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ORIGINAL ARTICLE

The identification of QTL controlling ergot sclerotia size in hexaploid wheat implicates a role for the Rht dwarfing alleles

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Abstract

Key message **Four QTL conferring resistance to ergot were identified in the UK winter wheat varieties 'Robigus' and 'Solstice'. Two QTL co-located with semi-dwarfing alleles at the *Rht* loci *Rht-1B* and *Rht-1D* implicating a role of these DELLA proteins in infection success of *Claviceps purpurea*.**

Abstract The fungal pathogen *Claviceps purpurea* infects ovaries of a broad range of temperate grasses and cereals, including hexaploid wheat, causing a disease commonly known as ergot. Sclerotia produced in place of seed carry a cocktail of harmful alkaloid compounds that result in a range of symptoms in humans and animals, causing ergotism. Following a field assessment of *C. purpurea* infection in winter wheat, two varieties 'Robigus' and 'Solstice' were selected which consistently produced the largest

differential effect on ergot sclerotia weights. They were crossed to produce a doubled haploid mapping population, and a marker map, consisting of 714 genetic loci and a total length of 2895 cM was produced. Four ergot reducing QTL were identified using both sclerotia weight and size as phenotypic parameters; *QCp.niab.2A* and *QCp.niab.4B* being detected in the wheat variety 'Robigus', and *QCp.niab.6A* and *QCp.niab.4D* in the variety 'Solstice'. The ergot resistance QTL *QCp.niab.4B* and *QCp.niab.4D* peaks mapped to the same markers as the known reduced height (*Rht*) loci on chromosomes 4B and 4D, *Rht-B1* and *Rht-D1*, respectively. In both cases, the reduction in sclerotia weight and size was associated with the semi-dwarfing alleles, *Rht-B1b* from 'Robigus' and *Rht-D1b* from 'Solstice'. Two-dimensional, two-QTL scans identified significant additive interactions between QTL *QCp.niab.4B* and *QCp.niab.4D*, and between *QCp.niab.2A* and *QCp.niab.4B* when looking at sclerotia size, but not between *QCp.niab.2A* and *QCp.niab.4D*. The two plant height QTL, *QPh.niab.4B* and *QPh.niab.4D*, which mapped to the same locations as *QCp.niab.4B* and *QCp.niab.4D*, also displayed significant genetic interactions.

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Introduction

The ergot fungus *Claviceps purpurea* infects the ovaries of many species of cereals and grasses, including the economically important cereals wheat, barley, oats, triticale, rye and millet (Tenberge 1999; Miedaner and Geiger 2015). Conidiospores and ascospores germinate on receptive stigma producing hyphae which grow down the lumen of the stigma towards the ovule tissue, following the route that would normally be taken by pollen tubes (Tudzynski and Schieffer 2004). Within 24 h of spore germination

the fungal germ tube enters the transmitting tissue of the ovule and continues to the ovule base, where the xylem and phloem enter from the rachis. Three to four days post-infection, when the hyphae have surrounded the ovary, hyphae become more branched. At 5 days post-infection there is complete host cell collapse and the fungus enters the sphacelial stage, where it becomes soft, white and porous, and begins to generate asexual conidiospores. Around 6–7 days post-infection an exudate, known as honeydew, is produced consisting of plant sap and *C. purpurea* asexual, haploid conidia which are believed to be dispersed by rain drops and insects (Tenberge 1999).

The infected ovary is eventually replaced by a purplish-black sclerotium, commonly referred to as an ergot, a hardened mass of white fungal mycelia that is covered with a purplish-black outer surface. These overwintering sclerotia eventually give rise to sexual reproductive structures, stroma or apothecia, from which wind-borne ascospores are produced (Mantle and Shaw 1976). Sclerotia contain a wide range of toxic alkaloids produced by the fungus, including ergometrine, ergotamine, ergosine, ergocristine, ergocryptine (which is a mixture of α- and β- isomers), ergocornine, and the corresponding -inine epimers. The alkaloids found within sclerotia can cause severe health problems in both humans and animals, responsible for the disease known as ergotism. Ergot alkaloids are classified as tryptophan derived alkaloids with physiological effects occurring from the absorption in the gastrointestinal tract. Toxicity associated with ergot alkaloids occurs through their action on neurotransmitters which can affect the nervous system (De Costa 2002) and at higher doses negatively impact the reproductive system in animals (De Groot et al. 1993).

Claviceps purpurea infection and sclerotia production therefore cause serious quality issues for cereal production. The European Union has imposed limits on the amount of ergot sclerotia allowed in grain destined for human and animal consumption to 0.01 % (1 g per kg) (Alexander et al. 2012). As *C. purpurea* enters the ovary via the stigma and style, incidents of infection are more common in open flowering cereals, with rye and Triticale often being heavily infected. As wheat is a closed-flowering cereal, the window of opportunity for infection is small. Consequently only 1–5 % of seed lots examined between 2002 and 2005 by the UK's Official Seeds Testing Station were found to contain ergot sclerotia. However, male sterility, whether genetic or as a consequence of environmental factors such as copper deficiency or drought, results in the flowers gaping open and presents an increased risk of infection. The production of hybrid cereal seed on cytoplasmic male sterile (CMS) mother plants is therefore particularly at risk.

Complete resistance to *C. purpurea* infection is unknown and a hypersensitive response to this fungus has not been observed in floral tissue (Tenberge 1999). A source of partial resistance was identified in the hexaploid wheat line 'Kenya Farmer' in the 1970s (Platford and Bernier 1970), which resulted in the production of smaller sclerotia (Platford and Bernier 1970; Pageau and Lajeunesse 2006). A reduction in sclerotial size may have an epidemiological impact, resulting in fewer ascospores germinating in the following season (Cooke and Mitchell 1966). Good partial resistance was identified in the tetraploid durum wheat line 9260B-172A, where there was both a reduction in sclerotia size and in the amount of honeydew produced (Menzies 2004). Variation in genetic resistance towards *C. purpurea* has also been reported in CMS genotypes of rye (Miedaner et al. 2010), while in sorghum nine QTL were identified that affect percentage infection by *C. africana* (Parh et al. 2008).

As few sources of resistance to *C. purpurea* have been reported in wheat, this study sets out to identify UK wheat varieties that differed in their ability to support sclerotia development and then determine the genetic components accounting for these differences. Significant differences were identified between winter wheat varieties in the average weights of sclerotia produced in manually inoculated florets. As the varieties 'Robigus' and 'Solstice' represented the extremes of this variation a doubled haploid population was made from their F1 cross. Phenotypic data were gathered over multiple years of replicated, inoculated field trials to identify the QTL responsible for the differences in sclerotia weight and size seen between 'Robigus' and 'Solstice'.

Materials and methods

Plant materials and doubled haploid mapping population

Fourteen wheat varieties were tested over multiple years (Table 1) as part of the HGCA-Defra LINK project LK0963, "Towards a sustainable whole-farm approach to the control of Ergot" (Bayles et al. 2008). In each year the wheat varieties were sown in replicate field trials at sites near NIAB, Cambridge, UK.

