1808 Fusarium wilt of Arabidopsis thaliana

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ABSTRACT

Rationale Host-specific strains of soil-borne *Fusarium oxysporum* are vascular pathogens and cause wilt diseases in variety of cultivated plants including cotton. For a number of hosts, a comprehensive literature describes the pathology of Fusarium wilt and the genetic and environmental factors contributing to disease severity. However, studying the molecular genetic basis of wilt disease in crop plants is constrained by inherent experimental limitations.

Objectives A tractable experimental pathosystem combines a host and pathogen that are amenable to routine molecular genetic analysis.

Methods Using a soil drench assay, pathogenic *F. oxysporum* isolates produce characteristic Fusarium wilt disease symptoms in *Arabidopsis thaliana*.

Results Disease severity among Arabidopsis accessions and mutants suggests that *F. oxysporum* promotes wilt disease as a biotroph. As well, the *F. oxysporum fmk1* insertion mutant exhibits an attenuate virulence in the Fusarium-Arabidopsis pathosystem.

Conclusions Fusarium wilt disease of Arabidopsis provides an experimental platform for gene discovery in both plant host and soil-borne pathogen. Ultimately, this pathosystem may provide a molecular description of disease outcome in terms of interactions among plant and fungal genes.

Keywords Fusarium oxysporum, *Arabidopsis thaliana*, pathosystem, wilt disease

INTRODUCTION

For the most part, research on *F. oxysporum* is motivated by the need to cope with plant disease and attendant economic implications. Diseases that are variously named wilts, yellows or root rots for the pronounced symptoms on infected plants may be caused by rare pathogenic isolates of *F. oxysporum*. Considering that the genetic relationship of virulent strains common to a host species is often monophyletic, the acquisition of virulence by *F. oxysporum* is thought to be a rare and consequential event (Kistler, 1997).

Whether any relationship among pathogenic forms that infect different hosts (formally called formae speciales) exists remains unclear. For instance, phylogenic analysis of common housekeeping genes among virulent *F. oxysporum* fails to make a correlation between the phylogeny of *F. oxysporum* formae speciales and the phylogeny of their respective host species. Thus, coevolution of pathogen and host appears to be insignificant for the pattern of host specialization. Indeed, the polyphyletic grouping of some formae speciales shows that host specificity may have multiple independent origins (Kistler, 1997).

In the laboratory, *Arabidopsis thaliana* is a host to pathogenic isolates from three related crucifers: *F. oxysporum* forma specialis (f.) *conglutinans* (from cabbage), F. *oxysporum* f. *raphani* (from radish) and *F. oxysporum* f. *matthioli* (from stock) (Diener and Ausubel,

2005). The wilt disease promoted by each of three formae speciales can be distinguished by characteristic symptoms. Moreover, symptoms in Arabidopsis are strikingly similar to symptoms that are seen in a natural host. Although infection in the laboratory is artificial, Arabidopsis is specifically susceptible to crucifer isolates and exhibits no disease symptoms 1 when the soil is infested with a nonpathogenic *F. oxysporum* or pathogen from a non-crucifer host.

Fusarium wilt disease of Arabidopsis can be a versatile model pathosystem. The plant host Arabidopsis is already the preeminent subject of plant molecular biology, including the molecular study of pathogen-plant interactions. Agrobacterium-mediated transformation of *F. oxysporum* makes both random insertional mutagenesis and targeted gene knockouts feasible and routine (Khang et al., 2005). Moreover, because Arabidopsis is susceptible to multiple and phylogenetically distinct formae speciales, it should be possible to decipher what features of pathogenesis are common, or conserved among *F. oxysporum*.

Implicit in such a model pathosystem is an expectation that valuable insights will be relevant to the interaction of other *F. oxysporum* formae speciales, such as f. *vasinfectum*, and their agricultural hosts, including cotton.

MATERIALS AND METHODS

Arabidopsis thaliana ecotypes Taynuilt-0 (Ty-0, CS6878) and St. Georgen-1 (Sg-1, CS6858) were provided by the Arabidopsis Biological Resource Center (Ohio State University, Columbus, OH). Arabidopsis was sown on Jiffy-7 peat pellets (Jiffy Products, Norwalk, OH), intermittently drenched with just water for approximately four weeks and then alternatively drenched with either half strength Hoagland's solution or water. Plants were grown under cool white fluorescent lighting at a photon density of 150 µE m-2 sec-1 for 12 h day-1.

