STRAIN-TYPING OF POTATO VIRUS Y ISOLATES FROM POTATO IN NIGERIA BY INFECTIVITY TESTS AND ELISA

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ABSTRACT

In a survey of potato viruses in Nigeria, five isolates of potato virus Y (PVY) were obtained. Two of these, PVY-BL and PVY-BL2, produced symptoms in indicator plants that resembled those caused by PVYN strains, particularly with their ability to incite systemic veinsial necrosis in Samsun tobacco. Since they reacted positively with monoclonal antibodies specific for PVYN, they were confirmed to belong to this strain group. Another isolate, PVY-RP reacted similarly in ELISA, but did not produce vein necrosis in Samsun tobacco. Two isolates, PVY-BK and PVY-KR, reacted positively in ELISA with polyclonal antibodies prepared against PVY-BL and also shared some pathogenic properties with PVYC and PVYO strain groups. They could, however, not be detected in ELISA using monoclonal antibodies specific for PVYC and PVYO strains, an indication that they may be strains that have not previously been described or are antigenic deviants of existing ones.

Key Words: Antibodies, ELISA, PVY strains, Solanum tuberosum

RÉSUMÉ

Dans une étude des virus de la patate au Nigéria, cinq isolats du virus de patates Y (PVY) ont été obtenus. Deux d'entre eux, le PVY-BL et le PVY-BL2, ont produs des symptomes dans les plantes témoins qui ressemblaient à ceux causés par les souches PVYN avec leur abilité particulière d'inciter la nécrose veinale systématique dans le tabac Samsun. Comme ils réagissaient positivement aux anticorps monoclonaux spécifiques pour le PVYN ils ont été confirmés comme appartenant à ce groupe de souches. Un autre isolat, le PVY-RP a réagi similaires dans ELISA, mais n'a pas produit de nécrose veinale dans le tabac Samsun. Deux isolats, le PVY-BK et le PVY-KR ont réagi positivement dans ELISA aux anticorps polyclonaux préparés contre le PVY-BL et partageaient certaines propriétés pathogéniques avec le groupes de souches PVYC et PVYO. Toutefois ils ne pouvaient pas être détectés dans ELISA en utilisant des anticorps monoclonaux spécifiques aux souches de PVYC et de PVYO une indication qu'il pourrait y avoir des souches qui n'ont pas été précédemment décrits ou des antigènes dérivés des antigènes existants.

Mots Clés: Anticorps, ELISA, souches de PVY, Solanum tuberosum
INTRODUCTION

Potato virus Y (PVY) is one of the most important viruses of potato (Solanum tuberosum L.) in many developing countries, especially in mixed cropping systems (Salazar and Accatino, 1990). Symptoms in potato may vary depending on the potato clone, virus strain and climatic conditions (DeBokx and Huttinga, 1981). The virus is also known to naturally infect other solanaceous crops, such as pepper, tomato and tobacco (Nelson and Wheeler, 1978; Sievert, 1978; Thomas, 1985).

PVY has been divided into three major strain groups based on reaction of certain indicator plants (DeBokx and Huttinga, 1981). The common strain (PVY*) is distributed worldwide while the tobacco veinal necrosis strain (PVYn) occurs in Europe, some parts of Africa and America. The stipple streak strain (PVYC) has been reported from India, Australia, United Kingdom and some parts of continental Europe. However, some strains do not fit into any of these three groups (Khan and Monroe, 1963; Heath et al., 1987).

Virus-like disease symptoms have frequently been observed in potato fields in Nigeria and these have been associated with reduced plant vigour and yield. During extensive surveys of farmers’ fields, several isolates reacted with antibodies to PVY, among others, in enzyme-linked immunosorbent assay (ELISA) both in the wet and dry seasons (Mih et al., 1993). In order to develop appropriate control measures and priorities in breeding programmes, it is necessary to determine the strain groups to which these isolates belong. This study was initiated to identify the strain types of the isolates by ELISA and infectivity tests.

