THE OCCURRENCE OF MIXED INFECTIONS OF TURNIP MOSAIC, CAULIFLOWER MOSAIC AND CUCUMBER MOSAIC VIRUSES IN WINTER OILSEED RAPE FROM THE TERRITORY OF UZBEKHISTAN

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Summary. – Turnip mosaic, cauliflower mosaic and cucumber mosaic viruses (TuMV, CaMV and CMV) were isolated and identified from winter oilseed rape plants from Uzbekhistan. This is the first record on the occurrence of CaMV and of mixed infection of rape with the three aforementioned viruses from the territory of the former Soviet Union.

Key words: turnip mosaic virus; cauliflower mosaic virus; cucumber mosaic virus; mixed infection; Brassicaceae

Introduction

Winter oilseed rape is a relatively new crop in Uzbekhistan and the area in which it is grown is recently rising in both the European and the Asian parts of the former Soviet Union. There are few reports from this territory on the occurrence of viruses in plants of the family Brassicaceae (Krylov *et al.*, 1981) and none in oilseed rape. In 1989, due to the courtesy of Dr. A. Ch. Vachabov (Institute of Microbiology, Academy of Sciences, Tashkent, Uzbekhistan), we obtained winter oilseed rape plants with symptoms of severe stunting and with different types of mosaic on leaves collected from a heavily infested field near Tashkent. This paper reports the results of the identification and characterization of causal agents of the disease.

Materials and Methods

Inoculation of test plants. Samples of rape leaves with symptoms of infection and of individual virus isolates were ground in a mortar in 0.1 mol/l phosphate buffer pH 7.0 and the sap was inoculated onto different test plants (Table 1). The symptoms were evaluated over a four week period after inoculation. Possible presence of virus infection in symptomless plants was checked by ELISA or by back inoculation to sensitive host plants.

Purification. The collected rape plants were stored at –40 °C and later purified using the method described by Hull *et al.* (1976). Part of the material was blended at 4 °C in a mixture of 0.5 mol/l potassium phosphate pH 7.5 and 0.5 % Na_2SO_3 (1:2 w/v). After filtration through a nylon cloth Triton X-100 and urea were added to final concentration of 0.5 % and 0.5 mol/l, respectively. The sap was stirred overnight at 4 °C and subjected to centrifugation at 8000 rpm for 15 mins in Beckman J-2-21 centrifuge. The supernatant was centrifuged in Beckman Ti 50.2 rotor at 30 000 rpm for 2 hrs (5 °C). the pellets were resuspended in 0.01 mol/l potassium phosphate buffer pH 7.5 and then centrifuged at 8000 rpm. After clarification by low speed centrifugation the virus suspension was layered on 10–40 % sucrose gradient in 0.01 mol/l potassium phosphate pH 7.2, and centrifuged for 3 hrs at 21 000 rpm in Beckman SW 27 rotor. The virus containing band was collected using UV scanner and fraction collector. The virus suspension in sucrose was diluted with 0.01 mol/l potassium phosphate pH 7.2 (1:3) and pelleted by centrifugation for 2.5 hrs in Ti 50.2 rotor at 30 000 rpm.

Electron microscopy. Virus particles were visualized in Hitachi 11 B electron microscope using preparations stained with 1 % uranyl acetate.

Isolation of viruses. CaMV was separated from the mixed infection with TuMV using the difference in thermal inactivation point which is 77 °C for CaMV and 58 °C for TuMV. Sap obtained from the original rape plants was heated at 60 °C for 10 mins,

