FUTURE CHALLENGES IN VIRUS TRANSMISSION BY *POLYMYXA*

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Summary

The incidence of phytoviruses transmitted by *Polymyxa graminis* and *P. betae* remains economically important in major crops such as sugar beet and cereals. Over the past 20 years, the use of varieties with resistance to *Beet necrotic yellow vein virus* or to cereal mosaic viruses such as *Barley yellow mosaic virus* or *Soil-borne cereal mosaic virus* has been the only way to achieve profitable yields on highly rhizomania- or mosaic-infested soils, respectively. The effectiveness of this strategy is limited, however, by the occurrence of resistance-breaking viral isolates or the absence of resistance in some plant species. In the context of integrated pest management, the development of control strategies against the vector would be useful, but this requires better knowledge of the soil ecology and an understanding of the molecular interaction between the vector *Polymyxa* and the host plant, as well as between *Polymyxa* and the virus it transmits. Recent studies have sought to develop new models that overcome the difficulties associated with the specific parasitism of *Polymyxa* spp. We describe here how the recent identification of a compatible *Arabidopsis thaliana-P. betae* interaction and the development of a dual *in vitro* culture system of *P. betae* on sugar hairy roots could help to improve knowledge in this area. The new approaches for investigating the virus acquisition and transmission processes by *Polymyxa* under controlled conditions, and for evaluating the specificity of the interaction between *Polymyxa* spp. and f.spp. isolates and the viruses vectored by them, are presented from the perspective of a risk assessment of the increased prevalence of viruses and vectors associated with distinct pathosystems.

Introduction

The transmission of viruses by *Polymyxa* confers particular epidemiological characteristics on the diseases caused by these viruses, such as dissemination through soil and very long survival in infested soils. Until now, genetic resistance against viral particles has been the only efficient way to control the diseases caused by these viruses. A strategy targeting the vector would limit the inoculum potential in the soil. Genetic resistance against the vector has been analyzed (Legrève *et al.*, 2000; McGrann *et al.*, 2007 and 2009), but the difficulty of growing the vector and the differences in host ranges between *Polymyxa*

populations has complicated these studies, and this strategy has therefore not been fully developed. The development of new strategies to control viral diseases transmitted by *Polymyxa* spp. requires a better understanding of the molecular interaction between the main actors in the pathosystems – i.e., the plant, the virus and the vector. Since *Polymyxa* is an obligate root endoparasite, studies are complicated by the fact that the genome of the vectors has been only partially described and by the difficulty of growing it in an axenic medium. In addition, the complete genome of major host plants (e.g., sugar beet, peanut, wheat and barley) is not yet known. New models have been developed to overcome these constraints, two of which are described here: a compatible interaction between *P. betae* and *A. thaliana*; and a dual culture system for *P. betae* in sugar beet hairy roots. The first model was developed in order to provide a useful tool for understanding the molecular interaction between *P. betae* and the host plant. Knowledge of the complete genome of *A. thaliana* enables the mechanisms involved in the interaction to be understood and contributes to knowledge about the *Polymyxa* genome. The second model was developed to enable *Polymyxa* in roots to be multiplied in an *in vitro* system without any other organism or root contaminant.

Another important issue regarding the epidemiology of viral diseases transmitted by *Polymyxa* relates to the specificity of virus transmission by *Polymyxa* spp. More than 17 viruses belonging to bymy-, furo-, peclu- and pomoviruses are transmitted by *Polymyxa* species. Several of them can occur in a same plant (e.g., *Beet necrotic yellow vein virus* [BNYVV], *Beet soil-borne virus* and *Beet virus Q*), and some soils are infested by species involved in several pathosystems (e.g., P. *graminis* f.sp. *temperata*, *P. graminis* f.sp. *tepida*, *Barley yellow mosaic virus* [BaYMV] and *Barley mild mosaic virus* [Vaianopoulos *et al*., 2007]*, and P. graminis* f.sp. *temperata* and *P. betae* associated or not with BaYMV or BNYVV, respectively). Given the host specificity of some of these species, some virus-vector interactions are unlikely. For example, the interaction between *Soil borne cereal mosaic virus* and *P. betae* is unlikely because the first infects cereals and the second, mainly, *Chenopodiaceae*. The recovery of the host ranges of other species, however, allows the question of the specificity of viral transmission by vector populations to be considered. The acquisition and transmission of *Peanut clump virus* (PCV) by different *formae speciales* of *P. graminis* on cereals has been studied under controlled conditions, using sugar cane as the common host for virus and vector (Dieryck *et al.*, 2011). This experiment demonstrated the role of *P. graminis* f.sp. *tropicalis* and *subtropicalis* zoospores in PCV transmission and clarified the issue of the specificity between virus, *Polymyxa* and plant species.

Materials and Methods

Detailed information on the materials and methods used in the studies described here is provided by Desoignies and Legrève (2011), Desoignies *et al.*

(2011) and Dieryck *et al.* (2011).

