Plant Height Affects Fusarium Crown Rot Severity in Wheat

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ABSTRACT

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Effects of plant height on Fusarium crown rot (FCR) disease severity were investigated using 12 pairs of near-isogenic lines (NILs) for six different reduced height (*Rht*) genes in wheat. The dwarf isolines all gave better FCR resistance when compared with their respective tall counterparts, although the *Rht* genes involved in these NILs are located on several different chromosomes. Treating plants with exogenous gibberellin increased FCR severity as well as seedling lengths in all of the isolines tested. Analysis of the expression of several defense genes with known correlation with resistance to FCR pathogens between the *Rht* isolines following FCR inoculation indicated that the better resistance of the dwarf isolines was not due to enhanced defense gene induction. These results suggested that the difference in FCR severity between the tall and dwarf isolines is likely due to their height difference per se or to some physiological and structural consequences of reduced height. Thus, caution should be taken when considering to exploit any FCR locus located near a height gene.

Additional keywords: *Fusarium pseudograminearum*.

Fusarium crown rot (FCR) is a serious cereal disease caused by multiple species of *Fusarium*, with *F. pseudograminearum* (*Gibberella coronicola*) and *F. culmorum* as two of the most predominant pathogens in Australia (1,5). In Australia, FCR is a chronic wheat disease inflicting yield losses of up to 89% (10). Surveys of wheat yield losses indicate a current loss of ca. $US72 millions per annum due to FCR (19). The disease has also become increasingly important in many other cereal growing regions including South Africa, Italy, Egypt, Turkey, Syria, Morocco, Argentina, and China (5,24). It has been reported that FCR could reduce wheat yield by up to 35% in the Pacific North-West of the United States (24). In addition to yield loss, FCR-infected wheat plants in glasshouse assays have been shown to contain mycotoxins in the heads and grain (18) which would potentially have an adverse affect if present in food and feed products.

Conflicting relationships between plant height and FCR resistance have been reported by different groups (12,28). Wallwork et al. (28) reported that taller plants gave better FCR resistance than shorter ones in a population that segregated for both plant height and FCR resistance. These authors mapped a quantitative trait loci (QTL) conferring FCR resistance near the reduced height (*Rht*) gene *Rht1* (*Rht-B1b*) on the short arm of chromosome 4B. Recent results from our laboratory also show an association between plant height and FCR severity in both wheat (12,16) and barley (13). However, contrasting to the previous report, it was the shorter plants that gave better FCR resistance in all three of these populations. As genetic materials, experimental conditions, inoculation methods, plant growth phases, and assessment criteria used in these studies are different, direct comparisons of plant height effects on FCR disease between them are difficult.

One common feature of the different reports to date on possible associations between plant height and FCR reaction is that they are based on studies using segregating populations. A major limitation in using such populations is that individuals have different genetic backgrounds in regard to the disease resistance loci under investigation. The different genetic backgrounds prevent accurate assessments of any genetic effect of the gene concerned. Further, all three of the existing studies on association between FCR reaction and plant height in wheat (12,16,28) involved populations segregating for the same *Rht* gene, *Rht1*. As different *Rht* genes have different effects on plant height as well as other traits of agronomic importance such as seedling vigor, coleoptile length, and leaf area (3,7,22), selecting appropriate *Rht* genes for particular environments has been a key consideration in breeding programs (7,11).

It is well known that exogenous gibberellin (GA) application increase plant height by promoting cell expansion and the levels of responses vary among different *Rht* genes (7,30). Recent results also showed that exogenous GA application may affect resistance to necrotrophic fungal pathogens in *Arabidopsis* (2). Several defense genes correlated with CR resistance have also been reported in wheat (6). These findings were exploited in the current study which was undertaken to examine possible effects of plant height on FCR resistance. To achieve this objective, differences in CR severity, response to exogenous GA treatment, and the expression of defense genes between the tall and dwarf isolines of several pairs of near-isogenic lines (NILs) were examined.

