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An assessment of the transmission rate of the *Tomato black ring virus* through tomato seeds

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Abstract: The *tomato black ring virus* (TBRV) infects a wide range of economically important plants, and is distributed worldwide. TBRV is transmitted by soil-inhabiting nematodes. However, long-distance dispersion is possible via the seeds and pollen. In this study, we provided evidence that the virus can be efficiently transmitted through tomato seeds. Three tomato varieties (Betalux, Grace and Moneymaker) and four genetically diverse TBRV isolates originally collected from different hosts were used in the experiments. The seedlings were grown in an insect-proof glasshouse and the presence of TBRV was verified by an immunoassay (ELISA). The seed transmission was significantly dependent on the tomato cultivar and virus isolate ranging from 1.69 up to 14.57%. The bioassays using *Chenopodium quinoa* plants confirmed the presence of the infectious virus in the seeds.

Keywords: ELISA; seed transmission; *Solanum lycoperscium*; TBRV

The *tomato black ring virus* (TBRV) is a member of the genus *Nepovirus* (subfamily Comovirinae, family Secoviridae, Picornavirales) that infects a wide range of economically important crops as well as many weed and ornamental species. Since 1957, there have been reports of significant damage caused by TBRV infections to several other important hosts: the strawberry, potato, celery, and artichoke (Hollings 1965; Gallitelli *et al.* 2004). In Poland, the presence of TBRV was confirmed in many plant species such as *Solanum tuberosum* (Linnaeus), *Solanum lycopersicum* (Linnaeus), *Cucumis sativus* (Linnaeus), *Robinia pseudoacacia* (Linnaeus), *Cucurbita pepo* convar. giromontiina (Linnaeus), *Sambucus nigra* (Linnaeus), and *Lactuca sativa* (Linnaeus) (Borodynko *et al*. 2001; Pospieszny *et al*. 2003, 2004; Pospieszny & Borodynko 2005; Rymelska *et al*. 2013; Hasiów-Jaroszewska *et al*. 2018). A collection of 49 TBRV isolates originating from different host species was established during 1998–2018 in the Department of Virology and Bacteriology of the Institute of Plant Protection-NRI in Poland. The TBRV population is

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highly diverse and the phylogenetic analysis based on the coat protein gene (CP) sequences of the Polish and other isolates described to date revealed the presence of distinct groups with the nucleotide sequence variability of the CP gene ranging from 0 to 19.7 %. Under natural conditions, TBRV is poorly transmitted mechanically by the nematodes *Longidorus elongates* and *Longidorus attenuates*, but effectively through the seeds. The virus is transmitted through the seeds of about 34 plant species in the range from a few percent to 100% (Harrison *et al*. 1961; Lister & Murant 1967). To our knowledge, the first information about the transmission of TBRV through tomato seeds was published over 50 years ago (Lister & Murant 1967). Since then, no data have been available describing this phenomenon. In recent years, many factors have changed which could have significantly affected the TBRV seed transmission: (*i*) the implementation of new agro-techniques in greenhouse tomato production that eliminated the presence of nematodes, (*ii*) the exchange of seed and plant materials around the world (*iii*) the introduction of new tomato varieties to the market, and (*iv*) the occurrence of new genetically diverse TBRV isolates has been described. The presence of the TBRV in a greenhouse with tomatoes planted on a mineral wool (Pospieszny 2000; Šneideris *et al*. 2012) suggested the introduction of the virus to the greenhouse via the seeds; however, the knowledge about this way of virus transmission is very limited. Seed transmission plays a significant role in the epidemiology of crop pathogens with the effect that even extremely low frequencies of seed transmission can result in severe epidemics (Simmons & Munkvold 2014). The TBRV is listed as a harmful organism in 64 countries (USDA-PCIT, 2016). Many of these countries are important trading partners including: Brazil, Canada, China, France, Germany, India, Japan, Mexico, Taiwan, and the UK. Considering all of the above, we aimed to analyse the transmission of TBRV through the seeds of different tomato varieties.

