Role of *Oxalis corniculata* L. as plant virus reservoir with special regard to Tomato Spotted Wilt Virus (TSWV-RB) strain occurrence in rock-wool cultivation in Hungary

Horn-Sauerklee (Oxalis corniculata L.) als Reservoir des Resistenz-überwindenden Stammes der Tomatenbronzefleckenkrankeit (TSWV-RB) in Steinwolle-basierendem Gemüsebau in Ungarn

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Abstract

The creeping wood sorrel (*Oxalis corniculata* L.) in Central Europe occurs mainly in anthropogenic areas, where it grows in parks and landfill sites or in protected cultivation sites like greenhouses and nurseries.

In Hungary *O. corniculata* has been spreading since the 19th century. In glass- and greenhouses it is the only weed species that was able to settle and flourish on hydroponic rock-wool and coconut fibre growing media.

Among virus pathogens of forced tomato (LYPES) and paprika crops (CPSAN) in Hungary the Tomato Spotted Wilt Virus (TSWV) has become an important one, severely affecting the plants and on occasions causing 100% yield losses. The spread of the virus was largely assisted by its effective vector the Western Flower Thrips (*Frankliniella occidentalis* Pergande). Initially the control of the disease was based on the control of the thrips, later, virus-resistant varieties were introduced. This however proved insufficient to fight the disease as it was discovered, quite recently, that in certain white pepper (CPSAN) varieties the virus was able to break up the resistance. According to data of the year 2012, those varieties that were considered resistant, showed up to 50% virus infection.

Considering the wide spread of *O. corniculata* and increasing occurrence of the resistance-breaking TSWV isolates, surveys in greenhouses were conducted to examine whether the weed could serve as reservoir for TSWV. Samples were collected of the virus infected crop plants and *O. corniculata* growing in the same coconut fibre cubes. The samples were examined using test-plants, serological and RT-PCR methods. In result it was found that the symptoms were indeed caused by resistance-breaking TSWV isolates. However, no virus was found in the suspected *O. corniculata* samples. Therefore it was concluded that in this particular case the *O. corniculata* cannot be considered reservoir for the virus, in the hydroponic culture.

Keywords: *Frankliniella occidentalis*, Hungary, invasive weed, pepper, TAS-ELISA, RT-PCR

Zusammenfassung

Der Horn-Sauerklee (*Oxalis corniculata* L.) kommt in Zentraleuropa vor allem in urbanen Habitaten vor, wie zum Beispiel in und um Gewächshäuser, in Parks oder auf Schuttflächen.

In Ungarn breitet sich *O. corniculata* seit dem 19. Jahrhundert aus. In Gewächshäusern ist der *O. corniculata* das einzige Unkraut, das sich auf Steinwolle- und Kokos-Substraten etabliert hat, und dort auch blüht. Bei Tomaten (LYPES) und Paprika (CPSAN) ist die Tomatenbronzefleckenkrankeit (Tomato Spotted Wilt Virus, TSWV) in Ungarn am problematischsten und kann zu 100 % Ertragsausfall führen. Die Virus-Übertragung erfolgt vor allem durch den Kalifornischen Blütenthrips (*Frankliniella occidentalis* Pergande). Deshalb wurde TSWV ursprünglich vor allem durch die Kontrolle der Thripse verhindert. Später wurden TSWV-resistente Sorten von Paprika (CPSAN) eingeführt. Kürzlich wurde jedoch ein Resistenz-überwindender Stamm des TSWV gefunden. Daten von 2012 aus Ungarn zeigen, dass die normalerweise resistenten Sorten nun bis zu 50 % befallen werden.

Deshalb wurden Erhebungen in ungarischen Gewächshäusern durchgeführt, um zu klären, in wieweit *O. corniculata* als TSMV-Reservoir dienen könnte. Paprikapflanzen mit Schadsymptomen wurden eingesammelt, als auch Horn-Sauerklee-Pflanzen wenn sie in dem gleichen Kokos-Substratblock wuchsen. Die Proben wurden serologisch und mit RT-PCR auf TSWV getestet. Ergebnisse zeigten, dass die Schadmuster auf den Paprikapflanzen tatsächlich vom TSWV herrührten. Auf den entsprechenden Horn-Sauerklee Proben wurde jedoch erstaunlicherweise kein TSWV gefunden. Darum kann der *O. corniculata* in diesem speziellen Fall als TSWV-Reservoir ausgeschlossen werden.

Stichwörter: *Frankliniella occidentalis*, invasives Unkraut, Paprika, RT-PCR, Ungarn, TAS-ELISA

Introduction

The creeping wood sorrel (*Oxalis corniculata* L.) was described by Linnaeus in 1753 from Sicily as Mediterranean species (EITEN, 1955). Currently it is spread in many parts of the world as cosmopolitan weed (PIGNATTI, 1982; EITEN, 1963).

