Virological survey of walk-in plastic tunnel grown pepper seedlings and forced pepper varieties in Western Hungary in 2023-2024

Nyugat-Magyarországon fóliasátorban nevelt paprikapalánták és hajtatott paprikafajták virológiai vizsgálata 2023-2024-ben

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Abstract: Peppers can be infected by approximately 50 plant viruses of which the most common species causing significant economic losses in Hungary are *Potato virus Y* (PVY) and *Cucumber mosaic virus* (CMV) in outdoor cultivation and *Tomato spotted wilt virus* (TSWV) and tobamoviruses in indoor cultivation. In addition to vectors' activity, the infected propagating material also plays a very important role in the spread of pepper infecting viruses. Thus, besides the use of virus-free propagating material and effective protection against vectors, the cultivation of virus-resistant varieties and application of hygiene regulations are essential in the control of pepper viruses. Even with the most precise cultivation techniques, virus infections can occur during indoor cultivation, not only in plantations which are already producing but also in seedling nurseries, therefore monitoring these is crucial to achieve virus-free fruiting pepper stand. In 2023–2024, virological survey of pepper seedlings and fruiting plants collected from two horticultural farms in Zala county and grown in walk-in plastic tunnel was performed using RT-PCR technique to test leaf and fruit samples. During the analysis only the presence of TSWV was identified in 13 samples, while infection of CMV, PVY and tobamoviruses was not detected. It is interesting to highlight that TSWV was detected, despite the black necrosis that developed, in the fruits of the TSWV resistance gene carrying pepper cultivars Antal F_1 and Zalkod F_1. These results indicate that the use of TSWV resistant pepper varieties or hybrids does not provide complete protection against TSWV damage as symptoms may also appear on resistant individuals not only on susceptible ones due to the vectors' activity. Therefore, monitoring the vectors that can transmit TSWV and timely control them are essential for indoor pepper cultivation. Without these even TSWV resistant plants will not be able to produce marketable crop.

Keywords: *Capsicum annuum, pepper, virus, RT-PCR, resistance*

Összefoglalás: Megközelítőleg 50 növényi vírus képes fertőzni a paprikát, melyek közül hazánkban szabadföldön a burgonya Y vírus (*Potato virus Y*, PVY) és az uborka mozaik vírus (*Cucumber mosaic virus*, CMV), hajtatásban pedig a paradicsom foltos hervadás vírus (*Tomato spotted wilt virus*, TSWV) és a tobamovírusok okozzák a legnagyobb problémát. A paprikát fertőző vírusok terjesztésében a vektorokon felül kiemelkedő szerepe van a fertőzött szaporítóanyagnak is. Így a paprikavírusok elleni védekezésben is elengedhetetlen a vírusmentes szaporítóanyag használata és a vektorok elleni hatékony védelem, ezeken felül pedig a vírusrezisztens fajták termesztése és a higiéniás rendszabályok betartása is fontos. Még a legprecízebb termesztéstechnológia ellenére is előfordulhatnak a zárt téri termesztés során is vírusfertőzések nem csupán a termő állományokban, de már a palántanevelőkben is, így ezek monitorozása kiemelten fontos a vírusmentes termő állományok kialakításához. 2023–2024-ben két Zala vármegyei kertészetből, fóliasátorból, paprika palántákról, illetve termő növényekről gyűjtött levél- és termésminták virológiai vizsgálatát végeztük el RT-PCR technikával. A vizsgálat során csak a TSWV jelenlétét tudtuk igazolni, 13 minta esetében kaptunk pozitív eredményt, míg a CMV, a PVY és tobamovírusok előfordulását egyetlen mintában sem tudtuk kimutatni. Érdekes megfigyelés volt, hogy a TSWV ellen rezisztenciagént hordozó Antal F_1 és Zalkod F_1 paprikafajták terméseiben a kialakult fekete nekrózisok ellenére is kimutattuk a TSWV-t. Eredményeink alapján megállapítható, hogy a TSWV rezisztens paprikafajták, illetve hibridek alkalmazása nem jelent teljes védelmet a TSWV kártételével szemben, ugyanis a vektorok kártételének következtében a TSWV rezisztens egyedeken is megjelenhetnek tünetek éppúgy, mint a rezisztencia gént nem tartalmazó, fogékony növényeken. Ezért a hajtatásban történő paprika termesztés alapvető feltétele a TSWV-t terjesztő vektorok monitorozása és a védekezés időben történő megkezdése. Ennek hiányában a TSWV rezisztens növények sem képesek értékesíthető termést hozni.

