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A proteomics survey on wheat susceptibility to *Fusarium* head blight during grain development

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Abstract

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The mycotoxigenic fungal species *Fusarium graminearum* is able to attack several important cereal crops, such as wheat and barley. By causing *Fusarium* Head Blight (FHB) disease, *F. graminearum* induces yield and quality losses and poses a public health concern due to *in planta* mycotoxin production. The molecular and physiological plant responses to FHB, and the cellular biochemical pathways used by *F. graminearum* to complete its infectious process remain still unknown. In this study, a proteomics approach, combining 2D-gel approach and mass spectrometry, has been used to determine the specific protein patterns associated with the development of the fungal infection during grain growth on susceptible wheat. Our results reveal that *F. graminearum* infection does not deeply alter the grain proteome and does not significantly disturb the first steps of grain ontogeny but impacts molecular changes during the grain filling stage (impact on starch synthesis and storage proteins). The differentially regulated proteins identified were mainly involved in stress and defence mechanisms, primary metabolism, and main cellular processes such as signalling and transport. Our survey suggests that *F. graminearum* could take advantage of putative susceptibility factors closely related to grain development processes and thus provide new insights into key molecular events controlling the susceptible response to FHB in wheat grains.

Keywords

Susceptibility factors; Wheat; *Fusarium graminearum*; FHB; Mycotoxin

Introduction

Pathogen-susceptible plants could be connected at least in part to host-susceptibility genes or more broadly, to host-susceptibility factors including a range of determinative factors able to favour pathogen growth (Li et al. 2013; González-Lamothe et al. 2012). Increasing evidence has already shown that lacking of these pivotal host genes/factors could hamper the infection process or even confer plant resistance while they could be essential for plant growth. More than 30 potential host susceptibility genes (S-genes) in different plant species have been reported in the literature (Pavan et al. 2010), depicting either that transient knock-down of such genes could increase the plant resistance or that the overexpression of other ones could lead to increasing susceptibility. For instance, among S-genes, *PMR6* and *MLO* genes were discovered as genes coding for susceptibility factors promoting growth of powdery mildews (Eckardt 2002), *ADH1* gene modulates the susceptibility of *hordei spp* plant to *Blumeria graminis* fungus (Pathuri et al. 2011) and recently *IAA-Asp* gene was identified as a susceptibility gene as it promotes the growth of *Botrytis cinerea* in *Arabidopsis thaliana* (González-Lamothe et al. 2012). Digging into S-genes or S-factors could provide a wealth of additional knowledge to understand plant susceptibility and could be a means to find new strategies aiming at sustainable plant resistance.

Fusarium Head Blight (FHB) is among the most devastating disease worldwide, affecting wheat and other small grains cereals (Goswami and Kistler 2004). This destructive and economically important disease is caused primarily by the mycotoxigenic ascomycetous fungus *Fusarium graminearum* (*Fg*). FHB causes not only lower yields, but also grain contamination by producing mycotoxins (e.g., deoxynivalenol, DON) which are very

harmful for human and animal supplies (Bottalico and Perrone 2002; Jansen et al. 2005; Ilgen et al. 2009). The plant symptoms and responses to *Fg* attack are diverse, tissue-specific and vary according to the plant species. Up to now, several resistant genotypes are used as a means to control FHB in field. Three main types of FHB resistance can be described: type I refers to the resistance to initial infection (Mesterházy 1995), type II depicts the resistance to the spread of pathogen within the spike (Schroeder 1963) and type III refers to the resistance to DON synthesis (Miller et al. 1985). To our knowledge, FHB determinism has been mainly assessed from resistant genotypes to trace the so-called resistance genes (R-genes). Resistance to FHB in wheat is quantitative and is governed by many QTLs which control different types of resistance (Bai and Shaner 2004). Among the one hundred FHB resistance QTLs identified in wheat, the *Fhb1* locus, which was located in chromosome 3BS, explains up to 60 % of the phenotype variation for type II FHB resistance and is seen as a major QTL for this disease (Cuthbert et al. 2006; Buerstmayr et al. 2009). Comparisons of protein expression profiles between the unique pair NILs contrasting in *Fhb1* alleles allowed the identification of nine types of either induced or up-regulated proteins that were associated with wheat FHB resistance in the *Fhb1*+ NIL. These differentially expressed proteins may be involved in multiple processes which make difficult the understanding of molecular events linked to FHB resistance (Zhang et al. 2013a). Likewise, many data have been accumulated about the defence mechanisms involved in wheat resistance to FHB (Zhou et al. 2002; Buerstmayr et al. 2003), either by using transcriptomic (Gottwald et al. 2012a; Kugler et al. 2013) or proteomic approaches (Zhou et al. 2005; Ding et al. 2011; Gunnaiah et al. 2012; Zhang et al. 2013b). Most of these studies were primarily focused on understanding the genetic basis of major QTLs (e.g., *fhb1*). Proteomic studies pointed diverse mechanisms of resistance such as oxidative burst, oxidative stress, response and induction of PR proteins, changes in hormones and secondary metabolism in barley (Yang et al. 2013) and in wheat (Hamzehzarghani et al. 2005; Yang et al. 2013). Another study combining proteomics and metabolomics approaches suggested that the *fhb1* resistance is mainly associated with cell wall thickening due to deposition of hydroxycinnamic acid amides, phenolic glucosides and flavonoides, but not with the conversion of DON to less toxic DON 3-O-glucoside (Gunnaiah et al. 2012). Furthermore, transcriptomics also revealed the activation of ABC transporters, UDP-glucosyltransferases and proteases in different FHB resistant genetic backgrounds (Gottwald et al. 2012b). The *Fhb1* QTL derived from the resistant genotype *sumai3*, showed greater accumulation of transcripts of Pathogen-Related proteins such as PR5, PR14, ABC transporter and JA signalling pathway (Xiao et al. 2013). In addition, Kugler et al. (2013) found that the G-protein coupled receptor kinase and biosynthesis genes for jasmonate and ethylene were earlier induced in plants including *Fhb1* QTL, and they found genes involved in biosynthesis and metabolism of riboflavin were more abundant in plants displaying *Qfhq.ifa-5A* QTL. These results show that many efforts have been undertaken to decipher the molecular determinism of plant resistance. Conversely, the understanding of plant susceptibility is still poorly documented in wheat while identification of susceptibility factors has already been reported using deletion mutants (Ma et al. 2006).

