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Potential Alternative Hosts and Transmissibility of *Potato virus Y*

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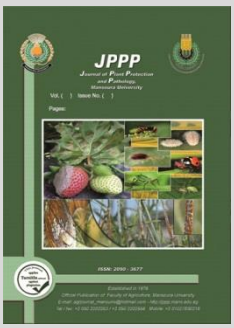

ABSTRACT

To study the potential alternative hosts to *Potato virus Y* (PVY), thirteen different plant species/cultivars, *Solanum tuberosum* cv. Spounta L., *S. tuberosum* cv. Cara L., *Datura stramonium* L., *D. metel* L., *Nicotiana tabacum* cv. White burley L., *Chenopodium amaranticolor* (H.J.Coste & A.Reyn.) H.J.Coste & A.Reyn., *Solanum lycopersicum* L., *S. melongena* L., *Capsicum annuum* L., *Phaseolus vulgaris* L., *Vicia faba* L., *Brassica oleracea* L. and *Lactuca sativa* L., belonging to five botanical families were tested. Nine of the above tested plant species/cultivars reacted to PVY isolate with different symptoms, while four of plant species *D. stramonium*, *V. faba*, *B. oleracea* and *L. sativa* did not react to PVY isolate. Mechanical transmission of PVY was confirmed from infected potato to healthy *D. metel* plants and back from PVY-inoculated *D. metel* to healthy potato plants with 100% success. Insect transmission was also confirmed from infected potato plants to healthy ones through aphid *Myzus persicae* with 80%. Virus particles of PVY were examined using transmission electron microscopy (TEM). Typical PVY particle size of 11 × 700 nm with flexuous filamentous shape was observed.

Keywords: PVY, DAS-ELISA, transmission, host range, stability, symptomatology

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a member of *Solanaceae* family. It ranks the fourth crop in importance worldwide after maize, rice and wheat. In Egypt, it is taking second place among vegetable crops after tomato. In 2018, Egypt ranked the sixteenth in the world and first in Africa with regard to potato production (FAOSTAT, 2020). *Potato virus Y* is a member of the *Potyvirus* genus that belongs to *Potyviridae* family. The family *Potyviridae* is the largest family of RNA plant viruses, having +ssRNA genome and flexuous filamentous shape with 680 – 900 nm long and 11- 20 nm width. The *Potyvirus* is the largest genus among plant viruses, containing about 162 species. Many species have limited host range and some members are transmitted by aphids. Also, some *Potyvirus* members could be seed-borne (ICTV, 2019). *Potato virus Y* has non-enveloped particles and flexible rod-shaped, with length of 984 nm in case of purified preparations, while 730 nm in leaf-dip preparations with 11 nm in width. The coat protein subunits are arranged in a helical symmetry (Zamora *et al*., 2017). *Potato virus Y* is one of the top 10 serious plant viruses based on economic importance and distribution in the world (Scholthof *et al*., 2011). *Potato virus Y* is the most dangerous virus infecting potato in the world, because it spread where the potato is cultivated and led to rejection of potato seeds by certification programs. It causes loss in potato yield estimated from 10 to 80% (Tsedaley, 2015). *Potato virus Y* on various potato cultivars under field conditions (natural infection) causes various symptoms, *i.e*., crinkle, mosaic, stunting, shriveling, deformation of leaves and necrosis (Christophe *et al*., 2017). *Potato virus Y* has a wide host range, including various species of plants belonging to more than nine botanical families, *i.e*., potato, tomato, pepper, tobacco, some ornamental plants and many weeds. While its experimental hosts are about 495 species in 72 genera of 31 botanical families, including 287 species of *Solanaceae*, 48 species of *Chenopodiaceae*, 25 species of *Fabaceae* and 11 species of *Asteraceae* (Edwardson and Christie, 1997; Jeffries, 1998). Powell *et al*. (2006) explained that aphids transmit viruses to plants in a mechanical manner (non-persistent/non-circulative). This phenomenon means that aphids are become a vector to virus once visiting an infected plant (acquisition feeding), then to a healthy plant (infectious feeding) during its probes, which done by short feeding on epidermis cells for evaluation the suitability of plant as a host. Abdel-Shafi *et al*. (2017) found that the transmission efficiency of PVY by mechanical means from infected potato to healthy was 90%, while from *Datura metel* L. to healthy potato plants was 75%, while by aphids was 85% and by grafting 100%. Stability of PVY showed that the thermal inactivation point (TIP) was between 55-60°C; dilution end point (DEP) was at 10^{-3} to 10^{-6} and longevity *in vitro* was between 24-48 h (Nasr-El-Din, 2007; Eid *et al*., 2008; Abdel-Shafi *et al*., 2017). Singh and Santos-Rojas (1983) reported that the PVY isolates were detected in naturally infected potato plants by ELISA tests. Ismail (1997) mentioned that the double antibodies sandwich (DAS)-ELISA was used to quantitative detection of PVY in infected potato by specific antibodies to PVY.

