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ORIGINAL PAPER

Capacity assessment of *Myzus persicae, Aphis gossypii* and *Aphis spiraecola* (Hemiptera: Aphididae) to acquire and retain PVY^{NTN} in Tunisia

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Abstract The study was carried out to investigate the ability of three aphids, *Myzus persicae, Aphis gossypii* and *Aphis spiraecola*, to acquire and retain the *Potato Virus Y* (PVY) isolate, PVY^{NTN}. Tobacco plants, *Nicotiana tabacum* var. Xanthi, were used as test plant for the virus inoculation and aphid acquisition. The serological test double-antibody sandwich enzyme-linked immunosorbent assay was applied for virus detection on the test plants and aphids. Furthermore, virus retention by aphids was also assessed using a monoclonal anti-PVY^{N}. Although a duration of 2 min was enough for the virus acquisition, the three tested aphids showed different capacities to retain PVY^{NTN}. The retention of PVY^{NTN} was 3 h for *M. persicae* and *A. spiraecola*, and 2 h for *A. gossypii*. This study provides basic information of the virus retention by potato-colonizing aphid species, which may increase our understanding of PVY epidemiology in Tunisia.

Keywords Acquisition · Aphids · PVY^{NTN} · Retention · Serological detection

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Introduction

Potato Virus Y (PVY) (Potyviridae:Potyvirus) is one of the most serious potato diseases worldwide, causing severe yield losses (Valkonen 2007; Kerlan and Moury 2008; Cervantes and Alvarez 2011). PVY is a non-circulative virus transmitted in a non-persistent manner by more than 70 aphid species involving colonizer and non-colonizer species on potato (Varveri 2000; Boquel et al. 2011; Buchen-Osmond 2002; Dorokhov et al. 2014). Such transmission mode is highly efficient causing a rapid spread of the disease in the field even with low densities of potato-colonizing aphids (De Bokx and Van der Want 1987; Boiteau et al. 1998). One aphid individual can transmit a *Potyvirus* after acquisition of 20 or 50 viral particles (Pirone and Thornburry 1983; Moury et al. 2007). PVY is characterized by short acquisition and transmission periods. The virus acquisition from infected plants and its transmission to healthy ones take a few tenths of seconds (Ferreres et al. 1992). PVY comprises a number of strains that mostly differ in host range, symptomology, serology and molecular characteristics (Boonham et al. 2002; Nie and Singh 2003; Crosslin et al. 2006). Although PVY is in constant evolution, the most common strains are classified into three major groups PVY^{O}, PVY^{C} and PVY^{N} (Chikh Ali et al. 2010). PVY^{N} includes also other strains PVY^{N}W, PVYN^{WI}, PVY$^{N:O}$ and PVY^{NTN} (Margaritopoulos et al. 2010; Ibaba and Gubba 2011). Currently, the latter one is expanding worldwide causing veinal necrosis in tobacco plant (Chikh Ali et al. 2008) and tuber necrotic ring spot disease in potato (Beczner et al. 1984; Boukhris-Bouhachem et al. 2007; Kogovsek et al. 2008).

In Tunisia, PVY^{NTN} is reported as an economically important virus affecting seed potato production (Boukhris-Bouhachem et al. 2010). Among 15 aphid species that have so far been reported to occur in potato fields in Tunisia, *Myzus persicae, Aphis gossypii* and *Aphis spiraecola* are the most abundant ones in yellow water traps (Boukhris-Bouhachem et al. 2007). A further study conducted by Boukhris-Bouhachem et al. (2011) demonstrated a key role of these aphid species in the establishment and transmission of PVY^{NTN} at a rate of 95, 73 and 68% for *M. persicae, A. gossypii* and *A. spiraecola*, respectively. Virus retention in the aphid's stylet is one of the important features to study virus epidemiology. However, studies related to the estimation of the time where the aphid becomes viruliferous remain not well documented. Such information will be important to improve the knowledge related to the spread of the virus disease in the field. Therefore, this work aims to study the PVY^{NTN} acquisition and retention times of *M. persicae, A. gossypii* and *A. spiraecola* by serological technique for a better understanding of the virus–vector relationship.

