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Transmission efficiency of five tobravirus strains by *Paratrichodorus teres*

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Abstract

Nematodes from a population of virus-free *Paratrichodorus teres* were allowed to feed on roots of *Petunia hybrida* and *Nicotiana tabacum* cv. 'White Burley' plants infected with five strains of tobacco rattle or pea early-browning tobraviruses and were subsequently tested for virus transmission to roots of virus-free *P. hybrida* and *N. tabacum* cv. 'White Burley' plants. Virus transmission was not correlated with serotypes. Two strains of TRV with different serotypes were efficiently transmitted, whereas two other strains of TRV and one strain of PEBV with serotypes similar to transmitted ones were not transmitted at all. The two efficiently transmitted strains had originally been obtained from *P. hybrida* roots which had been exposed to viruliferous *P. teres* in laboratory bait tests, whereas the three non-transmitted strains had been obtained from infected plant material collected in the field.

Introduction

The tobravirusgroup comprises three economically important plant viruses: tobacco rattle (TRV), pea early-browning (PEBV) and pepper ringspot (PRV). Currently, only one PRV serotype is known, PEBV contains at least three serologically distinct strains [Robinson and Harrison, 1985] and within TRV there is substantial serological diversity among strains [Robinson, 1989]. Several TRV isolates share extensive coat protein sequences with PEBV thus making it impossible to distinguish such isolates from PEBV by serological methods [Robinson, 1989]. However, they are readily identified as TRV by their symptomatology on differential host plants, by cDNA hybridization and by PCR techniques [Robinson and Harrison, 1978; Robinson, 1992].

The natural vectors of tobraviruses are root-ectoparasitic *Trichodorus* and *Paratrichodorus* nematode species (trichodorid nematodes) which frequently occur in light sandy to medium light loamy soils. Initial reports of specific associations between trichodorid vector species and TRV isolates [Harrison, 1966, 1967; Van Hoof, 1968] were later confirmed by Brown *et al.* [1989c] and Ploeg *et al.* [1991, 1992a, 1992b]. Testing naturally viruliferous individual nematodes, they reported associations between trichodorid species and serological variants (serotypes) of TRV. The hypothesis by Ploeg *et al.* [1992b] that the virus particle protein is a major factor in the recognition process between virus and vector is supported by results from a subsequent study indicating that the RNA-2 part of TRV, which contains the coat protein gene, carries the determinant for vector transmissibility [Ploeg *et al.*, 1993].

Although specific associations between vector species and TRV serotypes were apparent, it was also observed that some vector species were associated with serologically distinct TRV isolates [Ploeg *et al.*, 1992b]. Thus, one naturally viruliferous population of *Paratrichodorus teres* transmitted TRV serologically similar to the ORE-strain of TRV whereas a number of other *P. teres* populations transmitted TRV serologically similar to the Dutch strain of PEBV [Ploeg *et al.*, 1992b]. Similar results were also obtained by Jensen *et al.* [1974] who reported the transmission of ORE-serotype TRV in the USA and by Van Hoof [1962] who reported *P. teres* to be a vector of PEBV in The Netherlands. The object of this study was to determine whether *P. teres* nematodes, taken from

one virus-free population, are able to transmit several tobravirus strains. The nematodes were allowed to feed on virus-infected plants, and subsequently transferred to virus-free plants. Roots of the latter plants were tested for the presence of virus to establish if transmission had occurred. Two out of five tobravirus strains tested were transmitted.

Results and discussion

P. teres nematodes used in the transmission experiments were extracted according to a modified decanting and sieving technique [Brown and Boag, 1988] from field soil collected at a site in Wieringermeer, The Netherlands. An average of 655 *P. teres* were recovered per 250 g soil and no other trichodorid species were detected.

TRV strain PRN [Cadman and Harrison, 1959] was used as a positive control for the PRN-antiserum in ELISA tests. No antisera to strains PTW1 or ORY were available. However, Ploeg *et al.* [1992b] reported that ORE-serotype isolates could be distinguished from all other serotypes by their inability to react with PRN-antiserum and by their positive reaction to TY-antiserum. Therefore, the reaction of the tobravirus strains to PRN, TY- and N5-antisera was tested in $F(ab')_2$-ELISA as described by Ploeg *et al.* [1992b].