***Kulcsszavak**: Capsicum annuum, paprika, vírus, RT-PCR, rezisztencia*

1 Introduction

Sweet pepper (*Capsicum annuum* L.) is a herbaceous, thermophilic plant belonging to the *Solanaceae* family. It originates from the tropical areas of South America, where the indigenous Indians used it for various medicinal purposes (Barboza et al., 2005). It was probably introduced to Hungary by Turkish merchants in the 16th century. Hungarian pepper become world famous in 1937 with Albert Szent-Györgyi's research on vitamin C (Balázs, 1994).

Nowadays, approximately 24-25 million tons of pepper are harvested annually from an area of 2 million hectares worldwide. Hungary accounts for nearly 1% of the world's pepper production. In 2023, 90 thousand tons were harvested from 1281 hectares in Hungary. Outdoor pepper production area shows a decreasing trend, and currently pepper is typically grown in plant growing structures (Takácsné, 2017; KSH, 2024; FAO, 2024).

Peppers can be infected by approximately 50 plant viruses (Edwardson and Christie, 1997) of which the most common species causing significant economic losses in Hungary are *Potato virus Y* (PVY) and *Cucumber mosaic virus* (CMV) in outdoor cultivation and *Tomato spotted wilt virus* (TSWV) and tobamoviruses in indoor cultivation. PVY can be transmitted by more than 50, while CMV by more than 80 aphid species. The most important insect vector of TSWV is the western flower thrips (*Frankliniella occidentalis*), while in the case of tobamoviruses vector transmission is currently unknown. In addition to vectors' activity, the infected propagating material and disregarding of cultivation hygiene standards also play very important role in the spread of pepper viruses.

Thus, besides the use of virus-free propagating material and effective protection against vectors, the cultivation of virus-resistant varieties and application of hygiene standards are essential in the control of pepper viruses.

Even with the most precise cultivation techniques, virus infections can occur during indoor cultivation, not only in plantations which are already producing but also in seedling nurseries, therefore monitoring these is crucial to achieve virus-free fruiting pepper stand.

Between 2023 and 2024 a virological testing of 31 leaf and fruit samples from pepper seedlings and fruiting plants collected from two horticultural farms in Zala county and grown in walk-in plastic tunnel was carried out to determine the virus infection of the pepper samples.

2 Materials and Methods

In 2023–2024, pepper leaf and fruit samples from 27 pepper individuals - 17 seedlings and 10 fruiting plants - belonging to 11 different pepper varieties and hybrids (Table 1) grown in walk-in plastic tunnels in two horticultural farms (Kiskanizsa and Várfölde) in Western Hungary (Zala County) were collected. Overall 31 samples, 4 fruit and 27 leaf samples were tested. During sample collection, symptoms observed on the pepper plant parts and the presence of trips in the walk-in plastic tunnel were noted. In the case of seedlings only leaves (symptomatic or not) were sampled, while in the case of fruiting pepper, in addition to the symptomatic leaves, in some cases fruits were also sampled. 1-3 plant parts per plant were collected and stored at -70 ºC until use.

Total nucleic acid (TRNA) extraction was performed using a simplified CTAB method (Xu et al. 2004, Sáray et al., 2022). 0.2 g of plant tissue was used per sample for total nucleic acid extraction. The total nucleic acid extracts were visualized on a 1% TBE agarose gel containing a fluorescent dye and stored at -70 °C until further use.

