Fusarium graminearum and its interactions with cereal heads

studies in the proteomics era

Yang, Fen; Jacobsen, Susanne; Jørgensen, Hans Jørgen Lyngs; Collinge, David B.; Svensson, Birte; Finnie, Christine Susan

Published in:
Frontiers in Plant Science

DOI:
10.3389/fpls.2013.00037

Publication date:
2013

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Fusarium graminearum and its interactions with cereal heads: studies in the proteomics era

Fen Yang1,2*, Susanne Jacobsen2, Hans J. L. Jørgensen1, David B. Collinge1, Birte Svensson2 and Christine Finnie2

1 Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark
2 Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Frederiksberg C, Denmark

*Correspondence: Fen Yang, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Thørvilssensvej 40, 1871 Frederiksberg C, Denmark. e-mail: yangf@fle.ku.dk

INTRODUCTION

The pathogen Fusarium graminearum causes devastating head blight of small grain cereals including wheat and barley. Fusarium head blight (FHB), as a global problem, has great economic impact on the cereal industry due to the reduced grain yield and quality as well as to the contamination by diverse mycotoxins, including deoxynivalenol (DON) and zearalenone, which are harmful for humans and animals.

The disease (Figure 1A) is initiated by deposition of spores on or inside flowering spikelets (Bushnell et al., 2003). Fungal hyphae develop on the exterior surfaces of florets and glumes, rather than by direct penetration through the epidermis, prior to the colonization of anthers, stigmas, and lodicules (Bushnell et al., 2003). The fungus spreads in wheat from spikelet to spikelet through the vascular tissue in the rachis and rachilla (Trail, 2009) and this is associated with the production of DON, a virulence factor (effector molecule) causing tissue necrosis (Jansen et al., 2005).

In barley, spread of the disease is limited and virulence does not appear to be due to the presence of the toxin (Maier et al., 2006).

As a result of its devastating effects, F. graminearum has been under intense investigation for many years to understand the genetic basis of the life cycle, pathogenicity, evolution, and population biology. Availability of the full genome sequence (Ma et al., 2010) considerably revitalizes research of gene function in F. graminearum. In addition to classical biochemical, genetic, molecular biological, and plant pathology approaches, several "omics" techniques are employed in the studies of F. graminearum and its interactions with hosts. Transcriptome and metabolome analysis have been conducted in F. graminearum during the invasion of hosts, sexual development, and conidial germination, in response to azole fungicide and/or in F. graminearum mutants as well as in barley and wheat during infection to understand defense responses (reviewed by Kazan et al., 2012). In silico prediction of the secretome of F. graminearum has also been performed to identify potential pathogenicity factors and effectors (Brown et al., 2012). Proteomics, as the core technology in functional genomics, allows interpretation of gene function, determination of protein abundance, interactions, modifications, locations, and implications in development and environmental responses (Wright et al., 2012). In the present review, we focus on the recent progress made by using proteomics techniques to enhance the understanding of cellular and molecular mechanisms of F. graminearum pathogenicity and virulence as well as the host defense responses.

PROTEOMICS TECHNIQUES IN PHYTOPATHOGENIC FUNGI

Proteome analysis of phytopathogenic fungi and their interactions with hosts has increased dramatically over the last years, because of the technical development of "omics" and bioinformatic tools, and the growing number of fungal genomes being sequenced. Investigations in this area mainly are (i) identification of mycelial, conidal, and secreted proteins across a range of fungal species by establishing reference proteome maps of these fungal structures. Proteome profiles are conducted and/or compared between species, races, mutants, growth, development stages, and growth conditions (Gonzalez-Fernandez et al., 2010), in particular during spore germination, hyphal penetration, appressorium formation, toxin production, and secretion (van Kan, 2006), and (ii) plant-fungus interactions to study infection cycles, to identify pathogenicity factors and to study plant defense responses.
Analysis of proteins of some fungal species in planta is limited due to the fact that it is difficult to isolate fungal tissues from the infected hosts and that the fungal biomass constitutes a small portion of the total biological material resulting in the dominance of plant proteins. Besides fungi with agricultural interest, such as Botrytis cinerea, Sclerotinia sclerotiorum, and F. graminearum (reviewed by Gonzalez-Fernandez and Jorrin-Novo, 2012), important studies employing a diversity of proteomics techniques have been performed on major crops, including rice, maize, wheat, and barley interacting with fungal pathogens, in addition to Arabidopsis thaliana (reviewed by Gonzalez-Fernandez et al., 2010).