Thus, studying the potential alternative hosts, transmissibility and particle morphology of PVY was the main aim of the present work.

MATERIALS AND METHODS

Disease symptoms and sample collection

Naturally infected potato plants showing symptoms typical to those induced by PVY, were collected. Thirteen samples of potato plants were collected from different locations (7 samples from Talkha, 3 samples from Aga and 3 samples from Faculty of Agriculture's farm) in Dakahlia governorate, Egypt. All plant samples were tested with polyclonal antibodies specific to PVY obtained from German Collection of

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Microorganisms and Cell Cultures (GmbH Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSMZ GmbH) using DAS-ELISA to confirm the presence of PVY according to methods of Clark and Adams (1977), in the laboratory of Seed Pathology and Tissue Culture, Faculty of Agriculture, Mansoura University, Egypt.

Source and cultivation of indicator plants

Seeds of indicator plants *Nicotiana tabacum* cv. White burley were obtained from Romania, *D. stramonium* and *Chenopodium amaranticolor* obtained from Faculty of Science, Al-Azhar University, Egypt. The indicator plants were cultivated in pots in an insect-proof greenhouse conditions (at 28 ± 2°C and 16 h daylight). Etch pot was filled with a soil mix of clay, peat moss and sand (3:1:1 v/v/v, respectively). The soil mix was sterilized with formalin (1%) at 10 l/m^2, then was covered with plastic for 48 h, and then uncovered for 10 days before cultivation to get rid of formaldehyde gas.

Potential alternative hosts

To study the potential alternative hosts of PVY, thirteen different plant species/cultivars, *S. tuberosum* cv. Spounta, *S. tuberosum* cv. Cara, *D. metel*, *D. stramonium*, *N. tabacum* cv. White burley, *C. amaranticolor*, *S. lycopersicum*, *S. melongena*, *Capsicum annuum*, *Phaseolus vulgaris*, *Vicia faba*, *Brassica oleracea* and *Lactuca sativa* belonging to five botanical families were mechanically inoculated using 0.01 M phosphate buffer, pH 7.0 [1.781 g $Na_2HPO_2.2H_2O$ dissolved in 1 litter of dH_2O (A), 1.362 g KH_2PO_4 dissolved in 1 litter of dH_2O (B), then 51 ml from solution A mixed with 49 ml from solution B] with PVY (infectious sap of PVY-infected *D. metel* and *N. tabacum* cv. White burley). Three plants of each host were tested. All inoculated hosts were kept in insect-proof greenhouse at 28 ±2°C and 16 h daylight. Induced symptoms were observed 3-4 weeks post-inoculation.

Mechanisms of transmission

Mechanical transmission

Healthy potato cv. Spounta (20-30 days old) and alternative host plants were mechanically inoculated using PVY-infectious sap in 0.01 M phosphate buffer, pH 7.0 prepared as previously described. The inoculated plants were maintained in insect-proof greenhouse at 28 ±2°C and 16 h daylight for 20 days for further biological tests. The morphological symptoms were observed and tested by DAS-ELISA.

