Fungal Genetics and Biology 79 (2015) 24–28

Contents lists available at ScienceDirect

Fungal Genetics and Biology

journal homepage: www.elsevier.com/locate/yfgbi

Previous bottlenecks and future solutions to dissecting the *Zymoseptoria tritici*–wheat host-pathogen interaction

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ARTICLE INFO

Article history:
Received 6 January 2015
Revised 7 April 2015
Accepted 8 April 2015

Keywords:
Plant pathogen
Dothideomycete
Plant defence
Virulence

ABSTRACT

Zymoseptoria tritici (previously *Mycosphaerella graminicola*, teleomorph, *Septoria tritici*, anamorph) causes Septoria tritici blotch, one of the most economically important diseases of wheat (*Triticum aestivum*). The host pathogenic interaction, as currently understood, is intriguing, and may distinguish *Z. tritici* from many of the current models for plant pathogenic fungi. Many important questions remain which require a deeper understanding including; the nature and biological significance of the characteristic long latent periods of symptomless plant infection; how/why the fungus then effectively transitions from this to cause disease and reproduce? Elements of this transition currently resemble a putative "hijack" on plant defence but how is *Z. tritici* able to do this without any form of plant cell penetration? This commentary provides a summary of the recent history of research into the host-pathogen interaction, whilst highlighting some of the challenges going forwards, which will be faced by improved technologies and a growing research community.

This commentary on the *Zymoseptoria tritici* vs. wheat host-pathogen interaction seeks to address progress that has been made in the last decade of research, dating from approximately 2004. The main molecular genetic resources available at that time to study the susceptible interaction included two variable size fungal EST collections (Keon et al., 2005a; Kema et al., 2008), an approximate predicted one quarter genome cDNA microarray (Keon et al., 2005b), and ~300,000 wheat ESTs (mostly not from leaves), some of which were just being used to fabricate early wheat microarrays (http://www.plexdb.org/). However, some very useful experimental tools/procedures had already been developed, in particular the Agrobacterium-mediated fungal transformation procedure (Zwiers and De Waard, 2001), which facilitated the first direct identification of a virulence gene from *Z. tritici*, namely the ABC transporter, MgAtr4 (Stergiopoulos et al., 2003). For wheat, reverse genetics was not so "easy", and whilst stable transformation with RNAi constructs was being developed it was, and remains, a relatively time consuming methodology, which had not really addressed many pathogenic interactions.

This article covers only the susceptible disease interaction between *Z. tritici* and wheat and will not address resistant cultivar interactions, which are described elsewhere in this issue. For the "compatible" (or susceptible) interaction alone poses many intriguing questions still pertinent today. Many of these concern the extensive "latent period" of symptomless fungal colonisation, described as the period of time from which the fungus arrives on the host plant (inoculation) to the time when disease symptoms are macroscopically visible and sporulation has commenced (Leonard and Mundt, 1984). Long latent periods are now recognised as quite a conserved and peculiar feature of plant infection by most *Mycosphaerella* fungi, and can in some cases extend to several months. What purpose this serves, its genetic and biochemical basis, and why and how it suddenly ends with the induction of plant cell death remain some of the most scientifically interesting questions for this pathosystem, and others involving related *Mycosphaerella* fungi. This is even more remarkable when you also consider that *Z. tritici* does not appear to penetrate plant cells at any point during infection (Kema et al., 1996), instead exclusively colonising the intercellular spaces following initial entry through plant stomata right through to its exit, via the same route (Fig. 1). There is no such thing as "typical" for this system, but the symptomless latent period for *Z. tritici* on susceptible wheat is usually in the region of 7–14 days prior to leaf cells dying and the onset of fungal asexual sporulation. This suggests exquisite and dynamic communication mechanisms must exist throughout the interaction, and that the onset of wheat cell death is tightly regulated both temporally and spatially (Dean et al., 2012).

The overriding consensus a decade ago was that localised plant cell death in response to microbial pathogens functioned only as an exquisitely organised plant disease resistance response (Heath,

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http://dx.doi.org/10.1016/j.fgb.2015.04.005
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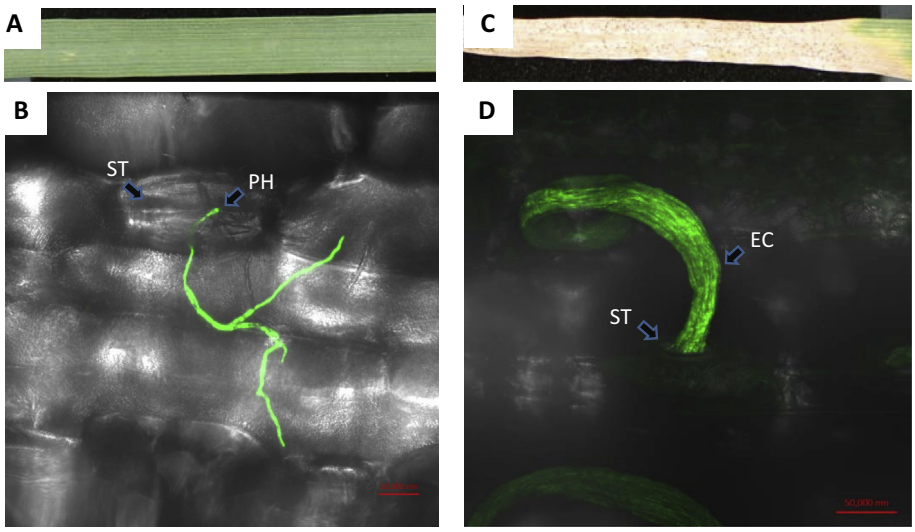

