Impact of Aphids and Host Weeds Interaction on the Dissemination of *Potato Virus* Y^N Strains

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ABSTRACT

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Weeds and volunteer plants susceptible to *Potato virus Y* (PVY) infection in different seed potato production sites were investigated in this study. Aphids occurring within these plants and identified as *Aphis fabae, A. gossypii*, and *Myzus persicae* were studied for possible interaction occurring between vectors and plant reservoirs of PVY. Out of 772 plants belonging to 12 different families (Solanaceae, Amaranthaceae, Chenopodiaceae, Papaveraceae, Urticaceae, Convolvulaceae, Asteraceae, Polygonaceae, Euphorbiaceae, Brassicaceae, Portulacaceae, and Compositae), 337 were found to be infected by PVY^N based on DAS-ELISA technique. Among these plants, *Solanum elaeagnifolium*, *Datura stramonium* and *Sonchus oleraceus* were found to be infected with the strain PVY^{NTN}. In addition to these reported weeds, *S. nigrum* seems to be an important host for PVY^N since this plant hosts aphid vectors. This investigation provides basic information about weeds and volunteer plants infected with PVY^N and aphid vectors. Such finding will increase knowledge of the PVY^N epidemiology in potato fields and consequently the possible management of this viral disease.

Keywords: Aphids, potato, PVY^N, PVY^{NTN}, volunteer plants, weeds.

Potato virus Y (PVY) of the family Potyviridae (Potyvirus) is one of the most economically important viruses infecting various host plants of different families such as the Solanaceae, Chenopodiaceae, Amaranthaceae, Euphorbiaceae, Fabaceae, and Brassicaceae. Several studies reported PVY infection in weeds.

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For example, weeds within *Solanaceae* and *Portulaca oleracea* were found to be a reservoir for PVY in France (Marchoux et al. 1976). In Argentina, PVY infection was found on *Physalis viscose* and *Solanum atriplicifolium* (Pontis and Feldman 1963). Later, a study from Israel reported that both *S. villosum* and *Hyoscyamus desertorum* were infected with PVY (Ucko et al. 1998). In Tunisia, the occurrence of the perennial plant, the silver nightshade, *Solanum elaeagnifolium* was reported (Mekki 2005) and registered as PVY host plant

(Boukhris-Bouhachem et al. 2007; Edwardson and Christie 1997).

Aphids (Hemiptera: Aphididae) are serious insect pest playing a key role in the establishment and the dissemination of plant viruses. More than 73 species of aphids, including colonizer and non-colonizer of potato crops, are known to transmit PVY through a non-persistent manner (De Bokx and Piron, 1990; Nanayakkara et al. 2012; Perez et al. 1995; Varveri 2000). Among the reported aphids, *Myzus persicae* was recognized as the most efficient vector of PVY^{NTN} the most abundant strain in Tunisia (Boukhris-Bouhachem et al. 2011; Mello et al. 2011; Verbeek et al. 2010). At spring season, winged aphids migrate to reach new secondary host plants such as potato. During this process, aphids may acquire PVY virions from infected weeds or volunteer plants which are considered as plant reservoirs. Such situation enhances the establishment and the increased dissemination of PVY^{NTN} which is the most abundant strain in Tunisian potato seed-producing fields.

Tunisian potato seeds production is an important sector often threatened by virus contaminations leading sometimes to 12% declassification of potato seed-producing areas (Boukhris-Bouhachem et al. 2015). This occurs when the virus infection level in surveyed fields exceeds 5%. The aim of this study is to highlight the potential host plant, weeds or crops, which may play a role as sources of PVY^{N}. Such finding will enhance knowledge about the dissemination of PVY^{N} in potato seed-producing fields.

MATERIALS AND METHODS

Plant sampling.

Sampling was carried out in four study areas (Fig. 1) namely Batan (Manouba), Sidi Mahmoud (Kairouan), Douala (Cap Bon), and Bousalem (Jendouba). Intensive sampling was performed during spring time from 2002 to 2007 in the site of Sidi Mahmoud and Douala. Occasionally, the investigation involved years 2013 to 2015. In all, 772 weed samples belonging to 12 different families (cultivated and volunteer Solanaceae, Amarantaceae, Chenopodiaceae, Papaveraceae, Urticaceae, Convolvulaceae, Asteraceae, Polygonaceae, Euphorbiaceae, Brassicaceae, Portulacaceae, and Asteraceae).

