Scientia Agricola
http://dx.doi.org/10.1590/0103-9016-2014-0197

Note

Biological and molecular characterization of Brazilian isolates of *Zucchini yellow mosaic virus*

David Marques de Almeida Spadotti[1], Débora Targino Wassano[1], Jorge Alberto Marques Rezende[1]*, Luis Eduardo Aranha Camargo[1], Alice Kazuko Inoue-Nagata[2]

[1]University of São Paulo/ESALQ – Dept. of Plant Pathology and Nematology, C.P. 09 – 13418-900 – Piracicaba, SP – Brazil.
[2]Embrapa Vegetables, Rod. BR-060, km 09 (Brasília/Anápolis), C.P. 218 – 70351-970 – Brasília, DF – Brazil.
*Corresponding author <jrezende@usp.br>

Edited by: Emerson Medeiros Del Ponte

Received June 03, 2014
Accepted August 08, 2014

ABSTRACT: *Zucchini yellow mosaic virus* (ZYMV) causes substantial economic losses in cucurbit crops. Although ZYMV has been present in Brazil for more than 20 years, there is little information about the biological and molecular characteristics of the isolates found in the country. This study aimed to characterize the experimental hosts, pathotypes and genetic diversity of a collection of eleven Brazilian ZYMV isolates within the coat protein gene. For biological analysis, plant species from *Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Solanaceae*, and *Pedaliaceae* were mechanically inoculated and pathotypes were identified based on the reaction of a resistant *Cucumis melo*, accession PI414723. All of the cucurbit species/varieties and *Sesamum indicum* were systemically infected with all isolates. The nucleotide sequence variability of the coat protein gene ranged from 82 % to 99 % compared to the corresponding sequences of ZYMV isolates from different geographical locations. No recombination event was detected in the coat protein gene of the isolates.
Keywords: Potyvirus, nucleotide sequence, genetic diversity

Introduction

Zucchini yellow mosaic virus (ZYMV) belongs to the genus *Potyvirus*, family *Potyviridae*; this positive sense single-stranded RNA virus has a genome of approximately 9,600 nucleotides contained in elongated and flexuous particles that are approximately 750 nm long and 12 nm in diameter. The viral RNA encodes a polyprotein, which generates eleven proteins, after cleavage by virus-encoded proteases. The coat protein (CP) has a molecular weight of 36 kDa (Lisa and Lecoq, 1984; Adams et al., 2012). ZYMV is experimentally transmitted by 26 species of aphids in a non-persistent manner (Katis et al., 2006).

ZYMV affects all cultivated cucurbit species [zucchini squash (*Cucurbita pepo* L. cv. Caserta, squash (*Cucurbita maxima* Duch. Ex Lam.), pumpkin (*Cucurbita moschata* Duch. Ex Poir.), cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), and watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai.)] worldwide. In Brazil, ZYMV was primarily detected in the states of São Paulo and Santa Catarina in the early 1990s (Vega et al., 1995; Kurozawa et al., 2005). It is likely that ZYMV is currently present in all cucurbit-growing regions of Brazil. Infected plants exhibit severe leaf mosaic, yellowing and eventually "shoestring" symptoms in the leaves. The fruits are stunted, twisted and deformed, resulting in reduced yield making the plants unmarketable, especially zucchini squash. In addition to cultivated species, ZYMV can systemically infect certain wild cucurbit species (Desbiez and Lecoq, 1997). The experimental evaluation of yield losses caused by ZYMV and the potyvirus *Papaya ringspot virus* - type W (PRSV - W) in zucchini squash showed that plants that were infected at 5, 15, and 20 days after emergence did not produce marketable fruits (Pereira et al., 2007).

Although ZYMV has been present in Brazil for over 20 years and is associated with significant yield losses in cucurbit crops, little is known about some of the biological and molecular traits of Brazilian isolates. Such information may be critical for the understanding of disease epidemiology and breeding for resistant varieties and hybrids. Therefore, this study aimed to characterize eleven Brazilian isolates of ZYMV on experimental hosts, the pathotype and genetic diversity of the coat protein gene.

