The Effect of the *Rht1* Haplotype on Fusarium Head Blight Resistance in Relation to Type and Level of Background Resistance and in Combination With *Fhb1* and *Qfhs.ifa-5A*

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Abstract

The semi-dwarfing reduced height alleles *Rht-D1b* and *Rht-B1b* have been deployed in modern wheat cultivars throughout the world, but they increase susceptibility to Fusarium head blight (FHB). Here, we investigated the impact of the *Rht1* genes on anther retention (AR) in relation to FHB resistance using four different sets of near isogenic lines (NILs) with contrasting levels and types of background FHB resistance. NILs were evaluated for FHB severity, plant height, and anther retention in three greenhouse and three field trials using artificial spray inoculation. *Rht-B1b* and *Rht-D1b* alleles increased AR and FHB susceptibility in all genetic backgrounds. The magnitude of the effects differed between NIL groups. Increased FHB susceptibility largely followed increased anther retention. Differences in FHB susceptibility between tall and dwarf haplotypes were largest in the NIL group with the highest changes in AR. In the most resistant NIL group, dwarfed lines had only slightly higher AR than tall lines and maintained good resistance, while both tall and dwarf lines had high levels of retained anthers in the most susceptible NIL group. We further investigated the effect of the *Rht1* genes in combination with the major Fusarium resistance QTL *Fhb1* and *Qfhs.ifa-5A*. *Qfhs.ifa-5A* enhanced anther extrusion in tall as well as semi-dwarf haplotypes whereas *Fhb1* did not affect AR. *Qfhs.ifa-5A* supported FHB resistance more efficiently than *Fhb1* in lines that were more responsive to AR, while both *Fhb1* and *Qfhs.ifa-5A* were equally efficient in NILs that had high background resistance and low response to AR.

Key Message

The effect of the *Rht1*-genes on FHB resistance depends on anther extrusion and level of background resistance. *Qfhs.ifa-5A* increases resistance and anther extrusion as efficiently as semi-dwarfing alleles decrease it.

Introduction

Fusarium head blight (FHB), primarily caused by members of the *Fusarium graminearum* species complex, is a highly destructive disease that affects wheat and small grains worldwide (Parry et al. 1995). Prolonged warm and humid conditions around flowering can cause severe FHB outbreaks that can drastically reduce grain yield and quality (McMullen et al. 2012). FHB is furthermore of great concern for food and feed safety as mycotoxins produced by *Fusarium* pathogens accumulate in infected grains and pose a health risk to humans and animals (Pestka 2010).

FHB resistance is a highly complex quantitative trait controlled by a multitude of genetic and non-genetic factors. Pheno-morphological traits, particularly plant height, spike architecture and flowering related traits, modify disease response (Buerstmayr et al. 2020; Zhu et al. 1999). Many studies have observed higher susceptibility to fungal entry (type 1 resistance) and reduced overall 'field resistance' of short-straw cultivars compared to tall ones when tested in the field. Of the QTL mapping studies that reported on both plant height and FHB resistance, 40% of the plant height QTL overlapped with QTL for type 1 resistance or overall 'field resistance' (Buerstmayr et al. 2020). The observed association between plant height and

FHB resistance may depend on several factors that affect the likelihood of infection and influence the microclimate at spike height. The pathogen overwinters saprophytically on plant debris that constitutes the primary inoculum for upcoming crops. FHB infection starts inside the florets with the plants being most sensitive around flowering (Parry et al. 1995). Spores enter the spikelets during anthesis when florets open or via degenerated anthers that are partially trapped between the glumes (Pugh 1933). Shorter plants are thus at greater risk for getting infected by rain-splash-dispersed spores (Jenkinson and Parry 1994). Heads on taller plants dry quickly, as they are exposed to wind and sun. In contrast, shorter plants are more affected by soil humidity and dew, where the dense canopy structure with the flag leaves close to the heads may reduce air circulation thereby increasing relative humidity and temperature around spikes that favor infection and disease development (Jones et al. 2018).

