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TOMATO SPOTTED WILT VIRUS ON PEPPER *(CAPSCIUM ANNUUM* L.) PLANTS IN HUNGARY

In Hungary resurgence of Tomato spotted wilt virus (TSWV) frequently causes heavy crop losses in pepper production since the mid nineties. Management of TSWV control was first directed against the thrips (using different insecticides or plastic traps), and against weeds as host plants of the virus and the thrips. Later on Tsw resistance gene was introduced into different types of pepper. In 2010 and 2011 sporadically, but in 2012 more frequently a resistance breaking strain of TSWV on resistant pepper cultivars was observed in the Szentes region (Hungary). It is supposed that outbreaks of TSWV infection was due to the fact that protection against Frankliniella occidentalis was neglected and some effective pesticides (like Unifos 50 EC) were withdrawn.

Key wods: Tomato spotted wilt virus, Frankliniella occidentalis.

Introduction. Tomato spotted wilt virus (TSWV) is the type member of the genus Tospovirus (family Bunyaviridae), causes an important disease of horticultural and agronomic crops. The virus distributed worldwide is having extremly broad host range and is now considered as one of the ten most economically destructive plant viruses [1, 6, 15]. TSWV is transmitted by thrips in a persistant manner [4]. The virion varies in size from 80 to 120 nm and has spherical enveloped character [5]. The genome of TSWV consists of three ssRNA segments: small (S) and medium (M) RNAs have ambisense coding strategies, whereas the large (L) RNA is of negative polarity.

In Hungary TSWV was described in 1972, but the virus was not considered as an important pathogen. In 1995 very severe damage of TSWV infection was observed in tomato and pepper production in the Szentes vegetable growing region (Hungary) (Fig.1). The introduction and spread of western flower thrips (*Frankliniella occidentalis*), an efficient TSWV vector, in that time certainly played an important role in TSWV emergence [5].

Fig.1. Symptoms of TSWV infection on susceptible pepper cultivar: chlorotic spots and rings on leaves (A) and fruits (B)

Management of TSWV control was first directed against the thrips using different insecticides or plastic traps, and against weeds as host plants of the virus and the thrips. Later on *Tsw* resistance gene [3] was introduced into different types of pepper (conical white, long pale green hot and sweet, tomato shape, spice pepper and blocky types) (Csilléry unpublished). Pepper cultivars carrying *Tsw* resistance gene upon TSWV inoculation show necrotic local lesions on the leaves or other parts of the plant without systemic infection (Fig. 2).

In 2010 and 2011 sporadically, but in 2012 more frequently systemic virus symptoms were observed on resistant pepper cultivars in Szentes region [2, 4, 12] (Fig. 3). The presence of new resistance breaking strain of TSWV was proved by virological (test-plant, serological and RT-PCR) methods. It was demonstated that TSWV can adapt very rapidly to plant resistance, and the *Tsw* resistance gene was broken down only a few years after its deployment in pepper crops [9, 11, 13, 14].

Fig. 2. Symptoms of TSWV infection on resistant pepper varieties: necrotic spots on stem (A) and fruit (B, C)

Fig. 3. Systemic symptoms of TSWV infection on resistant pepper cultivars

Materials and methods. *Virus isolates.* TSWV isolates originated from pepper cultivars susceptible and resistant against TSWV from Szentes region (South-east Hungary). Fruit samples were collected from plants exhibiting typical symptoms of virus infection such as stunting, mosaic, chlorotic and/or necrotic spots, rings and distortion on the leaves and fruits (Figure 3). The isolates were used for ELISA serological tests, RT-PCR and maintained by mechanical inoculation on *Nicotiana tabacum* cv. Xanthi-nc test plants.

*RNA extraction, RT-PCR.*Total RNA was extracted from leaves of *N. tabacum* cv. Xanthi-nc plants systemically infected by TSWV or from infected pepper fruits using the Spectrum Plant Total RNA Kit (Sigma) following the manufacturer's instructions. RT-PCR reactions for synthesis of first-strand cDNA were performed with Revert Aid H Minus First Strand cDNA Synthesis Kit (Thermo Science). Specific primers TSWV-185CPforward (5'-AAT TTC TCC GCA ATC TAT TTC AGT TG-3') and TSWV-183CP reverse (5'-GGG GAT CCA GAG CAA TTG TGT CAA TTTT-3') amplified 1720 bp fragment of N and the non-coding genomregions. PCR reaction was performed in 25µl – 50 µl final volume. Amplification consisted of one cycle at 94°C for 5 min, followed by 35 cycles of 30 sec of denaturation at 94°C, 1 min of annealing at 50°C; and 2 min of extension at 72°C; and then one cycle of final extension for 10 min at 72°C. PCR products were electrophoresed in 1% agarose and stained with ethidium bromide.

