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A new phenotype of *Polymyxa betae* in *Arabidopsis thaliana*

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Abstract The understanding of the molecular biology of *Polymyxa betae*, the protist vector of *Beet necrotic yellow vein virus,* remains limited because of the obligate nature of this root endoparasite and the limited data on the genome of *Beta vulgaris*, its most common host plant. The aim of this work was to assess the infection of *P. betae* in *Arabidopsis thaliana* in order to learn more about the *P. betae* genome and its interaction with the host. The susceptibility of a set of ecotypes of various origins to a monosporosorus and aviruliferous isolate of *P. betae* was analyzed in a series of bioassays conducted under controlled conditions. *P. betae* was detected in roots of *A. thaliana* using light microscopy and PCR. The infection severity was relatively low in this species compared with *B. vulgaris*, but the different stages of the life cycle were present. The phenotype of *P. betae* in *A. thaliana* root cells differed from the phenotype in *B. vulgaris:* the spore-forming phase was more prevalent in comparison with the sporangial phase, and the sporosori contained a lower number of spores. The compatible interaction between *P. betae* and *A. thaliana* obtained after the inoculation of zoospores and optimal conditions for the development of *P. betae* provide a new model system that can be used to improve the knowledge on the *P. betae* genome and on the mechanisms of the spore-forming phase of *P. betae*.

Keywords *Arabidopsis thaliana* · Host compatibility · Phenotype · Plant-pathogen interactions · *Polymyxa betae* · Spore-forming phase

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Introduction

The understanding of the complex interactions between obligate parasites and their host plants benefits from development of model systems that overcome the difficulties associated with their specific parasitism. *Arabidopsis thaliana* is one of the most studied model plants. The knowledge of the complete genome offers new molecular tools for studies of plant-pathogen interactions. The aim of this work was to develop an Arabidopsis-based experimental model that can offer new resources and tools to understand mechanisms involved in *Polymyxa* spp.-plant interactions. *P. betae* Keskin is a natural soil-borne parasite of the roots of Chenopodiaceae (Keskin 1964). It is classified within the plasmodiophorids, a monophyletic group of obligate biotrophic parasites that includes 10 genera previously considered to belong to the Fungi, but which are now included in the Protozoa (Braselton 2001). Its life cycle in the roots of *Beta vulgaris* is

divided into four stages: after the infection of the host cell by a zoospore (the mobile stage of *P. betae*), a plasmodium grows in the cell and differentiates into a sporosorus (a cluster of resting spores) or into a zoosporangium, releasing secondary zoospores that are able to cause new infections, depending on the sporogenic or sporangial part of the cycle (Keskin 1964).

P. betae is economically important because of its capacity to transmit *Beet necrotic yellow vein virus* (BNYVV), the causal agent of rhizomania disease (Fujisawa & Sugimoto 1976), and three other viruses, *Beet soil-borne mosaic virus* (BSBMV) also implicated in the rhizomania complex in sugar beet, the *Beet soil-borne virus* (BSBV), as well as the *Beet virus Q* (BVQ) (Wisler et al. 1994, Crutzen et al. 2009a, b). BNYVV and *P. betae* have a worldwide distribution (Peltier et al. 2008) and the disease occurs in all major sugar beet growing areas. The incidence of rhizomania can be reduced by cultural practices, but the most effective control measure is the use of tolerant sugar beets, which limits virus replication and spread (Rush et al. 2006; Pferdmenges et al. 2009), but allows the viruliferous vector to remain in the soil. The development of cultivars resistant to *P. betae* would constitute an alternative to limiting vector multiplication and thus the production of viruliferous inoculum in the soil. Although resistance to *P. betae* development has been found in wild *Beta procumbens* and *B. patellaris* (Barr et al. 1995), no cultivar of sugar beet with resistance derived from these wild species has yet been developed. An alternative approach to developing resistance to the vector would come from further knowledge (or studies) about the metabolic pathways involved in compatible and incompatible host-parasite interaction. However, the obligate nature of the parasitism and the relatively specific, restricted host range that allows the development of the sporangial and sporogenic phases of *P. betae,* complicate such studies. Knowledge about the genome of *P. betae* is still restricted. Parts of the ribosomal RNA, including the partial 18S ribosomal RNA gene, the internal transcribed spacer 1, the 5.8S ribosomal RNA gene, the internal transcribed spacer 2, and the partial 28S ribosomal RNA gene are sequenced (Ward and Adams 1998; Legrève et al. 2002; Bulman et al. 2001). A few other sequences from *P. betae* are found in the NCBI database: a partial sequence of the mRNA for glutathione-S-transferase (Mutasa-Göttgens et al. 2005), a RAPD-PCR amplified genomic *P. betae* DNA fragment, a *P. betae* Keskin EcoRI genomic DNA fragment (Obermeier 1998) and *P. betae* repetitive EcoRI-like fragments (Mutasa-Göttgens et al. 1993). Knowledge of these sequences has permitted phylogenetic analyses of the species to be undertaken and development of specific molecular detection and quantification tools facilitating studies of this parasite. *P. betae* is considered as separate from *P. graminis*, a species with the same morphology parasitizing mainly monocotyledonous species (Barr 1979) and differentiated into five *formae speciales*, depending on the specific combination of host range, temperature requirements and rRNA sequences (Ward and Adams 1998; Legrève et al. 2002, 2003). Apart from the *Polymyxa* sequences, the known coding sequences of plasmodiophorids are limited to actin and ubiquitin genes (Ward and Adams 1998; Bulman et al. 2001, Archibald and Keeling 2004), the trehalose-6-phosphate synthase gene from *Plasmodiophora brassicae* (Brodmann et al. 2002) and 76 *P. brassicae* gene sequences obtained after suppression subtractive hybridization between RNA from *P. brassicae*-infected and uninfected Arabidopsis tissue (Bulman et al. 2006)

The host–*P. betae* molecular interactions and *P. betae* genes involved in the interaction are not well known. Only a few studies have been conducted on such interactions, i.e., comparisons between hosts and non-hosts (McGrann et al. 2007; McGrann et al. 2009).