Materials and Methods

Virus isolates

ZYMV isolates were obtained from cucurbits grown in the states of Espírito Santo (ZYMV-ES), Pará (ZYMV-PA), Rio Grande do Norte (ZYMV-RN), Paraná (ZYMV-PR), Rio Grande do Sul (ZYMV-RS), São Paulo (ZYMV-RI and ZYMV-P), Mato Grosso (ZYMV-MT), and the Federal District (ZYMV-DF and ZYMV-Fe). A mild strain, named ZYMV-M, which was isolated by Rabelo and Rezende (2004), was also included in this study as a reference. All but the ZYMV-RI isolate, which was maintained *in vivo* for several months, were originally stored in dehydrated tissue at -20 °C. During the course of the host range experiments they were mechanically transmitted two or three times to zucchini squash plants. The isolates were maintained on zucchini squash plants by mechanical transmission. For mechanical inoculation, infected leaves were ground in a 0.02 M phosphate buf-

fer (pH 7.0) containing 0.02 M sodium sulfite and diluted 1:10 (w/v). The diluted inoculum was rubbed onto leaves that were dusted with carborundum.

Host range

The ZYMV isolates were mechanically inoculated onto two to six plants of various cucurbit species and other hosts listed in Table 1. The infection of test-plants was determined by examining the occurrence of local and/or systemic symptoms at 7, 14, and 21 days after inoculation. Infection was confirmed by plate trapped antigen – enzyme linked immunosorbent assay (PTA-ELISA) and reverse transcription – polymerase chain reaction (RT-PCR) analyses as described below. Whenever needed, the viral infection was also confirmed by back mechanical inoculation to *C. pepo* cv. Caserta.

Pathotype identification

The pathotype was determined based on the reaction of the ZYMV resistant *C. melo* accession PI414723 that was mechanically inoculated with ten severe isolates of ZYMV, as proposed by Pitrat and Lecoq (1984). *C. pepo* cv. Caserta plants were also inoculated as controls for infectivity of each isolate. The infection was assessed through symptom evaluation and by PTA-ELISA.

PTA-ELISA

The test plants were analyzed by plate-trapped antigen (PTA)-enzyme-linked immunosorbent assay (ELISA) (Mowat and Dawson, 1987) using polyclonal antiserum against purified virion of ZYMV. Appropriate negative and positive controls were included. The reaction was considered positive when the OD_{405nm} reading was at least three times that of the healthy sample.

RNA isolation and RT-PCR

Total RNA was extracted from leaf tissue according to Toth et al. (2002) and submitted to one-step RT-PCR in a thermocycler using the following parameters: 42 °C for 30 min; 94 °C for 2 min; 35 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 60 s; and a final elongation step at 72 °C for 10 min. RT-PCR was carried out using the primers ZY2 [5'-GCTCCATACATAGCTGAGACAGC-3'], which was derived from the NIb gene, and ZY3 [5'- TAGGCTTGCAAACGGAGTCTAATC-3'], which anneals to the 3' untranslated region (Thomson, 1995); to amplify a fragment of 1186 bp containing part of the NIb gene, the complete coat protein gene and most of the 3′ untranslated region.

The RT-PCR reaction mixture contained 12.5 µL of PCR Master Mix 2X (Fermentas), 1 µM of each primer, 1 unit of reverse transcriptase AMV (Avian myeloblastosis virus, Promega), 5.0 µL of RNA, and RNase-free water in a 25 µL final volume. The RT-PCR products were analyzed on a 0.8 % agarose gel that was stained with SYBR Safe DNA Gel Stain (Invitrogen) diluted 1:10,000 in a TBE buffer using the 1 Kb Plus DNA Ladder (Invitrogen) as the molecular size marker.

Nucleotide sequence analysis

The RT-PCR amplicons of each ZYMV isolate were purified using the Wizard SV Gel and PCR Clean-Up Sys-

Table 1 – Host range reaction of several species mechanically inoculated with 11 Brazilian ZYMV isolates and detection of the virus by PTA-ELISA.

