VIRUS DISEASES OF PEPPER (*CAPSICUM ANNUUM* L.) IN HUNGARY

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Abstract

Pepper (Capsicum annuum L.) is an important vegetable crop in Hungary. The virus diseases cause enormous losses in terms of quantity and quality of products. In 1970-80 years in the field production Cucumber mosaic virus (CMV), Potato virus Y (PVY), Broad bean wilt virus (BBWV), Potato X virus (PVX), Alfalfa mosaic virus (AMV), Tobacco mosaic virus (TMV) and Tomato mosaic virus (ToMV) were isolated. In the last 30-40 years pepper production has been intensified and basically changed and a wide selection of new pepper varieties were released. In the last decade main virus disease of pepper production in the field Cucumber mosaic virus, in forcing production 3 pathotypes of Pepper mild mottle virus and WT (normal) and RB (resistance breaking) strains of Tomato spotted wilt virus.

Key words: *pepper, pepper pathogen viruses, pathotypes, Hungary*

INTRODUCTION

Hungary - with 180-200 thousand tons production annually - is one of the biggest pepper producing countries in the European Union. Until mid-seventies pepper production was conducted mainly on the field (over 10000 ha while forcing production was negligible) and *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Potato X virus* (PVX), *Alfalfa mosaic virus*, (AMV) and *Broad bean wilt virus* (BBWV) were causal agents of virus diseases producing enormous crop losses (Tóbiás et al 1978, 1982a, 1982b). Later on - mainly in the beginning of nineties - forcing production in greenhouse and plastic tunnel became more and more important. In the last 3 years the ratio between them is stable; 950-1000 ha field and 1600 ha forcing pepper production, however virus disease as a damaging factor remained. In the last 20 years the main problem caused by tobamoviruses, *Tomato spotted wilt virus* (TSWV) and in some years on the field *Cucumber mosaic virus*. The most effective control against viruses seemed/proved to be the use of resistant cultivars in crop production. The first resistance gene *L 1* against TMV was introduced from *Capsicum annuum* L. (Holmes 1934, 1937), which triggered the emergence of new resistance breaking TMV isolates. Other resistance genes, like L3 and L4 were incorporated into new varieties from *Capsicum chinense* and *C. chacoense* (Boukema 1980, Csilléry 1985). Assortment of pepper varieties completely changed and varieties containing different resistance genes became prevalent. Consequently a wide range of tobamoviruses (*Tobbaco mosaic virus, Tomato mosaic virus, Obuda pepper virus, Pepper mild mottle virus and Tobbaco mild green mottle virus*) were isolated in Hungary.

The other pathogen *Tomato spotted wilt virus* (TSWV) was described in Hungary in 1972, but the virus was not considered as an important threat. In 1995 very severe damage of TSWV infection was observed in tomato and pepper production in the Szentes vegetable growing region. The introduction and spread of western flower thrips (*Frankliniella occidentalis*), an efficient TSWV vector, at the same time certainly played an important role in TSWV emergence (Gáborjányi et al. 1995). The *Tsw* resistance gene from *Capsicum chinense* PI152225 and PI159236 sources was incorporated into wide range of pepper varieties (conical white, long pale green hot and sweet, tomato shape, spice pepper and blocky types).). In Hungary few years after the resistant cultivars were introduced resistance breaking isolates of TSWV were observed first in conical white pepper cultivar (Bese et al 2012, Csilléry et al. 2012, Salamon et al. 2010). 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 (Roggero et al 2002)

In this paper our aim was to describe the virus symptoms observed in pepper, to determine the viruses and to characterize the molecular differences between normal and resistance breaking isolates.

MATERIALS AND METHODS

Plant samples (leaves and fruits) showing typical virus symptoms were collected in the main pepper growing regions of Hungary (Szentes, Újkígyós, Szegvár, Túrkeve, Zákányszék).

Test plants (*Nicotiana tabacum* cv. Xanthi-nc, *N. benthamiana*, *Capsicum annuum* cultivars "Albaregia", "Celtic", „Censor"`, „Carma", „Century", „Dimentio", „Skytia", „Karakter", „Brendon", „Bronson", „Bravia") were sap inoculated prepared from the samples and investigated by ELISA using TSWV, TMV, CMV, and PVY antibodies (Art. Ns. 190115, 190125; 190415, 190425; 160612, 160622; 112911, 112921, respectively, Bioreba AG).