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MATERIALS AND METHODS

Plant materials. Considering that different *Rht* genes may have different effects on plant height as well as on other traits of agronomic importance, an attempt was made in the current study to analyze all *Rht* NILs in our possession. A total of 19 genotypes consisting of 12 pairs of NILs for six different *Rht* genes (*Rht1*, *Rht2*, *Rht3*, *Rht4*, *Rht8*, and *Rht13*) were obtained for this study. The tall genotype Mironovskaya 808 (or M808) is the shared recurrent parent for six pairs of the NILs (Lan 1, Lan 2, Lan 3, Lan 4, Lan 8, and Lan 13, Table 1) and they were reported by Loskutova (15). The other six pairs of NILs were reported by Singh et al. (23). Three of the six *Rht* genes (*Rht1*, *Rht2*, and *Rht3*) are insensitive to applied GA (GA-insensitive) and the other three (*Rht4*, *Rht8*, and *Rht13*) are GA-sensitive (7). The six different *Rht* genes are located at five different chromosomes: *Rht1* and *Rht3* on the short arm of chromosome 4B (or 4BS), *Rht2* on 4DS, *Rht4* on 2BL, *Rht8* on 2DS, and *Rht13* on 7BS (7).

Measurement of plant height. Plant heights of the 19 genotypes used in this study were obtained from three trials conducted at three different times. Each trial contained two replicates. Five plants, each in a separate 2.0-liter pot, were used for each of the replicates. For each trial, the pots were arranged in a randomized complete block design in a glasshouse. Settings for the glasshouses were: 25/18 (±5)°C day/night temperature and 65/80 (±5)% day/night relative humidity, with natural sunlight levels and variable photoperiod depending on the time of year. The heights of the main tillers were measured at maturity and the average height from the five plants in each replicate was used for further analyses.

FCR assessment. The assessment of FCR was conducted using the *F. pseudograminearum* isolate CS3096. This isolate is highly aggressive based on an assessment of over 650 isolates collected in field surveys from Queensland and New South Wales (1). Fungal inoculum was prepared following the method described by Li et al. (14). The spores were harvested and the concentration of macroconidial suspension was adjusted to 1×10^6 spores/ml. Tween 20 was added to the spore suspension to a final concentration of 0.1% vol/vol prior to use in inoculation. Grains of all the 19 genotypes used for FCR assessment were selected by removing small and shriveled kernels. The selected grains were surface sterilized and germinated in petri dishes on three layers of filter paper saturated with water. Seedlings were inoculated when they reach between 0.5 and 1.0 cm in length by immersing in either distilled water (mock-inoculated controls) or the spore suspension for about 1 min as described by Li et al. (14). Ten FCR-inoculated seedlings or mock-inoculated controls were then wrapped and grown in a single piece of moist paper towel as one replicate. Experiments on FCR assessment were all carried out in a laboratory with constant temperature (23°C) and relative humidity (≈60%) with natural and fluorescent room lighting maintained for approximately 12 to 14 h daily. Seedlings were kept well watered for the first 48 h after inoculation and then watered only when the seedlings started to wilt by dipping the paper rolls into a water bath for 30 s. FCR severity was scored using a 0 to 5 scale as described by Li et al. (14), where 0 indicates no symptoms and 5 indicates whole seedling completely necrotic. A disease index (DI) was then calculated for each line following the formula of $DI = (\Sigma n_S S/5N) \times 100$, where the summation is over all possible scale values of S, n_S is the number of plants with score S, and N is the total number of plants assessed for each line.

The 12 pairs of NILs were assessed in four trials conducted at four different times. Each of the trials consisted of two FCR-inoculated and two mock-inoculated (controls) replicates. FCR severity was assessed when the most susceptible genotype Aconchi (tall) became severely necrotic (reached a score of 4 in the 0 to 5 scale used), which takes about 14 days after inoculation. At the same time of FCR assessment, lengths of the FCR-inoculated and mock-inoculated seedlings were measured for each of the lines tested.

Response of NILs in FCR reaction and seedling length to applied GA. All of the 12 pairs of NILs were assessed in four trials conducted at four different times. Each of the trials consisted of two GA-treated and two mock-treated (with distilled water) replicates. GA treatment was carried out by germinating grains in petri dishes on three layers of filter paper saturated with 25 ppm aqueous GA_3 (Sigma, Cat. G7645) solution. Seedlings were then inoculated when they reached between 0.5 and 1.0 cm in length by immersing in a spore suspension as described above. FCR assessment was carried out when the most susceptible line Aconchi (tall) became severely necrotic. Lengths of both the GA- and mock-treated seedlings were measured for each of the lines tested at the time of FCR assessment.