Acquisition and transmission experiments were similar to those described by Brown *et al.* [1989b]; The five tobravirus strains (Table 1) were propagated in *Nicotiana clevelandii* by inoculating the plants with sap of frozen infected *N. clevelandii* (strains ORY, PTW1, PTJ1, N5) or frozen infected pea seed cv. 'Rondo' (strain E116). Ten days after inoculation of the *N. clevelandii* plants each of the five strains was manually inoculated onto leaves of ten 8-wk-old *Petunia hybrida* plants. After another 10 days the *P. hybrida* plants were removed from potting compost and their roots thoroughly washed. A small sample of the root system of each *P. hybrida* plant was removed, homogenized with a mortar and pestle and manually inoculated onto carborundum-dusted leaves of a *Chenopodium amaranticolor* plant and primary leaves of a *Phaseolus vulgaris* cv. 'Bataaf' plant. For each tobravirus strain, five *P. hybrida* plants, shown to contain virus in their roots, were used as virus source plants for *P. teres*. In addition, groups of *P. teres* were given access to five *P. hybrida* plants which had not been inoculated. As *P. hybrida* appeared not to be equally suitable as a virus source for all five strains the experiment was repeated using *N. tabacum* cv. 'White Burley' as virus source plants.

Nematode suspensions containing *c.* 75 *P. teres* in 5 ml tap water were added to each of the virus source plants and the virus-free control plants. After a 3 week virus acquisition period at 17 °C the nematodes were extracted and transferred to virus-free *Petunia* or *N. tabacum* cv. 'White Burley' bait plants. After a 3 week bait period the nematodes were extracted, counted and the roots of the bait plants were tested for presence of virus by sap-inoculation onto *C. amaranticolor* leaves. Roots of the virus-free control plants were also tested for the presence of virus by sap-inoculation onto leaves of *C. amaranticolor* indicator plants. The serotype of the virus recovered from the bait plant roots was determined in $F(ab')_2$-ELISA.

After mechanical inoculation of tobravirus strains E116, PTW1, PTJ1 and N5 onto the leaves of *Petunia* plants the viruses readily systemically invaded the plant roots. Virus was detected 10 days after leaf inoculation in the roots of all 10 *Petunia* plants inoculated with one of these virus strains. Five *Petunia* plants were used with each of these four virus strains as virus sources for the nematodes. However, virus was not detected from the roots of 10 *Petunia* plants which had been inoculated with strain ORY ten days previously. All five strains systemically invaded the roots of *N. tabacum* cv. 'White Burley' plants 10 days after mechanical leaf inoculation (E116: 7 of 10 plants, ORY: 8 of 10 plants, PTW1: 7 of 10 plants, PTJ1: 10 out of 10 plants and N5: 9 of 10 plants). Inoculation of the viruses, obtained from the roots of the *P. hybrida* or *N. tabacum* cv. 'White Burley' source plants, onto *P. vulgaris* cv. 'Bataaf' resulted in the development of numerous pinpoint lesions in the inoculated leaves when source plants had been inoculated with TRV strains ORY, PTW1, PTJ1 or N5. However, larger lesions followed by systemic symptoms were observed when the source plants had been inoculated with PEBV strain E116. This result confirmed the respective TRV or PEBV nature of the tobravirus strains used. For each of the five virus strains 5 *N. tabacum* cv. 'White Burley' plants were used as virus sources for the nematodes. None of the 5 virus-free *P. hybrida* or *N. tabacum* cv. 'White Burley' control plants became virus-infected during the 3 week acquisition period confirming that the nematode population was not naturally viruliferous.

Results from the transmission tests are shown in Table 1.

