Wheat Resistances to *Fusarium* Root Rot and Head Blight Are Both Associated with Deoxynivalenol- and Jasmonate-Related Gene Expression

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ABSTRACT

Fusarium graminearum is a major pathogen of wheat causing *Fusarium* head blight (FHB). Its ability to colonize wheat via seedling root infection has been reported recently. Our previous study on *Fusarium* root rot (FRR) has disclosed histological characteristics of pathogenesis and pathogen defense that mirror processes of spike infection. Therefore, it would be interesting to understand whether genes relevant for FHB resistance are induced in roots. The concept of similar-acting defense mechanisms provides a basis for research at broad *Fusarium* resistance in crop plants. However, molecular defense responses involved in FRR as well as their relation to spike resistance are unknown. To test the

hypothesis of a conserved defense response, a candidate gene expression study was conducted to test the activity of selected prominent FHB defense-related genes in seedling roots, adult plant roots, spikes, and shoots. FRR was examined at seedling and adult plant stages to assess age-related pattern of disease and pathogen resistance. This study offers first evidence for a significant genetic overlap in root and spike defense responses, both in local and distant tissues. The results point to plant development-specific rather than organ-specific determinants of resistance, and suggest roots as an interesting model for studies on wheat–*Fusarium* interactions.

Fusarium graminearum is one of the most destructive fungal pathogens of wheat (*Triticum aestivum*) and the major causal agent of *Fusarium* head blight (FHB) (Trail 2009). We have recently demonstrated that root infection plays an important role in the life cycle of *F. graminearum*. The pathogen can effectively penetrate seedling roots and colonize the entire wheat plant. *Fusarium* root rot (FRR), caused by *F. graminearum*, leads to a significant decrease of root and seedling growth, followed by a pathogen migration into the stem base (stem segment at soil level), the upper stem internodes, the leaves, and ultimately the spikes. The majority of wheat genotypes (10 of 12) assayed for seedling reaction to FRR was susceptible, including FHB resistant cultivars Sumai 3, Ning 7840, and Frontana (Wang et al. 2015). Spike and seedling root infection represent different pathosystems, which gives reason to expect that *F. graminearum* has evolved different attack strategies. However, our results on the histopathology of FRR had disclosed pathogenic processes that are similar to the pathogenesis of FHB. On susceptible roots and florets spore germination, mycelial network formation and initial infection proceed similarly during the first 2 days after inoculation (dai) with minor host colonization, limited to subepidermal hyphal growth. With the beginning of main infection at 3 dai, runner hyphae, foot structures (bulbous infection hyphae), and infection cushions are formed on both organs, accompanied by necrotic lesions and sporulation structures. The progressive inter- and intracellular colonization of all root or floral cell types and the onset of disease spread into stem or spike rachis were similarly reported for the interval 4 to 5 dai (reviewed in Wang et al. 2015). Also, as an FRR pathogen, *F. graminearum* produces the mycotoxin deoxynivalenol (DON) (Wang et al. 2015), which in

the case of DON chemotypes is essential for the pathogenic process in spikes (Gunupuru et al. 2017; Kazan and Gardiner 2017). The role of DON in FRR pathogenesis is an open question, but the toxin significantly inhibits root growth (Masuda et al. 2007; Pasquet et al. 2016). The seedling resistance to FRR displayed by the cultivar Florence-Aurore has been histologically characterized as impaired colonization of cortical root cells with fungal hyphae restricted to the outer epidermal layer, and inhibition of fungal spread within the stem (Wang et al. 2015). In both the stem and the spike rachis vascular bundles are prominent sites of pathogen migration and wheat protection (Brown et al. 2010; Gunnaiah et al. 2012; Wang et al. 2015). These characteristics coincide with those reported for the main types of FHB resistance—Type I against penetration and Type II against fungal spread (Schroeder and Christensen 1963)—and indicate the rapid induction of wheat root defenses in response to pathogen attack.

Until today, the molecular defense response of wheat to *F. graminearum* has been primarily investigated in spikes, while the root response and its relation to the spike response are unknown. The abovementioned observations suggest that individual components of the wheat–*F. graminearum* interaction are shared in spikes and roots, for example infection structures, disease phases, or DON accumulation. Hence, it is interesting to ascertain whether a similarity exists in the molecular defense response (Kazan and Gardiner 2017). There are two opinions on this issue: (i) one suggests that disease components operating in a similar way in different pathosystems also trigger host defense components in a similar way (Jones and Dangl 2006; Nürnberger and Brunner 2002); (ii) the other one emphasizes the organ specificity in host–pathogen interactions (reviewed in Strugala et al. 2015). An associated aspect is the fact that FHB resistant genotypes tend to be susceptible to *Fusarium* infection at the seedling stages and vice versa (Li et al. 2010; Wang et al. 2015). This is reminiscent of the adult plant resistance, a common form of developmental resistance against biotrophic pathogens, and indicates plant or organ development-specific determinants of resistance efficiency (Chetoui et al. 2016; Develey-Rivière and Galiana 2007; Gao et al. 2013). The concept of broad-acting plant defense mechanisms will provide important

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implications for further research aiming at durable resistance of the crop plant. Such an overall resistance in wheat (cereals) could effectively help to control different *Fusarium* diseases and associated mycotoxin accumulations (Cheng et al. 2015; Liu et al. 2012).

Transcriptomic studies on FHB have revealed different types of defense-associated genes (Ding et al. 2011; Gottwald et al. 2012; Schweiger et al. 2013; Xiao et al. 2013). The full understanding of the genetic basis underlying resistance to FHB is challenging, particularly due to the genetic diversity of wheat cultivars and their various reactions to the same *Fusarium* disease. However, two features are common: (i) the relationship between resistance and DON detoxification, correspondingly the induction of detoxification associated genes (Li et al. 2017; Pasquet et al. 2016; Schweiger et al. 2016); and (ii) the relevance of jasmonate (JA), in particular JA signaling, for the rapid induction of defense responses (Ding et al. 2011; Gottwald et al. 2012; Li and Yen 2008; Qi et al. 2016; Walter et al. 2015). Therefore, we have hypothesized that prominent genes associated to these commonly induced defense mechanisms could represent candidates for a genetic overlap in root and spike defense programs. Four genes related to the DON detoxification mechanism in spikes, indeed, have demonstrated a similar involvement in root responses. As a result of DON application, they were either expressed in roots of FHB resistant wheat (*TaUGT3*, Lulin et al. 2010 and *TaABCC3.1*, Walter et al. 2015) or found to enhance root tolerance to the toxin (*HvUGT13248* in transgenic wheat, Li et al. 2015 and *Bradi5g03300* in *Brachypodium distachyon*, Pasquet et al. 2016). There is increasing evidence that JA signaling is the primary mechanism for regulating early spike defenses, in particular also for the FHB resistance mediated by the major QTL *Fhb1* (Sun et al. 2016; Xiao et al. 2013). DON detoxification and JA signaling seem to have relevance also for the seedling resistance to FCR caused by *F. pseudograminearum* (Powell et al. 2017). Moreover, root growth, seedling development, and interaction with soilborne microbes are JA-controlled processes (Wasternack and Hause 2013), aspects that are interesting with respect to the root–*F. graminearum* association.

