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STUDIES ON POTATO Y POTYVIRUS (PVY) AFFECTING POTATO IN AL DAKAHLIYA AND DAMMIETTA GOVERNORATES

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ABSTRACT

Two strains of potato virus Y "potyvirus" (PVY) were isolated from naturally infected potato *Solanum tuberosum* CV. Spunta plants from Al Dakahliya and Dammietta governorates showing rougosity, mosaic, and veinal necrosis on the leaves; stunting; stem canker; and palm sheep. The identification was based on the symptomatology, indicator plants, physical properties, serological tests "ELISA and immuno electron microscopy", molecular wieght, and negative staining with electron microscopy. The virus induced systemic and non systemic symptoms which appears on the indicator plants. *Datura stramonium* was resistant to both strains (PVY^{N}-W and PVY^{O}). On the other hand *Nicotiana tabacum* CV. Turkish, White Burley, Xanth, and Samsun., *S. tuberosum* CV. King Edward, Spunta., and *D. metel* showed various systemic symptoms. The thermal inactivation point (TIP) was between 53 ^{o}C and 56 ^{o}C, dilution end point (DEP) for the tested virus was between 10^{-3} and 10^{-4}, while longevity in vitro (LIV) was between 48 and 60 hr at room temperature (RT). Indirect - Enzyme Linked Immuno Sorbent Assay (I-ELISA) showed that there are nine positive samples from twenty fife samples. Electron microscopy showed separated particles of PVY with length of 600 nm , while using immuno electron microscopy showed PVY particles in aggregates. The molecular weight of purified PVY coat protein was 34 KDa through polyacrylamide gel electrophoresis test

Keywords: PVY Strains, Identification, Indicator Plants, Physical Properties, Serological Methods, ELISA, Molecular Weight, and Electron Microscopy.

INTRODUCTION

The potato (*Solanum tuberosum* L.) is one of the most important Solanacious crops in Egypt and in many countries of the world. In Egypt, the cultivated area was 238095 feddans in 2005 and it gave yield for approximately 2500000 tons, (FAO Stat. Database, 2006).

Potato is affected by many pests included fungal, bacterial and viral diseases. Potato is vegetative propagated; viruses constitute a permanent threat for seed potato growers because most of them induce systemic diseases and transmitted through seed tubers. Among viruses, potato virus Y (PVY), is considered the most economically important virus pathogens, Glais *et al.* (2005).

Potato virus Y has a high risk on potato production and its effect on potato yield up to 90%, (Salazar, 2003).

Virus diseases are still an international problem on potato production and its industry. This problem has rapidly increased during the last few years throughout the vegitative propagation. There are many potato viruses are resbonsible of annually loses among them is PVY potyvirus. Therefore this work was planned with the object to study the identification of PVY and its properties.

MATERIALS AND METHODS

Identification of Potato Virus Y

1.1. Isolation

Twenty five samples of naturally infected potato (*S. tuberosum*) plants were collected from AL Dakahliya and Dammietta governorates.

For isolation the infected potato leaves were ground in 0.01 M sodium citrate buffer pH 7.4 and the inoculum was prepared from infected potato leaves and applied to leaves of healthy plants of *Nicotiana tabacum* CV. Turkish and *Ch. amaranticolor* previously dusted with carborundum (600) mesh. The single local lesion technique was used for the strain PVY^{O} which produced local lesion on *Ch. amaranticolor*, while, the strain PVY^{N} which did not produced local lesion on *Ch. amaranticolor*, so we sure that these isolates were not mixed infection by using immuno electron microscopy. *N. tabacum* CV. Turkish and *N. glutinosa* were used for PVY^{O} and PVY^{N}-W propagation respectively.

1.2. Symptomatology and Indicator Plants

To study indicator plants and symptomatology of PVY^{N} ten hosts belong to two families were mechanically inoculated and observed for 4 weeks under green house conditions. The indicator plants were *S. tuberosum* CVs. Spunta, and King Edward, *D. stramonium, D. metel, Ph. floridana, N. glutinosa, N. tabacum* CVs. Turkish, White Burley, Xanth, and Samsun, *Ch. amaranticolor, Ch. album, Ch. quinoa*, *Cucumus sativus, Cucurbita pepo*, and *Gomphrena globos*a.

