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Short communication

FIRST REPORT OF *TOMATO BLACK RING VIRUS* (TBRV) IN THE NATURAL INFECTION OF ZUCCHINI (*CUCURBITA PEPO* L. CONVAR *GIROMANTIINA*) IN POLAND

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Abstract: In this paper we present the first identification of the *Tomato clack ring virus* isolated from zucchini with mosaic and deformation of leaves in Poland. Immunosorbent electron microscopy, ELISA test and IC-RT-PCR confirmed the identification of TBRV. RNA extracted from purified virus (size about 7.4 kb and 4.6 kb) was characteristic to this virus.

Key words: *Cucurbita pepo, Tomato black ring virus,* identification

Zucchini (*Cucurbita pepo* L. convar *giromantiina)* is not popular vegetable in Poland such as cucumber (*Cucumis sativus*) however, its production is steadily increasing mainly due to development of appropriate growing techniques, especially in the field.

Diseases, especially viral ones, are main problems in the production of cucurbit plants. Symptoms of viral infection on zucchini are mosaic, yellowing, stunting, leaf chlorosis and fruit deformations. In 2005, similar symptoms were observed on zucchini grown in the home garden and in the field. This paper presents the identification of the virus isolated from zucchini with mosaic and slight deformation of leaves, that was grown in the home garden, in Poznań, Poland.

Virus was maintained under greenhouse conditions on *Chenopodium quinoa* being a reservoir of the virus for studies on its host range and purification. The host range and symptoms for the studied virus were determined by mechanical inoculation of various plant species with the sap of infected *C. quinoa*.

Virus isolate from zucchini infected host range and induced symptoms similar to those caused by *Tomato black ring virus* (TBRV) isolates from cucumber (Pospieszny et al. 2003) and black elderberry (Pospieszny et al. 2004). Generally, mechanical inoculation of *Chenopodium* spp. resulted in chlorotic or necrotic local lesions and systemic deformation or necrosis of plant tops. Tobacco plants (*Nicotiana* spp.) reacted with local and systemic ring spots or/and pattern lines, characteristic for Polish isolates of TBRV (Pospieszny et al. 2003; Pospieszny et al. 2004).

Seven days after inoculation, *C. quinoa* plants were checked for the presence of virus by electron microscopy (EM) observations. Spherical virions and empty protein shells were observed in the sap (Fig. 1).

During centrifugation of the purified virus preparation in sucrose density gradient, virus particles sedimented as three opalescent zones in transmitted light. Top zone was of a low intensity and electron microscopy observation showed that this zone contained empty protein shells.

In immunosorbent electron microscopy the virus was strongly decorated with antiserum against TBRV (Fig. 2) and much weaker with *Beet ringspot virus*, BRSV. Virus was identified serologically also by ELISA test performed with antisera TBRV and BRSV. Only the first one gave a positive reaction with tested virus.

The identification was confirmed by immunocapture-reverse transcription polymerase chain reaction (IC-RT-PCR) with primers designed by Le Gall et al. (1995). These primers have been universal for detection of TBRV. The antibodies against TBRV were used for trapping TBRV particles from tested plant sap. The result of IC-RT-PCR for tested virus was 300-nucleotides product (Fig. 3), typical for TBRV from cucumber and TBRV from *Sambucus nigra* (Pospieszny et al. 2003; Pospieszny et al. 2004).

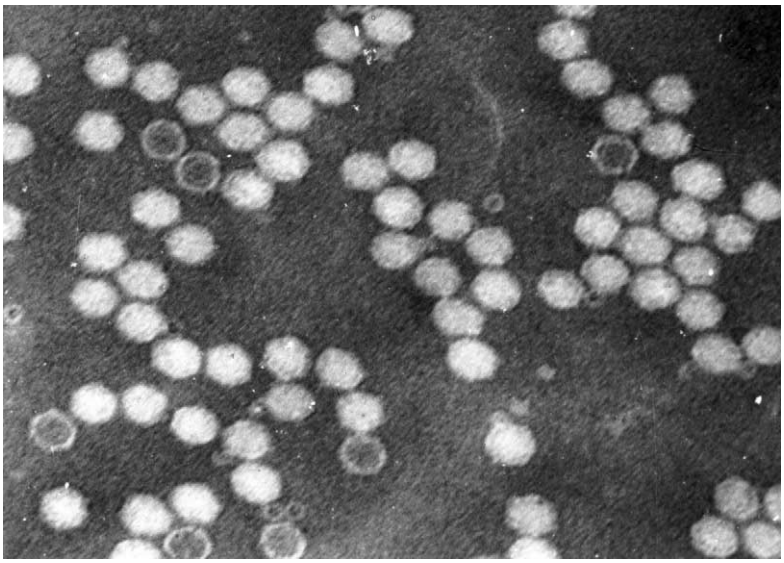

Fig. 1. Virions of *Tomato black ring virus* from *Cucurbita pepo*

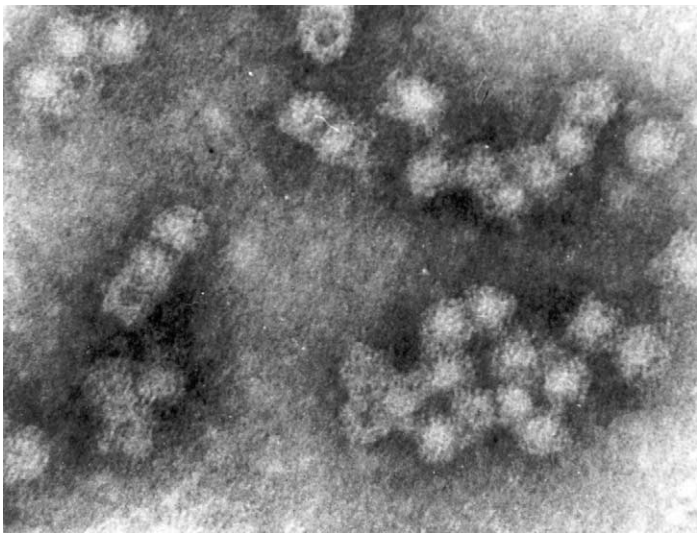

Fig. 2. Electron microscopy of *Tomato black ring virus* particles decorated with TBRV antiserum

