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New or Unusual Disease Reports

Molecular and serological detection of Parietaria mottle virus in *Phytolacca americana*, a new host of the virus

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Summary. Parietaria mottle virus (PMoV) is an emerging virus in Mediterranean countries, responsible for severe disease in tomato and pepper crops in the field and protected cultivation. The principal wild reservoir of PMoV is *Parietaria officinalis*, and only few additional wild plants have been described as natural reservoirs of the virus. During field survey in southern Italy, several plants of *Phytolacca americana* showing virus-like symptoms were collected. Serological and molecular assays showed that these plants were infected by PMoV. Sequence comparison of the movement protein gene of the PMoV isolate from *P. americana* showed the greatest similarity to the corresponding sequence from tomato plants growing nearby. These results indicate that *P. americana* is a new natural host of PMoV, and further investigation is warranted to establish the potential of this host as reservoir of the virus in the field.

Keywords. PMoV, alien species, American pokeweed, natural host, emerging viruses.

INTRODUCTION

Phytolacca americana L. (*Phytolaccaceae*), commonly known as “American pokeweed”, “pokeweed” or “dragonberries”, is a poisonous perennial geophyte herb native to North America. It was introduced in Europe where it is invasive in many countries, including Italy (Galasso *et al.*, 2018). This neophyte plant mainly spreads in anthropic and disturbed environments (Stinca and Motti, 2017; Bonanomi *et al.*, 2018). In July 2020, during a survey of tomato virus diseases in southern Italy (Campania region), several individuals of *P. americana* were noticed showing virus-like symptoms, comprising mosaic, chlorotic spots and lines and leaf distortions (Figure 1). The detected plants were growing close to a family garden, located in the Sorrento peninsula, where Parietaria mottle virus (PMoV) was detected in a local variety of tomato with high incidence. Distances from pokeweed plants to the tomato plants ranged between 5 to 20 m. The presence of thrips individuals on

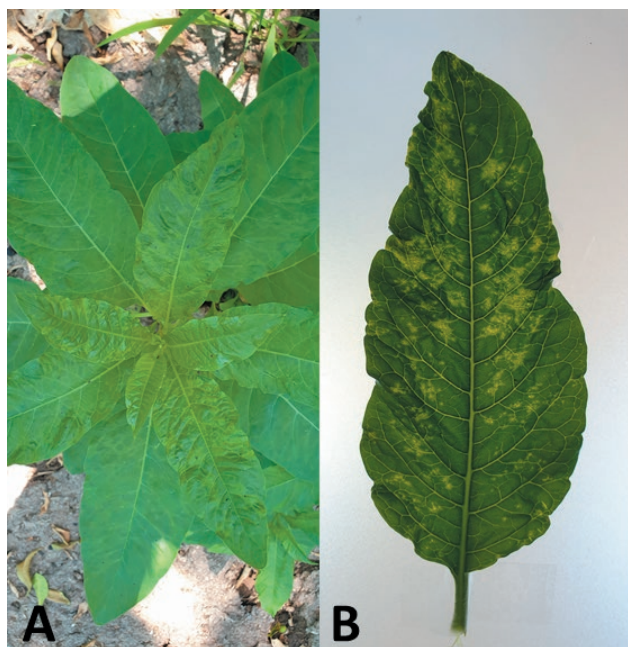


Figure 1. Symptoms associated to Parietaria mottle virus infection in *Phytolacca americana* L.: (A) mosaic on the apical leaves, and (B) details of symptoms on a leaf.

tomato and pokeweed plants was also observed. Overall, field observations suggested possible implication of PMoV as the cause of the symptoms observed in pokeweed plants.

MATERIALS AND METHODS

Leaf-sap from three symptomatic and two asymptomatic *P. americana* plants, and two symptomatic tomato plants, was mechanically inoculated to *Chenopodium quinoa* Willd., *C. amaranticolor* (H.J.Coste & A.Reyn.) H.J.Coste & A.Reyn., *Vigna unguiculata* (L.) Walp. 'Black eye' and *Nicotiana tabacum* L. 'Xanthi nc.'

The same samples were tested by antigen-coated-plate enzyme-linked immunosorbent assay (ACP-ELISA) with a specific PMoV polyclonal antibody (Lisa *et al.*, 1998), following the procedure of Parrella (2020). In addition, samples were tested by cucumber mosaic virus (CMV) and tomato spotted wilt virus (TSWV)-specific lateral flow (LF) kits (Pocket Diagnostic), since in tomato symptoms induced by PMoV resembling those induced by CMV and TSWV in single or mixed infections have also been reported (Ramasso *et al.*, 1997; Roggero *et al.*, 2000).