MATERIAL AND METHODS

The experiments were conducted from 2015–2018 using three tomato varieties: Betalux, Grace and Moneymaker and four genetically different TBRV isolates collected in Poland from the following hosts: TBRV-P1 (*S. lycopersicum*, 2000), TBRV-Pi (*S. lycoperscium*, 2011), TBRV-CK (*C. pepo* convar. giromontiina, 2004), and TBRV-S1 (*L. sativa*, 2013). The tomato plants were mechanically inoculated (at the two-leaf stage) with the TBRV isolates. The fragments of the plants were ground with a 0.05 M phosphate buffer (8.7 g/l of KH_2PO_4; pH 7.2) at a ratio of 1 : 5 (w/v). Mechanical inoculation was undertaken with gentle rubbing on lightly Carborundum-dusted (300 mesh grit powder) leaves of *S. lycopersicum* (two to three leaves stage) seedlings. The mock plants were inoculated with the buffer. The plants were grown to fruit maturity in an insect free greenhouse at 23–26°C and exposed to a 14 h light period. The seeds were manually separated from the tomato pulp and cleaned. The fruits were cut in half and all of the seeds were removed, transferred to a glass container and left to ferment for 24 h at 25–27°C. Next, the seeds were put in a sieve, washed with water and dried at room temperature. The seeds were stored in paper bags at 5°C for 4–5 weeks. The seeds were disinfected by incubating them in 10% (w/v) trisodium phosphate for 2 h, then rinsed with water for 30 min and dried. This treatment was carried out to minimise the possibility that any viral infection that occurred was not simply the result of the presence of the virus on the seed coat, but rather the result of an embryonic infection (Ling 2008). In order to determine the seed transmission rates of the tomato seedlings, the seeds from the infected tomatoes were sown in sterile, 24-well trays containing a sterilised substrate (2 : 1; peat : sand) with a single seed per well. The seedlings were grown in a greenhouse at temperatures ranging from 22 to 25°C with a 14 h photoperiod. Five to six weeks after sowing, each plant was individually tested by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and bioassayed in the *Chenopodium quinoa* plant. The leaf tissue collected from each seedling was homogenised in a plastic bag (BioReba, Switzerlasnd) with an extraction buffer (1 : 10 w/v) (Loewe, Germany) using a Homex 6 homogeniser (BioReba AG, Switzerland). The samples were tested in duplicate wells per sample. A DAS-ELISA test was carried out following the manufacturer's instruction using a TBRV-specific antiserum supplied by DSMZ (DSMZ, Germany) and 100 µl of the plant extract. The healthy and TBRV-infected tomato leaf samples were included in each ELISA plate to serve as negative and positive controls, respectively. The absorbance values (A_{405}) were measured using an immune plate reader (BioTek Instruments, USA). The values more than

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twice those for the healthy control plants were recorded as the infected samples. To confirm that the ELISA-negative samples contained no viable virus particles, a bioassay on *C. quinoa* was conducted. The mechanical inoculation was undertaken by gentle rubbing on the lightly Carborundum-dusted (300 mesh grit powder) *C. quinoa* leaves. The plants were maintained in an insect free greenhouse at 23–25°C and exposed to a 14 h light period. The symptoms were evaluated three weeks after inoculation and the presence of the TBRV in the infected plants was verified by ELISA as described above.

The frequency data of the infected seeds were analysed using a factorial generalized linear model (GLM) with binomial distribution and a logit link function (based on the lowest *BIC* -Bayesian information Criterion value among alternative models). The model incorporated the virus isolates and tomato varieties as orthogonal factors. The statistical significance of each main factor and their interaction was assessed using a likelihood-ratio test that asymptotically follows the χ^2 probability distribution.