Plant susceptibility and related susceptibility factors are expected to be part of the whole range of FHB responses found in susceptible genotypes. Therefore, to gain insights into

susceptibility putative responses in wheat grains, the plant molecular changes occurring during the compatible interaction between a mycotoxigenic *Fg* strain and the susceptible French wheat cultivar Recital were examined during 14 days. In wheat, the grain development involves three main steps: (i) the cell division occurs within 3–6 days after anthesis (daa), and is associated with cell wall initiation and intense mitotic activity; (ii) the differentiation step and endoduplication (7–10 daa) includes the formation of the main cell types (transfer cells, aleurone, starchy endosperm and cells surrounding the embryo); and (iii) the grain filling stage (12–28 daa) (Nadaud et al. 2010). By using proteomic tools (i.e. 2-DE combined to mass spectrometry), we assessed the dynamics of molecular events that occurred specifically in the grain tissue at these three key growth stages to provide for the first time a mapping of *Fg*-responsive proteins and to investigate FHB susceptibility factors during *Fg*-wheat compatible interaction.

Materials and methods

Host and fungal material

During 2009 and 2010, a winter wheat cultivar, so-called “Recital”, was grown in a greenhouse at the INRA Research Center of Clermont-Ferrand (France) without treatment and pesticide protection. Inoculation (i.e. infection) of wheat ears by *FgI* strain was done at the flowering stage (~14 weeks after sowing), especially at mid-anthesis, when about 50 % of the stamens were visible. The inoculum (10^5 spores per ml) was deposited by spraying about 50 µl on both sides of the ears. A mock treatment was applied by spraying water without spores and served as control. After spraying, mock- and *FgI*-treated ears were covered with a bag for 2 hours to maintain sufficient moisture and to promote attachment of the *FgI* spores in inoculated ears. The infection process was surveyed during 14 days at three key grain developmental stages in comparison to control plants: cell division, cell differentiation, and grain filling (Fig. 1a). As a whole, 10 infected and 10 control ears were randomly harvested at 3, 7, and 14 days after inoculation (dai) and immediately frozen in liquid nitrogen. The grains of control and inoculated ears were dissected and ground under liquid nitrogen. Samples were stored at –80 °C until proteomic analysis.

Fg biomass quantification and *FgTri-5* expression

Relative *F. graminearum* biomass quantification was performed on the genomic DNA by using qPCR. The DNA was extracted from 250 mg fresh material according to Rogers and Bendich (1994). DNA quality and concentration were inspected on a Nanodrop system (ThermoScientific, Bonn, Germany). For qPCR, the samples were prepared using the 480 Syber Green I Master Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer protocol and carried out according to the following protocol: denaturation at 95 °C for 5 min, 35 repeats of 95 °C for 10 s, 60 °C for 20 s and 72 °C for 20 s. A melting curve analysis was conducted from 55 °C–95 °C in 0.5 °C steps and 10 s dwell time to confirm the amplification of single amplicons. The amplicon size was confirmed on a 2 % agarose gel. The ratio of *F. graminearum* (*Fg*) to *T. aestivum* (*Ta*) gDNA was determined by qPCR by using primers amplified *FgIGS* (Inter Genic Spacer) and *Ta18S* (ribosomal DNA), respectively. The *FgTRI5* (gene coding for the trichodiene synthase) expression was followed to assess the induction of trichothecene pathway (mycotoxins biosynthesis) during