Insect vector transmission

Aphid transmission of PVY was tested using colonies of non-viruliferous aphid (*Myzus persicae*). Aphids were collected from potato fields and were identified by specialists in Plant Protection Department, Faculty of Agriculture, Mansoura University. The collected aphids were feeding on cabbage plants under insect-proof cages for one hour. The nymphal and adult stages of collected aphids were used for transmission of PVY in a non-persistent manner. Aphids were fasted for 15 min before acquisition feeding on PVY-infected potato cv. Spounta. The aphids were feeding on infected potato for 5, 10, 20 and 30 min. Then transferred to filter paper for one hour. After that, all insects were transferred to healthy potato plants cv. Spounta to feed for one hour, and then they were killed by systemic insecticide. All aphid-infected potato plants were kept in insect-proof cages in greenhouse at 28 ±2 °C and 16 h daylight till the development of external symptoms.

Virus stability

In order to study the stability factors of PVY *in vitro* (Thermal inactivation and dilution end points and Longevity *in vitro*), the indicator plants *C. amaranticolor* were mechanically inoculated with infectious sap obtained from *D. metel* infected with PVY. The inoculated plants were maintained in insect-proof greenhouse. Three plants of the indicator host were used as replicates. The number of local lesions developed on the inoculated leaves were determined as described by Walkey (1985) as the following:

Thermal inactivation point (TIP)

To determinate the TIP, one milliliter of infectious sap was placed in a test tube. The tubes were incubated in a water path for 10 min at 35, 40, 45, 50, 55, 60, 65, 70 and 75°C. The tubes were immediately cooled by dipping them in ice. All treated and non-treated infectious sap were used to inoculate the same number of indicator plants.

Dilution end point (DEP)

In order to determinate the DEP, six dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) of infectious sap were used to inoculate the same number of indicator plants.

Longevity *in vitro* (LIV)

To determinate the LIV, one milliliter of infectious crude sap was kept in small tubes (3 ml) at room temperature (about 20-25°C) for different periods (zero, 1, 6, 12 h, 1, 2, 3, 4, 5 and 6 days). After each period, one tube was used to inoculate the same number of indicator plants.

Particle morphology

In order to study the morphology of PVY isolate, infectious sap of PVY-infected *D. metel* leaves was homogenized in 0.01 M phosphate buffer, pH 7.0 (1:2 W: V). The infectious sap was clarified at 6000 rpm at 4°C for 15 min. A 50 µl of a clarified virus was dropped on a carbon-coated grid for 15 min, and excess liquid was removed with filter papers. Virus particles existed on carbon grids were stained with 2% phosphotungstic acid pH 7.0, then left to dry for 15 min. Finally, the grids were examined by transmission electron microscope (TEM) type JEOL JEM-2100 under voltage of 200 KV., at magnification of X 20000, Electron Microscope Unit, Faculty of Agriculture, Mansoura University, Egypt.

RESULTS AND DISCUSSION

Results

Symptomatology, locations and PVY-detection

From the thirteen samples (Table 1) collected from potato plants naturally infected with PVY from different locations in Dakahlia governorate, Egypt, six samples (3 samples of Talkha, 2 samples of Aga and one sample of Faculty of Agriculture farm) were PVY positive using DAS-ELISA test (Fig. 2).

Table 1. Locations of collected samples from Dakahlia governorate, Egypt.

Samples location	Sample code number
Talkha	1, 2, 3, 4, 5, 9 and 11
Faculty of Agriculture's farm	6, 7 and 8
Aga	10, 12 and 13

Field inspection during sample collection revealed that all potato plant samples showing symptoms such as mosaic, yellowing between veins, mottling, stunting, chlorosis and necrosis were considered typical to those induced by PVY infection (Fig. 1).

Figure 1. Potato plants: A) healthy, B) mottle and mild mosaic, C and D) necrosis and deformation

Potato virus Y was detected in potato plants naturally infected using the methods of DAS-ELISA with PVY-specific antibody. The obtained results showed that potato cv. Spounta naturally infected with PVY gave positive reaction with the specific antibody (Fig. 2). Those plants were selected and PVY was biologically isolated on *D. metel* plants.