RESULTS AND DISCUSSION

The four TBRV isolates used in this study were diverse in terms of their biological and genetic properties; they shared 97.2–99.6% of the CP nucleotide identity and they cause different symptoms on mechanically inoculated tomato plants. Generally, only TBRV-P1 and TBR-Pi display necrosis symptoms on the tomato leaves whereas the other two isolates induce mild symptoms or no symptoms (unpublished data). These symptoms slightly differ among the varieties tested: the most severe symptoms such as necrosis and leaf malformation can be observed for the Betalux variety whereas the infection of the Grace variety can be asymptomatic. None of the DAS-ELISA positively-detected seedlings exhibited symptoms of a virus infection; however, the presence of TBRV was confirmed on the very sensitive *C. quinoa* plants used in the bioassays. The characteristic symptoms of chlorotic spots followed by a systemic infection were clearly observed on the *C. quinoa* plants. The lack of symptoms is very important in the quarantine inspection for TBRV. The healthy-appearing seedlings might serve as the first source of the virus on the plantation and be involved in the spreading of the virus, especially when grafting is used.

Table 1 shows the frequency of the seed-transmitted infections, which are summarised in Figure 1. The statistical analysis showed: firstly, highly significant differences exist among the four viral isolates in terms of their capacity for vertical transmission (χ^2 = 284.205, 3 *df*, $P < 0.001$). As illustrated in Figure 1, on average, TBRV-Sl is the most efficiently transmitted isolate (11.96 ± 0.60%; error represent ± 1 SD), while TBRV-CK is the less efficiently transmitted one (2.13 ± 0.26%). Secondly, the seed transmission efficiency depends on the particular tomato variety being studied (χ^2 = 8.309, 2 *df*, P = 0.016). As can be seen in Figure 1, on average, the seed transmission is more efficient in the Grace variety (6.71 ± 0.45%), followed by the Moneymaker one (5.64 ± 0.40%) and the less efficient transmission takes place in the Betalux plants (4.96 ± 0.41%). Thirdly, a significant virus isolate-tomato variety interaction exists (χ^2 = 15.997, 6 *df*, P = 0.014), indicating that the transmission efficiency of each TBRV isolate is not the same in all the tomato varieties, but actually strongly depends on the variety being tested (Figure 1). For example, focusing on the most transmissible isolate, TBRV-Sl, its transmission frequency ranges from 9.58 ± 0.95% in the Betalux variety to 14.7 ± 1.12% in the Moneymaker one (Figure 1). In the case of TBRV-CK, the less efficiently transmitted isolate, the situation is somehow reversed: it is more efficiently transmitted by the Betalux variety (2.40 ± 0.53%) and less so by the Moneymaker one (1.70 ± 0.37%) (Figure 1). In the case of TBRV-Pl and -Pi, the seed transmission is most efficient in the Grace plants (Figure 1).

Table 1. The transmission rates of four diverse TBRV isolates through the tomato seeds of three different varieties

Isolate	Variety	Plants (pcs) infected/tested	Seed transmission (avg %)
TBRV-CK	Moneymaker	21/1236	1.69
	Grace	23/973	2.36
	Betalux	20/833	2.4
TBRV-P1	Moneymaker	46/995	4.62
	Grace	53/820	6.46
	Betalux	25/833	2.76
TBRV-Pi	Moneymaker	98/1192	8.22
	Grace	106/1024	10.35
	Betalux	71/841	8.4
TBRV-S1	Moneymaker	145/995	14.57
	Grace	128/1050	12.19
	Betalux	92/960	9.50

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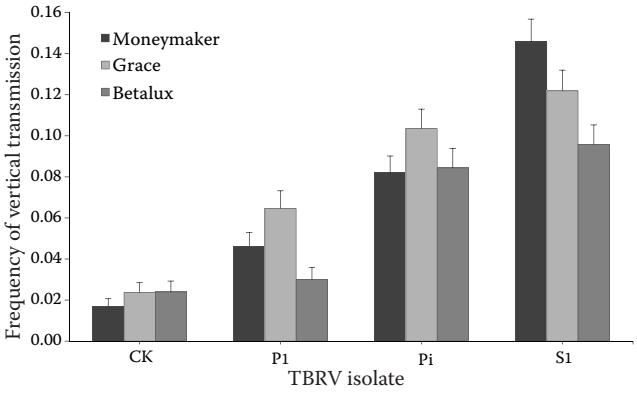

Figure 1. The frequency of the seed transmission observed for the four different TBRV isolates on each of the three tomato varieties used in this study. The error bars represent ± SD

The seed transmission serves as a major route for long-distance dissemination, provides an initial local source of inoculum for spreading by vectors, and through vertical transmission enables the virus survival at times when vector populations crash or go locally extinct (Sastry 2013). The seed transmission of the plant viruses is the outcome of a three-way interplay, the genetic components of the virus, the host and its progeny.

