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Short communication

Components of priming-induced resistance to Fusarium head blight in wheat revealed by two distinct mutants of *Fusarium graminearum*

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SUMMARY

Two mutants (*tri6Δ* and *noxABΔ*) of the fungal pathogen *Fusarium graminearum* were assessed for their ability to prime immune responses in wheat (cv. Roblin) against challenge with pathogenic *F. graminearum*. Priming treatments generated Fusarium head blight (FHB)-resistant wheat phenotypes and reduced the accumulation of fungal mycotoxins in infected tissues. Microarray analysis identified 260 transcripts that were differentially expressed during the priming period. Expression changes were observed in genes associated with immune surveillance systems, signalling cascades, antimicrobial compound production, oxidative burst, secondary metabolism, and detoxification and transport. Specifically, genes related to jasmonate, gibberellin and ethylene biosynthesis exhibited differential expression during priming. In addition, the induction of the phenylpropanoid pathways that lead to flavonoid, coumarin and hydroxycinnamic acid amide accumulation was also observed. This study highlights the utility of nonpathogenic mutants to both elicit and delineate stages of defence responses in wheat.

Keywords: Fusarium, *noxAB*, plant immunity, priming, resistance, *tri6*, wheat.

Fusarium head blight (FHB), caused by the fungal pathogen *Fusarium graminearum*, affects a number of economically important cereal crops. In addition to yield reductions, FHB causes the accumulation of trichothecene mycotoxins that present a health hazard to both animals and humans. Microbial biological control agents (BCAs) have been shown previously to reduce the severity of FHB and the accumulation of fungal mycotoxins in *Fusarium*-infected cereal heads (Khan and Doohan, 2009; Xue *et al*., 2009). Nonpathogenic *Fusarium* strains have been used effectively as BCAs, acting through various mechanisms, including nutrient and niche competition, and induced resistance in the host (reviewed by Alabouvette *et al*., 2009). Induced resistance, which occurs following a particular stimulus, leads to heightened resistance against subsequent pathogen attacks. This heightened physiological state, in which plants are able to more rapidly and/or better activate immune responses, is known as the 'primed state'. Nonpathogenic strains of *Fusarium* have been used to induce disease resistance in asparagus and tomato (Aimé *et al*., 2013; He *et al*., 2002), and priming has been shown to provide disease protection without incurring heavy fitness costs (Van Hulten *et al*., 2006). Previously, Petti *et al*. (2010) investigated the localized transcriptome associated with bacterial-induced priming in barley, and Aimé *et al*. (2013) investigated the expression of six defence response-related genes in various tissues during avirulent *Fusarium*-induced priming in tomato. To date, no study has described priming-induced resistance (PIR) against FHB in wheat. Here, we report two *F. graminearum* deletion mutants (*tri6Δ* and *noxABΔ*) that activate PIR against FHB in wheat (cv. Roblin), and the associated global transcriptional changes that accompany this phenotype.

The *F. graminearum tri6Δ* mutant strain contains a knockout in the *Tri6* gene which encodes a global transcriptional regulator found in the trichothecene gene cluster (Nasmith *et al*., 2011). This strain is unable to produce the mycotoxin deoxynivalenol (DON) in culture and is nonpathogenic (Nasmith *et al*., 2011; Scherm *et al*., 2011; Seong *et al*., 2009). The *F. graminearum noxABΔ* mutant strain has two genes related to the catalytic unit of NADH oxidase deleted (Wang *et al*., 2014). Unlike the *tri6Δ* mutant strain, high-performance liquid chromatography (HPLC) analysis showed that the *noxABΔ* mutant strain has the capacity to produce 15-acetyldeoxynivalenol (15-ADON) in culture (Wang *et al*., 2014). In pathogenicity assays, the *tri6Δ* strain is able to infect, but not spread beyond, the inoculated wheat spikelets, whereas, in wheat inoculated with the *noxABΔ* strain, the fungus managed a limited spread into wheat spikelets adjacent to those inoculated (Fig. 1a). With mutant strains, less DON/15ADON accumulated compared with wheat heads infected with pathogenic *F. graminearum* (Fig. 1a). To investigate the potential of the *tri6Δ* and *noxABΔ* strains to prime resistance in wheat and offer protection against

