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The ‘Green Revolution’ dwarfing genes play a role in disease resistance in Triticum aestivum and Hordeum vulgare

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Abstract

The Green Revolution dwarfing genes, Rht-B1b and Rht-D1b, encode mutant forms of DELLA proteins and are present in most modern wheat varieties. DELLA proteins have been implicated in the response to biotic stress in the model plant, Arabidopsis thaliana. Using defined wheat Rht near-isogenic lines and barley Sln1 gain of function (GoF) and loss of function (LoF) lines, the role of DELLA in response to biotic stress was investigated in pathosystems representing contrasting trophic styles (biotrophic, hemibiotrophic, and necrotrophic). GoF mutant alleles in wheat and barley confer a resistance trade-off with increased susceptibility to biotrophic pathogens and increased resistance to necrotrophic pathogens whilst the converse was conferred by a LoF mutant allele. The polyploid nature of the wheat genome buffered the effect of single Rht GoF mutations relative to barley (diploid), particularly in respect of increased susceptibility to biotrophic pathogens. A role for DELLA in controlling cell death responses is proposed. Similar to Arabidopsis, a resistance trade-off to pathogens with contrasting pathogenic lifestyles has been identified in monocotyledonous cereal species. Appreciation of the pleiotropic role of DELLA in biotic stress responses in cereals has implications for plant breeding.

Key words: Biotroph/necrotroph, DELLA; Green Revolution, Hordeum vulgare L., polyploidy, Reduced height (Rht), resistance trade-off, Slender 1 (Sln1), Triticum aestivum L.

Introduction

The introduction of the Reduced height (Rht) genes (Rht-B1b and Rht-D1b alleles) underpinned the increases in wheat yields that occurred during the ‘Green Revolution’ and they continue to be used in most modern varieties (Hedden, 2003). These alleles are less sensitive to the phytohormone, gibberellin (GA), than their wild-type counterparts (Rht-B1a and Rht-D1a) resulting in a reduction in stem elongation. The yield benefits associated with these alleles arise from increased resistance to lodging when additional nitrogen is applied to varieties carrying these alleles and an altered harvest index whereby a greater proportion of the plant biomass is partitioned into the grain. Rht-B1 and Rht-D1 encode DELLA proteins (Peng et al., 1999), which act to repress GA-responsive growth, and the Rht-B1b and Rht-D1b mutations are thought to confer dwarfism by producing constitutively active forms of these growth repressors (Peng et al., 1999). Most of our knowledge of DELLA genes derives from studies on Arabidopsis thaliana. The Arabidopsis genome contains five DELLA genes that encode distinct proteins (GAI, RGA, RGL1, RGL2, and RGL3; Peng et al., 1997; Silverstone et al., 1998; Wen and Chang, 2002). DELLA proteins are nuclear localized repressors of growth that are core components of the GA signal transduction pathway (Peng et al., 1997). In the presence of GA, DELLA interacts with

Abbreviations: DON, deoxynivalenol; ET, ethylene; FHB, Fusarium head blight; GoF, gain of function; GA, gibberellin; GS, growth stage; JA, jasmonic acid; LoF, loss of function; NILs, near-isogenic lines; qRT-PCR, quantitative real-time PCR; ROS, reactive oxygen species; SA, salicylic acid.

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the soluble GA receptor, GIBBERELLIN INSENSITIVE DWARF1, GID1 (Ueguchi-Tanaka et al., 2005) and the F-box protein SLY1/GID2, leading to the polyubiquitination, and subsequent degradation of DELLA protein by the 26S proteasome (Ueguchi-Tanaka et al., 2007). In addition to their role in plant development, recent studies in Arabidopsis have implicated DELLA proteins in resistance to biotic stress (Navarro et al., 2008), suggesting that DELLA encoding genes have a role in disease resistance.

Depending on their mode of infection, plant pathogens can be broadly classified into three trophic lifestyles; biotrophs, necrotrophs, and hemibiotrophs. Biotrophs derive nutrients from living cells whilst necrotrophs kill host cells in order to derive energy (Lewis, 1973). A hemibiotrophic pathogen requires an initial biotrophic phase before switching to necrotrophy to complete its life cycle (Perfect and Green, 2001). When subjected to pathogen attack, a plant is required to respond appropriately. An example, a trait which has been exploited by wheat breeders, for those for pathogen susceptibility, as has been demonstrated by Near-isogenic lines (NILs) of wheat (Triticum aestivum) varieties Mercia, Maris Huntsman, and April Bearded differing in the alleles at the Reduced height (Rht) loci on chromosome 4B and 4D were kindly supplied by Dr J Flintham of the John Innes Centre, Norwich, England. The semi-dwarf GoF alleles Rht-B1b and Rht-D1b (formerly Rht1 and 2) and the severe dwarf alleles Rht-B1c and Rht-D1c (formerly Rht3 and 10, respectively) are dominant Ga-insensitive (Ga) insensitive alleles which are suggested to accumulate DELLA to higher levels than the wild type (Peng et al., 1999) while the wild type (rht-tall) parental lines carry GA-sensitive alleles at all three homoeologous loci (Flintham et al., 1997). The severe dwarf allele, Rht-B1c, is a result of an insertion in Rht-B1 and Rht-D1c results from a gene duplication of Rht-D1 (Pearce et al., unpublished results), in both cases probably resulting in an increase in DELLA accumulation. Barley (Hordeum vulgare) variety Himalaya, the dwarf (GoF) mutant (M640), and the constitutive growth (LoF) mutant (M770) were kindly supplied by Dr P Chandler of CSIRO, Canberra, Australia. M640 carries a dominant GA-insensitive allele (Sln1d) at the Slender 1 (Sln1) locus that is orthogonal to Rht of wheat (Chandler et al., 2002). The LoF mutant M770 contains an early termination codon at the Sln1 locus resulting in a truncated protein lacking the COOH-terminal 17 amino acid residues. This loss of function allele has been designated sln1c. In the homozygous state, the loss of functional DELLA results in male sterility and therefore homozygous sln1c sln1c plants have to be selected in a 1:3 ratio from a heterozygous parent. All of the lines used in this study are summarized in Table 1.

