African Crop Science Journal, Vol. 3, No.1, pp. 99-104, 1995
Printed in Uganda.
1021-9730/94 $10.00+0.00

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

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(Received 7 September 1994; accepted 12 December, 1994)

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 PVY^N strains, particularly with their ability to incite systemic veinal necrosis in Samsun tobacco. Since they reacted positively with monoclonal antibodies specific for PVY^N, they were confirmed to belong to this strain group. Another isolate, PVY-RP reacted similarly in ELISA, but did not produce veinal 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 PVY^C and PVY^O strain groups. They could, however, not be detected in ELISA using monoclonal antibodies specific for PVY^C and PVY^O 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 produis des symptomes dans les plantes temoins qui ressemblaient a ceux causés par les souches PVY^N avec leur abilité particulière d'inciter la nécrose veinale systématique dans le tabac Samsun. Comme ils réagissaient positivement aux anticorps monoclonaux spécifiques pour le PVY^N, ils ont été confirmés comme appartenant à ce groupe de souches. Un autre isolat, le PVY-RP a réagi similairement 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 PVY^C et PVY^O. Toutefois ils ne pouvaient pas être détectés dans ELISA en utilisant des anticorp monoclonal spécifiques aux souches de PVY^C et de PVY^O 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 (De Bokx and Huttinga, 1981). The common strain (PVY') is distributed worldwide while the tobacco veinal necrosis strain (PVY^N) occurs in Europe, some parts of Africa and America. The stipple streak strain (PVY^C) 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) $MAbPVY^N$IF5 specific for PVY^N and $MAbPVY^{O+C}$5-9H specific for both PVY^O and PVY^C 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^RPVY-BL) and its alkaline phosphatase enzyme conjugate (Ab^RPVY-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 $well^{-1}$) 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 31min at 37°C. The MAbs, each diluted in PBST, were then applied to the plates (100 µl $well^{-1}$), one MAb $plate^{-1}$ and incubated for 3 hr at 37°C. GAM-PAL diluted in PBST was applied (100 µl $well^{-1}$) and incubated for 3 hr at 37°C.

The p-nitrophenyl phosphate substrate (1mg 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 Ab^{R}PVY-BL (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 Ab^{R}PVY-BL-PHL 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 preceeded by systemic vein-clearing in *N. tabacum* cv Samsun and *N. glutinosa.* In addition, PVY-BL and PVY-BL2 caused systemic veinal necrosis and epinasty in Samsun 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-BL, PVY-RP, and PVY-BL2 were detected in ACP-ELISA by MAbPVY^{N}IF5 (Fig. 1). The results were confirmed by TAS-ELISA and DAS-ELISA, in which a similar trend was observed when MAbPVY^{N}IF5 was used in conjunction with either

TABLE 1. Comparison of the reaction of potato virus Y (PVY) isolates from Nigeria and the type strains in selected host plants after mechanical inoculation[a]

Host plant	PVY test isolates					PVY type strain[b]		
	-BL	-BL2	-BK	-RP	-KR	PVY^{C}	PVY^{O}	PVY^{N}
Nicotiana tabacum Samsun	SVC, VN	SVC,M	SVC,M	SVC,M	SVC,M	SVC,M	SVC,M	SVC,M
N. debneyi	M	M	M	M	M	M	M	M
N. glutinosa	SVC, M	SVC,M	SVC,M	SVC,M	SVC,M	M	M	M
Physalis floridana	M	M	M	M	M	*SN*	*SN*	M
Datura stramonium	–	–	–	–	–	–	–	–
Capsicum annuum	–	–	–	–	–	M	M	M
Solanum nigrum	–	–	–	–	–	M	M	M
S. tuberosum	M	M	M	M	M	NLL	M	M
Chenopodium amaranticolor	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL

[a]SVC=Systemic vein clearing, M=Systemic mosaic/mottle, VN =Systemic vein necrosis, I=immune (No symptoms, negative in ELISA4), CLL = chlorotic local lesions, NLL = Necrotic local lesions.
[b]De Bokx and Huttinga (1981)