The tomato is a valuable crop worldwide. Most of the commercial hybrid tomato seeds are grown and harvested in one geographic site but shipped to other ones. For example, most tomato seeds sold in Europe are usually produced in China, Thailand, India, and Chile. The evidence from this study is that TBRV can be efficiently transmitted vertically has important implications for disease management and indicates that TBRV can be spread via the transportation of contaminated seed stocks between different countries.

References

Borodynko N., Palczewska M., Pospieszny H. (2001): Serologiczne zroznicowanie polskich szczepow wirusa czarnej pierscieniowej plamistosci pomidora [*Tomato Black Ring Nepovirus*/TBRV/]. Progress in Plant Protection/Postępy w Ochronie Roślin, 41: 696–698.

Gallitelli D., Rana G.L., Volvas C., Martelli G.P. (2004): Viruses of globe artichoke: An overview. Journal of Plant Pathology, 86: 267–281.

Harrison B.D., Mowat W.P., Taylor C.E. (1961): Transmission of a strain of Tomato black ring virus by *Longidorus elongatus* (Nematoda). Virology, 22: 480–485.

Hasiów-Jaroszewska B., Minicka J., Zarzyńska-Nowak A., Budzyńska D., Elena S.F. (2018): Defective RNA particles derived from Tomato black ring virus genome interfere with the replication of parental virus. Virus Research, 250: 87–94.

Hollings M. (1965): Some properties of celery yellow vein, a virus serologically related to *Tomato black ring virus*. Annals of Applied Biology, 55: 459–470.

Ling K.S. (2008): *Pepino mosaic virus* on tomato seeds: Virus location and mechanical transmission. Plant Disease, 92: 1701–1705.

Lister R.M., Murant A.F. (1967): Seed-transmission of nematode-borne viruses. Annals of Applied Biology, 59: 49–62.

Pospieszny H., Borodynko N. (2005): First report of *Tomato black ring virus* (TBRV) in the natural infection of zucchini (*Cucurbita pepo* L. convar giromontiina) in Poland. Journal of Plant Protection Research, 45: 321–325.

Pospieszny H., Borodynko N., Jończyk M. (2004): First report of *Tomato black ring virus* (TBRV) in the natural infection of *Sambucus nigra* in Poland. Journal of Plant Protection Research, 44: 373–376.

Pospieszny H., Jończyk M., Borodynko N. (2003): First record of Tomato black ring virus (TBRV) in the natural infection of *Cucumis sativus* in Poland. Journal of Plant Protection Research, 43: 11–18.

Pospieszny H. (2000): Dotychczasowy rozwój i dalsze perspektywy ograniczania strat powodowanych przez choroby roślin. Progress in Plant Protection/Postępy w Ochronie Roślin: 40, 84–92.

Rymelska N., Borodynko N., Pospieszny H., Hasiów-Jaroszewska B. (2013): Analysis of the biological and molecular variability of the Polish isolates of *Tomato black ring virus* (TBRV). Virus Genes, 47: 338–346.

Sastry K.S. (2013): Seed-borne plant virus diseases. Bangalore, Springer Science & Business Media, Springer.

Simmons H.E., Munkvold G.P. (2014): Seed Transmission in the Potyviridae. In: Gullino M.L., Munkvold G.P. (eds): Global perspectives on the health of seeds and plant propagation material. Plant pathology in the 21st century. Springer, Dordrecht, 6: 3–15.

Šneideris D., Zitikaitė I., Žižytė M., Grigaliūnaitė B., Sataniulis J. (2013): Identification of nepoviruses in tomato (*Lycopersicon esculentum* Mill.). Žemdirbystė=Agriculture, 99: 173–178.

USDA-PCIT (2016) Phytosanitary Export Database. Queried February 16, 2016. Available at https://pcit.aphis.usda.gov/pcit/

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