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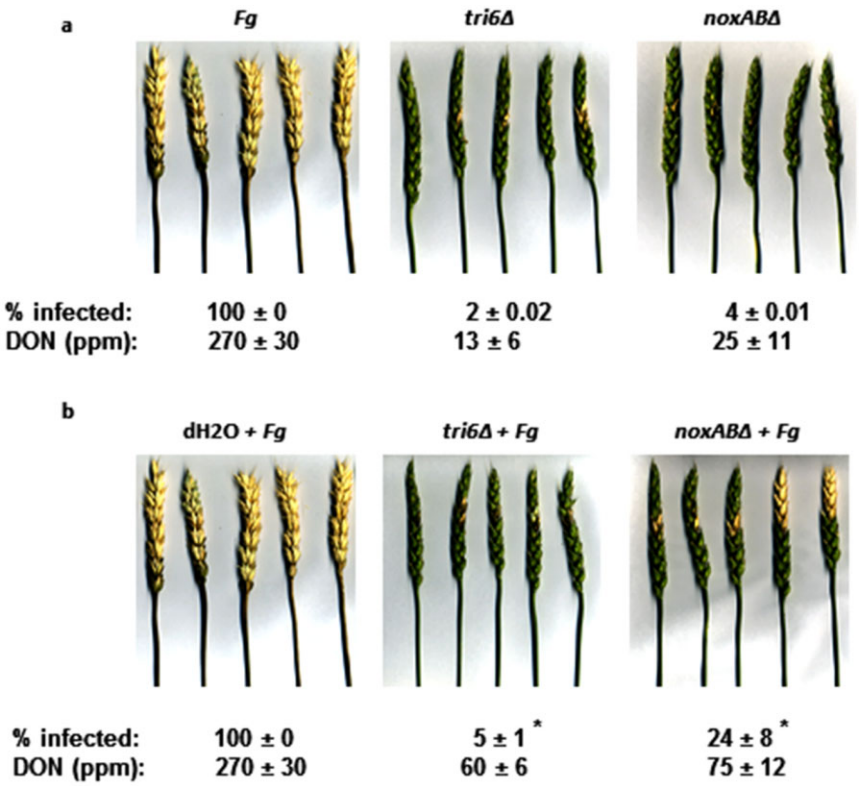

Fig. 1 Priming-induced resistance to Fusarium head blight in wheat (cv. Roblin). (a) Disease symptoms caused by pathogenic *Fusarium graminearum* (*Fg*; DAOM 233423; Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, ON, Canada) and the *F. graminearum* mutant strains *tri6Δ* and *noxABΔ* (*tri6Δ*, DAOM 237993; *noxABΔ*, DAOM 239571) were compared by point inoculation of 1000 spores to spikelet 0 (Fig. 2a). (b) The priming potential of *tri6Δ* and *noxABΔ* was evaluated by comparing mock treatments (dH_2O) with point inoculation treatments of 1000 spores of either *tri6Δ* or *noxABΔ* to spikelet 0, followed by point inoculation pathogen challenge treatment consisting of 1000 spores of pathogenic *F. graminearum* to spikelet 2 (Fig. 2a), 24 h later (*tri6Δ* + *Fg*; *noxABΔ* + *Fg*). Images were taken 21 days after application of initial treatments. The mean percentage of spikelet infection per head (% infected) is indicated ± standard deviation. Combined deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-ADON) levels in infected tissues were measured in an enzyme-linked immunosorbent assay (ELISA) and are presented as parts per million (ppm) ± standard deviation. Asterisks indicate a statistically significant difference between the two priming treatments in three independent experiments, as determined by Students's *t*-test ($P = 0.05$).

pathogenic *F. graminearum*, wheat spikelets were point inoculated with either *tri6Δ* or *noxABΔ* strain (spikelet 0) and subsequently challenged on an adjacent spikelet (spikelet 2) with pathogenic *F. graminearum* (Fig. 2a). Priming treatment with the *tri6Δ* strain consistently exhibited a more resistant phenotype than with the *noxABΔ* strain, as indicated by the level of infection and accumulation of DON in the wheat heads (Fig. 1b). This suggests that priming by the *Δtri6* mutant induces an early response by the host, whereas the *ΔnoxAB* mutant evokes a delayed response leading to more disease spread. Yet, disease symptoms and DON levels after both priming treatments were greatly reduced when compared with those in wheat heads infected only with pathogenic *F. graminearum* (Fig. 1b).

To characterize the genetic mechanisms underlying PIR against FHB in wheat, DNA microarray analysis was conducted using the Affymetrix Wheat GeneChip Array on RNA extracted from spikelet 2 at 8 h and 24 h post-challenge (Fig. 2a). All raw expression data can be accessed through http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE55653. In our analyses, probesets were considered to be differentially expressed if: (i) the fold change was at least two; (ii) the *P* value (Baldi and Long, 2001) was ≤0.001; and (iii) the false discovery rate (Benjamini and Hochberg, 1995) was ≤0.05. In cases in which multiple probesets quantified expression of the same transcript, we manually removed redundant probesets from the datasets. The priming phenotype only occurs when both the priming agent and the pathogen are present (Van Der Ent *et al*., 2009); therefore, we identified priming-associated transcripts (PATs) in primed and challenged (P&C) plant tissues showing differential expression relative to wheat spikelets that were (i) primed but not challenged (P&C vs. P) or (ii) challenged but not primed (P&C vs. C) at either of the 8 h or 24 h post-pathogen challenge time points (Table S1, see Supporting Information). This analysis identified 101 differentially expressed PATs during *noxABΔ*-induced priming (*noxABΔ* PATs), and 200