In Europe the weed can be found all over the continent, from Greece (KOVEOS *et al.*, 1999) up to the Scandinavian Peninsula (KARLSON, 1989; LIND, 1986). In Central Europe the weed occurs mainly in anthropogenic areas, where it grows in parks and landfill sites or in protected cultivation sites like greenhouses and nurseries (HANTZ, 1979; KRAWIECOWA, 1951). In Hungary the *O. corniculata* has been spreading since 19th century. Its presence is consistent with the rest of the area of distribution, and can be observed mainly in the cities where it affects parks, public green areas as well as in cultivated land of greenhouses, nurseries or gardens. It's definitely an unwanted plant in lawns and a noxious companion of potted balcony plants (ELMORE and CUDNEY, 2002).

In glass- and greenhouses it is the only weed species that was able to settle and flourish on hydroponic rock-wool and coconut fibre growing media (HODI *et al.*, 2010).

Among virus pathogens of forced tomato (EPPO Code: LYPES) and paprika crops (EPPO Code: CPSAN) in Hungary the Tomato Spotted Wilt Virus (TSWV) has become an important one, severely affecting the plants and on occasions causing 100% yield loss (GÁBORJÁNYI *et al.*, 1995). The spread of the virus was largely assisted by its effective vector the Western Flower Thrips (*Frankliniella occidentalis* Pergande). Initially the control of the disease was based on the control of the thrips, later, virus-resistant varieties were introduced. This however proved insufficient to fight the disease as it was discovered, quite recently, that in certain white pepper (EPPO Code: CPSAN) varieties the virus was able to break up the resistance (SALAMON *et al.*, 2010; BESE *et al.*, 2012).

The objective of this study was to examine whether the *O. corniculata* could serve as reservoir of the TSWV and as such contribute to outbreaks of the disease in Hungarian white pepper EPPO Code: CPSAN varieties grown on hydroponic substrates.

Material and Methods

The study was conducted in forced white so called TV[1]-paprika crop, where similarly to the precedent years, a natural outbreak of tomato spotted wilt was observed. Symptoms typical for TSWV-RB disease like leaf malformation, and chlorotic and necrotic ring spots were observed on over 50% of the plants. The variety was known to possess resistance against the wild TSWV. The coconut substrate, in which the crop was grown, was significantly populated by *O. corniculata*.

One sample of paprika plants and 15 samples of *O. corniculata* were collected for examination. The samples were then assessed using symptomatological, TAS-ELISA, RT-PCR and bioassay methods.

The TAS-ELISA analysis was conducted following the diagnostic protocol of EPPO, using the antibodies supplied by ADGEN GmbH and SIGMA (EPPO, 2004).

The purification of the total RNA for the molecular assay was done with the RNeasy Plant Mini Kit (Qiagen) and following the protocol recommended by the producer. The amplification of the cDNA was achieved by using TSWV primers specific for 276 base fragments of the L RNA segment (L1 TSWV 5' -AAT TGC CTT GCA ACC AAT TC-3' and L2 TSWV 5' -ATC AGT CGA AAT GGT CGG CA-3') (MUMFORD *et al.*, 1994). One tube RT-PCR reaction was performed with SuperScript III according to the general protocol. Programs of the thermal cycler were carried out as follows: cDNA synthesis for 30 min at 48 °C, denaturation for 10 min at 94 °C and followed by 40 cycles denaturations for 1 min at 94 °C, 1 min of annealing at 55 °C, extension for 1 min at 72 °C and the final extension step at 72 °C for 10 min. The amplified cDNA was then assessed on agarose gel of 1,5%-os concentration.

[1] TV in Hungarian "tölteni való" means "good/suitable for filling/stuffing"

For the purpose on the bio-assay an average sample of 1 g was taken from the *O. corniculata* samples. The samples were homogenised using a phosphate buffer 0.01M diluted in 1:10 proportion (pH7.0, containing 1% (w/v) sodium sulphite). The product was used for inoculating the cotyledons of *Capsicum chinense* PI152225 plants, dusted with silicon carbide. The plants were then placed in climate controlled and vector-free chambers. The occurrence of local and systemic symptoms of TSWV –RB was assessed on 4th, 7th, 14th and 28th day after inoculation.

The inoculation of *O. corniculata* was obtained by applying four plants collected from virus free area of the growing facility. The virus freedom was confirmed with the methods described above. The plants were kept in a virus free experimental glasshouse. The leaves were inoculated artificially with TSWV-RB isolate maintained on *Capsicum annum* L. using the infected plant sap. The effectiveness of the inoculation was assessed by presence of symptoms as well as using the TAS-ELISA and RT-PCR methods.