Analysis of defense gene expression by real-time quantitative polymerase chain reaction. Four pairs of NILs representing the two most widely used *Rht* genes worldwide, *Rht1* and *Rht2*, were analyzed in this experiment. Two of the NIL pairs (Galvez and Lan 1) were for the *Rht1* gene and the other two (Lan 2 and Pavon) for the *Rht2* gene. They were tested against nine defense genes with known correlation with resistance to FCR infection (6). Seven of the nine genes (*PR10*, *TaGLP2a*, *PR3*, *PR4*, *PR5*, *PR2*, and *PR1.1*) have previously been shown to be consistently up-regulated and the other two (WCI2 and WCI3) consistently down-regulated following FCR infection (6). As it is known that seedling ages may affect the expression of a given gene (6), FCR- or mock- inoculation was carried out at two different time points using either 2- or 14-day-old seedlings. Tissue samples were collected 2 days after inoculation (corresponding to 4- or 16-day-old seedlings, respectively) by removing the lower 2 cm of the seed-

TABLE 1. The 12 pairs of near-isogenic lines (NILs) used, their Fusarium crown rot (FCR) index, seedling length and plant height, and the estimated differences (Est)[a]

NIL pair		FCR index			Seedling length (cm)			Plant height (cm)		
Background	*Rht*	Tall	Dwarf	Est	Tall	Dwarf	Est	Tall	Dwarf	Est
Aconchi	*Rht1*	94.4	80.4	13.8**	19.2	12.2	6.3**	95.2	62.4	36.2**
Galvez	*Rht1*	89.8	67.0	22.3**	21.0	14.8	6.4**	96.4	73.2	24.2**
Kauz	*Rht1*	84.8	59.0	25.0*	17.8	14.6	3.0*	92.7	68.4	21.7**
Lan 1	*Rht1*	74.2	6.4	68.7**	28.9	19.6	9.6**	92.0	76.4	15.5*
Lan 2	*Rht2*	74.2	4.0	71.0**	28.9	20.9	8.5**	92.0	68.4	23.0**
Lan 3	*Rht3*	74.2	6.6	68.3**	28.9	15.2	14.4**	92.0	40.3	51.4**
Lan 4	*Rht4*	74.2	73.2	0.7	28.9	26.3	2.6*	92.0	79.3	12.3**
Lan 8	*Rht8*	74.2	34.6	39.7**	28.9	21.9	6.7**	92.0	60.7	30.3**
Lan 13	*Rht13*	74.2	17.0	57.6**	28.9	25.4	4.5**	92.0	56.8	35.3**
Nesser	*Rht1*	77.2	47.2	30.0**	22.6	13.6	9.3**	94.3	74.9	19.5**
Pavon	*Rht2*	91.0	75.8	15.5**	20.3	15.1	5.4**	107.3	83.0	24.3**
Seri	*Rht1*	90.2	71.8	18.9**	16.4	12.7	3.3**	97.0	78.3	18.8**

[a] * and ** represent significance at $P < 0.05$ and 0.01, respectively, based on Student's t test by fitting all data to one linear model.

ling base of all 10 seedlings in a replication and frozen immediately in liquid nitrogen and stored in –80°C freezers until processing. RNA was isolated using Promega Total RNA Isolation System kit (Promega Corporation, Madison, WI) following the manufacturer's protocol, and cDNA was synthesized with the SuperScript III First Strand Synthesis System from Invitrogen (Carlsbad, CA) using a combination of both 2.5 ng/µl random hexamers and 10 ng/µl oligodT for priming. Methods used for gene expression analysis by reverse transcriptase quantitative polymerase chain reaction (qPCR), including primer details and analysis of results, were as described by Desmond et al. (6). Specifically, real-time (RT)-qPCR was performed in 384-well plates using an ABI7900 HT Sequence Detection System. Each reaction contained 6 µl of 2× SYBR Green Master Mix reagent (Applied Biosystems, Warrington, UK), 250 nM of both forward and reverse gene specific primers, and 25 ng cDNA. Thermocycle conditions were as follows: 50°C for 2 min; 95°C for 10 min; 40 cycles of 95°C for 15 s and 60°C for 1 min. Data were analyzed using SDS2.2 software (Applied Biosystems). Baseline data were collected between the default settings of cycles 3 to 15 and subtracted from fluorescence in subsequent cycles. Exponential amplification was plotted on a logarithmic scale and the Rn was set to 0.32 for each RT-qPCR plate to obtain the cycle threshold (C_t) values. C_t values for all genes were then normalized to wheat β-tubulin (used as an internal control) to account for variation between PCR runs or different cDNA concentrations. Primer efficiencies were determined using the LinRegPCR program (21). Four RT-qPCR trials were conducted at four different times. Each