The workflow of proteomic analysis in phytopathogenic fungi is shown in Figure 1B. Experimental design and sampling reflect the aim of the study, i.e., whether it has focus on the fungus or the plant under the chosen conditions. The protein extraction protocol is a very critical step and determines which proteins are available for analysis. This step is particularly challenging for both plants and fungi, because of their robust cell walls in addition to proteases and different non-protein components which can interfere both with the population and quality of the proteins and their subsequent separation (Hurkman and Tanaka, 2007). Post-translational modifications (PTMs) can also be analyzed using proteomics, but require selective enrichment and purification strategies due to their reversible and labile nature and low stoichiometric abundances. Some PTMs, such as phosphorylation, glycosylation, acetylation, phenylation, S-nitrosylation, and ubiquitylation, are involved in signal transduction during plant-microbe interactions and have been analyzed by proteomics (Jayaraman et al., 2012).

Protein separation in the majority of earlier proteomics studies was based on two-dimensional gel electrophoresis (2-DE) coupled with conventional staining methods. Difference gel electrophoresis (DIGE), where samples are labeled differentially with fluorophores, allows distinction between proteins obtained in different samples that can be resolved on the same gel. This can address the issues of both sensitivity and gel variability in 2-DE (Wright et al., 2012). However, DIGE suffers from the same problems as traditional 2-DE, especially in relation to the resolution of hydrophobic proteins and proteins exhibiting extreme pls and molecular weights. Currently, gel-free techniques for separating peptides become standard for large-scale shotgun proteomics, which can overcome some of the limitations of the gel-based approach. The methods are based on the pre-fractionation of peptide mixtures by monodimensional LC or multidimensional protein identification technology (MudPIT) such as strong cation exchange (SCX) combined with reversed phase chromatography (Gilmore and Washburn, 2010).

Mass spectrometry (MS), consisting of an ion source, a mass analyzer, and a detector, is the most common technique for unbiased protein identification ( Aebersold and Mann, 2003). The various techniques for ionizing samples include matrix assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). The mass analyzers include time-of-flight (TOF), ion trap, quadrupole, orbitrap, and fourier transform ion cyclotron resonance. In MS/MS, specific precursor ions produced in the initial mass analyzer are chosen and fragmented, resulting in sequence-informative fragment ion spectra. Fragmentation methods can be collision-based (e.g., CAD and HCD) or electron-based (e.g., ECD and ETD) dissociation (Coon, 2009). Observed ion spectra are compared against databases containing known protein sequences by search algorithms (e.g., SEQUEST, Mascot, and OMSSA) for protein identification.
Comparative proteomics can be based on the traditional pre-staining of 2-DE gels such as Coomassie Blue staining, silver staining, and fluorescence staining and the modern label-free or labeling approaches at the MS stage, followed by the statistical, and bioinformatics analysis to determine the significance of data. Isotope-assisted quantification methods include in vitro chemical (e.g., ICAT, iTRAQ, TMT, and 18O) and in vivo metabolic (e.g., SILAC and ^15^N-labeling) labeling of biological samples. In chemical labeling, distinct protein samples are labeled with heavy and light isotopes or isobaric tags, pooled, and compared by MS. Stable isotope labeling of amino acids in cell culture (SILAC) or plants that are grown on media supplemented with heavy isotope-containing amino acids, allows for labeling of proteins as they are synthesized (Ong et al., 2002). The relative ratio of protein from different samples is determined by the ratios of signal intensities of the labeled peptides that are common to the samples in MS analysis. Label-free quantification compares samples based on the measurement of changes in peptide peak areas or peak heights in chromatography and peptide peak intensity in MS or the spectral counting of identified proteins after MS/MS analysis (Neilson et al., 2011).