Plants	ZYMV isolates										
	ES	PA	RN	PR	RS	RI	P	DF	MT	Fe	M
Cucurbita pepo cv. Caserta	M (+)	NLL, M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	NLL, M (+)	M (+)	M (+)	A (+)
C. moschata cv. Menina Brasileira	M (nt)	M (+)	M (nt)	M (nt)	M (nt)	M (nt)	M (nt)	M (nt)	nt (nt)	nt (nt)	M (nt)
C. maxima cv. Exposição	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	nt (nt)	M (+)
Cucumis sativus cv. Safira	M (+)	LLN, M (+)	M (+)	M (+)	M (+)	M (+)	LLN, M (+)	M (+)	M (+)	nt (nt)	nt (nt)
C. sativus cv. Aodai	M (+)	M (+)	M (+)	A (-)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)
Citrullus lanatus cv. Crimson Sweet	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	nt (nt)	M (+)
C. lanatus cv. Fair fax	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)
Cucumis melo cv. Ouro	A (-)	M (+)	LLN, M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	A (+)
C. melo cv. Caipira	M (+)	M (+)	LLN, M (+)	M (+)	LLN, M (+)	M (+)	M (+)	M (+)	M (+)	LLN, M (+)	LLN, M (+)
C. anguria	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)
Luffa cylindrica	M (+)	M (+)	CLL, M (+)	A (-)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)	M (+)
Chenopodium amaranticolor	CLL (nt)	CLL (nt)	CLL (nt)	CLL (nt)	CLL (nt)	CLL (nt)	CLL (nt)	A (nt)	nt (nt)	nt (nt)	A (nt)
C. quinoa	CLL (nt)	CLL (nt)	CLL (nt)	CLL (nt)	CLL (nt)	CLL (nt)	CLL (nt)	A (+)	nt (nt)	nt (nt)	A (nt)
Gomphrena globosa	A (-)	NLL (il+)	CLL (il+)	A (-)	A (-)	CLL (il+)	A (-)	A (-)	A (il+)	nt (nt)	A (il+)
Pisum sativum	A(il+)	CLL (il+)	A (il+)	A (-)	A (-)	A (il+)	CLL (il+)	A (-)	A (il+)	nt (nt)	A (-)
Phaseolus vulgaris	Nt (nt)	A (-)	nt (nt)	A (-)	A (-)	A (-)	A (-)	A (-)	nt (nt)	nt (nt)	nt (nt)
Nicotiana benthamiana	A (-)	A (-)	A (il+)	A (-)	M (+)	A (il+)	A (+)	A (-)	X (nt)	nt (nt)	A (-)
N. glutinosa	A (-)	X (nt)	A (-)	A (-)	A (-)	A (-)	A (-)	A (-)	A (-)	nt (nt)	A (-)
Sesamum indicum	A(+)	A (+)	A (+)	A (+)	A (+)	A (+)	A (-)	A (-)	M (+)	A (-)	A (-)

M: mosaic; CLL: chlorotic local lesion; NLL: necrotic local lesion; A: absence of symptoms; nt: not tested; -: ELISA negative; +: ELISA positive; X: dead plant; il: inoculated leaf.

tem and then directly sequenced in both directions using the same PCR amplification primers at Macrogen Inc., Seoul, Korea. The resulting nucleotide sequences of the coat protein gene were assembled using Electropherogram Quality Analysis software (http://asparagin.cenargen.embrapa.br/phph/) and then aligned using Clustal W (Thompson et al., 1994). The deduced amino acid sequences of the coat protein gene were obtained with the ExPASy proteomics server program (http://ca.expasy.org/tools/dna.html). The nucleotide and deduced amino acid sequences were compared with the corresponding sequences of 26 ZYMV isolates from different geographical regions that were deposited in the GenBank.

A phylogenetic analysis was conducted using the molecular evolutionary genetics analysis (MEGA) software version 5.0 (Tamura et al., 2011), and a phylogenetic tree was constructed using the neighbor-joining method and a maximum composite likelihood model with bootstrap analysis with 1000 repetitions. The nucleotide sequences of the coat protein gene of *Soybean mosaic virus* (SMV) and *Papaya ringspot virus* (PRSV) were used as out-groups. The nucleotide sequences of the coat protein gene of the Brazilian isolates of ZYMV were submitted to recombination analysis using the methods included in the RDP 3.0 package with default settings (Martin et al., 2010).

Results

Eleven ZYMV (ten severe and one mild) isolates that were collected from seven states in Brazil were mechanically inoculated into a range of 19 plant species belonging to *Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Solanaceae,* and *Pedaliaceae* (Table 1). Inoculated plants were maintained in a greenhouse without environmental control.