Fig. 1. Difference between the dwarf (*Rht1)* and tall (*rht1*) isolines of the genotype Galvez in their resistance to Fusarium crown rot infection. **A,** Mock-inoculated controls and **B,** inoculated with *Fusarium pseudograminearum* isolate CS3096, showing that the tall isoline (*rht*) was more severely infected than the dwarf isoline (*Rht*). Photos were taken at 10 days after inoculation.

of these trials consisted of three biological replicates. Ten FCR- and 10 mock- inoculated seedlings were used in each replicate. The relative abundance of transcripts between the dwarf and tall isolines was calculated as ratios of dwarf to tall. A ratio larger than 1 indicates that the gene was more strongly induced in the dwarf isoline compared to that in the tall isoline for a given pair of NILs, and vice versa. Relative abundance of the defense-related genes between FCR-inoculated (with *F. pseudograminearum* isolate CS3096) and mock-inoculated (with distilled water) controls were also calculated in the same manner.

Statistical models. To assess the differences in FCR severity between the tall and dwarf isolines of the 12 pairs of NILs, we used a general linear model with the following three covariates, T, G, and their interaction, $G \times T$. Here T is an indicator for tall ($T = 1$) or dwarf ($T = 0$) lines, G is an indicator of genotypes (12 categories). The same model was used for seeding length and plant height analyses. To assess the effects of exogenous GA on FCR disease or seeding length, we also used three covariates, T, G and their interaction, $G \times T$. In this analysis, T is an indicator for GA-treated ($T = 1$) or mock-treated ($T = 0$) plants, and G represents the genotypes (19 categories). All the analysis was done using the statistical software R (lm function). Significance tests were carried out using the t tests derived from the parameter estimates, their standard errors and the total number of parameters used. The Q-Q plot was produced to check any deviations from the normal assumption (27).

RESULTS

FCR severity and its relationship with plant height and seedling length in 12 pairs of *Rht* NILs. As shown for one of the *Rht1* NIL pairs (Fig. 1), all of the 12 dwarf isolines gave better FCR resistance when compared with their respective tall counterparts. The differences between the tall and dwarf isolines were statistically significant for 11 of the 12 NIL pairs. The only exception was for the NIL pair of Lan 4 differing for the *Rht4* gene. Of the 12 NIL pairs analyzed, the *Rht4* NIL pair was also the one with the smallest difference in plant height or seedling length (Table 1). Three types of differences, corresponding to FCR severity, seedling length and plant height, between the tall and dwarf isolines of the 12 pairs of NILs were analyzed for correlations. The correlations between changes in FCR severity and plant height or seedling length were $r = 0.38$ ($P = 0.22$) and $r = 0.68$ ($P < 0.01$), respectively, and the correlation between changes in plant height and seedling length was $r = 0.54$ ($P = 0.07$).

Six of the 12 NIL pairs analyzed were for the same dwarf gene, *Rht1*. Results from these six NIL pairs showed that the same *Rht* gene has different effects in the different genetic backgrounds on all of the traits measured. Their estimated differences in plant height varied from 12.3 (Lan 4) to 36.2 cm (Aconchi), seedling length from 2.6 (Kanz) to 14.4 cm (Lan 3), and FCR severity from 0.7 (Lan 4) to 71.0 (Lan 2) (Table 1).

Effects of exogenous GA application on FCR severity. As expected, seedling lengths of the 19 genotypes, carrying either GA-sensitive (*Rht4*, *Rht8*, and *Rht13*) or GA-insensitive *Rht* genes (*Rht1*, *Rht2*, and *Rht3*), were all increased following GA treatment (Table 2). The estimated increases in seedling length varied from 0.2 cm (Lan 3) to 7.7 cm (M808), with an average of 3.3 cm (Table 2). In these experiments, the estimated changes in FCR severity were strongly correlated with the estimated changes in seedling length at $r = 0.63$ ($P < 0.01$) across the 19 genotypes analyzed.