RT-PCR was carried out for the molecular detection of viruses. Viral cDNAs were synthesised by reverse transcription (RT) of the TRNAs extracted from pepper leaves or fruits using Random primer (Thermo Fisher Scientific) and RevertAid Reverse Transcriptase according to the manufacturer's instructions (Thermo Fisher Scientific). The success of the cDNAs synthesis were checked for each sample by PCR using primers designed for pepper actin gene (Capsicum_actin_for and Capsicum_actin_rev; Li et al., 2016).

Species-specific primer pairs were used for CMV (CMV_rev and CMV_for; Nemes and Salánki, 2020), PVY (PVY_rev and PVY_for; Nemes and Salánki, 2020) and TSWV (TSWV_rev and TSWV_for; Nemes and Salánki, 2020) and a universal genus-specific primer pair (UniTobamo5-for and UniTobamo3-rev; Kálmán et al., 2001) was used in the PCR for tobamoviruses. All PCRs were performed in a final volume of 25 µl. In addition to the nucleic acid, the reaction mixture contained 12.5 µl DreamTaq Green PCR Master Mix (Thermo Fisher Scientific), 10 pmol each of antisense and sense primers, and 10 µl of nuclease-free water. Sterile distilled water was used as a negative control, and the following parameters were set for each PCR: 95 °C for 5 min, for 35 cycles 95 °C for 30 sec, 60 °C for 30 min, 72 °C for 1 min, and finally 72 °C for 10 min. The PCR amplicons were visualized on a 1% TBE agarose gel staining with fluorescent dye.

Table 1 Tested pepper varieties and hybrids, and their resistances

Varieties and hybrids	Resistances
Armand F_1	*HR Tm 0*
Amy	-
Antal F_1	*HR Tm 3, IR TSWV*
Cassovia F_1	*HR Tm 2, IR TSWV*
Blumen	-
Hétvezér F_1	*HR Tm 0*
Eszter F_1	-
Promontor F_1	*HR Tm 0*
Rédei fehér	-
Senator	-
Zalkod F_1	*HR Tm 2, IR TSWV*

Legend: HR: High resistance; IR: Intermediate resistance; Tm 0: TMV resistance; Tm 2: TMV and ObPV resistance; Tm 3: TMV, ObPV and PMMoV resistance; TSWV: TSWV resistance.

3 Results

Among the 27 tested plant individuals, 26 showed some symptoms and one was asymptomatic. The symptoms observed on pepper samples were variable (Figure 1.). All the fruiting pepper plants were damaged by thrips, while the presence of thrips was not detected in the seedling nursery.

