30 min and incubated with blocking solution of 0.5% PBS plus 3% non-fat *Molico milk* under gentle agitation for 3 h. Subsequently, the membranes were placed in PBS solution containing specific antibodies for each isolated virus at a dilution of 1 µg/ml. The second antibody of goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma) was used at a dilution of 1:30 000 in 0.5%. For detection tests, BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) (Sigma FAST) substrates were used according to the manufacturer's protocol. The negative and positive controls (healthy plants and infected plants with virus WMV, CMV, ZYMV, PRSV-W and ZLCV) identified in CNPh were included in all assays).

Statistical analysis

The percentage of virus infection in the collected samples representative of each region of watermelon production in Tocantins State was evaluated by counting positive reactions by Dot-ELISA, being determined by the formula V% = (Number of positive samples in each region /Number of plants sampled) × 100, where V = percentage of viruses in the samples. All percentage data were obtained using the program Sigma Plot.

RESULTS AND DISCUSSION

PRSV-W was predominant in the Formoso do Araguaia Region, which represented 31% of the samples collected in this region (Fig. 1). This virus was also the most common in Gurupi and was detected in 12.5% of the samples. In the Lagoa da Confusão Region, WMV was found in 26.4% of the samples, followed by PRSV-W and ZLCV in 22% and 8.1% of the samples, respectively. In Porto Nacional, ZLCV virus was found in 13.8% of samples, followed by CMV (8.3%) and PRSV-W (10.3%) (Fig. 1).

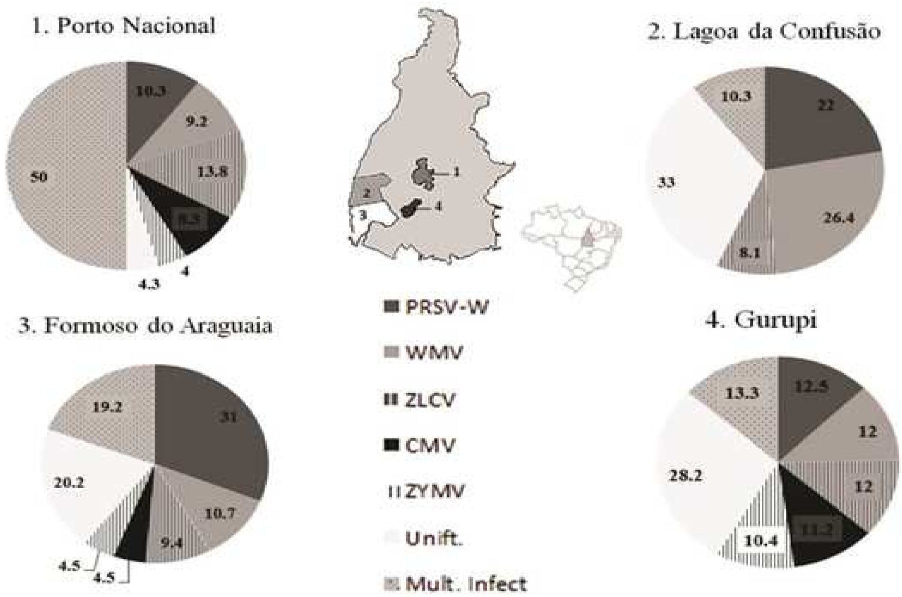

Figure 1 - Distribution space of viruses ZYMV, CMV, ZLCV, PRSV-W and WMV in Tocantins State, Brazil. Tocantins State Map indicating the four main watermelon-producing regions where the samples were collected with viral diseases symptoms.