grain colonization (Ilgen et al. 2009; Jurado et al. 2005). Total RNA was extracted from approximately 250 mg bulk grains using the phenol/chloroform/isoamyllic treatment (Bogorad 1983). Then, the RNA was precipitated using isopropanol. DNase I treatment was applied to remove contaminating genomic DNA. First strand cDNA was synthesized with 1 µg of purified RNA using the iScript reverse transcriptase (Biorad kit). The *TaRLI* (RNase L inhibitor protein) served as reference gene (Giménez et al. 2011). The primers used for qPCR in this analysis are listed in Table 1S. The ratio *FgIGS* to *Ta18S* and the ratio *FgTri5* to *TaRLI* were calculated using the Ct method (Pfaffl 2001).

Proteomics analysis and proteins identification by mass spectrometry (LC-MS/MS)

Protein samples were extracted using the TCA/acetone procedure described by Wang (Wang et al. 1996). The Bradford method was used to quantify protein concentration with BSA as standard (Bradford 1976). Two-dimensional electrophoresis was achieved by using 300 µg of solubilized protein sample. Following IEF and equilibration, the second separation was performed using 24-cm wide gels; the strips were positioned on top of a 14 % polyacrylamide gel in the presence of SDS and sealed with 0.5 % agarose. The gels were run for 30 min at 5 watts per gel followed by 6 h at 10 watts per gel. The gels were then stained using colloidal blue brilliant coomassie CBB G-250 (Sigma, St. Louis, MO, USA) according to Rabilloud (2002). As a whole, four 2-DE gels were run for each treatment from two protein extractions of the 10 pooled ears collected during each experimented year. Gels were scanned with a G-800 scanner (GE Healthcare, Uppsala, Sweden) at 300 dpi and 16-bit grayscale. Image analysis was achieved using Samespots software v4.1 (Nonlinear Dynamics, Newcastle, UK). After spot detection and spot matching, spot intensities were normalized in Progenesis PG240 software (Nonlinear Dynamics, Newcastle, UK) using the total quantity in valid spot method: the quantity of each spot in the gel is divided by the total quantity of all spots in the reference gel. An ANOVA test was then performed on the log-normalized quantities. Spots of interest that were deemed significant ($P \leq 0.05$) for any abundance changes were excised, destained and trypsin-digested as described in Nadaud et al. (2010). LC-MS/MS analysis was performed using the Ultimate 3000 RSLC (Thermo Fisher Scientific, Waltham, MA, USA) with 15 cm nanocapillary columns of an internal diameter of 75 µm (Acclaim Pep Map RSLC, Dionex). The solvent gradient was run from 4 to 50 % acetonitrile in 0.5 % formic acid at a flow rate of 300 nl min⁻¹ for 30 min. Peptides were electrosprayed into an LTQ Velos mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) through a nanoelectrospray ion source. The LTQ Velos was operated in a CID top 10 mode (i.e. one full scan MS from which ten major peaks are selected for MS/MS fragmentation). Xcalibur interface was used to monitor data-dependent acquisition of peptide ions. Database searches were achieved using X!Tandem (<http://www.thegpm.org/TANDEM>). For protein identification, the wheat and *Brachypodium* protein databases were combined with *Fg* database (*Fusarium graminearum* Genome Database V3.2, 2011/03/27; <http://mips.helmholtz-muenchen.de/genre/proj/FGDB/>) and completed to common contaminants. Protein identification was parsed and validated using the X!Tandem Pipeline tool when at least two peptides originating from a single protein were significant (<http://pappso.inra.fr/bioinfo/xtandempipeline/>).

Results

Fg development on wheat grain tissue

The *Fg*-induced responses were surveyed at three key grain developmental stages over a 14-days period (Fig. 1a). During the earliest stage at 3 dai, no obvious symptoms were observed on inoculated spikes while the *FgIGS* qPCR quantification enables a faint detection of *Fg* (Fig. 1b). However at 7 dai, a 3-fold increase of *Fg* biomass occurred along with the appearance of symptoms reaching some of the spikelets and some of the grains. A weak detection of *FgTri5* transcript was also observed, indicating the synthesis of the deoxynivalenol mycotoxin (DON) (Fig. 1c). Finally, at 14 dai, the ears were thoroughly infected and the grains were massively colonized by *Fg* (Fig. 1 and 1S); *FgIGS* quantification depicted abundance 12.000 times greater as 7 dai while *FgTri5* displayed a 15-fold increase.