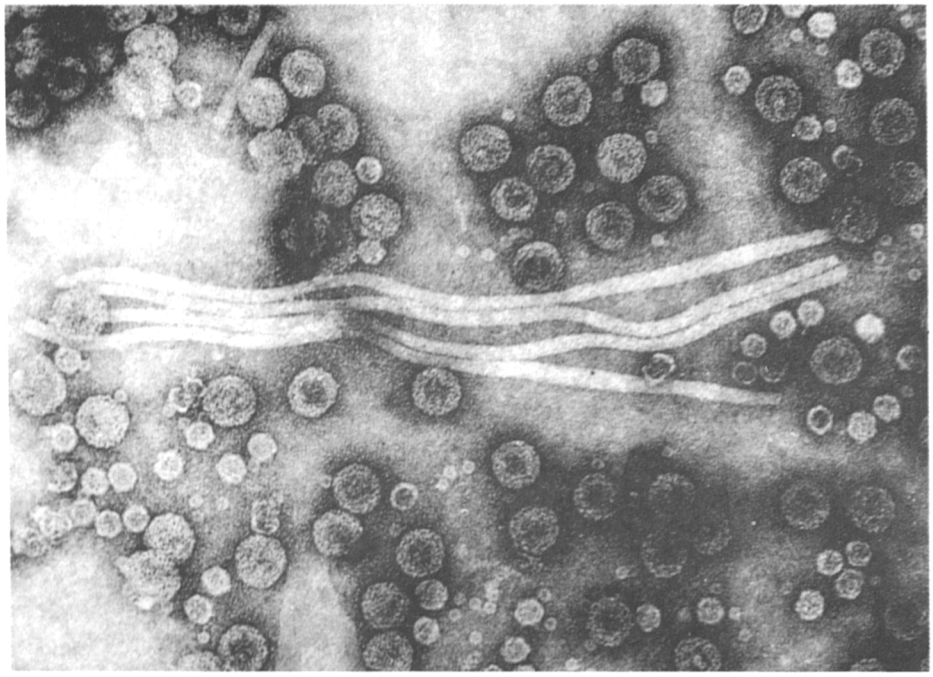

Fig. 1
Electron micrograph of viral particles in purified virus suspension from narurally infected plants of oilseed rape

cooled with tap water and rubbed immediately onto leaves of Chinese cabbage and *Nicotiana tabacum* L. cv. Samsun. Three weeks later when symptoms of vein mosaic appeared on the Chinese cabbage plants, sap from their leaves was reinoculated onto tobacco cv. Samsun plants as a control. TuMV was separated by inoculation of the sap obtained from the rape plants onto *N. tabacum* cv. Samsun. One of local necrotic lesions which appeared 7 days later was cut out, homogenized in the buffer and inoculated onto Chinese cabbage plants. The CaMV and TuMV isolates were retested for CMV by ELISA. The CMV isolate was separated by inoculation of the sap to cucumber plants.

ELISA. Double sandwich ELISA was performed according to Polák and Kříštek (1988) using horseradish peroxidase-conjugated IgG prepared against CaMV 1 isolate (Špak, 1989*b*), TuMV strain Ruzyně (Špak, unpublished data) and monoclonal antibodies against CMV (Novikov, unpublished data). For CaMV ELISA the samples were homogenized in PBS pH 7.2 extraction buffer containing 1 mol/l urea, 1 % PVP 25, 1 % bovine serum albumin and 0.05 % Tween 20 (Špak, 1989*c*).

Double diffusion test was performed in 0.9 % Difco Noble agar in 0.01 mol/l McIlvaine buffer pH 7.0 with 0.02 % sodium azide in a standard way.

Results

A positive reaction was obtained when the sap from stored plants was tested for CaMV, CMV and TuMV by double sandwich ELISA. Absorbance values for CaMV ranged from 0.453 to 0.571 in comparison to 0.035–0.057 for healthy controls. The values for TuMV and CMV varied from 0.398 to 0.684 and from 0.293 to 0.534, respectively, whereas healthy controls varied between 0.056 and 0.102.

When leaf sap was inoculated onto differential test plants, Chinese cabbage plants showed symptoms of severe vein mosaic and stunding. Plants of *N. tabacum* cv. Samsun developed local necrotic lesions resembling those caused by TuMV on leaves 7–10 days after inoculation.

The sap form naturally infected plants did not react in double diffusion test in agar with antisera against radish mosaic virus (Špak, 1992), erysimum latent virus (Špak *et al.*, 1993), or TuMV prepared in our laboratory.

