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Plant viruses and native vegetation in Mediterranean greenhouse areas

Estefanía Rodríguez[a,b,c,*], Jan van der Blom[b], Mónica González[c], Elena Sánchez[b], Dirk Janssen[d], Leticia Ruiz[d], Maria-Antonia Elorrieta[b]

[a] Department of Applied Biology, University of Almeria, Building: Science and Technology Building II - B. Ctra, Sacramento s/n. La Cañada de San Urbano, 04120 Almeria, Spain
[b] Departments of Phytopathology and Production Techniques, COEXPHAL, 04710 Almeria, Spain
[c] Cajamar Foundation, Experimental Station, Paraje las Palmerillas n° 25, 04710 Santa María del Águila El Ejido, 04710 Almeria, Spain
[d] IFAPA, Centro La Mojonera, Camino de San Nicolas, 1, La Mojonera, 04745 Almeria, Spain

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ABSTRACT

Semi-natural plantations around intensive horticulture production areas may contribute to biodiversity and act as phytosanitary barriers for pests and diseases that affect horticultural crops. However, native vegetation can also act as plant virus reservoirs, thus posing a risk for neighbouring crops. In this study, samples from 320 native perennial plant species, belonging to 20 botanical families were analyzed by enzyme-linked immunosorbent assay (ELISA) for the presence of the 10 most harmful viruses for horticulture in south-eastern Spain. Several species gave positive ELISA values for viruses. However, results of molecular tests and bioassays failed to confirm the presence of these viruses. Discrepancies between the results of the different types of analyses are discussed. Overall, this information indicates that the Mediterranean native flora study could be used for farmscaping in greenhouse areas.

1. Introduction

Intensive protected horticulture is located in the most arid regions of the Mediterranean Basin. Almeria (SE Spain) probably houses the most concentrated greenhouse area in the world, with over 25,000 ha of greenhouses concentrated in a total area of approximately 400 km^2. The region contains very little remnant native vegetation (Mota et al., 1996), making it extremely vulnerable to pest and disease attacks (van der Blom, 2010).

Non-crop vegetation may enhance the presence of natural enemies of pests by providing them alternative food or shelter resources, and may restrict the migration of pests between agricultural crops. More specifically, literature highlights the value of native vegetation as a source of beneficial insects, and much less so for agricultural pests (Fiedler and Landis, 2007; Schellhorn et al., 2010; Thomson and Hoffmann, 2010; Bianchi et al., 2013; Macfadyen and Muller, 2013). For that reason, planting of native hedgerows between greenhouses can be considered to be an effective preventive measure to minimise the pest pressure in horticulture areas with very limited biodiversity such us Almeria (Rodríguez et al., 2012).

However, to maximize the benefit of habitat management in greenhouse areas, the primary criterion to assess is to know if native vegetation is a risk for vegetable crops, since the plantings between the greenhouses may act as reservoir for plant diseases. Among plant viruses, insect-transmitted viruses, such as tomato spotted wilt virus (TSWV) (genus *Tospovirus*; family thrips *Frankliniella occidentalis*) transmitted by the thrips *Frankliniella occidentalis* (Pergande), and tomato yellow leaf curl virus (TYLCV) (genus *Begomovirus*, family *Geminiviridae*), transmitted by the whitefly *Bemisia tabaci* (Gennadius) are the main phytosanitary problems for horticultural production in Almeria (van der Blom, 2010). TSWV has a broad host-range consisting of more than 1000 species, including many weed species (Parrella et al., 2003). In contrast, host-range of TYLCV is short, including natural and experimental hosts (Polston et al., 1999; Jordá et al., 2001; Fanigliulo et al., 2007). Information on weed hosts of other common vegetable viruses are also reported in Spain: cucumber vein yellow virus (CVYV) (genus *Ipomovirus*, family *Potyviridae*) by Janssen et al. (2002), cucumber yellow stunting disorder virus (CYSDV) (genus *Crinivirus*, family *Closteroviridae*) by Ruiz et al. (2003), pepino mosaic virus (PepMV) (genus *Potexvirus*, family *Flexiviridae*) by Córdoba et al. (2004), and tomato torrado