The absence of reaction, ie, samples that did not respond to specific antisera in Lagoa da Confusão Region comprised 33% of the plants, Gurupi 28.2%, 20.2% and 4.3% in Formoso do Araguaia and Porto Nacional, respectively (Fig. 2). The occurrence of multiple infections was more pronounced in Porto Nacional (Fig. 1 and Table 1). However, double infections in Porto Nacional were predominantly PRSV-W + CMV with 21.6%, PRSV-W + WMV and ZLCV + CMV with 13.7% in both (Table 1). In Lagoa da Confusão Region only PRSV-W + WMV (43.8%), PRSV-W + ZLCV (39.1%) and ZLCV + WMV (17.4%) occurred as double infections. The double infections prevalent in Formoso do Araguaia were PRSV-W + ZLCV and PRSV-W + WMV with 16.3% in both, followed by ZLCV + WMV with 14.3% and PRSV-W + CMV with 12.2% (Table 1). In Gurupi, PRSV-W + WMV (31.6%) and PRSV-W + ZLCV (21.1%) were the most frequent double infections (Table 1).

The symptoms observed in greenhouse watermelon plants that received the inoculum of analyzed viruses did not differ significantly from the symptoms observed in the plants collected in field and later identified by serological test. The symptoms observed in the field and greenhouses were: mosaic, bubbles, narrowing leaf, leaf rolling, necrosis, spur and underdevelopment (Table 1 and Fig. 2). In addition, morphological changes were observed in fruit development, resulting in reduction of fruit size and external discoloration (Fig. 2). Some infections showed mild symptoms on plants with mosaic and stunted growth of the plant in those caused by CMV and PRSV-W. Normally, multiple infections caused severe symptoms such as leaf distortion and severe bumpy protrusions on the twigs of the plant.

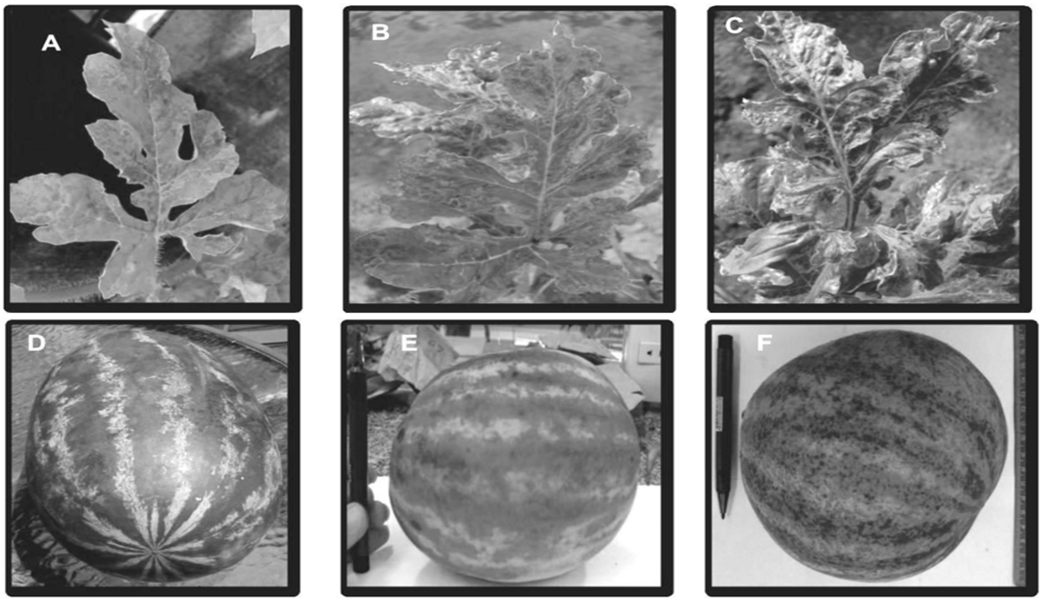

Figure 2 - Symptoms of viral infections of leaves and watermelon fruits. A. Mosaic with chlorotic rings. B. Bubbles. C. Narrowing leaf. D. Healthy fruit. E. Chlorosis of the shell. F. Deformation of the shell and chlorosis.

Table 1 - Average number of plants with single and multiple infections identified by serological test (Dot-ELISA) and expressed symptoms found in the main watermelon-producing regions in Tocantins.