'Robigus', a soft biscuit wheat (KWS UK, Ltd) and 'Solstice', a hard bread wheat (Limagrain UK) were crossed and a doubled haploid (DH) population of 159 lines produced by Saaten Union (Saaten-Union GmbH, Hovedisser Str. 92 D, 33818 Leopoldshoehe, Germany). This DH population was screened for *C. purpurea* resistance in field trials in 2009/10 and again in 2010/11 at sites near NIAB, Cambridge, UK.

Table 1 Winter wheat varieties screened for ergot resistance in inoculated field trials

Year of field trial	Number of ears per variety inoculated with *Claviceps purpurea*	Wheat varieties screened
2004/05	10 ears (2 reps) Variable number of florets inoculated per ear	Caphorn, Drifter, Paragon, Rialto, Robigus, Solstice, Tommi, Welford, Xi19
2005/06	3 ears (4 reps) 20 florets inoculated per ear	Apache, Caphorn, Drifter, Paragon, Rialto, Robigus, Solstice, Tommi, Welford, Xi19
2006/07	12 ears (2 reps) 10 florets inoculated per ear	Brompton, Cordiale, Glasgow, Mascot, Rialto, Robigus, Solstice, Xi19
2007/08	10 ears (1 rep) 20 florets inoculated per ear	Glasgow, Robigus, Solstice, Xi19

Table 2 *Claviceps purpurea* isolates used in inoculated field trials (Bayles et al. 2008)

Isolate name	Year of collection	Location	Plant host
04-02	2004	Cambridge	Blackgrass (*Alopecurus myosuroides*)
04-29	2004	Little Saxham, Suffolk	Wheat (cv. Hereward)
04-97	2004	Long Hoos IV, Rothamsted	Blackgrass (*Alopecurus myosuroides*)
03-20	2003	Elm Farm, Cirencester	Wheat (cv. Chablis)
03-43	2003	Elm Farm, Wakelyn's, Suffolk	Wheat (cv. Claire)

Preparation of *Claviceps purpurea* isolates

Claviceps purpurea conidia were obtained by culture on potato dextrose agar (PDA) and on wheat ears of the spring wheat variety 'Paragon'. Ergot sclerotia were surface sterilised for 4 min in 5 % sodium hypochlorite and washed four times in sterile distilled water. After surface sterilisation the sclerotia were cut in half and placed on 90-mm Petri plates with PDA (Merk 39 g/l) with 125 mg streptomycin/l. Colonies were sub-cultured on PDA, without antibiotic, after approximately 10 days of culture at 25 °C in the dark. The resulting *C. purpurea* colonies were confirmed based on conidial morphology and grown at 20 °C in the dark for 14 days to harvest conidia. Conidia were scraped from the centre of the colony and suspended in sterile distilled water.

Conidia, collected as honeydew, were produced by inoculating florets of the wheat variety 'Paragon'. 'Paragon' was grown in the glasshouse in Levingtons M2 compost with slow-release osmacote fertiliser, with a 16 h day length, 6000 Lux and day/night temperatures of 18/10 °C. Ears were inoculated before anthesis with a suspension of conidia from individual isolates (Table 2), using either a hypodermic needle to fill the space between the lemma and palea of each floret (approximately 0.025 ml of suspension at 10^6 conidia/ml), or by dipping wounded ears in the conidia suspension.

Honeydew produced by inoculating 'Paragon' was collected approximately 10 days after inoculation using either a Pasteur pipette or an inoculation loop and suspended in water. Honeydew was collected every 2–3 days and the suspensions stored at 5 °C between collections. Conidial

collections were bulked and stored as individual isolates in 10 % glycerol at −20 °C. Frozen suspensions were found to retain pathogenicity for at least two years.

Claviceps purpurea inoculated field trials

For the UK winter wheat variety field assessments equal amounts of five *C. purpurea* isolates (Table 2), at a final concentration of 10^6 conidia ml^{-1} were used. For the field inoculation of the 'Robigus' × 'Solstice' DH population one isolate, 04-97 was selected based on the differential ergot sclerotia size produced on 'Robigus' and 'Solstice' in glasshouse tests. A single spore culture was produced for isolate 04-97 (04-97.1) and used in all subsequent experiments. In 2009/10 only 105 of the DH lines, due to insufficient seed of some lines, and the parental varieties were sown in 1 m double-row plots. There were two replicates for all but 17 of the DH lines where seed quantity was limited. The ears from six primary tillers (six plants) per plot were inoculated with the *C. purpurea* isolate 04-97.1 between 1st and 7th June 2010. Twenty florets per ear were hand-inoculated with a conidia suspension by syringe. In 2010/11 three randomised blocks of all 159 DH lines and the parental varieties were sown in 1 m plots. Again 20 florets per ear, on the primary tiller of six plants were inoculated per line in each plot. Inoculations were carried out by hand between 18th and 27th May 2011.

Inoculum was prepared daily from newly emerging honeydew exuding from 'Paragon'. The honeydew was diluted with water to a concentration of 6×10^6 conidia/ml. Ears were inoculated when anthers in the middle third

of the ear had begun to turn yellow i.e. 1–2 days prior to anthesis. The first fully formed spikelet at the base of each ear was removed to identify the start of the inoculated region. The inoculum was delivered between the lemma and palea of the outer two florets of each spikelet using a 2 ml syringe and needle until the void was full of inoculum. Twenty florets on each ear were inoculated (ten on each side) and the ear labelled with a coloured tag, dated and initialled by the researcher who undertook the inoculation. Ears were harvested 7–8 weeks after anthesis and before the mature ergots began to drop from the ears. Ears were left to dry before removing ergot sclerotia. This inoculation protocol routinely achieved infection in 70–100 % of inoculated spikelets and was designed to minimise the chances for differences in ear morphology or effectiveness of pollen competition to influence the results, as we were more interested in post-infection resistance than 'escape' mechanisms.

Phenotypic assessment of ergot sclerotia development

Ergot sclerotia were removed by hand from the 20 inoculated florets of each ear and the following parameters measured:

1. Total sclerotia weight per ear—the weight of all sclerotia collected from one ear.
2. Average sclerotia weight per ear—the total sclerotia weight per ear/the total number of sclerotia collected from that ear.
3. Sclerotia size was determined using a numerical value according to the sclerotia's size within the seed cavity. The NIAB Ergot Scale index points are as follows (Online Resource 1): 0: dried-out ovule (ovule was infected, but no sclerotia formed); 1: Sclerotia <2 mm in length; 2: Sclerotia <3 mm in length; 3: <5 × 2 mm in size; 4: Sclerotia >5 × 2 mm in size; 5: >8 × 5 mm in size; 6: >10 × 5 mm in size; and 7: >17 × 5 mm in size.
4. Average sclerotia size was calculated as follows, where n_i = is the number of sclerotia assigned to each NIAB scale from 0 to 7 and n_j is the total number of sclerotia.

$$\frac{\sum (n_i \times \text{NIAB scale } [0 \text{ to } 7])}{n_j}$$

Plant height

Just before harvest plant heights were recorded for each of the DH lines of the 'Robigus' × 'Solstice' DH population. Plant height was measured as the height from the base of the plant to the bottom of the primary ear. These measurements were taken on ten random plants per line in each plot.