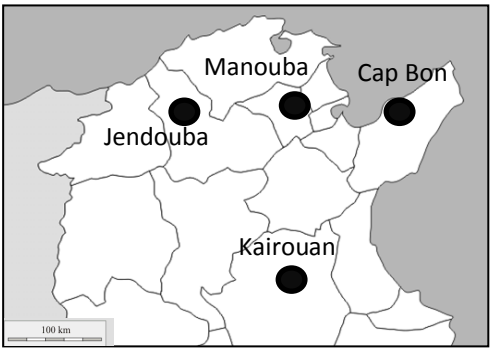

Fig. 1. Sites prospected for the survey of virus infections

Aphid identification

Aphids (alatae and aptera) were sampled randomly from weeds and volunteer plants occurring in potato fields. Aphid identification was done following both keys by Blackman and Eastop (2001) and Remaudière and Seco (1990).

Detection and characterization of PVY

All samples were tested by DAS-ELISA as described by Clark and Adams (1977), using a polyclonal anti-PVY (Bioreba) and anti-PVY^N serotype (Bioreba and INRA/FNPPPT). Molecular tests were only conducted on PVY^N positive samples from 11 *S. elaeagnifolium*, 6 *Datura stramonium* and 9 *Sonchus oleraceus* plants, considered as new host plants for PVY^N in Tunisia. Samples were grinded in extracted buffer of the ELISA kit (Bioreba), 100 µl were placed in the microplate. PVY^{NTN} strains detection was carried out by Immunocapture-RT-PCR. The reverse primer used for first-strand DNA synthesis (3'NTRC) was the same oligonucleotide used by Glais et al. (1998). The strain identity was based on the polymorphism in 5'NTR/P1 region amplified by specific primers (Glais et al. 2001). The Tunisian isolate PVY^{NTN} C1-3 maintained in the laboratory was used as a positive control. Amplified products were separated by electrophoresis on 1.5 agarose gels, stained with ethidium bromide and observed under UV.

RESULTS

Aphid identification.

Overall, four aphid species were encountered in this study namely *M. persicae, Aphis gossypii, A.* fabae, and *Hyperomyzus lactucae*. They were encountered on various weeds where *M. persicae* was observed on *S. elaeagnifolium*, *D. stramonium* and *C. arvensis*; *A. gossypii* was detected on *S. elaeagnifolium*; *A. fabae* was associated to *S. elaeagnifolium*, *S. nigrum*, and *A. retroflexus*; and *H. lactucae* was noted on *S. oleranceus*.

Virus detection.

From a total of 772 sampled plants, 337 were recorded to be infected with PVY^N (Table 1). A wide range of weeds from different families such as *S. elaeagnifolium*, *S. nigrum*, *S. oleraceus*, *Chenopodium album*, *Amaranthus hybridus*, *D. stramonium*, and *Convolvulus arvensis* have shown PVY^N infections. From Solanaceae family, 43.65% of weeds and volunteer plants were registered to be infected. A rate of 39.82% of *S. elaeagnifolium*, 93.33% of *D. stramonium* and 41.86% of *S. nigrum* were positive to PVY^N infection. *S. nigrum* and *D. stramonium* were the most frequently positive plants in Douala, while *S. elaeagnifolium* were mainly infected in Batan and Sidi Mahmoud. Two weeds belonging to Amaranthaceae were also positive for PVY^N infection. In fact, *Amaranthus* sp. was found positive to virus infection in all prospected sites while *A. retroflexus* was reported only in Douala and Batan. For *S. oleraceus*, samples were positive to PVY^N infection at the rates 13.63, 14.28 and 8.33% in the regions of Batan, Sidi Mahmoud, and Douala, respectively. It is necessary to highlight that this is the first report of PVY^N infection on *S. oleraceus* in Tunisia. Potato volunteer plants were also infected with PVY^N at the level of 74.2 and 85.44% in Batan and Douala, respectively.

Regarding the molecular investigations of the three tested weeds, *S. elaeagnifolium*, *D. stramonium* and *S. oleraceus*, the results showed that all samples were infected with PVY^{NTN} (Fig. 1).