**FUSARIUM GRAMINEARUM PROTEOME ANALYSES**

Proteomics studies conducted on *F. graminearum* have focused mainly on the secretome and impact of DON in pathogenicity. The first in vitro gel-based secretome study in *F. graminearum* was performed in a culture with a medium containing either glucose or hop cell walls. Here, 23 and 84 unique proteins were identified, respectively, mainly involved in cell wall polysaccharide degradation (Phalip et al., 2005). Using LC-MS/MS, 229 fungal proteins, mostly glycoside hydrolases and proteases, were identified in the secretome of *F. graminearum* during growth on 13 synthetic media (Paper et al., 2007). To closely mimic the nutritional situation of the fungus *in planta*, Yang et al. (2012) employed a gel-based proteomics approach to access the secretome in the growth cultures with barley or wheat flour as the sole nutrient source, resulting in the identification of 69 unique fungal proteins including enzymes involved in the degradation of cell walls, starch, and proteins. Secreted proteins differing in accumulation between wheat and barley flour media were mainly involved in fungal cell wall remodeling and the degradation of plant cell walls, starch, and proteins. To analyze the effect of DON production in host infection process, *F. graminearum* was grown on a medium promoting trichothecene biosynthesis (Taylor et al., 2008). Here, comparative proteomics showed 130 differentially expressed fungal proteins, of which proteins potentially involved in virulence were up-regulated, whereas down-regulated proteins were primary metabolic enzymes, chaperones, and proteins involved in translation.

Two phosphoproteome studies of *F. graminearum* under nitrogen limiting conditions and under conditions of unlimited nutrients have been published recently (Rampitsch et al., 2010, 2012). It was suggested that phosphorylation events are involved in the signaling pathways, leading to the activation of the trichothecene pathway, which is also activated in *F. graminearum* under nutrient stress (Rampitsch et al., 2010). A total of 348 phosphorylation sites localized to 301 peptides from 241 proteins including 10 protein kinases and seven transcription factors were identified during nitrogen starvation. When *F. graminearum* was grown *in vitro* without nutritional limitation, 2902 putative phosphopeptides with homologous matches to 1496 different proteins were identified (Rampitsch et al., 2012). Here, the majority of phosphoproteins were nuclear proteins with ATP-binding function and the phosphorylation sites were conserved in three phosphopeptides from transcription elongation factor 1β, acidic ribosomal proteins, and glycogen synthase.

Although it is very challenging to identify large numbers of *F. graminearum* proteins *in planta*, Paper et al. (2007) extracted *F. graminearum* secreted proteins from infected wheat heads by vacuum filtration, resulting in the identification of 120 fungal proteins including several cell wall degrading enzymes, of which 56% contained putative secretion signals. Additionally, proteomics analyses of *F. graminearum*-infected barley spikelets at maturity (Yang et al., 2010a) and 2 days after inoculation (dai; Yang et al., 2010b) as well as wheat spikelets from 1 to 3 dai (Zhou et al., 2006), revealed nine, one, and eight fungal proteins, respectively. The identification of fungal stress-related and antioxidant proteins *in planta* strongly suggests that the pathogen is exposed to stresses such as oxidation and starvation and that it attempts to overcome plant defense.

**PROTEOMICS STUDIES OF HOST DEFENSE TO FUSARIUM GRAMINEARUM**

Extensive proteomics studies have been conducted in *F. graminearum*-infected wheat, barley, and their wild relatives (Table 1). With the exception of one recent study (Gunnaiah et al., 2012) using shotgun proteomics, other studies have employed gel-based techniques to investigate the differentially expressed proteins of hosts with different levels of disease susceptibility at different time points after inoculation at anthesis or during germination (Table 1). Due to the use of different cultivars, inoculation methods, infection stages, growth conditions, and proteomic techniques, little overlap is apparent between the regulated proteins identified in these studies. In resistant and/or susceptible wheat in response to *F. graminearum* up to 5 dai, many proteins related to carbon metabolism and photosynthesis were down-regulated, whereas the up-regulated proteins could be involved in antioxidant, jasmonic acid, and ethylene signaling pathways, phenylpropanoid biosynthesis, antimicrobial compound synthesis, detoxification, cell wall fortification, defense-related responses, amino acid synthesis, and nitrogen metabolism. Wheat susceptibility likely reflected the delayed activation of the salicylic acid defense pathway (Ding et al., 2011). Moreover, distinct abundance patterns of different xylanase inhibitor forms and pathogenesis-related (PR) proteins were shown in the wheat ear in response to the *F. graminearum* ΔTri5 mutant at 5, 15, and 25 dai (Dornez et al., 2010).