Two species in Brassicaceae are known to be hosts of *P. betae* (Legrève et al. 2005) whereas *Plasmodiophora brassicae*, another plasmodiophorid close to *Polymyxa betae,* infects a high number of species in Brassicaceae. As a first step for further molecular analyses and improving the knowledge of *P. betae* and host-parasite interaction genes, we propose to develop a new study model of *P. betae* by assessing the potential of infection and multiplication of a non-viruliferous strain of *P. betae* in the model plant *Arabidopsis thaliana,* a member of Brassicaceae. During the past years, the tools available for Arabidopsis have been exploited in numerous studies on interactions between plants and pathogens. Microarray techniques permitted identification of key steps during pathogenesis (Lee et al. 2009; Siemens et al. 2006; Yuan et al. 2008), and mutants were used to study potentially interesting genes (Thatcher et al. 2009). Studies of the devel-

opment of *P. betae* in this model plant would allow the advantages of the model plant *A. thaliana* to be exploited. Distinct strategies and culture systems were tested to promote the four stages of *P. betae* within the roots of *A. thaliana*, with the focus of our approach lying in the confrontation between a *P. betae* monosporosoric strain and a wide spectrum of *A. thaliana* ecotypes. A range of 14 ecotypes were tested for their capacity to establish compatible interactions with *P. betae*, by successive mass inoculations. The presence of *P. betae* in the roots of *A. thaliana* was subsequently assessed by a specific PCR assay and microscope observations.

Materials and methods

P. betae inoculum

The aviruliferous monosporosorus strain A26-41 from a soil collected in a non-rhizomania-infested field at Opprebais in Belgium in 1987 was used in this study (Legrève et al. 1998). The multiplication of this strain was achieved by growing sugar beet (*Beta vulgaris* var. Cadyx) plants on a quartz-sporosori mixture using an automatic immersion system in an environmental cabinet at 20–25°C, as described by Legrève et al. (1998). Large quantities of zoospores were produced in the roots of young plants growing in this system for 2–3 weeks, with flooding for 6 h every 12 h. After this period, tubes with *Polymyxa*-infected plants were removed from the system and placed for 24 h under non-saturated conditions in order to synchronize zoosporangial maturation. The roots of the sugar beet plants were then removed from the tubes, rinsed in sterile water and immersed in fivefold diluted Hoagland solution, at 4°C in order to stimulate the release of zoospores. The zoospore concentration of the suspensions was determined using a Thoma counting chamber.

Plants

Fourteen ecotypes of *A. thaliana* of worldwide origin, able to grow under optimal conditions for *P. betae* (Table 1) and obtained from the European Arabidopsis Stock Centre, were tested for their compatibility with *P. betae*. Seeds were surface sterilized in 2.5% NaClO solution for five minutes and then rinsed three times in demineralised sterile water. Depending on the bioassay, sets of 4–5 seeds of each accession were sown in sterile quartz in six glass culture tubes (Fig. 1) and in six tubes adapted to an automatic immersion system (Legrève et al. 1998). The tubes were saturated with Hoagland solution and stratified for 48 h at 4°C. After this period, individual tubes were placed in a controlled environment room with a photoperiod of 12 h and at temperatures of 25–20°C. Four to five seedlings were obtained per tube.

The sugar beet cultivar 'Cadyx' (rhizomania sensitive) was used as a positive control of *P. betae* infection and also for *P. betae* multiplication and source of inoculum. The plants were placed in the automatic immersion system and renewed every 2 weeks.

Bioassays

The *A. thaliana* seedlings were co-cultured in the automatic immersion system, with or without sugar beet infected with *P. betae,* or placed in individual tubes in sterile sand, depending on the intended bioassay. The automatic immersion system was placed in controlled environmental cabinets at 20–25°C, with a photoperiod of 12 h. The automatic immersion system allowed alternate flooding-drainage periods (6 hours flooding/6 hours drainage) regulated by an electric timer.

Three different bioassays were conducted to assess the establishment of *P. betae* infection in *A. thaliana*. First, the encystment of zoospores on *A. thaliana* roots of plants grown in individual glass culture tubes (Fig. 1) was examined by microscopy: five 3-week-old plants of the Col-0 ecotype were carefully removed from the tubes and immersed for 3 h in 1.5 ml of a 700,000 zoospores/ml suspension. The roots were then stained with the fluorescent lipophilic stain 3,3′ dihexyloxacarbocyanine iodine $DiOC_6(3)$, following the method described by Barr et al. (1995). The $DiOC_6(3)$ was dissolved in 10% ethanol at a concentration of 0.5 mg/l and this stock solution was freshly diluted tenfold in milli-Q water before the roots were immersed in it for 15 min. The roots were rinsed in milli-Q water and observed by confocal microscopy, as described below.