The significance of anthers and their effect on FHB resistance has long been known (Pugh 1933) and has recently regained attention. Fully extruded anthers (Steiner et al. 2019) and complete cleistogamous flowering (Kubo et al. 2010) are associated with FHB resistance while susceptibility increases with the proportion of retained anthers or anthers partially trapped between palea and lemma (Buerstmayr and Buerstmayr 2015; Kubo et al. 2013). Among the mapping studies that collected data on anther retention (AR) or anther extrusion (AE), plant height and FHB resistance, 60% of the QTL for AR/AE coincided with FHB resistance QTL, whereas overlap of QTL for plant height and AR/AE were rare indicating that AR/AE and plant height are largely independently genetically controlled (Buerstmayr et al. 2020).

The implementation of the reduced height (*Rht*) alleles in wheat and rice breeding since the 1960s were key elements of the 'Green Revolution' (Borlaug 1968). Plants with the semi-dwarfing *Rht1* alleles are shorter, have an improved harvest index combined with higher grain yield and better resistance to lodging. This facilitated an intensification of farming systems and led to a substantial increase in grain yield worldwide (Hedden 2003). To date in many wheat growing regions modern wheat cultivars and breeding lines frequently possess one of the homeolog mutant semi-dwarfing alleles *Rht-B1b* or *Rht-D1b* located on the short arm of chromosome 4B and 4D, respectively (Evans 1998; Würschum et al. 2015). The *Rht1* genes encode DELLA proteins, which belong to a plant-specific family of transcriptional regulators and are involved in the signal transduction pathway of the phytohormone gibberellin (GA) with the wild type alleles being sensitive to GA (Achard et al. 2006). GA mediates the degradation of the growth repressing DELLA proteins allowing the plants to grow tall (Peng et al. 1997). The mutant *Rht-B1b* and *Rht-D1b* alleles have similar nucleotide substitutions in the N-terminal DELLA domain that result in premature stop codons. Due to translational reinitiation in the main open reading frame N-terminally truncated DELLA proteins are produced, which are resistant to GA-mediated degradation. This causes a lower reduction of the growth repressing DELLA proteins and plants remain short (Peng et al. 1999; Van De Velde et al. 2021). The *Rht1* genes influence a multitude of agronomically relevant traits that contribute to yield and grain quality, for instance coleoptile size and seed emergence, grain number per spikelet, seed size, protein content and grain yield (Acuña et al. 2001; Flintham et al. 1997). The semi-dwarfing alleles have a negative effect on anther extrusion (Boeven et al. 2016; Buerstmayr and Buerstmayr 2016; Lu et al. 2013; Muqaddasi et al. 2017). The combination of reduced height and high number of retained anthers particularly negatively impacts FHB resistance. This may explain the observed high effect of the *Rht1* loci

on type 1 and overall field resistance in almost all mapping populations that segregated for the *Rht1* alleles (Buerstmayr et al. 2020; Xu et al. 2020; Zhu et al. 2021).

The major FHB resistance QTL *Fhb1* and *Qfhs.ifa-5A* are the most extensively studied and best validated FHB resistance QTL and they have been successfully implemented in resistance breeding programs (Buerstmayr et al. 2020; McMullen et al. 2012; Steiner et al. 2017). *Qfhs.ifa-5A* confers resistance to fungal entry (type 1 resistance) (Buerstmayr et al. 2003) and increases anther extrusion (Steiner et al. 2019), while *Fhb1* acts against fungal spreading within the spike (type 2 resistance) (Waldron et al. 1999).

In our work we specifically investigated the effect of the *Rht1* genes on AR and on FHB resistance in *i)* four genetic backgrounds. These genetic backgrounds differed in types and levels of FHB resistance and *ii)* we investigated the effect of the *Rht1* genes in combination with the major FHB resistance QTL *Fhb1* and *Qfhs.ifa-5A*. For this we generated near isogenic lines (NILs) of spring wheat lines CM-82036 (superior type 1 and type 2 resistance, carrier of *Fhb1* and *Qfhs.ifa-5A*), C1 (moderate type 1 and moderate type 2 resistance, carrier of *Fhb1* and *Qfhs.ifa-5A*), E4-61 (high type 1 resistance), and Michael (susceptible) that were homozygous for either one of the two semi-dwarfing alleles (*Rht-D1b* or *Rht-B1b*) or the wild type alleles *Rht-B1a/Rht-D1a*. We furthermore developed and analyzed CM-82036 and C1 NILs with contrasting allele status at *Rht-B1*, *Rht-D1*, *Fhb1* and *Qfhs.ifa-5A*.