* Corresponding author at: Department of Applied Biology, University of Almeria, Building: Science and Technology Building II–B. Ctra, Sacramento s/n. La Cañada de San Urbano, 04120 Almeria, Spain. Tel.: +34 950 015476 +34 950 015027; fax: +34 950 015476.
E-mail addresses: e.rodriguez@ual.es, estefania.rodriguez@andaluciajunta.es (E. Rodríguez), jvdblom@coexphal.es (J. van der Blom), monicagonzalez@fundacioncajamar.com (M. González), dirk.janssen@juntadeandalucia.es (D. Janssen), mleticia.ruiz@juntadeandalucia.es (L. Ruiz), mariaantonia@coexphal.es (M.-A. Elorrieta).

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virus (ToTV) by Alfaro-Fernández et al. (2008). Therefore, in horticultural systems numerous species of weeds, often exotic, are known to harbour virus of crops, but there is a dearth of studies identifying native species that are not weedy which are not host of plant viruses (Morales et al., 2006; Cano et al., 2009).

There are several methods available for plant virus detection. Serological and molecular methods are the most commonly used. Among these, the enzyme-linked immunosorbent assay (ELISA) is the most frequently applied, since it is one of the most specific tests, easy and cheap, to obtain a rapid identification of viruses. The results are reliable when the outcome for a certain virus is negative, but in certain cases false positives can occur. PCR is more sensitive and specific than the ELISA method, but it is more expensive and, therefore, not always suitable for routine and large-scale sample detection in field surveys. PCR, however, is excellent to apply in the case of doubtful ELISA results. Finally, bioassays, using indicator plants, are indispensable tools to determine the pathogenic nature of plant viruses, in spite of the fact that these assays are often time consuming, especially in the case of viruses that are exclusively transmitted by specific vectors, as is the case of *Begomoviruses*. Ultimately, the test of choice to detect plant viruses in field surveys will depend on issues such as resources, facilities, skills, type, and number of samples to be tested, etc., (Albersio et al., 2012; Uehara-Ichiki et al., 2013).

The aim of this study was to record field infection of native Mediterranean plant species candidates for farmscaping, mainly not weedy, to viruses that affect horticultural systems.

2. Material and methods

Samples of 320 plants representing 28 species in 20 families were collected at a maximum distance of 100 m from the greenhouses in the most concentrate greenhouse area in Almeria (SE Spain). Heavy pest populations and severe virus damages are noticeable problems throughout this crop production area (Arnó et al., 2009). Consequently, it was assumed that there was a high probability that at least some of the perennial and old specimens had been exposed to the viruses.

All samples were tested by ELISA (enzyme-linked inmunosorbent assay) with commercial antisera (DSMZ, Braunschweig, Germany), according to manufacturer's instructions. Variations of the basic ELISA technique were performed, depending on the virus to be analyzed (Table 1).

In the cases that ELISA gave a positive result, suggesting the presence of the analyzed virus, a molecular detection assay and bioassay through mechanical inoculations on indicator plants were performed to confirm virus infection. This was the case of PepMV and TYLCV that were confirmed by RT-PCR (Hasiów et al., 2008) and PCR (Accotto et al., 2000), respectively. F5 and R5 specific primers (forward: F5 5′- GACTTCTCAAATCCTAATACAGC-3′; reverse: R5 5′-CACATCAGCATAAGCACGAGC-3′) described by Hasiów et al. (2008), amplify a 166 bp fragment of coat protein gene of PepMV. Total RNA from native plants was extracted using the Aurum Total RNA Mini Kit (Bio-Rad, CA, USA). Total RNA from infected tomato plants was used as positive control and total RNA from uninfected tomato plants were used as negative. RT-PCR mixtures and process were developed using GeneAmp EZ rTth RNA PCR Kit (Applied Biosystems, CA, USA) and following manufacturer's instructions. The reverse transcription and amplification were for 30 min at 60 °C, 2 min at 94 °C, 40 cycles of 1 min at 94 °C and 1 min at 60 °C, and a final step for 7 min at 60 °C. Internal RNA positive control provided by kit was included.