Total RNAs were extracted with the E.Z.N.A.[®] Plant RNA Kit (Omega Bio-tek), and RT-PCR using the PMoV specific primers PMoVMP1a and PMoVMP2b, encompassing the entire PMoV movement protein (Parrella *et al.*, 2016). Amplicons of the expected size (916 bp) obtained from symptomatic samples were cloned into pGEM-T vector (Promega), and were sequenced at Microsynth Seqlab (Göttingen, Germany). Blast analysis was performed online (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), and fifteen sequences of other PMoV isolates (Table 1) were downloaded and were used to determine

Table 1. Parietaria mottle virus isolates used in the present study.

Isolate	Host	Geographic origin	Year of collection	Accession No.
Pha1	<i>Phytolacca americana</i>	Campania, Italy	2020	MW248388
390	<i>Solanum lycopersicum</i>	Campania, Italy	2020	MW456562
391A	<i>Solanum lycopersicum</i>	Campania, Italy	2020	MW456563
Sar-1	<i>Solanum lycopersicum</i>	Sardinia, Italy	2019	MN782302
Ruc1	<i>Diplotaxis tenuifolia</i>	Campania, Italy	2016	KX866978
Ruc2	<i>Diplotaxis tenuifolia</i>	Campania, Italy	2016	KX866979
Fri-1	<i>Capsicum annuum</i>	Campania, Italy	2015	LT160068
Fri-2	<i>Capsicum annuum</i>	Campania, Italy	2015	LT160070
Pap-1	<i>Capsicum annuum</i>	Campania, Italy	2015	LT160069
M31A	<i>Solanum lycopersicum</i>	Catalonia, Spain	2006	AM182749
G34H	<i>Solanum lycopersicum</i>	Catalonia, Spain	2006	AM182744
AC1	<i>Solanum lycopersicum</i>	Catalonia, Spain	2006	AM182743
SD2	<i>Solanum lycopersicum</i>	Catalonia, Spain	2006	AM182745
RAMS1	<i>Solanum lycopersicum</i>	Catalonia, Spain	2006	AM182748
So7J	<i>Solanum lycopersicum</i>	Catalonia, Spain	2006	AM182746
JBT1	<i>Solanum lycopersicum</i>	Catalonia, Spain	2006	AM182747
CR8	<i>Solanum lycopersicum</i>	Catalonia, Spain	2001	FJ858204
T32	<i>Solanum lycopersicum</i>	Piedmont, Italy	1979	KT005245

the phylogenetic relationships among them and the pokeweed isolate (named Pha1). Multiple sequence alignment was performed using the ClustalW algorithm of MEGA 6.0 (Tamura *et al.*, 2013), with gap opening penalty of 15 and gap extension penalty of 6.66. A phylogenetic tree was constructed using the neighbor-joining (NJ) method with 1000 bootstrap replicates, and genetic distance was calculated by the Tamura three-parameter model which was determined as the best-fitting model of substitution using MEGA 7.0.

RESULTS AND DISCUSSION

Inoculated host plants showed the following symptoms after 1 to 3 weeks, consistent with symptoms caused by PMoV (Ramasso *et al.*, 1997; Marchoux *et al.*, 1999): large chlorotic local lesions followed by dieback and wilting in *C. quinoa*; large chlorotic local lesions followed by mosaic in *C. amaranticolor*; necrotic local lesions in *V. unguiculata*; and pinpoint necrotic local lesions followed by mosaic, leaf distortions, vein necrosis and necrotic patches on the apical non-inoculated leaves of *N. tabacum*. PMoV infection in symptomatic hosts was confirmed by RT-PCR. Host plants inoculated with leaf sap of the asymptomatic pokeweed plants showed no symptoms.

PMoV was detected by ACP-ELISA in all symptomatic plants, with an average optical density (O.D.) at 450 nm of 0.96 ± 0.60 ($n = 3$), and 0.12 ± 0.03 for asymptomatic plants ($n = 2$), after 1 h of substrate incubation. Tests for the potential additional presence of TSWV and CMV by LF were negative.