Comparative analysis of the wheat grain proteome during a time course of *Fg* infection

2-DE gels were analyzed at each developmental stage to detect grain proteins that were reproducibly regulated in response to *Fg* inoculation over the two experimental years. About 2000 protein spots were resolved on all proteome maps. Comparison of protein abundance patterns between control plants versus infected plants at the same developmental stage showed that most of the protein spots did not display any change in their abundance. As a whole, 22 protein spots displayed significant abundance changes at 3 dai, 32 were detected at 7 dai and 21 at 14 dai; noteworthy, each was specific to the developmental stage. Out of these 75 *Fg*-responsive protein spots (Fig. 2S), 73 were successfully identified by LC-MS/MS, 36 were up-regulated (7 at 3 dai, 25 at 7 dai and 4 at 14 dai) whereas 37 were down-regulated (13 at 3 dai, 7 at 7 dai and 17 at 14 dai) (Table 1). The identification results are listed in Table 1 (see Table 2S and 3S for details). Changes detected at 3 dai and 14 dai gathered mainly decreasing protein abundances (31 out of 43 spots) while changes induced at 7 dai gathered mainly increasing protein abundances (Fig. 3S).

To investigate which biological processes the differentially regulated proteins may be involved in, we classified proteins according to the functional categories previously defined in the functional catalogue recently published (Tasleem-Tahir et al. 2012). In our study, responsive proteins to *Fg* infection belonged to five main functional groups including primary metabolism (60 %), translation, cell cycle and DNA repair (19 %), stress and defence (15 %), transport (5 %) and signalling (1 %) (Table 1, Fig. 1d). Whatever the developmental stage, the primary metabolism group was the most widely impacted process including proteins involved in carbohydrate metabolism, storage proteins, energy metabolism, amino acids and fatty acids metabolism. Moreover, the primary metabolism group gathered 16, 26 and 18 % of the whole regulated protein set at 3 dai, 7 dai and 14 dai, respectively. At 3 dai, changes concerned both increasing and decreasing protein abundances, while 7 dai and 14 dai, the changes consisted of contrasted trends, including mostly up-regulation at 7 dai and mostly down-regulation in the latter stage (Fig. 1d). In the stress and defence protein group, 11 proteins displayed changes in their abundance during *Fg* infection. They gathered nearly 10 % of the whole regulated protein set detected at 3 dai and 14 dai, and less than 5 % at 7 dai. Most of them were down-regulated especially at 3 dai

and 14 dai while those detected at 7 dai were all up-regulated. Other proteins related to transport, signalling, translation and cell cycle control including DNA repair and cellular organization were also differentially regulated. Within these functional groups, most of the responsive proteins displayed increasing abundances during the whole infection kinetics and were specifically over-represented at 7 dai (Fig. 1d, Fig. 2).

To assess connections and discrepancies with previously published studies, we compared our results to three 2-DE works which have investigated the *Fg*/wheat interaction but under contrasting conditions (different wheat cultivars and *Fg* strains, other sampling points and different tissues) (Dornez et al. 2010; Ding et al. 2011; Yang et al. 2013) (Fig. 3) (Table 4Sa, 4Sb and 5S). Dornez et al. (2010) identified 39 protein spots differentially expressed during time course of infection (5, 15, and 25 days post anthesis) on susceptible grains of Bobwhite cultivar inoculated with Tri5*Fg* strain (no production of mycotoxins). Ding et al. (2011) used two contrasting genotypes (a resistant and a susceptible one) and identified 77 *Fg*-responsive proteins at 12 hpi; 64 were detected in the susceptible genotype and 65 in the resistant one including 52 common proteins. Finally, Zhang et al. (2013) focused on NILs contrasting in *Fhb1* alleles and identified 32 differentially regulated proteins in response to *Fg* inoculation. The comparison of our study with these three studies (Dornez et al. 2010; Ding et al. 2011 and Zhang et al. 2013) revealed only three common proteins (Glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase and elongation factor Tu). This low consistency can be explained by the fact that the plant genetic backgrounds (resistant and susceptible cultivars) and the harvested tissues (spikes, spikelets and grains) were different, as well as the *Fg* strain and the inoculation procedure (spraying or injection). However, the classification of these proteins into biological processes showed that several identified proteins were involved in the same biochemical pathways, including metabolism, stress and defence, DNA and gene regulation, and cell expansion. This result strengthens the possible involvement of these pathways in FHB responses. Regardless of the study, primary metabolism and stress proteins always gathered the largest number of proteins (Fig. 3). Noteworthy, transport proteins were over-represented only in our study; we observed also an impact on grain filling stage via alteration of storage protein and starch synthesis such as observed in Ding et al. (2011) for only starch synthesis.