Influence of DELLA alleles on resistance of wheat and barley to Blumeria graminis

Near-isogenic lines of wheat cvs Mercia (rht-tall, Rht-B1c, and Rht-D1c), Maris Huntsman (rht-tall, Rht-B1c, and the double mutant Rht-B1c+D1b), and April Bearded (rht-tall and the double mutant Rht-B1c+D1b), and barley cv. Himalaya WT, Sln1d, and sln1c plants were grown to growth stage (GS) 12 (Zadoks et al., 1974; i.e. second seedling leaf as long as the prophyll) in controlled environment cabinets under a 16 h photoperiod and a 17/12 °C day/night temperature regime. Detached sections (2.5 cm) of the consecutive leaves facilitating progress of the pathogen (the ‘ladder effect’, Bahat et al., 1980) and to alterations in canopy structure providing a micro-climate more favourable for pathogen establishment (Scott et al., 1982, 1985). However, not all plant height QTL are coincident with FHB resistance loci suggesting that they may have a pleiotropic effect on susceptibility to FHB (Srinivasachary et al., 2009).

The relative resistance of both wheat and barley lines, differing in DELLA status, against cereal fungal pathogens, representing each of the three classes of pathogen lifestyle, was assessed here. Barley was used to study the effect of DELLA (Sln1) gain of function (GoF) and loss of function (LoF) mutants in a diploid species. The effect of Rht in hexaploid wheat was also investigated using GoF mutants of differing severity originating from different sources. In combination the results suggest that DELLA confers a pleiotropic effect on disease resistance which may in part be due to DELLA’s role in the control of cell death.

Materials and methods

Plant material

The reduced height (Rht) gene of Triticum aestivum (bread wheat) and the Slender 1 (Sln1) gene of Hordeum vulgare (barley) are both orthologous to GA1 (Peng et al., 1999; Chandler et al., 2002). Mutations disrupting the conserved DELLA domain, essential for GID1 interaction, reduce the susceptibility of DELLA to GA-induced degradation (Peng et al., 1999; Chandler et al., 2002) and result in a dwarf phenotype, a trait which has been exploited by wheat breeders, for example, Rht-B1b and Rht-D1b.

Reduced height in wheat has been associated with increased susceptibility to splash dispersed pathogens (Vanbueningen and Kohli, 1990; Eriksen et al., 2003; Gervais et al., 2003; Draeger et al., 2007; Klahr et al., 2007). This was thought to be due to the reduced distances between consecutive leaves facilitating progress of the pathogen (the ‘ladder effect’, Bahat et al., 1980) and to alterations in canopy structure providing a micro-climate more favourable for pathogen establishment (Scott et al., 1982, 1985). However, not all plant height QTL are coincident with those for pathogen susceptibility, as has been demonstrated with fusarium head blight (FHB; Draeger et al., 2007), suggesting that resistance is not an effect of height per se but rather of linkage or pleiotropy. Significantly, the Rht-B1 and Rht-D1 loci on chromosome 4B and 4D, respectively, are coincident with FHB resistance loci suggesting that they may have a pleiotropic effect on susceptibility to FHB (Srinivasachary et al., 2009).

by guest on 25 July 2018
**Table 1. Description of plant material used**

| Species       | Locus | Allele | Pheno Type | Origin |
|---------------|-------|--------|------------|--------|
| *Triticum aestivum* Rht-B1 | rht-tall | Wild type | – | – |
| Rht-B1b       | Semi-dwarf | cv. Norin | 10–nucleotide substitutiona | – |
| Rht-B1c       | Severe-dwarf | cv. Tom | Thumb–insertionb | – |
| Rht-D1       | rht-tall | Wild type | – | – |
| Rht-D1b       | Semi-dwarf | cv. Norin | 10–nucleotide substitutiona | – |
| Rht-D1c       | Severe-dwarf | cv. Albian–gene | duplicationc | – |
| *Hordeum vulgare* Sln1 | WT | Wild type | – | – |
| Sn1d         | Dwarf | cv. Himalaya–point | (GoF) mutationd | – |
| sln1c        | Slender (LoF) | cv. Himalaya–point | mutationc | – |

*a* Peng et al., 1999.  
*b* Pearce et al., unpublished data.  
*c* Chandler et al., 2002.