Symptoms were recorded for 1-4 weeks after mechanical inoculation under green house conditions.

1.3. Physical Properties

Thermal inactivation point (TIP), dilution end point (DEP), and longevity in vitro (LIV) were performed using standard procedure as suggested by, **Noordam, 1973.** Leaves of infected *N. glutinosa* plants were used as a source for infectious crude sap. *Ch. amaranticolor* was used also as an assay host. At least five plants were used for each treatment. The number of local lesions which developed on the assay host leaves were calculated.

1.4. Serological Tests

1.4.1. Indirect -Enzyme Linked Immuno Sorbent Assay (I-ELISA)

Specific PVY antiserum was kindly donated from Danish Government Institution of Seed Pathology for Developing Countries (DGISP)

Twenty five samples of naturally infected potato leaf samples were tested using I-ELISA. The procedure was conduced according to the method of Clark & Bar-Joseph (1984). Naturally infected samples exhibited PVY symptoms were homogenized. ELISA plate wells were loaded with infected extract. The plates were incubated and washed with PBST. After washing the wells had loaded with primary antiserum then were incubated and washed, after that wells were loaded with secondary antiserum and were incubated

and washed. After that the wells were loaded with *ρ*-nitrophenyle phosphate (the substrate) and then incubated. NaOH was added for stop the reaction. Absorbance value was measured using Multiskan EX primary EIA ELISA Reader.

1.4.2. Direct -Enzyme Linked Immuno Sorbent Assay (D-ELISA)

Only three samples of infected tobacco were sent kindly to Dr. Jorg Schubert (Institute of Resistance Research and Pathogen Diagnostics, Federal Central for Breeding Research on Cultivated Plants, Germany). D-ELISA with monoclonal antibodies (PVY^{N} – McAbs cocktail, Bioreba) used for detection of isolated PVY strain (PVY^{N}-W). D-ELISA demonstrated by Clark and Adams (1977) was used.

1.4.3. Immuno Electron Microscopy (ISM)

The immuno electron microscopy (decoration method) were used to detect PVY^{N} particles from *N. glutinosa* exhibited systemic infection (21 days after virus inoculation). The method which described by, (Garg and Paul Khurana, 1991). Grids stained with 2% uranyl acetate (w/v) and then washed and lifted for 5 min to dry and then examined with JEOL (JEM 100 CXII) Transmission Electron Microscopy at magnification of X 57000.

1.5. Molecular Weight

The molecular weight of purified preparation of PVY^{N} was determined by sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 4% for stacking gel and 12% for resolving gel as described by, (Laemmli, 1970., and Shukla and Ward, 1988).

RESULTS

Identification of Potato Virus Y

1.1. Isolation

Results revealed that, the most frequent PVY symptoms were vienal necrosis on the leaves, palm sheep, and stunting (fig. 1 and 2); rougosity and mosaic, as shown as fig. (3). The percentage of PVY occurrence was in the range of 36 % in the potato samples which collected from growing areas.
The isolates of the two tested virus strains were designated as PVY^{N} and PVY^{O}.
PVY^{N} strain was used for further study, while the other PVY^{O} strain was used for local lesion assay on *Ch. amaranticolor*.

1.2. Symptomatology and Indicator Plants

The reactions of different indicator plants were tested using the mechanically inoculation of the infectious crude extract of the *N. glutinosa*. Data in table (1) showed the systemic and non systemic symptoms which appear on the indicator plants. *D. stramonium* was resistant to PVY^{N}-W and PVY^{O} strains. On the other hand *N. tabacum* CVs. Turkish, White Burley, Xanth, and Samsun., *S. tuberosum* CVs. King Edward, and Spunta., and *D. metel* showed various systemic symptoms. The symptomless indicators were confirmed by back inoculation into *N. glutinosa* which expressed no symptoms.