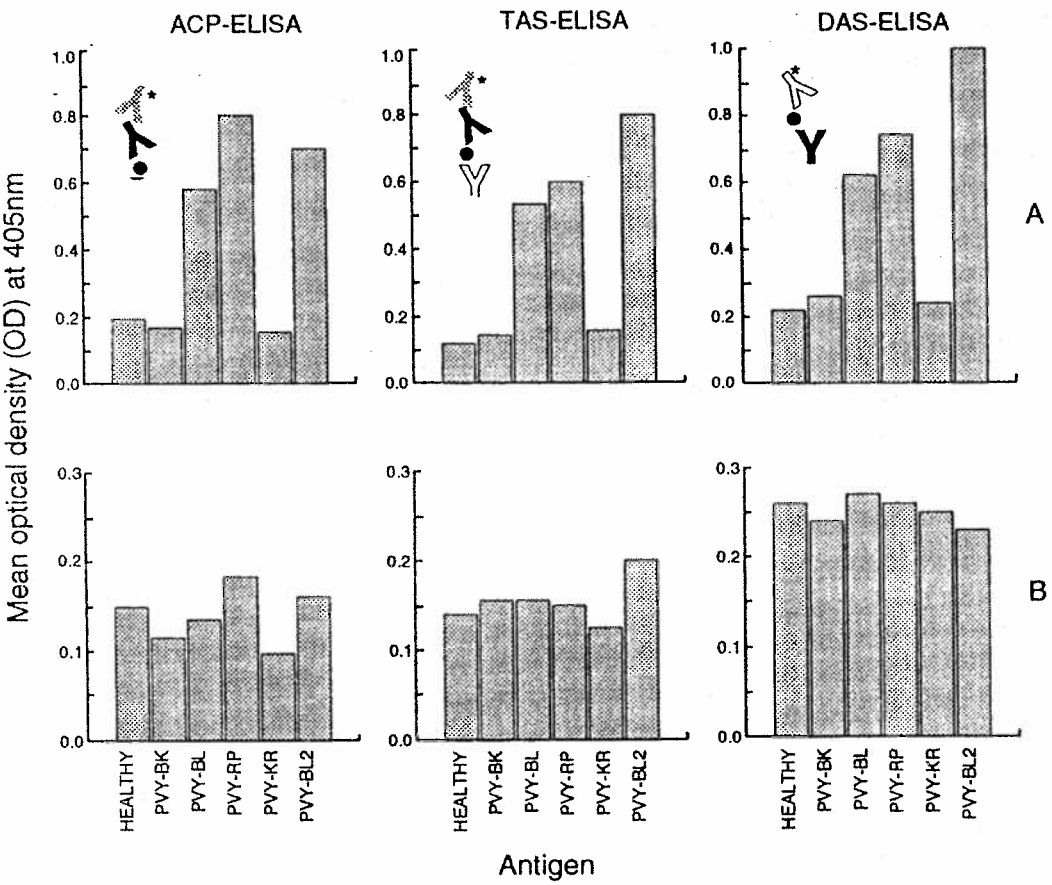

Figure 1. Determination of potato virus Y strains amongst PVY isolates from Jos Plateau: (A) PVY^N (B) PVY^O and PVY^C strains

GAM. PAL, • Antigen, Ab ^{R}PVY-BL, Ab ^{R}-Enzyme Conjugate,

MAb PVY^N 1F5 for (A) and MAb PVY^{O+C} 5–9H, for (B).

Ab^RPVY-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^{O+C}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 PVY^N, 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 PVY^N strain group was confirmed by the positive reaction of these isolates in ELISA to MAbPVY^N1F5 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 PVY^N 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 thePVY^N 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 PVY^N may not be a homogeneous strain group, since some PVY isolates which cause veinal 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 PVY^N did not cause symptoms in *C. amaranticolor* and *C. fruitescens* (McDonald and Krisjansson, 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 PVY^C and PVY^O 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.

ACKNOWLEDGEMENT

We wish to thank Drs L.F. Salazar (CIP, Peru) and P.J. Ellis (Agriculture Canada) for the provision of polyclonal antisera and MAbs, respectively. Second author is in part supported by a grant from the International Foundation for Science, Sweden.

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