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Potato virus X in Tunisian grapevines

by

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S u m m a r y : Two biologically distinct strains of potato potexvirus X (PVX) were recovered by sap inoculation from vines of cvs Carignan and Grenache in two different Tunisian localities. In a Grenache vineyard, PVX was detected by ELISA in about 4 % of the vines. Morphological, physico-chemical, serological and ultrastructural properties of both PVX strains from grapevine were the same as those of ordinary isolates of the type species, as shown by the results of comparative investigations. PVX seemed little pathogenic to grapevines, and was re-inoculated to grape rootlings with difficulty.

K e y w o r d s : Vitis, potexvirus, serology, diagnosis, ultrastructure.

Introduction

In the course of a routine survey for grapevine viruses in Tunisian vineyards, sap inoculations to herbaceous hosts from diseased plants of two different localities north (Ain-Ghellal) and south (Sidi Messaud) of Tunis, yielded two viruses that induced symptoms differing from those elicited by viruses commonly found in the area (BOULILA *et al.* 1991). As reported in the present paper, these viruses were investigated in detail and identified as two biologically different isolates of potato potexvirus X (PVX).

Materials and methods

V i r u s s o u r c e s , t r a n s m i s s i o n a n d c u l t u r e : Isolations were made from: (1) vines of cv. Grenache (Ain-Ghellal), some of which were apparently symptomless, whereas others showed vein banding symptoms (see BOVEY and MARTELLI 1992), occasionally accompanied by most unusual reddish ringspots; (ii) vines of cv. Carignan (Sidi Messaud) affected by typical yellow mosaic (see BOVEY and MARTELLI 1992).

Leaf tissues from donor vines were ground in the presence of phosphate buffer 0.01 M and 2.5 % nicotine and the extract was manually inoculated to a standard series of herbaceous hosts. Infected *Nicotiana glutinosa* and *Datura stramonium* were kept as virus cultures and used as inoculum source for host range studies.

Pot-grown rootlings of cv. Carignan were inoculated by subcortical injection of infected tissue extracts, and cv. Mission seedlings with purified virus preparations in Tunis and Bari, respectively.

An authentic PVX isolate from potato was used for comparative determination of serological and physico-chemical properties.

V i r u s p u r i f i c a t i o n a n d c h a r a c t e r i z a t i o n : Purification of viruses was from systemically infected leaves of *N. tabacum* cv. Samsun as described (CHABBOUH et al. 1990), and final separation from plant constituents was by centrifugation in 25 % continuous sucrose density gradients. RNA was extracted from purified virus preparations by the SDS-phenol

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method (DIENER and SCHNEIDER 1968) and electrophoresed in 1.2 % agarose gels after denaturation with 50 % formamide at 65 °C for 5 min. Reference marker was genomic RNA of tobacco mosaic (TMV) (mol. wt 2 x 10^6), and staining was with ethidium bromide.

Coat protein subunits obtained by boiling virus preparations in LAEMMLI's (1970) dissociation buffer, were electrophoresed in 12.5 % polyacrylamide slab gels using LAEMMLI's discontinuous buffer system. Staining was with Coomassie brilliant blue, and mol. wt. protein markers were from a commercial kit (Sigma MW-SDS-70L).

S e r o l o g y : Antisera were raised in rabbits by injecting purified antigen once intramuscularly and twice intravenously at weekly intervals. Collection of antisera began one week after the last injection, and titres were determined by immunodiffusion in agar plates.

For serological tests, immune electron microscopy (MILNE and LUISONI 1977), latex agglutination (BERCKS and QUERFURTH 1969) and ELISA (CLARK and ADAMS 1977) were used. Some of the tests were made with antisera to authentic PVX isolates obtained from Dr. G. FACCIOLI (anti PVX-PO) and the International Potato Center (anti PVX-CIP).

E l e c t r o n m i c r o s c o p y : Particle measurements were made from tobacco leaf dips in 2 % aqueous uranyl acetate using TMV virions as internal calibration standard. For thin sectioning, systemically infected and healthy (control) tobacco leaf tissues were processed and stained according to standard procedures (MARTELLI and RUSSO 1984). Observations were made with a Philips 201C electron microscope.

Results

H o s t r a n g e a n d s y m p t o m a t o l o g y : Virus isolates from the two grapevine sources differed remarkably in the reactions elicited in some herbaceous hosts. Isolates from Ain-Ghellal induced localized and systemic chlorotic/necrotic ringspots in all *Nicotiana* species, i.e. *N. tabacum* cvs White Burley, Xanthi and Samsun (Fig. 1 a), *N. occidentalis, N. glutinosa* and *N. clevelandii*. By converse, isolates from Sidi Messaud induced in some of the same hosts diffuse chlorotic local lesions followed by systemic vein clearing and mottling (Fig. 1 b). These reactions were similar to those evoked by the potato PVX isolate (PVX-PO).

