Fungi

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1 Introduction

Fungal diseases in plants are almost exclusively members of the phylum Ascomycota and subphylum Pezizomycotina. All common crop plants are susceptible to fungal disease of one kind or another. Spore production and dispersal is enormously efficient in fungi, and plants crowded together in monoculture provide an ideal environment for the appearance of virulent new forms, on occasion resulting in the loss of the entire crop. Fungal pathogens are conventionally categorised into three groups: obligate biotrophs, which obtain nutrients from living host tissue; necrotrophs, which induce cell death and maceration of tissue in their hosts; and hemibiotrophs, which are necrotrophs with an initial biotrophic phase but subsequently become necrogenic. Resistance to biotrophs, which include rusts and mildews, is normally qualitative and has been intensively studied in several pathosytems. Typically the genetics of resistance follows the well established race-specific gene-for-gene recognition system (Hammond-Kosack and Parker, 2003). Resistance to necrotrophs, by contrast, is often quantitative, poorly understood and relatively few resistance genes have been characterised. These include the race-specific *Hm1* gene in maize (Johal and Briggs, 1992), the *Pyrenophora tritici-repentis* ToxA-insensitivity *tsn1* (Faris *et al.*, 1996) and ToxB-insensitivity *tsc2* (Friesen and Faris, 2004) loci in wheat, and resistance to the *Fusarium graminearum* mycotoxin deoxynivalenol in wheat (Lemmens *et al.*, 2005).

2 Advantages of using *M. truncatula* to study resistance to fungal necrotrophs

Few pathogens were known in the best characterised plant model, *Arabidopsis*, prior to 1990 (Koch and Slusarenko, 1990). Intensive study of natural populations, and inadvertent and deliberate infection under disease-favouring conditions, has resulted in the current, rather limited list of fungal diseases. Amongst biotrophs, the most rapid progress was made with the powdery mildew species *Erysiphe cichoracearum and E. cruciferearum* (Xiao *et al.*, 2001). Only recently has a hemibiotroph been found to infect *Arabidopsis*, *Colletotrichum higginsianum* (Narusaka *et al.*, 2004). Among necrotrophs, just four species have been characterised; *Alternaria brassicicola* (Penninckx *et al.*, 1996), *Botrytis cinerea* (Govrin and Levine, 2002), *Fusarium oxysporum* (Mauch-Mani and Slusarenko, 1994), and *Sclerotinia sclerotiorum* (Dickman and Mitra, 1992). Of these, *Alternaria brassicicola* has been shown to be avirulent on all accessions tested to date and only shows symptoms on mutants compromised in their defense response (Penninckx *et al.*, 1996; Van Wees *et al.*, 2003). Some isolates of *Botrytis cinerea* and *Sclerotinia sclerotiorum* are opportunistic pathogens with unusually wide host ranges, and it is unclear whether the interactions studied represent a natural pathosystem in *Arabidopsis*. Similarly, resistance to *Fusarium* is not race-specific as has been found with some important crops (Diener and Ausubel, 2005).

Although gene expression and forward genetic approaches have helped elucidate different resistance mechanisms in *Arabidopsis*, gene expression experiments have also revealed important differences to those in crop species. For example in tomato, there is no support for the hypothesis developed from observations in *Arabidopsis* that the jasmonate response is involved in plant defense against necrotrophic pathogens but not against most biotrophic pathogens (Thaler *et al.*, 2004). Moreover, different families of classical R genes appear to have radiated among legumes (Zhu *et al.*, 2002). Thus, Medicago offers a more realistic model of genes involved in resistance among legume crops. As a genetically tractable model host, Medicago provides a platform to identify and isolate R genes and genes involved in resistance signaling pathways. Furthermore, questions such as whether common components of resistance are effective against different species of Dothideomycetes, and the extent to which the symbionts Rhizobium and arbuscular mycorrhizae confer protection against fungal pathogens (Fritz *et al.*, 2006; Rabie, 1998), can now be addressed.