When the proteomes of mature grains of susceptible barley infected by *F. graminearum* under two different levels of nitrogen fertilizers were analyzed, massive, fungus-induced degradation of the grain proteome was observed and increased *Fusarium* infection occurred with low N amount (Yang et al., 2010a). In contrast, Zantinge et al. (2010) observed no degradation of seed proteomes...
Table 1 | Original proteomics papers published on *F. graminearum* and its interactions with wheat and barley.

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>Sampling times</th>
<th>Sample materials</th>
<th>Proteomics techniques</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>In the growth media containing either glucose or hop cell walls</td>
<td>6 d, 9 d</td>
<td>Culture supernatants</td>
<td>1-DE, 2-DE, LC-MS/MS</td>
<td>Analysis of the fungal <em>in vitro</em> secretomes</td>
<td>Phalip et al. (2005)</td>
</tr>
<tr>
<td>In the synthetic media containing polysaccharide supplements</td>
<td>7 d</td>
<td>Culture supernatants</td>
<td>1-DE, LC-MS/MS</td>
<td>High-throughput analysis of the fungal <em>in vitro</em> secretomes</td>
<td>Paper et al. (2007)</td>
</tr>
<tr>
<td>In the wheat grains</td>
<td>Maturity</td>
<td>Fungal secretome</td>
<td>1-DE, LC-MS/MS</td>
<td>Analysis of the fungal <em>in planta</em> secretomes</td>
<td>Paper et al. (2007)</td>
</tr>
<tr>
<td>In the growth medium promoting trichothecene biosynthesis after 2-day growth in the rich medium</td>
<td>0, 4 d, 9 d, 12 d</td>
<td>Fungal tissues</td>
<td>iTRAQ, LC-MS/MS 2-DE, MS/MS</td>
<td><em>In vitro</em> time course study of the changes in fungal intercellular proteomes due to the induction of trichothecene production</td>
<td>Taylor et al. (2008)</td>
</tr>
<tr>
<td>In the growth medium containing only barley or wheat flour</td>
<td>7 d</td>
<td>Culture supernatants</td>
<td>2-DE, MALDI-MS/MS</td>
<td>Study of the fungal <em>in vitro</em> secretomes under growth conditions which mimic <em>in planta</em> nutritional situation</td>
<td>Yang et al. (2012)</td>
</tr>
<tr>
<td>In the growth medium with limited nitrogen after 2-day growth in the rich medium</td>
<td>0, 6 h, 12 h</td>
<td>Fungal tissues</td>
<td>2-DE, MALDI-MS, 1-DE, IMAC, TiO₂, LC-MS, SAX, IMAC, LC-MS/MS</td>
<td>Analysis of the fungal phosphoproteomes under the <em>in vitro</em> growth condition that activates trichothecene pathway</td>
<td>Rampitsch et al. (2010)</td>
</tr>
<tr>
<td>In the growth medium with unlimited nutrients</td>
<td>1 d</td>
<td>Fungal tissues</td>
<td>SCX, IMAC, LC-MS/MS</td>
<td>Analysis of the fungal <em>in vitro</em> phosphoproteomes</td>
<td>Rampitsch et al. (2012)</td>
</tr>
<tr>
<td>Virus-free and -infected strains grown in the complete medium</td>
<td>5 d</td>
<td>Fungal tissues</td>
<td>2-DE, LC-MS/MS</td>
<td>Study of the fungal proteomes in response to viral infection</td>
<td>Kwon et al. (2009)</td>
</tr>
<tr>
<td>In the resistant wheat spikes</td>
<td>6 h, 12 h, 24 h</td>
<td>Wheat spikes</td>
<td>2-DE, MALDI MS</td>
<td>Study of the differential expressed wheat proteins in response to fungal infection</td>
<td>Wang et al. (2005)</td>
</tr>
<tr>
<td>In the susceptible and resistant wheat spikes</td>
<td>5 d</td>
<td>Wheat spikelets</td>
<td>2-DE, LC-MS/MS</td>
<td>Study of the differential expressed wheat proteins in response to fungal infection</td>
<td>Zhou et al. (2005)</td>
</tr>
<tr>
<td>In the susceptible wheat ears</td>
<td>1 d, 2 d, 3 d</td>
<td>Wheat spikelets</td>
<td>2-DE, LC-MS/MS</td>
<td>Identification of wheat proteins regulated by the fungus and fungal expressed proteins <em>in planta</em></td>
<td>Zhou et al. (2006)</td>
</tr>
<tr>
<td>In the susceptible wheat ears</td>
<td>5 d, 15 d, 25 d</td>
<td>Wheat ears</td>
<td>DIGE, MALDI MS/MS</td>
<td>Investigation of the changes in xylanase inhibitors (iso)forms of wheat due to fungal TirB mutant infection</td>
<td>Dornez et al. (2010)</td>
</tr>
<tr>
<td>In the susceptible and resistant wheat spikes</td>
<td>12 h</td>
<td>Wheat spikes</td>
<td>2-DE, MALDI MS</td>
<td>Study of the differential expressed wheat proteins and genes in response to fungal infection</td>
<td>Ding et al. (2011)</td>
</tr>
<tr>
<td>In the moderate resistant wheat spikes</td>
<td>48 h</td>
<td>Wheat spikes</td>
<td>2-DE, MALDI MS</td>
<td>Study of the differential expressed wheat proteins in response to fungal infection</td>
<td>Shin et al. (2011)</td>
</tr>
</tbody>
</table>