The susceptibility of the different ecotypes of *A. thaliana* to infection by *P. betae* was assessed in a

Table 1 Detection of *P. betae* infection in *A. thaliana* ecotypes and *B. vulgaris* after three periods of incubation[A]

Ecotype	Origin	21 dai			33 dai			45 dai		
		PCR[B]	Microscopy[C]		PCR[B]	Microscopy[C]		PCR[B]	Microscopy[C]	
			S	OBS		S	OBS		S	OBS
A. thaliana										
Cvi-0 (N902)	Cape Verde Islands	1/2	+	P	1/2	++	P, S	2/2	++	P, S
Bd-0	Germany	0/2	–	–	0/2	–	–	2/2	+	P
Bla-3	Spain	2/2	+	P, S	1/2	+	P, S	2/2	++	P, S
Bs-1	Switzerland	2/2	+	P, S	1/2	+	P, S	2/2	++	S
Co-2	Portugal	0/0	nt	nt	0/0	nt	nt	2/2	+	P, S
Col-0	USA (Columbia)	0/2	–	–	1/2	–	–	1/2	+	P, S
Cvi-0 (N1096)	Cape Verde Islands	2/2	++	P	2/2	++	P, S	2/2	++	P, S
Gr-1	Austria	2/2	++	P, S	1/2	++	P, S	1/2	++	P, S
Ita-0	Morocco	0/0	nt	nt	0/2	nt	nt	0/2	–	–
Kas-1	India	2/2	+	P, S	2/2	+	P, S	2/2	+	P
Kil-0	UK	0/0	nt	nt	0/2	–	–	1/2	+	S
Mh-0	Poland	1/2	+	P, S	0/2	–	–	1/2	+	P, S
Tu-1	Italia	0/0	nt	nt	0/0	nt	nt	2/2	+	P, S
Van-0	Canada	1/2	–	–	1/2	+	P, S	2/2	+	S
B. vulgaris Cadyx	SES Vanderhave	2/2	+++	P, Z, S	2/2	+++	P, Z, S	2/2	+++	P, Z, S

[A] for Col-0 the different times were 15, 30 and 45 days after inoculation (dai)

[B] Detection by PCR: number of infected root systems (Ninf) out of number of tested root systems (Nt)

[C] Detection by light microscopy: severity of infection (S) was evaluated by counting the total number of *P. betae* structures observed in root systems : (−) no structures; (+) 0–10 structures; (++) 10–100; (+++) more than 100 structures; nt—not tested. The observed stages of the life cycle (OBS) are annotated as following: P—plasmodia ; S—Sporosori ; Z—zoosporangia

second bioassay. Six tubes with 15-day-old healthy plants of each *A. thaliana* accession and sugar beet (as the control) were placed in the automatic immersion system next to tubes containing sugar beet plants heavily infected with *P. betae* zoosporangia. Zoospores released from these plants into the nutrient solution served as inoculum to other plants in the system during the flooding periods. The root systems of two tubes per accession were harvested at 21, 33 and 45 days after inoculation (dai). The tubes containing Arabidopsis plants, which were removed from the system, were replaced by tubes with healthy sugar beet plants to promote the multiplication of *P. betae* in its host for a continuous production of zoospores.

Tubes containing plants of each *A. thaliana* ecotype (one tube/ecotype) were also placed in the automatic immersion system in the absence of sugar beet and *P. betae* inoculum, as the negative control. After incubation, each root system was separated into two parts. *P. betae* infection was assessed by light microscopy for one part and by PCR for the second part.

The infection of *A. thaliana* was scored by combining incidence and severity in the different ecotypes. The infection severity was assessed as described by Legrève et al. (2000): scores were given depending on the number of *P. betae* structures observed in roots, i.e., 0 if no structure is observed, 1 for 0–10 structures in the observed root system; 2 for 10 to 100 structures and 3 for more than 100 structures. The degree of infection was calculated for each accession over the times, as $\frac{\sum_{i=1}^{3}(N\mathrm{inf}_t.S_t)}{Ntot}$, where $N\mathrm{inf}_t$ is the number of infected root systems at time t, S_t the infection severity at time t and N_{tot} the total number of tested root systems.

A third assay was performed in order to assess the ability of each ecotype to release *P. betae* zoospores. *Arabidopsis thaliana* plants were sown in individual

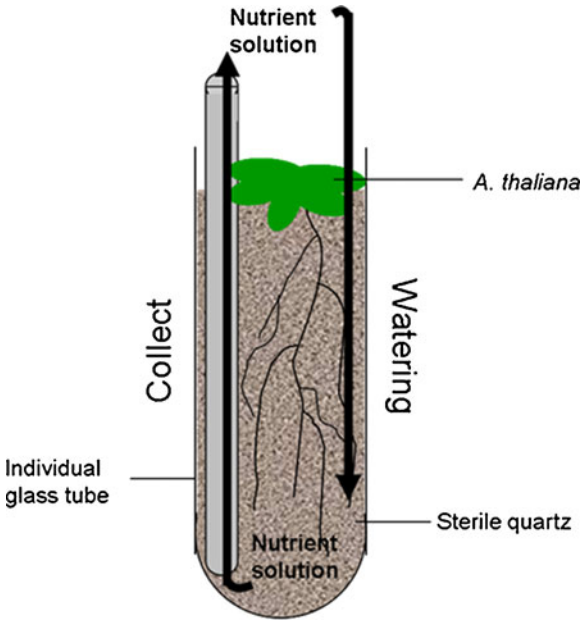

Fig. 1 Individual glass tube system for *A. thaliana* culture. Seedlings are cultivated in sterile quartz and watered with Hoagland solution. The inner glass pipe allows to sample nutrient solution in order to detect *P. betae* zoospores