*Bgh* inoculated barley leaves were collected at 48 h and 60 h post-inoculation (hpi) for microscopic analysis. Leaf tissue was cleared and fungal structures were scored for papillae defence and host cell death response at each time point as described by Boyd et al. (1994a). One-hundred interaction sites were observed across four leaves per genotype. Fungal structures and plant cellular autofluorescence were observed using a Nikon Microphot-SA with Nomarski (DIC) and a fluorescence filter; FITC (450–490 nm) and Cy5 (660–740 nm). In this study, a successful interaction was scored if the fungus developed an haustorium. The stages of *Bgh* development are well defined (Boyd et al., 1994b).

The stage to which infection progressed was scored for each form within the epidermal cells and hyphal development follows. Symptoms were assessed at 15 dpi as the percentage of leaf area covered with lesions. This experiment was repeated three times with the addition of *sln1c* lines and five were inoculated with *O. yallundae*. The experiment was repeated once to confirm the findings.

In a third experiment, Himalaya WT, *Sln1d*, and *sln1c* lines were inoculated with *O. acuformis*. Due to the elongated nature of the *sln1c* mutant line, the method described by Chapman et al. (2008) was modified by using longer tubes to contain the inoculum. This experiment was arranged in a randomized block design with five blocks as described above.

**Influence of DELLA alleles on Type 1 resistance of wheat heads to Fusarium graminearum**

Two independent experiments were carried out to assess resistance to initial infection [Type 1 resistance *sensu* Schroeder and Christensen (1963)]. In experiment 1, Mercia and Maris Huntsman NILs (*rht-tall*, *Rht-B1b*, and *Rht-B1c*) were grown in the field. Experiment 2 was conducted in an unheated polytunnel, only Mercia NILs (*rht-tall*, *Rht-B1b*, and *Rht-B1c*) were tested. In both experiments, lines were inoculated by spraying until run-off with a conidial suspension of *F. graminearum* (*1×10^6* conidia ml^-1^) at GS 65 (Zadoks et al., 1974) as described previously by Gosman et al. (2005). Disease severity was visually assessed as the percentage of spikelets infected at 14 dpi. Experiment 1 was conducted in a randomized complete block design field trial with three replicate plots per line. Experiment 2 was conducted in a randomized complete block design consisting of four blocks within which were seven plants of each line.

**Influence of DELLA alleles on Type 2 resistance of wheat and barley heads to Fusarium graminearum**

Mercia and Maris Huntsman NILs (*rht-tall*, *Rht-B1b*, and *Rht-B1c*) were phenotyped in independent experiments for resistance to *F. graminearum* at GS 65 (Zadoks et al., 1974) by point inoculation with 50 μl of conidial suspension (*1×10^6* ml^-1^) of a deoxynivalenol (DON) producing isolate of *F. graminearum* (UK1), injected into a single floret within the central portion of each spike. High humidity was maintained for 72 hpi by misting. Disease severity was measured as the number of diseased spikelets 14 dpi.

**Influence of DELLA alleles on foliar disease resistance of wheat and barley to Fusarium graminearum**

Plants of wheat cv. Maris Huntsman (*rht-tall* and *Rht-B1c* NILs) and barley cv. Himalaya (WT and *Sln1d*) were grown to GS 12 in controlled environment cabinets under a 16 h photoperiod and a 15/12 °C day/night temperature with 70% relative humidity. Sections (5 cm) of the second leaf were inoculated with *F. graminearum* (*5 μl* of *1×10^6* conidia ml^-1^) as described by Chen et al. (2009). Leaves were returned to the growth cabinet and lesion areas were measured after 6 d using ImageJ (Abramoff et al., 2004).

**Influence of DELLA on resistance of wheat heads to deoxynivalenol**

Maris Huntsman (*rht-tall*, *Rht-B1b*, and *Rht-B1c*) NILs were tested for resistance to DON in an unheated polytunnel. DON was kindly supplied by Dr M Lemmens (IFA-Tulln, Austria). At GS 65, two spikes per plant were treated with DON according to the method of Lemmens et al. (2005) with the following modification; DON solution was applied to a single clipped spikelet on each wheat head instead of two. Pots containing individual plants were arranged in a randomized complete block...
design of four replicates of seven plants per line. Following treatment, the number of damaged spikelets per head was assessed at 14 dpi.

**Influence of DELLA on root growth of wheat and barley in response to deoxynivalenol**

Seed of wheat cv. Maris Huntsman (rht-tall, Rht-B1b, and Rht-B1c NILs) and barley cv. Himalaya (WT and Slhl) were germinated on 0.7% water agar and transferred to 90 mm Petri-dishes of water agar containing 25 μM DON or to new water agar dishes (controls) and incubated at 5 °C in the dark. Samples consisted of three replicate dishes of 20 germinated seeds per line. The lengths of primary roots were measured after a period of 10 d (experiment 1) or 14 d (experiment 2). The root length of seedlings grown on DON was expressed as a percentage of the average root length of their respective controls (mean relative length).