Materials and methods

Test plant

Nicotiana tabacum var. Xanthi seeds were sown and grown inside aphid-free cages of growth chambers at the National Agricultural Research Institute of Tunisia. The virus-free tobacco plant material was used for the inoculation experiment.

Virus isolate, source and inoculation

The PVY^{NTN} isolate C1-3 was obtained from potato cv. 'Spunta' field sampled from Cap Bon region (North East of Tunisia) in 2003. PVY^{NTN} infection was previously confirmed by RT-PCR by Boukhris-Bouhachem et al. (2010). PVY^{NTN} C1-3 storage was performed according to the technique given by Bos (1969) and periodically grown on *N. tabacum* to test and keep the virulence of the isolate. Virus inoculation was performed mechanically on *N. tabacum* and placed in controlled condition chamber at 23 °C and 16:8 day: night cycle. Infected plant leaves were crushed in a sodium phosphate buffer (0.01 M phosphate and sodium sulfite 0.4%, pH 7.5) to be used as inoculum. The inoculation procedure was performed by placing the leaf extract on four-leaf stage tobacco plants. Two weeks later, the infected plants showed distortion of leaves and vein necrosis. Once the virus infection was confirmed by ELISA test, the plants were used for the virus aphid acquisition.

Aphid sampling and rearing

Aptera of *M. persicae, A. gossypii* and *A. spiraecola* used for virus acquisition and retention studies were chosen according to their high efficiency in the transmission of PVY^{NTN} in Tunisia (Boukhris-Bouhachem et al. 2010, 2011). Sampling of the target aphids was performed in the Cap Bon region. Both *M. persicae* and *A. gossypii* were sampled from potato leaves, while *A. spiraecola* from citrus leaves. Aphid identification was performed under binocular following keys by Blackman and Eastop (2000). *M. persicae* and *A. gossypii* rearing was done from a single parthenogenetic adult, while *A. spiraecola* individuals were bred from mixed populations. Aphid rearing was performed at 23 °C under L:D 18:6.

PVY detection after Acquisition Access Period (AAP)

Twenty aphids from each species were placed in empty plastic Petri dishes for a 2-h period to assure the fasting of the individuals. Afterwards, the aphids were released on a PVY^{NTN}-infected tobacco leaves for a 2-min virus acquisition. The probing assays were conducted in Petri dishes of 90 mm diameter. *M. persicae* reared on infected tobacco plant were taken as a positive control and the same species free of virus as a negative control. Four repetitions of five aphids were gathered in Eppendorf tube to be homogenized in 200 µl extraction buffer. Then, 100 µl of each tube was transferred to previously PVY^{N}-coated ELISA plate following the Clark and Adams (1977) protocol. The detection of viral particles in the aphid species was performed using a microplate reader at the absorbance 405 nm.

Virus retention time estimation

After 2 min of virus probing period, 120 aphids from each species were considered for this assay. The different aphids were allocated in 6 groups of 20 individuals in order to measure the virus retention time after 2-, 15-, 30-, 60-, 120- and 180-min period. For each group and time, two aphid individuals were separately transferred in Eppendorf tube, to be homogenized in the sodium phosphate buffer then tested by DAS-ELISA as described by Clark and Adams (1977) and Carlebach et al. (1982), using a monoclonal anti-PVY^{N} (INRA/FNPPPT). In all, 10 repetitions for each species were performed for each time. Although the adopted DAS-ELISA technique was performed to detect PVY^{N}, the different hypothesis and results of this study were built based on PVY^{NTN} C1-3 infection.