Virus	------- **Watermelon producing regions**[1] ------				Sympt.[2]
	Porto Nacional	Lagoa da Confusão	Formoso do Araguaia	Gurupi	
		- Average Number of Infected plants -			
PRSV-W	11	51	82	19	M, Bl, Nl, Lr, Sp.
WMV	09	61	28	18	M, Bl, Nl.
ZLCV	14	19	25	18	M, Ne, Ud.
CMV	08	0	12	17	M, Nl, Lr, Ud.
ZYMV	04	0	12	16	M. Bl.
Unifected	04	76	53	42	-
Multiple infection	53	24	51	20	-
Total samples	105	232	265	150	
		- Average number of plants with multiple infection -			
PRSV-W+ZYMV	4.56 ± 1.66	-	4.16 ± 1.56	1.30 ± 0.66	M, Bl, Nl, Lr, Ud, M, Nl,
PRSV-W+ZLCV	5.01 ± 1.69	9.1 ± 2.66	8.09 ± 2.01	4.11 ± 1.21	Ne, Sp,Lr, Ud
PRSV-W+CMV	11.24 ±2.61	-	6.20 ± 1.15	2.22 ± 0.86	M, Ud.
PRSV-W+WMV	7.23 ± 1.66	10.5 ± 3.26	8.33 ± 2.86	6.12 ± 1.32	M, Bl, Ne.
ZYMV+ZLCV	2.22 ± 0.62	-	3.12 ±1.56	-	M, Bl, Ne.
ZYMV+CMV	1.96 ± 0.68	-	1.54 ± 0.26	-	M, Nl.
ZLCV+CMV	6.13 ± 1.96	-	5.20 ± 2.08	-	M, Lr, Ud.
ZLCV+WMV	7.76 ± 2.66	4.4 ± 1.47	7.20 ±2.90	2.17 ± 0.23	M, Bl, Ne, Ud.
CMV+WMV	6.24 ± 1.66	-	4.16 ± 1.03	1.03 ± 0.34	M, Bl, Nl, Ne, Sp, Ud.
Total mean	53.4 ± 1.56	24 ± 2.46	51.12 ± 1.65	20 ± 0.77	

[1]Tocantins state Regions (Brazil); [2]Symptoms (M=Mosaic, Bl=Bubbles, Nl=Narrowing leaf, Lr=Leaf rolling, Ne=Necrosis, Sp=Spur, Ud=Underdevelopment); Average ± SD.