glass tubes filled with sterile quartz and containing a little glass pipe to allow drainage of the solution from the rhizosphere to the outer tube (Fig. 1). The plants in each tube were inoculated three times, i.e., after 26, 33 and 45 days, with 40,000 zoospores in 5 ml at each inoculation time. Five tubes without plants were inoculated with the same suspensions and placed under the same conditions, as a negative control. In addition, plants from each ecotype were grown under the same conditions without inoculum, as a second negative control. The plants were watered with nutrient solution every 2 days.

Six and nine days after the last inoculation, about 1 ml of the solution in each tube was collected via the glass pipe and the presence of *P. betae* zoospores was examined by microscopy without staining, in order to observe the typical behaviour of the biflagellate *P. betae* zoospore and also after immobilization with iodine. The presence of zoospores was examined in the two samples. In order to ensure that the observed zoospores were those of *P. betae*, a specific PCR was performed directly on the solution, as described below. Furthermore, PCR was also performed on the roots, under the same conditions. The identification of organisms present in solution was done by amplifying and sequencing a part of the ribosomal DNA region including internal transcribed spacers (ITS) 1 and 2 using universal primers (ITS1 and ITS4).

Detection of *P. betae* in roots

For light microscopy, roots were observed using differential interference contrast microscopy after lactophenol blue staining (Legrève et al. 1998).

For confocal microscopy, roots were stained with the fluorescent lipophilic stain 3,3′ dihexyloxacarbocyanine iodine $DiOC_6(3)$ and analyzed using a Zeiss LSM 5 exciter (Carl Zeiss AG, Jena, Germany) confocal microscope, with a 488 nm excitation laser and a 505 nm barrier filter.

For the immunolocalization of *P. betae* in roots of *A. thaliana*, roots were prepared and embedded in Technovit 8100 (Kulzer, Wehrheim, GE), following the manufacturer's protocol. Sections of 5 µ were cut and placed on a microscope polylysine slide in a water drop and warmed in a water bath. The slides were then incubated with 0.01% trypsin in $CaCl_2$, pH 7.8, for 5 min at 37°C. The specific immunolocalisation of *P. betae* was carried out as described by Ruzin (1999): the slides were incubated in a blocking solution (phosphate buffered saline with bovine serum albumin 3%, pH 7.2) at room temperature for 30 min, then rinsed with the PBS washing solution and treated with 10 µl of 100-fold diluted primary polyclonal antibody specific to *P. betae* and *P. graminis*, developed by Delfosse et al. (2000). After 90 min of incubation at 37°C, the slides were rinsed in PBS three times. Then 10 µl of 100-fold diluted Goat-Antirabbit (H + L) Cy3™ (LOEWE, Sauerlach, GE) was added to the slides, which were incubated at 37°C for 90 min. The slides were then rinsed twice in PBS for 15 min and in distilled water for 10 min. The slides were analyzed by confocal microscopy using the Zeiss LSM 5 Exciter with a 543 nm lazer, with a filter LP 560 nm and a beam splitter HFT 543 nm.

PCR detection

Roots were removed from the automatic immersion system or individual tubes and were rinsed in demineralised water in order to eliminate possible external presence of *P. betae*. The total DNA of the root system was extracted using the FAST DNA© kit

(Q.BIOGENE, Ca, USA) following the manufacturer's protocol. The roots were crushed in the CLS-Y buffer using the Fast Prep™ FP120 instrument (Q.BIOGENE, Ca, USA) for 40 s at speed 6. Extracted DNA was diluted 10 times in DEPC treated water. A volume of 2.5 µl of the tenfold diluted extract was tested in a total volume of 25 µl: each 25 µl mixture was prepared with 15.5 µl DEPC water, 2.5 µl 10X DNA polymerase buffer (Promega, Madison, USA), 2.5 µl of $MgCL_2$ 25 mM (Promega), 0.5 µl of each primer, 0.75 µl DNTPs and 0.25 µl of Taq DNA Polymerase (Promega) 5u/µl. The specific primers for *Polymyxa* detection, Psp1 (5′-TAGACGCAGGTCATCAACCT-3′) and Psp2rev (5′-AGGGCTCTCGAAAGCGCAA-3′), developed by Legrève et al. (2003), were used to assess the presence of *P. betae* in the *A. thaliana* roots. The PCR was performed in an MJ Mini thermocycler (Bio-rad, Ca, USA). An initial denaturation was carried out at 94°C for 2 min, followed by 35 cycles, including 30 s denaturation at 94°C, annealing at 60°C for 30 s and elongation for 30 s. A final elongation was completed at 72°C for 7 min. The samples were loaded onto a 1.2 % agarose gel in a Tris borate ethylenediaminetetraacetic acid buffer at pH 8 and the electrophoresis was performed using the Sub-Cell GT-Agarose Gel electrophoresis Systems (Bio-Rad). After migration, the bands were visualized using a Gel Doc 2000 (Bio-Rad).