Data analysis

Data observed in the acquisition experiments were analysed in SPSS 20 software. Mean ELISA optical density (OD) values were compared using Duncan's test. The test was significant when $p \leq 0.05$. Retention time assessments were transformed by Statistica 9.0 using the exponential decay function of time model $J = \alpha\ e^{\beta t}$ (where β is a regression coefficient R^2, $\alpha = 1/\beta$, t: time) followed by ANOVA analyses of variance.

Results

Acquisition access period (AAP)

The PVY^{NTN} diagnostic was confirmed for each sample of aphid species with monoclonal antibody ELISA, PVY^{N} of the Bioreba kit (Fig. 1). The optical density (OD) values of the ELISA readings of each sample were estimated after 2-min probing preceded by 2 h of fasting. The measurements of the OD implicitly revealed the concentration of the virion acquired by the tested aphids. The OD ranged from 2.1 to 2.98 according to aphid species. A significant high OD was observed for *A. spiraecola*. However, no significant difference between *M. persicae* and *A. gossypii* was recorded. Based on this result, 2-min period was sufficient for the aphids to ingest several virus particles, which makes them potential vectors of PVY^{NTN}. During this period, the aphid stylet penetrates into the leaf cell membranes to reach the phloem, where the virus particles could be ingested.

Retention time assessment

All aphids tested by DAS-ELISA showed the capacity to retain the virus for a certain period (Fig. 2). The ANOVA detected significant differences between times 2′, 15′, (30′, 60′) and (120′, 180′). At 2 min, the maximum of virions were conserved on aphids. Then a constant decrease in PVY^{NTN} particles was registered for the three tested aphid species after 15 min from the probing experiment. Up to 60 min, the three aphid species remained viruliferous and were able to transmit PVY^{NTN}. Both *M. persicae* and *A. spiraecola* retained the virus for more than 120 min to lose this ability at 180 min. However, *A. gossypii* expressed shorter period by losing its retention capacity at 120 min.

Discussion

The efficiency of *Potyvirus* transmission by aphids is mainly related to a probing behaviour (Powell 2005; Powell et al. 2006; Fereres and Moreno 2009; Stafford et al. 2012). The three aphid species, *M. persicae, A. gossypii* and *A. spiraecola*, can acquire PVY^{NTN} within 2 min of probing on infected tobacco plants. Such a period seems to be optimum for the acquisition of virions leading to the transformation of non-viruliferous aphids to viruliferous ones and its transmission without a latent period.

As presented earlier, PVY^{NTN} is a non-persistent and non-circulative virus. Such description is important to highlight during the probing process since it may define the time between the virus acquisition and its inoculation to another plant. Blanc et al. (2014) have reported that the virus is most likely retained at the very tip of the maxillary stylets where the food and salivary canal fuse to form