Expression of defense-related genes between tall and dwarf isolines. Significant differences in basal gene expression in the mock-inoculated seedlings were detected not only within a given pair of NILs (between the tall and dwarf isolines) but also between NIL pairs for majority of the NIL genotype-gene combinations. This was the case for both of the time points analyzed (Supplementary Figs. 1 and 2). Transcripts for none of the nine genes were more abundant in all four dwarf lines analyzed compared with those in their respective tall counterparts. However, one of the nine genes, *TaGLP2a*, was more strongly expressed in all of the four tall isolines in the 4-day-old seedlings.

A consistent pattern of transcript abundances was again not detected following FCR infection. Most of the genes expressed more strongly in the majority of the genotypes analyzed. Of the 72 combinations (eight lines by nine genes), only 20 had less transcripts in the inoculated 4-day-old seedlings than in the mock-inoculated counterparts (Fig. 2) and the number was further halved (9 out of the 72) in 16-day-old seedlings (Supplementary Fig. 3). However, two of the genes, *PR3* and *PR4*, were more strongly expressed in all of the eight genotypes in 4-day-old seedlings following FCR infection (Fig. 2). These two, together with another three genes, *PR2*, *PR5*, and *PR10*, were more strongly expressed across all of the eight lines analyzed in 16-day-old seedlings following FCR infection (Supplementary Fig. 3). Similar to those in mock-inoculated controls, transcripts

TABLE 2. Observed and estimated differences (Est) in Fusarium crown rot (FCR) index and seedling length of the 19 genotypes following gibberellin or mock treatment (CK)[a]

		FCR severity			Seedling length (cm)		
Genotype	*Rht*	CK	Gibberellin	Est	CK	Gibberellin	Est
Aconchi tall	*rht1*	82.1	90.0	8.0**	19.8	24.2	4.4**
Aconchi 89	*Rht1*	39.1	45.3	6.5*	12.3	13.3	0.6
Galvez tall	*rht1*	76.3	95.8	19.9**	21.2	26.5	5.3**
Galvez dwarf	*Rht1*	49.6	65.2	15.5**	15.0	17.6	2.7**
Kauz tall	*rht1*	69.4	78.0	9.0**	17.6	21.2	3.7**
Kauz dwarf	*Rht1*	47.8	48.6	0.5	13.4	14.0	0.5
Nesser tall	*rht1*	46.8	67.1	20.9**	20.5	25.0	5.2**
Nesser dwarf	*Rht1*	35.9	39.6	4.4	13.4	15.6	2.4**
Seri tall	*rht1*	68.1	81.1	2.7**	17.3	22.6	5.2**
Seri dwarf	*Rht1*	38.9	46.1	6.9*	12.7	13.4	0.7
M808	*rht*	68.3	80.9	13.1**	27.5	35.3	7.7**
Lan 1	*Rht1*	18.8	24.9	6.5*	19.2	20.6	1.4**
Lan 2	*Rht2*	16.0	21.5	5.4	20.0	21.0	0.8
Lan 3	*Rht3*	26.2	30.1	3.6	13.8	14.1	0.2
Lan 4	*Rht4*	56.0	67.5	11.0**	24.6	30.3	5.7**
Lan 8	*Rht8*	21.6	30.9	9.4**	22.8	27.8	4.8**
Lan 13	*Rht13*	47.0	66.4	19.5**	22.7	28.7	5.9**
Pavon tall	*rht2*	68.8	82.5	13.8**	20.6	24.7	3.9**
Pavon dwarf	*Rht2*	50.8	53.4	3.0	15.5	17.8	2.5**

[a] * and ** represent significance at $P < 0.05$ and 0.01, respectively, based on Student's t test by fitting all data to one linear model.

for none of the nine genes were more abundant in all four dwarf lines analyzed compared with those in their tall counterparts for both of the time points analyzed (Fig. 2; Supplementary Fig. 3). *TaGLP2a* was again more strongly expressed in all of the four tall isolines in the 4-day-old seedlings.

