TWO BIOLOGICALLY DISTINCT ISOLATES OF ZUCCHINI YELLOW MOSAIC VIRUS LACK SEED TRANSMISSIBILITY IN CUCUMBER

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Summary. – The seed transmission of the Zucchini yellow mosaic virus (ZYMV) was studied in cucumber using two isolates unrelated in their biological characteristics. Although the virus could be readily detected in mature seeds harvested from infected cucumbers, the seedlings obtained from infected germinated seeds tested negative for ZYMV using both ELISA and RT-PCR assays. No evidence was obtained for transmission of two ZYMV isolates through seeds.

Key words: Cucumis sativum; seed transmissibility; Zucchini yellow mosaic virus

Introduction

ZYMV belonging to the genus *Potyvirus* is a harmful pathogen responsible for serious losses to cucumber crops worldwide. In a field, the natural spread of ZYMV is caused by several aphid species, which transmit the virus in a non-persistent manner (Desbiez and Lecoq, 1997). The seed transmission, i.e. vertical transfer of virus from seeds to seedlings, is an intrinsic characteristic of some plant viruses and presents an important epidemiological phenomenon. Approximately 10% of plant viruses are considered as seed-transmissible (Mink 1993; Johansen *et al*., 1994). These viruses can be spread to distant locations by contaminated seeds and cause serious economic losses.

Some species of the genus *Potyvirus*, such as Pea seed-borne mosaic virus, Bean common mosaic virus or Lettuce mosaic virus are seed-transmissible (Johansen *et al*., 1994). In some cases seed transmission of ZYMV, although at very low rate, has been also described (Greber *et al*., 1988; Schrijnwerkers *et al*., 1991; Tobias and Palkovics, 2003). On the other hand, many studies have failed to demonstrate seed transmission of ZYMV in its natural hosts (Orozco-Santos *et al*., 1994; Gleason and Provvidenti, 1990; Desbiez and Lecoq, 1997).

The aim of the present study was to assess the seed transmissibility of two ZYMV isolates, asymptomatic SE04T and symptomatic ZYMV-H, which were different in their biological properties.

Material and Methods

Viruses. The isolate ZYMV-H was isolated from squash (*Cucurbita pepo* L.) in Czech Republic (Svoboda and Polák, 2002) and the isolate SE04T was isolated from squash in Slovakia (M. Glasa, unpublished). The viruses were mechanically transmitted to zucchini plants cv. Diamant. Systematically infected leaves were stored at -80°C and used as inoculum.

ELISA. The presence of viral antigens in leaves, fruits, seeds or young seedlings was detected by DAS-ELISA (Clark and Adams, 1977) using commercially available antibodies (Loewe, Cat. No. 07090).

Western blot analysis. The samples were analyzed by PAGE using 10% gel and transferred to the Immobilon P membrane (Serva). The standardized procedure (Nováková *et al*., 2006) was used for analysis of blots using polyclonal antibody against ZYMV kindly provided by Dr. H. Lecoq (INRA, Avignon, France).

Immunocapture reverse transcriptase PCR (IC-RT-PCR). Two-step IC-RT-PCR was performed as described previously (Glasa *et al*., 2002). Briefly, extracts of plant tissues were prepared by grin-

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Abbreviations: IC-RT-PCR = immunocapture reverse transcriptase PCR; ZYMV = Zucchini yellow mosaic virus

ding of the leaves, cotyledons, roots, whole seed, seed coat, and endosperm and diluted 1:10 (w/v) in PBS containing 0.05% (v/v) Tween-20 and 2% (w/v) polyvinyl pyrrolidone. The clarified extract in volume 100 µl of was incubated in tubes coated with 1 mg/ml anti-ZYMV IgG overnight at 4°C. The complementary DNA synthesis was carried out using AMV reverse transcriptase (Promega) and the reverse primer ZY8841R for 45 mins at 41°C. PCR was performed using the Go Taq® DNA polymerase (Promega) under following cycling conditions (Techne Genius, Merck): initial denaturation at 94°C/5 mins, 35 cycles of 94°C/1 min, 54°C/45 secs, 72°C/1 min, and final extension at 72°C/10 mins. The primers ZY8282F (5´-ACAGAGGCTATTTGCGCTGCG-3´, forward) and ZY8841R (5´-TGCTGATGAGACGCTCGTGTG-3´, reverse) amplified a 560 bp fragment spanning the (Cter)NIb-(Nter)CP region.

Seed transmission experiments. A group of 10 healthy cucumber plants (*Cucumis sativum*, cv. Nora F1) grown singly in pots were mechanically inoculated at the cotyledonary stage with the respective ZYMV isolate. Ten days p.i. the virus presence in cucumber plants was checked by DAS-ELISA. Infected plants were transplanted to larger pots with sterile garden soil and kept under insect-proof conditions with regular fertilisation. After picking from the ripe fruits, the seeds were washed in sterile water. The first part of the seeds was tested for the virus presence, the second part was allowed to germinate in sterile perlit**,** and the third part was sowed in a growth chamber with sterile garden soil.

Results and Discussion

We found that the isolates ZYMV-H and SE04T showed different behavior in the infected cucumber. While ZYMV-H-infected cucumber displayed severe symptoms and growth dwarfing, the SE04T-infected cucumber remain unaffected, without visible symptoms. We have obtained 15 ripe fruits from SE04T-infected cucumbers. Conversely, only 4 mature fruits could be collected from cucumber plants infected with ZYMV-H due to the devastating effect of this isolate. The presence of both viruses was confirmed in the skin and flesh of collected fruits by Western blot analysis and IC-RT-PCR.

