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The First Report of Polyclonal Antibody Production of a Syrian Isolate of *Potato virus Y*

By

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Summary : *Potato virus Y* (PVY) is reported to be the main virus occurring in potato fields and it increases the cost of potato production in Syria. Producing and utilizing of certified seed potatoes with minimum PVY infection is the main control key that requires efficient virus detection methods. Enzyme linked immunosorbent assay (ELISA) using specific antisera is the most common method for the detection of potato viruses. In this study we report the production of PVY antiserum in Syria for the first time. This antibody was produced against a Syrian PVY isolate and its reliability was confirmed by testing a panel of PVY infected samples collected from Syria. The results of ELISA using this antibody were consistent with those obtained using Agdia PVY antibody and no significant differences were found. In addition this antibody was highly specific to PVY with undetectable cross reaction to plant proteins. Moreover it was shown to be very sensitive and PVY infection could be detected in highly diluted plant saps. Since this antibody was produced against a Syrian isolate of PVY it has advantages over imported PVY specific antiserum.

Key words : Seed potatoes, ELISA, *Potato virus Y,* Antibody, Syria

Introduction

In Syria, potato (*Solanum tuberosum* L.) is one of the main crops, grown in 24,789 ha with a total production of 486,605 tons[1].

Potato virus Y (PVY) was reported to be the main virus infecting potato crops in Syria[2]. PVY is the type member of the genus *Potyvirus,* family *Potyviridae*[3–4]. PVY has two main serological groups : $PVY^{O,C}$ serotype (O serotype) and PVY^{N} serotype (N serotype)[5] that can be identified using monoclonal antibodies, whereas polyclonal ones are not able always to differentiate them[6]. PVY causing 10 to 80% potato yield losses is one of the most important viruses affecting potato production worldwide[7].

Stable supply of healthy seed potatoes is a basic procedure to control and minimize PVY incidence and consequently depress the impact on potato production[8]. This cannot be performed without rapid and accurate PVY detection. Such useful identification methods of PVY allow the early tracking of seed potato stocks with higher infection than the pre-described thresholds and therefore would help to remove such stocks from the seed potato production system.

The appearance of new variants of PVY that cause very mild symptoms or are symptom-less and the developing of new potato cultivars that can be infected latently limit the effectiveness of field inspection and rouging to control this virus. Therefore the laboratory tests are becoming more important than before. Many detection and identification methods can be used to detect potato viruses including inoculation test on indi-

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cator plants, the polymerase chain reaction (PCR), and immunological tests. Inoculation test is time consuming and requires a green house. Although PCR is a very sensitive method, it is too expensive and laborious. Therefore, it only can be applied on a limited number of samples[9]. Enzyme linked immunosorbent assay (ELISA) is the most common method for the detection of potato viruses. It is a sensitive, reproducible, and practical virus detection method that can be used in large scale tests. This method is being used wherever seed potato production is carried out[9].

In Syria, the General Organization for Seed Multiplication (GOSM) of the Ministry of Agriculture and Agrarian Reform is in charge of seed potato importation, production and multiplication. In GOSM, ELISA is used to test potato plants at the various seed potato production steps. However the technology of producing antisera was introduced only recently. In this study we report the production of PVY specific antiserum against a Syrian isolate of PVY in Syria for the first time. The reliability of this antiserum was tested using a significant number of Syrian PVY isolates.

Materials and Methods

(1) Virus isolates

To produce antiserum, a Syrian PVY isolate, labeled SYR-Wi-11 collected from Syria in 2002 was used. Single local lesion isolation was carried out using *Chenopodium quinoa*. Serological and molecular typing revealed that SYR-Wi-11 coat protein (CP) belongs to the O serotype of PVY[10]. This isolate was propagated and maintained in *Nicotiana sylvestris*. Inoculated plants were kept in a green house at 20℃ and normal day length. To evaluate the efficiency of the new antibody, 77 PVY-infected samples were used. These samples were collected from Syria during the period from 2002 to 2004[2] and in the autumn season of 2006. Five healthy potato plants and one tobacco plant were also tested.