Separate analyses of the two different *Rht1* genes also failed to reveal any consistent pattern in gene expression either with (Supplementary Figs. 1 and 2) or without (Fig, 2; Supplementary Fig. 3) FCR infection. Transcript abundances for the nine genes vary between the tall and dwarf isolines for a given NIL pair as well as between NIL pairs at both of the time points (4- or 16-day-old seedlings) analyzed.

DISCUSSION

In the experiments reported in this study we observed that the dwarf isolines always gave better resistance to FCR infection and that both seedling lengths and FCR severities of all genotypes were increased following GA treatment. Together with the lack of consistent pattern in defense gene expression between the tall and dwarf isolines, these results point to the likelihood that height per se may affect FCR resistance in wheat. One of the implications of these results is that caution needs to be taken when considering to exploit any FCR locus located near a height gene (28).

Two of the previous mapping studies showed co-locations of QTL for FCR resistance and the *Rht1* locus on the short arm of chromosome 4BS (16,28). As different *Rht* genes are desirable for different environments (7,11), we therefore investigated the possible effects of plant height on FCR by exploiting 12 pairs of NILs for six different *Rht* genes in wheat using a seedling inoculation assay. Interestingly, the six different *Rht* genes appeared to show effects on FCR reactions in the different genetic backgrounds among the 12 pairs of NILs analyzed that were similar to their effects on plant height and seedling length. When compared to their respective tall counterparts, all the dwarf lines showed better resistance to FCR infection. As the six different *Rht* genes have different chromosomal locations (7), it would be very unlikely that each of these *Rht* genes by chance is linked with a different locus conferring FCR resistance.

A major difference between dwarf and tall isolines is their different cell densities, as *Rht* genes are known to predominantly reduce cell length not cell number or width (4,17,20). The correlation detected between the differences in FCR severity and seedling length between the tall and dwarf isolines ($r = 0.68$, $P < 0.01$) showed strong associations between these two traits, although no causal-and-effect can be implied at this stage. The hypothesis that cell density may affect FCR reaction was further supported by the results from the experiments on exogenous GA treatment, as the growth response of wheat seedlings to exogenous GA application is due to cell expansion not cell division (9,29,30).

Results from previous studies showed that GA and GA signaling pathways may be important in resistance to necrotrophic fungal pathogens in *Arabidopsis* (2). In *Arabidopsis* the DELLA genes are negative regulators of GA responses but appear to promote resistance to necrotrophic fungi by potentiating the jasmonate-dependent defense response. Application of GA to plants leads to the degradation of DELLA proteins and in *Arabidopsis* increased susceptibility to the fungal necrotroph *Alternaria brassicicola* (8). The rice pathogen *Gibberella fujikuroi* is therefore thought to secrete GA as a virulence factor to promote disease development (2). The wheat pathogen *F. pseudograminearum*, used in the present study, is a necrotrophic pathogen and it has been shown previously that FCR disease development by this pathogen is sensitive to jasmonate-regulated defenses (6). Therefore, one would predict that application of GA should lead to increased disease development and this was observed with all GA-treated lines showing increased FCR symptoms.

The possibility that defense gene expression is probably not related to the effect of reduced plant height on FCR was further