In the present study, we aimed to gain insights into the FRR resistance of wheat at the molecular level, based on the hypothesis of similar defense response in root and spike. For this purpose, we conducted a candidate gene expression study, analogous to the concept of candidate gene association studies introduced for disease research (Patnala et al. 2013). This approach focused on the selection of genes that have been associated with the above mentioned key FHB defense mechanisms. Neither the FRR defense transcriptome nor the wheat response dynamics (timing and magnitude) to root infection were known at the beginning of this study. Thus, the candidate gene approach was applied as a tool allowing for studying disease stage-related changes in gene expressions. During FHB, the gene expression dynamics proved to be a key feature that distinguishes resistant from susceptible responses (Cirilini et al. 2014; Gottwald et al. 2012; Ma et al. 2015; Makandar et al. 2006; Muhovski et al. 2014). A set of 11 candidate genes was tested in an experimental system that combined reliable root infection and disease assessment with time-course expression analysis. In a first experiment, FRR assays for seedling and adult plant stages were established to examine age-related effects on disease progress and wheat response. In a second experiment, two gene expression assays were designed to study candidate genes (i) for the resistance to disease spread (DON resistance) in seedling and adult plant roots, and (ii) for the resistance to initial infection (JA-mediated resistance) in seedling roots and spikes. Finally, in a case study on FRR-induced responses selected genotypes and genes were analyzed in healthy seedling/adult plant shoots. We present first evidence that *F. graminearum* induces overlapping sets of genes in resistant seedling root and spikes, and that root infection leads to systemic expression of FHB defense-related genes. Our results show that root and plant development are important determinants of local and systemic resistance rather than organ

specificity, providing important hints to the understanding of wheat–*F. graminearum* interactions. The results of this candidate gene expression study will serve as a basis for a translational research at broad and sustainable disease resistance in wheat and other cereal crops.

MATERIALS AND METHODS

Plant and fungal material. The hexaploid spring wheat genotypes Florence-Aurore, Sumai 3, Frontana, and Ning 7840 representing a diverse spectrum of responses to FRR at seedling stages and to FHB at adult plant stages were investigated in this study (Table 1). Macroconidia of *F. graminearum* isolate ‘IFA 65’ (IFA, Tulln, Austria) were grown on synthetic nutrient agar medium (Spezieller Nährstoffarmer Agar, SNA) (Leslie and Summerell 2006) at 20°C under cool-white and near-UV light illumination. The macroconidia suspension was prepared according to Gottwald et al. (2012). The final concentration was determined with a hemocytometer adjusted to 5×10^4 conidia ml⁻¹ for root inoculations (Wang et al. 2015) and 5×10^5 conidia ml⁻¹ for spike inoculations (Gottwald et al. 2012).

Root-dip inoculation and plant tissue sampling. Seed sterilization, plant cultivation until treatment and root-dip inoculation with *F. graminearum* and distilled water (control treatment) have been described by Wang and Gottwald (2017). For the seedling assay, plants were treated at the first true leaf stage, respectively at Zadoks stage (Z) 11 (Zadoks et al. 1974). After root-dip inoculation seedlings were planted in pots (7.5 × 7.5 × 8.0 cm) with noninfested, autoclaved sand. For the adult plant assay, plants were treated at the early stem elongation stage (Z32 to 33). After root-dip inoculation plants were cultivated in pots (11.5 × 11.5 × 12 cm) with a 1:2 (vol/vol) mixture of noninfested, autoclaved silica sand and soil (Fruhstorfer Erde, HAWITA Group, Vechta, Germany). The following procedures were similarly applied to the seedling and adult plant assay. Plants were transferred to the greenhouse according to the random principle and cultivated with a day/night 16/8 h photoperiod at 22°C/18°C and 60% humidity. To avoid additional stress factors, each pot was regularly watered and weekly fertilized with 100 ml (seedlings) and 250 ml (adult plants) Wuxal Super (Manna, Düsseldorf, Germany) according to the manufacturer’s specifications. Four independent experiments were carried out, each with 10 seedlings or adult plants per genotype/treatment/timepoint. The experimental design is in accordance with the general guidelines for agronomic studies recently published by Casler et al. (2015). Inoculated and noninoculated root samples were either collected at 1, 3, 5, 7, 10, 14, and 21 dai (21-day time-course assay for seedling and adult plant roots) or at 2, 4, 8, 12, 16,

TABLE 1. Characteristics of the analyzed spring wheat genotypes

Genotype	Origin	Pedigree ^a	FRR ^b	FHB ^c
Florence-Aurore	France	Florence/Aurore	R	HS
Sumai 3	China	Funo/Taiwan-Xiaomai; Jingzhou/Sumai 2; Funo/Taiwanmai	S*	HR**
Frontana	Brazil	Fronteira/Mentana	S	R***
Ning 7840	China	Avrora/Anhui11/Sumai 3	HS	HR**

^a Pedigrees were obtained from the Wheat Pedigree database (<http://genbank.vurw.cz/wheat/pedigree/>).

^b Information on the response to Fusarium root rot (FRR) was obtained from Wang et al. (2015). Special features of FRR response: *, moderate fungal growth in roots, but disproportionate high disease severity. R, resistant; HS, highly susceptible; and S, susceptible.

^c Information on the response to Fusarium head blight (FHB) was obtained from Badea et al. (2008) and Buerstmayr et al. (2009). Special features of FHB response: **, inhibition of fungal spread and deoxynivalenol (DON) content; ***, inhibition of fungal penetration and moderate susceptibility to fungal spread and DON content. HR, highly resistant; R, resistant; and HS, highly susceptible.

24, 48, and 72 hours after inoculation (hai) (72-hai time-course assay for seedling roots). Root, stem, and leaf tissues were separated after the measurement of plant growth, and the respective samples were immediately frozen in liquid nitrogen and stored at -80°C . Root tissues of five plants per genotype/treatment/timepoint were randomly selected to be used for fungal and wheat root biomass quantification (Wang et al. 2015).

Spike inoculation and plant tissue sampling. For Sumai 3 and Florence-Aurore plants, plant cultivation in the greenhouse and single floret inoculation with *F. graminearum* or distilled water (control treatment) were carried out as described by Gottwald et al. (2012). At early anthesis (Z60 to 61), six florets per spike (from six central spikelets) were inoculated. Treated spikes were moistened by spraying with water and thereafter covered in plastic bags for 24 h to provide humid conditions favorable for pathogen infection. Three independent experiments were carried out, each with six plants per genotype/treatment/timepoint. Tissues of *F. graminearum* inoculated and control spikelets were collected at 2, 4, 8, 16, 24, 48, 72, and 96 hai and immediately frozen in liquid nitrogen and stored at -80°C .

Real-time quantitative PCR (qPCR) measurement of fungal and root gDNA. Plant and fungal gDNA extraction, qPCR analysis, and calculation of relative fungal biomass were done as described by Wang et al. (2015). Analyses were performed on a 7500 Fast Real-Time PCR System (Applied Biosystems). The primers Fg16 N were used to amplify a *F. graminearum* specific fragment. Wheat root DNA was quantified by amplification of the wheat *Ubiquitin* gene (DQ086482/Ta.28553.1.S1_s_at) with the primers TaUbi. The Fg16 N and TaUbi primer sequences are listed in Supplementary Table S1 and information on the respective *F. graminearum* and wheat specificity were provided earlier by Wang et al. (2015). The reference gene-based DNA quantification method (Brunner et al. 2009) was used to calculate the average amount of *F. graminearum* in wheat roots and the infection percentage of root samples (relative fungal biomass). For each tissue sample, six technical replicates were carried out (three replicates

per plate and two qPCR experiments). Relative fungal biomass data of both PCR experiments were inspected for similar results. Four independent inoculation experiments (biological replicates) were done. An average “technical replicate” value for each of the biological replicates has been calculated.

Disease severity assessment and FRR disease index (FDI). At each timepoint root biomass (obtained from reference-gene-based qPCR), root length, and shoot length were measured for inoculated and noninoculated control plants, and the percentage change (D) between both treatments was calculated: $D = (\text{control} - \text{inoculated}) / \text{control} \times 100$. At adult plant stages, the stem bases of wheat show a natural (nonpathogenic) browning of leaf sheaths, reminiscent of pathogenic necrosis symptoms. Hence, necrosis was unusable as disease trait in a comparative analysis between different wheat developmental stages. Briefly, the disease severity data observed for seedling stages were in accordance with those previously published by Wang et al. (2015). The FDI incorporates the disease traits relative fungal biomass, percentage change in root biomass, root length, and shoot length. The FDI was calculated as described in Wang et al. (2015), and the FDI based genotypic variation observed for seedling stages was in accordance with this publication. The measured values of each severity parameter were transferred into dimensionless susceptibility index (SI) values to allow normalization and direct comparison between different types of value. SI values were calculated using the equation $SI = (XT_x)/(Y)$, where X represents the measured value for each genotype and timepoint (T_x), and Y represents the corresponding overall mean calculated over all assayed genotypes and timepoints. The respective FDI values for each genotype and timepoint has been calculated as the sum of SI values.