**Influence of DELLA on deoxynivalenol-induced expression of negative cell death regulators**

Maris Huntsman (rht-tall, Rht-B1b, and Rht-B1c) NILs were incubated in darkness at 20 °C for 5 d on moist filter paper. Roots were submerged in water or DON solution (50 μM) and incubated for 8 h prior to RNA extraction. Total RNA was isolated using Qiagen RNA easy spin columns from 100 mg of root tissue, ground in a pestle and mortar under liquid nitrogen. DNase treatment was carried out using the Turbo DNA-free kit (Ambion) and cDNA was synthesised from 5 μg of RNA using SuperScript III (Invitrogen) following the manufacturer’s instructions with the addition of random nonamers (50 μM, Invitrogen). RNA was digested with RNase-H (Invitrogen) from the RNA-DNA duplex to leave single-strand cDNA. cDNA was diluted 1:20 for qRT-PCR. qRT-PCR reactions were carried out using a DNA engine Opticon2 Continuous Fluorescence Detector (MJ Research Inc., Alameda, CA, USA). Amplification was carried out using SYBR Green Jumpstart Taq ready mix with gene-specific primers (BAX INHIBITOR-I fwd – TACATGGTAGTACGCACACGA and rev – GTCCATGTCGCCGTGG (accession: HM447649) and RADICAL-INDUCED CELL DEATH 1 fwd – GCGTCTGTCTGATATCCTGAATCTGC and rev – TGTGGATGGACAAAAACCAA (Walter et al., 2008)). An initial activation step at 95 °C for 4 min was followed by 40 cycles of 30 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C. Target gene expression was calculated relative to the expression of the reference gene, 18S rRNA (fwd – AGTAA-GCGCGAGTCATCAGCT and rev – CATTCAATCGGTAG-GAGCGAC) using the ΔΔCT method (Pfaffl, 2001). cDNA was diluted 1:100 for the quantification of the expression of 18S rRNA.

**Statistical analysis**

All statistical analyses were performed using GenStat for Windows, 12th edition (Payne et al., 2009). For count data, a Poisson regression and for percentage data a logistic regression was used in a generalized linear model (GLM) to estimate variance attributable to replicate, experiment, and genotype. Means were compared using t-probabilities calculated within the GLM.

### Results

**Interaction between DELLA and Blumeria graminis**

*B. graminis* f. sp. *tritici* (*Bgt*) and f. sp. *hordei* (*Bgh*) are obligate biotrophic fungi that infect wheat and barley, respectively, causing powdery mildew. The DELLA GoF barley line, *Shl1d*, showed a significant (*P* < 0.001) increase in susceptibility to *Bgh* compared with the Himalaya wild type (Fig. 1A). Whilst the LoF DELLA mutant line, *shl1c*, was significantly (*P* < 0.01) more resistant compared with the Himalaya wild type, which itself expressed a high level of resistance relative to the susceptible cultivar Golden Promise. The *shl1c* line exhibited a spreading hypersensitive cell death phenotype (Fig. 1B), which is absent in the other lines. The wild type and severe dwarf (*Rht-B1c* and *Rht-D1c*) NILs of Mercia and Maris Huntsman were equally infected by *B. graminis* (Fig. 2A). However, lines containing two copies of mutant alleles (the semi-dwarf and severe dwarfing alleles: *Rht-B1c* and *Rht-D1b*) were significantly (*P* < 0.05) more susceptible (assessed as colonies cm⁻²) than the respective wild-type plants (Fig. 2B, C).

Cytological analysis of the barley lines showed that a significantly (*P* < 0.001) higher proportion of spores infected plant host epidermal cells in *Shl1d* (57%) compared with WT Himalaya (18%), and none of the spores infected the cells of *shl1c* leaves. In the *shl1c* line, a high proportion (73%) of the unsuccessful interactions were due to host cell death. In *Shl1d* leaves, by contrast, a significantly
remaining spores not being advanced enough to elicit either response. Interestingly, while the B. graminis-associated cell death differed significantly in a DELLA-dependent manner, restriction of the fungus by papillae formation alone was independent of the DELLA status of the line. Furthermore, the fungal colonization process was more rapid in the Sln1d interaction than in the wild type. By 60 hpi, all successfully infecting spores reached the hyphal stage on Sln1d whilst only 17% of successfully infecting spores had reached this stage on wild-type leaves, with most (83%) only at the balloon/digitate haustorium stage.

Interaction between DELLA and Ramularia collo-cygni

The responses of barley lines, differing in DELLA status, to the hemibiotroph Ramularia collo-cygni were assessed. R. collo-cygni is a barley infecting pathogen which exhibits a long biotrophic, endophytic phase before switching to a necrotrophic lifestyle late in infection (Stabentheiner et al., 2009). The DELLA GoF mutant, Sln1d was significantly (P <0.001) more susceptible (assessed as % diseased leaf area) to R. collo-cygni than the wild-type line. In a third experiment, the LoF DELLA mutant line, sln1c was included. Confirming the role of DELLA in increasing susceptibility to R. collo-cygni infection, the DELLA LoF mutant line was found to be significantly (P=0.03) more resistant than the wild-type line (Table 2).