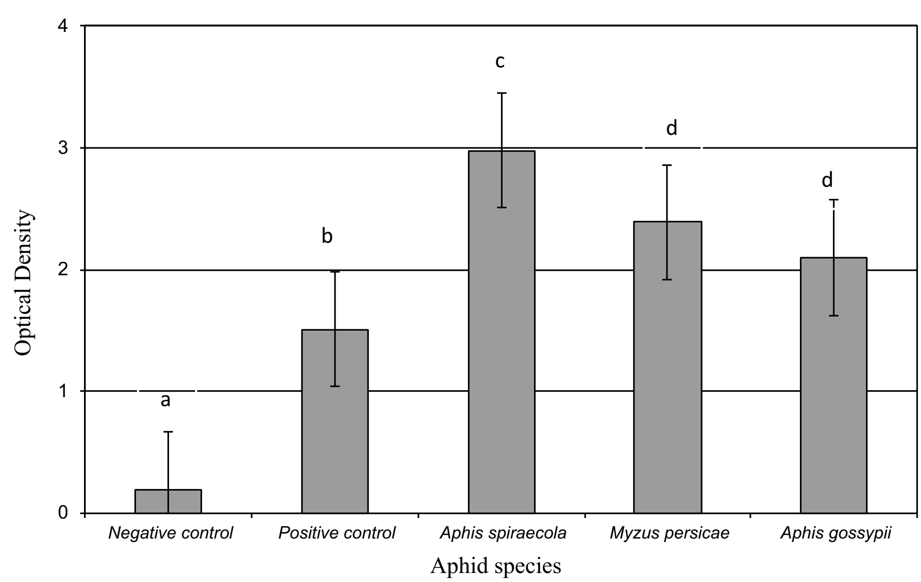
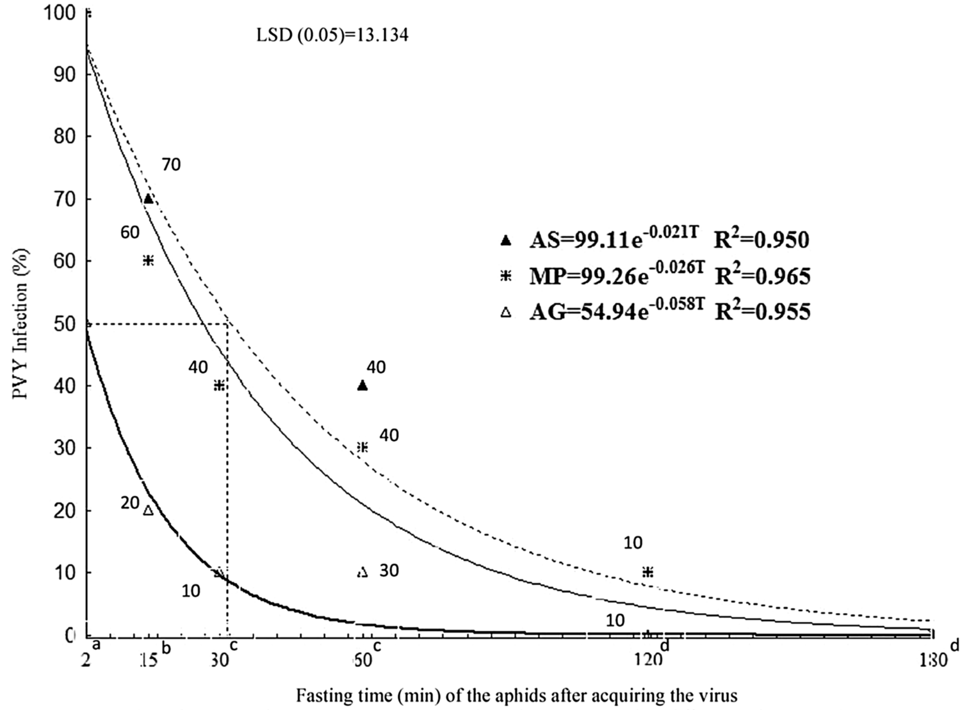


Fig. 1 PVY detection (OD values) in three aphid species after 2-min acquisition period on infected tobacco leaves. (*Error bars* represent standard deviations; OD with *different letters* is significantly different at $p \leq 0.05$, according to Duncan's test)

Fig. 2 PVY retention time in three aphid species



AS: *Aphis spiraeola*, MP: *Myzus persicae*, AG: *Aphis gossypii; when differences between LSD means value ≥13.134 the test is significant*

the common duct. This situation is in favour of two majors facts: (1) the “non-circulative” transmission of this virus on the viruliferous vector (Kalleshwaraswamy and Kumar 2008) and (2) the easy establishment of PVY inocula during the first phase of the aphid feeding through the injection of the watery saliva into the cytoplasm of the plant cell (Martin et al. 1997; Powell 2005; Blanc et al. 2014).

This study has shown that *A. spiraecola* presents the highest capacity to acquire PVY^{NTN} in comparison to *M. persicae* and *A. gossypii*. This ability may be explained by the difference in the concentration of the acquired virions in the vectors occurring in the fields. Such result may explain the great ability of *A. spiraecola* to propagate PVY^{NTN} as reported by Boukhris-Bouhachem et al. (2011).

