Note

BREAKDOWN OF RESISTANCE IN SWEET PEPPER AGAINST *Pepper yellow mosaic virus* IN BRAZIL

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ABSTRACT: Plants of *Capsicum annuum* cv. Magali R, resistant to *Pepper yellow mosaic virus* (PepYMV), which showed severe yellow mosaic, leaf malformation and stunting were observed during the 2003/04 growing season in Lins, São Paulo State, Brazil. Potyvirus-like particles observed in leaf sap from infected plants under the electron microscope reacted with an antiserum against PepYMV in PTA-ELISA. In addition to *C. annuum* cv. Magali R, this potyvirus also infected systemically the resistant *C. annuum* cv. Rubia R. The nucleotide sequence of part of the *CP* gene of this potyvirus shared 96–98% identity with that of other PepYMV isolates. The partial nucleotide sequence of the 3' NTR showed 94–96% identity with that of PepYMV. These data indicate that this potyvirus is a resistance-breaking isolate of PepYMV.
Key words: Potyvirus, RT-PCR, ELISA, resistance gene

QUEBRA DA RESISTÊNCIA EM PIMENTÃO CONTRA O *Pepper yellow mosaic virus*

RESUMO: Plantas de *Capsicum annuum* cv. Magali R, resistentes ao *Pepper yellow mosaic virus* (PepYMV), exibindo sintomas severos de mosaico amarelo, malformação foliar e subdesenvolvimento foram encontradas em plantios na região de Lins, SP, Brasil, em 2003/04. Partículas semelhantes àquelas do gênero *Potyvirus* foram observadas em extrato foliar de planta infectada examinado em microscópio eletrônico de transmissão. O extrato foliar também reagiu com anti-soro contra o PepYMV em PTA-ELISA. Além de *C. annuum* cv. Magali R, esse potyvirus também infectou sistemicamente *C. annuum* cv. Rubia R, que é resistente ao PepYMV. A seqüência de nucleotídeos de parte do gene da proteína capsidial (*CP*) desse potyvirus apresentou 96–98% de identidade com a de outros isolados do PepYMV. A seqüência parcial de nucleotídeos da região 3' não traduzida (3' NTR) apresentou 94–96% de identidade com a do PepYMV. Esses resultados são indicativos de que o potyvirus que quebrou a resistência em pimentão é um isolado do PepYMV.
Palavras-chave: Potyvirus, RT-PCR, ELISA, gene de resistência

INTRODUCTION

During the 1960s, *Potato virus Y* (PVY) was the most prevalent and economically important virus on sweet pepper (*Capsicum annuum* L.) crops in Brazil (Nagai, 1983). The disease caused by this potyvirus was controlled after the introduction of several PVY-resistant cultivars in the 1970s (Kurosawa et al., 2005). However, during the early 1990s, the occurrence of a PVY isolate that was able to overcome the resistance of those cultivars was reported. This isolate, then referred to as PVY^{m} or $PVY^{1,2}$ (Boiteux et al., 1996) was recognized as a new species of potyvirus, named *Pepper yellow mosaic virus* (PepYMV) (Inoue-Nagata et al., 2002).

As this virus became prevalent in sweet pepper crops in Brazil, the introduction of virus-resistant hybrids such as Magali R and Rubia R were landmarks for the sweet pepper crop because of their resistance to PVY strains 0, 1, and 1,2, and PepYMV. As the resistance to PVY strains is conferred by the *Pvr4* gene from *C. annuum* 'Serrano Criollo de Morelos 334

('SCM 334'), which also confers resistance to *Pepper mottle virus* (PepMoV) (Palloix & Kyle, 1995; Dogimont et al., 1996; Andrés et al., 1998; Echer & Costa, 2002), it is supposed that the same gene might be responsible for the resistance to PepYMV.

During the 2003/04 growing season, 80% of Magali R plants from a greenhouse planting in São Paulo State, Brazil were found exhibiting severe mosaic. Preliminary electron microscopic analysis of extracts from diseased plants showed the presence of potyvirus-like particles, which were further characterized as a strain of PepYMV, hereby designated as PepYMV-Lins.

MATERIAL AND METHODS

Leaf samples from *C. annuum* cv. Magali R, which exhibited stunting, severe yellow mosaic, and malformed leaves, were collected from a commercial crop in Lins, São Paulo State, Brazil, for virus isolation. Leaf extracts of these plants, prepared in 0.02 M phosphate buffer, pH 7.0, were rub-inoculated on leaves of five *C. annuum* cultivars, and other species listed in Table 1. The original isolate of PepYMV used by Inoue-Nagata et al. (2002) to describe this species of potyvirus was also inoculated as control.

Aphid transmission tests of PepYMV-Lins were carried out with *Aphis gossypii* and *Myzus persicae* raised on *Nicotiana tabacum* and *Raphanus raphanistrum*, respectively. Aphids were fasted for 1 h and then transferred onto *C. annuum* cv. Magali infected with PepYMV-Lins, for a ten min acquisition access period and were transferred to healthy Magali R plants (20 aphids per plant) for a 24 h inoculation access period. Ten and 15 plants were inoculated with *M. persicae* and *A. gossypii*, respectively.

