OCCURRENCE AND SIGNIFICANCE OF THE TEMPERATE RIBOTYPES OF *POLYMYXA GRAMINIS*

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Summary

A number of subgroups of *Polymyxa graminis* have been separated on the basis of their rDNA sequence. Isolates of *P. graminis* found on temperate cereals can similarly be divided into two subgroups known as ribotypes I and II. Nothing is known about the biological significance of these ribotypes with regard to host range and virus transmission. Initial studies have been directed towards testing of *P. graminis* associated with virus infested soils especially those from an agricultural site in Wiltshire, England known to be infested with Soil borne cereal mosaic virus (SBCMV). A number of wheat cultivars and wild wheat relatives were grown in this soil under controlled environment conditions. Initial results show that both ribotypes of *P. graminis* are present in this soil but that ribotypes may exhibit host specificity.

Introduction

Polymyxa graminis is a root-infecting commensalistic organism known to infect a number of grass and cereal species. It is unusual in the fact that it transmits plant pathogenic viruses from at least five different genera. These viruses are of agronomic importance around the world causing severe yield losses. Little is known about *P. graminis* and the biological significance of the recently identified subgroups of the species (Kanyuka *et al*., 2003). However there is already some evidence to suggest that these ribotypes differ in their ability to transmit SBCMV (Ward *et al*., 2005). Initial studies were conducted on wheat and wild wheat relatives grown in soil from a site in Wiltshire, UK, known to be infested with SBCMV. The plants were then analysed by ribotype-specific PCR assays to determine which of the two ribotypes of *P. graminis* were present in their roots. Results from these experiments are presented and their implications discussed.

Materials and Methods

Wheat cultivars and grass species (Table 1) were sown in a 1: 2 mix of soil and sharp sand from a SBCMV infected field in Wiltshire, England. The plants were grown for 5 weeks with a 14 hr photoperiod at 20°C (night period 14°C) to allow development of viral symptoms. Plants were then tested for the presence of SBCMV in leaves and roots using an indirect $F(ab)_2$ ELISA (Chen *et al*., 1991). In addition, total DNA was extracted from root tissue using the method described by Ward *et al.* (2005). Conventional PCR using two *Polymyxa graminis* specific primer sets PgF1/R1 (Pg.F1: 5'-AAC ATG TGG ATT GTG GGC TAT GTG-3', PgR1: 5'-AAC TCC CAT TCT CCA CAA CGC AA-3') and PgF2/R2 (PgF2:5'-ATG TGG ATC GTC TCT GTT GCT GGA-3', PgR2: 5'-CCT CAT CTG AGA TCT TGC CAA GT-3'*)* was then used to determine the ribotypes of *Polymyxa graminis* present in the root tissue (Ward *et al.*, 2005). These assays are specific for their respective ribotypes and amplify products of 292 (type I) or 430 (type II) bp (Fig. 1).

Results

When tested by ELISA, SBCMV was detected in roots of the resistant control *Triticum aestivum* cv. Cadenza and significant, but smaller, signals were obtained from leaves of the susceptible control cv. Avalon and from one sample of cv. Nikoniya. It seems unlikely that any of the other cultivars or wild relatives were infected with SBCMV (Table 1).

In PCR tests, both types of *P. graminis* were detected but type II was more common. One sample was infected with both types and some samples appeared not to be infected (Table 1 and Fig. 1).

Table1. Absorbance values in ELISA for the detection of SBCMV and *Polymyxa graminis* ribotype detection by PCR using samples of wheat cultivars and wild relatives grown in Wiltshire soil

Triticum, Aegilops, and Dasypyrum species	ELISA[1]			*P. graminis*[2] Ribotype
	tissue	1	2	
Triticum spelta v. *caeruleum* UA 0300074,IR00127	root	0.013	0.017	II
Aegilops geniculata 26/93	root	0.022	0.025	-
Aegilops tauchsii	root	0.126	0.122	I,II
Aegilops tauchsii	root	0.041	0.044	II
Triticum aestivum cv. Cadenza (SBCMV resistant)	root	2.080	2.159	nt
Triticum aestivum cv. Nikoniya	leaf	-0.02	0.012	II
Triticum aestivum cv. Nikoniya	leaf	0.926	0.821	II
Dasypyrum villosum	root	0.018	0.019	-
Dasypyrum villosum	leaf	0.006	0.008	-
Triticum boeticum k1814/96	leaf	-0.01	0.007	-
Triticum aestivum cv. Kuyalnik	leaf	-0.01	-0.01	-
Triticum aestivum cv. Kolumbiya	leaf	-0.01	-0.01	-
Triticum aestivum cv. Donskaya	leaf	-0.01	0.019	-
Triticum aestivum cv. Avalon (SBCMV susceptible)	leaf	0.278	0.298	II

[1] ELISA (A_{405}) values after *c.* 1h incubation with substrate. Values (1 and 2) are from two separate plates (means of two replicate wells)

[2] Deduced from PCR banding patterns of root samples I, ribotype I; II, ribotype II; -, negative; nt, not tested