Expression analysis of genes associated with FHB resistance. Wheat genes were selected for analysis using the following criteria: (i) association with JA-mediated defense (biosynthesis, signaling, or defense response); (ii) association with resistance to the mycotoxin DON; (iii) localization in a FHB resistance QTL region; and (iv) repeated detection in different FHB resistant

TABLE 2. Information on selected candidate genes tested for their activities in wheat roots challenged with *Fusarium graminearum*

Gene	Gene product	FHB resistant wheat genotype	Ref. ^a	Putative or proven role in <i>Fusarium</i> resistance	Ref. ^a
Deoxynivalenol (DON) resistance mechanism (against spread of infection); test assay ‘seedling versus adult plant roots’					
<i>CYP709C1</i>	Cytochrome P450	Sumai 3, Ning 7840	1, 2	<i>Fusarium</i> - and DON-induced; putative role in JA signaling and detoxification	
<i>TaUGT3</i>	UDP-glucosyltransferase	Dream, Sumai 3, Wangshuibai	3, 4	Enhanced tolerance to DON	4
<i>TaUGT12887</i>	UDP-glucosyltransferase	Dream, Sumai 3, Wangshuibai, CM82036	3, 5, 6	Closest wheat homolog to the barley gene <i>HvUGT13248</i> with proven ability to detoxify DON	6, 10
<i>TaMDR1</i>	MDR-like ABC transporter	Dream, Sumai 3, CM82036	3, 6	Putative trichothecene detoxification and transport	
JA-mediated resistance mechanism (against initial infection); test assay ‘seedling roots versus spikes’					
<i>TaLox2</i>	Lipoxygenase2	Dream, Sumai 3	3, 6, 7	Key JA biosynthesis gene	11
<i>TabHLH041</i>	Basic helix-loop-helix TF	Dream, Wangshuibai, BW278	3, 8, 16	Putative chitin-inducible gene; [physical colocation] QTL-Fhb2-mediated resistance to disease spread and DON in spike rachis	12, 19
<i>TaJAZ9</i>	Jasmonate ZIM domain	Wangshuibai	8	Key JA signaling gene	
<i>TaPR-4b</i>	Pathogenesis-related protein	Sumai 3	7, 9	Wheatwin protein; [genetic colocation] QTL-6BL2 associated with JA mediated resistance	13, 17
<i>TaPR-14</i>	Pathogenesis-related protein	Wangshuibai	8	Nonspecific lipid transfer protein1; JA defense signaling; QTL-Fhb1 mediated resistance to disease spread and DON	14, 18
No association to a specific resistance mechanism; test assays ‘seedling versus adult plant roots’ and ‘seedling roots versus spikes’					
<i>WZF1</i>	Wheat zinc finger transcription factor	Dream, Sumai 3	3, 7, 9	Putative role in defense response regulation	15
<i>WFhb1-c1</i>	Wheat <i>Fhb1</i> candidate gene1	Sumai 3	7	Proven to have anti-FHB activity; [genetic colocation] QTL-Fhb1-mediated resistance to disease spread and DON	

^a Multiple literature associations were obtained by BLAST (bl2seq) analysis of respective published genes. References (Ref.): 1, Li et al. (2010); 2, Kong et al. 2005; 3, Gottwald et al. (2012); 4, Lulin et al. (2010); 5, Walter et al. (2008); 6, Schweiger et al. (2013); 7, Zhuang et al. (2013); 8, Xiao et al. (2013); 9, Li and Yen (2008); 10, Li et al. (2015); 11, Feussner and Wasternack (2002); 12, Fonseca et al. (2014); 13, Bertini et al. (2009); 14, Boutrot et al. (2007); 15, Kagale and Rozwadowski (2011); 16, Dhokane et al. (2016); 17, Derksen et al. (2013); 18, Sels et al. (2008); and 19, Kielbowicz-Matuk (2012).

genotypes and/or global transcriptome profiling studies. Detailed information on the selected genes is presented in Table 2.

For each independent experiment, the roots/stems/leaves of five and the spikelets of six plants were biologically averaged (pooled) per genotype/treatment/timepoint. This has become necessary since a single seedling does not provide sufficient root biomass for RNA extraction. To ensure consistency, biological averaging was generally applied to all experiments according to Chen et al. (2014) and Kendzierski et al. (2005). RNA extraction and cDNA synthesis were done as described by Gottwald et al. (2012). Total RNA was extracted using the guanidinium thiocyanate-phenol-chloroform method, and cDNA was synthesized with 1.0 µg of total RNA and 0.5 µg of oligo(dT)18 primers using the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas, Germany). Gene expression analysis was performed by using the ABI Step One Plus real-time PCR system (Applied Biosystems). For each reaction 1 µl of template cDNA (1:10) was mixed with 5 µl Roche FastStart Universal SYBR Green Master (Roche Diagnostics GmbH, Mannheim, Germany), 3 µl of sterile water, forward and inverse primer 1 µl (each 10 pmol/µl). PCR was performed according to the following cycling protocol. Initial denaturation for 10 min at 94°C was followed by 44 cycles with 45 s at 94°C, 45 s at 60°C, and 45 s at 72°C. The final elongation was executed for 5 min at 72°C. Fluorescence was determined during the annealing step of each cycle. Following amplification, the melting curves were acquired by heating the samples to 95°C for 15 s, cooling to 60°C for 1 min, and then slowly increasing the temperature from 60°C to 95°C at a rate of 0.5°C 10 s⁻¹, with continuous measurement of the fluorescence, cooling to 60°C for 15 s. Melting curves were analyzed using Dissociation Curves Software (Applied Biosystems) to exclude that primer dimer or unspecific fragments have been amplified (Livak and Schmittgen 2001; Paolacci et al. 2009).

All cDNA samples of each treatment were amplified simultaneously in a single PCR plate, and target gene expression was quantified using the comparative 2^{-ΔΔC_t} method (Livak and Schmittgen 2001). For each primer pair, the amplification efficiency was determined using 10-fold cDNA dilution series (dilution method) to check the accuracy of quantification results. Generally, efficiency of reaction values between 90 and 110% are considered acceptable for qPCR reactions (Taylor et al. 2010). The expression of each target gene is presented as fold change normalized to the reference gene *Ubiquitin* (DQ086482/Ta.28553.1.S1_s_at) and relative to the untreated control sample. For each gene, six technical replicates were carried out (three replicates per plate and two qPCR experiments). Gene expression data of both PCR experiments were inspected for similar results. Four independent inoculation experiments (biological replicates) were done. An average “technical replicate” value for each of the biological replicates has been calculated.

Statistical analyses for disease assessment. All statistical analyses were performed by using the software SPSS 20 (IBM SPSS Statistics 20; IBM Corp.). For FRR disease traits, the repeated measures analysis of variance (rANOVA) was used to assess the time-course of FRR disease progression. Since the Mauchly's test for sphericity violated the equality assumption, the Greenhouse-Geisser correction was applied to adjust the degrees of freedom appropriately. For multiple comparisons, the Bonferroni adjustment (correction for Type I error) was applied. The mean profile plots (estimated marginal means) were applied to interpret significant time–treatment interactions. Two-way rANOVA was performed to test treatment and time effects on the traits root biomass, root length, and shoot length. Mixed rANOVA was performed to test for time effects (changes) in the disease progress in terms of fungal growth and reduction in root/shoot biomass of disease seedlings.