Plant Material

Development of *Rht1*-NIL groups C1, CM, E4 and MI

Rht-D1b (donor cultivar Monsun) or *Rht-B1b* (donor cultivar Bobwhite) were each five times back-crossed to spring wheat recipient lines 'C1', 'CM-82036', 'E4-61', or 'Michael' leading to an expected recovery of ~ 99% for the recipient genome. All four recipient lines possess the tall wild-type alleles (*Rht-B1a/Rht-D1a*) of the *Rht1* genes but differ in their resistance to FHB. 'CM-82036' originates from the cross Sumai 3/Thornbird-S. CM-82036 has outstanding type 1 and type 2 resistance and contains the FHB resistance QTL *Fhb1* and *Qfhs.ifa-5A* (Buerstmayr et al. 2002; 2003). Line C1 possesses moderate type 1 and type 2 resistance. It contains the resistance alleles of *Fhb1* and *Qfhs.ifa-5A* embedded in the susceptible background of the cultivar 'Remus' (Schweiger et al. 2013). Line E4-61 was selected from a doubled haploid population developed from a cross of Frontana and Remus (Steiner et al. 2004). E4-61 shows good type 1 resistance but lacks type 2 resistance. Michael is a high yielding commercial spring wheat released by Saatzzucht Bauer GmbH & Co KG, Germany. Michael is highly susceptible to FHB.

Rht-B1 and *Rht-D1* markers as described by Ellis et al. (2002) or allele specific KASP assays retrieved from the Cerealsdb database (www.cerealsdb.uk.net/cerealgenomics/CerealsDB/Excel/MAS_data_May_2013.xls) were used to ensure the transmission of the *Rht-D1b* and *Rht-B1b* allele through the five backcrossing cycles. Per backcross stream two heterozygous BC₅F₁ plants were selfed and plants homozygous for the dwarf or tall alleles at

the *Rht1* genes were selected from BC₅F₂ plants. The thus developed plant material comprises four *Rht1*-NIL groups defined by their recurrent parent (abbreviations for NIL groups: C1, CM, E4, MI), each containing five, two and two NILs of the *Rht1*-haplotypes *B1aD1a*, *B1bD1a*, and *B1aD1b*, respectively (Table S1).

Development of C1 and CM *QTL*-by-*Rht1* NILs

Each semi-dwarfing *Rht1*-NIL of group C1 and CM were crossed with Remus or CM-NIL51, respectively. Remus (near isogenic to C1) and CM-NIL51 (near isogenic to CM) have the tall *Rht-B1a/Rht-D1a* haplotype and the susceptible alleles at *Fhb1* and *Qfhs.ifa-5A* (Schweiger et al. 2016). Per cross all possible *QTL*-by-*Rht1* combinations were selected (Table S2) using allele specific KASP assays for *Rht-B1*, *Rht-D1* and *Fhb1* (Su et al. 2018) and SSR markers gwm304 (Röder et al. 1998) and wmc705 (Somers et al. 2004) that flank the *Qfhs.ifa-5A* *QTL* interval.

Fusarium inoculation and trait assessments

Green house experiments

Three greenhouse experiments were conducted with two replicates per genotype in January 2020 and in December 2020 and an un-replicated experiment in February 2021. Seeds of *Rht1*-NILs and recurrent parents were germinated in seedling starter trays on a mixture of recycled compost and sand and vernalized at 4°C with a 12h day/night light regime for 1 week. Twelve seedlings per NIL genotype were replanted into pots (21cm diameter, 23 cm height) filled with 6.5 l of potting soil (75% heat-sterilized recycled compost, 23% peat, 2% silica sand) and transferred to the greenhouse.