Statistical data analysis

Analysis of variance (ANOVA) of total sclerotia weight per ear, average sclerotia weight, average sclerotia size and plant heights were undertaken using restricted maximum likelihood (REML). Predicted means for the DH lines were extracted from the REML analyses for each phenotypic data set. Where required, data were transformed using log or square root transformation to achieve near normality and independence of the means and variances. The effects of test replications and genotypes were accounted for in the model. For the 2010 field trial data each inoculated ear was treated as a replicate to derive the predicted means. All analyses were performed using the statistical package Genstat for Windows, release 12 (2009). The variance components from the REML analyses were used to calculate heritability (Holland et al. 2010) using the following formula:

$$h^2 = V_g/(V_g + V_e)$$

V_g = Estimated genetic variance between lines V_e = The mean environmental and residual error calculated using:

$$V_e = \frac{\text{Environmental error from DH. reps}}{\text{Rep no}} + \frac{\text{Residual error}}{(\text{Reps} \times \text{Plants})}$$

Variance components for V_g and V_e were calculated by REML analysis in Genstat where DH lines were inserted into the random model along with DHline × repno.

Genotyping of the 'Robigus' × 'Solstice' Doubled Haploid population

Genomic DNA was extracted from leaf tissue using a modified Tanksley method (Fulton et al. 1995), which included an RNase digestion step. Genomic DNA was genotyped by Diversity Arrays Technology Ltd (Akbari et al. 2006) using the DArT array version 2 and by Victoria Agri-Biosciences Centre, Australia for SNPs using the Infinium iSelect 9 k chip. In addition, the DH population was screened with two chromosome 4D Kaspar markers, 10920_kasp9 and HV132-1_kasp9 developed for improving marker coverage of the 4D short arm by Dr Chris Burt, and two PCR markers developed for the SNPs causing the *Rht-B1b* and *Rht-D1b* allele mutations (Wilhelm 2011).

Construction of a DNA marker linkage map for the 'Robigus' × 'Solstice' DH population

MapDisto version 1.7.5 Beta 4 (Lorieux 2012) was used to construct a linkage map from 700 DArT, 1902 Infinium iSelect 9 k chip SNP markers and the four additional Kaspar/PCR markers. Markers that significantly differed from the expected 1:1 segregation ratio were removed from the

genotype file before mapping. The Haldane mapping function, with a maximum recombination fraction of 0.3 and LOD of 6, was used to create the map. Several rounds of remapping were undertaken, where co- segregant markers were removed. The "order sequence" function was run using ripple option and bootstrap analysis.

In order to assign linkage groups to physical chromosomes linkage groups were aligned with existing wheat consensus maps. In total 78.4 % of the mapped DArT markers were assigned a chromosomal location (218 out of 278) by reference to the consensus DArT map (Huang et al. 2012) and by searching the DArT BIN maps on the CerealsDB website (Wilkinson et al. 2012). For the Infinium iSelect SNPs markers 72.7 % (314 out of 432) could be assigned a chromosomal location by comparison with the Wang consensus map (Wang et al. 2014).

QTL analyses

QTL analyses were carried out using the QTL package R/qtl, version 1.33-7 (Broman et al. 2003). Predicted means were obtained for each phenotypic data set from the REML analysis, and used as additional data sets in the QTL analyses. Single marker regression (SMR), interval mapping (IM) and composite interval mapping (CIM) were performed with all ergot phenotypic data sets and plant height. 1000 permutation tests were performed for each phenotypic data set to obtain the 5 % LOD significance thresholds. Peak markers identified by SMR and IM were used as co-factors in CIM analyses. Any significant additional QTL were noted and were examined further using a two-dimensional, two-QTL approach.

Two-dimensional, two-QTL scans

A multiple QTL model, using two-dimensional two-QTL scans (Broman and Sen 2009) was carried out for the *C. purpurea* and plant height traits to identify QTL interactions. The scantwo function in R/qtl considers all possible two-locus QTL models in a two-dimensional genome scan, enabling the identification of additive and/or epistatic-interactions between those loci. In addition, this analysis confirms the presence of two or more loci, especially where loci have a modest effect.

LOD_f measures the improvement in the fit of the full model, considering all possible QTL, over the null model. LOD_i measures the improvement in the fit of the full model over that of the additive model. Thus it indicates evidence for interaction.

LOD_{av1} measures the improvement in the fit of the two-locus additive model to the single-QTL model. Thus it indicates evidence for a 2nd QTL assuming no epistasis.

LOD_{fv1} measures the improvement in the fit of the full model to the single-QTL model. Thus it indicates evidence for a 2nd QTL allowing for the possibility of epistasis.

All phenotypic traits analysed by the scantwo function were subjected to 1000 permutation tests, and instead of thresholds, significance *p* values are presented.

Results

Field assessment of ergot resistance

Fourteen UK winter wheat varieties were assessed for resistance to *C. purpurea* in inoculated field trials over four growing seasons from 2005 to 2008. Between four and ten varieties were tested every year, some over more than one growing season (Table 1). The total and average sclerotia weight for the wheat varieties 'Xi-19', 'Robigus', 'Solstice', 'Glasgow' and 'Rialto' are shown in Fig. 1.

While there were some differences in ergot sclerotium formation across the different growing seasons (year: total weight *F* value 17.61; average weight *F* value 18.60, both with a prob. <0.001) the wheat varieties showed consistent, significant differences in total and average sclerotial weights, implying an underlying genetic response. The variety 'Robigus' produced significantly lower total and average sclerotia weights to 'Solstice' (*t* test prob. <0.001)', 'Rialto' (*t* test prob. <0.001) and 'Xi1' (*t* test prob. =0.001), but not 'Glasgow' (total weight, *t* test prob. =0.263 and average weight, *t* test prob. =0.347). As the biggest statistical difference in ergot sclerotia weights was between 'Robigus' and 'Solstice' these varieties were selected for genetic mapping to locate loci influencing ergot sclerotial development in hexaploid wheat.

Frequency distributions across the 'Robigus' × 'Solstice' DH population indicated a normal, quantitative distribution for average sclerotia size, while the frequency distributions of total and average sclerotia weight were positively skewed (Fig. 2). Plant height was bi-modally distributed and confirms the postulated presence of different *Rht* dwarfing alleles in 'Robigus' and 'Solstice', 'Robigus' carrying the *Rht-B1b* allele at locus *Rht-B1* on chromosome 4B, while 'Solstice' carries the *Rht-D1b* allele on chromosome 4D (Fig. 2).