The majority of cucurbit species was systemically infected with all isolates and showed the typical mosaic symptoms, ranging from mild to severe in intensity (Table 1). The symptoms also included, in some cases, leaf narrowing, blistering, wilting, systemic necrosis and plant death. The ZYMV-M isolate caused mild symptoms, as expected, on all of the cucurbit species tested and caused an asymptomatic infection of *C. pepo* cv. Caserta and *C. melo* cv. Ouro. The local reaction of the cucurbit cotyledons after mechanical inoculation varied according to the species/variety and the virus isolate (Table 1; Figure 1A-C).

The ZYMV isolates ES, PA, RN, PR, RS, RI and P induced chlorotic local lesions on the inoculated leaves of *Chenopodium amaranticolor* (Figure 1E) and *Chenopodium quinoa,* whereas the mild isolate M did not induce symptoms. ZYMV-DF was asymptomatic on *C. quinoa*, but the virus could be serologically detected (Table 1). The isolates ZYMV- ES, PA, RN, RI, P, and MT infected *Pisum sativum*, but the infection was confined to the inoculated leaves as evidenced by chlorotic local lesions only for the isolates ZYMV-P (Figure 1D) and PA. ZYMV-PA induced necrotic local lesions on *Gomphrena globosa,* whereas ZYMV-RN and RI induced chlorotic local lesions.

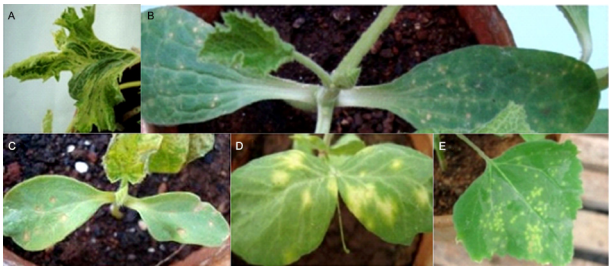

Figure 1 – Symptoms of Brazilian ZYMV isolates on different hosts. A) Mosaic, leaf deformation and stunting in *C. pepo* cv. Caserta infected with ZYMV- ES; B) Necrotic local lesions induced by ZYMV- DF in cotyledons of *C. pepo* cv. Caserta; C) Necrotic local lesions caused by ZYMV- RN in cotyledons of *C. melo* cv. Ouro; D) Chlorotic local lesions induced by ZYMV- P in *P. sativum* leaves; E) Chlorotic local lesions caused by ZYMV- PA in *C. amaranticolor*.

The isolates ZYMV-M and MT did not induce any symptoms in *G. globosa*, but the virus was detected in the inoculated leaves using PTA-ELISA and by back inoculation test. *S. indicum* plants were systemically infected by seven isolates of ZYMV, although only those inoculated with ZYMV-MT were symptomatic. *Nicotiana benthamiana* plants were systemically infected by ZYMV-RS and P, but only ZYMV-RS induced symptoms. ZYMV-RN and RI were asymptomatic in *N. benthamiana*. *Phaseolus vulgaris* and *Nicotiana glutinosa* plants were not infected by any ZYMV isolate. The ten severe ZYMV isolates did not infect *C. melo* PI414723; therefore, these isolates were classified as belonging to pathotype 0.

The ZY2 and ZY3 primers amplified a fragment of approximately 1186 bp for all of the Brazilian isolates containing the complete coat protein gene of the virus. The PCR products were directly sequenced, and the electropherograms were clear with a single peak with high confidence for all of the samples (data not shown). The coat protein gene of all of the isolates comprised 837 nucleotides, encoding a 279 amino acids protein. The nucleotide sequences were compared pairwise, and the nucleotide identity of the coat protein gene ranged from 93 % to 100 % among the Brazilian isolates and from 92 % to 94 % with isolate TW-TN3 (NC_003224) used as reference.

The coat protein sequence from the eleven Brazilian isolates and 26 isolates from different parts of the world were aligned, and a phylogenetic tree was constructed (Figure 2). The ZYMV isolates formed three distinct groups, named A, B, and C; group A was divided into four subgroups, as proposed by Coutts et al. (2011). The Brazilian isolates were all grouped within subgroups I and II of group A. No recombination event was detected in the coat protein gene of any of the Brazilian ZYMV isolates using seven independent RDP3 methods.