Table 1: Indicator Plants of PVY strains and their reaction

Family	The Tested Hosts	Strain (PVY^N-W)	Strain (PVY^o)
Chenopodiacae	*Chenopodium amaranticolor* Coste & Reyn.	NR	LL
	Chenopodium album L. *Chenopodium quinoa* Wild.	NR	-
Solanaceae	*Datura stramonium* L.	NR	NR
	Datura metel L.	Mo	Mo
	Nicotiana tabacum CV. Turkish, White Burley, Xanthi, and Samsun	VN, SC, PS	M
	N. glutinosa L.	VC and Mo	Mo
	Physalis floridana L.	Mo	LL
	Solanum tuberosum CV. King Edward	VN, PS	M
	Solanum tuberosum CV. Spunta	VN, PS	M

(-) = Not Tested, (LL) = Local Lesion, (M) = Mottling, (Mo) = Mosaic, (NR) = NO Reaction , (PS) = Palm Sheep, (SC) = Stem Canker, (SN) = Systemic Necrosis, (VC) = Vein Clearing, (VN) = Vein Necrosis.

1.3. Physical Properties

Data in table (2) revealed that thermal inactivation point (TIP) was between 53 °C and 56 °C, dilution end point (DEP) was between 10^{-3} and 10^{-4}, while longevity in vitro (LIV) was between 48 hr and 60 hr at room temperature (RT).

Table 2: Stability of PVY^O strain using *Ch. amaranticolor* plants as local lesion diagnostic host.

Thermal inactivation point		Dilution end point		Longevity in vitro	
Temp. (°c)	No. of NLL/leaf	Dilutions	No. of NLL/leaf	Storage (hr.)	No. of NLL/leaf
Unheated	133	Crude sap	130	Immediatel	129
40	75	10^{-1}	118	12	107
50	25	10^{-2}	55	24	41
53	9	10^{-3}	12	36	17
56	0	10^{-4}	0	48	7
59	0	10^{-5}	0	60	0
60	0			72	0
70	0				

1.4. Serological Tests of PVY

1.4.1. I-ELISA (Indirect -Enzyme Linked Immuno Sorbent Assay)

Using I-ELISA method, nine of the collected samples gave positive reaction and its incidence was 36% from the collected samples.
Data presented in the table (3) showed the absorbance values of I-ELISA method for detection of PVY in Spunta potato leaves of 25 samples plus the healthy control.

Table 3: The absorbance value average at 405 nm of I-ELISA method for potato leaves samples.

Sample No.	Absorbance Value Average at 405 nm		Result
	After 60 min	After 120 min	
1	0.489	0.931	+
2	0.869	1.759	+
3	0.906	1.828	+
4	0.032	0.142	-
5	0.042	0.073	-
6	0.052	0.123	-
7	0.516	0.995	+
8	0.049	0.154	-
9	0.034	0.116	-
10	0.541	1.045	+
11	0.032	0.128	-
12	0.042	0.080	-
13	0.053	0.134	-
14	0.314	0.599	+
15	0.046	0.089	-
16	0.048	0.071	-
17	0.044	0.117	-
18	0.371	0.764	+
19	0.040	0.104	-
20	0.041	0.117	-
21	0.053	0.138	-
22	0.042	0.097	-
23	0.275	0.434	+
24	0.037	0.092	-
25	0.236	0.399	+
(H)	0.038	0.099	

(H) = Healthy

1.4.2. D-ELISA (Direct-Enzyme Linked Immuno Sorbent Assay)

Two samples of *N. tabacum* CV. Samsun plants infected with PVY^N gave positive reaction with PVY^N McAbs by using D-ELISA (table 4), whereas one sample of *N. tabacum* CV. Samsun infected with PVY^N gave negative reaction with the same antiserum (PVY^N McAbs), so these isolates were called Wilga type (N-strain) tobacco veinal necrosis strain (PVY^N-W).

Table 4: Detection of PVY^N-W in *N. tabacum* CV. Samsun leaves infected with the tested isolates using D-ELISA.

Sample No.	Absorbance Value After 60 min at 405 nm	Result
1	4.00	+
2	3.87	+
3	0.07	-
(H)	0.05	-

(H) = Healthy, (1) and (2) = PVY^N-W, (3) = PVY^o

Fig. 1. *Solanum tuberosum* CV. Spunta leaf naturally infected with PVY^N -W strain exhibited vein necrosis and silvering.