Results. The collected samples showed typical symptoms of Tomato spotted wilt virus infection. The virus was transmitted by mechanical inoculation onto test plants. On *Nicotiana tabacum* cv *Xanthi*-nc plants chlorotic and necrotic spots and rings on inoculated leaves and systemic mosaic or necrotic rings or necrosis were observed (Fig. 4). Slight differences on symptoms were observed among different isolates independently whether originated from TSWV susceptible or resistant pepper cultivars. Samples for ELISA serological test were taken from the original fruits and from test plants. In all samples TSWV were detected.

Fig. 4. TSWV symptoms on *Nicotiana tabacum* cv *Xanthi*-nc plants

TSWV specific PCR-product was amplifyed by RT-PCR method (Fig. 5). Our results confirmed the presence of Tomato spotted wilt virus both in TSWV susceptible and resistance cultivars in Hungary. Our results confirmed the presence of the resistance breaking isolate of Tomato spotted wilt virus in Hungary. Further investigations needed to characterize the resistance breaking TSWV isolates from Hungary.

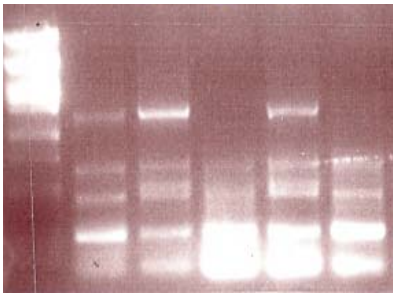

Fig. 5. Separation of amplified RT-PCR products of TSWV infected pepper plants on 1 % agarose gel stained with ethidium bromide. 1 – DNA lenght marker Pst I digested λDNA, Lane 2,3 and 5 TSWV infected pepper plants, Lane 4 – uninfected pepper plant, Lane 6 – healthy pepper plant

Earlier experiments predicted the determinant for breakdown *Tsw* resistance locating on RNA S of TSWV [7, 8]. For this reason our aim is in the future to characterize the S RNA of Hungarian resistance breaking isolates of TSWV and to compare them to other TSWV isolates.

It is supposed that outbreaks of TSWV infection in Szentes vegetable growing region is due to the fact that protection against *Frankliniella occidentalis* was neglected and some effective pesticide (like Unifos 50 EC) were withdrawn.

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ВІРУС ПЛЯМИСТОГО В'ЯНЕННЯ ТОМАТУ НА РОСЛИНАХ ПЕРЦЮ СОЛОДКОГО ІРУС (*CAPSICUM ANNUM* L.) В УГОРЩИНІ

З середини 1990-х років спалахи захворювання, спричиненого вірусом плямистого в'янення (бронзовості) томату (Tomato spotted wilt virus (TSWV)) в Угорщині, часто призводять до значних втрат врожаю комерційних посівів перцю солодкого. Початково зусилля при боротьбі з TSWV спрямовувалися на контролювання векторів вірусу – трипсів (шляхом використання різних інсектицидів чи пластикових пасток) та бур'янів, які виступають хазяями для вірусу та трипсів. Пізніше були створені різноманітні трансгенні сорти перцю з інтродукованим геном стійкості до даного вірусу – Tsw. Починаючи з 2010-2011 рр. у регіоні Сентеш в Угорщині траплялися поодинокі, а з 2012 р. – все частіші випадки появи нової форми TSWV, яка була здатна до подолання стійкості трансгенних рослин перцю. Вважається, що спалахи інфекції, викликаної TSWV, спричинені невиконанням рекомендацій щодо контролю трипса Frankliniella occidentalis та припиненням використання деяких ефективних пестицидів (наприклад, Unifos 50 EC). Дана робота присвячена вивченню цієї проблеми.

Ключові слова: вірус плямистого в'янення томату, трипс Frankliniella occidentalis.