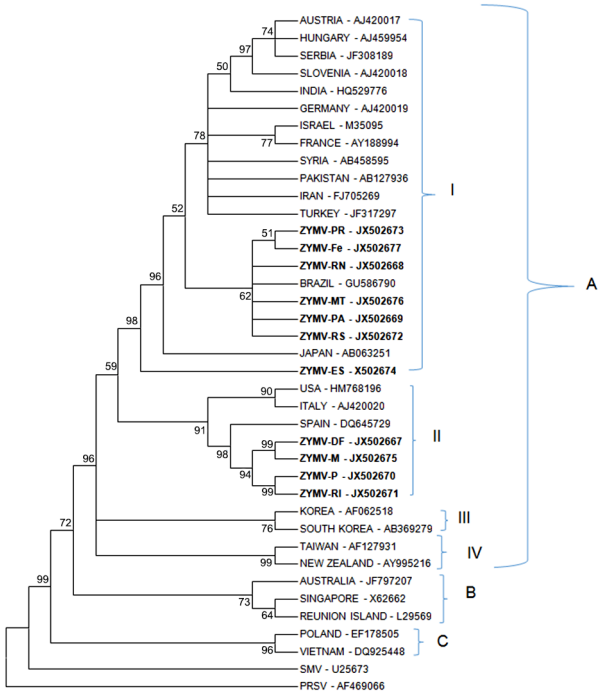

Figure 2 – Phylogenetic tree of ZYMV isolates generated based on the coat protein gene nucleotide sequences and the neighbor-joining method based on 1000 bootstrap replications. The numbers after the names represent the respective GenBank accession numbers. SMV and PRSV were designated as the out-group.

Discussion

The cucurbit species tested in this study were systemically infected with the ten severe isolates of ZYMV and exhibited symptoms of severe mosaic, followed by growth reduction, foliar malformation, and reduced plant growth. Interestingly, *C. melo* cv. Ouro was not infected by ZYMV-ES, and *Cucumis sativus* cv. Aodai and *Luffa cylindrica* were not infected by ZYMV-PR. A large variety of symptoms have been reported in plants inoculated with the ZYMV isolates collected from France (Lisa and Lecoq, 1984), USA (Provvidenti et al., 1984), and Australia (Coutts et al., 2011), similar to the results of our study. The ZYMV-M isolate caused only mild or asymptomatic infections of cucurbit species, confirming its potential for cross protection.

Among the non-cucurbit plants, *S. indicum* was the only species that was systemically infected with different ZYMV isolates. In Sudan, a ZYMV isolate causing severe mosaic and leaf deformation in *S. indicum* was reported in 1997 (Mahgoub et al., 1997). This is the first record of ZYMV infection of *S. indicum* under experimental conditions in Brazil. This infection, however, was mostly asymptomatic, except for isolate ZYMV-MT. *P. sativum* was systemically infected by ZYMV isolates from Lebanon and Israel, although the plants were asymptomatic (Lesemann et al., 1983; Antignus et al., 1989), showing only localized infection on the inoculated leaves for some Brazilian isolates of the virus. These results suggested that cucurbits are the most important virus source in the field, which is relevant in Brazil due to the numerous cucurbit species present in cultivated and non-cultivated areas. *S. indicum* could be a potential source of the virus, but further testing is required to test this hypothesis.

The nucleotide sequence identity of the coat protein gene among the Brazilian isolates of ZYMV ranged from 93 % to 100 %, and the deduced amino acid sequence identity ranged from 97 % to 100 %. Compared to the corresponding nucleotide sequences of ZYMV isolates from different geographic origins, the identity varied from 82 % to 99 %, whereas that of the deduced amino acid sequences varied from 87 % to 99 %.

The phylogenetic tree (Figure 2) constructed using the nucleotide sequences of the coat protein gene of Brazilian and 26 other ZYMV isolates from different regions of the world showed three well-supported groups (A, B and C) and four subgroups within group A, as proposed by Coutts et al. (2011). Clearly, the Brazilian isolates belonged to group A, subgroups I and II. The ZYMV-PR, Fe, RN, MT, PA, and RS isolates clustered in group A, subgroup I, along with a Brazilian isolate previously collected in the state of São Paulo State (GU586790) and with isolates from Asia and Europe, whereas isolates ZYMV-DF, M, P, and RI were in group A, subgroup II, with isolates from North America and Europe.