Potential alternative hosts to PVY

Thirteen different plant species/cultivars belonging to five botanical families shown in Table (2) were tested to determine their response to infection with PVY. Nine of the tested plant species/cultivars (*S. tuberosum* cv. Spounta, *S. tuberosum* cv. Cara, *D. metel*, *N. tabacum* cv. White burley, *C. amaranticolor*, *S. lycopersicum*, *S. melongena*, *C. annuum*, *P. vulgaris*) were reacted with PVY isolate in different symptoms, but four of plant species (*D. stramonium*, *V. faba*, *B. oleracea* and *L. sativa*) did not react with PVY isolate.

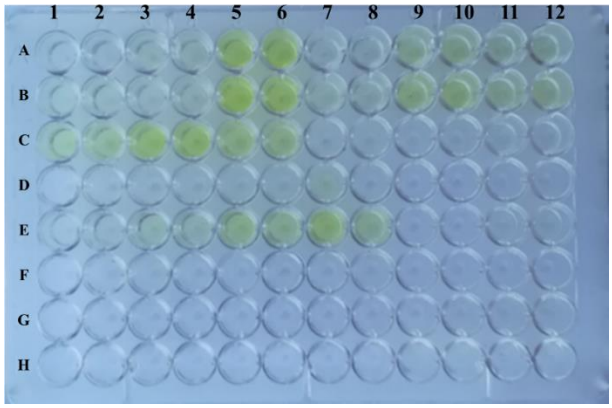

Figure 2. Detection of PVY in naturally infected potato plants using DAS-ELISA technique: negative sample (A1, 2); Talkha location 1 (A5, 6); Faculty of Agriculture farm 7 (B5, 6); Talkha location 9 (B9, 10); Talkha location 11(C1, 2); Aga location 12 (C3, 4); Aga location 13 (C5, 6); blank sample (C11, 12)

Table 2. Reaction of some potential alternative hosts to PVY isolate using mechanical inoculation.

No.	Plant name	Scientific name	Symptoms[a]
1	Potato cv. Spounta	*Solanum tuberosum* cv. Spounta L.	M, N, D, Mo
2	Potato cv. Cara	*Solanum tuberosum* cv. Cara L.	MM, SM, D
3	*D. metel*	*Datura metel* L.	M, D
4	*D. stramonium*	*Datura stramonium* L.	NS
5	Tobacco	*Nicotiana tabacum* cv. White Burley L.	M, Mo
6	*C. amaranticolor*	*Chenopodium amaranticolor* L.	Lch.
7	Tomato	*Solanum lycopersicum* L.	MM, D
8	Eggplant	*Solanum melongena* L.	Mo
9	Pepper	*Capsicum annuum* L.	M, LC, CP
10	Common bean	*Phaseolus vulgaris* L.	M, VB
11	Faba bean	*Vicia faba* L.	NS
12	Cabbage	*Brassica oleracea* L.	NS
13	Lettuce	*Lactuca sativa* L.	NS

[a]M = Mosaic, MM= Mild Mosaic, SM= Severe Mosaic, D= Deformation, N= Necrosis, Mo = Mottling, Lch= Local chlorotic lesion, LC= Leaf crinkle, CP= Cup shape, VB= Vein banding NS= No symptoms.

Mode of transmission

Potato virus Y isolate was successfully transmitted by mechanical inoculation from infected potato plants to healthy *D. metel* plants, and vice versa with 100% success. However, it was transmitted from infected potato plants to healthy ones by green peach aphid *Myzus persicae* with 80% transmission success (Table 3).

Virus stability

The infectious crude sap used for stability assays was obtained from infected *D. metel* plants, while *C. amaranticolor* plants were used as an assay host. At least three plants were used for each treatment. The local lesions observed on *C. amaranticolor* plants were counted. Data in Table (4) show that thermal inactivation point (TIP) was between 55-60°C; dilution end point (DEP) was 10^{-4} and longevity *in vitro* was between 2 and 3 days.

Particle morphology

Transmission electron microscope examination of a partially purified preparation from infected *D. metel* leaves negatively stained with phosphotungstic acid showed that the size of PVY was about 11×700 nm with flexuous filamentous shape (Fig. 3).