Interaction between DELLA and Oculimacula spp.

O. acuformis and O. yallundae infect the stem base of cereal hosts and present contrasting pathogenic lifestyles. The former is considered a necrotroph whilst there is evidence that the latter exhibits a short biotrophic phase of establishment before switching to necrotrophic nutrition (Blein et al., 2009). Wheat GoF severe dwarfing mutant lines, Rht-B1c and Rht-D1c, showed significantly greater resistance to both O. acuformis and O. yallundae (P <0.01 and P <0.05, respectively) compared with rht-tall lines (Fig. 4A). Interestingly, the disease resistance conferred by DELLA stabilisation was greater for the fully necrotrophic species O. acuformis than for O. yallundae which exhibits a short initial biotrophic phase. GoF mutants in barley also exhibited significantly (P <0.001) increased resistance to both forms of the disease relative to wild-type plants and in further support of the role of DELLA in disease resistance, the LoF DELLA mutant line sln1c exhibited significantly (P <0.001) greater susceptibility to O. acuformis (Fig. 4B).

Interaction between DELLA and FHB caused by Fusarium graminearum

Fusarium graminearum is one of the predominant causes of FHB. While F. graminearum was, for a considerable period, thought to be entirely necrotrophic in lifestyle, it is now considered to exhibit a short biotrophic phase during the infection of wheat heads (Brown et al., 2010). Schroeder and Christensen (1963) describe two main components of resistance to FHB: resistance to initial infection (Type 1)
and resistance to spread within the head (Type 2). These components can be broadly dissected using different inoculation techniques (Miedaner et al., 2003), namely spray or point inoculation.

Spray inoculation of wheat heads is generally used to assess the combined effects of Type 1 and Type 2, but scoring disease symptoms before the fungus begins to spread within the head provides a measure of Type 1 resistance, which includes the biotrophic phase of the interaction. Following establishment, *F. graminearum* switches to a necrotrophic mode during which it produces the mycotoxin deoxynivalenol (DON) that functions as a virulence factor in FHB of wheat facilitating the spread of the fungus within the head (Bai et al., 2002). Point inoculation of individual spikelets, which bypasses the plant’s defence against initial infection, is used to assess resistance to disease spread (Type 2 resistance).

Near-isogenic wheat lines were subjected to spray or point inoculation with conidia of *F. graminearum*. Following spray inoculation, *rht-tall* showed the greatest resistance to initial infection in both field and polytunnel experiments (Table 3). In both experiments the severe dwarf *Rht-B1c* NIL showed significantly greater (*P* <0.009) susceptibility to initial infection compared with the semi-dwarf *Rht-B1b* line.

Following point inoculation, the wild-type line of Maris Huntsman was highly susceptible to the spread of *F. graminearum* with an average of 10.0 spikelets showing disease at 14 dpi (Fig. 5A, B). *Rht-B1b* NILs showed significantly less (*P* <0.001) symptom spread than the wild-type line, with an average of 8.9 spikelets showing disease at this stage. The reduction in symptoms was even greater for the *Rht-B1c* NILs with only 5.2 spikelets exhibiting disease (*P* <0.001). A similar trend was observed with Mercia wild-type, *Rht-B1b*, and *Rht-B1c* NILs (data not shown).

**Table 2.** The effect of *Sln1* alleles on resistance to *Ramularia collo-cygni* assessed as % Diseased leaf area

| Genotype | % Diseased leaf area | SEM | *P*-value |
|----------|----------------------|-----|-----------|
| WT       | 6.4                  | ±1.0|           |
| *Sln1d*  | 14.1                 | ±1.0| <0.001    |
| *sln1c*  | 1.0                  | ±2.2| 0.033     |

Influence of DELLA on lesion development induced by *F. graminearum* on leaves of wheat and barley

Wound inoculation of leaves with *F. graminearum* also bypasses defence against initial infection, and thus disease development can be used to assess resistance to fungal spread independently of resistance to initial infection. Following wound inoculation of leaves, the zone of cell death about the inoculation point at 6 dpi was significantly greater (*P* =0.006) in Maris Huntsman wild type than the *Rht-B1c* NIL (Fig. 5D, E). Similarly, the zone was significantly greater (*P* <0.001) in Himalaya wild type than the *Sln1d* line (Fig. 5D, E). Cell death was not observed beyond the point of inoculation in wheat or barley following wounding alone (data not shown).