Fig. 1. *Z. tritici* uses a strictly extracellular mode of plant pathogenesis with a long latent period for disease development. (A) Susceptible wheat leaf infected with a GFP expressing isolate of *Z. tritici* at 1 day post surface inoculation (1-dpi). (B) Stereomicroscope image of GFP tagged hyphal filaments developing on the leaf surface and penetrating the leaf through a stomatal aperture. Image taken at 1-dpi. ST = stomatal aperture; PH = penetrating hyphae. (C) Susceptible wheat leaf infected with a GFP expressing isolate of *Z. tritici* at 21 day post inoculation (21-dpi). (D) Stereomicroscope image of GFP tagged asexual spore masses exuding within a cirrus from below a leaf stomata. Image taken at 21-dpi. ST = stomatal aperture; EC = extracellular oozing cirrus containing new asexual pycnidiospores.

2000), which it probably is against biotrophs and some hemibiotrophs. In contrast cell death occurring during infection by necrotrophs and/or other hemibiotrophs was just some form of random disorganised collapse under the attack of arsenals of pathogen-derived hydrolytic enzymes and toxins. The aforementioned work from Kema and associates (1996) on the histology of infection had already shown that *Z. tritici* did not extensively penetrate plant cells during infection. But it still elicited plant cell death somehow from its extracellular location, and also took over a week to do it. This excellent study was performed using traditional scanning and transmission electron microscopy and first identified some form of host cell perception of fungal hyphae taking place around the onset of plant cell death. This was associated with specific sub-cellular alterations including the movement of particular organelles towards hyphae and irregular enlargement of chloroplast structures. This suggested that the plant cells had begun to "recognise" and respond to something associated with the encroaching extracellular fungal hyphae. Exactly what factors might be recognised is still unknown although a consensus is emerging in several labs that perhaps host selective protein toxins produced in a temporally regulated manner on the switch to necrotrophy might play a role. This would represent a slightly modified model to what has been shown for the wheat pathogens *Stagonospora nodorum* and *Pyrenophora tritici-repentis* in particular (Oliver and Solomon, 2010; Winterberg et al., 2014). This model is currently being tested for *Z. tritici* (see Ben M'Bareck et al., 2015 and Gohari et al., 2015), but it is clear for the plant side of the interaction that the transition to disease symptoms involves very specific changes in gene expression and the activation of signalling pathways which are more commonly associated with plant "defence" (Keon et al., 2007; Rudd et al., 2008, 2015; Yang et al., 2013). Wheat leaf cells ultimately appear to undergo a form of regulated programmed cell death (PCD) in the vicinity of *Z. tritici* hyphae (Keon et al., 2007), in response to these as yet unidentified pathogen cues. The fact that the pathogen effectively reproduces (asexual sporulation) in this environment suggests that the plants effort to "defend" itself has in some way been manipulated or "hijacked" by the pathogen to support its asexual reproduction. This is an attractive model but one which admittedly requires further testing.

Arguably the most significant recent progress has been made in the area of *Z. tritici* genomics. The first publically available genome resource for *Z. tritici* resulted from the actions of a research community led by Dutch and US scientists who lobbied for some time to get a reference genome of *Z. tritici* sequenced and assembled. The case was supported, eventually, by re-emphasizing the point that *Z. tritici* belonged to one of the largest groups of plant pathogenic fungi, the *Dothideomycetes*, and that numerous *Mycosphaerella* species within this group were responsible for causing many of the world's most important crop diseases (Goodwin, 2004). Despite this there were few available sequenced genomes covering these organisms at that time (Goodwin, 2004). Moreover the sequencing and assembly, done in collaboration with the United States Department of Energy-Joint Genome Institute (US DOE-JGI) was such a great success that the project was extended to produce a "finished" genome of the reference isolate, IPO323 (Goodwin et al., 2011). The high quality of this reference genome (http://genome.jgi-psf.org/Mycgr3/Mycgr3.home.html) has since facilitated comparative studies with sequences from closely related species (Stukenbrock et al., 2011), along with members of the larger *Dothideomycete* class in general (Ohm et al., 2012) with the variable aims of understanding host adaptation, evolution and virulence mechanisms. Of the many notable observations arising from these genome sequences was that *Z. tritici,* and *Mycosphaerella* species in general, have relatively low numbers of predicted secreted plant cell wall attacking enzymes (Goodwin et al., 2011; do Amaral et al., 2012). This is intriguing and may have evolved as either a cause or a consequence of their extracellular lifestyles on their hosts.

It is arguably the search for new virulence and pathogenicity genes which will potentially gain most from the new tools for *Z. tritici* which are described in the range of accompanying articles. Relatively speaking, and despite its agricultural importance, the number of genes which have been shown to contribute to pathogenicity and virulence of *Z. tritici* is small (Table 1 lists 17 to date). Moreover this list is largely made up of global regulators of metabolism and cell signalling including components of mitogen-activated protein kinase pathways and cyclic nucleotide signalling for example. Only one secreted protein effector currently makes this list, the chitin binding protein 3LysM (Marshall et al., 2011). Many, if not all, of these genes have already been ascribed