MATERIALS AND METHODS

Virus isolates. Five virus isolates were assayed from field-grown potato plants showing yellow mosaic symptoms in the Jos Plateau of Nigeria. The isolates were identified by the locations (in parenthesis) from which they were collected: PVY-BL and PVY-BL2 (Barakin Ladi), PVY-RP (Ropp), PVY-KR (Kirang), and PVY-BK (Bokkos). The isolates were each passed through three single lesion transfers in Chenopodium amaranticolor Coste et Reyn., and subsequently maintained in Nicotiana benthamiana Domin. by regular mechanical inoculation of young plants.

Infectivity assay. A range of frequently reported experimental and natural hosts of PVY were mechanically inoculated with each of the virus isolates. Inoculum was obtained by triturating systemically infected leaves of N. benthamiana in 0.01M phosphate buffer containing 0.001M cysteine and 0.01M ethylene diamine tetraacetic acid disodium salt (EDTA) at the rate of 1 g leaf/4 ml buffer. Carborundum powder (600 mesh) was used as abrasive. Control plants were inoculated with buffer only. Test plants were maintained in the screenhouse (29-33°C) and symptomless infections were checked by back inoculation to C. amaranticolor and ELISA.

ELISA protocols. Three ELISA protocols were performed in polystyrene microtitre plates. Two monoclonal antibodies (MAbs) MAbPVY*IF5 specific for PVY* and MAbPVY*C5-9H specific for both PVYO and PVYC strains were kindly supplied by Dr P. J. Ellis of Agriculture Canada, Vancouver. They were used at dilutions of 1/10000 and 1/5000, respectively. The polyclonal antibody (Ab*PVY-BL) and its alkaline phosphatase enzyme conjugate (Ab*PVY-BL-PAL) were prepared against PVY-BL and were used at dilutions of 1/1000 and 1/500, respectively. Goat antimouse IgG alkaline phosphatase conjugate (GAM-PAL) (BIO-RAD Laboratories) was used at a dilution of 1/3000.

In the antigen-coated plate ELISA (ACP-ELISA), crude sap from leaves of N. benthamiana, systemically infected with each of the isolates, was diluted (1/5) in 0.05M carbonate buffer of pH 9.6, containing 2% polyvinyl pyrrolidone (PVP). Sap from healthy leaves was similarly diluted and included as a control. The diluted sap was used to coat 10 wells in each of two plates (100 µl well') and incubated overnight at 4°C. Blocking solution (freshly prepared 3% skim milk in phosphate buffered saline containing 0.05% Tween 20, PBST) was applied to the wells and incubated for 31 min at 37°C. The MAbs, each diluted in PBST, were then applied to the plates (100 µl well'), one MAb plate' and incubated for 3 hr at 37°C. GAM-PAL diluted in PBST was applied (100 µl well') and incubated for 3 hr at 37°C.
The p-nitrophenyl phosphate substrate (1 mg ml⁻¹ 10% diethanolamine, pH 9.8) was applied at the rate of 200 μl well⁻¹ and plates were incubated for 1 hr at room temperature after which they were read with a Dynatech model MR5000 ELISA reader. Except after blocking, plates were washed three times for 4 min at the end of every incubation period before substrate application.

In the triple antibody sandwich ELISA (TAS-ELISA) plates were coated with Ab⁸PVY-GL (diluted in carbonate buffer) for 4 hr at 37°C followed by incubation with test samples (diluted in PBST + 2% PVP) overnight at 4°C, the MAbS (diluted in PBST) for 3 hr at 37°C, and GAM-PAL (diluted in PBST) for 4 hr at 37°C. Blocking, washing, substrate application and plate reading were as described for ACP ELISA.

The coating pattern in double antibody sandwich ELISA (DAS-ELISA) was MAbS for 4 hr at 37°C, test samples overnight at 4°C and Ab⁸PVY-GL-PH for 3 hr at 37°C. Similarly, blocking, washing, substrate application and plate reading were as described for ACP-ELISA. In each case, a positive reaction was considered as one in which the 7 optical density (O.D.) at 405 nm is at least twice that of the healthy control. Each test was replicated three times.