Discussion

To gain new knowledge about the FHB susceptibility, we traced plant molecular changes especially in the wheat grain tissue. We examined the dynamics of protein abundance changes induced by *Fg* infection and its outcome during grain development (i.e. grain formation). FHB-responsive proteins were surveyed over three pivotal growth stages of the wheat grain (cell division, cell differentiation and grain filling) as described by Nadaud et al. (2010) (Fig. 1a). The grain growth is controlled by a deep remodelling of protein patterns (Rogers and Bendich 1994; Evers and Millar 2002; Nadaud et al. 2010). Therefore, FHB-induced changes were examined within a specific fluctuating molecular context which includes: (i) increasing amounts of proteins involved in cell wall initiation and energy production at the early cell division stage, (ii) varying abundances of proteins involved in carbohydrate metabolism and stress responses during cell differentiation stage, and (iii) accumulation of starch and storage proteins upon the latter stage consisting in grain filling

(Nadaud et al. 2010). As already reported in *Fg*-infected barley grains (Zantinge 2010; Yang et al. 2010a, 2010b), we noticed that grain protein patterns were slightly impacted by *Fg* inoculation at the three ontogeny stages studied here. This result suggests that the infectious process did not involve dramatic modification in grain development or even preserved grain ontogeny in spite of an established biotic stress. Among significant grain proteome remodelling, *Fg*-induced changes depended mainly on grain ontogeny and all were connected directly or indirectly with grain developmental process. This supports the idea that FHB-related responses summon up some specific biochemical processes according to the molecular context of the grain formation. Interestingly, the stage of cell differentiation (7 dai) appeared as a pivotal step during the FHB plant response. This stage gathered the largest number of protein abundance changes, along with the highest tri5 gene expression relative to *Fg* biomass amount and it marked the detection of visible symptoms.

Additionally, the number of proteins which were up-regulated was larger than the number of down-regulated proteins while the two other stages (3 dai and 14 dai) included mainly down-regulated ones. Cell differentiation stage is a hinge step to reach grain filling (Tasleem-Tahir et al. 2012) and obviously a key step in the infectious process. Most of the metabolic changes and cell process-related adjustments (transport, translation DNA processing and oxidative stress) triggered by *Fg* were detected at this stage (Fig. 2) and noticeably, usual processes involved in grain growth such as carbohydrate metabolism and stress responses were maintained. This finding provides evidence that FHB establishment is closely embedded with wheat grain ontogeny. A similar result was obtained by 2-D DIGE which showed that the expression of wheat xylanase inhibitor protein families during *Fg* infection is dependent on the development stage of the grain (Dornez et al. 2010) which were less abundant at the early stages of the grain development.

Despite the detection of *Fg* by qPCR method (Fig. 1b) and the induction of Tri5 gene involved in TCT biosynthesis (Fig. 1c), no differentially regulated *Fg* protein was detected in our study. At the early stages of infection (day 3 to day 7), this could be due to the very low amount of *Fg* proteins compared to grain proteins that did not reach the requirements of 2-DE methodology. At the late stage of infection (day 14), *Fg* proteins could probably be detected but obviously their changing abundance did not reach a sufficient level of statistical significance to be further identified by MS/MS. In infected grains, primary metabolism was overall deeply altered by *Fg* infection. Our results suggest that during the infection process, *Fg* induced specific changes in the regulatory pathways that control the primary metabolism at each stage of the grain development. By favouring *Fg* development in grain tissues, these responses could involve putative susceptibility factors tightly connected with grain ontogeny. Several studies investigating plant-pathogen interactions have already highlighted reprogramming of primary metabolism of the host plant (Jubault et al. 2013). Together with the concomitant down-regulation of proteins involved in starch biosynthesis and the storage proteins as well as the up-regulation of proteins involved in starch degradation (Fig. 2), these data suggest that the grain filling stage is impacted by the FHB infection and possibly controlled by *Fg* to fulfill its own requirements. While in a healthy grain, this stage is characterized by starch protein accumulation with the involvement of sucrose synthase, ADP glucose pyrophosphorylase, starch branching enzyme and starch synthesis (Kang et al. 2006), *Fg* could take advantage of starch and storage protein remobilization to sustain the

establishment of the carbon metabolic sink in infected wheat grains. Similar observations were reported in a proteomic study focusing on the grains of naked barley infected by *Fg* (Yang et al. 2010a, 2010b). Furthermore, *Fg* secretome analysis using growth culture with barley or wheat flour as the sole nutrient source in order to mimic the nutritional situation of the fungus in *planta* revealed 69 unique fungal proteins involved in starch and protein degradation (Yang et al. 2012). Likewise and possibly for the same reason, energy production mechanisms were also changed upon *Fg* inoculation including the up-regulation of cellular respiration and the induction of glycolysis. The increase of energy metabolism was also reported in susceptible barley inoculated by *Fg* 48 h after inoculation (Yang et al. 2010a, 2010b). Together, these data do not negate the hypothesis that the wheat grain primary metabolism alteration could be either the result of pathogen manipulation (Yang et al. 2012), or an intrinsic plant host response (Kugler et al. 2013) or some combination of both (Jubault et al. 2013) that refers to a susceptibility factor.