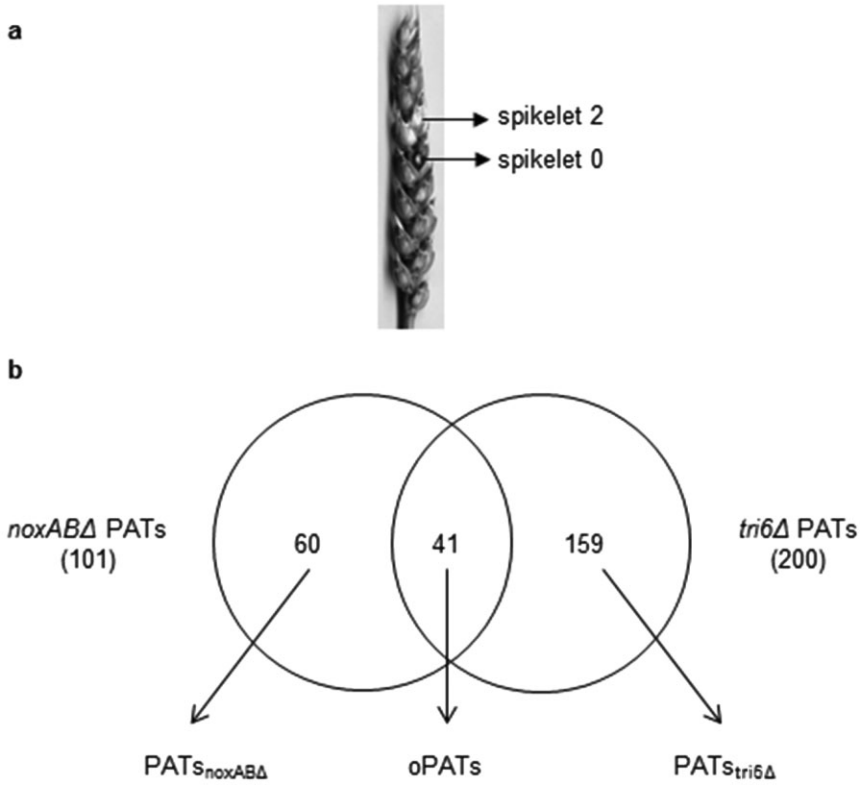

Fig. 2 Analysis of wheat spikelets primed with *Fusarium graminearum* mutant strains. (a) Site of point inoculation for priming experiments on wheat (cv. Roblin) heads. Priming or mock priming treatments were applied to spikelet 0 and pathogen challenge or mock challenge treatments were applied to spikelet 2. RNA samples were isolated from spikelet 2 for microarray analysis, as described previously (Nasmith *et al*., 2011; Wang *et al*., 2010). Microarray analysis was performed with three biological replicates and, for each experiment, RNA was extracted from eight spikelets. (b) Venn diagram of the number of transcripts showing two-fold or greater change in abundance at either 8 h or 24 h post-pathogen challenge, relative to primed but not challenged as well as challenged but not primed controls. The dataset of priming-associated transcripts (PATs) differentially expressed during both *noxABΔ*-induced priming (*noxABΔ* PATs) and *tri6Δ*-induced priming (*tri6Δ* PATs) is defined as overlapping PATs (oPATs), and datasets with nonoverlapping PATs specific to either *noxABΔ*- or *tri6Δ*-induced priming are defined as $PATs_{noxAB\Delta}$ and $PATs_{tri6\Delta}$, respectively.

differentially expressed PATs during *tri6Δ*-induced priming (*tri6Δ* PATs; Fig. 2). Furthermore, we compiled a dataset of 41 overlapping PATs (oPATs) composed of differentially expressed transcripts found in both the *noxABΔ* and *tri6Δ* PATs (Fig. 2). As the *tri6Δ* and *noxABΔ* strains differ in their relative virulence, but both generate priming phenotypes, the transcripts in the oPATs are more likely to highlight the essential transcriptional changes associated with priming. We also defined datasets containing PATs that were either uniquely primed by *noxABΔ* ($PATs_{noxAB\Delta}$) or *tri6Δ* ($PATs_{tri6\Delta}$) mutant stains (Fig. 2). We identified 60 $PATs_{noxAB\Delta}$ and 159 $PATs_{tri6\Delta}$ (Fig. 2b; Table S1). Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of selected PATs was used to support our analysis (Table S2, see Supporting Information).