Results

The potential infection of *A. thaliana* roots by *P. betae* was initially evaluated by testing the capacity of the zoospores released from sugar beet to encyst on seedling roots of ecotype Col-0. Three hours after contact between seedling roots and zoospores suspension, more than twenty zoospores were found encysted per *A. thaliana* root, mainly on the root hairs, as illustrated in Fig. 2.

The compatibility between *P. betae* and 14 accessions of *A. thaliana* was studied in a second bioassay in comparison with *B. vulgaris*, the most common host of *P. betae*. No *P. betae* was detected by PCR and no *Polymyxa*-like structures were observed in *A. thaliana* and *B. vulgaris* plants grown under the same conditions, in the absence of *P. betae* (data not shown).

Twenty-one days after initiation of the co-cultures of *P. betae* and *A. thaliana*, *P. betae* infection was detected by PCR in eight accessions of *A. thaliana* (Table 1). Except for the Van-0 ecotype, this PCR detection was confirmed by microscopy. *P. betae*-like plasmodia and often resting spores, were observed in the *A. thaliana* roots (Table 1). The severity of infection was very low, with less than 10 infected cells for the Cvi-0 (N902), Bla-3, Bs-1, Kas-1 and Mh-0 ecotypes and between 10 and 100 infected cells for the Cvi-0 (N1096) and Gr-1, and much lower than the severity of infection observed in *B. vulgaris*. One month after the co-culture was started, the infection was confirmed in these accessions, except for Mh-0, by PCR and microscopy. *P. betae* was present in one of two analyzed root systems for Cvi-0 (N902), Bla-3, Bs-1, Gr-1 and Van-0, with plasmodia and spore-like structures being observed. At this time, the presence of *P. betae* was also detected in Col-0 by PCR only. Two weeks later, *P. betae* was detected using both methods in all tested ecotypes except for Ita-0, the Moroccan ecotype. The severity of infection was higher than 15 days earlier for Cvi-0 (N902) and Bs-1, and remained high for Cvi-0 (N1096) and Gr-1. Plasmodia and resting spores were observed in almost

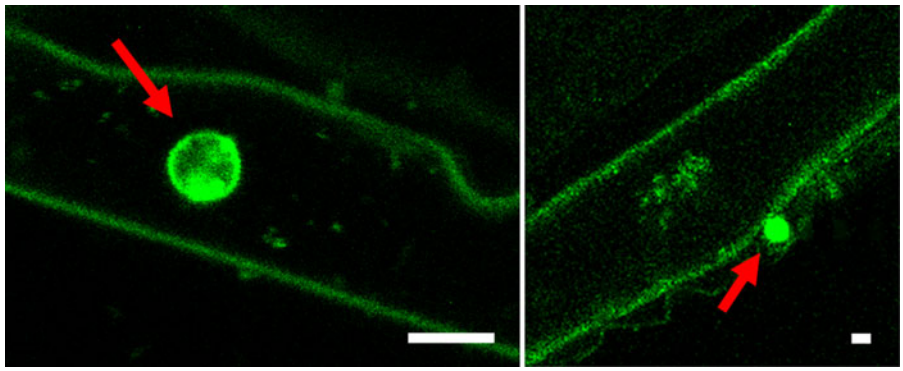

Fig. 2 Zoospores encysted on an *A. thaliana* roots cells, observed after staining with $DiOC_6(3)$ with a confocal microscope. Bars=5 µ

all these accessions, except for Bd-0 and Kas-1, where only a few plasmodia were visible, and Bs-1, Kil-0 and Van-0, where only spores were detected. The degree of infection was calculated for each accession over the times in Table 1 (Fig. 3). Although *P. betae* infection was detected in all but one accession, differences in the degrees of infection, depending on the accessions, were observed, with the highest values for ecotype Cvi-0 (N1096) and the lowest for Bd-0 (Germany), Col-0 (Columbia), Kil-0 (UK), Mh-0 (Poland), and Van-0 (Canada). The infection degree depends on the incidence and severity of infection, and provides information on the compatibility of the host-parasite interaction. For example, *P. betae* was detected in root systems of accessions Co-2 (Portugal) and Cvi-0 (N1096) (Cape Verde), but the degree of infection in Cvi-0 was two times higher than in Co-2.

In *B. vulgaris*, the degree of infection increased over time and exceeded 1,000 infected cells 21 days after initiation of the co-culture between *P. betae* and *B. vulgaris*. In the roots of *B. vulgaris*, the three life stages of *P. betae* (formation of plasmodia, zoosporangia and sporosori) were visible in all the observed root systems, with the sporangial part of the cycle being predominant at the first observation date and the sporogenic part of the cycle at the later dates. As shown in Table 1, no zoosporangia were observed in the root system of *A. thaliana*, whatever the accession. Although the shapes of the observed plasmodium structures in *A. thaliana* and *B. vulgaris* root epidermis cells or root hairs look very similar (Fig. 4), the resting spore arrangement was clearly different, depending on the host plant species. In *B. vulgaris*, the spores were grouped in clusters of tens to hundreds of spores. In *A. thaliana*, the resting spores were similar in size and morphology to the spores produced in *B. vulgaris*, but the spores appeared in the root cortical cells either isolated or grouped along a string or in a loose cluster (Fig. 4). The confirmation of the identity of these spores observed in light microscopy as *P. betae* spores was obtained by immunodetection using a *Polymyxa*-specific antibody revealed by confocal microscopy (Fig. 5).