The chaperones proteins involved in stress response were mainly found as down-regulated while they are usually mobilized to counter stressful physiological states. These proteins (such as Hsp90) play an important role in triggering immunity of plants (Hubert et al. 2003). So their down regulations could delay or prevent the activation of defence mechanisms (Hubert et al. 2003) and support also their possible involvement as susceptibility factors. Ding et al. (2011) suggest that the wheat susceptibility to *Fg* infection is related to the delay of defence mechanisms activation via the shift in the salicylic acid pathway activation. In this study, we showed that several proteins involved in oxidative stress such as the cell redox homeostasis proteins increased in infected grains. The same work in barley infected by *Fg* fungus revealed the enhancement of the oxidative stress in the susceptible genotypes (Yang et al. 2010a, 2010b) but no changes in proteins associated with oxidative response were observed in resistant genotypes (Geddes et al. 2008). Considering our results, we suggest that the enhancement of the oxidative stress contributes actively to the grain susceptibility to *Fg* infection. Furthermore, the down regulation of phenylalanine ammonia lyases (PAL), which catalyze the transformation of phenylalanine to trans-cinnamate in the first dedicated step in the biosynthesis of phenylpropanoids and lignin suggests that the susceptibility to FHB could be linked to the prevention of cell wall thickening in the infected grains which facilitates the degradation of plant cell wall and fungal penetration. Although found in fewer proportion, *Fg*-induced changes involved also other cell processes including transport, signalling, translation, cell cycle, stress response and DNA replication. Noteworthy, some of these changes did not happen as they could be expected during abiotic stress or during healthy grain growth which presume specific patterns in changes induced by *Fg* infection on the wheat grains.

In conclusion, understanding the molecular and physiological basis of wheat susceptibility to *Fg* infection represents a major challenge in FHB control. In the present study, we provide new data on the susceptible wheat response to FHB during different stages of the grain development. Modifications in primary metabolism (starch metabolism, energy production, storage proteins, amino acids metabolism), in the host cellular processes (transport, translation, DNA processing) and singular molecular patterns involved in stress and defence mechanisms (triggering immunity, thickening of cell wall) provide further

informations on the molecular determinism controlling FHB susceptibility in wheat. This first proteome dynamics of the interaction between wheat grain and *Fusarium graminearum* fungus will be combined with a deeper analysis of cellular expression pattern, the characterization of different mutants and biochemical investigations to specifically trace wheat-responsive proteins involved in FHB susceptibility in grains. Finally, our results strengthen the hypothesis that FHB susceptibility factors allow *Fg* to create an appropriate metabolic environment in the grain tissue to favour its own development and further, to support its dissemination.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Impact of *Fusarium graminearum* on wheat grain tissue. **(a)** Symptoms observed during susceptibility responses to *Fusarium graminearum* at the three time points of infection in wheat grains (3, 7, 14 days after inoculation; 3, 7, 14 dai), **(b)** and **(c)** *F. graminearum* biomass quantification and FgTri5 relative gene expression assessed at 3, 7 and 14 dai by using qPCR method, **(d)** distribution of the differentially regulated proteins according to the biological processes

Fig. 2. Schematic representation of the main FHB-related changes in proteome patterns. Magnitudes of changes are depicted for each biological process at the three time points of *Fusarium graminearum* infection in wheat grains (3, 7, 14 days after inoculation; 3, 7, 14 dai). In red: Up regulated pathway, In green: down regulated pathway

Fig. 3.

Comparative analysis of three studies published in 2010, 2011 and 2013 on the Fg / wheat interaction. Identified proteins were grouped into five biological processes. The histograms show the number of proteins which were up (*in red*) or down (*in green*) regulated in each biological process

Table 1

List of grain proteins displaying significant changes in their abundance as a consequence of *Fg* inoculation. U: up-regulated proteins, D: down regulated proteins after *Fg* inoculation