The three datasets were analysed with respect to what is currently known about host responses during *F. graminearum* infection, general plant–microbe interactions and the observed priming phenotype. Transcripts were assigned to functional categories based on annotations provided by HarvEST Wheat1 chip version 1.59 (http://harvest.ucr.edu). A large proportion (41%) of the *noxABΔ* PATs overlapped with the *tri6Δ* PATs, whereas only 21% of the *tri6Δ* PATs overlapped with the *noxABΔ* PATs (Fig. 2). The majority of the PATs were up-regulated: 90% in oPATs, 92% in $PATs_{tri6\Delta}$ and 65% in $PATs_{noxAB\Delta}$ (Table S1).

Transcripts found in the oPATs, $PATs_{noxAB\Delta}$ and $PATs_{tri6\Delta}$ are associated with a wide range of roles in plant immunity (Tables 2–4, and complete datasets in Table S1). Pathogen recognition plays a critical role in the activation of plant immune responses (reviewed in Dodds and Rathjen, 2010). Recognition events occurring at the plant cell surface are based on the perception of various molecular stimuli, known as pathogen-, microbe- or damage-associated molecular patterns (PAMPs, MAMPs or DAMPs, respectively) by membrane-bound pattern recognition receptors (PRRs). Many PRRs are receptor-like kinases (RLKs) responsible for the recognition of fungal PAMPs, such as chitin and xylanase, and PAMP recognition activates an immune response known as PAMP-triggered immunity (PTI) (reviewed in Deslandes and Rivas, 2012; Zipfel, 2009). There are several transcripts annotated as membrane-bound RLKs in the $PATs_{tri6\Delta}$ (Table 4). However, membrane-bound RLK transcripts are not found in the oPATs or $PATs_{noxAB\Delta}$. Indeed, $PATs_{tri6\Delta}$ contains the largest proportion of transcripts associated with signal transduction (5%; Table 1), suggesting an important role for these processes in *tri6Δ*-induced priming. Consistent with this interpretation, signalling modules known to function downstream of PTI activation are exclusive to $PATs_{tri6\Delta}$. For example, mitogen-activated protein kinase (MAPK) and calcium/calmodulin-dependent protein kinase (CAMK) signalling systems are both associated with PAMP recognition and PTI activation (Bhardwaj *et al*., 2011; Gao *et al*., 2013), and transcripts with annotations related to both of these signalling systems are exclusive to $PATs_{tri6\Delta}$ (Table 4). Furthermore, MAPK cascades are hypothesized to direct the expression of WRKY transcription factors, which are important mediators of plant defence

Table 1 Functional categorization of the 60 PATs$_{noxAB\Delta}$, 41 overlapping priming-associated transcripts (oPATs) and 159 PATs$_{tri6\Delta}$ from Fig. 2. The percentage of transcripts allocated to each category is indicated.

Functional category	PATs$_{noxAB\Delta}$	oPATs	PATs$_{tri6\Delta}$
Cellular component organization, biogenesis and development	5.0%	2.4%	3.8%
Energy	1.7%	0.0%	1.9%
General metabolism	1.7%	14.6%	6.9%
Hormone metabolism	3.3%	2.4%	6.9%
Other	6.7%	7.3%	6.3%
Photosynthesis	0.0%	0.0%	0.0%
Protein degradation	1.7%	2.4%	0.0%
Protein synthesis	1.7%	0.0%	1.9%
Redox homeostasis, detoxification and secondary metabolism	8.3%	12.2%	4.4%
Signal transduction	1.7%	0.0%	5.0%
Stress	8.3%	14.6%	7.5%
Transcription	6.7%	0.0%	8.8%
Transport	3.3%	9.8%	7.5%
Unclassified	50.0%	34.1%	39.0%

Functional categories were based on MAPMAN (Thimm *et al*., 2004) classifications.

responses, and are also exclusively in PATs$_{tri6\Delta}$ (Table 4; Eulgem, 2006; Ross *et al*., 2007). Similarly, transcription factors belonging to the Myb family, which are known to be involved in defence responses to fungal pathogens, are exclusive to PATs$_{tri6\Delta}$ (Table 4; Liu *et al*., 2013). These results suggest that mutants varying in pathogenicity and inducing the priming response differentially (Fig. 1) may be used to tease out stages of plant defence responses.

