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CUCUMBER MOSAIC VIRUS AND SOME OTHER VIRUSES ON TOBACCO IN PAKISTAN

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A tobacco disease in Pakistan, characterized by mosaic, vein clearing, necrosis and reduction in plant height, was found to be caused by a complex of three viruses two of them reported previously as tobacco mosaic virus and potato virus Y. A spherical component was identified by host range, particle morphology and serology as cucumber mosaic virus. This is the first report of the occurrence of CMV in tobacco from Pakistan.

Key words: Cucumber, Mosaic, Virus, Tobacco.

Introduction

Pakistan is a producer of flue-cured Virginia, air-cured Virginia, burley and white patta (leaf) tobacco, used in cigarette manufacture, for domestic and export purposes. More than 100 viruses are known for which *Nicotiana* spp. serve as propagative and indicator hosts. Numerous viruses are reported to infect tobacco worldwide, cucumber mosaic virus (CMV) is one of them. It is common on tobacco and where ever the crop is grown but particularly it is very severe in Japan [1].

In Pakistan losses caused by virus diseases in tobacco have been reported previously [2], but few have been studied in detail [3]. In recent years potato virus X(PVX), potato virus Y(PVY), and tobacco mosaic virus (TMV) were reported to infect tobacco [4]. So far CMV has been reported from eggplant [5] and its occurrence in the country in other vegetable crops is suspected. In this paper we report the identification of CMV from tobacco on the basis of host range, particle morphology and serology.

Materials and Methods

Virus source. The virus was isolated from a tobacco field near Mangora (Bandi) in the province of N.W.F.P. Infected plants were showing mosaic, chlorosis, narrowing of leaves, vein clearing and necrosis. Height and leaf area of diseased plants was reduced as compared to healthy ones. The isolate was maintained by periodic sap transfer in *N. tabacum* cv. Samsun NN and *N. glutinosa* in the glass-house at 22-25°.

Transmission and host range. Inoculum was prepared by grinding infected tobacco leaves in chilled pestle and mortar with 0.02M phosphate buffer pH 7.0. Three plants of each species were inoculated to determine the host range, and an equal number of uninoculated plants were kept as control. The reaction of indicator plants were observed daily for 3-6 weeks.

Electron microscopy. Electron microscope grids were prepared from field samples and from inoculated indicator plants by the leaf dip method and stained with 2% PTA (phosphotungstic acid) pH 6.8. Partially purified virus preparations were stained either with 2% PTA or 2% uranyl acetate (UA) and examined with a JOEL 100-CXII electron microscope.

Virus purification. Partial virus purification was according to Lot [6] with some modifications. Systematically infected leaves of *N. tabacum* cv. Samsun were homogenized in 0.5 M sodium citrate buffer pH 6.5 (1ml/gm tissue), containing 0.5% thioglycolic acid (TGA). The mixture was emulsified with one volume of chloroform and broken at 10,000 rpm for 10 mins. The supernatant was passed through Whatmans filter paper No. 4. Virus was precipitated with 10% polyethleneglycol (PEG), M.Wt. 6000 followed by two cycles of differential centrifugation (high at 27,000 rpm for 3 hrs and low at 8,000 rpm for 10 mins.). All low and high speed centrifugations were carried out in Beckman JA-10, JA-20 and Type 30 rotors in a J2-21 and L8-80 centrifuge respectively.

Serology . Double-diffusion tests in gel [7] and ELISA were performed for serological identification of the viruses. Sap from naturally infected tobacco plants and inoculated indicator plants was used as antigen in gel-diffusion tests. Partially purified sonicated preparations were also included in gel-diffusion tests. Sonication, to break up the elongated particles, was carried out in a Titertek Ultrasonic cleaner for 10 mins for easy diffusion in gel [8]. Reagents used in ELISA (immunoglobulins-IgG and IgG- conjugates) for PVX and PVY were from Bioreba Ag, Switzerland and that of TMV and CMV were from Agdia, Indiana-46545, USA. Samples were prepared at 1:10 dilutions (w/v) and tests performed as described [9]. The IgG and conjugate dilutions for PVX and PVY were 1:1000, and for CMV 1:200 and 1:400, respectively. In each plate positive and negative controls were also included. Reactions were evaluated visually or photospectrometrically in Titertek Multiscan Model MC type 340 at 405 nm for PVX and PVY and at 490nm for TMV and CMV. Samples giving double the value of negative controls were considered as positive [10].