(A) (B)

Fig. 2. *Solanum tuberosum* CV. Spunta plant naturally infected with PVY^N –W strain, exhibited palm sheep and stunting (A),

Fig. 3. *Solanum tuberosum* CV. Spunta naturally infected with PVY^o strain exhibited rougose mosaic.

(A) (B)

Fig. 4. Reaction of PVY° strain on artificially inoculated *Chenopodium amaranticolor* 10 days after virus inoculation, exhibited chlorotic local lesion (B), healthy leaf (A).

Fig. 5. Reaction of PVY^N-W strain artificially inoculated *Solanum tuberosum* CV. Spunta after thirteen days of virus inoculation, exhibited vein necrosis.

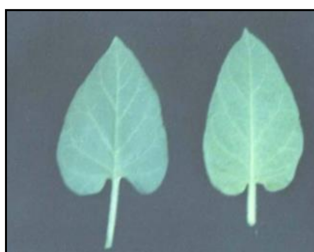
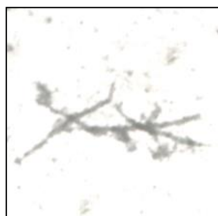
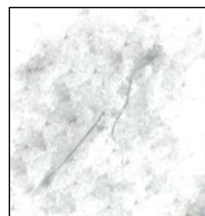


Fig. 6. Reaction of PVY^N-W strain artificially Inoculated *Nicotiana glutinosa* L., twenty one days after virus inoculation, exhibited mild mosaic, vein clearing, and mosaic “the left leaf as a control”.

Fig. 7. Reaction of PVY^N-W strain artificially inoculated *Nicotiana tabacum* CV. Turkish, twenty eight days after virus inoculation, showing palm sheep.

Fig. 8. Reaction of PVY^O strain artificially inoculated Nicotiana tabacum C.V. Turkish,wenty one days after virus inoculation, showing mottling.





(A) (B)

Fig. 9. Electron micrograph of PVY^N-W particles (aggregates) trapped on a grid coated with 1: 200 diluted IgG and decorated with the same diluted IgG (A), individually PVY^N-W particles (B). (X 57000). Transmission Electron Microscopy (Jeol JEM 100 CX II).

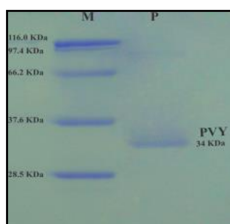


Fig. 10. Electropherogram of purified preparation of potato virus Y after Sucrose density gradient centrifugation. (M) = Marker and (P) = Purified virus

1.4.3. Immuno-electron Microscopy

Immuno electron microscopy was used for the detection of the potato virus Y and for emphases that the samples were not mixed infected. The decorated virus particles were appeared in {fig. (9). A}. The virus particle had 600 nm long as in {fig. (9). B}

1.5. Molecular Weight

Data illustrated in fig. (10) showed that the molecular weight of purified PVY coat protein was approximately 34 KDa when it estimated by electrophoresis (12%) and stained with coomassie brilliant blue.

DISCUSSION

The present study was carried out to isolate and identify the causal virus from naturally infected potato plants in Al Dakahliya and Dammietta governorates - Egypt using different identification methods and its incidence was determined.

The potato virus Y potyvirus has been identified on the basis of symptoms reaction of specific indicator hosts, physical properties of the causal pathogen, ELISA, electron microscopy negative staining technique, immuno electron microscopy and molecular weight of causal virus. The results showed that the two strains of PVY were PVY^{N}-W and PVY^{O} . The obtained reactions of the tested diagnostic hosts were different slightly from those previously obtained by other investigators. The two strains of PVY infected tested species and reacted as systemic and non systemic symptoms which appear on the indicator plants. *D. stramonium* was resistant to PVY^{N}-W strain, and to PVY^{O} strain also. On the other hand *N. tabacum* CV. Turkish, White Burley, Xanth, and Samsun, *S. tuberosum* CV. King Edward, Spunta., and *D. metel* were expressed various systemic symptoms.These results were similar to that obtained by, (Mc Donald and Singh, 1996; Boonham et al, 1999; and El Mohsen 2003).