The colonization of host plants requires pathogens to overcome PTI. To this end, many pathogens secrete effector proteins that interfere with PTI responses. Accordingly, plants have evolved to recognize these effector proteins and activate a second branch of immune responses, known as effector-triggered immunity (ETI; reviewed in Dodds and Rathjen, 2010). Effector recognition is mediated either directly or indirectly by the disease resistance (R) proteins. Indirect interaction with R proteins requires a deployment of proteins that guard the actual targets of the effectors (reviewed in Dodds and Rathjen, 2010). For example, in *A. thaliana*, RPS2 is an R protein that activates ETI through indirect interaction with a bacterial effector protein (reviewed in Xin and He, 2013). In the *Pseudomonas–Arabidopsis* interaction, RIN4 (an important regulator of ETI) is targeted for degradation by the bacterial effector AvrRpt2 (Liu *et al*., 2009). Degradation of RIN4 leads to the activation of RPS2, and the onset of ETI (Mackey *et al*., 2003). A transcript annotated as *RPS2* is found in PATs$_{tri6\Delta}$ (Table 4). This finding is surprising, as no effector proteins, nor associated race-specific resistance ETI phenotypes, have been identified in any *F. graminearum* pathosystem, although both of these phenomena have been identified in multiple *F. oxysporum* pathosystems (Thatcher *et al*., 2012). It is possible that the wheat transcript annotated as *RPS2* represents a cognate receptor for a yet-to-be identified *F. graminearum* effector protein, or may guard other wheat proteins targeted by these putative effector proteins. Interestingly, a transcript annotated as Mlo3, a guard protein targeted by effectors, is found in the oPATs; (Table 2; Panstruga, 2005). In barley, the transmembrane protein Mlo3 interacts with the Ca^{2+} sensor calmodulin and negatively regulates cell death during powdery mildew infection (Miklis *et al*., 2007). Importantly, loss-of-function *Mlo* alleles in barley confer complete resistance to biotrophic powdery mildews, whilst conferring enhanced susceptibility to necrotrophic *F. graminearum* (Jansen *et al*., 2005). Overall, the discovery of genes encoding guard proteins in oPATs suggests that effector recognition and the activation of PTI/ETI are important in PIR against FHB in wheat.

Many PATs were associated with hormone metabolism (Tables 2, 3 and S1), including jasmonic acid (JA), gibberellic acid (GA) and ethylene (ET). PATs$_{tri6\Delta}$ contained the largest proportion of transcripts associated with this functional category (6.9%), compared with either PATs$_{noxAB\Delta}$ (3.3%) or oPATs (2.4%; Table 1). JA and its derivatives are important systemic signalling molecules that regulate plant immunity, and are produced in response to abiotic cues, such as wounding, as well as to biotic cues, such as pathogen challenge, symbiosis and priming (Aimé *et al*., 2013; De Geyter *et al*., 2012; Kazan and Manners, 2008; Memelink, 2009; Petti *et al*., 2010). Transcripts associated with JA biosynthesis are found in all three PATs datasets, including 12-oxo-phytodienoic acid reductase 1 (OPR1), alpha-dioxygenase (α-DOX2), allene oxide synthase (AOS) and jasmonate-ZIM-domain protein 1 (JAZ1) (Tables 2–4). There are two major recognized branches of the JA signalling pathway, one of which is controlled by MYC-type transcription factors that are negatively regulated by JAZ repressor proteins (Van der Does *et al*., 2013). A transcript annotated as JAZ1 is an up-regulated oPAT (Table 2); JAZ transcripts themselves are positively regulated in a MYC2-dependent manner (Chini *et al*., 2007; Thines *et al*., 2007); therefore, the up-regulation of JAZ transcripts during PIR against FHB in wheat may be indicative that MYC2-dependent JA signalling is important to this phenomenon.

The second major branch of the JA signalling pathway requires both JA and ET signalling (Van der Does *et al*., 2013). All ET-regulated responses begin with ET biosynthesis, and 1-amino-cyclopropane-1-carboxylic acid synthase (ACC synthase; ACS) is an essential enzyme in this process. A transcript annotated as ACS is an up-regulated PATs$_{tri6\Delta}$ (Table 4).

GAs are plant hormones that regulate plant growth as well as immune responses (Kazan and Manners, 2012). Various nodes in GA signalling are up-regulated PATs$_{noxAB\Delta}$ and PATs$_{tri6\Delta}$, including scarecrow-like (SCL) transcription factors 5 and 9, as well as GID1 (GA insensitive dwarf1) (Tables 3 and 4). In *A. thaliana*, SCL transcription factors belong to a family of proteins that positively regulate GA signalling (Zhang *et al*., 2011), and GID1 is a gibberellin receptor that activates GA signalling (Sun, 2010). It is

Table 2 Selected overlapping priming-associated transcripts (oPATs) with predicted functions related to recognition and signalling, defence response or detoxification and transport.