F. oxysporum strains (see Table 1) originally from Paul 1 H. Williams were provided by H. Corby Kistler (Kistler et al., 1991). Stocks of Fusarium isolates were stored at -80°C in 50% glycerol. Stocks were thawed on Czapek-Dox (CzD, Remel, Inc., Lenexa, KS) agar plates, and liquid CzD cultures are initiated using a streak 5 from a CzD plate culture. To obtain conidia, a CzD liquid culture was shaken at 300 revolutions m-1 at 28°C for one wk; after which, the grown culture was filtered through sterile bandage gauze. Conidia were repeatedly settled by centrifugation and resuspended in sterile water. Conidial density was determined using a hemacytometer, and conidia were diluted into water to obtain an appropriate conidial density for soil drench. The disease index is a progressive grade of symptom development: 5 = unaffected (no symptoms); 4 = rosette leaf stunting; 3 = more stunting and mild chlorosis in older leaves; 2 = chlorosis and premature senscence of older leaves; 1 = severe stunting of young leaves and senescence of older leaves; and, 0 = dead (Diener and Ausubel, 2005). The rosette radius is the mean length of three rosette leaves from one plant.

Standard molecular biology techniques were used for southern blot hybridization of *F. oxysporum* genomic DNA (Ausubel et al., 1998). The *MAT1-1* and *MAT1-2* DNA probes were previously described pCR subclones of the *MAT1* locus amplified with primers Fo14 and Fo25 (Arie et al., 2000). The PKS1, PKS4 and PKS9 DNA probes were generated from three PCR products among others that encode homology to the ketosynthase domain of polyketide synthases, were amplified from *F. oxysporum* f. *conglutinans* race 2 genomic DNA with

degenerate primers KS1 5'-GGRTCNCCIARYTGIGTICCIGTICCRTGIGC-3' and KS2 5'-MGIGARGCIYTIGCIATGGAYCCICARCARMG-3' and were 1 subcloned in pGEM-T Easy (Promega Corp., Madison, WI).

A genomic clone of the *FMK1* locus was isolated from a genomic library, which was constructed from *Sau*AI-partial digestion of *F. oxysporum* f. *conglutinans* race 1 DNA and cloned into the T-DNA region of binary vector pPZP621 (Hajdukiewicz et al., 1994). The *FMK1* gene in the genomic clone was disrupted by blunt-end cloning a *Xba*I- and *Sal*I-digestion fragment of pDH25 (a hygromycin resistance marker, provided by H. Corby Kistler, University of Minnesota, MN) into the sole *Eco*RV restriction site that is found in *FMK1* in both f. *conglutinans* and f. *lycopersici* (Genbank accession AF286533). Using Agrobacterium-mediated transformation *FMK1* was targeted for disruption in f. *conglutinans* (Khang et al., 2005). The *fmk1* insertional disruption was confirmed by southern blot hybridization.

RESULTS AND DISCUSSION

In Table 1, three representative Arabidopsis ecotypes (or accessions) are differential hosts to *Fusarium oxysporum* and distinguish the four races of three formae speciales (f.). As previously described, only pathogenic *F. oxysporum* isolates from related crucifer plants (1) produce wilt disease with distinct symptoms in Arabidopsis and (2) are distinguished by the relative susceptibility of a variety of Arabidopsis ecotypes (Diener and Ausubel, 2005). Interestingly, representative isolates of the two races of f. *conglutinans* were distinguished by ecotype Ty-0, which displayed resistance to race 1 and susceptibility to race 2. The virulence of either f. *conglutinans* race isolate was confirmed by infection of ecotype Sg-1. The three crucifer-specific formae speciales appear 1 to be from distinct phylogenic lineages of *F. oxysporum*. In Table 1, the genetic dissimilarity of the formae speciales is indicated by differences in vegetative compatibility group, the *MAT1* idiotype or the presence of DNA sequences encoding homology to polyketide synthases (PKS), which are associated with secondary metabolism.