Table 1. Transmission efficiency of tobravirus strains and their serotypes to *Petunia hybrida* or *Nicotiana tabacum* cv. 'White Burley' by *Paratrichodorus teres*

Tobravirus source strain	Serotype	Number of bait plants (n = 5) infected	
		N. tabacum source and bait plants	*P. hybrida* source and bait plants
Virus-free control	n.a.	0	0
PEBV-E116[a]	PEBV-D	0	0
TRV-ORY[b]	ORE	0	n.d.
TRV-PTW1[c]	ORE	5	5
TRV-PTJ1[d]	PEBV-D	5	5
TRV-N5[e]	PEBV-D	0	0

[a] Isolated from field infected pea seed in The Netherlands [Bos and van der Want, 1962].
[b] Isolated from field infected potato tubers in Oregon, USA [Lister and Bracker, 1969].
[c] Isolated from *P. hybrida* bait plant root, transmitted by *P. teres* [Ploeg *et al.*, 1992b].
[d] Isolated from *P. hybrida* bait plant root, transmitted by *P. teres* [Ploeg *et al.*, 1991].
[e] Isolated from field infected narcissus leaf in Scotland [Harrison *et al.*, 1983].
n.a. = not applicable.
n.d. = not done.

The number of *P. teres* recovered after the bait period varied from 34 to 57 and from 25 to 49 per bait plant when *P. hybrida* and *N. tabacum* cv. 'White Burley' were each used as bait- and source plants respectively. Nematode recovery was not significantly different for the different virus strains (data not shown).

The 20 virus isolates which were recovered from the roots of the bait plants gave similar reactions to their source strains in $F(ab')_2$-ELISA. Isolates transmitted to bait plants by the nematodes which had been allowed to feed on source plants inoculated with TRV-PTJ1 reacted with N5-antiserum (A_{405} of tested isolates >10 times A_{405} of healthy sap) but not with PRN- or TY antisera (A_{405} of tested isolates slightly lower than A_{405} of healthy sap) and could not be serologically distinguished from the original PTJ1-strain, from the N5-strain or from PEBV-D strain E116. Similarly, isolates transmitted to bait plants by nematodes which had been allowed to feed on virus source plants inoculated with strain PTW1 reacted with TY-antiserum (A_{405} of tested isolates >10 times A_{405} of healthy sap) but not with PRN- or N5-antisera (A_{405} of tested isolates similar to A_{405} of healthy sap) and could not be serologically distinguished from the original PTW1-strain or the ORY-strain.

The results from this study clearly show that *P. teres* can transmit two serologically distinct strains of TRV and confirms the observation by Ploeg *et al.* [1991] that *P. teres* is naturally associated with the PEBV-D and ORE-serotypes of TRV. However, it was also demonstrated that differences in transmissibility by *P. teres* occur within these two serotypes. Strains E116 and N5 were not transmitted although serologically similar to the efficiently transmitted isolate PTJ1. Also, *P. teres* did not transmit strain ORY which is serologically similar to the efficiently transmitted strain PTW1. Similar results were obtained from studies on the transmission of nepoviruses by longidorid vector species, where specificity of transmission is also thought to be largely based on differences in the coat proteins of the transmitted viruses [Harrison *et al.*, 1974]. Thus, *Xiphinema diversicaudatum* was shown to transmit strawberry latent ringspot virus and two serologically distinguishable strains of arabis mosaic virus [Brown, 1986; Lister, 1964] and Brown *et al.* [1989a] demonstrated that efficiencies of transmission of serologically similar strains of tomato black ring virus by *Longidorus attenuatus* varied from 3% to 78%. As a result of these findings Brown [1989] concluded that the observation by Harrison [1964] that serologically distinguishable nepovirus strains appear to be associated with specific longidorid vector species requires to be qualified. A similar conclusion may be drawn from the results from our study with regard to the specificity of tobravirus transmission by trichodorid nematodes.

The failure of *P. teres* to transmit TRV strains ORY and N5 and PEBV-D strain E116 may have resulted from the repetitive subculturing of these strains e.g. by mechanical inoculation in the glasshouse (strains ORY, N5), by seed transmission (strain E116) or by vegetative propagation of plant material (strain N5) in field conditions for several years without vector transmission. The transmissible strains PTW1 and PTJ1

each had been recently obtained from roots of *Petunia hybrida* plants which had been exposed to viruliferous *P. teres* [Ploeg *et al.*, 1991].

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