The isolate ZYMV-ES from the state of Espírito Santo is distantly related to subgroup I. The results suggest that ZYMV isolates may have been introduced into Brazil on at least three occasions, potentially with infected seeds from abroad. ZYMV-infected seeds may act as effective viral reservoirs and partially accounting for the current geographic distribution of the virus (Simmons et al., 2011). Coutts et al. (2011) cautiously suggested that absence of local lesion in *C. quinoa* might be a way to assign ZYMV isolates to A-II group. In the present study, of the four A-II isolates (ZYMV-DF, M, P, and RI), ZYMV-P and RI induced chlorotic local lesions in this species, that did not concur with previous reports (Coutts et al., 2011). The results also suggested that the coat protein gene sequence might not be associated with symptomatology in this host.

The *C. melo* PI414723 accession is the only source of resistance to ZYMV (Daning-Poleg et al., 2002) and is widely used in breeding programs not only because of its resistance to this virus but also because of its resistance to *Papaya ringspot virus*, the aphid *Aphis gossypii*, and the powdery mildew fungus *Podosphaera xanthii* (Pitrat and Lecoq, 1983; McCreight et al., 1992). Resistance to ZYMV is controlled by the Zym-1 gene (Périn et al., 2002) and possibly by two additional genes of minor genetic effects (Danin-Poleg et al., 1997). The reaction of PI414723 to ZYMV, however, is not uniform which led Lecoq and Pitrat (1984) to classify isolates of the virus into pathotypes based on three possible reactions of this

accession: no symptoms (pathotype 0), systemic necrosis (pathotype 1), and yellowing, mosaic and deformation of the leaves and wilting (pathotype 2). As the ten severe ZYMV isolates used in this study were assigned to pathotype 0, it is concluded that muskmelon hybrids carrying the Zym-1 resistance gene of this wild accession may be effective in controlling ZYMV in different melon-producing regions of Brazil.

References

Adams, M.J.; Zerbini, F.M.; French, R.; Rabenstein, F.; Stenger, D.C.; Valkonen, J.P.T. 2012. Family Potyviridae. p. 1069-1089. In: King, A.M.Q.; Adams, M.J.; Carstens, E.B; Lefkowitz, E.J., eds. Virus taxonomy: classification and nomenclature of viruses; Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA, USA.

Antignus, Y.; Raccah, B.; Gal-On, A.; Cohen, S. 1989. Biological and serological characterization of zucchini yellow mosaic and watermelon mosaic virus - 2 isolates in Israel. Phytoparasitica 17: 289-298.

Coutts, B.A.; Kehoe, M.A.; Webster, C.G.; Wylie, S.J.; Jones, R.A.C. 2011. *Zucchini yellow mosaic virus*: biological properties, detection procedures and comparison of coat protein gene sequences. Archives of Virology 156: 2119-2131.

Danin-Poleg, Y.; Paris, H.S.; Cohen, S.; Rabinowitch, H.D.; Karchi, Z. 1997. Oligogenic inheritance of resistance to *Zucchini yellow mosaic virus* in melons. Euphytica 93: 331-337.

Danin-Poleg, Y.; Tadmor, Y.; Tzuri, G.; Reis, N.; Hirschberg, J.; Katzir, N. 2002. Construction of a genetic map of melon with molecular markers and horticultural traits, and localization of genes associated with ZYMV resistance. Euphytica 125: 373-384.

Desbiez, C.; Lecoq, H. 1997. *Zucchini yellow mosaic virus*. Plant Pathology 46: 809-829.

Katis, N.I.; Tsitsipis, J.A.; Lykouressis, D.P.; Papapanayotou, A.; Margaritopoulos, J.T.; Kokinis, G.M.; Perdikis, D.C.; Manoussopoulos, I.N. 2006. Transmission of *Zucchini yellow mosaic virus* by colonizing and non-colonizing aphids in Greece and new aphid species vectors of the virus. Journal of Phytopathology 154: 293-302.

Kurozawa, C.; Pavan, M.A.; Rezende, J.A.M. 2005. Cucurbit diseases. = Doenças das cucurbitáceas. p. 293-302. In: Kimati, H.; Amorim, L.; Rezende, J.A.M.; Bergamin Filho, A.; Camargo, L.E.A., eds. Plant pathology = Manual de fitopatologia. Agronômica Ceres, São Paulo, SP, Brazil (in Portuguese).