This is believed to be the first report of the occurrence of potyviruses (PRSV-W, ZYMV and WMV), tospovirus (ZLCV) and cucumovirus (CMV) in watermelon crops in Tocantins State. PRSV-W, ZLCV and WMV were generally prevalent in the watermelon-producing regions in Tocantins in single and mixed infections. However, virus distribution differed between the regions. Several studies have shown that PRSV-W has a high incidence in cucurbit crops in Northeastern Brazil (Lima et al. 1997; Oliveira et al. 2000; Moura et al. 2001). According to those authors, this dominance may have been influenced by climatic factors inherent in the region, spontaneous vegetation and vector species, and the high virulence of this virus for the cucurbit family. The results observed here (Table 1) and those of similar studies in other regions of Brazil (Cruz et al. 1999; Oliveira et al. 2000; Yuri et al. 2000; Moura et al. 2001) and other parts of the world (Ullman et al. 1991, Luis-Arteaga et al. 1998) demonstrated that the incidence of virus on cucurbit species grown is very dynamic, varying with the virus strains and their climatic conditions, population and migration vectors and cultured species and natural reservoirs of virus in the predominant plant variety.
Studies performed in the Mato Grosso do Sul State found that PRSV-W was also predominant in cucurbits, followed by ZLCV and ZYMV (Stangarlin et al. 2000). This result was similar to that found in São Paulo and Roraima States, because among the viruses analyzed (PRSV-W, ZYMV, ZLCV, CMV and WMV), PRSV-W and ZYMV were the most frequently found viruses (Yuki et al. 2000). According to similar results presented in different regions, is suggested that the spread of virus in the field is more strongly related to its infective capacity and its specificity for the host species and its vector than to the environmental conditions.
The association of PRSV-W + WMV was the most frequent, representing 21.8% of the multiple infections. The associations between PRSV-W + ZLCV, ZLCV + WMV and PRSV-W + CMV were also significant, showing respective values of 18.3%, 14.1% and 13.4%. The results observed in this study suggested that the absence of this reaction in the leaf may be associated with infection by other viruses that commonly infect cucurbits. In this context, Alencar et al. (2012) reported the presence of SqMV in a cucurbits sample in Tocantins State, but this study did not identify SqMV infections due to the lack of this virus antiserum.
The occurrence of multiple infections under natural conditions is associated with factors such as virus type, host plant and insect population, which directly influences the virus predominance in a crop system. In this case, the lowest level of infection was observed in ZYMV, CMV and ZLCV (Table 1). In general, virus predominance has been associated with various factors, indicating an important ecological role in the virus maintenance in the crop system (Sánchez et al. 1999; Davino et al. 2006; Gomez et al. 2009). Several studies have reported differences in the interactions between virus in the laboratory and field conditions associated with the host plant and interaction with the insect vector (Gal-on and Shiboleth 2006).
The predominant occurrence of PRSV-W in our samples was probably due to its virulence and interaction with the host plant species and their vectors, when compared with other viruses (Fabre et al. 2010). The widespread presence of viruses in watermelon-producing areas of Tocantins may have been favored by the presence of crop residues, absence of watermelon cultivars with resistance to the potyvirus group, and the adoption of ineffective control measures. Fayad-André et al. (2011) have observed that high incidence of single and multiple infections of potyvirus, carlavirus and allexivirus in garlic-producing regions was associated with the low level of technology used by producers in the field. The rapid spread, coupled with the difficulty of vector control, could be minimized by adopting integrated pest management along with disposal of debris or inoculum sources (Lima et al. 2009). Therefore, in the context of research, this is a difficult problem when already installed. There are no curative measures for the control of viruses, and the sooner the plant is infected, the more severe the symptoms are and also the greater the production losses. Accordingly, we recommend the adoption of preventive measures to prevent or reduce the occurrence of viruses in the plants and their dissemination area.

CONCLUSIONS

Study of the distribution of PRSV-W, ZYMV, ZLCV, WMV and CMV in watermelon-producing areas in Tocantins State is important for establishing integrated management of viral diseases, as well as for studying population dynamics of insect vectors and interaction among prevalent viruses. Such information will serve to assist in the study of breeding to obtain watermelon cultivars that are resistant or tolerant to these viruses, ensuring continued productivity of watermelon in the Tocantins State.

REFERENCES

Alencar NE, Figueira AR, Almeida JEM, Lucas MA, Santos LB, Nascimento IR. Identificação biológica e molecular de vírus detectados em espécies de cucurbitáceas provenientes do estado do Tocantins. *J Biot and Biodiversity*. 2012; 3: 32-37.

Banttari EE, Goodwin BH. Detection of potato viruses S, X and Y by enzyme-linked immunosorbent assay on nitrocellulose membrane (Dot ELISA). Pl Disease. 1985; 69: 202-205.

Borém A, Milach SCK. Plant Breeding in the Turn of the Millenium. *Braz Arch Biol Technol.* 1998; 3: 278-283.

Cruz ES, Paz CD, Pio-ribeiro G, Batista DC, Pereira filho GG, Andrade GP. Levantamento de viroses em melancia e melão no Submédio São Francisco. *Summa Phytopathologica*. 1999; 25: 21.

Davino S, Napoli C, Davino M, Accotto GP. Spread of Tomato yellow leaf curl virus in Sicily: partial displacement of another geminivirus originally present. *Eur J P Pathology*. 2006; 14: 293-299.

Eiras M, Colariccio A, Chaves ALR. Isolated from cucumber mosaic virus obtained from banana in São Paulo belongs to subgroup Ia. *Fit Brasileira*. 2001; 26: 53-59.

Fabre F, Chadoeuf J, Costa C, Lecoq H, Desbiez C. Asymmetrical over-infection as a process of plant virus emergence. *J Theory Biology*. 2010; 265: 377-388.