In *A. thaliana,* no typical zoosporangia were observed. In order to check the ability of *P. betae* to produce its sporangial phase in *A. thaliana,* the release of zoospores from *A. thaliana* roots was tested in a third bioassay by analyzing the presence of zoospores in the solution surrounding the roots of living *A. thaliana* inoculated with *P. betae* and grown in the glass culture system (Fig. 1). Bi-flagellate zoospores were detected at 6 and/or 9 dai in the solution surrounding living plants of all *A. thaliana* accessions, except for Bd-0 and Gr-1. The identification of these zoospores as *P. betae* zoospores was confirmed by specific PCR and ITS sequencing. To confirm that the zoospores observed in the solution surrounding the plant roots were not the originally inoculated zoospores, a control test was performed on the solution collected in three tubes inoculated with

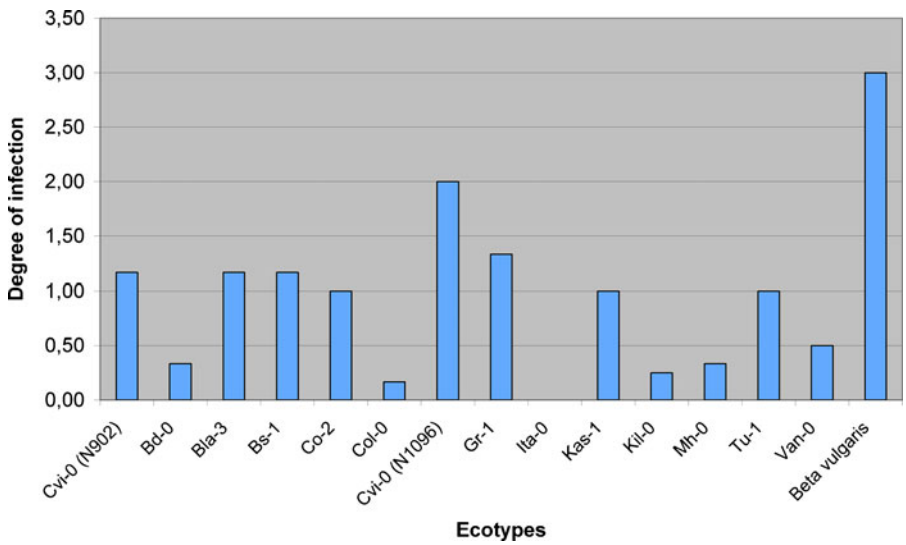

Fig. 3 Degree of infection of 14 ecotypes of *A. thaliana* and of *B. vulgaris* by *P. betae*. Ecotypes compatibility (degrees of infection) was calculated by combining the susceptibility (the ratio of the infected plants on tested plants) and degree of infection, depending on the severity of infection (number of cells infected)