3 Fungal pathogens of *M. truncatula*

Medicago spp. have been intensively and commercially grown as pastures species for a century. The barrel medic, *M. truncatula* Gaertn, is estimated to be grown on over 4.5 million hectares in Australia (Pearson *et al.*, 1997). Biotrophs such as *Erysiphe pisi* cause disease on *M. truncatula* (see Chapter " Inoculation and growth with leaf pathogenic fungi, section 5"). However, the significance of *M. truncatula* lies in its susceptibility to a range of known necrotrophic pathogens. Most necrotrophs belong to the order Dothideomycetes, which includes, for example, species of *Ascochyta*, *Botrytis*, *Colletotrichum*, *Leptosphaerulina* and *Phoma* that respectively cause blight on chickpeas, grey mould on faba beans, anthracnose on lupins, black stem on alfalfa, and black spot on peas. A list of

pathogens known to cause differential disease responses in *M. truncatula* accessions is given in Table 1. Among other pathogens identified, *Rhizoctonia solani* (AG8) causes hypocotyl rot in *M. truncatula*, however no significant differences were observed among 100 *M. truncatula* SARDI core collection accessions in their response to this pathogen (Lichtenzveig and Anderson, *unpublished data*). Compatible interactions were observed between *M. truncatula* and *Fusarium solani* (Salzer *et al.*, 2000), but to the best of our knowledge no extensive evaluation of natural variation in resistance has been undertaken. The majority of these pathogens are also known to infect lucerne (http://www.apsnet.org/online/common/names/alfalfa.asp), and as *M. truncatula* is a close relative, one might expect it to share other pathogens known in that species. For example; *Alternaria solani*, *Botrytis cinerea*, *Cercospora medicaginis*, *Pseudopeziza medicaginis*, *Sclerotinia trifoliorum*, *Stemphylium botryosum*, and *Verticillium albo-atrum*. Pathogens exhibiting a broad host range (e.g. *A. solani*, *B. cinerea*, *R. solani*, and *S. botryosum*) are of particular interest because of the numbers of crop species affected. Details fungal isolation, storage, and inoculation can be found in Chapters "Inoculation and growth with root pathogenic fungi" and "Inoculation and growth with foliar pathogenic fungi".

Table 1. Fungal species causing a differential response among *M. truncatula* accessions

Species	Original host	Disease name	Reference or Section
Ascochyta lentis	*Lens culinaris*	Ascochyta blight	T. Pfaff, *unpublished*
A. rabiei	*Cicer arietinum*	Ascochyta blight	T. Pfaff, *unpublished*
Colletotrichum trifolli	*M. sativa*	Anthracnose	O'Neill and Bauchan, 2000
C. coccodes	*Lupinus albus*	Anthracnose	T. Pfaff, *unpublished*
Fusarium oxysporum	*M. sativa*	Fusarium wilt	J. Lichtenzveig, *unpublished*
Leptosphaerulina trifolii	*M. truncatula*	Leaf spot	Barbetti, 1995
Mycosphaerella pinodes	*Pisum sativum*	Black spot	Moussart *et al.*, 2006
Phoma medicaginis	*M. truncatula*	Spring blackstem and leaf spot	Ellwood *et al.*, 2006
Stagonospora meliloti	*M. sativa*	Leaf spot and root rot	T. Pfaff, *unpublished*

4 New pathogens and isolate validation

DNA-based identification of fungi has gained in importance in recent years for several reasons: traditional phenotype-based identification methods utilized in plant pathology rely on the production and recognition of reproductive structures. For many species, identification is difficult or impossible when these structures are not observed. To classify

new isolates, DNA-based characterisation allows researchers without expertise in morphological taxonomy to determine the placement of fungi within larger taxonomic groups. In addition, this technique allows confirmation of the identity of cultures obtained from collections which may occasionally be contaminated or incorrectly identified.

The most commonly used DNA region used in isolate identification is the rDNA operon composed of ITS1 (Internal Transcribed Spacer 1), the ribosomal 5.8S subunit, and ITS2, defined by primers ITS1 and ITS4 (White *et al.*, 1990). This region normally provides resolution at the species level. Other gene regions may be examined to further distinguish isolates, for example the β-tubulin, calmodulin, and EF-1α gene regions described by Carbone and Kohn, 1999. For some genera and species, specific, hypervariable gene regions may be employed to distinguish isolates; for example in *Fusarium* (http://fusarium.cbio.psu.edu,Geiser *et al.*, 2004), *Ascochyta* (Barve *et al.*, 2003) and *Phoma medicaginis* (Ellwood *et al.*, 2006).

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