In the greenhouse within each block NILs for each NIL group and the respective recurrent parent were grouped together. Per NIL group semi-dwarfing and tall NILs were furthermore organized into sub-groups whereby NILs within sub-groups were fully randomized. This design allowed to adjust the distance between wheat heads and green house lamps during the inoculation and scoring period so that heads of dwarf and tall lines had the same distance of approximately 80 cm to the greenhouse lamps (Philips MASTER SON-T PIA Plus 400W) and were therefore equally affected by radiant heat emitted by the lamps. Temperature in the greenhouse was set to 18/12°C (day/night) from tillering to heading with 12–14 h of light. Shortly before anthesis, the greenhouse conditions were set at 22/18°C (day/night) with a 16 h photoperiod at 15,000 lx and settings were kept constant until the end of the experiments. Heads were inoculated individually when they were flowering by spraying three ml of a *F. graminearum* conidial suspension (*F. graminearum* isolate IFA66, spore concentration of 5×10^4 ml⁻¹ and 1 ml Tween 20 per liter) using a hand-held sprayer. Inoculated heads were covered with translucent polyethylene bags for 30 hours assuring high humidity for optimal fungal development. AR was evaluated on five basal florets per inoculated head five days after inoculation (dai). A floret was scored as retained when at least one anther remained inside the floret or was trapped between lemma and palea. Number of infection sites per

inoculated head were assessed at 8, 12 and 16 days after inoculation. Infection sites are defined as clearly separated starting points of *Fusarium* infection within a head. Number of diseased heads and number of symptomatic spikelets per spike were assessed at 8, 12, 16, 20 and 24 dai. The total number of spikelets of inoculated heads were counted. The percentage of symptomatic spikelets over the total number of inoculated spikelets per scoring date was calculated and used to determine the area under the disease progress curve (AUDPC) as described by Buerstmayr et al. (2000). AR was expressed in percent of florets with at least one anther retained. Number of inoculated heads per pot ranged from 10 to 22, with an average of 14 inoculated heads per pot. Plant height per pot was measured in cm. Each pot was considered as an experimental unit. Scoring data were averaged per pot over the total number of inoculated heads.

Field experiments

All field experiments were conducted at IFA Tulln, Austria (16°04,16'E, 48°19,08'N, 177 m above sea level) with four replicates per genotype and year. C1, CM, E4 and MI NILs along with their recurrent parents were tested in years 2019, 2020 and 2021 and C1 and CM *QTL-by-Rht1* NILs were tested in 2020 and 2021. Within each replication NILs with the same near isogenic background (including recurrent parents) were organized into semi-dwarfing and tall subgroups that were planted next to each other. NILs within tall and dwarf sub-groups were fully randomized. This field design aimed to minimize potential height effects of neighboring plots. Plots consisted of double rows of 1 m length with 17 cm spacing. Sowing time was early spring in all years. Artificial spray inoculation started when the first plot reached mid-anthesis and was repeated every other day until 2 days after the last plot reached mid-anthesis. About 100 ml m⁻² of freshly diluted macro-conidial suspension of the *F. culmorum* single-spore isolate 'Fc91015', prepared as described by Buerstmayr et al. (2000), was sprayed onto the heads using a battery-driven backpack sprayer. Inoculations were carried out in the late afternoons from 4:00 to 6:00 p.m. An automated mist-irrigation system triggered by leaf wetness measurements was used to ensure high humidity during the first 20 h after each inoculation. FHB incidence scoring was assessed individually per plot 21 days after mid flowering as the percentage of heads showing ≥ 1 symptomatic spikelets out of 40 and 50 randomly chosen heads in 2019 and 2021, respectively. FHB severity was visually estimated as the percentage of infected spikelets within each plot at 10, 14, 18, 22 and 26 days after anthesis and used for calculating the AUDPC as described by Buerstmayr et al. (2000). The percentage of retained anthers was assessed five days after anthesis on 30 florets by inspecting three basal florets of ten randomly chosen heads. Plant height (cm) was measured in all plots of all years.

Phenotypic data analysis

Statistical analysis was performed in R version 4.0.3 (R Core Team 2020) and was done for each NIL group and for field and greenhouse experiments separately. Variance components were determined by the restricted maximum likelihood (REML) method, assuming a general linear mixed model using the R package lme4 (Bates et al. 2015):

For *Rht1*-NIL groups C1, CM, E4 and MI:

$$Y_{ikl} = \mu + t_i + e_k + te_{ik} + b_l(e_k) + \varepsilon_{ikl}$$

where μ denotes the overall mean, t_i the effect of *Rht1* haplotype i , e_k the effect of the experiment k , te_{ik} the interaction between *Rht1* haplotype i and experiment k , $b_l(e_k)$ the effect of the block l nested in the experiment k , and ε the residual effect.