Spot Id	Protein description	Log ₂ Ratio (±SD)			Functional category	Accession number
		3 dai	7 dai	14 dai		
Primary metabolism						
174	Pyruvate, orthophosphate dikinase	-1,17(±0,01)	NS	NS	Pyruvate metabolism	Bradi2g25745.1
215	plastid alpha-1,4-glucan phosphorylase (Pho1)	0,97(±0,07)	NS	NS	Polysaccharides catabolism	B2LXU4
219	plastid alpha-1,4-glucan phosphorylase (Pho1)	0,98(±0,07)	NS	NS	Polysaccharides catabolism	B2LXU4
322	Glutaminyl-tRNA synthetase	-1,26(±0,09)	NS	NS	AA metabolism	Bradi2g05460.1
358	HMW-glutenin By subunit	NS	-0,95(±0,07)	NS	Storage protein	Q6UJY7
367	methionine-tRNA ligase activity, ATP binding	NS	NS	0,66(±0,007)	AA metabolism	Bradi1g69880.1
402	ATP synthase subunit alpha, mitochondrial	NS	0,61(±0,05)	NS	Energy metabolism	P12862
487	Starch synthase 1, chloroplastic amyloplastic	NS	NS	-1,01(±0,01)	Starch synthesis	Q43654
502	Transketolase, thiamine diphosphate binding domain	NS	NS	-0,6(±0,01)	Energy metabolism	Bradi1g51670.1
506	Transketolase, thiamine diphosphate binding domain	NS	NS	-0,61(±0,07)	Energy metabolism	Bradi1g51670.1
684	Phosphoglucomutase	NS	NS	-1,58(±0,02)	Glucose metabolism	Q8VYX48
715	ATP synthase subunit alpha, chloroplastic	NS	0,86(±0,0001)	NS	Energy metabolism	P12112
731	Succinate-CoA ligase activity	NS	NS	-0,713(±0,05)	C3 and C4 cycle	Bradi1g07020.1
770	Methylenetetrahydrofolate reductase	NS	0,91(±0,001)	NS	AA metabolism	A6XMY8
823	glutathione-disulfide reductase activity	NS	-0,59(±0,05)	NS	Energy metabolism	Q6UQ06
829	Acetohydroxyacid synthase	NS	0,91(±0,07)	NS	Energy metabolism	Q84U05
842	ketol-acid reductoisomerase activity	NS	NS	-1,2(±0,16)	AA metabolism	Bradi2g15790.1
870	Pyruvate, orthophosphate dikinase	-0,4(±0,0001)	NS	NS	Pyruvate metabolism	Bradi2g25745.1
959	Cytosolic glutathione reductase	NS	NS	-0,62(±0,07)	Energy metabolism	Q6UQ06
980	Glucose-1-phosphate adenylyltransferase	NS	NS	-0,92(±0,04)	Starch synthesis	Q7XJA9
1032	glutathione-disulfide reductase activity	NS	0,9(±0,0)	NS	Energy metabolism	Q6UQ06
1061	Phosphoglycerate kinase, cytosolic	NS	NS	-0,81(±0,03)	C3 and C4 cycle	P12783
1134	Glucose-1-phosphate adenylyltransferase	NS	0,63(±0,001)	NS	Starch synthesis	P12299
1392	Low molecular weight glutenin subunit	NS	-0,86(±0,01)	NS	Storage protein	B2Y2Q1
1400	Glutamine synthetase	NS	NS	-0,8(±0,02)	AA metabolism	O6RUJ1