		Fold change†							
		tri6Δ				*noxABΔ*			
		PC vs. C		PC vs. P		PC vs. C		PC vs. P	
Probeset	Predicted function	8 h	24 h	8 h	24 h	8 h	24 h	8 h	24 h
	Recognition and signalling								
Ta.9507.2.S1_at*	Jasmonate-ZIM-domain protein 1 (JAZ1)		2.58	0.44	8.80	2.67	3.00		2.35
Ta.27345.1.S1_at*	Mlo3		2.08		10.16		2.28		5.29
	Defence response								
Ta.26340.1.A1_at	Chitinase		2.06		8.82		2.11		3.79
Ta.18497.1.S1_at	Peroxidase precursor		3.49		8.92		3.39		5.44
Ta.8483.1.S1_at	Blue copper protein		2.10		4.19		2.02		7.38
TaAffx.29050.1.S1_s_at	Agmatine coumaroyltransferase		3.08		5.96		2.80		3.03
Ta.21061.3.S1_at	*trans*-Cinnamate 4-monooxygenase (C4H)		3.09		7.28		3.64		3.65
TaAffx.28302.2.S1_at	Dirigent-like protein		2.36		3.96		2.10		6.49
Ta.7022.2.S1_at	Phenylalanine ammonia-lyase		2.09		4.92	2.10	2.38		3.42
Ta.8559.1.S1_at	Polyphenol oxidase		0.36		0.45		0.36		0.35
Ta.5610.1.S1_at	Nonsymbiotic haemoglobin		3.28		2.26		2.51		13.10
	Detoxification and transport								
Ta.23833.3.S1_x_at*	Plasma membrane intrinsic protein 1;3 (PIP1;3)		0.46		0.49		0.38		0.47
Ta.28728.2.S1_x_at	Plasma membrane intrinsic protein 2;4 (PIP2;4)		0.41		0.48		0.30		0.46
TaAffx.29128.1.S1_at	ABC transporter-like		2.18		2.19		2.18		5.25
TaAffx.113701.1.S1_s_at	Pleiotropic drug resistance protein		2.29		3.91		2.75		2.02

*Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) data available (Table S2).
†Data shown for probesets exhibiting two-fold greater or two-fold lower (values less than unity) change in expression at either 8 h or 24 h post-pathogen challenge, relative to primed but not challenged (PC vs. P) as well as challenged but not primed (PC vs. C) controls.
Predicted function shows the best significant BLASTX database hit from HarvEST.

Table 3 Selected PATs$_{noxAB\Delta}$ with predicted functions related to recognition and signalling, defence response or detoxification and transport.

		Fold change†			
		noxABΔ			
		PC vs. C		PC vs. P	
Probeset	Predicted function	8 h	24 h	8 h	24 h
	Recognition and signalling				
TaAffx.31548.1.S1_at*	Gibberellin receptor GID1L2		2.51		3.34
Ta.1207.1.S1_at	12-Oxophytodienoate reductase (OPR1)		2.13		2.28
Ta.22602.1.S1_a_at	Alpha-dioxygenase (α-DOX2)		2.26		4.28
	Defence response				
Ta.9336.2.S1_x_at	Blue copper-binding protein		2.19		3.37
TaAffx.45277.1.S1_x_at	Phenylalanine ammonia-lyase	2.22	2.32		4.36
Ta.14545.1.S1_at	*O*-Methyltransferase ZRP4		2.50		8.26
Ta.82.1.S1_at	Peroxidase precursor	0.46			3.28
	Detoxification and transport				
Ta.21281.1.S1_at	Pleiotropic drug resistance (PDR)-type ABC transporter		2.15		2.25
Ta.30922.1.S1_at	Glutathione *S*-transferase		2.29	0.49	4.49

*Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) data available (Table S2).
†Data shown for probesets exhibiting two-fold greater or two-fold lower (values less than unity) change in expression at either 8 h or 24 h post-pathogen challenge, relative to primed but not challenged (PC vs. P) as well as challenged but not primed (PC vs. C) controls.
Predicted function shows the best significant BLASTX database hit from HarvEST.

of particular interest that both JA and GA signalling pathways are represented in the PATs, as antagonistic and synergistic interactions are known to occur between these pathways (Kazan and Manners, 2012). For example, low GA levels can prevent JAZ repression of MYC2, leaving JA signalling unencumbered (Hou *et al*., 2010). However, when GA levels reach a certain threshold, JAZ proteins actively repress MYC2, leading to suppression of MYC2-dependent JA responses. Conversely, another mechanism

Table 4 Selected PATs$_{tri6\Delta}$ with predicted functions related to recognition and signalling, defence response or detoxification and transport.

