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Assessing aphids potato virus Y-transmission efficiency: A new approach

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A B S T R A C T

In order to develop an alternative method to optimize the relative efficiency factor (REF) assessment, the efficiency of transmission of *Potato virus Y* (PVY) by seven aphid species was examined. *In vitro* micro-propagated potato plantlets were used to experiment on phenotypically and genetically homogeneous material. Species-specific acquisition access period (AAP) on a PVY-infected plantlet was assessed for each aphid species using electrical penetration graph (EPG) technique.

Aphid probing behaviour determined by EPG showed that *Macrosiphum euphorbiae* and *Myzus persicae* exhibited the shortest AAPs (15 and 11 min, respectively) whereas *Rhopalosiphum padi*, *Sitobion avenae*, *Brevicoryne brassicae* and *Acyrthosiphon pisum* exhibited the longest ones (more than 30 min). The transmission rate obtained for *M. persicae* (83.3%) was higher than the ones reported in the literature. REFs assessment showed that *A. pisum* and *B. brassicae* were poor efficient vectors while *M. euphorbiae* and *S. avenae* seemed to be efficient ones even though their respective REF were significantly lower than that of *M. persicae*. The species *R. padi* and *A. fabae* did not transmit PVY.

The hypothesis assessed for *M. euphorbiae* and *S. avenae* and consisting in the compensation of a weak PVY-transmission efficiency by a higher number of vectors, was not supported. The use of this new method for REF evaluation and the need to consider aphid behaviour for such an assessment was discussed.

1. Introduction

Potato virus Y (PVY, *Potyviridae*: *Potyvirus*) has a huge economic impact on the potato (*Solanum tuberosum* L.) production system (Sigvald, 1992) as it can cause tuber necrosis leading to unmarketable harvest. Under field conditions, PVY is transmitted efficiently in a non-persistent manner by potato colonizing aphids such as the green peach aphid *Myzus persicae* (Sulzer) and the potato aphid *Macrosiphum euphorbiae* (Thomas) (Radcliffe and Ragsdale, 2002). Some non-colonizing potato aphid species have also been reported to transmit PVY despite lower transmission rates. Field samples showed high virus infection rates in potato plots even when very low densities of potato colonizing aphids were reported (Boiteau et al., 1998) and it has been suggested that in the absence of potato colonizing species the massively trapped non-colonizing aphids may be responsible for the spread of PVY (DiFonzo et al., 1997).

Many studies measuring and estimating PVY vector efficiency revealed intraspecific variations (Harrington and Gibson, 1989; Sigvald, 1984; van Harten, 1983; van Hoof, 1980; Verbeek et al., 2010). However, all of the investigations agreed to consider *M. persicae* as the most efficient PVY vector and used it as a reference. The transmission efficiency of other aphid vectors is therefore expressed as a relative efficiency factor (REF) related to that of *M. persicae* set to 1 (van Harten, 1983). Within an aphid species, the variability of REF reported in the literature can result from the aphid biotype and the number of aphids used to infect plants experimentally (Halbert et al., 2003; Lupoli et al., 1992; Moreno et al., 2007; Verbeek et al., 2010), the PVY strains and isolates (Verbeek et al., 2010), the target plant species and cultivar (Fereres et al., 1993; Hamm et al., 2010; Singh and Boiteau, 1986), the plant phenological state (Woodford, 1992) and the virus concentration in the plant source (Pirone and Megahed, 1966). Finally, REF variability can rely on the aphid probing behaviour as efficient acquisition and inoculation are realised during brief intracellular punctures (3–6 s) performed during the first steps of host plant colonisation process (Powell et al., 2006). Therefore, successful vectoring depends firstly on the acquisition access period (AAP) that corresponds to the time elapsed from the aphid contact with the infected plant to the first intracellular puncture.

Abbreviations: AAP, acquisition access period; ELISA, enzyme-linked immunosorbent assay; EPG, electrical penetration graph; IAP, inoculation access period; MS, Murashige and Skoog; PCR, polymerase chain reaction; PVY, potato virus Y; REF, relative efficiency factor; RT, reverse transcription.

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Basically, two methods have been used to assess vector efficiency. First, aphids are placed in a closed arena containing both infected and healthy plants (Kanavaki et al., 2006; Katis et al., 2006). However, such a set-up does not allow us to distinguish the contribution of aphid behaviour in the transmission process (vector activity) from their intrinsic ability to transmit viruses (vector efficiency). The second experimental approach aims to control the transmission process by placing the aphid vector on an infected plant for an AAP, and then transferring it to a healthy plant for an inoculation access period (IAP). While the latter method minimizes vector activity contribution as the vectors are artificially placed on the source and then on the target plant, they are usually performed with short AAPs that can induce bias in the vector efficiency assessment. Indeed, it has been demonstrated that AAP varies depending on the aphid species and is modulated by the healthy or infected status of the plant (Boquel et al., 2011).

The main objective of this paper was to develop, on the aphid vector – PVY – potato pathosystem, an alternative method to optimize REF assessment by minimizing the part of the source and target plants and the part of vector. *In vitro* micropropagated potato plantlets were used to get phenotypically and genetically homogeneous plant material (Acquaah, 2007; Hu and Wang, 1983) and minimize potato plant defence responses (Péros et al., 1994; Petrovič et al., 1997), and clones of seven aphid species (*M. euphorbiae*, *M. persicae*, *Rhopalosiphum padi*, *Sitobion avenae*, *Brevicoryne brassicae*, *Aphis fabae* and *Acyrthosiphon pisum*) were used to minimize intraspecific variability. To ensure the vector a sufficient contact time with the plant to perform intracellular puncture and acquire virus particles, electrical penetration graph (EPG) technique was used to define a specific AAP for each aphid species. Finally, this method was used to test the hypothesis suggested by DiFonzo et al. (1997) who postulated that a weak vector efficiency could be compensated by high vector densities in fields.