(2) Virus purification

Virus purification was carried out according to LOEBENSTEIN *et al.*[9] with some modifications. In brief, leaves (2,150 g) were triturated with four volumes (v/w of leaf tissue) of 0.1 M Citrate buffer (pH 7.2) containing 0.01 M EDTA, 0.04 M Sodium sulfite, 0.2% mercaptoethanol, and 0.1% Triton X-100. Then this was mixed with cold chloroform : charbontetrachloride (1 : 1) to 50% of total tissue and buffer volume. Mixture was centrifuged at low speed (10,000 g) and aqueous phase was stirred with 4% PEG and 0.4 M NaCl for 1 h followed by low speed centrifuge (10,000 g). Pellet was dissolved in 0.02 M Tris-HCl (pH 7.2) containing 0.1% mercaptoethanol, 0.01 M EDTA and 0.1% Triton X-100 and mixed with cold chloroform and finally centrifuged at low speed (10,000g). Aqueous phase was centrifuged at high speed (110,000 g) and the resultant pellet was dissolved in 0.02 M Tris-HCl, pH 7.2 containing 0.01 M EDTA. Dissolved pellet was layered on (10–40%) sucrose gradients and recovered virus zone was diluted in 0.02 M Tris-HCl, pH 7.2 containing 0.01 M EDTA and centrifuged at high speed (112,000 g) for 90 min at 4℃. Purified virus was dissolved in NaCl (0.85%) solution and stored at −80℃.

(3) Immunization of rabbit

A rabbit was immunized by a combination of two injection methods. A combination of 3 intramuscular and 5 intravenous injections were applied in a series for 10 weeks.

(4) Antibody purification

Antibody purification was carried out according to HAMPTON *et al.*[11]. Fractionated IgG was stored at −80 ℃.

(5) Alkaline phosphatase enzyme conjugation

Alkaline phosphatase enzyme conjugate preparation was carried out as described by AVRAMEAS[12].

(6) ELISA

ELISA was conducted in a double antibody sandwich (DAS) form according to HAMPTON *et al.*[11]. IgG was used at dilution 1 : 1000 in carbonate buffer. Leaf sap was prepared at dilution rate 1 : 10 in a general extraction buffer [1xPBST, 1.3 g L^{-1} Na_2SO_3, 20 g L^{-1} polyvinylpyrrolidone (PVP) MW 24–40000, 2.0 g L^{-1} bovine serum albumin (BSA), 20.0 g L^{-1} Tween-20 ; pH 7.4]. Enzyme conjugated antibody was used at dilution rate of 1 : 500 in ECI buffer (1xPBST, 20 g L^{-1} PVP MW 24–40000, 2.0 g L^{-1} BSA ; pH 7.4). Samples were considered positive when their absorbance values (at 405 nm) were 5 times of greater than those of corresponding negative control after incubation for 1 h at room temperature. Plates were kept in humid boxes during all incubation periods.

The results of ELISA using polyclonal antibody produced in this study were compared with those of a PVY monoclonal antibody (Mab) 4C3 that detects all PVY isolates (Catalog number SRA 20001/0500, Agdia, USA). When Mab 4C3 was used, ELISA was performed according to the manufacturer's instructions.

The antiserum produced in GOSM and reported in this study was used after almost two-year storage at

temperature range 4–10℃.

Results

(1) Virus purification

Of 2,150 g PVY infected fresh leaves of *Nicotiana sylvestris,* 10.61 mg purified PVY was obtained. Virus concentration was measured using a spectrophotometer (extinction coefficient = 2.3).

(2) Antibody purification

Total yield of crude antiserum was 30 m*l* and the total yield of IgG was about 8 mg/m*l*. The PVY polyclonal antibody produced in this study was designated as Pab (pvy010100).

(3) ELISA

Pab (pvy010100) could detect PVY in 77 PVY-infected samples (data not shown) and 67 samples among them were tested using both Pab (pvy010100) and Mab 4C3. Both antibodies reacted similarly to all samples tested (Table 1). All healthy control samples reacted negatively to Mab 4C3 were negative to Pab (pvy010100) (Table 1).