Analysis of variance indicated significant differences (*F* prob. <0.001) between the parents and the DH lines for all ergot sclerotial phenotypes. Transgressive segregation was apparent for all sclerotial phenotypes, with five DH lines having significantly (*t* test prob. <0.001) lower sclerotial weights than 'Robigus' and 13 lines showing smaller sclerotia at a *t* test probability of <0.001. Heritability values indicated that all three sclerotia phenotypes and plant height had a relatively high genetic component contributing to the phenotypes. The highest h^2 values were obtained for

Fig. 1 Inoculated field trial data showing the mean across replicates for total sclerotia weight per ear and average sclerotia weight for five UK winter wheat varieties. Data were collected from replicated field trials over 4 years, from 2005 to 2008. *Error bars* show the standard error between replicates, apart from 2006, where all replicates of sclerotia were pooled together before weighing
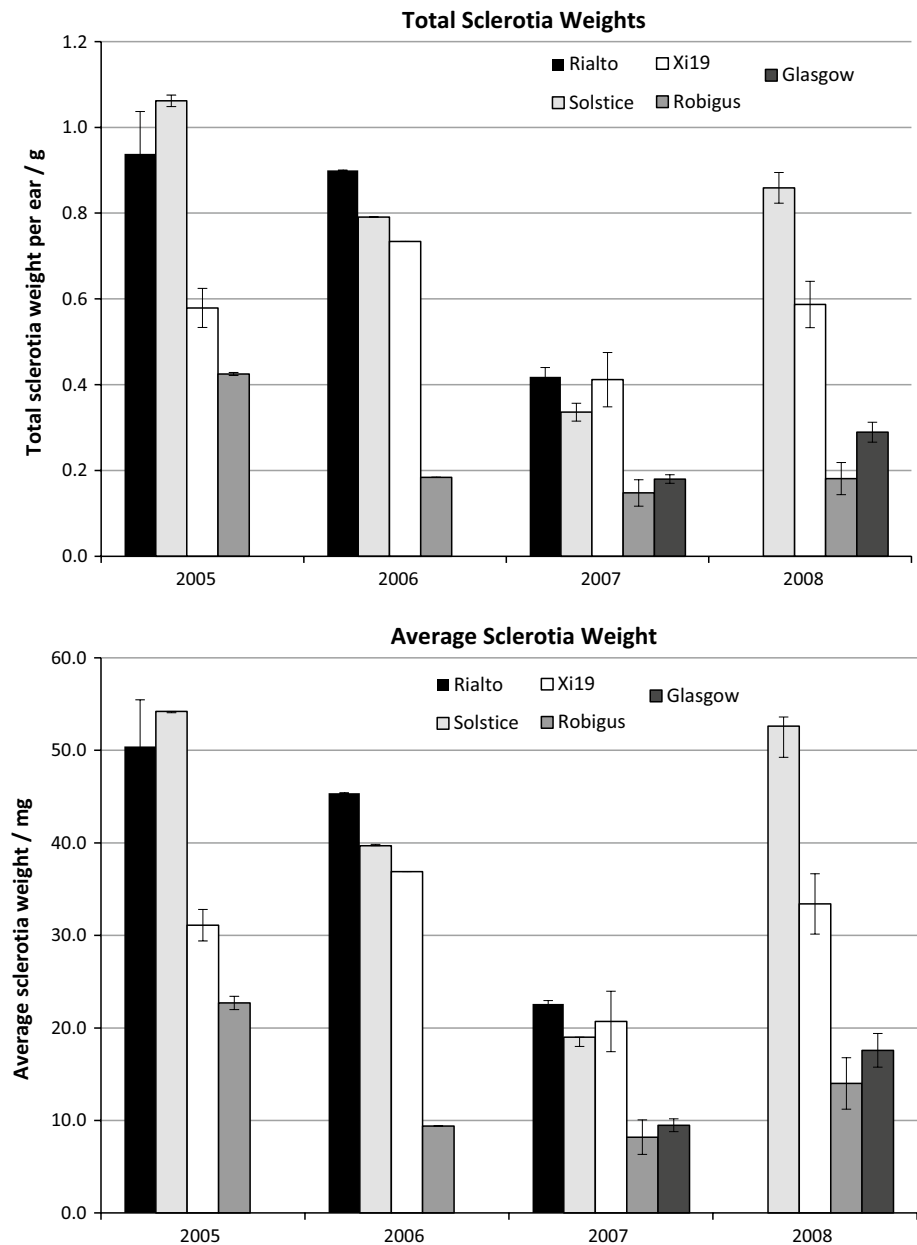
average sclerotia weight ($h^2 = 0.7616$) and average sclerotia size ($h^2 = 0.7242$), with total sclerotia weight having a slightly lower h^2 value of 0.6212 (all values shown calculated using 2011 field data). Plant height had an h^2 value of 0.9138. The full set of h^2 values can be found in Online Resource 2.

Identification of QTL underlying the response to ergot infection in the 'Robigus' × 'Solstice' cross

A genetic marker map, total length 2895 cM, consisting of 38 linkage groups and representing all 21 chromosomes of hexaploid wheat was constructed for the

'Robigus' × 'Solstice' cross. The map consisted of 714 loci; 432 Infinium iSelect SNPs and 278 DArTs markers, plus two PCR markers which distinguished the *Rht* mutant alleles from the wild-type; *Rht-D1a/b* and *Rht-B1a/b* (Wilhelm 2011), and two additional SNP markers tightly linked to the *Rht* locus on 4DS. Details of all markers and their positions can be found in Online Resources 3 and 4.

The QTL reported are those identified using the predicted means and having a LOD value above the 5 % global thresholds given in Table 3. Three QTL, *QCp.niab.2A*, *QCp.niab.4B* and *QCp.niab.4D* were consistently identified in 'Robigus' on chromosomes 2A, 4B and 4D, respectively using the phenotypic data sets for average sclerotia size