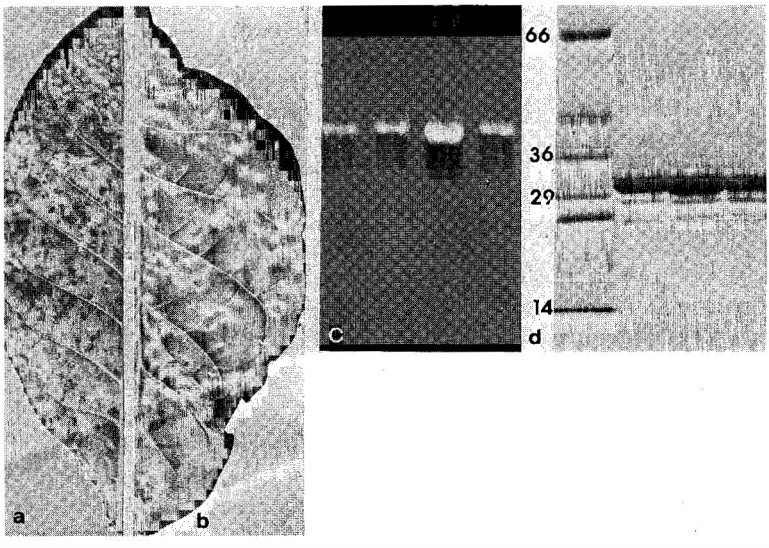

Fig. 1: **a, b** Differential reaction of tobacco cv. Samsum to infection by the RS and the MO strain of PVX recovered from Tunisian grapevines; **c** Electropherogram of RNA preparations from (left to right) TMV, PVX-RS, PVX-MO and PVX-PO; **d** Electropherogram of markers dissociated coat protein of PVX-RS, PVX-MO and PVX-PO. Protein markers are in lane 1 (mol. wt of some markers expressed in kDa are shown).

No differences were observed in the reactions of *N. benthamiana* (severe systemic necrosis), *Chenopodium quinoa* (necrotic local lesions), and *Gomphrena globosa* (necrotic local lesions with reddish halo) to infection by the two viral isolates.

Multiple sequential transfers from highly diluted extracts from single local lesions of both viral isolates gave consistent reproduction of symptoms, indicating that the isolates were stable and there were no mixed infections. Virus isolates thus obtained, referred to as PVX-RS and PVX-MO, were multiplied separately and used for subsequent characterization.

Grapevine rootlings inoculated by subcortical injections became infected. The distal part of inoculated shoots necrotized but the virus could be recovered from newly produced leaves although they were symptomless or, at the most, showed faint chlorotic spots or vein banding. No infection was obtained in any of the manually inoculated grape seedlings.

Properties of virus isolates: PVX-RS and PVX-MO had properties similar to those reported in the literature for ordinary PVX strains (KOENIG and LESEMANN 1989). Both were readily purified from tobacco leaves in large quantities (up to and above 100 mg nucleoprotein per 100 g of tissue) and sedimented as a broad single band in density gradient centrifugation.

In polyacrylamide gel electrophoresis, their nucleic acids migrated as a single species with an estimated mol. wt of about 2×10^3 kDa. The rate of migration was practically the same for the three virus isolates and TMV RNA (Fig. 1 c).

Dissociated coat protein of the three isolates migrated as a major band with an estimated mol. wt of about 30 kDa and two minor components (Fig. 1 d), likely to represent partly degraded subunits (KOENIG et al. 1970).

Serology: Antisera to PVX-RS and PVX-MO had both a titre of 1:256, as determined in immunodiffusion, and did not give visible reactions with healthy plant extracts. In immunodiffusion tests these antisera, and those to PVX-CIP and PVX-PO, reacted with homologous and heterologous antigens with single precipitin lines that merged at the junction

Fig. 2: **a** PVX-RS particles from a tobacco leaf dipmounted in uranyl acetate; **b** PVX-RS particles decorated by an antiserum to a potato PVX isolate; **c** Paracrystalline aggregates of virions in the cytoplasm of a tobacco leaf infected by PVX-RS; **d** Laminate inclusions (LI) and disorderly accumulations of virus particles (V) in a tobacco cell infected with PVX-MO. Bars = 200 nm.

without forming spurs. Moreover, the antisera recognized and decorated equally well homologous and heterologous virus particles (Fig. 2 b).

In latex-agglutination tests, the antiserum to PVX-CIP detected viral antigens in extracts from grapevine and tobacco (positive control) leaves, but not in supposedly healthy grapevines.

Electron microscopy: Virus particles from leaf dip preparations of PVX-RS and PVX-MO appeared as slightly flexuous filaments about 520 nm long (Fig. 2 a). Thin sectioned cells contained large accumulations of virions and the beaded laminate inclusions (Fig. 2 d) that are specifically induced by PVX (Shalla and Shepard 1972). Whereas in cells infected by PVX-MO virus particles were in massive but disorderly aggregates (Fig. 2 d), in PVX-RS local lesions virions tended to become organized into paracrystalline arrays (Fig. 2 c).

Virus distribution in the field: Of 180 samples, each from an individual vine, collected at random at Ain-Ghellal, 7 (about 4 %) gave clear-cut reaction in ELISA. Positive samples were either from apparently symptomless vines or from plants that showed vein banding, sometimes accompanied by reddish rings.

Discussion

The results of the present investigation provide experimental evidence that the viruses recovered by inoculation of sap from Tunisian grapevines are two biologically distinct strains of PVX.

The differential reactions observed in *Nicotiana* species had prompted a more detailed study of the two strains, which has shown that both have morphological, physico-chemical, serological and ultrastructural properties indistinguishable from those of ordinary isolates of the type species (SHALLA and SHEPARD 1972; KOENIG 1988; KOENIG and LESEMANN 1989).

This is not the first record of PVX in grapevines, because the occurrence of this virus in a single vine of cv. Barbera, was reported years ago from Italy (GIUNCHEDI 1973). As in the present instance, the Italian PVX isolate was not associated with distinct symptoms, nor could it be back-inoculated to grapevine seedlings (GIUNCHEDI 1973). Although this may be taken as an indication that PVX is little pathogenic to *Vitis*, the Tunisian data on virus distribution in the field seem to support the notion that PVX infections in grapes may no longer be regarded as a mere scientific curiosity.

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