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ВИРУС ПЯТНИСТОГО УВЯДАНИЯ ТОМАТА НА РАСТЕНИЯХ ПЕРЦА СЛАДКОГО (*CAPSICUM ANNUM L.*) В ВЕНГРИИ

С середины 1990-х годов вспышки заболевания, вызванного вирусом пятнистого увядания (бронзовости) томата (Tomato spotted wilt virus (TSWV)) в Венгрии, часто приводит к значительным потерям урожая коммерческих посевов перца сладкого. Изначально усилия при борьбе с TSWV направлялись на контроль векторов вируса – трипсов (путем использования различных инсектицидов или пластиковых ловушек) и сорняков, которые выступают хозяевами для вируса и трипсов. Позже были созданы разнообразные трансгенные сорта перца с интродуцированным геном устойчивости к данному вирусу – Tsw. Начиная с 2010-2011 гг в регионе Сентеш в Венгрии случались единичные, а с 2012 г. – все чаще случаи появления новой формы TSWV, которая была способна к преодолению устойчивости трансгенных растений перца. Считается, что вспышки инфекции, вызванной TSWV, вызванные невыполнением рекомендаций по контролю трипса Frankliniella occidentalis и прекращением использования некоторых эффективных пестицидов (например, Unifos 50 EC). Данная работа посвящена изучению этой проблемы.

Ключевые слова: вирус пятнистого увядания томата, трипс Frankliniella occidentalis.

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EFFICIENCY OF SEROLOGICAL KITS OF DIFFERENT MANUFACTURERS IN DEFECTION OF ANTIBODIES AGAINST PRRVS CIRCULATION IN UKRAINE

Stadejek et al. have studied the efficiency of five serological test kits, which are in wide use across the world, showing that their sensitivity differs in relation to the geography of PRRSV isolation. Karniychuk et al. confirmed an essential genetic and antigenic distinctions between East European PRRSV isolates and West European and North American strains of the virus. Our results on differences in the sensitivity of serological kits to detect anti-PRRSV antibodies comply with the data published earlier. When using a BIONOTE kit false positive results were received whereas the application of a CIVTESTsuisPRRS kit might lead to the significant number of false negatives questioning the expediency to use these test kits. Our findings lead us to conclude that a serological IDEXX HerdCheck PRRS 3XR ELISA kit is the most sensitive to Ukrainian PRRSV strains while an Ingezim PRRS Universal test kit may also be used to detect antibodies against this virus in Ukraine.

Key words: PRRVS, strains, serological kits, antibodies.

Introduction. A porcine reproductive and respiratory syndrome virus (PRRSV) is a member of Arterevirus genus, Artereviridae family and Nidovirales order [1]. Virus isolates are usually attributed either to the North American and European strains that have some distinctions in genome. The fact that these genetically different types of the virus appeared practically simultaneously presents one of the today's mysteries [2]. The analysis of their nucleotide sequences and antigen properties demonstrated that the North American (type 2) and European (type 1) PRRSV strains are only 63% identical at genome level [1]. Evolutional variability of this virus is suggested to be the highest among RNA viruses [3].

Until 2010 it was thought that the ORF7 is the most conservative gene of PRRSV. That is why it was widely used in diagnostic in test kits based on RT-PCR and real time PCR techniques [4]. However, Stadejek et al. analyzing European isolates of the virus found out the significant genetic variability among the ORF7 sequences which was especially high in East Europe where four main virus subgroups were revealed [4, 5]. Such high variability makes significantly harder to diagnose the related disease correctly, to develop efficient and safe vaccines as well as to control the disease.

Clinical signs of PRRSV infection vary depending on the virus virulence, immune status of the herd and age of infected animals. Viremia leads to clinical manifestation of the disease. PRRSV capable to cross transplacentary barrier and infect fetus causing abortions, stillbirths and births of weakened piglets [6, 7].

The International Epidemiological Bureau marks out, as the most efficient, several techniques of PRRSV diagnostics: virus isolation, serological tests, PCR and real time PCR. Serological tests are a potent and sensitive approach used in schemes of PRRSV control [3, 8]. Seroconversion can be identified 7-11 days after animals been infected using proven, highly sensitive and specific serological kits [9]. An analysis is performed in serum sampled from animals of an infected herd belonging to different age groups.

Blood serum specimens are tested with time intervals (for example, at the time of clinical signs development and then in 2–3 weeks) providing the basis for serological diagnostics. Such approach is also applied to control the results of vaccination. It is important to take into account the presence of maternal anti-PRRSV antibodies in serum specimens. The level of these antibodies gradually decreases up to 9th week of animal life [10]. According to literature the number of blood serum specimens sampled from a single