



Transmission Efficiency of *Potato Leafroll Virus* by Four Potato Colonizing Aphid Species in Tunisian Potato Fields

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Abstract This study investigated the transmission efficiency of *Potato leafroll virus* (PLRV) by four potato colonizing aphid species, *Myzus persicae*, *Macrosiphum euphorbiae*, *Aphis gossypii* and *Aphis fabae*, reported from leaves and yellow water trap. *Physalis floridana* was used as a test plant for virus transmission. DAS-ELISA was used for virus screening of samples as well as virus detection on the test plant after transmission experiment. A 2-h period was sufficient for the tested aphids to acquire PLRV virions. However, a difference in the transmission potential occurred according to the aphid species. The highest potential was recorded for *M. persicae* and *M. euphorbiae*, at 90 and 80%, respectively. For the first time, the study revealed the PLRV transmission efficiency of *A. fabae*, estimated at 50%. The lowest potential rate of 30% was recorded for *A. gossypii*. This study highlights the PLRV transmission capacities of four potato colonizing aphids suspected to play a key role in the spread of PLRV in potato seed production sites.

Keywords Acquisition rate · Aphid prevalence · *Potato leafroll virus* · Transmission efficiency

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Introduction

Potato leafroll virus (PLRV) (*Luteoviridae: Polerovirus*) has a worldwide distribution and is economically one of the most important pathogens infecting potato crops (Nikan 2014). Wales et al. (2008) highlighted annual potato yield losses of 20 million tons due to PLRV infections. PLRV causes several damaging symptoms such as leaf rolling, phloem necrosis and plant stunting (Chavez et al. 2012). In Tunisia, the potato seed production sector is prone to PVY and PLRV infections (Chérif and Boudhir 1990). Potato yield losses were estimated at 50% for PVY and from 27 to 35% for PLRV (Chérif and Boudhir 1990).

Virus transmission by vectors is among the most important studies that may elucidate the infection process of phytoviruses. Therefore, knowing the potential of aphid vectors occurring in potato fields is necessary to understand the virus epidemiology. PLRV virions are transmitted by several aphid species in a persistent and circulative non-propagative manner (Gildow 1999; Robert and Bourdin 2001; Saguez et al. 2013). Most of the studies conducted to investigate PLRV epidemiology used *Myzus persicae*, and only rarely other potato colonizing aphids (Yardımcı et al. 2015). The potential of *Aphis gossypii* and *Macrosiphum euphorbiae* to transmit PLRV has been investigated (Robert 1971; Sertkaya and Sertkaya 2005), whereas no reports have dealt with *Aphis fabae* as a potential vector despite its presence in potato fields. This study was therefore performed to elucidate the potential of the most common potato colonizing aphid species to acquire and transmit PLRV in Tunisia. Epidemiological features such as acquisition and transmission were investigated to enhance knowledge about PLRV establishment and dissemination.

Materials and Methods

Aphid Sampling

Aphids were picked from 100 leaves taken from a potato field at the Soliman (36° 42' 53" N, 10° 29' 00" E), Cap Bon region, North-East Tunisia, from March to May 2015. The samples were placed in ventilated plastic boxes and taken to the laboratory for aphid identification. In addition, an inventory was made of winged aphids occurring in the prospected potato field. Winged aphids were captured in a yellow water trap (Moericke 1955), placed in the middle of the field at 0.7 m above soil level. Aphid identification was performed under binoculars following keys by Remaudière and Remaudière (1997).

Virus Screening and Selection of PLRV Source

Potato leaves (cv. Spunta) showing symptoms of virus infection were also sampled from March to May 2015 at Soliman. A total of 112 samples were tested for the five most important viruses, PVY, PLRV, PVA, PVS and PVX. The direct Double Antibody Sandwich Enzyme Linked Immuno Sorbent Assay (DAS-ELISA) was adopted for virus detection, using the protocol of Clark and Adams (1977) and the guidelines of commercial diagnosis Kit “Bioreba”. The same test was performed for PLRV

acquisition on plants and aphids respectively. After incubation for 2 h at room temperature, the occurrence of each virus was determined using a microplate reader (Multiskan Ascent Labsystems, Waltham, MA, USA) by measuring the absorbance at 405 nm. Reactions were considered positive when the detected ratio was at least twice the mean value of the negative controls. The virus screening was performed in order to select single infection with PLRV.