Results

The symptoms of the virus infection were not found on any of the collected *O. corniculata* samples. The paprika plant sample in turn showed green mosaic, leaf malformation and necrotic rings. Similarly, the laboratory tests were negative in terms of virus presence in the *O. corniculata* samples, and confirmed virus infection of the paprika sample. The bio-assay only confirmed these findings. The plants of *C. chinense* did not express any virus symptoms, while the paprika sample showed only systemic symptoms (i.e., leaf malformation and chlorosis) on 14th day after inoculation (Tab. 1).

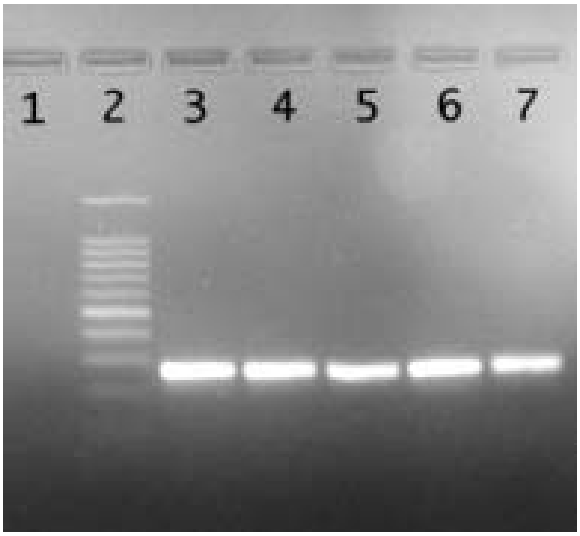
Tab. 1 Results of assays conducted on plant samples from *O. corniculata* and paprika EPPO Code: CPSAN collected from a site of TSWV outbreak in Hungary.

Tab. 1 *Untersuchungsergebnisse der Pflanzenproben von Horn-Sauerklee und Paprika, welche in TSWV-infizierten Standorten in Ungarn gesammelt wurden.*

Collected sample	Symptoms	Result of TAS-ELISA	Result of RT-PCR	Result of biotest
1. *O.corniculata*	-/-[1]	negative	negative	-/-
2. *O.corniculata*	-/-	negative	negative	-/-
3. *O.corniculata*	-/-	negative	negative	-/-
4. *O.corniculata*	-/-	negative	negative	-/-
5. *O.corniculata*	-/-	negative	negative	-/-
6. *O.corniculata*	-/-	negative	negative	-/-
7. *O.corniculata*	-/-	negative	negative	-/-
8. *O.corniculata*	-/-	negative	negative	-/-
9. *O.corniculata*	-/-	negative	negative	-/-
10. *O.corniculata*	-/-	negative	negative	-/-
11. *O.corniculata*	-/-	negative	negative	-/-
12. *O.corniculata*	-/-	negative	negative	-/-
13. *O.corniculata*	-/-	negative	negative	-/-
14. *O.corniculata*	-/-	negative	negative	-/-
15. *O.corniculata*	-/-	negative	negative	-/-
16.*Capsicum annum*	-/M[2], Ldef[3], NSRi[4]	TSWV positive	TSWV positive	-/Ldef, Ch[5]

[1] Local symptoms/systemic symptoms
[2]M: mosaic
[3]Ldef: leaf deformation
[4]NSRi: necrotic systemic rings
[5]Ch: chlorosis

The Oxalis plants collected for the purpose of the artificial inoculation were clearly symptom-free before the inoculation. This has been confirmed by the TAS-ELISA, RT-PCR and bio-assays. Several necrotic lesions occurred on the inoculated leaves between 7th and 14th days following the inoculation. Their serological, molecular (Fig. 1) and bioassay analysis detected infection by TSWV-RB isolate. However, systemic symptoms were not observed.



Legend: 1 - negative sample, 2 - 100 bp DNA marker, 3 -. positive control, 4 to 7 - *O. corniculata* samples

Fig. 1 Results of RT-PCR after artificial inoculation of *Oxalis corniculata* with TSWV.

Abb.1 *Ergebnisse der RT-PCR Analyse von Horn-Sauerklee-Proben, die künstlich mit TSWV geimpft wurden.*

Discussion

According to the scientific literature (PARRELLA *et al.*, 2003) *O. corniculata* is considered a natural host plant for Tomato Spotted Wilt Virus. Our study conducted on paprika infected by TSWV-RB isolate did not confirm this statement. Though some local necrotic lesions occurred on *O. corniculata* plants artificially inoculated with the virus, the infection remained local, without further escalation into a systemic one. Therefore in this particular case the weed which grew on the coconut substrate could not be confirmed the source of the TSWV outbreak in the paprika crop. Nevertheless, it is important for the hygiene of the crop that *O. corniculata* should be controlled as a potential reservoir of virus diseases.

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