Fig. 2 Distribution of total sclerotia weight per ear, average sclerotia size, average sclerotia weight and plant height phenotypes in the 'Robigus' × 'Solstice' double haploid population. *Arrowheads* indicate the phenotypic averages of the parents
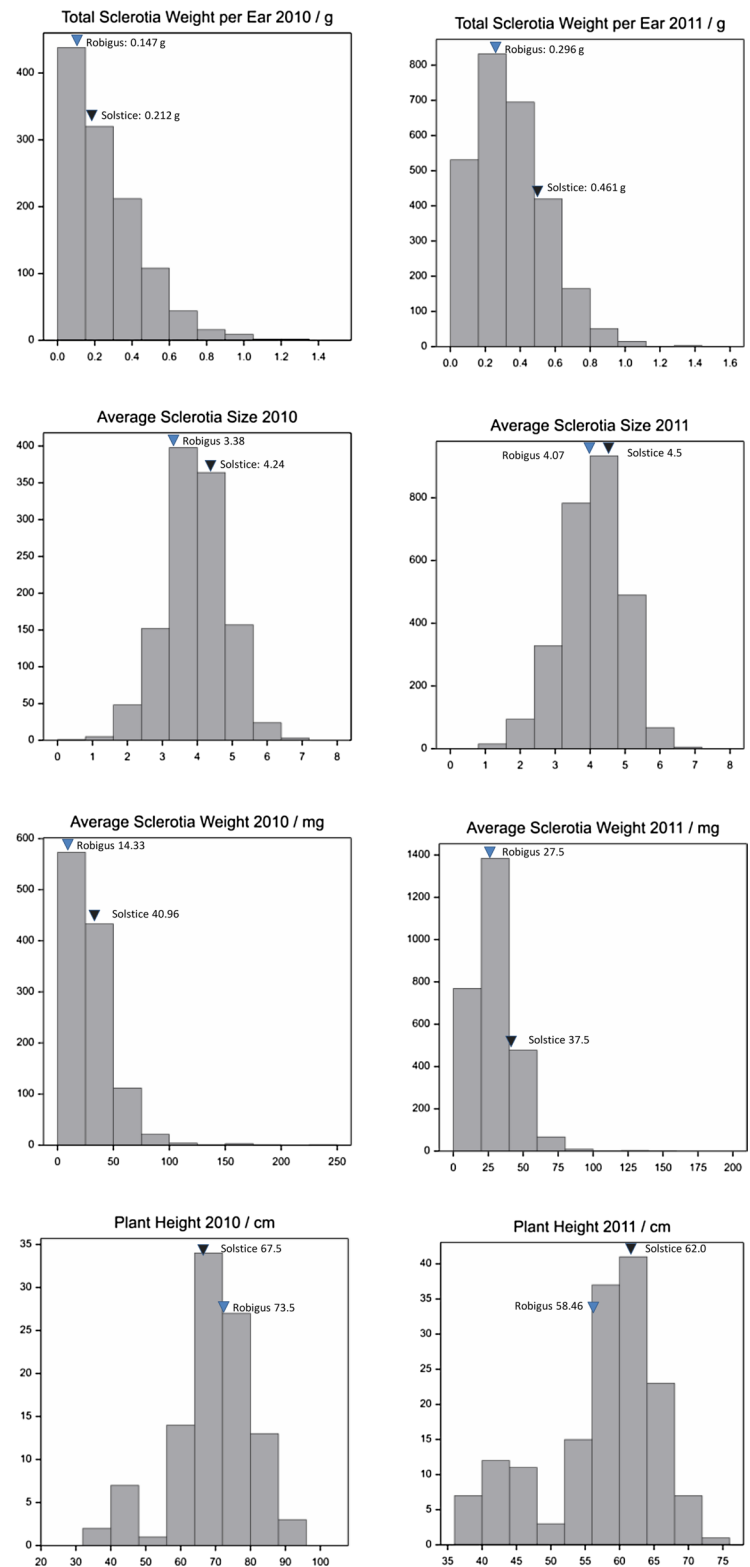
and average sclerotia weight (Fig. 3). The QTL regions on 4B and 4D were also detected using total sclerotia weight per ear. In general, the percentage phenotypic variance explained by individual QTL was relatively small, ranging

from a maximum of 4.76 % for *QCp.niab.2A*, 5.88 % for *QCp.niab.4B* and 13.94 % for *QCp.niab.4D*. While some QTL were not significant using IM in either 2010 or 2011, all three QTL were significant in both years using

Table 3 QTL identified in the 'Robigus' × 'Solstice' cross for ergot sclerotia development and plant height

QTL designation	Chromosome location	Peak marker	Position (cM)	LOD value	%Variance explained	[a]Parental allele	[b]Phenotypic data set	[c]QTL analysis
QCp.niab.2A	2A	wsnp_BQ168780B_Ta_2_1	108	4.631	4.17	R	2010-SS	SMR, IM, CIM
		wsnp_Ex_c2337_4379619	134	3.899	2.47		2011-SS	SMR, IM, CIM
		wsnp_BQ168780B_Ta_2_1	108	5.28	4.76		2010-SW	SMR, IM, CIM
QCp.niab.4B	4B	Rht-B1b	72.76	4.827	4.35	R	2010-SS	CIM
		wsnp_CAP12_c13_8078	70.9	6.497	4.11		2011-SS	SMR, IM, CIM
		Rht-B1b	72.0	6.527	5.88		2010-SW	CIM
		wsnp_CAP12_c13_8078	70.9	8.52	5.39		2011-SW	SMR, IM, CIM
		wsnp_CAP12_c13_8078	70.9	5.1	4.62		2010-TW	CIM
QPh.niab.4B		Rht-B1b	72.75	11.67	6.95		2010-PH	CIM
		Rht-B1b	72.75	24.25	24.0		2011-PH	SMR, IM, CIM
QCp.niab.4D	4D	Rht-D1b	13	13.53	12.19	S	2010-SS	SMR, IM, CIM
		Rht-D1b	8	3.45	2.19		2011-SS	CIM
		Rht-D1b	14	15.4	13.94		2010-SW	SMR, IM, CIM
		Rht-D1b	12	12.07	10.87		2010-TW	SMR, IM, CIM
QPh.niab.4D		Rht-D1b	9	23.16	13.79		2010-PH	SMR, IM, CIM
		Rht-D1b	9	32.01	31.70		2011-PH	CIM
QCp.niab.6A	6A	wPt-665636_NA	0	3.49	3.14	S	2010-TW	SMR
QPh.niab.6A		wPt-665636_NA	0	4.76	2.83		2010-PH	SMR, IM

[a] The contributing parental alleles, *R* 'Robigus' and *S* 'Solstice' confer smaller sclerotia and shorter plants

[b] Phenotypic data codes: *SS* average sclerotia size, *SW* average sclerotia weight, *TW* total sclerotial weight per ear, *PH* plant height

[c] QTL analyses were performed using single marker regression (*SMR*), interval mapping (*IM*) and composite interval mapping (*CIM*). The position of the QTL is shown in centimorgans (cM). The 5 % LOD thresholds for CIM analysis (unless indicated otherwise) are as follows 2010-SS: 3.50, 2011-SS: 3.38, 2010-SW: 3.58, 2011-SW: 3.40, 2010-TW: 3.76, 2010-PH: 3.69, 2011-PH: 3.42, 2010-TW-SMR: 3.13, 2010-PH-SMR: 3.06

CIM (Table 3). A smaller effect QTL, *QCp.niab.6A,* was detected with the 2010 total sclerotia weight per ear data set in 'Solstice' on chromosome 6A. *QCp.niab.6A* had a LOD of 3.49 and a percentage variance of 3.14 %.

As 'Robigus' and 'Solstice' were known to carry different semi-dwarfing alleles, 'Robigus' having *Rht-B1b* (located on chromosome 4B) and 'Solstice' having *Rht-D1b* (located on chromosome 4D), plant height was mapped in the 'Robigus' × 'Solstice' DH population. CIM identified two QTL for plant height, *QPh.niab.4B* and *QPh.niab.4D* on chromosomes 4B and 4D, respectively, where the PCR markers Rht-B1b and Rht-D1b were the peak markers. In 2010 *QPh.niab.4B* and *QPh.niab.4D* accounted for 6.95 and 13.79 % of the phenotypic variance in plant height, while in 2011 they accounted for 24.01 and 31.70 %, respectively. Interestingly, a QTL for plant height

was also identified with the 2010 plant height data set in the same region as *QCp.niab.6A,* with the height-reducing allele and the resistance allele both coming from 'Solstice' (Online Resource 5).