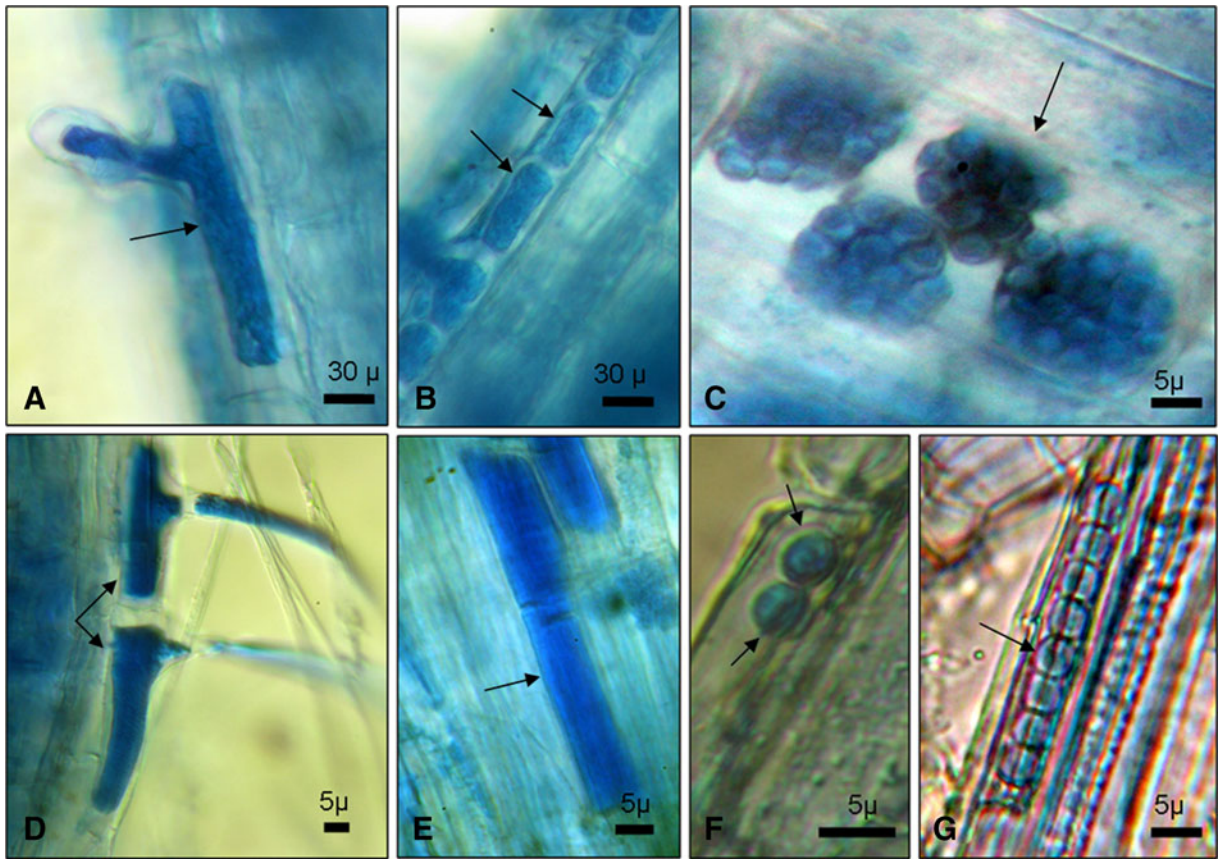

Fig. 4 Different structures of the life cycle of *P. betae* on sugar beet (**a**-**c**) and *A. thaliana* (**d**-**g**) roots. **a**,**d**) Plasmodia. **b**) Zoosporangia. **e**) Differentiating plasmodium. **c**,**f**,**g**) Resting spores, gathered in clusters (**c**) or in chains (**f**,**g**)

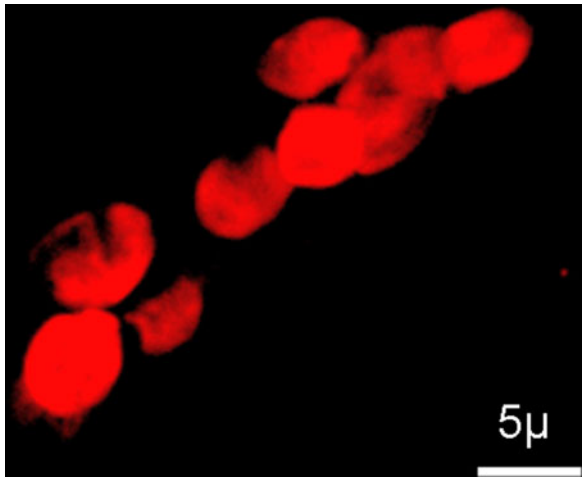

Fig. 5 Confirmation of the identification of *P. betae* resting spores in *A. thaliana* roots by immunolocalisation using a *Polymyxa*-specific antibody

the same number of zoospores, but without plants. No zoospores could be detected in these control tubes.

Discussion

Compatible interactions between *P. betae* and *A. thaliana* would be a useful tool for understanding the molecular interaction between *P. betae* and its host by providing a system for identifying both host and parasite genes induced during infection. Although the host range of *P. betae* was first considered to be restricted to Chenopodiaceae (Keskin 1964), studies have shown that species from two other families, Amaranthaceae and Caryophyllaceae, might also be heavily infected (Barr 1979; Abe and Ui 1986; Barr and Asher 1992). More recently, molecular tools have allowed *P. betae* to also be detected in some

Asteraceae, Papaveraceae, Poaceae and Urticaceae, as well as in two species in Brassicaceae, i.e., *Capsella bursa-pastoris* and *Thlapsi arvense* (Legrève et al. 2005). Although *P. betae* has not yet been reported to infect *A. thaliana*, the detection of this parasite in roots of Brassicaceae and the high rate of multiplication of other plasmodiophorids (*Plasmodiophora brassicae*) on *A. thaliana* (Koch et al. 1991, Desoignies et al. 2009) led us to assess the compatibility between *P. betae* and distinct *A. thaliana* ecotypes under optimal conditions for the development of *P. betae*, using a monosporogenic strain of *P. betae* as inoculum.

We observed that zoospores are able to detect the presence of *A. thaliana* roots and to encyst on them. This stage of the infection does not appear to prevent establishment and development of *P. betae* on *A. thaliana*. This result is in accordance with the observations of Barr et al. (1995), who observed encysted *P. betae* zoospores on *Beta patellaris* and *B. procumbens* roots despite lack of further infection in these species.

Polymyxa betae was detected in all but one of the 14 tested ecotypes, using PCR as well as microscopy. The differences in the degree of infection and in the observed stages, depending on the *A. thaliana* accessions, revealed differences in compatibility. The higher degree of infection observed for ecotypes Cvi-0 (N1096) and Gr-1 suggests a higher degree of compatibility than for the other ecotypes. Especially the ecotypes Bd-0, Col-0, Kil-0 and Mh-0 harboured a lower degree of infection. The absence of sporosori in the roots of the ecotypes from the Cape Verde Islands [Cvi-0 (N902) and Cvi-0 (N1096)] at 21 dai, on which only plasmodia were present, combined with a relatively high degree of infection at 33 and 45 dai, could suggest that the sporangial phase of *P. betae* is promoted in these ecotypes rather than the spore-forming phase, at least during the first 3 weeks after inoculation. In contrast, the detection of sporosori in the less susceptible Bs-1, Van-0 and Kil-0 ecotypes 45 dai revealed that the sporogenic phase had already initiated. The factors determining the sporangial vs sporogenic development from the plasmodial stage still remain unknown (Braselton 1995; Littlefield et al. 1998). However, from the dynamics of the development of sporangia and sporosori observed for *P. betae* on sugar beet and for *P. graminis* on cereals (Legrève et al. 1998), the sporangial phase appeared to occur in environments favourable to the multiplication of *Polymyxa* sp. In contrast, the survival stage was initiated when the infection became high or the conditions were not optimal. This behaviour has been reported for other soil microfungi (Grishkan et al. 2003) and could explain why, in a less susceptible host, the sporogenic phase is activated earlier than in a compatible interaction. Interestingly, the ecotype most compatible with *P. betae* [Cvi-0 (N1096)] is also known to be particularly susceptible to the infection and the multiplication of *Plasmodiophora brassicae* (Kobelt et al. 2000).