Table 1. List of weeds and plant volunteer showing a PVY infection according to DAS-ELISA, 2002-2015

Sampling site		Batan	Bousalem	Sidi Mahmoud	Douala	PVY infection[N]
Weeds		N+/N	N+/N	N+/N	N+/N	
Amaranthaceae	*Amaranthus* sp.	4+/19	3+/7	2+/6	2+/16	11+/48
	Amaranthus retroflexus	2+/17	-	-	1+/10	3+/27
	Amaranthus hybridus	1+/1	-	-	-	1+/1
Chenopodiaceae	*Chenopodium album*	7+/30	0+/1	8+/57	1+/5	16+/93
	Chenopodium amaranticolor	1+/5	-	-	0+/1	1+/6
	Beta vulgaris	4+/9	0+/1	-	-	4/+10
Convolvulaceae	*Convolvulus arvensis*	9+/14	3+/12	18+/29	6+/7	36+/62
Portulacaceae	*Portulacaoleracea*	-	-	-	1+/1	1+/1
Solanaceae	*Solanum nigrum*	6+/17	--	2+/4	10+/22	18+/43
	Solanum elaeagnifolium	7+/13	1+/5	37+/95	-	45+/113
	Datura stramonium	1+/1	-	-	13+/14	14+/15
	Whithenia somnifera	-	-	3+/16	-	3+/16
Compositae	*Sonchus oleraceus*	9+/66	0+/7	1+/7	1+/12	11+/92
Papaveraceae	*Papaver rhoeas*	-	0+/1	-	-	0+/1
Polygonaceae	*Emex spinosa*	0+/2	-	-	-	0+/2
Urticacae	*Urtica urens*	1+/2	-	0+/3	1+/2	2+/7
Asteraceae	*Calendula arvensis*	-	0+/1	-	-	0+/1
	Erigeron sp.	-	-	-	1+/1	1+/1
	Picris echioides	-	-	0+/1	-	0+/1
	Xanthium sp.	-	-	1+/1	-	1+/1
Brassicaceae	*Sinapis arvensis*	-	0+/1	-	-	0+/1
Euphorbiaceae	*Euphorbia* sp.	-	-	-	1+/13	1+/13
Volunteer solanaceous plants						
Tomato	*Lycopersicum esculentum*	-	-	4+/11	2+/5	6+/16
Pepper	*Capsicum annuum*	-	-	4+/9	1+/6	5+/15
Potato	*Solanum tuberosum*	69+/93	-	-	88+/103	157+/196
Total		**121+/279**	**7+/36**	**80+/239**	**129/218**	**337+/772**

N: Number of positive plants (+); Nt: Total number of tested plants

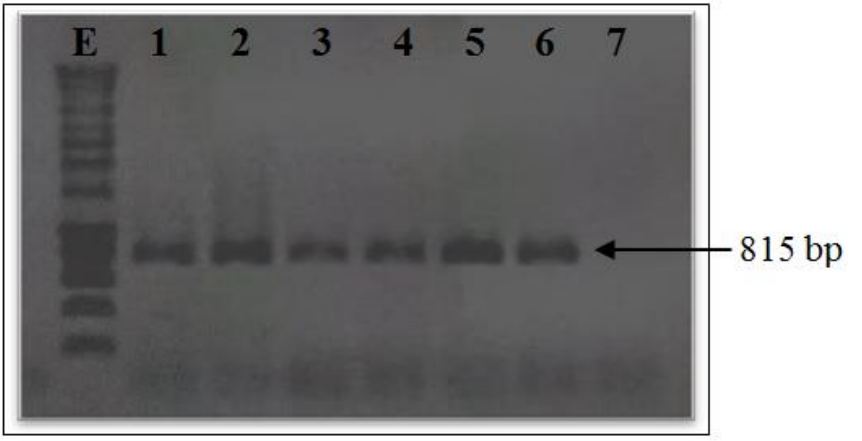

Fig. 1. Amplification of P1 region by RT-PCR.E: Smart Ladder (100 bp, EuroGentec); 1: PVY^{NTN}-C1-3, 2-3: *Solanum elaeagnifolium*; 4-5: *S. oleraceus*; 6: *Datura stramonium*; 7: Negative control.