Table 1 The comparison between ELISA results obtained using Pab (pvy010100) and Mab 4C3.

ELISA Reaction	Mab 4C3	Pab (pvy010100)
-	6	6
+	3	4
++	11	7
+++	27	21
++++	20	29
Total	67	67

-, absorbance values <5 times of the negative control; +, greater than 5 times of the negative control and less than 0.5; ++, 0.5-1.0; +++, 1.0-2.0; ++++, >2.0.

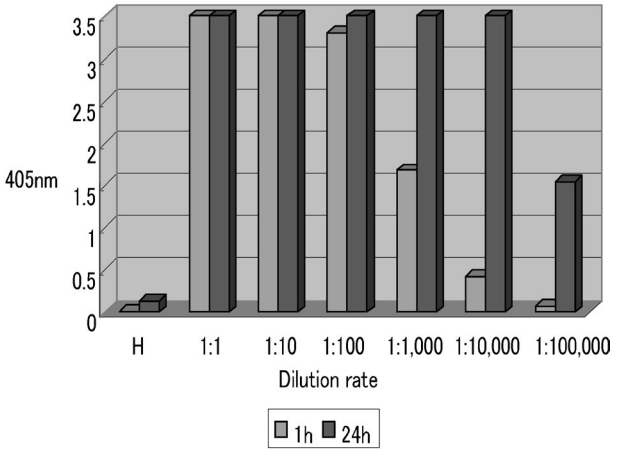

Fig. 1 The absorbance values of healthy and PVY infected saps at different dilution rates in general extraction buffer after 1 h and 24 h incubation with substrate ; H, healthy control.

The discrepancy between the absorbance values of PVY infected saps and those of the healthy controls started to become significant (>5 times) after 15 min of incubation at room temperature (data not shown).

In the end dilution point test of PVY infection, Pab (pvy010100) could detect PVY infection at a dilution rate up to 1 : 100,000 (Fig. 1).

Since Pab (pvy010100) was produced against a Syrian isolate of O serotype, it is of importance to test its ability to detect PVY isolates of N serotype. Therefore, 5 PVY isolates of N serotype (un-published data) were tested and all were detected with high absorbance values (Table 2). Fig. 2 shows the relation between development of absorbance values and successive incubation periods with substrate for two PVY isolates belonging to the serotypes O and N along with potato and tobacco negative controls.

The non-specific reaction to plant proteins was very low and plates could be read after 1, 2, and 3 h of incubation at room temperature followed by a 24 h incubation at 4℃ (Fig. 2).

Discussion

In seed potato certification systems, virus infection rate is one of the most important values that determine the grade of seed potatoes. Field inspection is carried out to estimate the level of virus infection based on visible virus-like symptoms. However, virus infection can go latently without any visible symptoms and moreover late infections in the growing season cannot be detected by visual means. Therefore, field inspec-

Table 2 The reaction of the isolate SYR-Wi-11 and PVY isolates of the N serotype with Pab (pvy010100)

PVY isolate	Serotype	absorbance values*
S8	N	1.29
S10	N	1.37
13	N	1.832
27	N	1.827
6-9	N	1.252
SYR-Wi-11	O	3.500

*At 405nm after 1 h incubation with substrate at room temperature.