Lecoq, H; Pitrat, M. 1984. Strains of *Zucchini yellow mosaic virus* in muskmelon (*Cucumis melo* L.). Journal of Phytopathology 111: 165-173.

Lesemann, D.E.; Makkouk, K.M.; Koenig, R.; Natafji Samman, E. 1983. Natural infection of cucumbers by *Zucchini yellow mosaic virus* in Lebanon. Phytopathologische 108: 304-313.

Lisa, V.; Lecoq, H. 1984. *Zucchini Yellow Mosaic Virus.* CMI/AAB, Kew, UK. (Descriptions of Plants Viruses, 282).

Mahgoub, H.A.; Desbiez, C.; Wipfscheibel, C.; Dafalla, G.; Lecoq, H. 1997. Characterization and occurrence of *Zucchini yellow mosaic virus* in Sudan. Plant Pathology 46: 800-805.

Martin, D.P.; Lemey, P.; Lott, M.; Moulton, V.; Posada, D.; Lefeuvre, P. 2010. RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics 26: 2462-2463.

McCreight, J.D.; Bohn, W.; Kishaba, A.N. 1992. Pedigree of PI 414723 melon. Cucurbit Genetics Cooperative Report 15: 51-52.

Mowat, W.P.; Dawson, S. 1987. Detection of plant viruses by ELISA using crude sap extracts and unfractionated antisera. Journal of Virological Methods 15: 233-247.

Pereira, M.J.Z.; Sussel, A.A.B.; Silva, R.F.; Kuhn, O.J.; Domingues, F.; Rezende, J.A.M. 2007. Evaluation of yield losses caused by ZYMV and PRSV-W in zucchini squash. Summa Phytopathologica 33: 192-194 (in Portuguese, with abstract in English).

Périn, C.; Hagen, L.S.; De Conto, V.; Katzir, N.; Danin-Poleg, Y.; Portnoy, V.; Baudracco-Arnas, S.; Chadoeuf, J.; Dogimont, C.; Pitrat, M. 2002. A reference map of *Cucumis melo* based on two recombinant inbred lines populations. Theoretical and Applied Genetics 104: 1017-1034.

Pitrat, M.; Lecoq, H. 1983. Two alleles for watermelon mosaic virus 1 resistance in melon. Cucurbit Genetics Cooperative Report 6: 52–53.

Pitrat, M.; Lecoq, H. 1984. Inheritance of *Zucchini yellow mosaic virus* resistance in *Cucumis melo* L. Euphytica 33: 57-61.

Provvidenti, R.; Gonsalves, D.; Humaydan, H.S. 1984. Occurrence of *Zucchini yellow mosaic virus* in cucurbits from Connecticut, New York, Florida, and California. Plant Disease 68: 443-446.

Simmons, H.E.; Holmes, E.C.; Gildow, F.E.; Bothe-Goralczyk, M.A.; Stephenson, A.G. 2011. Experimental verification of seed transmission of *Zucchini yellow mosaic virus*. Plant Disease 95: 751-754.

Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M.; Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731-273.

Thompson, J.D.; Higgins, D.G.; Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673-4680.

Thomson, K.G.; Dietzgen, R.G.; Gibbs, A.J.; Tang, Y.C.; Liesack, W.; Teakle, D.S.; Stackebrandt, E. 1995. Identification of *Zucchini yellow mosaic* potyvirus by RT-PCR and analysis of sequence variability. Journal of Virological Methods 55: 83-96.

Toth, I.K.; Hyman, L.J.; Berteau, Y.; Fréchon, D. 2002. Methods for the Detection and Quantification of *Erwinia carotovora* subsp. *atroseptica* (*Pectobacterium carotovorum* subsb. *atrosepticum*) on Potatoes: A Laboratory Manual. Scottish Crop Research Institute, Dundee, Scotland.

Vega, J.; Rezende, J.A.M.; Vuki, V.A. 1995. Detection of *Zucchini yellow mosaic virus* in Brazil: partial characterization of an isolate found in São Paulo. Fitopatologia Brasileira 20: 72-79 (in Portuguese, with abstract in English).