(Continued)
### Table 1 | Continued

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>Sampling times</th>
<th>Sample materials</th>
<th>Proteomics techniques</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>In wheat carrying either resistant or susceptible alleles at the Fhb 1 locus</td>
<td>72 h</td>
<td>Wheat spikelets</td>
<td>LC-MS/MS, spectral counting</td>
<td>Identification of mechanisms of resistance governed by the FHB resistance locus Fhb 1</td>
<td>Gunnaiah et al. (2012)</td>
</tr>
<tr>
<td>In emmer heads and co-colonization with Fusarium culmorum</td>
<td>Maturity</td>
<td>Emmer grains</td>
<td>2-DE, LC-MS/MS</td>
<td>Study of the differential expressed emmer seed proteins in response to fungal infection</td>
<td>Eggert et al. (2011)</td>
</tr>
<tr>
<td>In the spikes of six barley genotypes of varying resistance</td>
<td>3 d</td>
<td>Barley spikelets</td>
<td>2-DE, LC-MS/MS</td>
<td>Study of the differential expressed barley proteins in response to fungal infection</td>
<td>Geddes et al. (2008)</td>
</tr>
<tr>
<td>In the susceptible barley spikes grown under different N fertilizers</td>
<td>Maturity</td>
<td>Barley seeds</td>
<td>2-DE, MALDI MS/MS</td>
<td>Investigation of effect of nitrogen fertilizer mounts on the severity of FHB and identification of fungal proteins in planta</td>
<td>Yang et al. (2010a)</td>
</tr>
<tr>
<td>In the susceptible barley spikes</td>
<td>2 d</td>
<td>Barley spikelets</td>
<td>2-DE, MALDI MS/MS</td>
<td>Definition of infection levels correlated to fungal induced plant proteome degradation and identification of the differential expressed barley proteins in response to fungal infection</td>
<td>Yang et al. (2010b)</td>
</tr>
<tr>
<td>In the susceptible barley seeds</td>
<td>3 d</td>
<td>Germinating barley seeds</td>
<td>2-DE, MALDI MS/MS</td>
<td>Study of the differential expressed barley seed proteins in response to fungal infection during germination</td>
<td>Yang et al. (2011)</td>
</tr>
<tr>
<td>In the spikes of eleven barley genotypes of varying resistance</td>
<td>Maturity</td>
<td>Barley seeds</td>
<td>2-DE, LC-MS/MS</td>
<td>Study of the differential expressed barley seed proteins in response to fungal infection</td>
<td>Zantinge et al. (2010)</td>
</tr>
<tr>
<td>In the naked barley heads and co-colonization with Fusarium culmorum</td>
<td>Maturity</td>
<td>Naked barley grains</td>
<td>2-DE, MALDI MS, LC-MS/MS</td>
<td>Identification of the differentially expressed seed proteins in response to fungal infection and to growing location of the plant</td>
<td>Eggert and Pawelzik (2011)</td>
</tr>
</tbody>
</table>
A lack of wheat and barley genome sequences, although barley and these genes have not been fully investigated, except the PR-proteins identified proteins or genes is required to elucidate their roles in enhanced resistance. The reasons can be that some proteins of interest are actually not found by proteomics due to low abundance in enhanced resistance. The reasons can be that some proteins of interest are actually not found by proteomics due to low abundance.
Proteome analysis of Fusarium infection in emmer grains (Triticum durum). 
Plant Pathol. 60, 918–928.