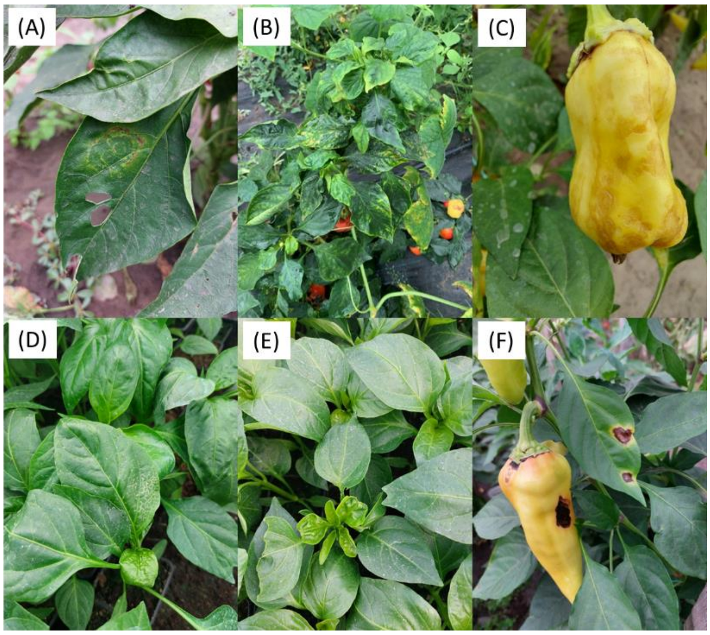

Figure 1 *Symptoms on pepper plants: (A) Chlorotic necrotic rings on Armand F1 hybrid leaf (Photo: Fehér), (B) Chlorosis on Eszter F1 hybrid leaf (Photo: Fehér), (C) Ringspots on Hétvezér F1 hybrid fruit (Photo: Szendrei), (D) Vein lightening on Amy seedling leaf (Photo: Szendrei), (E) Two-pointed leaf on Promontor(6) seedling (Photo: Szendrei), (F) Dark necrotic areas on Zalkod F1 hybrid fruit and leaf (Photo: Szendrei)*

Actin test gave positive results for all 31 pepper samples. In the cases of all samples the ~230 bp long DNA fragments were amplified during RT-PCR. In the case of control water samples negative results were obtained. In the RT-PCR assays for virus testing using the virus species-specific and genus-specific primer pairs positive results were obtained only when using the TSWV-specific primer pair. In the case of 13 samples the ~350 bp long PCR products were detected. While for the control water samples, and RT-PCR tests for CMV, PVY, and tobamoviruses gave negative results and no PCR products was detected. All the TSWV positive plants showed symptoms, while none of the monitored viruses was detected in the asymptomatic seedling. 43.33% of the symptomatic samples, 9 leaf and 4 fruit samples were proved to be infected with TSWV. The presence of TSWV was detected in at least one tested sample of 8 tested varieties out of the 11. In the case of Cassovia F_1, Amy and Senator varieties none of the tested samples were found to be infected with any of the tested viruses. There were no varieties for which all samples tested were infected with TSWV, although one sample [Promontor (2)] was asymptomatic. The highest infection rate (75%) was observed in the case of Armand F_1. Three out of four leaf samples collected from four different Armand F_1 hybrid seedlings were found to be infected with TSWV (Table 2).

Table 2 Results of RT-PCR tests

No.	Pepper sample	Plant part	Symptom	Presence of vector	CMV	PVY	TOBAMO	TSWV	*C. annuum* actin
1.	**Armand(1)**	leaf	+	+	-	-	-	+	+
2.	*Armand(2)*	leaf	+	-	-	-	-	-	+
3.	*Armand(3)*	leaf	+	-	-	-	-	+	+
4.	*Armand(4)*	leaf	+	-	-	-	-	+	+
5.	*Amy*	leaf	+	-	-	-	-	-	+
4.	**Antal(1)***	leaf	+	+	-	-	-	-	+
5.	**Antal(2L)***	leaf	+	+	-	-	-	-	+
6.	**Antal(2F)***	fruit	+	+	-	-	-	+	+
7.	**Antal(3)***	leaf	+	+	-	-	-	-	+
9.	*Blumen(1)*	leaf	+	-	-	-	-	-	+
10.	*Blumen(2)*	leaf	+	-	-	-	-	+	+
11.	**Cassovia***	leaf	+	+	-	-	-	-	+
12.	**Hétvezér(1)**	leaf	+	+	-	-	-	-	+
13.	**Hétvezér(2L)**	leaf	+	+	-	-	-	+	+
14.	**Hétvezér(2F)**	fruit	+	+	-	-	-	+	+
15.	**Eszter(1)**	leaf	+	+	-	-	-	-	+
16.	**Eszter(2L)**	leaf	+	+	-	-	-	-	+
17.	**Eszter(2F)**	fruit	+	+	-	-	-	+	+
18.	*Promontor(1)*	leaf	+	-	-	-	-	+	+
19.	*Promontor(2)*	leaf	-	-	-	-	-	-	+
20.	*Promontor(3)*	leaf	+	-	-	-	-	+	+
21.	*Promontor(4)*	leaf	+	-	-	-	-	+	+
22.	*Promontor(5)*	leaf	+	-	-	-	-	-	+
23.	*Promontor(6)*	leaf	+	-	-	-	-	-	+
24.	*Rédei(1)*	leaf	+	-	-	-	-	-	+
25.	*Rédei(2)*	leaf	+	-	-	-	-	-	+
26.	*Rédei(3)*	leaf	+	-	-	-	-	-	+
27.	*Rédei(4)*	leaf	+	-	-	-	-	+	+
29.	*Senator*	leaf	+	-	-	-	-	-	+
30.	**Zalkod(L)***	leaf	+	+	-	-	-	-	+
31.	**Zalkod(F)***	fruit	+	+	-	-	-	+	+