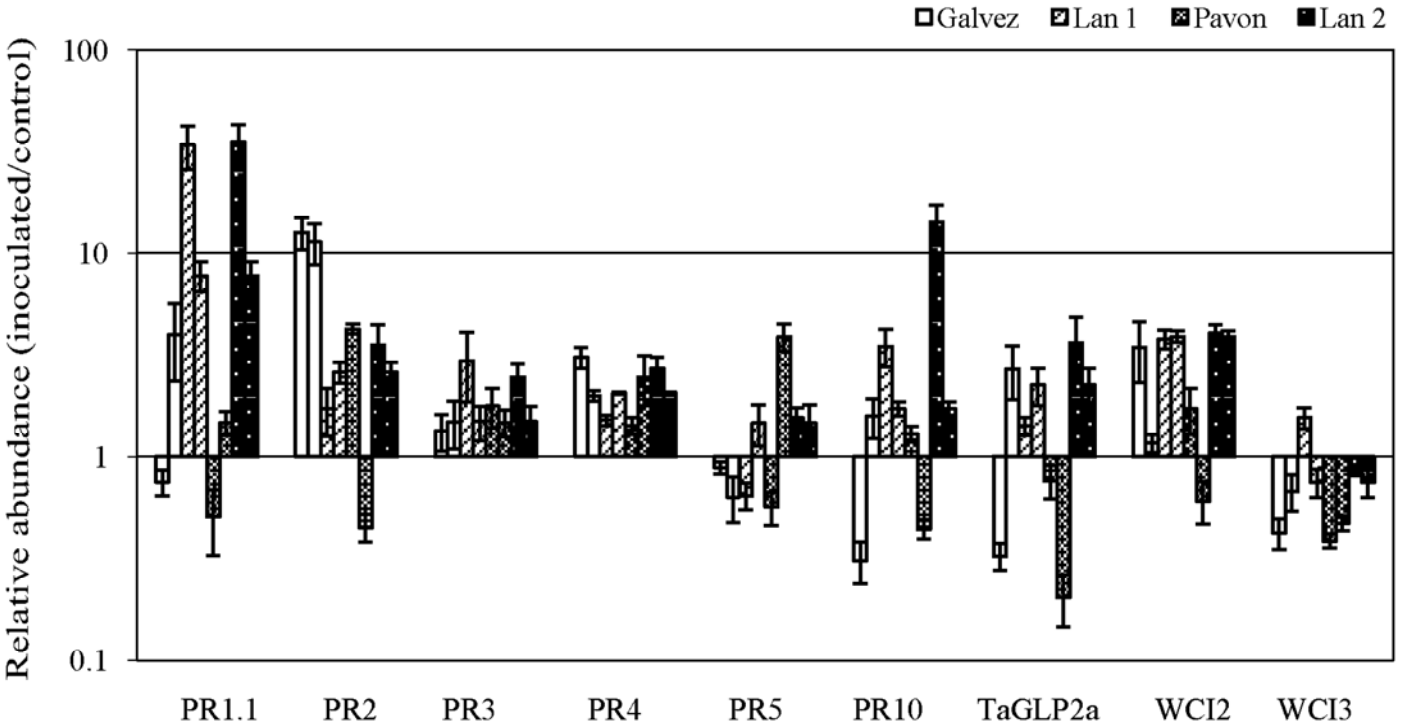

Fig. 2. Relative abundance of nine defense-related genes between Fusarium crown rot (FCR) inoculated (with *F. pseudograminearum* isolate CS3096) and mock-inoculated controls in 4-day-old seedlings of eight genotypes consisting of four pairs of near-isogenic lines (NILs) for two different *Rht* genes. Abundance of gene transcripts for the eight genotypes was normalized to β-tubulin, and the relative abundance of transcripts between the FCR-inoculated and mock-inoculated control was then calculated as ratios. Thus, a ratio larger than 1 indicates that the gene was more strongly induced in the FCR-inoculated treatment compared with that in the mock-inoculated control for a given genotype, and vice versa. For each set of the NILs, the first column represents the dwarf isoline and the second the respective tall isoline. Columns represent average abundance of three biological replicates (±SE) and are plotted on a logarithmic scale.

exemplified by our study of selected wheat defense genes. Although these genes all showed strong correlation with FCR resistance in a previous study (6), none of them was more strongly induced in the more resistant dwarf isolines across all of the NILs analyzed. Clearly, the results from only nine genes could not rule out the possible involvement of other defense genes. It has also been shown recently that increased defense responses are not necessarily always associated with increased expression of resistance (26). The *TaGLP2a* gene, encoding a germin-like protein, showed an increased expression in 4-day-old seedlings of both the mock-inoculated and the *Fusarium*-inoculated tall plants of all the four NIL pairs. These genes are thought to be involved in reactive oxygen generation which may stimulate host cell death and this may merit closer investigation. Nevertheless, the lack of a consistent pattern of stronger induction in the more resistant dwarf isolines for any of the nine genes analyzed conforms to the hypothesis that plant height per se affects FCR severity in wheat, although possible linkages between *Rht* genes and FCR susceptibility loci may exist in some genotypes such as the one reported by Wallwork et al. (28).

We have not identified the molecular mechanisms by which reduced plant height may affect FCR disease development. The infection mechanism for *F. pseudograminearum* in FCR disease is poorly understood but has been recently studied in the closely related fungus *F. graminearum* (25). During infection there is substantial upregulation of fungal genes encoding cell wall depolymerases and it is possible that tissues with higher cell density and reduced cell elongation may be more recalcitrant to cell wall breakdown.

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