		Fold change†			
		tri6Δ			
		PC vs. C		PC vs. P	
Probeset	Predicted function	8 h	24 h	8 h	24 h
	Recognition and signalling				
TaAffx.103209.1.S1_at	Resistant to *Pseudomonas syringae* 2 (RPS2)		2.04		3.39
TaAffx.53376.1.S1_at*	Receptor-like protein kinase 2		2.36	2.23	2.74
TaAffx.84014.1.S1_at*	Cysteine-rich receptor-like protein kinase 6 precursor (CRK6)		2.02		2.58
Ta.25487.1.S1_at	Cysteine-rich receptor-like protein kinase 10 (CRK10)		2.12		3.03
Ta.8585.1.S1_at	Gibberellin receptor GID1B		2.40		10.76
Ta.5671.1.S1_at	Scarecrow-like transcription factor 5 (SCL5)		2.27		2.03
TaAffx.128684.1.S1_x_at	12-Oxophytodienoic acid reductase (OPR1)		2.46		9.11
Ta.18630.1.A1_x_at	Allene oxide synthase (AOS)		2.10		3.32
TaAffx.71241.1.A1_at*	1-Amino-cyclopropane-1-carboxylic acid synthase (ACC synthase, ACS)		2.58		3.14
Ta.236.1.S1_at	Mitogen-activated protein kinase 5 (MPK5)		2.01		2.59
Ta.16181.1.S1_at	Calcium/calmodulin-dependent protein kinase (CAMK)		2.05		3.79
Ta.4725.1.S1_at	WRKY14		2.24		3.60
Ta.5405.1.S1_x_at	MYB family transcription factor		2.23		5.71
Ta.11849.1.S1_at	Myb transcription factor		0.24		0.42
	Defence response				
Ta.4328.1.S1_at	Pathogenesis related protein 10 (PR-10)		2.08		4.58
Ta.24544.1.S1_at	Hs1 (HsPro homologue)		2.16		2.65
Ta.21556.1.S1_at	Wheat-induced resistance 1B (WIR1B)		2.09		4.48
Ta.10133.1.S1_x_at	Protease inhibitor/seed storage/lipid transfer protein precursor		2.06	0.43	
Ta.9220.1.S1_a_at	Putative phenylalanine ammonia-lyase (PAL)		2.27		6.73
Ta.25703.2.A1_s_at	Flavonol synthase/flavanone 3-hydroxylase		6.48		3.29
	Detoxification and transport				
Ta.6990.1.S1_at	Pleiotropic drug resistance protein		2.26		4.12
Ta.2793.1.S1_at	Multidrug resistance protein 1		2.40		41.50
TaAffx.120297.1.S1_at	ABC transporter family protein		2.13		5.29
Ta.12715.1.S1_s_at	Heavy metal transport/detoxification protein		2.24		2.32
TaAffx.110629.1.S1_at	Glutathione *S*-transferase		2.73		2.97
TaAffx.111585.1.S1_at*	UDP-glycosyltransferase/sinapate 1-glucosyltransferase		2.72		31.74
Ta.12887.1.S1_at	UDP-glucuronosyl/UDP-glucosyl transferase		2.89	2.13	28.11

*Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) data available (Table S2).
†Data shown for probesets exhibiting two-fold greater or two-fold lower (values less than unity) change in expression at either 8 h or 24 h post-pathogen challenge, relative to primed but not challenged (PC vs. P) as well as challenged but not primed (PC vs. C) controls.
Predicted function shows the best significant BLASTX database hit from HarvEST.

allows high GA levels to directly de-repress MYC2-dependent JA responses (Hong *et al*., 2012). It has been proposed that underlying physiological conditions determine the outcome of JA–GA interactions, resulting in local and systemic changes in gene expression co-ordinated specifically to changing environmental circumstances (Hong *et al*., 2012). The simultaneous up-regulation of JA and GA signalling pathways during PIR against FHB in wheat implies a synergistic rather than antagonistic interplay between these two hormone pathways, and it is tempting to speculate that the JA, ET and GA pathways contribute to the systemic activation of the priming phenotype in wheat.

In addition to recognition and signalling, PIR against FHB in wheat is associated with the differential expression of transcripts with roles in defence responses. For example, chitinases, which directly attack fungal structures, are up-regulated oPATs (Table 2). The expression of other defence-related transcripts is idiosyncratic to *tri6Δ*-induced priming. For example, the WIR1 family of proteins (Wheat-induced resistance 1), lipid transfer proteins (LTPs) and PR-10 (pathogenesis-related class 10) are all exclusive to PATs$_{tri6\Delta}$ (Table 4). WIR1 proteins are hypothesized to be membrane-spanning proteins that enhance the adhesion of the plasma membrane to the cell wall during pathogen attack (Bull *et al*., 1992); they are induced in wheat and barley in response to a number of

microbial pathogens (Tufan *et al*., 2012). Similarly, some LTPs are speculated to play a role in reinforcing the cuticle through the incorporation of fatty acids, and have been reported previously to be key factors in priming in barley and FHB resistance in wheat (Petti *et al*., 2010; Schweiger *et al*., 2013). PR-10 has functions related to secondary metabolism and antimicrobial activity, and is positively correlated with resistance to *F. graminearum* in cereals (Bernardo *et al*., 2007; Makandar *et al*., 2006; Mohammadi *et al*., 2011).