All test-plants were maintained in a greenhouse for evaluations based on disease expression and virus detection in inoculated and newly emerged leaves by PTA-ELISA (Mowat & Dawson, 1987). Diluted (1:1000) polyclonal antiserum against PepYMV (Inoue-Nagata et al., 2002) was used. Leaf extracts from healthy and PepYMV (original isolate) infected pepper plants were used as negative and positive controls, respectively.

PepYMV-Lins was purified from fresh symptomatic leaves of experimentally infected cv. Magali R plants, as described by Lane (1992). RNA was extracted from virus suspensions using phenol/chloroform (1:1 v/v), followed by precipitation with 100% ethanol. The pellets were washed with 70% (v/v) ethanol and resuspended in 20 mL diethyl pyrocarbonate-treated, sterile water. cDNA was amplified using the PV1 universal potyvirus primer (5'-GAT TTA GGT GAC ACT ATA GT_{16}-3') (Gibbs & Mackenzie, 1997), which anneals to the 3' poly (A) tract of potyviruses and WCIEN primer (5'-ATG GTT TGG TGY ATY GAR AAT-3') (Mota et al., 2004), which anneals to the WCIEN motif of the coat protein of potyviruses. This pair of primers amplify a fragment of approximately 800 bp. Nucleotide sequences directly obtained from purified PCR product were compared with those of other potyviruses deposited in GenBank using the Blastn program (http://www.ncbi.nlm.nih.gov/BLAST) and Clustal W (http://www.ebi.ac.uk/Tools/clustalw/index.html).

Table 1 - Reactions of species/cultivars to PepYMV and PepYMV-Lins isolated from Lins, São Paulo State, Brazil.

Species/cultivars	PepYMV	PepYMV-Lins
C. annuum (cv. Magali)	M*	M
C. annuum (cv. Magali R)	NLL	M/ S/ LM/ TN
C. annuum (cv. Magda)	YM	M
C. annuum (cv. Rubia R)	-	M/ S/ LM/ TN
C. annuum (cv. Ikeda)	M	M
Chenopodium amaranticolor	-	-
Cucurbita pepo (cv. Caserta)	-	-
Datura metel	NLL/SN	NLL/SN
D. stramonium	-	-
Gomphrena globosa	-	-
Lactuca sativa	-	-
Lycopersicon esculentum	-	-
Nicotiana benthamiana	M/LM	M/ LM
N. clevelandii	M	M
N. rustica	M	M
N. tabacum	-	-
Petunia hybrida	-	-
Solanum tuberosum cv. Monalisa	-	-
S. tuberosum cv. Agata	-	-
S. tuberosum cv. Atlantic	-	-
S. tuberosum cv. Aracy	-	-

*SN, systemic necrosis; NLL, necrotic local lesion; M, mosaic; YM, yellow mosaic; LM, leaf malformation; S, stunting; TN, tip necrosis; –, absence of symptoms.

RESULTS AND DISCUSSION

PepYMV and PepYMV-Lins induced the same reaction in the majority of the inoculated test-plants, except on *C. annuum* cvs. Magali R and Rubia R, in which PepYMV-Lins caused mosaic, leaf malformation, necrosis on apical leaves and plant stunting (Table 1). *A. gossypii* and *M. persicae* transmitted PepYMV-Lins to 80% and 100% of the inoculated plants, respectively. Infection of all test-plants was confirmed by PTA-ELISA.

Nucleotide sequence of the 3' terminal of the coat protein (*CP*) region (378 nt) of PepYMV-Lins had 98 and 96% identity with the corresponding region of two isolates of PepYMV deposited in GenBank (AF348610 and EF488081), respectively. Deduced amino acid sequence identities for *CP* were 100 and 99%, respectively. Nucleotide identities for the 3' nontranslated region (NTR) (258 nt) were 96 and 94%, respectively. Collectively these data indicate that the potyvirus that infected the sweet pepper plants was a resistance-breaking isolate of PepYMV.

The importance of potyvirus diseases in sweet pepper crops is recurrent because of the emergence of new strains or species, which overcome resistance genes incorporated in commercial sweet pepper cultivars. This is a phenomenon that has been reported for other potyviruses such as *Lettuce mosaic virus* (Krause-Sakate et al., 2002) and *Soybean mosaic virus* (Choi et al., 2005).

The PepYMV-Lins strain may have been selected from the natural virus population after large-scale cultivation of resistant hybrids. Although the present data do not allow the identification of the origin of this strain, the presence of PepYMV-Lins is a threat to the sweet pepper crop in Brazil because it can be disseminated by aphids. Rapid identification of new sources of resistance genes is necessary for incorporation into new cultivars and hybrids. At present, however, a management of the different existing resistant and susceptible cultivars may be an alternative to slow the selection and spread of these virulent PepYMV strains.

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