RESULTS

Infectivity of virus isolates. All virus isolates incited systemic mosaic or mottle in Nicotiana species, Physalis floridana, and S. tuberosum (Table 1). This was usually preceded by systemic vein-clearing in N. tabacum cv Samson and N. glutinosa. In addition, PVY-GL and PVY-GL2 caused systemic vein necrosis in Samson tobacco. All five isolates did not infect Datura stramonium, S. nigrum, and Capsicum annuum, but produced chlorotic local lesions on C. amaranticolor. The symptoms of the main strains as described by DeBokx and Huttinga (1981) are included for comparison.

Serological reaction. Three isolates, PVY-GL, PVY-RP, and PVY-GL2 were detected in ACP-ELISA by MAbPVY⁸IF5 (Fig. 1). The results were confirmed by TAS-ELISA and DAS-ELISA, in which a similar trend was observed when MAbPVY⁸IF5 was used in conjunction with either
Figure 1. Determination of potato virus Y strains amongst PVY isolates from Jos Plateau: (A) PVY<sup>N</sup> (B) PVY<sup>o</sup> and PVY<sup>c</sup> strains

- GAM. PAL, ○ Antigen, ▼ Ab<sup>R</sup>PVY-BL, ▶ Ab<sup>R</sup>-Enzyme Conjugate, Y MAb PVYN<sup>1</sup>F5 for (A) and MAb PVY<sup>o+c</sup>5-9H, for (B).

Ab<sup>P</sup>PVY-BL or its enzyme conjugate. Both PVY-KR and PVY-BK were not detected in any of the three ELISA protocols (Fig. 1A). The ELISA results in the three protocols using MAbPVY<sup>o+c</sup>5-9H were all negative (Fig. 1B).

**DISCUSSION**

The symptoms produced by both PVY-BL and PVY-BL2 in some frequently reported host plants were similar to those reported for PVYN, particularly with respect to their ability to cause systemic veinal necrosis in Samsun tobacco (DeBokx and Huttinga, 1981). The fact that these two strains belong to the PVYN strain group was confirmed by the positive reaction of these isolates in ELISA to MAbPVYN<sup>1</sup>F5 specific for the group. PVY-RP also reacted similarly in ELISA but, unlike the former two, it failed to produce systemic veinal necrosis in Samsun tobacco. Pirone and Nesmith (1994), in a survey of Kentucky tobacco, found a single plant infected with a PVYN isolate that failed to incite necrosis in tobacco. None of the isolates used in this study infected D.
stramonium, in agreement with the observation that this host is diagnostically immune to PVY (Buschen-Osmund, 1990).

The Nigerian isolate of PVY also differed from other strains in infectivity tests. For example, PVY-BL, PVY-BL2 and PVY-RP, which were confirmed to belong to the PVYN strain group in all three ELISA protocols, did not infect C. annuum and S. nigrum. In contrast, an isolate of PVY in southern France caused mild mottle symptoms in S. nigrum (Marchoux et al., 1975), while the virus is known to cause various symptoms on pepper (DeBokx and Huttinga, 1981). Thus, Heath et al. (1987) observed that PVYN may not be a homogeneous strain group, since some PVY isolates which cause veinral necrosis in Samsun tobacco failed to infect potato systemically. A similar variation in this strain group has been observed in Canada where four isolates of PVYN did not cause symptoms in C. amaranaticolor and C. frutescens (McDonald and Krisjanson, 1993).

Both PVY-BK and PVY-RP did not react with any of the MABs, although they were detected by polyclonal antibodies prepared against PVY-BL (Mih, 1994). Although the symptoms of these isolates in some test plants closely resembled those caused by isolate 18 of Heath et al. (1987), they differed in that the present isolates infected potato systemically while isolate 18 did not. Both PVY-BK and PVY-KR share some properties with PVYc and PVYb strain groups. However, MABs specific for these strain groups could not detect these two isolates. Thus, the isolates may be strains that have not been previously described, since it has long been realised that some strains may not easily be classified into any of these strain groups (Khan, and Monroe, 1963; Heath et al., 1987). On the other hand, they could be deviants in which pathogenic properties were not seriously affected. Further studies are in progress to establish these.

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