We observed several PATs with annotations related to reactive oxygen species (ROS), known to play key roles in plant immunity (Nanda *et al*., 2010). For example, superoxides and hydroxyl radicals may damage fungal structures directly (Hemetsberger *et al*., 2012). ROS produced by peroxidases during the synthesis of lignin, suberin and during pathogen-induced cell wall modifications have been hypothesized to slow the influx of fungal toxins and efflux of plant nutrients (Kang and Buchenauer, 2000), and several oPATs and PATs$_{noxAB\Delta}$ are annotated as peroxidases (Tables 2 and 3). In addition, several transcripts found in the oPATs and PATs$_{noxAB\Delta}$ datasets are annotated as blue-copper proteins, which function as electron transporters during redox processes, are induced during stress and wounding, and have previously been associated with disease resistance in wheat (Tables 2 and 3; Coram *et al*., 2010; Li *et al*., 2012).

Secondary metabolism also plays a major role in the development of the priming phenotype in wheat; multiple oPATs, PATs$_{noxAB\Delta}$ and PATs$_{tri6\Delta}$ are annotated as part of the general phenylpropanoid pathway, including phenylalanine ammonia-lyase, polyphenol oxidase and *trans*-cinnamate 4-monooxygenase (Tables 2–4). In addition, one oPAT was annotated as agmatine coumaroyltransferase (ACT), an enzyme that catalyses the biosynthesis of hydroxycinnamic acid amides (HCAAs), and one PAT$_{tri6\Delta}$ was annotated as flavonol synthase (Tables 2 and 3; Held *et al*., 1993). Flavonoids, coumarins and HCAAs can function as phytoalexins, but can also be deposited in plant cell walls as structural reinforcements (reviewed in Vogt, 2010). Recently, resistance to FHB associated with the wheat *Fhb1* locus has been shown to be associated with the up-regulation of flavonoid and HCAA biosynthesis, and the specific deposition of these compounds into the plant cell wall (Gunnaiah *et al*., 2012). Consistent with our previous observations, many secondary metabolic pathways are known to be regulated in a JA- and GA-dependent manner (De Geyter *et al*., 2012; Hong *et al*., 2012).

During infection, *F. graminearum* produces a number of toxic secondary metabolites that disrupt host cell functions. Accordingly, the ability of the host to mitigate the effects of these toxins, either through detoxification or export, is critical for disease resistance. For example, glycosyltransferases (GTs) are known to play important roles in general detoxification, and some UDP-glycosyltransferases (UGTs) are specifically associated with DON detoxification (Lulin *et al*., 2010; Poppenberger *et al*., 2003; Schweiger *et al*., 2013). There are multiple transcripts annotated as UGTs in PATs$_{tri6\Delta}$ (Table 4). Additional genes with predicted function in transport and stress tolerance were also found in the three PAT datasets (Tables 2–4).

We have successfully demonstrated the utility of nonpathogenic strains of *F. graminearum* to induce resistance in wheat heads and have profiled the global transcriptome during this phenomenon. The early arrest of the pathogen induced by the treatment with the *tri6Δ* mutant indicated that genes involved in the early defence response might be responsible for this phenotype. The expression of RLKs and MAPKs only in the PATs$_{tri6\Delta}$ dataset supports this hypothesis.

The wide array of transcripts associated with plant immunity during PIR against FHB probably reflects the importance of each of the multiple battlegrounds on which the *F. graminearum*–wheat interaction takes place. PIR in wheat is associated with the active transcription of genes that are involved in immune surveillance, signalling, reinforcement of the plant cell wall, and detoxification and/or export of fungal toxins. JA, ET and GA pathways are all suggested to play roles in the development of this phenotype, and we have highlighted transcripts encoding the phenylpropanoid pathway, detoxification/transport systems as well as PRR proteins that warrant further study regarding the genetic improvement of wheat through either traditional or advanced molecular means. Furthermore, our results support the use of nonpathogenic *F. graminearum* mutant strains as a management strategy for FHB in wheat.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Priming-associated transcripts (PATs) differentially expressed during priming-induced resistance to Fusarium head blight in wheat.

Table S2 Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of selected priming-associated transcripts (PATs). Relative levels of expression were measured as described previously (Wang *et al*., 2010).