Thermal inactivation point (TIP) was between 53 °C and 56 °C, dilution end point (DEP) was between 10^{-3} and 10^{-4}, while longevity in vitro (LIV) was between 48 hr and 60 hr at room temperature (RT). These results were nearly agreement with that obtained by, (Buchen Osmond, 1987).

Results of the virus survey indicated that PVY was detected from naturally infected potato and this virus was associated with the collected samples and its incidence was 36% from the collected samples.

Antigenic typing of PVY^{N}-W- McAbs suggested that the isolates No.1 and No.2 were more closely related to PVY^{N}-W. Typical result was obtained by, (Kerlan et al 1999., and Ounouna et al 2002). Data of immuno electron microscopy examination showed the particles of potato virus Y decorated with homologus antibodies. This result was similar to that obtained by, (Garg and Paul Khurana 1991., and Roy and Ramachandra 1998). The virus particles showed clumps (groups) by the antibodies (IgG) in immuno electron microscopy. While, without using IgG the virus particles stilled individually

and had 600 nm long. This referred to using the IgG and the technique it self. Typical result was obtained by, (Walkey, 1991).

PVY particles were flexuous filamentous. This result was similar to that obtained by, (Garg and Paul Khurana 1991., and Roy and Ramachandra 1998).

The molecular weight of purified potato virus Y^N coat protein is 34 000 Da when estimated it by using SDS-PAGE. This result was similar to that obtained by (de Bokx and Huttinga 1981., and Hiebert and 1973).

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دراسات على فيروس البطاطس واى (Potato virus Y) المؤثر على محصول البطاطس فى محافظتى الدقهلية ودمياط.

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استهدفت هذه الدراسة تعريف فيروس البطاطس واى باستخدام كلاً من الاعراض المرضية للعوائل المفرقة، الخصائص الفيزيائية فى العصير، اختبار الاليزا، الميكروسكوب الإليكتروني (الصبغ السالب) ،الميكروسكوب الإليكتروني المناعي، وكذلك الوزن الجزيئى للغطاء البروتينى للفيروس المختبر. وقد تم عزل وتعريف سلالتين من فيروس البطاطس واى هم (PVY^{O} ، PVY^{N}-W) من نباتات البطاطس المصابة طبيعيا والتى تنمو فى محافظتى الدقهلية ودمياط فى اعوام ٢٠٠٣،٢٠٠٤ .ولقد دلت النتائج على إن الفيروس المعزول هو فيروس البطاطس واى حيث تفاعل مع العوائل المفرقة و أعطى اعراضا مماثلة تماما لهذا الفيروس. حيث أظهرت سلالة فيروس البطاطس واي PVY^{N} المختبرة إصابة جهازيه في أصناف الدخان زانثاى، تركى، هويت بيرلى و أعطت موت العروق، بينما السلالة PVY^{O} أعطت تبرقش على نفس أصناف الدخان. باستخدام اختبارات الخواص الفيزيائية فى العصيرالمعدى وجد ان الفيروس فقد القدرة على احداث العدوى ما بين ٥٣- ٥٦ درجة مئوية، وكذلك فقد القدرة على احداث العدوى عند تخفيف ما بين 10^{-3}- 10^{-4} و فقد القدرة على احداث العدوى ما بين ٤٨- ٦٠ ساعة من طحن العينة. باستخدام اختبار الاليزا تم الكشف عن خمسة وعشرون عينة ظهر عليها اعراض الاصابة بفيروس البطاطس واى (PVY^{O}، PVY^{N}-W) تسعة عينات فقط أعطت تفاعلا ايجابياً. باستخدام الميكروسكوب الإلكتروني المناعي تم ملاحظة تجمع من جزيئات فيروس البطاطس واى ولوحظ عليها الأجسام المضادة بعكس استخدام الميكروسكوب الإلكتروني (الصبغ السالب) بدون استخدام الانتيسيرم حيث لم يلاحظ فيها الا جزيئات الفيروس وكان طول جزيئات الفيروس ٦٠٠ نانوميتر .تم معرفة الوزن الجزيئى للغطاء البروتينى للفيروس النقى وكان ٣٤ كيلو دالتون باستخدام الهجرة الكهربية للفيروس النقى خلال اختبار البولى اكريلاميد چيل.