Transgenic expression of polygalacturonase-inhibiting proteins in Arabidopsis 
and wheat increases resistance to the flower pathogen Fusarium graminearum. 

Differential expression of proteins in response to the interaction between 
the pathogen Fusarium graminearum 
and its host, Hordeum vulgare. 
Proteomics 8, 545–554.

Advances in shotgun proteomics 

Contribution of proteomics to the study of plant 
pathogenic fungi. J Proteome Res. 11, 3–16.


Comparative proteomics of the exoproteome of 
Fusarium graminearum 
infected with Fusarium graminearum virus D2K1. Virus Res. 144, 96–106.

Expression of a radiaph defenser in transgenic wheat confers increased resistance 
to Fusarium graminearum and Rhizoctonia cerealis. 
Funct. Integr. Genomics 11, 63–70.

Comparative genomics reveals mobile pathogen 
genome characteristics in Fusarium. 

Overexpression of defense response genes in transgenic wheat 
enhances resistance to Fusarium 

Involvement of tri-cholesterenic in fusarios of wheat, 
barley and maize evaluated by gene 
disruption of the trichodiene syn- 
thase (Tri7) gene in three field isolates of different 
cheletype and vir- 

Genetically engineered resistance 
to Fusarium head blight in wheat 
by expression of Arabidopsis NPR1. 
Mol. Plant Microbe Interact. 19, 123–129.

Neilson, K. A., Ali, N. A., Murakihara, S., Mirzaei, M., Mariandi, M., 
Assadourian, G., et al. (2011). Less label, more free: approaches in label- 
free quantitative mass spectrometry. 
Proteomics 11, 535–553.

Ong, S. E., Blagoev, B., Kratchmarova, I., Korsbet, D., Steen, H., Panze, A., 
and Mann, P. (2002). Stable isotope labeling of 
proteins for human and murine 
proteomics by stable isotope labeling 
itoty acids in cell culture, SILAC, as a simple and accurate 
approach to expression proteomics. 

Comparative proteomics of extracellular proteins in vitro 
and in plants from the pathogenic fungus Fusarium graminearum. 
Proteomics 7, 3171–3183.

Fusarium ear blight (scab) in small grain cereals: a review. 
Plant Pathol. 44, 207–238.

exoproteome of Fusarium graminearum grown on 

The phosphopro- 
teome of Fusarium graminearum 
at the onset of nitrogen starvation. 
Proteomics 10, 124–140.

Phosphoprote- 
tome profile of Fusarium gramin- earum grown in vitro under non- 
limiting conditions. Proteomics 12, 1002–1005.

Shin, K. H., Kamal, A. H. M., Cho, K., 
Defense proteins are induced in 
wheat spikes exposed to Fusarium 
graminearum. Plant Mol. 4, 270–277.

Shin, S., Mackintosh, C. A., Lewis, J., Heinen, S. I., Radimer, L. D., 
Transgenic wheat 
expressing a barley class II chitinase 
genase gene has enhanced resistance against 

Proteomic analy- 
yses of Fusarium graminearum grown under mycotoxin-inducing condi- 
tions. Proteomics 8, 2256–2265.

The International barley Genome 
Sequencing Consortium (2012). 
A physical, genetic and functional 
sequence assembly of the barley 

Trail, F. (2009). For blighted waves of 
grain: Fusarium graminearum in the 
postgenomics era. Plant Physiol. 149, 103–110.

vanKan, J. A. L. (2006). Licensed to 
kill: the life style of a necrotrophic 

Volpi, C., Janni, M., Lionetti, V., 
expression of a pectin methyl 
esterase inhibitor gene increases pectin 
methyl esterification and limits fungal 


Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 November 2012; paper pending published: 04 January 2013; accepted: 12 February 2013; published online: 28 February 2013.


This article was submitted to Frontiers in Plant Proteomics, a specialty of Frontiers in Plant Science.

Copyright © 2013 Yang, Jacobsen, Jørgensen, Collinge, Svensson and Finnie. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.