To confirm the presence of CaMV, CMV and possibly other viruses, we chose the relatively gentle purification method of Hull *et al.* (1976). Three types of virus particles were then seen in electron microscopic preparations. The pellets contained spherical particles of about 50 nm in diameter, typical for CaMV, and "empty" and "full" angular particles of about 30 nm diameter. There were found also flexuous particles of about 12–13 nm diameter with a modal length of 774 nm corresponding to TuMV (98 particles measured) (Fig. 1).

The reaction of different test plants to the CaMV, TuMV and CMV isolates is presented in Table 1. The virulence of the CaMV, TuMV and CMV isolates was very similar to that of earlier isolates from Czechoslovakia (Špak, 1989*a*; Špak and Polák, 1985).

Table 1. Reactions of the test plants on the inoculation with the TuMV, CaMV and CMV isolates

Plant species	Virus					
	TuMV		CaMV		CMV	
	I	II	I	II	I	II
Brassicaceae:						
B. napus L. cv. Jet Neuf	cL	M	cL	VM,Dw	–	–
B. nigra (L.) Koch	nL	M,Dw	–	VM,Dw	–	–
B. pekinensis (Lour.) Rupr. cv. Nozaki	–	M	–	M	–	–
B. rapa L. var. rapa cv. Albína	nL	M,Dw	cL	VM,Dw	–	–
B. oleracea L. var. gongylodes cv. Gigant	–	–	–	VM	–	–
B. oleracea L. var. botrytis cv. Bolero	–	–	–	VM	–	–
Sinapis alba L. cv. Přerovská	–	M,Dw	–	M,Dw	–	–
Raphanus sativus L.	–	M	–	VM	–	–
Non-*Brassicaceae*:						
Nicotiana glutinosa L.	nL	M	–	–	–	–
N. clevelandii Gray	nL	M	–	–	cL	M
N. tabacum L. cv. Samsun	nL	–	–	–	–	N
Chenopodium quinoa Willd.	cL	–	–	–	cL	–
Reseda odorata L.	cL	M	–	–	–	–
Datura stramonium L.	–	–	nL	–	–	M
Papaver somniferum L.	–	M	–	–	–	–
Cucumis sativus L.	–	–	–	–	–	M

I – symptoms on inoculated leaves
II – systemic symptoms
nL – local necrosis lesion
cL – local chlorotic lesion
VM – vein mosaic
N – necrosis
M – mosaic
Dw – dwarf

Discussion

A critical review of the obtained micrographs revealed that the angular 30 nm particles do not represent CMV, but more likely they are a comovirus (radish mosaic virus) or a fabavirus (broad bean wilt virus) (R. G. Milne, personal communication). Our tests for radish mosaic virus, Turnip yellow mosaic virus and erysimum latent virus which occurr frequently in *Brassicaceae* were all negative and, unfortunately, this unknown virus was lost during the process of isolation of individual viruses.

The occurrence of mixed infections in rape has been observed by several authors earlier. Shukla and Schmelzer (1973) reported seriously diseased fodder rape infected with TuMV, CMV and broad bean wilt virus (BBWV) or CaMV. TuMV and CaMV have also been found infecting field crops of winter oilseed rape with other viruses in Great Britain by Walsh and Tomlinson (1985) and in Czechoslovakia by Špak and Polák (1985) and by Kvíčala (1974).

Horváth (1969) isolated CMV from naturally infected winter oilseed rape showing mosaic symptoms, deformation of leaves, stems and pods, and stunting of plants. He regarded CMV as an important pathogen of rape because of the 50 % lower yield. As an overwintering host, rape was shown to play an important role in the epidemiology of CMV in Hungary. However, most of strains and isolates of CMV used by Shukla and Schmelzer (1973), Walsh and Tomplinson (1985) and Špak and Polák (1985) induced either a very faint mottle or no symptoms in infected rape cultivars. The failure of the CMV isolate to infect any

Brassicaceae plants in our present study seems to support previous results and namely the assumption that CMV is spread in these plants mostly by simultaneous transmission with other viruses by aphids.

Our results show the necessity to obtain further information on the occurrence of *Brassica* viruses of the territory of the former Soviet Union. They indicate a possibility of serious damage to rape by viruses under the warm climate conditions supporting the transmission and apread of such disease complexes by aphids.

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