*Legend: Samples marked with * collected from TSWV resistant plants; samples from fruiting plants are marked in bold, samples from seedlings are marked in italics; +: positive result, - negative result.*

4 Discussion

Virus infection of more than 40% of the symptomatic samples were determined during virological testing of pepper samples, however, of the four monitored viruses that most commonly infect pepper in Hungary (CMV, PVY, TSWV, tobamoviruses), only the presence of TSWV was detected.

In a virological survey, Sáray et al. (2021) found that more than half of the tested greenhouse pepper samples (58%) were infected with viruses and most of the positive samples (67%) was infected with TSWV, which is in line with our results. However, in the contrary to our results they also confirmed the presence of tobamoviruses (19%), CMV (12%), and PVY (2%) in the tested samples.

The pepper samples were collected from varieties and hybrids with or without virus resistance against plant viruses. The leaves and fruits of the TSWV-resistant Antal F_1, Cassovia F_1 and Zalkod F_1 hybrids showed symptoms. The necrotic spots observed on Antal F_1 and Zalkod F_1 plants clearly indicated the presence of the resistance gene against TSWV and that the normal strain of TSWV was present in the walk-in plastic tunnel (Tóbiás et al., 2014). However, in the fruits of Antal F_1 and Zalkod F_1 the presence of TSWV was determined despite the presence of TSWV resistance gene and black necroses that developed. It is interesting to note that in the case of these two varieties the presence of TSWV was only detected in the fruit but not in the leaf sample showing necrotic symptoms and originating from the same pepper individual. TSWV infection was also identified only in the fruit but not in the leaf sample of Eszter F_1 hybrid collected from the same symptomatic plant individual.

Although symptoms were observed on all plants except for one seedling we were unable to detect the monitored viruses in more than half of the samples, which confirms the well-known finding that macroscopic symptoms observed on plants can easily be confused with changes caused by certain abiotic factors, such as lack of micro- or macroelements or heat stress. However, it cannot be excluded that other viruses that were not examined might infect these symptomatic pepper plants.

Nearly half of the samples collected from a plant growing structure where thrips were noticed, while the other half collected from a walk-in plastic tunnel where the presence of thrips were not observed. Thrips, especially the western flower thrips, is the main vector of TSWV and is responsible for the main virus transmission (Whitfield et al., 2005). However, the virus can also be transmitted mechanically and by seed (Wang et al., 2022) which may have contributed to that TSWV was detected in leaf samples from locations where thrips were not detected.

5 Conlusion

Based on our results it can be concluded that the use of TSWV resistant pepper varieties or hybrids does not provide complete protection against TSWV damage as symptoms may also appear on resistant individuals due to vectors' activity. Therefore monitoring vectors transmitting TSWV and timely control them are essential for indoor pepper cultivation. Without these even TSWV resistant plants will not be able to produce marketable crop. Mechanical and seed transmission may also have a significant role in spread of TSWV. Thus continuous disinfection of the tools used during cultivation and growing seedlings from virus-free seeds are also essential conditions for successful cultivation.

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