Results and Discussion

Identification of a compatible *Arabidopsis thaliana-P. betae* interaction:

The potential of infection of 14 *A. thaliana* accessions by *P. betae* was evaluated by testing the capacity of the zoospores released from sugar beet to infect and parasitise the roots (Desoignies *et al.*, 2011). *Polymyxa betae* infection occurred in all but one accession (Ita-0 from Morocco), but there were differences in the level of infection, depending on the accession. The greatest infection was observed in the ecotype Cvi-0 (N1096) from Cape Verde Island. In this accession, some plasmodia of the protist were detected in the roots 21 days after inoculation; 12 and 24 days later the same structures, and sporosori, were visible in the roots. In the accessions Cvi-0 (N902) from Cape Verde Islands, Bla-3 from Spain, Bs-1 from Switzerland, Kas-1 from India, Gr-1 from Austria and Tu-1 from Italia, *P. betae* plasmodia and sporosori were also detected, but the infection level was lower than in Cvi-0 (N902). In the other tested accessions (Bd-0 from Germany, Co-2 from Portugal, Col-0 from USA, Kil-0 from UK, Mh-0 from Poland and Van-0 from Canada), the infection was slight and sporadic. Although *P. betae* infection was observed in all but one *A. thaliana* tested accession, the infection level was lower than in the *P. betae-Beta vulgaris* interaction, and the sporogenic phase of the life cycle was favored over the sporangial phase. In addition, the phenotype of *P. betae* in *A. thaliana* differed from the one in *B. vulgaris*; the dimensions of the plasmodia were smaller and the resting spores were not grouped, or only a few spores were grouped. This phenotype could be related to the host cell. The compatible interaction between *P. betae* and *A. thaliana* has also recently been reported in a study by Smith *et al*. (2011). These authors described the infection of *A. thaliana* by *Polymyxa* spp. after growth on *Polymyxa*-infested soils. The compatible interactions highlighted in two independent studies offers new possibilities for studies on plant-parasite interactions.

Dual *in vitro* culture system of *P. betae* on sugar beet hairy roots:

A dual *in vitro* culture system of *P. betae* on sugar beet hairy roots was developed by inoculating fragments of the roots transformed by *Agrobacterium rhizogenes* with *P. betae* zoospores and transplanting the inoculated fragments on Gamborg B5 liquid medium with sucrose (Desoignies & Legrève, 2011). The zoosporangia-infected roots, produced in the automatic immersion device classically used to culture *P. betae* (Legrève *et al.,* 1998), were disinfected in $Ca(ClO)_2$ solution and used to inoculate sugar beet hairy roots. After 24 h incubation in the presence of inoculum, the roots were transplanted into the liquid medium and incubated in the dark at 18-23°C under sterile conditions. Ten weeks after incubation, *P. betae* was detected by microscopy and specific PCR in half of the inoculated roots. Typical *P. betae* plasmodia and sporosori

were observed and the severity of infection in parts of roots after inoculation revealed the occurrence of a secondary infection in the system. This new *in vitro* culture system will be useful for multiplying and conserving *P. betae* strains/isolates. The production of an axenic inoculum of *P. betae* will also be useful for the molecular characterization of the species.

Study on the specificity of the transmission of viruses by *Polymyxa* spp.:

A new way of studying PCV transmission by the protist vector *P. graminis* on cereal under controlled conditions was developed. Using sugar cane as common host for this virus and distinct *formae speciales* of the vector, viruliferous zoospores of *P. graminis* f.sp. *temperata, tropicalis* and *subtropicalis* were produced in systemically PCV-infected plants grown in automatic immersion devices. The same numbers of viruliferous zoospores of each special form were then inoculated in distinct cereal species. Effective PCV transmission by *P. graminis* f.sp. *tropicalis* occurred in pearl millet (44%), barley (13%), sorghum (4%) and wheat (6%), and by *P. graminis* f.sp. *subtropicalis* in barley (6%) and wheat (10%), but not in pearl millet and sorghum. No PCV transmission by *P. graminis* f.sp. *temperata* was observed, although PCV had been detected by RT-PCR in the zoospore suspensions released from PCV-infected sugar cane and used to inoculate cereal plants. The experiment therefore demonstrated the role of *P. graminis* f.sp. *tropicalis* and *subtropicalis* zoospores in PCV transmission. It also demonstrated differences in transmission efficiency depending on the *P. graminis formae speciales*, highlighting the specificity of the interaction between the virus and its vector. Although molecular interaction between PCV and *P. graminis* has not yet been identified, a co-evolution mechanism has been hypothesized to explain the specificity of the PCV-*P. graminis* interaction based on the origin of viral and vector isolates used in this study. The absence of PCV transmission by *P. graminis* f.sp. *temperata*, despite the virus being detected in the zoospores suspension, suggests that the mechanism involved in the acquisition of the virus by the vector could differ from that involved in the release of the virus by the vector after the infection. The molecular characterization of such interaction will clarify such mechanisms.

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