

Solanum americanum: reservoir for *Potato virus Y* and *Cucumber mosaic virus* in sweet pepper crops

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ABSTRACT

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Weeds can act as important reservoirs for viruses. *Solanum americanum* (Black nightshade) is a common weed in Brazil and samples showing mosaic were collected from sweet pepper crops to verify the presence of viruses. One sample showed mixed infection between *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY) and one sample showed simple infection by PVY. Both virus species were transmitted by plant extract and caused mosaic in tomato

(*Solanum lycopersicum* cv. Santa Clara), sweet pepper (*Capsicum annuum* cv. Magda), *Nicotiana benthamiana* and *N. tabaccum* TNN, and local lesions on *Chenopodium quinoa*, *C. murale* and *C. amaranticolor*. The coat protein sequences for CMV and PVY found in *S. americanum* are phylogenetically more related to isolates from tomato. We conclude that *S. americanum* can act as a reservoir for different viruses during and between sweet pepper crop seasons.

Additional keywords: Weeds, *Cucumovirus*, *Potyvirus*

RESUMO

Moura, M. F.; Soman, M.; Mituti, T.; Pavan, M. A.; Krause-Sakate, R. *Solanum americanum*: reservatório de *Potato virus Y* e *Cucumber mosaic virus* em áreas de cultivo de pimentão. *Summa Phytopathologica*, v.40, n.1, p.78-80, 2014.

As plantas daninhas podem atuar como importantes reservatórios de vírus. *Solanum americanum* (Maria Pretinha) é uma planta daninha comum no Brasil e amostras com mosaico foram coletadas em áreas produtoras de pimentão, para verificar a presença de vírus. Uma amostra apresentou infecção mista com *Cucumber mosaic virus* (CMV) e *Potato virus Y* (PVY) e uma amostra apresentou infecção simples com PVY. As duas espécies virais foram transmitidas por extrato vegetal e ocasionaram mosaico em tomate (*Solanum*

lycopersicum cv. Santa Clara), pimentão (*Capsicum annuum* cv. Magda), *Nicotiana benthamiana* e *N. tabaccum* TNN, lesões locais em *Chenopodium quinoa*, *C. murale* e *C. amaranticolor*. As sequências de proteína capsial para o CMV e PVY encontradas em *S. americanum* estão filogeneticamente mais relacionadas com isolados de tomate. Conclui-se que *S. americanum* pode atuar como reservatório de diferentes vírus, durante e entre as épocas de cultivo de pimentão.

Palavras-chave adicionais: plantas daninhas, *Cucumovirus*, *Potyvirus*

Solanum americanum Mill. (black nightshade) is an annual plant classified into the family Solanaceae. In Brazil, this species is commonly found in tomato and pepper crops. Species of begomoviruses (1), tospoviruses (8), *Potato virus Y* (PVY) (5) and *Cucumber mosaic virus* (CMV) (9) have already been reported for that host.

S. americanum plants showing mosaic symptoms and leaf distortion were found naturally occurring in sweet pepper crops in São Paulo State (Lins region, October 2011) and the presence of viruses was investigated (Figure 1).

Double-antibody sandwich (DAS)-ELISA was carried out for the presence of potyvirus genus by utilizing antiserum anti-potyvirus (Agdia Inc., Elkhart, IN) (11). Two potyvirus positive samples (71d and 72d) were also analyzed by leaf dip, using Uranyl acetate (3%) as contrast solution, under an electron microscope (CM100 - Phillips), and 800nm filamentous particles, of potyvirus type, were observed for both

samples. Presumed isometric particles were observed on sample 72d.

For the biological tests, sap from the leaf samples 71d and 72d was prepared in 0.02 M phosphate buffer, pH 7.0, carborundum as abrasive, and inoculated into *Nicotiana tabaccum* L. "TNN", *N. benthamiana* Domin, *Chenopodium quinoa* Willd., *C. amaranticolor* Coste and Reyn, *C. murale* L., *Capsicum annuum* L. cv. Magda and *Solanum lycopersicum* Mill cv. Santa Clara. Mottle on *C. annuum*, *N. benthamiana*, *N. tabaccum* cv. TNN and *S. lycopersicum*, and local lesions on *C. quinoa*, *C. murale* and *C. amaranticolor* were observed for samples 71d and 72d.