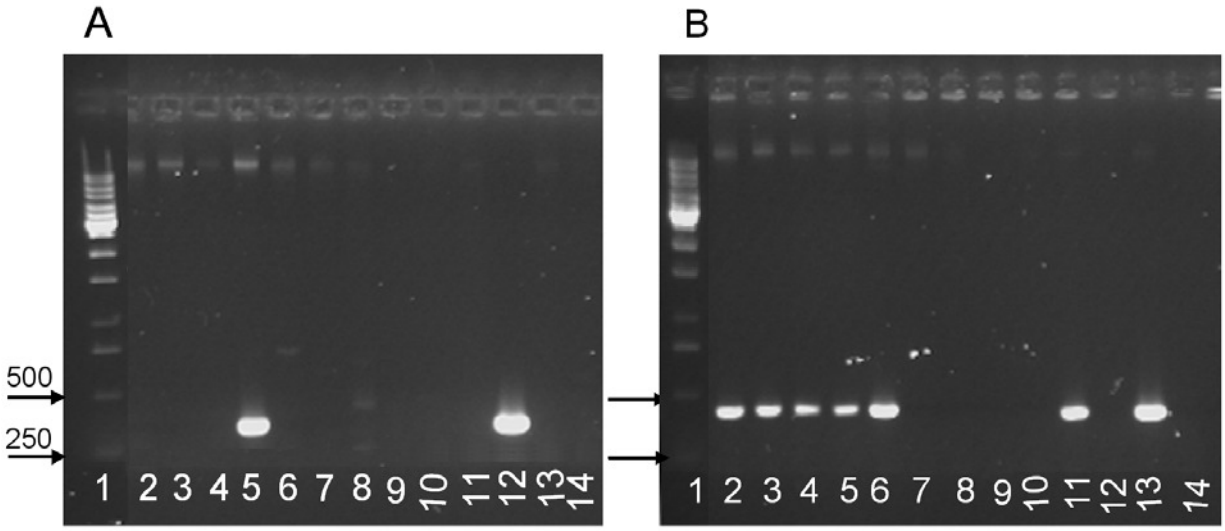

Fig. 1. Agarose gels showing bands amplified from roots of plants grown in Wiltshire soil using **A** *P. graminis* ribotype I specific primers Pg F1/R1 (expected band size 292 bp) or **B** ribotype II specific primers Pg F2/R2 (expected band size 430 bp). Lane 1**:** 1kb ladder (Promega); lane 2: *Triticum aestivum* cv. Nikoniya; lane 3: *T. aestivum* cv. Nikoniya; lane 4 : *T. spelta*; lane 5: *Aegilops tauschii*; lane 6: *A. tauschii*; lane 7: *A. geniculata*; lane 8: *Dasypyrum villosum*; lane 9: *T. boeticum*; lane 10: *D. villosum*; lane 11: *T. aestivum* cv. Avalon; lane 12: Type I DNA from infected root *Hordeum vulgare cv.* Maris Otter; lane 13: Type II DNA from infected root *T. aestivum cv*. Avalon; Lane 14: DNA from non-infected root *T. aestivum* cv. Mercia.

Discussion

This preliminary study shows that both ribotypes of *P. graminis* are present in the soil at the Wiltshire site as both can be found in the roots of these lines. However both ribotypes are not present in all lines. Ribotype II *P. graminis* was present in some *Triticum* species but not all. *Aegilops tauchsii* was the only line to have both types present in the roots of one of the plants, although the other plant in this test only had type II present. This probably reflects the natural variation of inoculum present in the soil.

The strongest ELISA signal for the detection of SBCMV was from the roots of the resistant cultivar Cadenza. This is not unexpected because the resistance in this cultivar is mediated by a mechanism preventing movement from roots to shoots (Kanyuka *et al*., 2004; Hunger *et al*., 1985).

Further, much larger scale experiments are needed to confirm these results as only one or two plants were tested in each case. If these results are borne out, there may be implications for virus epidemiology. *Aegilops tauchsii*, the D genome donor to *Triticum aestivum,* is susceptible to infection by both types of *P. graminis*.

These data from the Wiltshire site are similar to data from a site in Kent, UK also infested with SBCMV. In this soil both ribotypes of *P. graminis* are also present but ribotype II was always found in plants infected with SBCMV. Ribotype I was sometimes found along with ribotype II in SBCMV infected plants but not always (Ward *et al*., 2005).

In work by Ratti *et al*., 2004, hexaploid wheat varieties grown in SBCMV-infested soil in the UK were analysed using our Pxfwd1/ Pxrev7 conventional PCR assay (Ward *et al*.,1998), which allows discrimination between *P. betae* (265bp product), *P. graminis* ribotype type I (280bp) and

P. graminis ribotype II (320bp). The authors report that the assay consistently gave a 280bp product but made no comment about *P. graminis* ribotypes. If this reported size is accurate, it would indicate that all of their samples (including those containing SBCMV) contained only *P. graminis* ribotype I whereas our results suggest that SBCMV is always associated with ribotype II. Unfortunately, their paper does not provide any gel images, but their comment that the assay 'distinguishes between *P. betae* and *P. graminis* by producing PCR products of 265 and 280bp respectively' indicates that they may not have appreciated the difference in size between the ribotypes. However, it is possible that the size of the band may not have been reported accurately. It would be interesting if the samples were retested alongside known ribotype I and ribotype II samples to clarify which ribotypes were present.

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