Two-dimensional, two-QTL scans were undertaken to identify pair-wise interactions between QTLs (Online Resource 6). These analyses identify additive effects between QTL, where the two genes have a straightforward additive effect on the phenotype. Epistatic-interactions are identified when the effect of two genes together is greater (or lesser) than the effect of either gene, bearing in mind that for some QTL the effect of one can only be seen in the presence of the other usually explained by their sequential action in the same pathway. These analyses were undertaken using the average sclerotia size and plant height data sets. Additive effects were seen with the
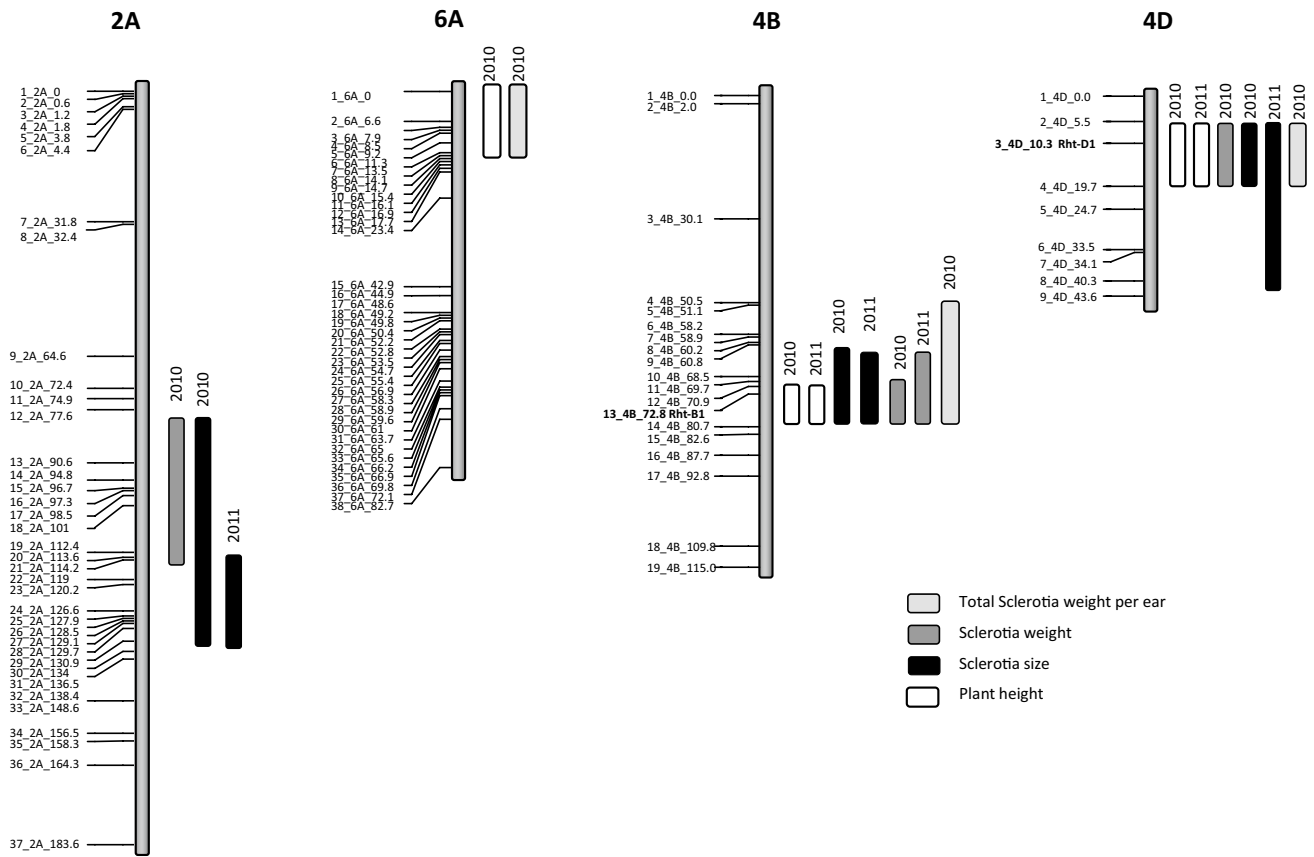
Fig. 3 QTL positions for ergot sclerotia total and average weights, sclerotia size and plant height in the 'Robigus' × 'Solstice' cross following CIM and/or IM analysis. QTL positions are shown to the right of the linkage maps on chromosomes 2A, 4B, 4D and 6A. QTL *bars* represent 1.5 LOD support intervals around the peak score marker.

Marker locations are shown to the *left* of each linkage map, along with marker number, chromosome identifier and position in centimorgans from the top of the linkage group. The full marker names can be found in Online Resource 3

QTL for average sclerotia size, the largest effect being seen with the 2010 data set between QTL *QCp.niab.4B* and *QCp.niab.4D* (LOD$_{av1}$ = 6.81; p < 0.001). With the 2011 average sclerotia size data set, the same QTL interaction had a LOD$_{av1}$ = 3.01; however this was just over the significance threshold with a p-value of 0.172. An additive interaction was also seen between *QCp.niab.2A* and *QCp. niab.4B*, with both the 2010 (LOD$_{av1}$ = 3.03; p = 0.072) and 2011 (LOD$_{av1}$ = 3.93; p = 0.037) data sets, however no interaction was identified between *QCp.niab.2A* and *QCp.niab.4D*. To visualise the QTL additive effects for average sclerotia size dot plots of the phenotypic averages of the lines carrying the QTL *QCp.niab.2A*, *QCp.niab.4B* and *QCp.niab.4D* alone or in combination are presented (Fig. 4).

Two-dimensional, two-QTL scans suggested that there is a significant interaction between the plant height QTL *QPh.niab.4B* and *QPh.niab.4D* (2010, LOD$_i$ = 16.01 and LOD$_{av1}$ = 23.97; p < 0.001 and 2011, LOD$_i$ = 7.84 and LOD$_{av1}$ = 11.79; p < 0.001; Online Resource 6). A height

QTL, in addition to *QPh.niab.4B* and *QPh.niab.4D* was identified using two-dimensional two-QTL analysis on chromosome 2B, designated *QPh.niab.2B*, which shows a significant interaction with *QPh.niab.4B* (LOD$_i$ = 7.37; p < 0.001), further reducing the height of *Rht-B1b* carrying plants (Online Resource 5). The dependence of expression of a phenotype for *QPh.niab.2B* on the presence of *QPh.niab.4B* may also indicate why *QPh.niab.2B* was not detected by IM.