DISCUSSION

A screening of virus plant reservoirs was performed during 9 years in four Tunisian regions. Our study provides a list of weeds belonging to 25 species and 12 families that host PVY^N infection. Aphid identification on infected weeds revealed the high occurrence of *M. persicae*, qualified as the most efficient vectors of PVY^N. All identified aphids on weeds were previously reported to be present on potato leaves (Boukhris-Bouhachem et al. 2011), and could be therefore involved in the secondary infection cycle.

Based on our study, 39.82% samples of *S. elaeagnifolium*, recently introduced in Tunisia, were infected with PVY^N. Except for Douala, *S. elaeagnifolium* infected with PVY^N were registered in all prospected areas. The investigation of aphid species occurring on *S. elaeagnifolium* revealed the presence of *M. persicae*, *A. gossypii*, and *A. fabae*. These aphids have been previously reported to be vectors of PVY^{NTN} in potato fields with a transmission rate estimated at 95, 82 and 43% for *M. persicae*, *A. gossypii* and *A. fabae*, respectively (Boukhris-Bouhachem et al. 2011). Our results showed that infection of the silver nightshade with PVY^N was often detected from Sidi Mahmoudas reported by Boukhris-Bouhachem et al. (2007). Features such as the abundance of this weed, its ability to survive during the winter, the high infection rate within samples, and the presence of aphids suggest the silver nightshade as a plant reservoir for PVY^N.

Previous studies have reported that several aphid species, including *M. persicae* and *A. gossypii*, play an important role in the transmission of viruses such as *Alfalfa Mosaic Virus* (AMV), *Cucumber Mosaic Virus* (CMV) and PVY by feeding on these weeds (Graham et al. 1979; Harris et al. 2001). Another study reported the high incidence of PVY^N in potato seed-producing fields has led to the decline of this crop (Boukhris-Bouhachem et al. 2015). In fact, despite the regular management of these plots (early removal of diseased plants, chemical treatments, haulm destruction ...), this situation occurred in Sidi Mahmoud where *S. elaeagnifolium* was relatively important (Boukhris-Bouhachem 2007). This observation may suggest that the occurrence of *S. elaeagnifolium* in the agricultural sites of the region of Sidi Mahmoud were the source of infection of potato fields by aphid vectors of PVY^N.

In Douala, the important frequency of PVY^N infection revealed on both *S. nigrum* and *D. stramonium* and the occurrence of aphid vectors may increase more the impact of these plants as potential alternative hosts for virus dissemination. In comparison to the four studied regions, Bousalem showed the lowest PVY^N infection on weeds.

The high levels of PVY^N infection detected in plant reservoirs proved their important impact for the certified potato seed-producing crops. Agronomic and environmental context such as the proximity often observed in the same area of potato crops for commercial and seed production and the presence of other solanaceous crops such as pepper, commonly infected with PVY virus (Gorsane et al. 1999) and the occurrence of weeds provide an unfavorable environment for the production of potato seeds. Indeed, this situation preserves the virus inocula in the fields which may be a risk of PVY infection in potato crops. However, little is known about the possible cross transmission of PVY^N strains between pepper, tomato, and potato. Some PVY^N isolates from tomato are similar to PVY^C strain infection on

potato or to isolates of PVY^{NTN} variant, which may induce necrotic systemic infections under laboratory conditions (Moury et al. 2007). In this study, PVY^{NTN} infection was reported for the first time on *S. elaeagnifolium*, *D. stramonium* and *S. oleraceus* in Tunisia. This finding is not a surprise and was already reported by Boukhris-Bouhachem et al. (2010) where PVY^{NTN} was reported as the most abundant variant on potato seed-producing regions. This situation makes the eradication of these weeds, an important management to control PVY^{NTN} propagation. However, it is imperative to highlight that not all infected weeds may contribute in PVY^{N} dissemination. Therefore, additional assays should be performed to study the role of these infected weeds in PVY^{N} dynamics.

The investigated weeds were shown to be a virus source since they may cumulate their effects and to be a risk for the sanitary quality of potato seeds. Under these conditions, it becomes important to inform and increase knowledge of farmers about these plant reservoirs and to build a strategy to discard them from the field. Treatment against aphids that are known to be PVY^{N} vectors should be also conducted since they play important role in the virus infection establishment. As alternatives to chemical insecticides, the use of mineral oils (Boukhris-Bouhachem et al. 2015) or plant essential oils against insect pest seems to hold a promising future within integrated pest management concept (Isman 2000). In addition, it will be necessary as prevention measurements to search for new potato seed-producing areas far from the regions recognized to hold a risk for virus infection.