The ACP-ELISA results were confirmed with reverse transcription (RT)-PCR. The expected 916 bp fragment was obtained from symptomatic pokeweed (Figure 2) and tomato plants tested, while no amplicon was

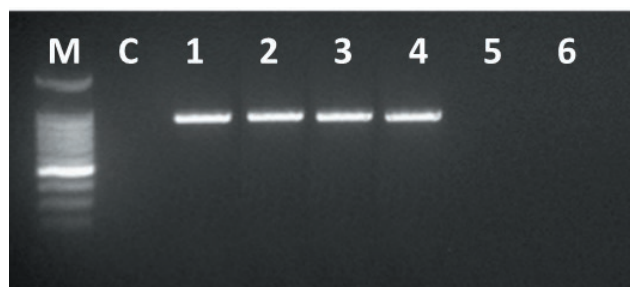


Figure 2. RT-PCR detection of Parietaria mottle virus in symptomatic *Phytolacca americana* L. leaves. Lanes M, 100 bp DNA ladder; C, control without total RNAs; 1, Positive control (PMoV infected tomato plants); 2 to 4, symptomatic *P. americana* leaves from three different plants; 5 and 6, asymptomatic *P. americana* leaves from two different plants.

obtained from asymptomatic plants. Sequences obtained from symptomatic *P. americana* shared 100% nucleotide similarity, and the corresponding sequence of the isolate Pha1 was deposited in GenBank (Accession n. MW248388). The Pha1 sequence shared greatest nucleotide similarity with sequences of the two PMoV tomato isolates from hosts growing nearby: 99.67% with the tomato isolate 390 (Accession n. MW456562) and 99.78% similarity with isolate 391A (Accession n. MW456563). This indicated virus movement between the American pokeweed and tomato hosts. These three isolates also clearly grouped in the phylogenetic tree, forming a distinct clade together with the isolates Ruc1, identified previously from *Diploaxis tenuifolia* (L.) DC., and Fri-2, from *Capsicum annuum* L., in the same Italian region (Parrella *et al.*, 2017; Parrella *et al.*, 2016). Nevertheless, overall phylogenetic grouping showed no clear correlation with host or geographic origin, although the existence of four distinct phylogenetic groups of PMoV isolates was indicated based on the MP gene (Figure 3). These results confirm those previously obtained and based on the coat protein (CP) phylogenetic relationships of PMoV isolates (Galipienso *et al.*, 2015).

PMoV is an emerging virus, due to its increasing incidence in tomato and other cultivated plants, such as *Cap-*

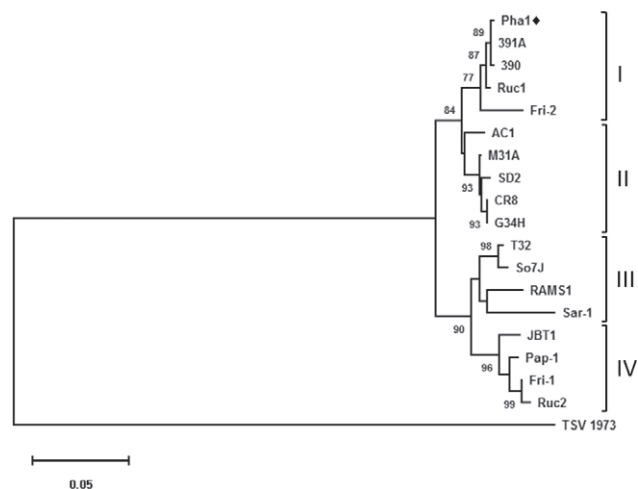


Figure 3. Phylogenetic analysis based on the movement protein (MP) gene of the Parietaria mottle virus (PMoV) isolates examined in this study (Pha1, 390 and 391A), and 15 other PMoV isolates available in GenBank. The accession numbers, isolate names, hosts, geographic origins and years of sampling for each isolate are listed in Table 1. The isolate from *Phytolacca americana* (Pha1) is indicated in the tree with the symbol \blacklozenge . The phylogenetic tree was constructed using the neighbor joining (NJ) method, with 1000 bootstrap replicates, and genetic distances were calculated by the Tamura three-parameter model using MEGA 7.0. The isolate of tobacco streak virus TSV 1973 (Accession n. JX463336) was used as an outgroup.

sicum annuum, *Diplotaxis tenuifolia*, and the ornamental plant *Mirabilis jalapa* L. (Parrella, 2002; Janssen *et al.*, 2005; Parrella *et al.*, 2016; Parrella *et al.*, 2017; Caruso *et al.*, 2018; Parrella *et al.*, 2020). The discovery of a new natural host of PMoV, i.e. *Phytolacca americana*, confirms the tendency of the virus to occupy new ecological niches. Within Caryophyllales, *Phytolaccaceae* are closely related to *Nyctaginaceae*, the plant family to which *Mirabilis jalapa* belongs, and this host is another unusual natural host of PMoV (Rettig *et al.*, 1992; Cuénoud *et al.*, 2002; Lee *et al.*, 2013). After the present discovery, further investigations would be useful to increase knowledge of the possible role of American pokeweed as a reservoir of PMoV, and the impacts of epidemics of this pathogen on susceptible crops such as tomato and pepper.

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