2. Materials and methods

2.1. Virus isolate and in vitro plantlets

The PVY^{NTN} isolate was obtained from potato tubers collected in a potato field (Cambrai, France). Healthy and PVY^{NTN}-infected *in vitro* potato (cv. Bintje) lines were obtained from germ fragments (2–3 cm), respectively, collected from a healthy and an infected tuber, washed during 20 min with a 7% calcium hypochlorite solution, dried with blotting paper and deposited in a Murashige and Skoog (1962) (MS basal medium with sucrose and agar, 42.4 g L^{-1}) medium for development. For micropropagation, explants were isolated on MS medium in a small glass vial (5 mL) placed in a sterile culture glass tube (25 mm × 150 mm) in a growth chamber at 20 ± 1 °C, 60 ± 5% relative humidity and 16:8 h day:night cycle. Each small glass vial containing one 15 day-old healthy or PVY-infected *in vitro* plantlet was withdrawn from the glass tube for experiments. Immunocapture RT-PCR technique was used to determine the healthy or infected status of the plantlet according to Glais et al. (1998).

2.2. Aphids

Aphid species used for virus transmission bioassays were chosen according to their abundance in potato fields in northern France or their ability to transmit PVY (Harrington and Gibson, 1989; Sigvald, 1984; van Harten, 1983; van Hoof, 1980). Aphids were obtained from different French localities or laboratories: *M. persicae* (potato field, Loos-en-Gohelle), *M. euphorbiae* (INRA-INSA, Villeurbanne), *A. fabae* (eggplant greenhouse, Amiens), *S. avenae* and *R. padi* (INRA, Le Rheu), *A. pisum* and *B. brassicae* (INRA-INSA-CIRAD, Montpellier). Each aphid clone, started from a virus free single apterous parthenogenetic female, was reared on a healthy host plant enclosed in a ventilated Plexiglas® cage (360 mm × 240 mm × 110 mm) in a growth chamber (20 ± 1 °C, 60 ± 5% relative humidity, 16:8 h day:night cycle). *M. persicae* and *M. euphorbiae* were reared on potato (*S. tuberosum* L.), *A. fabae* and *A. pisum* on broad bean (*Vicia faba* L.), *S. avenae* and *R. padi* on wheat (*Triticum aestivum* L.) and *B. brassicae* on broccoli (*Brassica oleracea* L.). All experiments were performed at 20 ± 1 °C with alates synchronized in host plant seeking phase according to the set-up described by Brunissen et al. (2009).

2.3. Evaluation of a species-specific acquisition access period (AAP)

The DC-electrical penetration graph (EPG) technique (Tjallingii, 1978, 1988) was used to determine the necessary time for each aphid species to perform the first potential drop (intracellular puncture) on a PVY-infected *in vitro* potato plantlet. To insert one aphid and one plant in an electrical circuit, a thin gold wire (20 μm diameter, 2 cm long) was stuck with conductive water based silver glue (EPG-systems, Wageningen, The Netherlands) on the aphid's dorsum and a second electrode was inserted into the agar-based MS medium. The aphid was then connected to the DC-EPG amplifier and placed carefully on a PVY-infected *in vitro* plantlet into a Faraday cage at an ambient temperature of 20 ± 1 °C. For each aphid species 20 replicates were done. The recordings were performed during daytime for 8 continuous hours. Acquisition and analysis of the EPG waveforms were done with PROBE 3.5 software (EPG-Systems, Wageningen, The Netherlands) and AAP, defined as the mean time from start of the monitoring (when the aphid was deposited on the leaflet) to the first potential drop added to its standard error, was calculated using the EPG-Calc 4.9 software (Giordanengo, 2009).

2.4. Transmission experiments and relative efficiency factors (REFs) evaluation

For each aphid species, 5 synchronized alates were deposited on a PVY-infected *in vitro* potato plantlet placed in a Plexiglas® box (120 mm × 90 mm × 50 mm) during a period equal to their specific AAP. Then, aphids were deposited individually on a healthy *in vitro* plantlet grown in the culture glass tube for a 24 h IAP. Finally, after IAP, aphids and their possible progeny were removed and *in vitro* plantlets were placed in a growth chamber for a 15-day-incubation period before checking for PVY infection by immunocapture RT-PCR. For each aphid species, 30 individuals were tested.

The REF of each aphid species was calculated by dividing the number of infected plants obtained with the considered species by the number of infected plants obtained with *M. persicae* used as referent species.

2.5. Number vs efficiency

DiFonzo et al. (1997) hypothesized that the high numbers of non-colonizing aphids could compensate their weak virus transmission efficiency. In accordance to the obtained results in transmission experiments, this hypothesis was tested with the two aphid species that ranked, respectively, second and third in the calculated REF. The number of potentially viruliferous aphids deposited simultaneously per target plantlet for IAP was calculated according to the equation: number aphid to use = REF *M. persicae*/REF tested species. Then, transmission efficiency experiments were realised according to the method described above.