At the morphological level, our observations indicated that the morphology of *P. betae* in *A. thaliana* is distinct from the *P. betae* structures observed in the roots of *B. vulgaris*. As shown in Fig. 4, the plasmodia formed on both species are similar except that the plasmodial dimensions are smaller in *A. thaliana* than in sugar beet. The size of the plant cells in the two distinct plant species could influence the size of the plasmodia. In addition, the morphology of sporosori in these two species is different: in sugar beet, *P. betae* resting spores are grouped into clusters of numerous spores to form sporosori whereas in *A. thaliana*, only a few individual resting spores are present in a cell, sometimes forming strings or disconnected groups of a few resting spores. The observation of particular phenotypes of *P. betae* resting spores, being dependent on the host cell, is in accordance with reports by Barr (1979) who also observed distinct forms of *Polymyxa* spp. in different host plants, and concluded that the resting spore clusters might assume the shape of the host cell. It is interesting to note that the morphology of *P. betae* spores, shown in Fig. 4 on *A. thaliana*, is very close to the morphology of *P. brassicae* resting spores produced on the same plant (Koch et al. 1991). This phenotype might be related to a specific interaction between two plasmodiophorid species and *A. thaliana.*

Although no typical zoosporangia could be identified in the roots of *A. thaliana*, the observation of zoospores in the nutrient solution surrounding the rhizosphere for all accessions except ecotypes Bd-0 and Gr-1 showed that zoospores were produced and thus zoosporangia present. The low number of zoospores indicated that zoosporangia are scarce in *A. thaliana* compared with sugar beet, even in

susceptible accessions of *A. thaliana* in which the degree of infection is considerably lower than in *B. vulgaris*. The absence of zoospores for ecotypes Bd-0 and Gr-1 reflects the absence of zoosporangia in their roots at the time of the analysis. Zoospores were detected in the rhizosphere of the Moroccan ecotype Ita-0 and *P. betae* was detected in plants grown in the glass culture tube system, although no infection was detected in plants grown and inoculated in an automatic immersion system. This discrepancy could be explained by the differences in culture systems between the automatic immersion system and the individual tubes, or by the limited number of tested plants of this ecotype due to the low germination rate. The lack of detection of typical zoosporangia in the roots of *A. thaliana*, although zoospores are produced, could be explained by differently shaped zoosporangia in the roots of this species. The small size of the cells might inhibit the division of zoosporangia into several segments, as usually observed on sugar beet, and makes it difficult to distinguish between the plasmodial and zoosporangial stages. The different levels of granularities observed for plasmodia in the microscope observations could be linked to the degree of differentiation of zoosporangial plasmodia: a higher level of granularity could correspond to zoosporangia. The plasmodial structures shown in Fig. 4 could correspond to *P. betae* zoosporangia.

The ability of *P. betae* to re-infect sugar beet after one life cycle was not tested, but Legrève et al. (2005) showed that inoculum from some alternative hosts could re-infect sugar beet. It would be interesting to test the ability of *P. graminis* to develop on *A. thaliana*. Indeed, both pathogen species are close genetically (Ward and Adams 1998; Legrève et al. 2002) and *P. graminis* has been shown to infect some dicotyledonous species (Legrève et al. 2000).

We obtained compatible interactions between *P. betae* and *A. thaliana* under conditions favourable for *P. betae* growth and found differences in *P. betae* infection degree between the tested ecotypes. These results may be used in the development of a new model system to study interactions between *P. betae* and its hosts. The ecotype Cvi-0 (N1096) appeared to be particularly interesting in the development of this new model system because *P. betae* infection in this ecotype was higher than in the others ecotypes. The *P. betae* genome is still largely unknown because of the obligate nature of this parasite and because the complete genome of sugar beets is not known. Use of the *A. thaliana-P. betae* interaction as a new model will allow the use of the molecular tools available for this plant to investigate mechanisms underlying plant-parasite interactions. A transcript analysis of differentially expressed genes after *P. betae* infection will be possible. Identification of *P. betae* genes will be possible using a bioinformatics approach. Separation of host and parasite genes will be possible since *A. thaliana* is full-genome sequenced. However, *P. betae* infection in roots of *A. thaliana* is lower than in the natural host and the phenotype is slightly different, indicating that this plant-parasite interaction may be host-specific. Therefore, even though this model offers new possibilities, it should be considered as complementary to the *P. betae-B. vulgaris* system.

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