Spot Id	Protein description	Log ₂ Ratio (±SD)			Functional category	Accession number
		3 dai	7 dai	14 dai		
1425	Aspartate aminotransferase/Glutamic oxaloacetic transaminase AAT1/GOT2	-0,8(±0,15)	NS	NS	AA metabolism	Bradi3g09050.3
1474	Glyceraldehyde-3-phosphate dehydrogenase	NS	0,54(±0,05)	NS	Glycolysis	C7C4X1
1478	Glyceraldehyde-3-phosphate dehydrogenase	NS	0,82(±0,03)	NS	Glycolysis	C7C4X1
939	transcription corepressor activity, phosphopyruvate hydratase activity	NS	NS	-1,08(±0,01)	Glycolysis	Bradi3g22550
1496	dTDP-glucose 4-6-dehydratase/UDP-glucuronic acid decarboxylase	-0,74(±0,03)	NS	NS	Xylose biosynthesis	Bradi1g66440.1
1548	Malate dehydrogenase	NS	0,98(±0,13)	NS	C3 and C4 cycle	A3KLL4
1549	Aestivum stearyl-ACP desaturase	NS	0,61(±0,06)	NS	Fatty acid metabolism	E9N9R5
1577	High-molecular-weight glutenin subunit	NS	NS	-0,56(±0,08)	Storage protein	A9ZMG8
1594	Fructose-bisphosphate aldolase	-0,86(±0,06)	NS	NS	Glycolysis	C1J9S9
1602	LMW-GS	NS	NS	-0,7(±0,07)	Storage protein	H6VLQ4
1662	NAD-dependent malate dehydrogenase	-0,86(±0,03)	NS	NS	C3 and C4 cycle	Bradi2g15740.1
1733	S-malonyltransferase	0,72(±0,06)	NS	NS	Energy metabolism	Bradi1g65140.2
1931	Globulin 1 OS	NS	-0,89(±0,08)	NS	Storage protein	QQQ5D4
1991	SUGAR-1-PHOSPHATE GUANYL TRANSFERASE	NS	0,43(±0,06)	NS	Lipopolysaccharide biosynthesis	Bradi1g19770.1
2034	SUGAR-1-PHOSPHATE GUANYL TRANSFERASE	NS	-1(±0,02)	NS	Lipopolysaccharide biosynthesis	Bradi1g19770.1
2064	oxidoreductase activity/NAD or NADH binding	NS	0,67(±0,03)	NS	Energy metabolism	Bradi2g20370.1
2227	Acid-reductone-dioxygenase-like protein	NS	0,65(±0,08)	NS	AA metabolism	C4PK50
2346	lactoylglutathione lyase/metalion binding	0,8(±0,09)	NS	NS	Energy metabolism	Bradi5g03830.1
2448	Triticin	0,61(±0,01)	NS	NS	Storage protein	B2CGM6
Stress and defense						
352	Molecular chaperone (HSP90 family)	-1,22(±0,06)	NS	NS	Protein folding	Bradi4g06370.1
379	Chaperone HSP104 and related ATP-dependent Clp proteases	-1,09(±0,15)	NS	NS	Protein folding	I11A06
435	Molecular chaperone (HSP90 family)	-0,91(±0,03)	NS	NS	Protein folding	Bradi4g32941.1
602	Phenylalanine ammonia lyase (PAL)	NS	NS	-1,37(±0,005)	Stress and defense	Q9LD25
628	70 kDa peptidyl-prolyl isomerase	NS	NS	-0,72(±0,08)	Protein folding	Q43207
641	Heat shock protein 70	NS	NS	-1,44(±0,05)	Protein folding	C3V133
907	Chaperonin complex component, TCP-1 beta subunit (CCT2)	-1,09(±0,17)	NS	NS	Protein folding	I1HGE3
1173	Putative PDI-like protein	NS	0,58(±0,04)	NS	Cell redox homeostasis	D8L9B4
2517	Peptidyl-prolyl cis-trans isomerase	NS	1,03(±0,005)	NS	Protein folding	A7XDG5
2598	thioredoxin peroxidase activity/oxidoreductase activity/antioxidant activity	NS	NS	0,67(±0,02)	Stress and defense	Bradi2g46380

Spot Id	Protein description	Log ₂ Ratio (±SD)			Functional category	Accession number
		3 dai	7 dai	14 dai		
2678	Response to stress	NS	0,62(±0,054)	NS	Stress and defense	Bradi2g23560.1
414	Ribonucleotide reductase, barrel domain/ATP binding	Translation, cell cycle and DNA repair			DNA replication	Bradi1g48170.1
1021	Alpha tubulin-3A	NS	NS	-0,75 (±0,005)	Cytoskeleton Structure	A4K4Y4
1178	Mitochondrial translation elongation factor Tu	NS	0,59(±0,09)	NS	Translation	I1IB68
1222	ATP-dependent 5'-3' DNA helicase activity	NS	0,97(±0,04)	NS	DNA replication	I1GwP1
1513	Mitotic spindle checkpoint protein BUB3, WD repeat superfamily	NS	0,51(±0,18)	NS	Cell Cycle	gj357121328
1634	Eukaryotic translation initiation factor 2 subunit beta	-0,75(±0,07)	NS	NS	Translation	O24473
1797	Eukaryotic translation initiation factor 3 subunit G	-0,65(±0,06)	NS	NS	Translation	I1IDB7
2464	Eukaryotic translation initiation factor 5A3	NS	0,62(±0,03)	NS	Translation	Q3S4H9
2471	Eukaryotic translation initiation factor 5A3	0,73(±0,05)	NS	NS	Translation	Q3S4H9
2576	Uncharacterized protein	1,07(±0,03)	NS	NS	Translation	I1I0C2
2620	small subunit ribosomal protein S12e	NS	0,52(±0,03)	NS	Translation	I1GMJ6
3052	small subunit ribosomal protein S21e	NS	NS	1,14(±0,04)	Translation	I1GMJ6
2118	laminin receptor activity	Signaling			Signaling	Bradi1g60310.1
744	Vesicle trafficking protein Sly1 (Sec1 family)	Transport			Transport	Bradi5g24240.1
1083	hydrogen-exporting ATPase activity, phosphorylative mechanism	NS	0,79(±0,18)	NS	Transport	Bradi1g34170
2069	Voltage dependent anion channel (VDAC)	NS	0,89(±0,01)	NS	Transport	Q41591
2139	Clathrin adaptor complex	NS	0,61(±0,01)	NS	Transport	Bradi3g34620.1
1728	Polypeptide: ORF	No annotation			uncharacterized	Bradi5g12080.1
		NS	-0,89(±0,04)	NS		

NS Non-significant

Up regulated protein

Down regulated protein