Statistical analyses for gene expressions. All statistical analyses were performed on the log₂-transformed fold change values. Differences between wheat genotypes were statistically

tested at each timepoint by one-way ANOVA. As the homogeneity of variance was not given for all data (Levene's test), the one-way ANOVA was complemented by the Welch's *t* test. A significant difference was assumed if confirmed by both tests. In case ANOVA predicted significant differences, the posthoc pairwise comparisons (Bonferroni adjustment) were considered to elucidate intergenotype variation. In addition, the mixed rANOVA was performed to test gene expressions for significant time–genotype interactions. Disease severity and gene expression data are presented as mean standard deviation (+SD) of four biological replicates (seedling and adult plant root assay) or three biological replicates (spike assay). SD is a measure of data variability around mean of a sample of population.

RESULTS

FRR proceeds similarly in wheat seedlings and adult plants, but wheat responses can differ between both stages.

To study the interaction between wheat root and *F. graminearum* at different developmental stages, the roots of four spring wheat genotypes with varying response to seedling FRR and FHB were inoculated either at the seedling stage Z11 (emergence of third leaf) or at the adult plant stage Z32 to 33 (stem elongation). The virulence of *F. graminearum* was not notably affected by the developmental wheat stage, neither in terms of root infection and colonization nor in terms of effects on wheat development (Fig. 1). Seedling and adult plant root infection had a severe impact on the formation of root biomass, leading to mean reduction for all genotypes of 61.0% (seedlings) and 59.0% (adult plants). Correspondingly, also root and shoot length was strongly reduced by FRR. The mean reduction of roots was 27.0% (seedlings) and 17.0% (adult plants), and suppression of shoots was 16.0% (seedlings) and 11.0% (adult plants). Significant treatment effects on seedlings as well as adult wheat plants were observed for all three traits in the time-course experiment (Tables 3 and 4). In addition, the rANOVA showed a significant time–treatment interaction, meaning that the FRR disease had a significant effect on the seedling and adult plant growth over time. At both the seedling and the adult plant stages, FRR-treated and nontreated wheat plants showed a diverging development, characterized by lowered growth rates of diseased roots and shoots (Supplementary Fig. S1).

The partial seedling resistance of Florence-Aurore was characterized by a low and stable level of fungal growth (mean over seven timepoints, MoT 0.56%) associated with the lowest measured reduction of root biomass (MoT 31.5%), root and shoot length (MoT 9.4% and 8.6%) of diseased seedlings (Fig. 1A and B). However, in adult plant roots the fungal biomass was on average about sevenfold higher than at the seedling stage, coinciding with an about twofold increase in plant biomass loss. In adult Florence-Aurore plants the growth pattern of *F. graminearum* peaked at 21 dai at a level of 8.65% (Fig. 1A), reminiscent of the pattern observed in seedlings of Ning 7840. Fungal accumulation in the adult plant roots of Ning 7840 (Fig. 1C) was considerably lower (MoT 3.4%) than in seedling roots (MoT 8.3%). However, between 1 to 10 dai the pathogen developed comparable levels of biomass in seedling and mature roots. But, from 14 dai onwards seedling roots showed a sudden threefold increase in fungal growth which was not found in the adult plant roots of Ning 7840. Nevertheless, disease severity was almost reduced by half compared with the FRR-treated seedlings (Fig. 1D). On the contrary, the cultivars Frontana (Fig. 1E and F) and Sumai 3 (Fig. 1G and H) showed at both wheat stages an almost similar level of relative susceptibility to root infection. Interestingly, although *F. graminearum* accumulated at different rates in seedling roots (MoT 1.6%) and mature roots (MoT 3.4%) of Sumai 3, the fungal growth pattern showed a similar quadratic trend that peaked at 5 dai (Fig. 1G).

The progressive nature of FRR disease was demonstrated by significant time effects on all four severity traits, indicating an

increasing disease impact over time (Tables 3 and 4). Three distinct disease phases could be distinguished in both wheat stages; posthoc pairwise comparisons showed significant changes in fungal growth between 1 to 3 dai ($P < 0.001$) and between 10 to 14 dai (seedlings

$P = 0.001$, adult plants $P < 0.001$). Finally, the developmental stage of wheat had obviously no impact on pathogen growth and disease progress, but had an effect on the wheat response to FRR. Significant time $\times$ genotype interactions (mainly at $P < 0.001$, but in

Fig. 1. Comparison of *Fusarium* root rot severity in seedling and adult wheat roots. Relative fungal biomass in cultivars **A**, Florence-Aurore; **C**, Ning 7840; **E**, Frontana; and **G**, Sumai 3: *Fusarium graminearum* biomass was measured by reference gene-based qPCR and calculated as fungal versus wheat root biomass (DNA) ratio. Growth impairment in cultivars **B**, Florence-Aurore; **D**, Ning 7840; **F**, Frontana; and **H**, Sumai 3. The level of reduction was calculated as percent difference in root biomass (measured by qPCR), root length, and shoot length between inoculated and noninoculated plants. All data are presented as mean ($\pm$ SD) of $n = 4$ biological replicates. A graphic representation (profile plot) showing the performance of inoculated and noninoculated plants over the time in a comparison is provided in the supplementary material. Abbreviations: dai, days after root inoculation; RBr, root biomass reduction; RLr, root length reduction; and SLr, shoot length reduction.

all cases at $P < 0.05$) were actually found for most assayed traits (Tables 3 and 4). This finding demonstrates that, besides pathogenic virulence, the wheat genotype is another major factor that affects FRR severity and progression in all growth stages of wheat. The observed genotypic responses were combined by calculating a FRR disease index (FDI) including all four severity traits (Fig. 2). The ranking illustrates for Florence-Aurore a partial seedling resistance (minimum FDI) and an adult plant susceptibility to FRR (maximum FDI, similar to Ning 7840 seedlings), corresponding to the FHB susceptibility of this genotype. Moreover, the FDI ranking shows that none of the genotypes expressed adult plant resistance to root infection. This has been a rather unexpected result, even though Ning 7840, Sumai 3, and Frontana are resistant to FHB. The adult plant FDI of Ning 7840 was lower than the seedling value, but still closer to the FDI values of Sumai 3 and Frontana than to those of resistant Florence-Aurore seedlings. This demonstrates an adult plant response of Ning 7840 which can be defined as moderate susceptibility rather than as partial resistance to FRR.

Seedling resistance to root colonization is associated with expression of FHB resistance-related genes in roots. Seedling resistance to FRR leads to a significantly lowered *F. graminearum* growth during main infection and root colonization (Fig. 1A). To study this resistance on the molecular level, six defense-related genes (*TaUGT12887*, *TaUGT3*, *CYP709C1*, *WZF1*, *WFhb1-c1*, and *TaMDR1*) were selected, previously associated with the FHB resistance against DON and fungal invasion of host tissue. Detailed information on these genes is presented in Table 2, including references for expression patterns during FHB. Gene expression was analyzed in the seedling roots of Florence-Aurore, Ning 7840, Sumai 3, and Frontana in the interval 1 to 21 dai. The results demonstrate that all six genes were up-regulated in response to *F. graminearum* root infection (Figs. 3A to C and 4A to C). For all genes the rANOVA showed significant time-genotype interactions, meaning that the factor genotype had an effect on the *F. graminearum* induced gene expression over time (Supplementary

Table S2, including rANOVA results for the subsequent time-course analyses). A much faster defense response was triggered in the seedling roots of resistant Florence-Aurore as compared with the FRR susceptible varieties Ning 7840, Frontana, and Sumai 3. For Florence-Aurore, the ANOVA statistics demonstrated a significantly different response—in terms of higher relative expression rates—in the period 1 to 5 dai. This applied to the UDP-glucosyltransferase (UGT) genes *TaUGT12887* (Fig. 3A) and *TaUGT3* (Fig. 3B), the cytochrome P450 gene *CYP709C1* (Fig. 3C), *WZF1* encoding a wheat zinc finger transcription factor (TF) protein (Fig. 4A), and *WFhb1-c1*, a genetic component of the major FHB resistance QTL *Fhb1* (Fig. 4B). Solely the ABC transporter gene *TaMDR1* showed a distinct expression pattern with a maximal up-regulation at 10 dai in Florence-Aurore roots and at 14 dai in Frontana roots (Fig. 4C). The Florence-Aurore-specific response at 1 to 5 dai coincided with an overlap in time between main infection and root colonization in susceptible interactions associated with accelerated fungal growth and increasing disease severity (Wang et al. 2015). The seedling roots of Ning 7840, Frontana, and Sumai 3 responded considerably slower (not before 7 dai) and typically at lower levels compared with the early response in Florence-Aurore. From 7 dai on, gene expressions were rather balanced between the four genotypes, meaning that the statistical grouping no longer suggested a clear difference between genotypes.