For C1 and CM *QTL*-by-*Rht1* groups:

$$Y_{ijkl} = \mu + t_i + q_j + tq_{ij} + e_k + r_l(e_k) + te_{ik} + qe_{jk} + tqe_{ijk} + \varepsilon_{ijkl}$$

where μ denotes the overall mean, t_i the effect of *Rht1* haplotype i , q_j the effect of *QTL* haplotype j , tq_{ij} the interaction between *Rht1* haplotype i and *QTL* haplotype j , e_k the effect of the experiment k , te_{ik} the interaction between *Rht1* haplotype i and experiment k , tqe_{ijk} the interaction between *Rht1* haplotype i , *QTL* haplotype j and experiment k , $r_l(e_k)$ the effect of the replication l nested in the experiment k , and ε_{ijkl} the residual effect. *Rht1* and *QTL* haplotypes and *QTL*-by-*Rht1* interaction were considered as fixed effects, all other factors were considered as random effects.

Contrasts between haplotypic groups were assessed with the `glht` function (method: Tukey, p adjustment: Bonferroni) of the `multcomp` package (Hothorn et al. 2008).

Results

Effects of *Rht1* haplotypes on plant height, anther retention and FHB resistance

Rht1 haplotype had a strong and significant effect on plant height in all NIL groups in greenhouse and field experiments (Table S3). The *Rht1* haplotype effect on AR was significant in greenhouse experiments for NIL groups C1, CM and E4 and in field experiments for C1, CM and MI and marginally significant in the green house for MI ($p = 0.052$) and in field tests for E4 ($p = 0.086$). The impact of *Rht1* haplotype on FHB incidence and AUDPC was significant in all NIL groups under field conditions. *Rht1* haplotypes had a significant effect in greenhouse experiments on AUDPC and on the number of infection sites in group C1. *Rht1* significantly influenced the number of infection sites in group E4 and had a weak impact in group MI ($p = 0.062$) under greenhouse conditions.

Comparing *Rht1* haplotype groups

Plant height: Wildtype lines (*B1aD1a*) of group E4 had the tallest plant phenotype (≥ 100 cm) and were significantly taller than wildtype lines of groups C1 (90 cm) and MI (88 cm) and of groups C1 (86 cm) and CM (92 cm) in greenhouse and field experiments, respectively (Figs. 1, 2; Tables S4, S5). Plant height reduction of introgressed dwarfing alleles was significant in all four recurrent NIL groups and was highest in E4 NILs, followed by MI, and C1, and was lowest in CM (Figs. 1, 2; Tables S4, S5).

Rht-B1b and *Rht-D1b* dwarfing alleles led to similar decreases in height within NIL groups.

Anther retention: Average AR% of wildtype haplotypes in NIL groups C1 (31% or 33%) and CM (37% or 13%) was markedly lower than in E4 (54% or 58%) and was highest in MI (88% or 93%) in respective greenhouse or field experiments. There were significantly more anthers retained in dwarfing NILs in field and greenhouse experiments in all groups, but the difference between tall and semi-dwarfing *B1b* NILs was below significance in field experiments of NIL group E4 and in greenhouse experiments of NIL group MI (Figs. 1, 2). The *Rht1-D1b* allele had a significantly stronger effect compared to the *Rht-B1b* allele in NIL group C1 while in the NIL groups CM, E4 and MI no differences were observed between *B1b* and *D1b* isolines. The increase in AR in the dwarfed versus tall lines was highest in NIL group C1, followed by E4 and CM and was smallest in group MI. However, since the tall MI haplotypes had already nearly complete anther retention, there was little room for further increase.

FHB resistance traits: Generally, within NIL groups no significant differences in FHB susceptibility were found between the semi-dwarfing *Rht-B1b* and *Rht-D1b* haplotypes, although there was a trend towards higher susceptibility for *D1b* than for *B1b* carriers. Differences between tall and dwarfing lines were more pronounced under field conditions and were particularly high among NILs of groups C1 and E4 (Fig. 2). *Rht1* haplotype did not affect FHB resistance level of CM and MI NILs in the greenhouse and had a relatively small impact under field conditions (Figs. 1, 2). The effect of the dwarfing alleles was low in the highly FHB resistant CM background and the dwarfed NILs remained highly resistant in greenhouse and field experiments. FHB severity in all MI NILs was high, regardless of height. The FHB severity levels in MI NILs were particularly high under field conditions. Infection of E4 NILs was relatively low in the greenhouse but high in the field.