Interaction between DELLA and deoxynivalenol induced lesion development

Production of DON by *F. graminearum* is required for disease spread in wheat heads (Bai et al., 2002). To determine whether DELLA accumulating lines are more resistant to DON, bleaching symptoms induced by this mycotoxin were
assessed following point inoculation with DON. In the wild-type Maris Huntsman line, bleaching symptoms spread an average of 4.3 spikelets from the point of inoculation at 14 dpi (Fig. 5A, C), whereas in the Rht-B1b line, symptom spread was significantly less (P < 0.01) than in the wild-type line, with an average of 3.1 spikelets exhibiting symptoms at 14 dpi. Most strikingly, following injection of the Rht-B1c line, symptoms were restricted to the inoculated spikelet (Fig. 5A, C). Overall, these symptoms closely resembled those appearing following point inoculation with a DON-producing isolate of *F. graminearum* (Fig. 5A, B).

**Influence of DELLA on deoxynivalenol-induced inhibition of root elongation of wheat and barley**

DON has been demonstrated to inhibit root growth (*Eudes et al., 2000*). To assess the effect of DELLA on root growth in the presence of DON, treated and untreated roots of Maris Huntsman NILs (rht-tall, Rht-B1b, and Rht-B1c) and Himalaya WT and *Sln1d* were measured. DON-induced root inhibition was significantly less (P < 0.001) for Rht-B1c than for the wild type (rht-tall) or Rht-B1b lines of Maris Huntsman (Table 4). The mean growth of DON-treated roots, relative to untreated roots across experiments was 56.4% for Rht-B1c, 40.0% for Rht-B1b, and 37.7% for the rht-tall line of Maris Huntsman. Similarly, the effect of DON on root growth of Himalaya WT and *Sln1d* also differed significantly (P < 0.001; Table 1). The mean growth of DON-treated roots, relative to untreated roots across experiments was 44.4% for *Sln1d*, and 36.8% for the Himalaya wild-type line.

**Influence of DELLA on deoxynivalenol-induced expression of negative cell death regulators**

Deoxynivalenol has been demonstrated to induce H$_2$O$_2$ production and to promote host cell death (*Desmond et al., 2008*). To gain an insight into the mechanism of DELLA-conferred tolerance to DON, the expression of two candidate negative regulators of cell death, *BAX INHIBITOR-1 (BI-1)* and *RADICAL INDUCED CELL DEATH 1 (RCD1)*, was quantified in rht-tall, Rht-B1b, and Rht-B1c Maris Huntsman NILs following exposure to DON (Fig. 6).

Expression of *BI-1* differed between the untreated rht-tall, Rht-B1b, and Rht-B1c NILs: expression was significantly lower in the Rht-B1b and Rht-B1c lines than in the rht-tall line (P=0.01 and P=0.003, respectively) while expression levels were similar for all three lines following treatment with DON (Fig. 6A). Treatment with DON did not significantly alter expression of *BI-1* in the rht-tall line. By contrast, treatment with DON resulted in a significant increase in expression of *BI-1* in both the Rht-B1b (P=0.048) and Rht-B1c (P=0.001) lines.

Expression of *RCD1* in the untreated roots also differed between the rht-tall, Rht-B1b, and Rht-B1c NILs. Although it was not significantly different, expression was lower in the Rht-B1b and Rht-B1c lines than in the untreated rht-tall line (Fig. 6B). No significant difference was observed in the rht-tall lines following DON treatment while, by contrast, DON treatment resulted in a significant increase in
expression of RCD1 in both the Rht-B1b (P<0.001) and Rht-B1c (P<0.001) lines. Following DON treatment the expression of RCD1 was significantly (P<0.001) greater in both the Rht-B1b or Rht-B1c lines than in the rht-tall line.

Discussion

The reduced GA sensitivity of the DELLA Rht-B1b and Rht-D1b semi-dwarfing alleles in wheat were central to enhanced crop yields achieved as part of the Green Revolution (Hedden, 2003). However, research using Arabidopsis has indicated that DELLA may have pleiotropic effects, including an altered response to pathogens. Using DELLA mutants in Arabidopsis, Navarro et al. (2008) showed that DELLAs promote susceptibility to biotrophs and resistance to necrotrophs. Such findings are potentially significant in agriculture but information relating to potential pleiotropic effects in monocotyledonous species is currently lacking.

In the current study, the role of DELLA proteins in response to pathogens was investigated in two important monocotyledonous crop species. In contrast to Arabidopsis, H. vulgare (barley) is a diploid species which contains a single DELLA-encoding gene. Both GoF and LoF mutants are available in barley arising from point mutations enabling us to observe the effect of Sln1 without the gene redundancy present in Arabidopsis and wheat. Using these mutants, it was demonstrated that DELLA confers a resistance trade-off to a range of fungal pathogens with differing trophic lifestyles that are responsible for some economically important diseases of cereals. T. aestivum (bread wheat) is a polyploid species originating from the hybridization of three diploid progenitors and as such contains three DELLA-encoding genes. Using GoF mutants in polyploids, a correlation between dwarfing severity and