Adult plant susceptibility to FRR leads to age-related changes in gene expression pattern. While investigations at the seedling stage allow testing genotypic response patterns to FRR, the age-related patterns of defense reactions can be studied by testing different growth stages. Therefore, we have studied the gene expression patterns in response to adult plant root inoculation. Of particular note was that the transition from seedling resistance to adult plant susceptibility observed in Florence-Aurore (Fig. 2) correlated with changes in the expression of all six genes assayed (Figs. 3D to F and 4D to F). While the seedling resistance of Florence-Aurore was characterized by maximum gene expression

TABLE 3. Repeated measures analysis of variance (rANOVA) on *Fusarium graminearum* and root biomass at seedling and adult wheat stages^a

Stage	Relative fungal biomass				Root biomass			
	df	F	P	E	df	F	P	E
Seedling stage								
Treatment ^b	–	–	–	–	1.0	181.14	<0.001	0.92
Time ^b	–	–	–	–	1.4	30.44	<0.001	0.67
Treatment × time ^b	–	–	–	–	2.1	12.85	<0.001	0.46
Time ^c	3.9	163.92	<0.001	0.93	3.2	25.81	<0.001	0.68
Time × genotype ^c	11.6	99.90	<0.001	0.96	9.6	9.22	<0.001	0.70
Adult plant stage								
Treatment ^b	–	–	–	–	1.0	32.39	<0.001	0.68
Time ^b	–	–	–	–	3.5	5.13	0.039	0.26
Treatment × time ^b	–	–	–	–	3.7	4.04	0.007	0.21
Time ^c	2.5	8.70	0.001	0.42	3.4	4.15	0.010	0.26
Time × genotype ^c	7.4	4.81	0.001	0.55	10.1	2.51	0.019	0.39

^a In all analyses, no sphericity could be assumed ($P < 0.05$, Mauchly's test). Results were therefore corrected based on the Greenhouse-Geisser F test. No differences were observed in the significance of F values between all three corrections (Sphericity assumed, Greenhouse-Geisser, Huynh-Feldt) provided by the tests of within-subjects effects table. df, degrees of freedom; F , F value; P , P value; E , effect size (partial eta squared statistic), which is the strength of associations between main effect/interaction and dependent variable is given as effect size (partial eta squared statistic).

^b Two-way rANOVA on root biomass in infected and uninfected seedlings/adult plants, based on two within-subjects factors time and treatment.

^c Mixed rANOVA on disease progression in terms of fungal growth and decrease of root biomass, based on within-subjects factor time and between-subjects factor genotype.

TABLE 4. Repeated measures analysis of variance (rANOVA) on root and shoot response at seedling and adult wheat stages^a

Stage	Root length				Shoot length			
	df	F	P	E	df	F	P	E
Seedling stage								
Treatment ^b	1.0	275.00	<0.001	0.78	1.0	192.22	<0.001	0.78
Time ^b	4.5	341.10	<0.001	0.81	3.7	362.72	<0.001	0.87
Treatment × time ^b	3.8	29.66	<0.001	0.27	3.9	17.10	<0.001	0.24
Time ^c	4.9	10.10	<0.001	0.16	4.7	6.74	<0.001	0.12
Time × genotype ^c	14.9	3.00	<0.001	0.15	14.2	2.23	0.007	0.11
Adult plant stage								
Treatment ^b	1.0	566.02	<0.001	0.88	1.0	206.61	<0.001	0.72
Time ^b	2.5	35.78	<0.001	0.31	4.1	11.32	<0.001	0.13
Treatment × time ^b	4.0	65.72	<0.001	0.45	5.2	23.10	<0.001	0.23
Time ^c	5.1	160.35	<0.001	0.68	3.9	72.26	<0.001	0.49
Time × genotype ^c	15.3	20.35	<0.001	0.45	11.9	6.43	<0.001	0.20

^a In all analyses, no sphericity could be assumed ($P < 0.05$, Mauchly's test). Results were therefore corrected based on the Greenhouse-Geisser F test. No differences were observed in the significance of F values between all three corrections (Sphericity assumed, Greenhouse-Geisser, Huynh-Feldt) provided by the tests of within-subjects effects table. df, degrees of freedom; F , F value; P , P value; E , effect size (partial eta squared statistic), which is the strength of associations between main effect/interaction and dependent variable is given as effect size (partial eta squared statistic).

^b Two-way rANOVA on root biomass in infected and uninfected seedlings/adult plants, based on two within-subjects factors time and treatment.

^c Mixed rANOVA on disease progression in terms of fungal growth and decrease of root biomass, based on within-subjects factor time and between-subjects factor genotype.

levels covering main infection and root colonization (1 to 5 dai), this was no longer present in adult plant roots. Although the factor genotype generally had a significant effect on the gene expression over time and high-level transient up-regulations were observed in adult plant roots, no statistically significant gene expression pattern was found for any of the genotypes tested. Expression of both UGT genes at 1 dai (Fig. 3D and E) and the P450 gene *CYP709C1* at 1 to 7 dai (Fig. 3F) were significantly different between Ning 7840 and the other three genotypes. This was a notable change with respect to the gene expressions found in Ning 7840 seedling roots. Nevertheless, the observed gene expression pattern corresponds to the absence of a resistant phenotype at the adult plant stages (Fig. 2), and suggests that timing and amplitude of gene expressions may play a role for the transition from susceptibility to resistance.

FHB resistance-related gene expression during the early stages of FRR resistance. Besides root cortex invasion, initial infection is also reduced in resistant seedling roots (Fig. 1). Hence, we have studied the early infection events in seedling roots and spikes in relation to the expression pattern of five wheat genes (*TaLox2*, *TaPR-4b*, *TaJAZ9*, *TabHLH041*, and *TaPR-14*) selected as components of a JA-mediated resistance to initial FHB infection. Detailed information on these genes is given in Table 2. The gene *WZF1* was included in this study since it had not been allocated to a specific type of resistance, and *WFhb1-c1* as it had been related to the early spike response (8 to 24 hai) controlled by the major resistance QTL-*Fhb1* (Table 2). An inoculation experiment was conducted for the interval 2 to 72 hai and gene expression was analyzed in Florence-Aurore, Ning 7840, Sumai 3, and Frontana. For all genes the rANOVA statistics demonstrated a significant genotype impact on the expression over time. All seven genes tested showed an increased transcript accumulation at 24 to 72 hai in the seedling roots of Florence-Aurore (Figs. 5A to D and 6A to C). This response was significantly different from the susceptible genotypes, in terms of higher relative expression rates. Three genes showed a sudden maximal transcript accumulation at 24 hai: the lipoxygenase gene *TaLox2* (Fig. 5A), the putative pathogenesis-related (PR) genes *TaPR-4b* (Fig. 5B) and *WZF1* (Fig. 5C). Another three genes demonstrated ascending expression with induction peaks at 72 hai, coinciding with the first acceleration of *F. graminearum* growth during susceptible interactions (Fig. 1). This group comprised *TaJAZ9* encoding a *Jasmonate ZIM-domain* protein (Fig. 6A),

named due to protein sequence similarity with rice gene *OsJAZ9*, *TabHLH041* encoding a basic helix-loop-helix TF (Fig. 6B), and *TaPR-14* encoding a nonspecific (ns) LTP1 precursor (Fig. 6C). Taking into account the 21-day time-course, the results suggest for *WZF1* a Florence-Aurore-specific expression response at relative maximal levels from 0.5 to 10 dai (Figs. 4A and 5C), and for *WFhb1-c1* from 0.5 to 5 dai (Figs. 4B and 5D). Briefly, for both genes transcript accumulations agreeing between the 21-dai and 72-hai assay were observed. Finally, in the interval 2 to 12 hai, no significant gene expression pattern was found if compared with the sustained high-level expressions between 12 to 72 hai in Florence-Aurore roots. Even though, temporary gene expression in susceptible roots occasionally exceeded those of the resistant roots. Considering the coincidence in time between gene up-regulation and histologically observed fungal processes, the expression dynamics associated with root infection complies with those observed for the genes assayed as components of a resistance to root colonization.