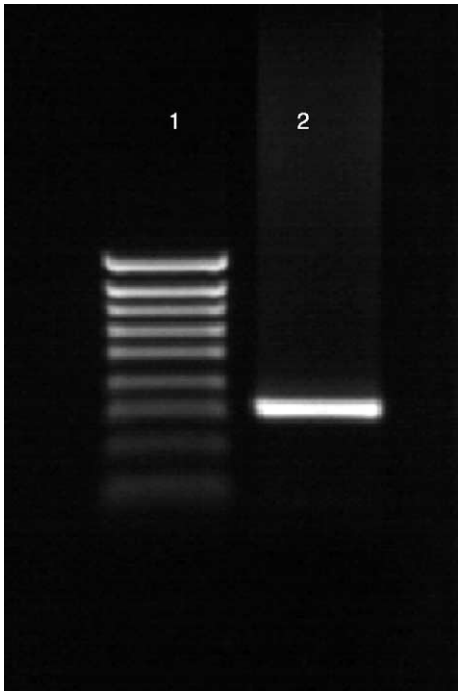

Fig. 3. Electrophoretic mobility of IC-RT-PCR product
Lane 1. DNA HyperLadder IV (1000, 800, 700, 600, 500, 400, 300 200, 100)
Lane 2. TBRV – zucchini

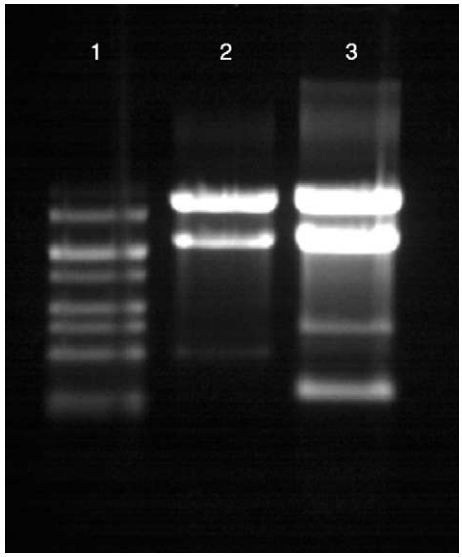

Fig. 4. Electrophoretic separation of viral RNA on 1% agarose gel
Lane 1. RNA leader (6, 4, 3, 2, 1.5, 1, 0.5, 0.2 kb)
Lane 2. TBRV – zucchini
Lane 3. TBRV – cucumber

From purified virus preparation RNA was isolated and separated by electrophoresis in 1% agarose gel. The electrophoresis of zucchini isolate RNA showed an expected size of genomic RNAs (about 7.4 kb and 4.6 kb) and appearance of one additional, small RNA of the size ca. 1000 nucleotides. Additional, small RNAs of size ca. 500 and 1200 nucleotides also were observed during the electrophoresis RNA of TBRV cucumber isolate (Fig. 4). The RNAs expected for TBRV satellite were about 1350 nucleotides in length (Fritsch et al. 1984, 1993) and the observed bands were smaller.

This is first record of TBRV from zucchini in Poland. Earlier, we collected several different TBRV isolates from various plant species: tomato (Pospieszny and Borodynko 1999), cucumber (Pospieszny et al. 2003), black elderberry (Pospieszny et al. 2004) and black locust (Borodynko et al. 2001; Borodynko 2004). For the most of them high biological and molecular variations were shown (Borodynko 2004; Jończyk et al. 2004).

These above may indicate that in Poland various plant species might serve as natural hosts for different TBRV isolates but the occurrence of the virus is still not prevalent.

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POLISH SUMMARY

PIERWSZE DONIESIENIE O WYSTĘPOWANIU WIRUSA CZARNEJ PIERŚCIENIOWEJ PLAMISTOŚCI POMIDORA (*TOMATO BLACK RING VIRUS*, TBRV) W CUKINII W POLSCE

Z rośliny cukinii z objawami mozaiki i zniekształconymi liśćmi wyizolowano wirusa, którego na podstawie zakresu porażanych roślin gospodarzy, jak i objawów na nich powodowanych, wstępnie zaklasyfikowano do rodzaju *Nepovirus*. Testy serologiczne (immunoelektronomikroskopia, ELISA) wykazały obecność wirusa TBRV w testowanej roślinie. Następnie identyfikacja została potwierdzona IC-RT-PCR, w którym otrzymano produkt wielkości 300 nt, podobnie jak otrzymywano wcześniej dla izolatów TBRV z ogórka czy bzu czarnego.

Z oczyszczonego preparatu wirusowego wyizolowano RNA, które następnie rozdzielono na 1% żelu agarozowym. Otrzymano charakterystyczne dla TBRV dwa genomowe RNA, wielkości ok. 7,4 kb i 4,6 kb oraz małe, niegenomowe RNA wielkości 1 kb.