Discussion

Ergot is a plant disease of considerable importance to human and animal health due to the toxic effects of alkaloids present in the sclerotia (De Costa 2002). Few sources of genetic resistance to *C. purpurea* have been reported, with the best source of resistance identified to date being found in a durum wheat genotype (Menzies 2004). In this study we report genetic variation in the response of hexaploid wheat
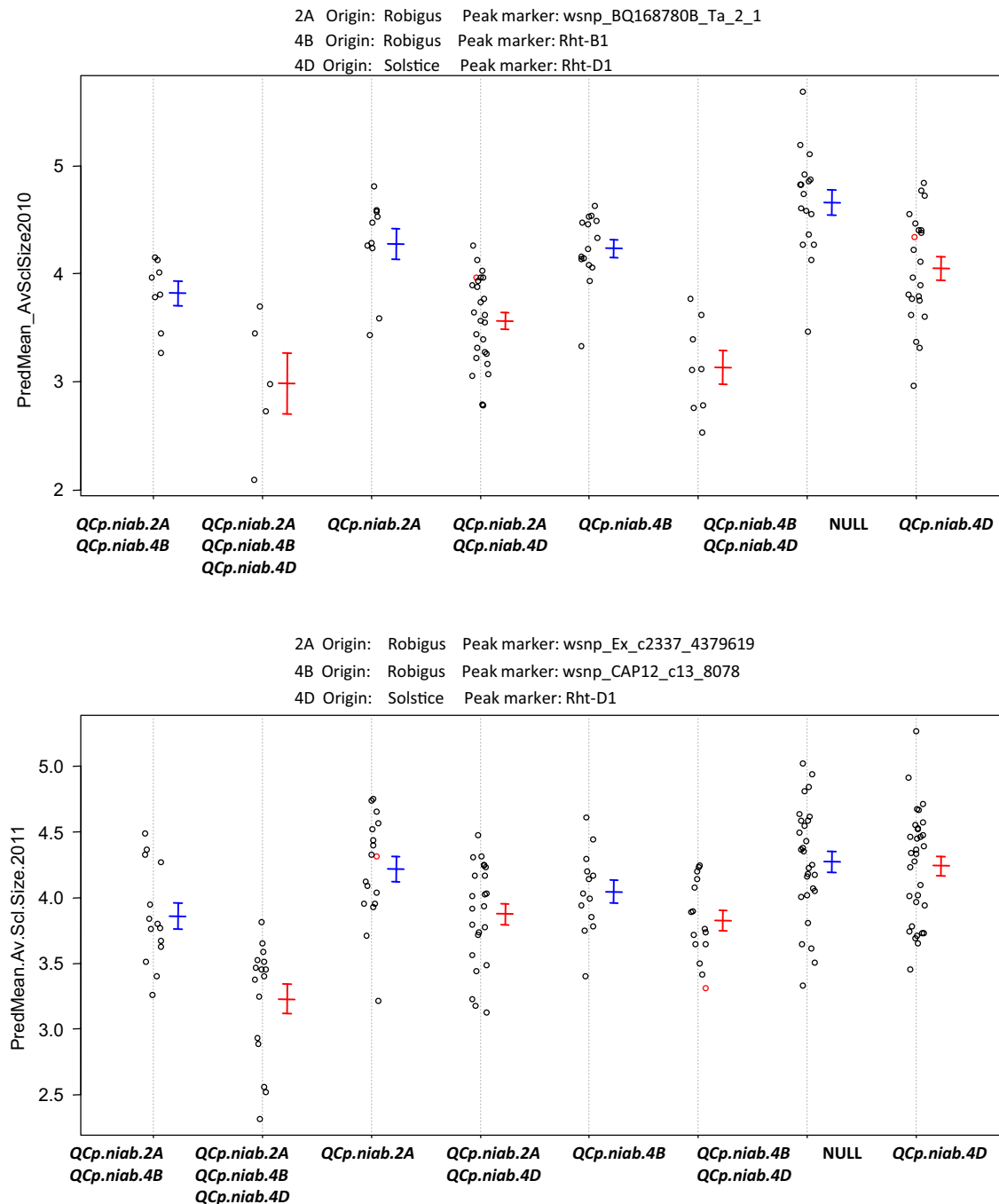
Fig. 4 Phenotypic distribution of sclerotia size in 'Robigus' × 'Solstice' doubled haploid lines carrying one or more of the three major QTL contributing to reduced sclerotia size, *QCp.niab.2A*, *QCp.niab.4B* and *QCp.niab.4D*. The peak marker used to identify each QTL, the QTL parental origin and chromosomal location is indicated above each plot. Phenotypic data from 2010 and 2011 field trials are shown. *Error bars* are ±1 standard errors

varieties to *C. purpurea* and identify interacting QTL in the varieties 'Robigus' and 'Solstice' that influence the size and weight of sclerotia produced by *C. purpurea*.

Together the three QTL *QCp.niab.2A*, *QCp.niab.4B* and *QCp.niab.4D* at best only explained 24.6 % of the phenotypic variation for sclerotial weight (2010 data set), while dividing the percentage phenotypic variance by its corresponding heritability value ($h^2 = 0.78$) gave a maximum genetic variation of 31.7 %. This implies that the majority of the genetic variation affecting ergot

Fig. 5 Relationship between ergot sclerotia size and plant height in doubled haploid lines from the cross 'Robigus' × 'Solstice', 2011 data. The *Rht-B1* and *Rht-D1 a* (wild-type) and *b* (dwarf mutant) alleles carried by each line are indicated. The 'Robigus' genotype is *Rht-B1b/Rht-D1a* and the 'Solstice' genotype is *Rht-1Ba/Rht-1Db*
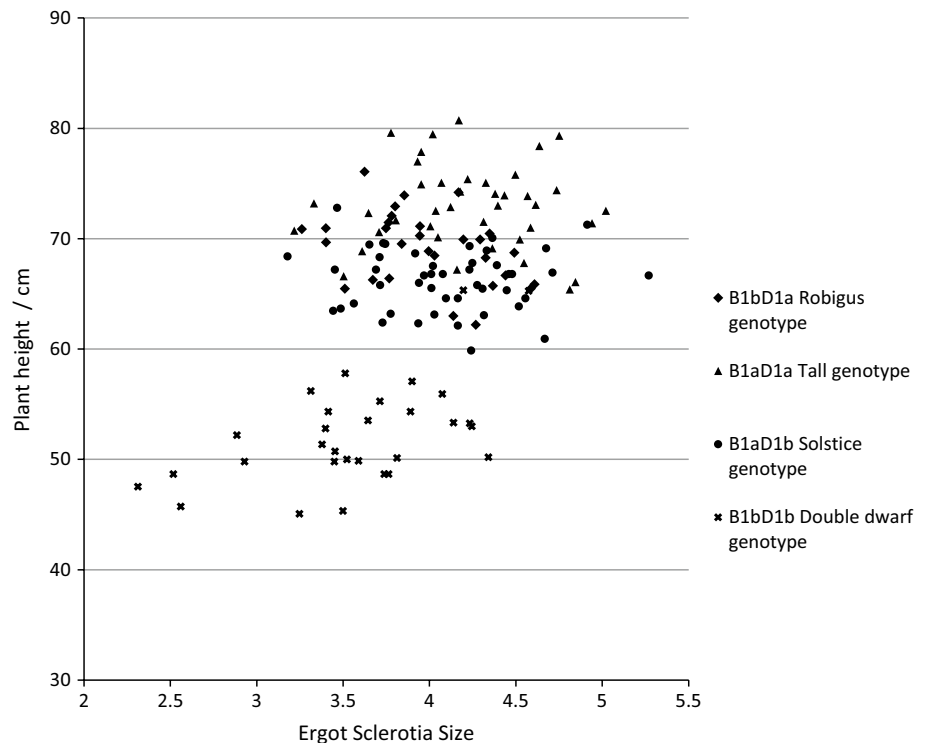
development and sclerotium formation in the 'Robigus' × 'Solstice' population was not detectable. This is likely due to the presence of many small genetic influences affecting sclerotial development, each genetic locus producing a phenotypic effect below the current resolution of QTL analysis. The small size of the 'Robigus' × 'Solstice' population may also have limited the QTL detection power of the population.