Gene expression pattern in Florence-Aurore and Sumai 3 related to early spike infection by *F. graminearum*. To compare early gene activity in response to seedling root and floret infection, we have used a spike assay applied to the genotypes Sumai 3 and Florence-Aurore, which differ in their response to FRR and FHB. The spike study covered the whole course from 2 to 96 hai. All seven genes assayed were induced in *F. graminearum*-inoculated florets during resistant and susceptible interactions (Fig. 5E to H and 6D to F). Both JA pathway genes, *TaLox2* (Fig. 5E) and *TaJAZ9* (Fig. 6D), were expressed in Sumai 3 and Florence-Aurore spikes. However, *TaLox2* was significantly earlier up-regulated in Sumai 3 spikes, and *TaJAZ9* was 1 day earlier and two- to fourfold higher expressed in Sumai 3 than in Florence-Aurore. *TaJAZ9*, encoding a key regulator of JA signaling, showed remarkably similar expression patterns in roots and spikes, suggesting that *F. graminearum* infection triggers the same JA-mediated responses in both organs. The gene *PR-14*—supposed to be involved in JA defense signaling (Table 2)—showed notable expression values (greater than twofold change) exclusively in resistant spikes at 4 and 24 hai (Fig. 6F). The rapid up-regulation at 4 hai in Sumai 3 spikes is shared by the genes *PR-14* and *Lox2* (Fig. 5E). *TaPR-4b* was increasingly up-regulated in both genotypes (Fig. 5F). However, in Sumai 3 the gene showed a notable expression (greater than twofold induction) 2 days earlier and was significantly higher up-regulated between 24 to 72 hai. *WZF1* was significantly higher expressed in Sumai 3 than in Florence-Aurore at 48 to 72 hai (Fig. 5G), and *TabHLH041* solely at 48 hai (Fig. 6E). As an exception, rANOVA suggested a nonsignificant genotype impact on *WZF1* expression over time. *WFhb1-c1* was almost exclusively expressed in Sumai 3 with sevenfold change values between 8 to 48 hai and a 74-fold induction peak at 16 hai (Fig. 5H). A similar resistance-specific activity was found in the seedling roots of Florence-Aurore at 12 to 72 hai (Fig. 5D), suggesting that *WFhb1-c1* may have a relevant role in both the root and the spike resistance to *F. graminearum*. Finally, the observed similarity in early molecular events between seedling resistance (Florence-Aurore) and adult plant resistance (Sumai 3) demonstrates that the ability of wheat to respond to *F. graminearum* infection is subjected to major changes during plant development.

FRR-induced expressions of defense-related genes in stem and leaf tissues. The induced systemic expression of defense-related genes in healthy wheat shoots as a result of local root infection by *F. graminearum* has, to our knowledge, not been reported to date. The present case study addresses the gene expression in healthy aboveground stem and leaf tissues of FRR-treated seedling and adult plants. Analyses were conducted in Florence-Aurore and Ning 7840 (Supplementary Fig. S2), which differ clearly in their respective seedling and adult plant responses to FRR (Fig. 2). The DON resistance-related genes *TaUGT12887* and *CYP709C1*, the JA signaling gene *TaJAZ9*, and the transcription factor gene *WZF1* were subjected to time-course expression analyses during 1 to 7 dai,

Fig. 2. Genotypic variation in the wheat response to Fusarium root rot (FRR) at seedling and adult plant stages. Genotypic responses are given as mean FRR disease index (FDI) over seven measurement timepoints. The respective FDI scores are given as dimensionless susceptibility index (SI) values, and are ranked in an ascending order where a lower FDI is taken as higher relative resistance, while a higher FDI indicates a higher relative susceptibility. For all genotypes the reactions to seedling FRR and Fusarium head blight (FHB) are listed: HR, highly resistant; R, resistant; HS, highly susceptible; and S, susceptible.

coinciding with the most critical events during the course of FRR disease. In the seedlings, all four genes were expressed at 3 to 7 dai in stem and leaf tissues of the FRR resistant Florence-Aurore, while no expression was observed in FRR susceptible seedlings of Ning 7840. The systemic response of the regulating genes *TaJAZ9* and *WZF1* was induced already at 1 dai in both organs and thus, more immediate compared with the DON resistance-related genes. In the adult plants, it was noticeable that none of the genes assayed was induced in the stem, while all four genes were expressed at 1 to

7 dai in the leaves. Another striking aspect was that the genes now responded much earlier and/or at higher fold change values in Ning 7840, compared with the adult plant leaves of Florence-Aurore. Although the presented observations are preliminary in terms of the limited number of genotypes, genes, and timepoints, they already demonstrate a notable coincidence with the local responses in diseased roots as well as with the finding of age-related changes in the wheat response to *F. graminearum* infection. Further experiments should aim at the confirmation of induced systemic

Fig. 3. Comparative expression analysis of **A and D**, *TaUGT12887*, **B and E**, *TaUGT3*, and **C and F**, *CYP709C1* between seedling and adult wheat roots. Transcript levels were monitored by qPCR in root samples of Florence-Aurore (seedling resistance/adult plant susceptibility to Fusarium root rot [FRR]), Sumai 3, Frontana, and Ning 7840 (varying levels of seedling/adult plant susceptibility to FRR). An overview on the genotypic variation in response to FRR is given in Figure 2. Fold increases were calculated relative to the internal standard gene (*Ubiquitin*) and a water control sample using the comparative cycle threshold (Ct) method. Data are presented as mean ($\pm$ SD) of $n = 4$ biological replicates. Gene expressions indicated by the same letter are not significantly different between genotypes (at least at $P \geq 0.05$; one-way analysis of variance).

resistance in wheat, and study the age-related changes and control mechanisms (for example root-shoot signaling) associated with these responses.

DISCUSSION

Studies on root resistance are generally limited by the difficulty to identify genotype-specific interactions in below-ground

pathosystems (Okubara and Paulitz 2005). Therefore, the discovery of partial seedling resistance to root infection by *F. graminearum* in the wheat cultivar Florence-Aurore provided a unique opportunity to gain insights into a previously unknown type of *Fusarium* resistance in wheat. Since then the Florence-Aurore resistance has been validated in several experiments, including phenotypic and histological studies (Wang et al. 2015). To gain insights into the molecular basis of this resistance and its relation to FHB reaction,

Fig. 4. Comparative gene expression analysis of **A and D**, *WZF1*, **B and E**, *WFhb1-c1*, and **C and F**, *TaMDR1* between seedling and adult wheat roots. Transcript levels were monitored by qPCR in root samples Florence-Aurore (seedling resistance/adult plant susceptibility to Fusarium root rot [FRR]), Sumai 3, Frontana, and Ning 7840 (varying levels of seedling/adult plant susceptibility to FRR). An overview on the genotypic variation in response to FRR is given in Figure 2. Fold increases were calculated relative to the internal standard gene (*Ubiquitin*) and a water control sample using the comparative cycle threshold (Ct) method. Data are presented as mean ($\pm$ SD) of $n = 4$ biological replicates. Gene expressions indicated by the same letter are not significantly different between genotypes (at least at $P \geq 0.05$; one-way analysis of variance).