The relative resistance to disease progression in Arabidopsis mutants revealed the biotrophic nature of *F. oxysporum* as a vascular pathogen. In terms of pathogenic lifestyle, *F. oxysporum* is ambiguously described as either a biotroph, hemi-biotroph or necrotroph. As reported previously, Arabidopsis mutants with reduced salicylic acid (SA) accumulation in response to virulent pathogen exhibit increased susceptibility to *F. oxysporum* f. *conglutinans* (Diener and Ausubel, 2005). In contrast, the *jin1-7* mutant, was more resistant to f. *conglutinans* as shown in Figure 1 (Anderson et al., 2004). Whereas most wild type plants displayed stronger symptoms of disease index 1 and 2, all *jin1-7* mutants had the milder symptoms indicated by disease index 3 and 4. Indeed, the wild type rosette leaves were more stunted than *jin1-7* leaves. *jin1* is characterized by an insensitivity to exogenous jasmonic acid (JA) (Lorenzo, 2004).

Attenuated virulence of the *F. oxysporum* f. *conglutinans fmk1* mutant, which is analogous to the loss of virulence seen with the homologous *F. oxysporum* f. lycopersici *fmk1*, suggests that pathogenesis in Arabidopsis and tomato share common genetic mechanisms (Di Pietro, 2001). *FMK1* encodes a mitogen22 associated protein kinase that is conserved and required for full virulence in many fungal pathogens. Whereas the ecotype Sg-1 is highly susceptible to wild type f. *conglutinans* and succumbs to a soil drench with a low conidial 1 density of 103 mL-1, Sg-1 is resistant to a soil drench of *fmk1* conidia at 106 mL-1.

The Fusarium-Arabidopsis pathosystem shares characteristics that are common to Fusarium wilt disease of field-grown crops. Diversity among Arabidopsis accessions provides a range of susceptibility to *F. oxysporum* and distinguishes pathogen races. Wilt disease in Arabidopsis, like Fusarium wilt of tomato, is compromised by infection with a loss-of-function *fmk1 F. oxysporum* mutant. Unless the infection modes for three distinct lineages of *F. oxysporum* crucifer pathogens are each dissimilar to all other Fusarium wilt diseases, Arabidopsis should make a comprehensive molecular genetic description of wilt disease attainable. Moreover, what is common to the diseases promoted by the three crucifer pathogens is anticipated to be largely common to all other Fusarium wilts, including the diseases of cotton.

Like many plant pathogens, *F. oxysporum* has an ambiguous lifestyle. Superficial observation of debilitating symptoms and necrosis suggests that *F. oxysporum* is a necrotroph. Nevertheless, *F. oxysporum* ramifies within a healthy living plant, and host response can provide strong qualtitative resistance: characteristics of a biotrophic interaction. Because JA response and SA accumulation respectively antagonize and facilitate resistance to biotrophs, Arabidopsis mutant analysis indicates that *F. oxysporum* has a biotrophic lifestyle.

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Table 1: Crucifer isolates of *F. oxysporum*

Forma specialis	Race	Natural host	Isolate[a]	VCG[a]	*MAT1*[b]	PKS1	PKS4	PKS9	Ecotype reaction[d]		
									Col-0	Sg-1	Ty-0
conglutinans	1	*Brassica oleracea* (cabbage)	777	0101	*MAT1-1*	(+)[c]	(-)	(+)	I	S	R
conglutinans	2	*Brassica oleracea* (cabbage)	808	0101	*MAT1-1*	(+)	(+)	(+)	I	S	S
raphani	NA	*Raphanus sativa* (radish)	815	0102	*MAT1-2*	(-)	(-)	(-)	I	I	I
matthioli	2	*Mathiola incana* (stock)	726	0103	*MAT1-2*	(-)	(-)	(+)	R	I	S

a from Kistler et al., 1991

b Although aesexual, *F. oxysporum* isolates harbor either of two *MAT1* idiotypes.

c A cross-hybridizing restriction fragment is either present (+) or absent (-) in genomic DNA.

d Ecotypes are either resistant (R), partially resistant (I) or susceptible (S) at 10^6 conidia mL^{-1}.

CAPTION FOR FIGURE

Figure 1. Arabidopsis *jin1-7* is resistant to Fusarium wilt disease (A) Arabidopsis wild type (*JIN1*) and *jin1-7* plants were grown in checkerboard fashion in 5 x 10 flats. Plants were code-tagged and randomized before disease evaluation. The disease index gauges the symptom severity from unaffected (5) to dead (0): see Materials and Methods. (B) The rosette radius is a measure of rosette leaf stunting. For *JIN1*, n = 20 plants and, for *jin1-7*, n = 30 plants. Error bars represent the standard deviation.

Figure 1