For long time storage, samples were kept in a deep freezer (at -70 °C).

RT-PCR. Total nucleic acid (TNA) was extracted from small pieces of fruit flesh or leaves by the method of White and Kaper (1989).

Tobamovirus specific primers:For 5' GATCGCGCGAGTCGTGATTCGTATTTAAATATG-3' and Rev 5'-TGGGCCGCCTACCGCGGCGG-3' amplified 700 nt product from the coat protein region. The following conditions were used for the PCR: initial denaturation at 95 ºC for 5 minutes, 30 cycles of 95 ºC for 30 s, 60 ºC for 30 s and 72ºC for 1 min and a final extension at 72 ºC for 10 min.

The PCR amplification of the 1404 bp fragment of TSWV NSs region was carried out with the primers NSs-Forward (50-GG CTGTAG CAG AGA GCA ATT GTG TCA TAA TTT T-30) and NSs-Reverse (50-GGA CAT AGC AAG ATT ATTTTG ATC CTG-30). The amplification consisted of 5 min at 94 ºC followed by 35 cycles of 1 min of denaturation at 94 ºC, 30 s of annealing at 51ºC, and 3 min of extension at 72 ºC, and a final extension cycle for 5 min at 72 ºC.

Two subgroups of CMV was tested with the following primer pairs:

1. subgroup: CMV-RS RNS3, 1374forw 5'-TTCGCGACTTAATAAGACGTTAGCAGC-3'
   CMV SG. I reverse 5'-GCGGATCCTGGTCTCCTTTTGGAGGCCC-3'
2. subgroup: Trk3-1274 forw 5'-CGTCGTCGCCCGCGTAGAGG-3'
   Trk 3' PstI rev 5'-GGCTGCAGTGGTCTCCTTATGGAGAACCTGTGG-3'

The PCR product was purified by High Pure PCR Purification Kit (Roche) and sequenced (Baygen, Szeged) or cloned into p-GEM T-Easy Vector (Promega, USA) and sequenced.

RESULTS AND DISCUSSION

Symptoms on pepper plants.

In the field most frequently two types of symptoms are observed, one is the necrotic rings, spots and oak leaf pattern (Fig. 1A), and the other is chlorosis and chlorotic mosaic symptoms on leaves, which show various degree of deformation with protruding primary veins (Fig 1/B.). The infected plants are stunted and the fruits are malformed, sometimes develop ringspotting (Fig. 1/C., 1/B.).



A B C D

Figure 1. Virus symptoms on pepper caused by *Cucumber mosaic virus* infection. A – necrotic rings and pattern, B - chlorosis and chlorotic mosaic with various degree of leaf deformation and protruding primary veins, C - chlorosis and chlorotic mosaic with stunting, D – ring spotting on fruit.

In plant samples collected from the field only CMV was detected. According to the RT-PCR results CMV isolates belonged almost equally into subgroups I. and II. It was a change to earlier experience in mid-seventies, where the majority of CMV isolates belonged into subgroup II.In forcing pepper production tobamovirus infection causes obvious and marked symptoms on fruits as chlorotic or necrotic spots and sometimes deformation (2. /A, /B, /C). Leaves are mainly symptomless or mild chlorosis or mosaic could be noticed.



A B C

Figure 2. Virus symptoms on pepper fruits caused by tobamovirus infection. A – necrosis, B – malformation and deformation on fruit, C - chlorotic or necrotic spots on fruits.

In the last 15-20 years tobamoviruses isolated from pepper fruits originated from forcing production belonged to *Pepper mild mottle virus* (PMMoV) while *Tobacco mosaic virus* (TMV) and *Tomato mosaic virus* (ToMV) only in few cases were found. PMMoV isolates were classified into P_o (infecting only pepper without resistance gene), $P_{1,2}$ (infecting pepper varieties containing L_1 resistance gene) and $P_{1,2,3}$ (infecting pepper varieties containing L_3 resistance gene). Majority of PMMoV isolates belonged to $P_{1,2}$ pathotype, but P_o and $P_{1,2,3}$ pahtotypes are also present (Kálmán 2003, Nemes et al 2016). In 2015 first time *Tobacco mild green mottle virus* was isolated in Hungary as a new tobamovirus infecting pepper (Nemes et al 2016).