The QTL with the largest effect, *QCp.niab.4B* and *QCp.niab.4D,* mapped to the same location as the Reduced Height (*Rht*) loci *Rht-B1* and *Rht-D1* (Peng et al. 1999), with the resistance effect co-segregating with the dwarfing alleles *Rht-B1b* (from 'Robigus') and *Rht-D1b* (from 'Solstice'), respectively. As a result, the general trend was for shorter DH lines to produce smaller sclerotia, although there was significant overlap in the sclerotial size distributions of tall, semi-dwarf and double-dwarf plant size classes indicating the presence of additional genes, not linked to height, influencing sclerotia size (Fig. 5).

Rht-B1 and *Rht-D1* encode for DELLA proteins (Peng et al. 1999). In *Arabidopsis* DELLA proteins have been shown to be nuclear-located and are putative transcriptional regulators that interact with the gibberellic acid receptor protein GID1 (GA-Insensitive Dwarf), resulting in growth repression. In the presence of gibberellic acid (GA) the DELLA proteins are degraded via the 26S proteasome SCF complex, resulting in subsequent stimulation of plant growth (Dill et al. 2004). The gain-of-function dwarfing alleles *Rht-B1b* and *Rht-D1b* produce GA-insensitive

mutant DELLA proteins, resulting in a partially stable pool of DELLA protein that continues to suppress plant growth, producing semi-dwarfed plants (Pearce et al. 2011).

The association between *QCp.niab.4B* and *Rht-B1b*, and *QCp.niab.4D* and *Rht-D1b* may be pleiotropic, i.e. the same locus affects both sclerotium formation and plant height, or could be due to close genetic linkage of functionally independent genes. In the latter case, height and ergot resistance should be genetically separable if a sufficiently high resolution mapping approach was brought to bear. If the former is the case, attention should be devoted to testable hypotheses that might flow from a postulated pleiotropy between wheat DELLA function and ergot sclerotia size. Although neither scenario can be ruled out, some further observations lend more weight to the pleiotropy scenario.

Firstly, wheat *DELLA/Rht* genes have been demonstrated to have potential pleiotropic effects on disease resistance to multiple pathogens, with gain-of-function mutant alleles conferring increased susceptibility to biotrophic pathogens and increased resistance to necrotrophic pathogens (Saville et al. 2012). In *Arabidopsis*, DELLA mutants have been shown to suppress hypersensitive cell death, leading to increased resistance to necrotrophic pathogens and increased susceptibility towards pathogens with a biotrophic life stage (Navarro et al. 2008). In the wheat-Fusarium Head Blight (FHB) pathosystem, the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* were associated with decreased resistance to primary infection (Type I resistance) of wheat florets by *Fusarium* species, with

the early stages of *Fusarium* infection currently thought to represent a biotrophic phase (Hilton et al. 1999; Draeger et al. 2007; Srinivasachary et al. 2009). However, while *C. purpurea* is considered a biotroph, in the present study the *Rht* gain-of-function mutant alleles were associated with increased resistance and not susceptibility. The *Rht* alleles therefore have contrasting effects on the infection of florets and ovules by *C. purpurea* and *Fusarium* species.

Secondly, the association between ergot resistance and GA-regulated plant height may not be confined to the *Rht-D1* and *Rht–B1* loci, as the small effect QTL *QCp.niab.6A* was detected with both the plant height and total sclerotia weight per ear phenotypic data sets, with the 'Solstice' allele being responsible for both reduced sclerotia and reduced height. Previous studies have identified plant height genes on chromosome 6A (Watanabe 2008), the alleles *Rht14*, *Rht16* and *Rht18* at this locus conferring GA-sensitive reduced plant height. Unfortunately, we were not able to confirm a common location for *QCp.niab.6A* and the plant height QTL reported on chromosome 6A (Watanabe 2008).

Thirdly, there seems to be a more general association between hormone signalling and quantitative development of ear diseases in wheat. A QTL for Type I FHB resistance has also been reported on chromosome 2A, however common markers were not available to compare the position of this QTL with the ergot resistance QTL *QCp.niab.2A* found in this study (Lin et al. 2006; Diethelm et al. 2014). Characterisation of the peak marker for the 2A FHB QTL suggests that the gene underlying the QTL may be a wheat homologue of NPR1 (non-expresser of pathogenesis related protein 1) (Diethelm et al. 2014). NPR1 is at the heart of hormonal cross-talk between the salicylic acid (SA) and jasmonic acid (JA) pathways, being involved in the SA-mediated suppression of the JA pathway (Spoel et al. 2003). The functional annotation of transcripts differentially expressed between ovaries inoculated with *C. purpurea* versus mock-inoculated ovaries in RNA-Seq experiments also highlights hormone signalling as one of the most responsive functional categories (unpublished data).

Gibberellins play a crucial role in many aspects of plant development. Active forms of these phytohormones are known to control processes such as seed germination, stem elongation, floral development and anther extrusion (Cheng et al. 2004; Peng and Harberd 1997). *C. purpurea* may have evolved an ability to manipulate the endogenous host hormone signalling pathways to command the resources necessary to produce sclerotia many times the fresh weight of a fully developed grain (Tudzynski 2005). Mutations in key hormone signalling pathway genes, such as the *Rht-B1b* and *Rht-D1b* alleles, are therefore attractive candidates that could explain how the ability to re-programme host resource

allocation is partly compromised in the absence of any symptoms resembling a typical immune response. In the case of the *Rht* mutants one may speculate that perturbed GA levels may interact with normal mechanisms by which *C. purpurea* colonise floral tissues, resulting in altered levels of colonisation. In semi-dwarf and dwarf lines GA levels increase in expanding leaf tissues (Appleford and Lenton 1991; Wu et al. 2011), but to our knowledge GA levels in ovule tissue pre-pollination have never been measured and are a potential avenue of further investigation.

Compliance with ethical standards

All authors have consented to submit this paper. All authors have followed scientifically good practice as outlined in the Instructions for Authors. This work did not involve human or animal participants.

Conflict of interest The authors declare that they have no conflict of interest.

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