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RESUME

Boukhris-Bouhachem S., Ben Fekih I., Rouzé-Jouan J., Souissi R. et Hullé, M. 2017. Impact de l'interaction pucerons-adventices hôtes dans la dissémination des souches du PVY^{N}. Tunisian Journal of Plant Protection 12: 41-48.

Dans le contexte d'une étude épidémiologique du virus Y de la pomme de terre (PVY), une recherche des plantes adventices susceptibles d'héberger ce virus a été réalisée. Ces plantes peuvent être des sources d'infection par le PVY par le biais des pucerons vecteurs pour les plants prévus à la production de semences. En effet, trois espèces de pucerons ont été identifiées sur ces adventices dont *Aphis fabae, A. gossypii* et *Myzus persicae*. Ceci a permis d'étudier l'interaction possible entre les vecteurs et les réservoirs du PVY^{N}. Sur un total de 772 plantes appartenant à 12 familles différentes (*Solanaceae, Amaranthaceae, Chenopodiaceae, Papaveraceae, Urticaceae, Convolvulaceae, Asteraceae, Polygonaceae, Euphorbiaceae, Brassicaceae, Portulacaceae* et *Compositae*), 337 plantes se sont révélées infectées par le PVY^{N} moyennant la technique DAS-ELISA. Parmi ces plantes, on cite *Solanum elaeagnifolium*, *Datura stramonium* et *Sonchus oleraceus* qui sont infectées par la souche PVY^{NTN}. En outre, ces trois adventices en plus de *S. nigrum* semblent être des plantes hôtes importantes du PVY^{N} vu qu'elles sont aussi infestées par les pucerons vecteurs. Ces informations de base sur les mauvaises herbes et les repousses qui hébergent le PVY^{N} et les pucerons vecteurs permettraient de mieux connaître l'épidémiologie du PVY^{N} dans les champs de pomme de terre et de trouver les méthodes appropriées pour gérer cette maladie virale.

Mots clés: Adventices, pomme de terre, puceron, PVY^{N}, PVY^{NTN}, repousses

ملخص

بوخريص-بوهاشم، سنية وابتسام بن فقيه وجويل روزي-خوان ورابحة السويسي وموريس هولاي. 2017. تأثير تفاعل حشرات المن والأعشاب الضارة العائلة في انتشار سلالات فيروس PVY^N.
Tunisian Journal of Plant Protection 12: 41-48.

في نطاق الأبحاث حول فيروس البطاطا (PVY) قمنا بدراسة حول الأعشاب الضارة العائلة والنبات المتطوع العائل اللذان يمكن أن يكونا مصدرا لإصابة بذور البطاطا وانتشار فيروس PVY بواسطة حشرات المن *Aphis fabae* و*Aphis gossypii* و*Myzus persicae*. تبين أن من بين 772 عينة من الأعشاب الضارة التي تم اختبارها بالاعتماد على تقنية الأمصال (DAS-ELISA)، 337 عينة كانت تحمل الفيروس PVY^N. تنتمي النباتات المذكورة إلى 12 عائلة مختلفة هي Solanaceae, Amaranthaceae, Chenopodiaceae, Papaveraceae, Urticaceae, Convolvulaceae, Asteraceae Polygonaceae, Euphorbiaceae, Brassicaceae, Portulacaceae, Compositae. ومن أهم هذه النباتات نذكر *Solanum elaeagnifolium* و*Datura stramonium* و *Sonchus oleraceus* وهي تحمل سلالة الفيروس PVY^{NTN}. إذن، تلعب الأعشاب دورا هاما في انتشار الفيروس PVY^N على بذور البطاطا خاصة وأن أنواع حشرات المن الناقلة للفيروس تتكاثر عليها. لذا، يتعين على مكثري البذور القضاء على هذه الأعشاب لتحسين الحالة الصحية للبذور ومقاومة هذا المرض.

كلمات مفتاحية:أعشاب الضارة،بطاطا، نبات متطوع،منّ، PVY^{NTN}، PVY^N

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