Total RNA was extracted from the original plants and from the inoculated plants by using the protocol described by (3). RT-PCR was performed with the primer pairs WCIE (5' ATG GTT TGG TGY ATY GAR AAT 3') (12) and PV1 (5' GAT TTA GGT GAC ACT ATA GTT TTT TTT TTT TTT 3') (7), general primers for *Potyvirus* genus.



Figure 1. Mosaic symptoms and leaf distortion on *Solanum americanum* collected from the field: isolate 71d (A) and isolate 72d (B).

To detect CMV, the primer pairs 035-3 (5' GCC GTA AGT GGAT GG ACA A 3') and 038-8 (TAT GAT AAG AAR CTT GTT TCG CG 3') were employed (4) in a one-step reverse transcription (RT)-PCR assay, using the cycle 42°C/30 min, 95°C /2 min and 35 cycles of 94°C/ 1 min, 55°C/ 2 min and 72°C/ 2 min, ending with 72°C / 5 min.

For the potyvirus fragment, a nucleotide identity of 94% was observed with PVY (GenBank Accession No AJ439545.1). To amplify the complete coat protein of PVY, the primer pairs PepNib (5' GWTSGYYGMMTTGGATGATG 3') and PepUTR (5' AGTAGTACAGGAAAAGCC 3') were used as described by (11) and the sequence was compared with those deposited in GenBank by using the programs Blastn (<http://www.ncbi.nlm.nih.gov/BLAST>) and

Clustal W (<http://www.ebi.ac.uk/Tools/clustalw/index.html>). For the complete CP, a nucleotide sequence identity of 97% was observed with PVY (GenBank Accession No HQ594528.1) for sample 72d (GenBank Submission No 1602271). A mixed infection between PVY (GenBank Submission No. 1602256) and CMV (GenBank Submission No. 1602253) was observed for sample 71d and confirmed by the nucleotide identity of 99% with PVY (GenBank Submission No. 1602256) and 96% with CMV (GenBank Accession No. JN054635.1).

Both CMV and PVY found in *S. americanum* are closely related to isolates collected from tomato (Figure 2 and 3), even though this isolate has been collected from sweet pepper crops. The biological experiments confirmed that CMV and PVY from *S. americanum* can