Specific primers for TYLCV and tomato yellow leaf curl Sardinia virus (TYLCS) amplify the nucleotide 580 bp (forward: TY(-) 5′ GG(A/G)TTAGA(A/G)GCATG(A/C)GTAC-3′; reverse: TY(+) 5′ GCCCATGTA(T/C)CG(A/G)AAGCC-3′) (Accotto et al., 2000). Total DNA from each group of native plants was extracted and subjected to PCR using the conditions described by Accotto et al. (2000). Blank was used as negative control of PCR reaction.

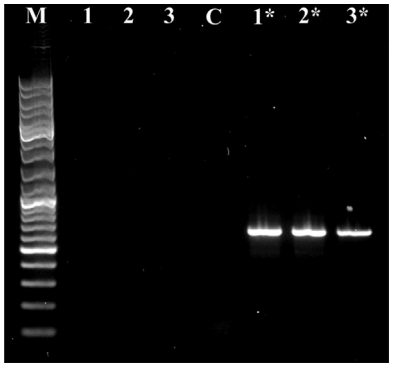

Fig. 1. Image of a UV-illuminated agarose gel after electrophoresis. Lanes 1, 2, and 3 are negative reactions of PCRs of suspected TYLCV-infected native plants (nonspiked samples). Lanes 1*, 2*, and 3* are positive reactions of spiked samples with DNA-TYLCV isolates from native plants. Lane C is a negative blank. M is marker.

Moreover, plants tested in this study were native perennial shrubs that are well adapted to semiarid conditions, which may have substances that can produce any type of inhibition of PCR. In order to assure an accurate and reliable PCR diagnosis and identify possible false positives from ELISA assays, a 'spike' controls were incorporated when running PCR. In order to obtain the spike controls, DNA from TYLCV-tomato infected plants was added to half of the native plant material suspected to be infected by the virus (spiked samples 1*, 2*, 3*) and the other half was analyzed without adding DNA material (nonspiked samples 1, 2, 3).

Finally, ELISA positive outcomes with respect to PepMV and PMMoV were submitted to confirmation trials by means of inoculation with plants extracts into susceptible tomato plants (*Solanum lycopersicum*) for detection of PepMV and *Nicotiana glutinosa* for detection of *Tobamoviruses*. Since, a bioassay for TYLCV would imply whitefly-mediated inoculation, only molecular assay were performed to confirm TYLCV infections.

3. Results and discussion

Out of all combinations, the only positive ELISA outcome was found for *Olea europaea* L. (*Oleaceae*) and *Lavandula multifida* L. (*Lamiaceae*) with respect to PepMV and for *Maytenus senegalensis* (*Celastraceae*) (Lam.) Exell. with respect to TYLCV. *L. multifida* also yielded positive result to PMMoV. However, ELISA positives could be not confirmed by molecular methods or bioassays (Table 2).

In particular, only an amplicon of the expected size (166 bp) was obtained from PepMV positive control tomato plant. No other significant amplification product was visible and no amplification was obtained from each PepMV-sample of *L. multifida*, or *O. europaea*, or healthy tomato plant (control) (Table 2). Similarly, some plants of *M. sengalensis* were found positive for TYLCV by means of ELISA. However, PCR showed a negative reaction in nonspiked samples of *M. senegalensis* (1, 2, 3), like in the blank (C) (Fig. 1), whereas spiked samples of *M. sengalensis* (1*, 2*, 3*) produced amplicons of the expected size (580 bp) in PCR (Fig. 1). Positive reactions from spiked samples suggested that the PCR was free of reaction-inhibiting contaminants, so the presence of the virus could not be confirmed.

Finally, bioassays by means of mechanical inoculations on test plants did not confirm virus infectivity. None of the seedlings of *N. glutinosa* inoculated with *L. multifida* plant extracts expressed symptoms of PMMoV. Similarly, tomato seedlings inoculated with