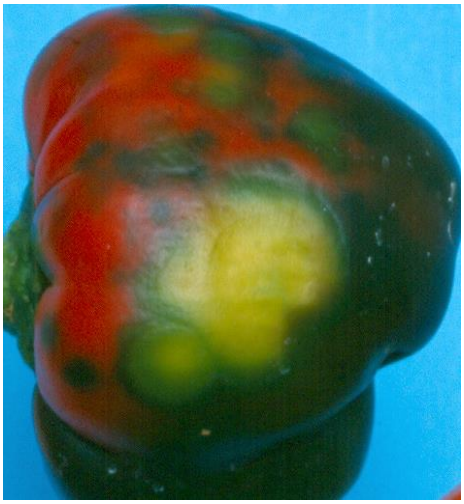
Tomato spotted wilt virus infection is the major yield-limiting factor to pepper production in Hungary. Symptoms on leaves and fruits are chlorotic spots, ringspots and patterns often necrotizing (Fig. 3/A,3./B,3./C, 3/D). Early infection/Infection at an early plant stage often leads to severe stunting of the plants. On pepper varieties containing *Tsw* resistance gene in case of TSWV infestation necrotic spots and rings were observed (Fig. 4/A, 4/B). In 2010 and 2011 sporadically, but in 2012 more frequently systemic virus symptoms were observed on resistant pepper cultivars in Szentes region (Fig. 4/C). The presence of new resistance breaking strain of TSWV was proved by virological (test-plant, serological and RT PCR) methods.



A



B



C



D

Figure 3. Chlorotic spots, ringspots and patterns on leaves (A and B) and discoloration on fruits (C and D) caused by TSWV infection.



A B C D

Figure 4. Virus symptoms on pepper varieties containing *Tsw* resistance gene. Hypersensitive (HR) symptoms caused by TSWV-WT (normal) strain infestation (A and B) and systemic symptoms caused by TSWV-RB (resistance breaking) strain infection (C and D).

The rapid adaptation of TSWV to pepper resistance and breakdown of the *Tsw* resistance gene facilitated the determination of the avirulant factor (Avr).In case of TSWV the gene silencing suppressor NSs protein was identified as the Avr determinant (Margaria et al. 2007). Comparing the NSs protein, the Hungarian RB and WT strains of TSWV differed only in two amino acid (aa) positions, at aa 104 and 461. Threonine in TSWV-WT NSs changed to alanine in case of TSWV-RB strain. The aa alterations between the WT and RB strains of TSWV are at different positions according to the various geographical region (Table 1).

Table 1. Amino acid differences in NSs protein of TSWV-WT and TSWV-RB strains in various geographical regions.

Amino acid positions	Brazilian TSWV-WT	Brazilian TSWV-RB
174	Isoleucine (I)	Methionine (M)
255	Lysine (K)	Asparagine (N)
	Spanish TSWV-WT	Spanish TSWV-RB
84	Aspartic acid (D)	Asparagine (N)
407	Threonine (T)	Isoleucine (I)
	North Italian TSWV-WT	North Italian TSWV-RB
424	Phenylalanine (F)	-
	South Italian TSWV-WT	South Italian TSWV-RB
427	Glycine (G)	-
	Hungarian TSWV-WT	Hungarian TSWV-RB
104	Threonine (T)	Alanine (A)
461	Threonine (T)	Alanine (A)

Based on the phylogenetic analysis of the NSs protein of several TSWV pepper strains, it can be concluded that cluster differentiation relies mostly on the geographic origin (Fig. 5). The phylogenetic analysis supported the hypothesis that TSWV RB strains has been developed locally, and the worldwide trade and transport of plant propagating material seem not to contribute to the expansion of RB strains.