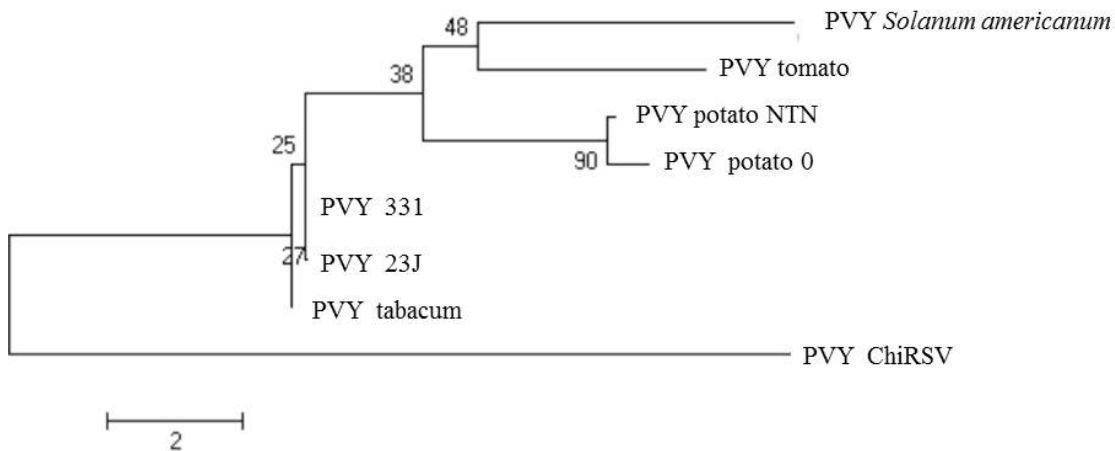


Figure 2. Phylogenetic tree obtained by the program Mega 5.0, bootstrap value 2000 replicates based on the nucleotide sequences of the CP coding region: PVY (Isolate 71d), PVY tomato (GenBank Access: JF 804783), PVY potato NTN (GenBank Access: JQ 924285), PVY potato 0 (GenBank Access: JQ 924285), PVY capsicum 331 (GenBank Access: HG 594528), PVY capsicum 23J (GenBank Access: 594529), PVY tabacum (GenBank Access: JN 711118). Outgroup *Chilli ringspot virus* (ChiRSV) (GenBank Access: DG 925438).

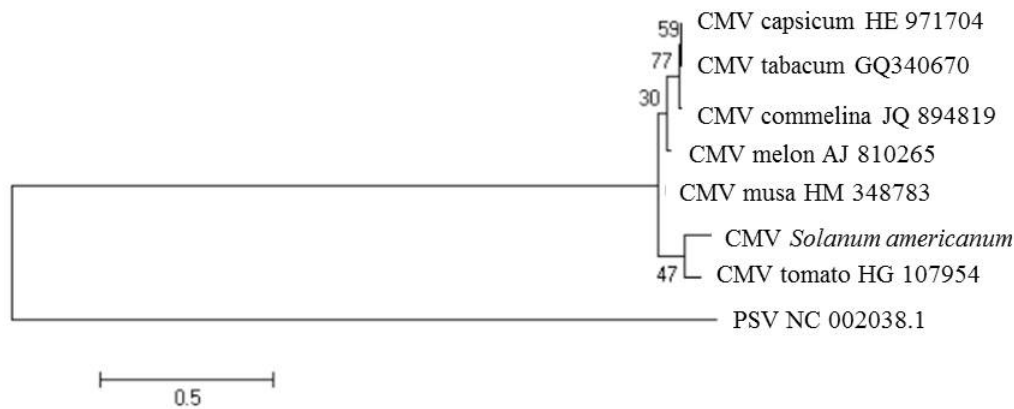


Figure 3 Phylogenetic tree obtained by the program Mega 5.0, bootstrap value 2000 replicates for the nucleotide coding region of the coat protein. CMV tomato (GenBank Access: HQ 107954), CMV melon (GenBank Access: AJ 810265), CMV capsicum (GenBank Access: HE 971704), CMV tabacum (GenBank Access: GQ 340670), CMV commelina (GenBank Access: JQ 894819), CMV musa (HM 348783). Outgroup *Peanut stunt virus* (PSV) (GenBank Access: NC 002038.1).

infect tomato and sweet pepper, and the latter host is a reservoir for these viruses during and between the sweet pepper crop seasons. *S. americanum* has been reported as an important reservoir for PVY in sweet pepper crops (9).

In São Paulo, PepYMV is the most frequently found potyvirus infecting sweet pepper, and infections by CMV and PVY (pathotype zero) can be observed occasionally (10). The increased use of resistant pepper material for PVY can explain the low incidence of PVY for this culture (10). Understanding the epidemiological role played by reservoir plants in the maintenance of viral diversity and in the acquisition of novel or enhanced virulence towards the cultivated host can help improve the management of the resistance gene.

In different cases, an emerging disease appears when a pathogen “jumps” to a host which was not infected by it (or no longer infecting) and adapts (or readapts) to it (2). RNA viruses, such as CMV and PVY, are likely to evolve in a reasonable experimental time because they have large population size, rapid replication rates and a high mutation rate (6).

Thus, periodically observing the incidence of *Potyvirus* and *Cucumovirus* in Solanaceae producing areas is important in order to verify a possible re-emergence of PVY and CMV, since these viruses are still present in the field, preferentially in weed species and not in cultivated plants. This result shows the high adaptability of important viruses in weeds such as black nightshade. We conclude that *S. americanum* is a natural reservoir for viruses and can act as a mixing vessel for different viruses affecting solanaceous in Brazil.

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