Fig. 5. Comparative gene expression analysis between wheat seedling roots and spikes: **A** and **E**, *TaLox2*, **B** and **F**, *TaPR-4b*, **C** and **G**, *WZF1*, and **D** and **H**, *WFhb1-c1* with peak expression at 24 h after root infection. The seedling root assay comprises the Fusarium root rot (FRR) resistant cultivar Florence-Aurore, and the FRR susceptible cultivars Sumai 3, Frontana, and Ning 7840. The spike assay comprises the Fusarium head blight (FHB) susceptible cultivar Florence-Aurore, and the FHB resistant cultivar Sumai 3. Transcript levels were monitored by qPCR; and fold increases were calculated relative to the internal standard gene (*Ubiquitin*) and a water-treated control sample (mock) of the respective sampling time using the comparative cycle threshold (Ct) method. Seedling root data are presented as mean (+SD) of $n = 4$ biological replicates; and those spikes data are presented as mean (+SD) of $n = 3$ biological replicates. Gene expressions indicated by the same letter are not significantly different between genotypes (at least at $P \geq 0.05$; one-way analysis of variance).

we have employed a candidate gene/pathway approach by testing a collection of 11 prominent FHB defense-related genes for differential expression. The results demonstrate that the FRR resistance phenotype (Florence-Aurore) is associated with a more rapid, high-level expression of these genes, specifically covering the period when main infection and seedling root colonization overlap (Wang et al. 2015). In contrast, the susceptible phenotype

(Ning 7840, Sumai 3, and Frontana) shows much lower expression values during this period, and expression levels similar to the early Florence-Aurore response are delayed and temporary. This finding indicates recognition of *F. graminearum* by the wheat root, which triggers the induction of a set of genes originally identified as related to spike resistance. This overlap comprises marker genes for two spike defense mechanisms (DON and JA-mediated resistance)

Fig. 6. Comparative gene expression analysis between wheat seedling roots and spikes: **A and D**, *TaJAZ9*, **B and E**, *TabHLH041*, and **C and F**, *TaPR-14* with peak expressions at 72 h after root infection. The seedling root assay comprises the Fusarium root rot (FRR) resistant cultivar Florence-Aurore, and the FRR susceptible cultivars Sumai 3, Frontana, and Ning 7840. The spike assay comprises the Fusarium head blight (FHB) susceptible cultivar Florence-Aurore, and the FHB resistant cultivar Sumai 3. Transcript levels were monitored by qPCR, and fold increases were calculated relative to the internal standard gene (*Ubiquitin*) and a water-treated control sample (mock) of the respective sampling time using the comparative cycle threshold (Ct) method. Seedling root data are presented as mean (+SD) of $n = 4$ biological replicates; and those spikes data are presented as mean (+SD) of $n = 3$ biological replicates. Gene expressions indicated by the same letter are not significantly different between genotypes (at least at $P \geq 0.05$; one-way analysis of variance).

that are common to a variety of wheat genotypes (as summarized in Table 2). However, whether these features also apply to infections in other plant organs was hitherto unknown. Important implications for the applied candidate gene approach came from the revealed resemblance of *F. graminearum* pathogenesis in roots and spikes. Chronology and pathogenic processes underlying root infection and FRR disease progression were similar to those reported for FHB (Wang et al. 2015). This is supported by the detection of several overlapping virulence genes and metabolites induced by *F. graminearum* during seedling coleoptile (FCR) and spike infection (Stephens et al. 2008; Zhang et al. 2012). Until now, no detailed study has been conducted to determine the pathogenicity factors released by *F. graminearum* during root infection. However, the fungal gene *FgABC1*, encoding an ABC drug resistance transporter, was proven to be equally vital for the pathogenicity of *F. graminearum* during FHB, FCR, and FRR (Gardiner et al. 2013).

The seedling resistance to FRR observed in this study was associated with the rapid and sustained expression of three genes (*TaUGT12887*, *TaUGT3*, and *CYP709C1*) that have been detected as components of spike resistance against the mycotoxin DON, and two genes (*WZF1* and *WFhb1_c1*) associated with protection against the pathogen spread from initial site of infection (Table 2). Common to these genes were transcript levels that continuously increased from 1 to 5 dai (Figs. 3A to C and 4A to C). This expression dynamics is consistent with reports on FHB (Gottwald et al. 2012; Ma et al. 2015; Muhovski et al. 2014) as well as with the increase of DON detoxification rate from 1 to 5 days in Sumai 3 (Cirlini et al. 2014) and in transgenic wheat spikes expressing *HvUGT13248* (Li et al. 2015). *TaUGT12887* is the hitherto closest wheat homolog of the barley UGT gene *HvUGT13248*. The barley gene has a proven ability to inactivate the mycotoxins DON (Schweiger et al. 2010) and nivalenol (Li et al. 2017), and was found to significantly lower the spread of *F. graminearum* in spikes when expressed in transgenic wheat (Li et al. 2015, 2017). Notably, wheat seedlings expressing *HvUGT13248* display higher tolerance to DON-inhibited root growth compared with the controls (Li et al. 2015). The same observations were made in spikes and seedling roots of the model grass species *B. distachyon* by overexpressing *Bradi5g03300*—a UGT gene with a proven ability to detoxify DON in planta. In addition, evidence was found that DON is involved in the infection process of *F. graminearum*, which would explain the observed detoxification rates already at 1 dai (Pasquet et al. 2016). Our results agree with this concept and indicate a direct, coordinated counter-response to root infection, DON production and *F. graminearum* growth—disease events that were similarly assigned to the interval 1 to 5 dai for FRR (Wang et al. 2015) and FHB (Boenisch and Schäfer 2011). The UGT product of *TaUGT12887* has weak detoxification ability compared with *HvUGT13248*. However, *TaUGT12887* is highly responsive to DON and an indicator of activated toxin resistance. Hence, uncharacterized paralogous of *TaUGT12887* may perform primary detoxification function (Schweiger et al. 2013). Until today, no wheat UGT involved in DON detoxification has been functionally characterized (Powell et al. 2017).

The maximum rate of *F. graminearum* gene induction in planta was found during the early infection process until about 72 hai (Harris et al. 2016; Seong et al. 2008; Stephens et al. 2008; Zhang et al. 2012). Interestingly, for the period <12 to 72 hai the largest difference of resistance-related transcript and metabolite accumulations was found between resistant and susceptible wheat spikes. A function of these responses might be to interfere with the onset of necrotrophic disease spread (Paranidharan et al. 2008; Zhuang et al. 2013). Accordingly, all seven FHB defense-related genes selected to study the early wheat responses (Table 2) were expressed between 12 to 72 hai in the roots of FRR resistant Florence-Aurore (Figs. 5A to D and 6A to C). A similar resistance-related expression dynamic was observed in the parallel Sumai 3 spike assay (Figs. 5E to H and 6D to F), whereby the genes *TaLox2* and *TaPR-14* were induced already at 4 hai. *F. graminearum* infects the root by direct

cell penetration. Initially, hyphopodia-like infection structures are formed by the vegetative mycelium, resembling that of “typical” root pathogens. Later, bulbous infection hyphae and infection cushions originate from runner hyphae, reminiscent of that formed in florets (Wang et al. 2015). Against this background, it was interesting that wheat transcript accumulations correlated with changes in the pathogen behavior: either with the start of initial penetration at 24 hai, or with the onset of main infection and first root necrosis at 72 hai.

The JA signaling pathway has a central role in FHB defense transcriptome activation (Sun et al. 2016). JA application reduces both the *F. graminearum* and the symptom spread during FHB (Sun et al. 2016; Qi et al. 2016) and FCR (Desmond et al. 2006; Powell et al. 2017). Correspondingly, our results demonstrate the activation of genes (*TaLox2*, *TaJAZ9*, *TaPR-4b*, and *TaPR-14*) associated with the JA pathway in both the FRR and the FHB resistant phenotype. For example, inoculation of wheat coleoptiles, roots, or germinating seeds with the *F. graminearum* relative *F. culmorum* has triggered the induction of *PR4* genes, whose wheatwin protein products significantly inhibit the hyphal growth of several pathogens (Bertini et al. 2003; Caruso et al. 1999; Roberti et al. 2008).