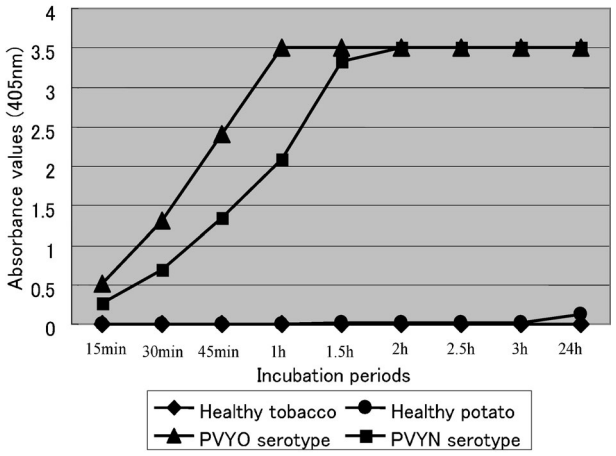

Fig. 2 Binding of Pab (pvy010100) to saps of tobacco PVY infected samples of serotypes O and N and healthy controls of tobacco and potato plants by DAS-ELISA at different incubation periods. Incubation was at room temperature for the first 3h and then plates were moved to 4℃ and delete read after 24 h.

tion is to be complemented by specific and sensitive virus detection methods that are suitable for large scale application.

ELISA is the method of choice to detect and identify plant viruses in large scale sample tests. As an example, in the Netherlands alone about 5×10^6 ELISA tests are conducted annually on seed potatoes[8]. In GOSM, ELISA test is being used to check potato plants for virus infections in seed potato production system including *in-vitro,* green house, and screen house derived plant materials. In addition, it is used in the post harvest indexing of seed stocks. Six viruses are tested regularly including PVY, *Potato leafroll virus, Potato virus A, Potato virus M, Potato virus S* and *Potato virus X*. However, GOSM has to import the specific antisera of these viruses, which increases the cost of virus test and consequently the cost of seed potato production.

Producing of specific antisera against Syrian isolates of potato viruses in GOSM would decrease the cost of ELISA test through elimination of the high cost of imported antisera. In addition, the successful production of these antisera would build the confidence of GOSM staff and encourage the continuous production of new antisera against other common potato viruses and as a result may lead to the complete determination of antisera import.

In this study we report the production of specific polyclonal antibody, labeled Pab (pvy010100) to a local PVY isolate for the first time in Syria. The reliability, specificity, sensitivity, and stability of this antibody were tested through a series of confirmatory tests. Pab (pvy010100) could detect all PVY isolates tested. The results of ELISA using this antibody were consistent with those obtained using Agdia PVY antibody and no significant differences were found, which insures its reliability. In addition, Pab (pvy010100) was highly specific to PVY with undetectable cross reaction to plant proteins and ELISA plates could be read even after 24 h of incubation with the substrate. This makes the use of this antiserum convenient as the substrate is added and then plates can be read the next day without worrying about the non-specific discoloration of the healthy controls. Moreover this increases the sensitivity of this antiserum as shown in Fig. 1, where the differences between absorbance values of the highly diluted sample (1 : 100,000) and the healthy control became clearly significant after 24h of incubation with the substrate, even though it was significant enough after 1h incubation. This high sensitivity of Pab (pvy 010100) makes it suitable to detect a single PVY infected sample in compound samples which decreases the cost of virus test.

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シリア産ジャガイモ Y ウイルスに対するポリクローナル抗体の初作製

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要約：ジャガイモ Y ウイルス（PVY）はシリアのジャガイモ生産における主要ウイルスの一つとされ，ジャガイモ生産の阻害要因となっている。PVY 感染の無い種イモの生産と供給が主要な防除手段であるが，そのためには，効果的なウイルス検査法が必要である。酵素結合抗体法（ELISA）は複数種類のジャガイモウイルス検出には最も一般的な技術であるが，それぞれのウイルスに対して特異的な抗血清が利用できることが必須条件となる。本研究では，シリアに発生した PVY 分離株を純化し，ウサギに免疫することにより抗血清をはじめて作製した。この抗血清を利用し多くのシリア産 PVY を用いた ELISA による検討では，市販の抗血清と同等の検出能力を有し，両抗血清間に結果の相違は認められなかった。また，非特異的反応も生じなかった。さらに，検出限界は高かった。以上より，本研究ではシリアで分離された PVY に対して初めてシリア国産抗血清を得ることができ，抗血清の恒常的輸入を不要にしたことに大きな意義があると考えられる。

キーワード：種ジャガイモ，ELISA，ジャガイモ Y ウイルス，抗血清，シリア

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