Results and Discussion

Virus was readily transmitted mechanically from field samples to a large number of indicator plants causing local and systemic infection. Two types of systems were produced on *Datura stramomium* and *N. glutinosa* i.e. necrotic and chlorotic local lesions, resembling the symptoms caused by CMV and TMV. From *D. stramonium* these viruses (TMV and CMV) were transferred to *N. glutinosa* on which TMV produces necrotic local lession and CMV moves systemically [11]. PVY was eliminated since it does not infect *D. stramonium* [12]. Symptoms, characteristic of CMV were induced on different indicator plants. The symptoms observed were local lesions on *Chenopodium amaranticolor, C. quinoea, D. stramonium, Vigna sinensis, V. unguiculata*, and systemic infection in *Cucumis sativus* cv. National Pickling, *C. melo, N. glutionosa* and *N. tabacum* cvs. Samsun NN, White Burley, Xanthi NC, NC-95, *Lycopersicon esculentum* cvs. Rutger and Sahiwal, *Capsicum annuum, Gomphrena globosa* and *Citrullus vulgaris*. *N. glutinosa* and tomato plants developed typical shoestring symptoms 20-30 days after inoculation. The reactions of indicator plants were identical to those reported for CMV strains by other workers [13-16].

Electron microscopy revealed three types of virus particles in field samples and in inoculated 'Samsun' plants. They were polyhedral, rigid and flexuous rods, resembling those of CMV, TMV and PVY, respectively. The number of polyhedral particles were greater than those of the rigid rods, followed by flexuous ones. Grids prepared from indicator plants with biologically isolated CMV and with partially purified preparations showed only polyhedral particles resembling those of CMV, and these gave positive ELISA reactions.

TMV was readily detected in crude extracts from naturally infected plants in gel diffusion tests, but no reaction occurred in such sap with antiserum to PVY or PVX. Partially purified sonicated preparations, containing a mixture of the viruses when used as antigen, showed clear reactions also to PVY. In double- antibody sandwich ELISA, field samples showed positive reactions to TMV, PVY and CMV. PVX did not show any reaction, confirming the presence of three viruses in field samples.

The purification method gave satisfactory results. In partially purified virus preparations large numbers of polyhedral virus particles with an average diameter of 30 nm were seen (Fig. 1). The preparation was infectious on all tested indicator plants.

All these criteria indicate that the mosaic disease of tobacco was caused by three viruses i.e. PVY, TMV and CMV. This was confirmed through host-range studies, particle morphology and serology. This is the first report of CMV occurrence on tobacco in Pakistan.

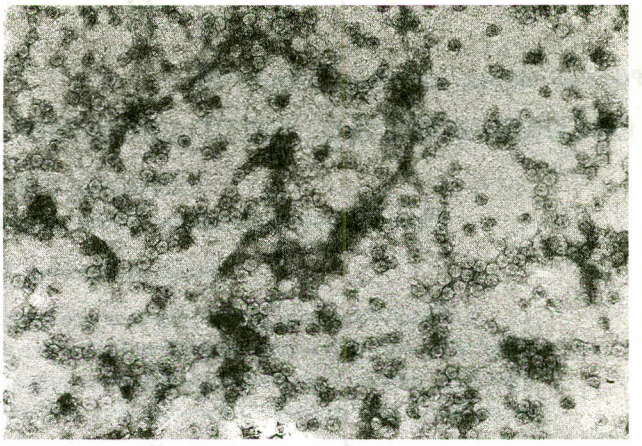

Fig. 1. Electronmicrograph of partially purified preparation of cucumber mosaic virus stained with 2% uranyl acetate pH 4.2.

It is assumed tha TMV spread is through previous year plant debris in the field or with cutting knives. CMV and PVY are not seed transmitted in tobacco, but a large number of aphids are known to transmit these viruses in the field. Although at present incidence of CMV is low, the cultivation in Pakistan of tobacco near other crops, including tomato, pepper, cucumber and other solaneceous hosts may lead to a further increase of CMV and PVY, and of its aphid vectors in tobacco and other solanaceous hosts. Crop rotation together with phytosanitary measures and vector control are required for control.

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