Effects of *Rht1* haplotypes in combination with FHB resistance QTL *Fhb1* and *Qfhs.ifa-5A* on plant height, anther retention and FHB resistance

Analysis of variance revealed a significant effect of the *Rht1* haplotype on plant height, AR%, FHB incidence and AUDPC in group C1 and on plant height and AUDPC in group CM. The status of the FHB resistance alleles significantly affected AR%, FHB incidence and AUDPC in group C1, and AR% and AUDPC in group CM. Significant *QTL-by-Rht1* interaction was revealed for plant height in group CM and for FHB incidence and AR% in group C1 and CM (Table S6).

Comparing QTL-by-*Rht1* haplotypic groups

C1 and CM wild type *Rht1*-haplotypes were consistently taller than their corresponding *Rht-B1b* or *Rht-D1b* variants, while only minor height differences were observed among NILs with contrasting QTL combinations at *Fhb1* or *Qfhs.ifa-5A* (Figs. 3, S1, S2; Table 1). Generally, a higher proportion of anthers was retained in semi-dwarfing NILs compared to their tall variants (Figs. 3, S1, Table 1). The resistance alleles at *Qfhs.ifa-5A* clearly reduced AR in tall as well as semi-dwarf haplotypes. Differences were particularly high and always significant between *Qfhs.ifa-5A* carriers and non-carriers within *Rht1* subgroups of C1 lines, while the differences were smaller in CM NILs and only significant within the tall and the semi-dwarfing *Rht-D1b* haplotypes (Fig S1). *Fhb1* had no effect on AR. Tall NILs without the

Qfhs.ifa-5A resistance allele had 10 to 30% more anthers retained than semi-dwarfing NILs with the *Qfhs.ifa-5A* resistance allele.

Table 1
Best linear unbiased estimators (BLUES) across experiments of NIL group C1 and CM grouped by *Rht1* haplotype and QTL combination

				NIL group C1		NIL group CM	
Trait	<i>Rht1</i> haplotype	FHB resistance QTL		BLUES ^a		BLUES ^a	
Plant height	<i>B1aD1a</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	83.4	b	90.2	b
	<i>B1aD1a</i>	-	<i>Qfhs.ifa-5A</i>	85.0	b	93.0	b
	<i>B1aD1a</i>	<i>Fhb1</i>	-	82.0	b	90.5	b
	<i>B1aD1a</i>	-	-	82.2	b	89.0	b
	<i>B1bD1a</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	71.6	a	79.7	a
	<i>B1bD1a</i>	-	<i>Qfhs.ifa-5A</i>	70.6	a	79.7	a
	<i>B1bD1a</i>	<i>Fhb1</i>	-	68.1	a	79.1	a
	<i>B1bD1a</i>	-	-	69.4	a	77.8	a
	<i>B1aD1b</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	67.5	a	81.6	a
	<i>B1aD1b</i>	-	<i>Qfhs.ifa-5A</i>	68.1	a	82.0	a
	<i>B1aD1b</i>	<i>Fhb1</i>	-	66.6	a	76.6	a
	<i>B1aD1b</i>	-	-	67.2	a	77.5	a
Anther retention	<i>B1aD1a</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	27.8	a	13.4	a
	<i>B1aD1a</i>	-	<i>Qfhs.ifa-5A</i>	29.8	a	10.7	a
	<i>B1aD1a</i>	<i>Fhb1</i>	-	71.9	cde	27.7	abc
	<i>B1aD1a</i>	-	-	78.0	def	22.0	ab
	<i>B1bD1a</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	56.2	bc	26.0	abc
	<i>B1bD1a</i>	-	<i>Qfhs.ifa-5A</i>	46.5	ab	25.2	abc
	<i>B1bD1a</i>	<i>Fhb1</i>	-	87.1	eg	43.0	bd
	<i>B1bD1a</i>	-	-	85.3	eg	41.3	bd
	<i>B1aD1b</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	63.6	bcd	20.0	ab
	<i>B1aD1b</i>	-	<i>Qfhs.ifa-5A</i>	60.3	bcd	15.6	a
	<i>B1aD1b</i>	<i>Fhb1</i>	-	96.9	g	47.7	cd
	<i>B1aD1b</i>	-	-	96.3	fg	56.7	d
FHB incidence	<i>B1aD1a</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	56.5	a	15.4	a