A total of 57 and 387, respectively, of fully developed germinable seeds were harvested from both ZYMV-H and SE04T-infected cucumber plants. To obtain information about virus distribution in the seed, the 30 seeds of the isolate SE04T and 10 seeds of the isolate ZYMV-H were divided into seed coat and endosperm and tested separately by IC-RT-PCR. In case of both ZYMV isolates, the virus was detected in individual whole seeds, in the seed coat, but not in the endosperm (Fig. 1). Occasionally, viral antigens were detected in the dry seeds even after 5 months of storage by DAS-ELISA (data not shown) and Western blot analysis (Fig. 2).

Next, 20 infected seeds of each virus isolate were allowed to germinate in sterile perlit. Three days post germination, the roots and cotyledons were tested separately by ELISA and IC-RT-PCR. The virus was not detected in any case. Finally, the presence of the virus in 3-week-old seedlings from infected seeds was tested by DAS-ELISA. Similar to previous results, the virus transmission in seeds was not detected in 337 (isolate SE04T) and 27 (isolate ZYMV-H) seedlings examined.

Rarely, the seed transmission of ZYMV was detected in squash (Greber *et al*., 1988; Schrijnwerkers *et al*., 1991) and oil pumpkin (Tobias and Palkovics, 2003). However, even

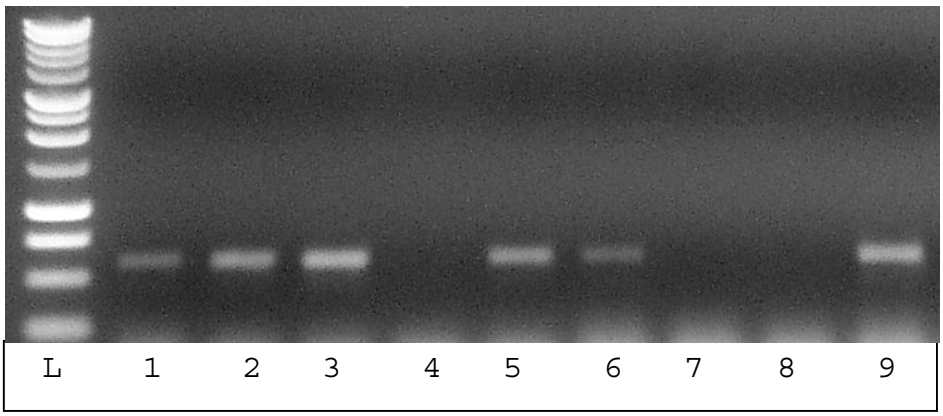

Fig. 1

IC-RT-PCR of ZYMV-H and SE04T-infected freshly harvested whole seeds (lanes 2, 5), seed coat (lanes 3, 6), and endosperm (lanes 4, 7)

1kb DNA ladder (Promega) (lane L), infected (lanes 1, 9) and healthy (lane 8) leaf controls.

a very low rate of seed transmission might be sufficient to spread the virus to a new location and to initiate epidemics. ZYMV is readily transmitted to cucumbers by aphid vectors and sowing the seed stocks infected by seed-transmissible virus should generate primary infection sources to initiate the disease cycle. Several factors, such as virus isolate or host genotype might interfere with the seed transmission rates. In general, the ZYMV-infected plants produce only few viable seeds (Desbiez and Lecoq, 1997). However, the existence of mild, weak or symptomless ZYMV isolates was reported (Lecoq *et al*., 1991; Wang *et al.*, 1991). Infection by these isolates does not affect the growth and production of fruits and seeds. Thus, much attention should be paid to the seed transmission studies of such isolates. Although ZYMV was detected in coat of mature seeds, it was concluded that both ZYMV isolates tested are not seed-transmissible in cucumber.

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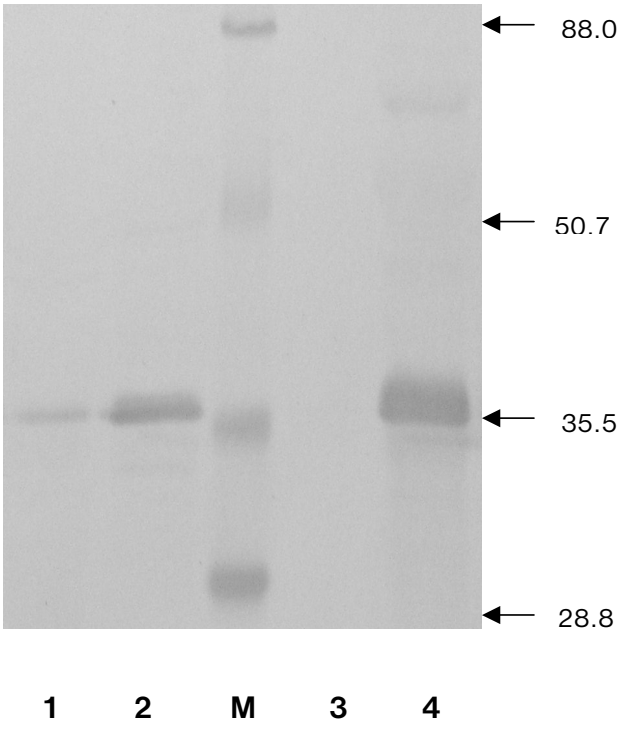

Fig. 2

Western blot analysis of coat proteins SE04T-infected three (lane 1) and ten (lane 2) dry seeds stored for 5 months

Molecular weight markers (M), coat proteins of healthy (lane 3) and infected (lane 4) leaf controls.

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