Transmission of PLRV by Aphids

Aphid Rearing

Aphid rearing was performed in cages in growth chambers at 23 °C, 60% RH and 16:8 h light:dark. The target aphid species were *M. persicae*, *M. euphorbiae*, *A. fabae* and *A. gossypii*, known to be potato colonizing species. Tobacco plants (*Nicotiana tabacum*) were used as plant support for *M. persicae*, whereas *M. euphorbiae*, *A. fabae* and *A. gossypii* were maintained on potato, faba bean and tomato plants, respectively.

Test Plant for PLRV Maintenance and Transmission

The downy ground-cherry (*Physalis floridana*) considered the most suitable indicator plant for PLRV indexing (Syller 1996) was chosen as the test plant. Virus-free *P. floridana* plants were maintained in growth chambers at 23 °C under 16-h light and 8-h dark conditions. *P. floridana* was then used as a plant source for the maintenance of a single infection with PLRV using *M. persicae*. The same plant was used as a support for the transmission experiment. Both experiments were performed on a four-leaf stage *P. floridana*.

Transmission Assay

Twenty-five individuals from each aphid species were placed separately in empty plastic petri dishes for a 2-h fasting period. Later on, the individuals were picked up and released on an infected *P. floridana* plant for a 2-h period, named the Acquisition Access Period (AAP). Following the probing period, the 25 individuals from each species were divided into 5 groups of 5 individuals to be released and maintained on healthy *P. floridana* for 3 days, corresponding to the inoculation access period (IAP). Three weeks later, PLRV infection symptoms on *P. floridana* were investigated through DAS-ELISA.

To confirm PLRV acquisition by the tested aphids, 5 individuals were taken from each plant and tested by DAS-ELISA. Aphids were collected in an Eppendorf tube and homogenized in 200 µl extraction buffer. Then, 100 µl from each tube was transferred to a previously coated ELISA plate.

Statistical Analyses

SPSS 20.0 software was used for all statistical analyses. Aphid transmission rates were compared by ANOVA test. Means were separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Results

Aphid Prevalence

From a total of 405 sampled aphids, 9 species infesting potato leaves were found during the sampling period (Table 1). Only 5 aphid species were recognized to be potato colonizers, *A. gossypii*, *M. persicae*, *M. euphorbiae*, *A. fabae* and *A. spiraecola* with a prevalence of 46.9, 16.3, 8.9, 8.4 and 3.4%, respectively.

From the yellow water trap, 3757 winged aphids were captured and 54 aphid species were identified (Table 2) of which 5 were reported to be potato colonizers. The major species were *A. spiraecola* (non-colonizer) and *A. gossypii* (colonizer). The other colonizers were *A. fabae*, *M. persicae*, *M. euphorbiae* and *Aulacorthum solani*.

Potato Virus Screening

Virus screening of the 112 sampled leaves revealed both single and mixed infections (Table 3). For the 42 single infections, PVY was the dominant virus (24 samples), followed by PVS (16 samples). Single infection with PLRV was reported only on 2 samples. For the mixed infections, 4 double-infection types were reported: PVY/PVS, PVY/PLRV, PVY/PVX and PLRV/PVS on 43 samples in total. Interestingly, the most abundant mixed infection was registered for PVY/PVS on 37 samples. Furthermore, 5 samples were reported to be infected with 3 viruses (PLRV, PVY and PVS).

The serological characterization of the two PLRV infected leaves showed differences in the optical density (OD) (Table 4). The sample with the highest PLRV viral concentration was used for the transmission experiments.

PLRV Acquisition and Transmission

Investigation of PLRV acquisition was performed on the different aphid species once the transmission experiment was conducted. The results showed that all tested species became viruliferous with an estimated acquisition rate of 95, 90, 75 and 50% for

Table 1 Prevalence of the aphid species occurring on 100 potato leaves at Soliman from March to May 2015

Aphid species	Prevalence (%)
<i>Acyrtosiphon pisum</i>	0.2
<i>Aphis fabae</i>	8.4
<i>Aphis gossypii</i>	46.9
<i>Aphis</i> sp.	12.8
<i>Aphis spiraecola</i>	3.4
<i>Brachycaudus cardui</i>	1.2
<i>Macrosiphum euphorbiae</i>	8.9
<i>Myzus persicae</i>	16.3
<i>Uroleucon sonchi</i>	1.7

Total = 405 individuals on potato leaves

Table 2 Aphid species captured in yellow water trap at Soliman from March to May 2015