Fayad-André MS, Dusi AN, Resende RO. Spread of viruses in garlic fields cultivated under different agricultural production systems in Brazil. *T P Pathology*. 2011; 36: 341-349.

Gal-on A, Shiboleth YM. Cross-protection. In: Loebenstein G, Carr JP (Eds) Natural resistance mechanisms of plants to viruses. *S Dordrecht.* 2006; 261-288.

Giampan JS, Rezende JAM, Piedade SMS. Damage caused by Zucchini lethal chlorosis virus (ZLCV) on production of commercial fruits of zucchini squash "Caserta". *S Phytopathologica*. 2009; 35: 223-225.

Gomez P, Sempere RN, Elena SF, Aranda MA. Mixed infections of Pepino Mosaic Virus strains modulate the evolutionary dynamics of this emergent virus. *J Virol*. 2009; 83: 12378-12387.

Lima JAA, Vale CC do, Oliveira VB. Viruses that infect cucurbits in the northeast of Brazil. *Viruses: Reviews & Research J Braz Soc for Virology*. 1997; 2: p.202.

Lima MF, Nagata T, Neves FM, Inoue-Nagata AK, Moita AW, Sousa C, Della Vecchia M, Rangel MG, Dias RCS, Dutra LS, Ávila AC. Detecção por sorologia de Melon yellowing associated virus (MYaV) em áreas produtoras de melão no Nordeste brasileiro. *H Brasileira*. 2009; 27: 478-483.

Luis-Arteaga M, Alvarez JM, Alonso Prados JL, Bernal JJ, Garcia-arenal F, Laviña A, Battle A, Morinoes E. Ocurrence, distribution, and relative incidence of mosaic viruses infecting field-grown melon in Spain. *Plant Disease*. 1998; 82: 979-982.

Moura MCCL, Lima JAA, Oliveira, VB, Gonçalves MFB. Serological identification of virus species infecting curcubitáceas producing areas of Maranhão. *Fit Brasileira*. 2001; 26: 90-92

Oliveira VB, Lima JAA, Vale CC, Paiva WO. Biological and serological characterization of potyvirus isolates obtained from cucurbits in northeastern Brazil. *Fit Brasileira*. 2000; 25: 628-636.

Resende RO, Pozzer L, Nagata T, Bezerra IC, Lima MI, De Brito Giordano L, Kitajima EW, Avila AC. New tospovirus found in Brazil. *A Horticulturae*. 1996; 43: 78-89.

Rezende JAM, Pacheco DA. Stability isolated weak premunizantes mosaic virus - watermelon strain. *Fit Brasileira*. 1997; 22: 64-68.

Rodrigues SP, Lindsey GG, Patricia MBF. Biotechnological approaches for plant viruses resistance: from general to the modern RNA silencing pathway. *Braz Arch Biol Technol*. 2009; 52, 795-808.

Sá PB, Kitajima EW. Characterization of an isolate of watermelon mosaic virus 2 (WMV-2) from Brazil. *Fit Brasileira*. 1991; 16: 217-233.

Sánchez-Campos S, Navas-Castillo J, Camero R, Soria C, Díaz JA, Moriones E. Displacement of tomato yellow leaf curl virus (TYLCV)-Sr by TYLCV-Is in tomato epidemics in Spain. *Phytopathology.* 1999; 89: 1038-1043.

Stangarlin OS, Dias PRP, Rezende JAM. Levantamento das viroses em cucurbitáceas no Estado do Mato Grosso do Sul, Brasil. In: XXIII Congresso Paulista de Fitopatologia, Campina SP, S Phytopathologica (Resumos). 2000; 26: 132.

Ullman DE, Cho JJ, German TL. Occurrence and distribution of cucurbit viruses in Hawaiian Islands. *Plant Disease.* 1991; 75:367-370.

Yuki WA, Rezende JAM, Kitajima EW, Barroso PAV, Kuniyuki H, Groppo GA, Pavan MA. Occurrence, distribution and relative incidence of five viruses infecting cucurbits in the state of São Paulo, Brazil. *P Disease*. 2000; 84: 516-520.

Received: June 26, 2014;
Accepted: August 31, 2014.