Fig. 5. The effect of GoF mutant alleles on resistance to Fusarium disease spread. (A) Number of damaged spikelets following point inoculation with either F. graminearum or deoxynivalenol (DON) of the ears of wheat cv. Maris Huntsman NILs allelic at the Rht-B1 loci at 14 dpi. Black bars represent F. graminearum inoculated ears. *** Significant difference (P<0.001) from rht-tall. Grey bars represent DON treated ears. ††, ††† Significant difference (P<0.01 and P<0.001, respectively) from rht-tall. Bars ±1 SEM. (B, C) Typical symptom spread and contained phenotype in rht-tall and Rht-B1c lines following point inoculation with F. graminearum and DON, respectively. Injected spikelet is arrowed. (D) Mean cell death lesion area (cm²) following F. graminearum inoculation of the leaves of wheat and barley lines 6 dpi. **, *** Significant difference (P<0.01 and P<0.001) from the corresponding wild type. Bars ±1 SEM. (E) Representative lesions on leaves of wild-type and mutant plants corresponding to labels in (D) stained with trypan blue to detect cell death. The central circles show inoculation points.
influence on resistance was also demonstrated. In addition, the effect of polyploidy has been assessed, observing how disease resistance is influenced by DELLA alleles conferring semi-dwarf and severe-dwarf phenotypes, functioning in the presence of background wild-type homoeologous DELLA genes. The results reported in this study using diverse DELLA mutants in two cereal species indicates that the effect on disease resistance to pathogens with differing lifestyles is due to pleiotropy rather than linkage. Thus our findings support those previously reported in dicot species (Navarro et al., 2008) that suggested a pleiotropic role of DELLA in disease resistance.

Biotrophic pathogens derive their nutrients from living host cells. All obligate biotrophic pathogens possess specialist feeding structures, known as haustoria, which penetrate the cell wall. Recognition of the invading pathogen results in an increase in reactive oxygen species (ROS) production leading to the hypersensitive response (HR), a type of programmed cell death which deprives biotrophic pathogens of their food source. Studies in Arabidopsis have implicated DELLA proteins in processes leading to cell death. Achard et al. (2008) demonstrated that DELLA delay ROS-induced cell death and Navarro et al. (2008) reported that DELLA suppress the accumulation of salicylic acid (SA) and cell death in response to infection by Pseudomonas syringae pv. tomato. In the present study, microscopic analysis of the host defence response in the barley-B. graminis (biotroph) interaction showed that the DELLA GoF line is hypersensitive to cell death resulting in complete resistance, whilst the frequency of hypersensitive cell death was reduced in the DELLA LoF line, resulting in a higher number of successful haustorial establishment events (Fig. 3). As in Arabidopsis DELLA accumulating mutants, the barley DELLA GoF line may exhibit a delay in ROS-induced cell death which, in turn, reduces the effectiveness of the HR thereby increasing susceptibility both to an obligate biotroph, B. graminis (Figs 1, 2) and to a hemibiotroph with a long biotrophic or endophytic phase, R. collo-cygni (Table 2).

The height reduction associated with the Sln1d (GoF) allele in barley is similar to that associated with Rht-B1c and Rht-D1c alleles in wheat (c. 50% of the respective wild type). However, in contrast to the significantly enhanced susceptibility to B. graminis of the barley Sln1d line (Fig. 1), wheat lines carrying single mutant alleles, Rht-B1c or Rht-D1c did not exhibit an equivalent increase in susceptibility. The combination of dwarf and semi-dwarf mutant alleles (Rht-B1c+Rht-D1b) in a single line, however, did enhance susceptibility. In polyploid species like wheat, the presence of wild-type homoeologous copies of the Rht genes might buffer the negative effect of a single mutation on susceptibility to B. graminis. It is conceivable that DELLA accumulation must pass a threshold in order significantly to delay cell death induced by B. graminis and that this threshold is exceeded in Rht-B1c+Rht-D1b (dwarf+semi dwarf) NILs carrying mutations in two of their three Rht (DELLA) homoeologues but not in lines carrying a mutation in only a single homoeologue. Further investigation with other biotrophic pathogens is necessary to determine whether this phenomenon is specific to B. graminis or common to other biotrophs.

The combination of dwarf and semi-dwarf mutant alleles in two cereal species indicates that the effect on disease resistance to pathogens with differing genotypes needs to be assessed in a wider range of biotrophic pathogens. Further investigation with other biotrophic pathogens is necessary to determine whether this phenomenon is specific to B. graminis or common to other biotrophs.
mesophyll cells (Sutton and Waller, 1988; Stabentheiner et al., 2009). The barley lines carrying the GoF or LoF DELLA mutations showed differential responses to *R. collo-cygni* with the GoF mutant being significantly more susceptible and the LoF mutant significantly more resistant than the wild type (Table 2). These results suggest that DELLA-mediated prevention of cell death benefits the initial biotrophic phase of *R. collo-cygni* infection and supports the current view that *R. collo-cygni* has a long biotrophic phase (Walters et al., 2008).