Table 3. Success percentage of PVY transmission by mechanical inoculation and aphid transmission.

No. of plants	Mechanical inoculation success	Aphid transmission success
-control	-	
+control	+	
1	+	+
2	+	+
3	+	-
4	+	+
5	+	+
6	+	+
7	+	+
8	+	-
9	+	+
10	+	+
%	100%	80%

Table 4. Stability of PVY isolate in infectious sap at room temperature using local lesion assay.

Thermal inactivation point (TIP)			Dilution end point (DEP)			Longevity *in vitro* (LIV)		
Temperature (°C)	No. local lesions	Infectivity (%)	Dilutions	No. local lesions	Infectivity (%)	Longevity	No. local lesions	Infectivity (%)
Control	80	100	Control	80	100	Control	80	100
35	73	91.25	10^{-1}	75	93.75	1 h	67	83.75
40	68	85	10^{-2}	58	72.5	6 h	55	68.75
45	52	65	10^{-3}	20	25	12 h	40	50
50	28	35	10^{-4}	5	6.25	1 day	25	31.25
55	15	18.85	10^{-5}	0	0	2 days	5	6.25
60	0	0	10^{-6}	0	0	3 days	0	0
65	0	0				4 days	0	0
70	0	0				5 days	0	0
75	0	0				6 days	0	0

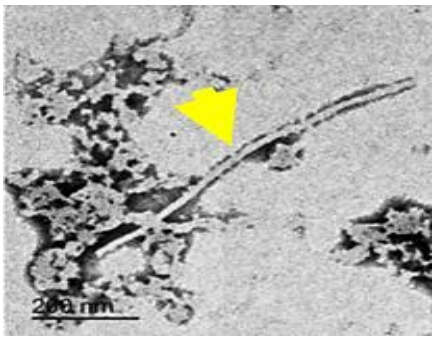

Figure 3. Transmission electron micrographs showing the morphology of PVY particles (arrow-head)

Discussion

Studying the PVY symptomatology, potential alternative hosts, mechanisms of transmission, stability, and particle morphology was carried out. These assays were achieved based on the methods explained by Matthews (2001); Abdel-Shafi *et al*. (2017); Christophe *et al*. (2017); Hamza *et al*. (2018). Diagnosis of potato plants naturally infected with viral diseases was achieved and plants showing viral symptoms were collected. Many symptoms related to PVY infection such as mosaic, yellowing between veins, mottling, stunting, chlorosis, and necrosis were observed. *Potato virus Y* was detected in potato plants naturally infected and exhibited typical symptoms of PVY infection, by the methods of DAS-ELISA using specific antibody of PVY. The obtained results showed that potato plants cv. Spounta naturally infected with PVY gave positive reaction with the specific antibody. Those plants were selected and PVY was biologically isolated on *D. metel* plants. Typical systemic symptoms of mosaic, mottling and leaf crinkle were observed after 15 days from inoculation. These observed symptoms and biological isolation were similar to those reported by Abdel-Shafi *et al*. (2017); Hamza *et al*. (2018). For biological isolation of PVY, *C. amaranticolor* plants were mechanically inoculated from infected *D. metel* plants. The chlorotic local lesions were observed 15-20 days after inoculation. For virus propagation, *N. tabacum* cv. White burley and *D. metel* plants were inoculated with single chlorotic local lesion from infected *C. amaranticolor*. Typical systemic symptoms of mosaic, mottling and leaf deformation were observed 35 days post-inoculation. These results are typical with those reported by Eid *et al*. (2008); Hamza *et al*. (2018); Nasr-Eldin *et al*. (2018). Symptomatology of PVY were studied through inoculation of *D. metel* plants, which exhibited typical systemic symptoms of mosaic, mottling and leaf crinkle. Also, *N. tabacum* cv. White burley showed typical systemic symptoms of mosaic, mottling and leaf deformation. For studying the host range, thirteen different plant species/cultivars belonging to five botanical families, including two cultivars of potato (Spunta and Cara), two species of *Datura*, tobacco (cv. White burley), *C. amaranticolor*, tomato, eggplant, pepper, common bean, faba bean, cabbage, and lettuce were tested to determine their response to infection with PVY. Nine tested plants (potato, *D. metel*, tobacco, *C. amaranticolor*, tomato, eggplant, pepper and common bean) were reacted exhibiting different symptoms, but four plants (*D. stramonium*, faba bean, cabbage and lettuce) did not react to PVY isolate. Similar results were obtained by Jeffries (1998); Eid *et al*. (2008); Abdel-Shafi *et al*. (2017). Mechanical transmission was 100% successful from infected potato plants to healthy *D. metel* plants and vice versa but it was 80% from infected potato plants to healthy ones via *Myzus persicae*. These results agree with those of Ragsdale *et al*. (2001); Abdel-Shafi *et al*. (2017). Stability assay of PVY proved that the TIP was between 55-60°C; DEP was 10^{-4} and longevity *in vitro* was between 2 and 3 days. Similar results were reported by Mahfouz (2003); Nasr-El-Din (2007); Eid *et al*. (2008); Abdel-Shafi *et al*. (2017). Transmission electron microscope examination of a partially purified preparation from infected *D. metel* leaves negatively stained with phosphotungstic acid showed that the size of PVY was around 11×700 nm with flexuous filamentous shape as