Notwithstanding that *A. spiraecola* has shown greater capacity to acquire PVY^{NTN} particles than the tested aphids, it reveals same retention time estimated at 3-h period as for *M. persicae*. Interestingly, *M. persicae* seems to catch less virus particles than *A. spiraecola*. This finding does not necessarily make *A. spiraecola* as the best vector of the virus. Previously, Boukhris-Bouhachem et al. (2011) have confirmed the transmission efficiency of PVY^{NTN} by *A. spiraecola, A. gossypii* and *M. persicae* and other aphids, whether they are potato colonizer or not. The same author has reported that the most efficient vector was *M. persicae* followed by *A. spiraecola* and then *A. gossypii*.

The retention time is generally about a few minutes to a few hours, where the aphids usually lose their infectivity rapidly. The limited period to retain the virion is mainly explained by the transmission process. In this step, the viruliferous vector gradually becomes non-viruliferous by losing the viral particles retained in their stylet. In fact, the period where the aphid remains viruliferous is reported to be very short due to the easy loss of the viral particles after the acquisition period even without a feeding behaviour (Palacios et al. 2002; Kalleshwaraswamy and Kumar 2008; Froissart et al. 2010). The rate at which infectivity is lost depends on many factors, including temperature, whether they are held on plants or under some artificial conditions (Bradley and Rideout 1953) or the time of feeding on infected plants (Kostiw and Trojanowska 2011). Similar study performed by Trojanowska (2004) on *M. persicae* has shown that the retention of PVY^{N} and PVY^{NTN} in the stylets of this aphid species was only for 16 min. However, other reports related to PVY retention by aphids, without considering a specific strain, exhibit different time ranges. In fact, Kostiw (1987) revealed that winged specimens of *M. persicae* may be active vectors for PVY for a period of 128 min without being in contact with the plant. The same author reported that activity of *M. persicae* as PVY vectors may be extended to 17 h in case the winged aphid comes in contact to virus source plant.

The current investigation reports two key aspects involved in the virus epidemiology: acquisition and retention time. *M. persicae, A. gossypii* and *A. spiraecola* were recognized worldwide to be potato-colonizing aphid species (Saguez et al. 2013). Previously, Boukhris-Bouhachem et al. (2011) have reported the occurrence of *M. persicae* and *A. gossypii* from both potato leaves sampling and yellow water traps placed in potato fields. Considering these results of the previous transmission experiment and its correlation with our finding, we can suggest the tested aphids play an important role in the spread of PVY^{NTN} in the field. Both colonizing aphid species, *M. persicae* and *A. gossypii*, are likely involved in the establishment and transmission of the PVY^{NTN}. Furthermore, *A. spiraecola* may play an important role in the spread of PVY^{NTN} even if it was not observed in potato leaves. In fact, *A. spiraecola* was massively found only in the yellow water trap which may be explained by the important areas of citrus orchards in the studied region. Therefore, besides its capacity to acquire the virions, *A. spiraecola* seems to ensure a long-distance transport and spread of PVY^{NTN}.

In conclusion, the present research study provides PVY^{NTN} quantification of the particles in the aphid stylets and its retention by three aphid species to expand knowledge about the transmission and dispersion of PVY^{NTN}. These results may contribute to the development of suitable methods to control aphid vectors. So far, sanitation based on the removal of infected weeds and volunteer plants, the use of certified seeds and/or resistant potato cultivars is the most used approach to prevent seed potato production from PVY infections (Karasev and Gray 2015; Rowley et al. 2015). Within this context, the use of mineral oil seems promising to inhibit the virus acquisition by aphid vectors (Martín-López et al. 2006; Al-Mrabeh et al. 2010; Boukhris-Bouhachem et al. 2015). However, the mechanism and durability of the mineral oil is not fully covered, which led to develop further studies to explore other effects of the oil as a means to control viral infection.

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