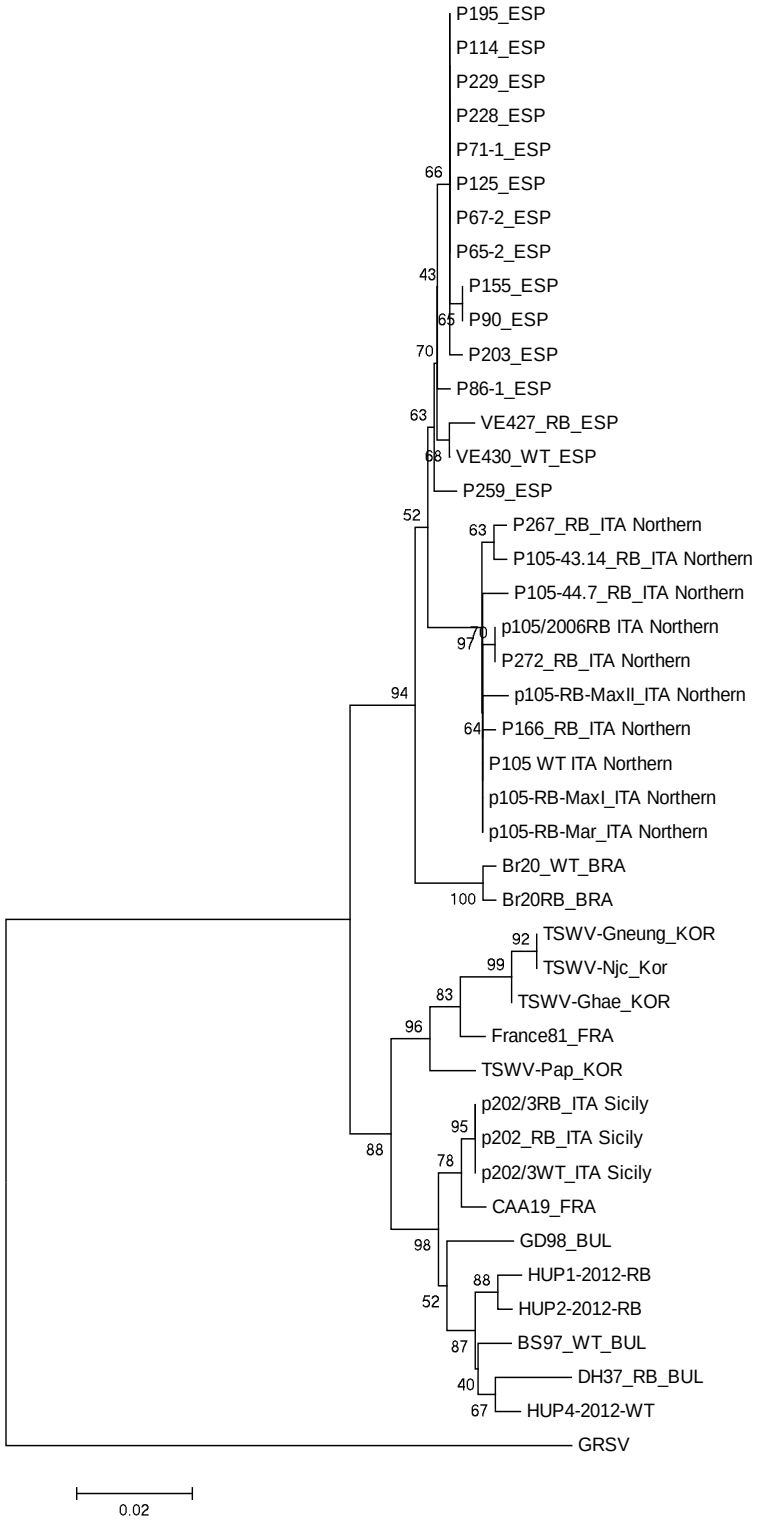


Figure 5. Phylogenetic tree on the basis of TSWV NSs gene.

In the last 30-40 years pepper production has been intensified and basically changed as forcing production became dominant form. Enormous transformation was observed in the wide selection of pepper varieties containing different resistance genes. Consequently, introducing resistance genes induced the evolution/emergence of new virulent strains or pathotypes, as it can be seen in case *of Pepper mild mottle virus* and *Tomato spotted wilt virus*. Currently we can conclude, that in pepper production in field *Cucumber mosaic virus*, and in forcing production 3 pathotypes of *Pepper mild mottle virus* and WT (normal) and RB (resistance breaking) strains of *Tomato spotted wilt virus* are the most important viruses in Hungary.

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