As for other plant-pathogen interactions, different plant hormones play a role in resistance of wheat to *F. graminearum*, including JA, salicylic acid (SA), ethylene, or abscisic acid. For instance, both JA and SA accumulate in wheat spikes infected by *F. graminearum*. SA may have a direct effect on fungal growth and DON production rather than a regulative function (Qi et al. 2012; Zhang et al. 2017). There is also evidence that SA signaling plays a critical role in the early (genotype-independent) basal resistance against FHB (Ding et al. 2011; Makandar et al. 2012). JA signaling seems to play a dichotomous role within the complex SA–JA interaction. Methyl-JA (MeJA) application at the time of fungal inoculation was found to attenuate the early (<12 hai) SA-mediated resistance in *TaNPR1*-expressing wheat (cultivar Bobwhite), while MeJA application after inoculation (≥ 2 dai) led to an enhanced resistance in FHB susceptible (nontransgenic) Bobwhite plants (Makandar et al. 2012). It is yet unknown whether a corresponding SA–JA interaction exists in wheat roots. During the period 2 to 12 hai, none of the JA pathway-related genes assayed in seedling roots showed an expression pattern that could be associated with a genotype-specific response to FRR as compared with the period 24 to 72 hai. In this context, the very early stage (<12 hai) is associated with the establishment of FRR disease on root surface (spore germination, formation of mycelium network), but not with the penetration of root epidermis (Wang et al. 2015).

The expression dynamics found for the JA signaling gene *TaJAZ9*, which was detected as component of the FHB resistance in the landrace Wangshuibai (Xiao et al. 2013), was remarkable. In both, the roots of FRR resistant Florence-Aurore and spikes of the FHB resistant Sumai 3, *TaJAZ9* showed a sustained increase in transcript accumulation from 24 hai on (Fig. 6A and D). Constant expression was also observed for the FRR induced systemic responses at 1 to 7 dai in healthy stems and leaves of Florence-Aurore seedlings. JAZ proteins are central components in the regulation of plant defense and development (Liu et al. 2015), because they link JA perception with transcriptional changes (Santner and Estelle 2009). Their roles in monocot resistances to fungal (*Fusarium*) pathogens are little understood (Campos et al. 2014). Six *TaJAZ* genes previously associated with *Fusarium* and/or DON resistance in wheat (named according to their closest rice homologs) are listed in Supplementary Table S3.

The continuous induction of *TaJAZ9*, unique among the seven genes assayed in Sumai 3, corresponds to the expression dynamics reported for stress-responsive JAZ genes, presumably required for a sustainable alteration of JA (Demianski et al. 2012; Sherif et al. 2015). In this context, it has been demonstrated in wheat seedling roots and shoots that *TaJAZ9* expression is not induced solely by MeJA at 24 to 72 dai (Liu et al. 2016). Therefore, *F. graminearum*

infection may account for the *TaJAZ9* expressions that we observed in seedling roots, shoots and spikes. It has been demonstrated in wheat and *B. distachyon* spikes that the DON mycotoxin induces the expression of *TaJAZ6* (assayed at 24 hai) and *TaJAZ13* (assayed at 96 hai), and that the mycotoxin modulates JA biosynthesis and signaling to promote disease (Desmond et al. 2008; Pasquet et al. 2014). In susceptible wheat genotypes, the DON phytotoxicity leads to an impaired seedling root growth (Masuda et al. 2007) and grain development (Siou et al. 2014), both effects are significantly lowered by an enhanced DON detoxification process (Li et al. 2015). The *F. graminearum*- and DON-mediated up-regulation of *JAZ* gene transcripts suggests the suppression of the MYC2-induced JA signaling (Pasquet et al. 2014)—a branch in the JA pathway that negatively affects the resistance to necrotrophic pathogens (Kazan and Manners 2013). The majority of *JAZ* proteins found in *Arabidopsis* repress the MYC2-dependent signaling, and deactivation of AtMYC2 has been correlated with enhanced resistance in different pathosystems (de Torres Zabala et al. 2015; Kidd et al. 2011; Ralhan et al. 2012; Thatcher et al. 2009). A corresponding relationship between the expression of AtMYC2 wheat homologs and FHB susceptibility has been reported in wheat (Ding et al. 2011; Xiao et al. 2013), while *TaJAZ9* expression was related to resistance (Xiao et al. 2013). For resistant seedling roots we found the sustained up-regulation of *TaJAZ9* during the entire time-course 0.5 to 21 dai, with a maximum 284-fold induction at 5 dai (Supplementary Fig. S3). This expression pattern coincides, among others, with those of the prominent DON resistance-related genes *HvUGT12887* and *CYP709C1* (Fig. 3A and C).

F. graminearum is a systemic pathogen that infects root tissue and travels to the stem vasculature to cause disease in the stem and leaf tissue. An important feature of seedling resistance to FRR is inhibition of stem colonization, particularly the protection against hyphal growth into the stem vasculature (Wang et al. 2015). We have selected the genes *TaJAZ9*, *HvUGT12887*, *CYP709C1*, and *WZF1* to test for FRR-induced systemic responses. Systemic responses were an important defense associated with *Arabidopsis* root infections by *F. oxysporum* (Kidd et al. 2011). However, the induced systemic resistance (ISR) is less explored in monocot than in dicot host plants (Heil and Bostock 2002). At seedling stages, all four genes assayed in our study showed high expressions at 1 to 7 dai in the stem and leaf tissues of Florence-Aurore (FRR seedling resistance), while the shoots of Ning 7840 (FRR susceptible) did not show gene expressions. Hence, ISR can be considered as a mechanism of *F. graminearum* resistance, possibly being involved in the FRR resistance-related reduction of disease spread within the shoot (Wang et al. 2015). It would be interesting to study and compare the stem and the spike rachis resistance, the latter characterized by cell wall enforcement and detoxification of DON (Dhokane et al. 2016; Gunnaiah et al. 2012). For example, the transcription factor *bHLH041* (Fig. 6B and E; Table 2) was recently identified as a candidate resistance gene localized in the QTL-Fhb2 region, associated with high rachis resistance in BW287, a descendant of Sumai 3 (Dhokane et al. 2016). At adult plant stages, all four genes were equally expressed in the leaves of Ning 7840 (adult resistance to FHB), while no expression was observed in leaves of Florence-Aurore (adult plant susceptibility to FRR and FHB) and stem tissues of both genotypes. These preliminary results point to plant age-related changes in the systemic response of Florence-Aurore and Ning 7840, in accordance with their FHB phenotypes. Corresponding changes in gene expression were observed in adult plant roots of Florence-Aurore (Figs. 3 and 4). However, we have never observed (molecular and phenotypic) adult plant resistance to FRR comparable to FHB resistance (Buerstmayr et al. 2009) or seedling resistance to FRR (Fig. 2).

Generally, our results show a wheat response in which besides the *F. graminearum* infection also the plant developmental stage influences the host–pathogen interaction. On the one side, the candidate gene expression analysis has revealed that different

wheat organs utilize similar defense mechanisms in response to *F. graminearum* infection. This suggests that *Fusarium* resistance in wheat may be regulated by common (pathogen induced) signal transduction pathways, as previously postulated by Gao et al. (2013). On the other side, these signaling pathways seem to integrate endogenous molecular signals associated with plant development. A stage-specific reprogramming of gene expression may explain that the same genotype can display adult plant resistance to FHB but susceptibility to seedling attack, or vice versa (Li et al. 2010; Wang et al. 2015). It is conceivable that root defenses are significantly reduced by root cortex cell death. For example, leaf senescence can lead to susceptibility, even if major resistance genes are present (Millett et al. 2009). This aspect suggests that the expression of adult plant resistance to FHB is associated with an enhanced protection against root infection. Interestingly, this was not the case, even though the systemic leaf response showed an age-related change in gene expression. These observations point to physiological features of root aging that impair adult plant resistance. Indeed, root aging in wheat and barley is associated with the nonpathogenic root cortex cell death. Therefore, adult roots may favor the colonization by root pathogens, which are capable for a parasitic and/or saprophytic way of life (Henry and Deacon 1981) such as *Fusarium* pathogens (Goswami and Kistler 2004).

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