found in previous work of Delgado-Sanchez and Grogan (1966). Method of DAS-ELISA was used and confirmed the presence of PVY in tested plants using polyclonal antibodies specific to PVY (Carlebach *et al*., 1982; Singh and Santos-Rojas, 1983; Ismail, 1997; Eid *et al*., 2008).

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العوائل البديلة المحتملة وإمكانية نقل فيروس البطاطس Y

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لدراسة العوائل البديلة المحتملة لفيروس البطاطس Y (PVY) ، تم اختبار ثلاثة عشر نوعًا/صنفاً نباتيًا تنتمي إلى خمس عائلات نباتية مختلفة، وكانت الأنواع/الاصناف كالتالي: بطاطس صنف اسبونتا *Solanum tuberosum* cv. Spounta L.، بطاطس صنف كارا *S. tuberosum* cv. Cara L.، داتوره ميتل *Datura metel* L.، داتوره استرامونيم *D. stramonium* L.، الدخان *Nicotiana tabacum* cv. White burley L.، الزربيح *Chenopodium amaranticolor* (H.J. Coste & A. Reyn.) H.J. Coste & A. Reyn.، الطماطم *Solanum lycopersicum* L.، الباذنجان *S. melongena* L.، الفلفل *Capsicum annuum* L.، الفاصوليا *Phaseolus vulgaris* L.، الفول البلدي *Vicia faba* L.، الكرنب *Brassica oleracea* L. والخس *Lactuca sativa* L.. وقد أظهرت النتائج أن تسعة من الأنواع/الاصناف النباتية التي تم اختبارها تفاعلت مع عزلة PVY بإظهار أعراض مختلفة، في حين أن أربعة أنواع منها (الداتوره استرامونيم، والفول البلدي، والكرنب، والخس) لم تتفاعل مع نفس العزلة من الفيروس. كما تم تأكيد النقل الميكانيكي لـ PVY من نباتات البطاطس المصابة إلى نباتات الداتوره ميتل *D. metel* السليمة وإعادة النقل من الداتوره ميتل *D. metel* المصابة بـ PVY إلى نباتات البطاطس السليمة بنسبة نجاح 100 ٪. كما تم تأكيد نقل الفيروس من نباتات البطاطس المصابة إلى النباتات السليمة عن طريق حشرة من الخوخ الأخضر *Myzus persicae* بنسبة 80٪. كما تم ايضاً فحص جزيئات فيروس PVY باستخدام الميكروسكوب الإلكتروني النافذ (TEM)، وأظهرت نتائج الفحص أن حجم الفيروس 11×700 نانومتر، يتطابق تماماً مع الحجم والشكل الخيطي المرن الخاص بجزيئات فيروس PVY.