Necrotrophic pathogens derive their nutrients from dead host cells. These pathogens have evolved a number of strategies to kill host cells including the secretion of toxins, cell-wall-degrading enzymes, and eliciting ROS production. If, as reported, DELLA prevents ROS-induced cell death then it would be predicted that plants with increased DELLA accumulation would be more resistant to necrotrophs than their tall counterparts. *O. acuformis* is considered to be a typical necrotrophic pathogen while the closely related species *O. yallundae* exhibits a very short non-necrotrophic initial phase during the infection of wheat (Blein et al., 2009). Both species cause eyespot disease on the stem base of cereals and are considered a serious disease of winter wheat in northern Europe and north-west USA (Fitt, 1992). GoF mutations of DELLA in both wheat and barley resulted in significantly increased resistance to both *Oculimacula* species as might be anticipated given their necrotrophic lifestyles. Supportive of DELLA increasing resistance to necrotrophs, the barley LoF mutant exhibits enhanced susceptibility to eyespot caused by *O. acuformis*.

Interestingly, the effect on the GoF mutation in wheat was greater for *O. acuformis* than for *O. yallundae*. It is speculated that this reflects the different growth habits of the two species. Whilst *O. acuformis* exhibits an invasive growth habit, *O. yallundae* penetrates the coleoptile in a more ordered, intramura1 manner (Daniels et al., 1991), with no sign of host cell death (Blein et al., 2009). Once at the first leaf sheath the pathogen initiates the formation of an infection plaque from which hyphae grow and penetrate the first, then successive leaf sheaths facilitated by the secretion of cell-wall-degrading enzymes (Mbwaga et al., 1997). These observations suggest that *O. yallundae* may be considered to be a hemibiotroph with a very short initial phase of biotrophic growth before switching to a necrotrophic phase once the pathogen reaches the first leaf sheath (Blein et al., 2009) while *O. acuformis* exhibits the traits of a conventional necrotroph.

*F. graminearum* was originally considered to be entirely necrotrophic. However, evidence is accumulating to indicate that this may not always be the case. Analysis of the interaction at the cellular level shows that *F. graminearum* exhibits extracellular growth during the early stages of infection (Pritsch et al., 2000) and sub-cuticular growth reminiscent of *O. yallundae* (Rittenour and Harris, 2010). It appears that *F. graminearum* requires a transient biotrophic phase of establishment before switching to necrotrophic nutrition during the infection of wheat leading to FHB (Goswami and Kistler, 2004). This view is supported by studies on the interaction of FHB with the SA and JA signalling pathways. SA content and expression of the SA-inducible PRI gene in *Arabidopsis* inoculated with *F. graminearum* showed that SA signalling is activated in the early stages of infection (Makandar et al., 2010). Furthermore, over-expression of a gene that regulates SA signalling (NPR1), in wheat and *Arabidopsis*, increases resistance to *F. graminearum* (Makandar et al., 2006). Methyl jasmonate (MJ), however, has dichotomous effects on the susceptibility of *Arabidopsis* to *F. graminearum*. The application of MJ during the early stages of infection enhanced disease severity, presumably due to JA attenuating SA signalling, whilst, when applied at later stages of infection, MJ reduced disease severity (Makandar et al., 2010).

Short wheat varieties tend to be more susceptible to FHB in the field than tall ones and it has been demonstrated that semi-dwarf lines carrying the *Rht-B1b* allele are more susceptible to initial infection than those carrying the wild-type allele (Srinivasachary et al., 2009). In the present study, it has been demonstrated that both *Rht-B1b* and *Rht-B1c* NILs exhibit increased Type 1 susceptibility (initial infection) relative to the wild type, and that this is associated with the severity of the DELLA effect on plant height. By contrast, following point inoculation, *Rht-B1b* and *Rht-B1c* NILs exhibited increased resistance to disease spread relative to the wild type and this also correlates with the DELLA effect on plant height. DELLA GoF dwarf lines of both wheat and barley also showed enhanced resistance to cell death following wound-inoculation of leaves with *F. graminearum*. Overall, these results indicate that, whilst DELLA accumulating lines are more susceptible to the initial establishment phase they are more resistant to the later colonization phase. It is proposed that these contrasting differential susceptibilities reflect the different trophic modes of growth employed by *F. graminearum* and support the evidence that *F. graminearum* exhibits an initial biotrophic phase during FHB infection prior to entering a longer necrotrophic phase.

The trichothecene mycotoxin DON functions as a virulence factor for *F. graminearum* during colonization (Bai et al., 2002). The up-regulation of trichothecene biosynthetic genes and subsequent accumulation of DON in head tissues have been observed 48 hpi (Boddu et al., 2006). This is thought to signal the switch from biotrophic to necrotrophic growth. DON has been shown to induce *H₂O₂* production in the host, promoting cell death (Desmond et al., 2008). In addition, *in vitro* experiments have demonstrated that *H₂O₂* induces DON production by the fungus (Ponts et al., 2006) leading to a cycle that ultimately favours necrotrophy. Plant lines with enhanced capabilities of alleviating oxidative stress would be anticipated to exhibit increased resistance to *F. graminearum* and DON. This view is supported by a number of studies. For example, increases in the activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) positively correlated with FHB resistance in a set of wheat varieties inoculated with DON-producing isolates (Chen et al., 1997). Similarly, the *Arabidopsis RADICAL-INDUCED CELL
on 25 July 2018

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