Aphids	Species	March	April	May
Potato colonizing species	<i>Aphis fabae</i>	0	66	16
	<i>Aphis gossypii</i>	1	26	806
	<i>Aulacorthum solani</i>	0	5	0
	<i>Macrosiphum euphorbiae</i>	0	4	7
	<i>Myzus persicae</i>	2	15	17
Species not colonizing potato	<i>Acyrtosiphon pisum</i>	0	9	22
	<i>Aphis craccivora</i>	1	11	8
	<i>Aphis</i> sp.	0	18	92
	<i>Aphis spiraeicola</i>	5	251	2027
	<i>Aploneura lentisci</i>	0	12	46
	<i>Brachycaudus cardui</i>	0	8	0
	<i>Dysaphis tulipae</i>	1	16	9
	<i>Hyperomyzus carduellinus</i>	2	15	4
	<i>Hyperomyzus lactucae</i>	2	66	13
	<i>Metopolophium dirhodum</i>	0	48	4
	<i>Brevicoryne brassicae</i>	1	0	4
	<i>Capitophorus elaeagni</i>	1	1	4
	<i>Dysaphis apiifolia</i>	0	3	2
	<i>Dysaphis</i> sp.	0	6	0
	<i>Hyalopterus pruni</i>	0	0	5

Thirty-four aphid species were identified with an abundance < 5: *Acyrtosiphon malvae*, *Amphorophora idaei*, *Amphorophora rubi*, *Aphis nerii*, *Aphis pomi*, *Brachycaudus helichrysi*, *Brachycaudus prunicola*, *Brachycaudus rumexicolens*, *Cavariella aegopodii*, *Cinara costata*, *Dysaphis foeniculi*, *Dysaphis pyri*, *Forda* sp., *Geoica lucifuga*, *Hyalopterus amygdali*, *Hyperomyzus picridis*, *Lipaphis erysimi*, *Macrosiphum rosae*, *Microlophium* sp., *Myzus* sp., *Nasonovia ribisnigri*, *Protaphis* sp., *Phylloxera* sp., *Rhodobium porosum*, *Rhopalosiphum maidis*, *Rhopalosiphum padi*, *Schizaphis graminum*, *Sipha maydis*, *Sitobion fragariae*, *Smynthuroides betae*, *Thelaxes suberi*, *Therioaphis trifolii*, *Uroleucon sonchi* and *Uromelan* sp.

M. persicae, *M. euphorbiae*, *A. fabae* and *A. gossypii*, respectively. Such results showed that the 2-h probing period was sufficient for the aphids to acquire PLRV virions. Three weeks later, the infected *P. floridana* plants expressed symptoms of chlorosis, curling and stiffness on their leaves. Both *M. persicae* and *M. euphorbiae*

Table 3 Investigation of the main potato viral infections from March to May 2015

Viruses	PVY	PLRV	PVA	PVS	PVX
PVY	24				
PLRV	3	2			
PVA	0	0	0		
PVS	37	1	0	16	
PVX	2	0	0	0	0

Table 4 ELISA optical density values of two PLRV isolates with a single infection

Samples	PLRV	PVY	PVA	PVS	PVX
I ₁	1.300	0.432	0.090	0.292	0.601
I ₂	1.188	0.418	0.076	0.330	0.652
(C+)	2.975	2.416	2.413	2.885	2.410
(C-)	0.360	0.423	0.092	0.257	0.613

I₁ and I₂: single infected PLRV isolates; (C+): positive control; (C-): negative control

were significantly more efficient at transmitting PLRV (90 and 80%, respectively) than *A. fabae* (50%) and *A. gossypii* (30%), which was least effective (Table 5).

Discussion

The current study highlights two important features of PLRV epidemiology, acquisition and transmission. Five aphid species, *M. persicae*, *M. euphorbiae*, *A. fabae*, *A. gossypii* and *A. spiraeicola*, were identified on potato leaves and in yellow water traps. They are recognized worldwide as potato colonizing aphid species (Saguez et al. 2013). Previously, Boukhris-Bouhachem et al. (2011) reported the prevalence of these aphid species from the same region of Tunisia based on potato leaves and yellow water traps. In this study, *A. spiraeicola* (non-colonizing) was the most prevalent species in the yellow water trap, which may be explained by the region's agricultural context, where citrus is an important crop. However, it is important to emphasize that these samples of *A. spiraeicola* were exclusively winged. According to Boukhris-Bouhachem et al. (2011), *A. spiraeicola* is found only in yellow water traps. During this study, *A. gossypii* (colonizing) was found to be one of the predominant sampled species, which is in accordance with Boukhris-Bouhachem et al. (2011). Besides, both studies have shown a low prevalence of *A. fabae*.

Three weeks after the transmission experiment, typical symptoms of PLRV were reported showing distinct interveinal chlorosis within the leaves as described by De Bokx and Van der Want (1987). This result proved that the 4 tested colonizing aphid species had successfully transmitted PLRV, despite the differences noticed in their

Table 5 PLRV transmission efficiency by four potato aphids

Aphid species	Transmission efficiency (%)
<i>Myzus persicae</i>	90 ^a ± 10
<i>Macrosiphum euphorbiae</i>	80 ^a ± 14
<i>Aphis fabae</i>	50 ^b ± 10
<i>Aphis gossypii</i>	30 ^c ± 10

A 2-h period was used for both aphid fasting and PLRV acquisition from plant source. Seventy-two hours was the adopted period to transmit PLRV on *Physalis floridana* by all aphids. ANOVA was used to compare the efficiency between aphid species. Transmission efficiency values (%) with different letters were statistically different ($P = 0.05$) according to Duncan's Multiple Range Test

transmission potential. Our results highlight an important potential of both *M. persicae* and *M. euphorbiae* to transmit PLRV. Concerning the high potential of *M. persicae*, these findings correspond to the results obtained by Rouze-Jouan et al. (2001) and Djilani Khouaja et al. (2011). Such a high potential enhances the role of *M. persicae* as a vector model to study PLRV transmission (Bosque-Pérez and Eigenbrode 2011; Rajabaskar et al. 2014). For *M. euphorbiae*, however, this study has, for the first time, reported the high potential of this aphid species to transmit PLRV. This is in contrast with Robert (1971), Roberts and Harrison (1979) and Tamada and Harrison (1981), who found a low efficiency of this species compared to *M. persicae*. According to Robert (1971), PLRV transmission efficiencies by the 4th stage larva of *M. persicae* and *M. euphorbiae* were found to be 70.9 and 12.8%, respectively. Within the same context, Roberts and Harrison (1979) proved that PLRV particles, detected in *M. persicae* by immunosorbent electron microscopy, were 10 to 30 times higher than those in *M. euphorbiae*. Later on, Woodford (1992) reported the efficiency of *M. persicae* and some clones of *M. euphorbiae* as PLRV vectors on *Nicotiana clevelandii* and on potato. Such results suggest that through the passage of time, *M. euphorbiae* is in constant evolution, which may increase its potential as a PLRV vector. Therefore, the high potential of *M. euphorbiae* recorded in this study was compatible with Woodford (1992). Concerning *A. gossypii*, a low transmission potential was found, which is in accordance with Sertkaya and Sertkaya (2005), who registered a similar transmission rate of the virus once transmitted from potato to other solanaceous test plants (*Capsicum annum* L., *Lycopersicon esculentum* L., *P. floridana* Rydb. and *Solanum tuberosum* L.). Despite the low efficiency rate of *A. gossypii*, its high prevalence in the field may be of greater importance than a fewer number of efficient vectors.

This is the first investigation performed to elucidate the potential of *A. fabae* to transmit PLRV. Interestingly, this study reports a moderate potential to transmit PLRV by *A. fabae*, which may improve our knowledge about the role of this aphid species in the establishment and dissemination of this virus.

PLRV acquisition on the tested aphids after the transmission assay was based on the persistent and circulative characteristics of the virus. The OD measurements of the ELISA readings of each sample confirmed that the different species had acquired the viral particles. Therefore, the 2-h acquisition period used in this investigation was enough to acquire the PLRV virions. This 2-h period is compatible with the interval range of 30 min to 6 h previously mentioned by Kotzampigikis et al. (2010). In contrast, the studies of Rouze-Jouan et al. (2001) and Djilani Khouaja et al. (2011) reported that 2 to 3 days are considered the appropriate period for the virus acquisition.

Conclusion

In conclusion, this research updates the aphid species colonizing potato leaves, and their transmission capacities, in Tunisian potato fields. It adds *A. fabae* to *M. persicae*, *M. euphorbiae* and *A. gossypii*, as known PLRV vectors. A period of 2 h was enough for aphids to acquire the PLRV virions. These results increase our knowledge about the epidemiology of PLRV and may help in the development of ways to prevent PLRV infections in seed potato production sites.

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