				NIL group C1		NIL group CM	
	<i>B1aD1a</i>	-	<i>Qfhs.ifa-5A</i>	65.9	a	43.3	b
	<i>B1aD1a</i>	<i>Fhb1</i>	-	90.8	bc	40.6	b
	<i>B1aD1a</i>	-	-	93.4	bc	82.5	d
	<i>B1bD1a</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	82.3	b	35.8	ab
	<i>B1bD1a</i>	-	<i>Qfhs.ifa-5A</i>	88.5	bc	82.3	d
	<i>B1bD1a</i>	<i>Fhb1</i>	-	98.0	c	52.3	bc
	<i>B1bD1a</i>	-	-	97.8	c	90.5	d
	<i>B1aD1b</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	88.5	bc	51.0	bc
	<i>B1aD1b</i>	-	<i>Qfhs.ifa-5A</i>	94.3	bc	73.3	cd
	<i>B1aD1b</i>	<i>Fhb1</i>	-	99.0	c	71.0	cd
	<i>B1aD1b</i>	-	-	99.0	c	92.0	d
AUDPC	<i>B1aD1a</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	300.7	a	40.6	a
	<i>B1aD1a</i>	-	<i>Qfhs.ifa-5A</i>	406.6	ab	119.2	ac
	<i>B1aD1a</i>	<i>Fhb1</i>	-	687.9	cd	95.5	ab
	<i>B1aD1a</i>	-	-	796.8	cf	339.6	ac
	<i>B1bD1a</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	454.1	ab	70.9	ab
	<i>B1bD1a</i>	-	<i>Qfhs.ifa-5A</i>	584.3	bc	276.8	ac
	<i>B1bD1a</i>	<i>Fhb1</i>	-	804.4	cf	123.7	ac
	<i>B1bD1a</i>	-	-	896.1	df	459.6	bc
	<i>B1aD1b</i>	<i>Fhb1</i>	<i>Qfhs.ifa-5A</i>	609.0	bc	91.6	ab
	<i>B1aD1b</i>	-	<i>Qfhs.ifa-5A</i>	741.4	cde	260.3	ac
	<i>B1aD1b</i>	<i>Fhb1</i>	-	915.9	ef	206.8	ac
	<i>B1aD1b</i>	-	-	997.3	f	528.9	c
^a BLUEs of groups with different letters are significantly different (Bonferroni <i>p</i> -value adjustment, <i>p</i> < 0.05)							

The introgression of *Qfhs.ifa-5A* alone or in combination with the *Fhb1* resistance allele into the C1 background decreased FHB incidence and AUDPC in tall and dwarf haplotypes (Figs. 3, S1). The ranking of subgroups within *Rht-B1aD1a*, *Rht-B1bD1a* or *Rht-B1aD1b* haplotypes was constant whereby NILs with

Qfhs.ifa-5A and *Fhb1* were most resistant, followed by NILs containing *Qfhs.ifa-5A* only, and NILs carrying *Fhb1* alone were almost as susceptible as NILs without any resistance QTL. Differences between QTL subgroups were larger within the tall *Rht1* lines (Fig. 3, Table 1). QTL subgroups that had both QTL introgressed were usually significantly more resistant than lines containing solely *Fhb1* or no resistance QTL but were in most cases not different from NILs which contained only the *Qfhs.ifa-5A* resistance QTL (Fig S1). Semi-dwarfing NILs containing the *Qfhs.ifa-5A* QTL alone or in combination with *Fhb1* were consistently slightly more resistant than tall variants without the *Qfhs.ifa-5A* QTL.

Rank order of QTL subgroups within *Rht1* haplotypic groups of CM NILs was consistent, whereby CM NILs having both QTL combined performed best, second were *Fhb1*, closely followed by *Qfhs.ifa-5A* NILs, and lines without a resistance QTL were most susceptible (Figs. 3, S1, Table 1). Resistance to FHB incidence was significantly better in lines with both QTL combined compared to NILs without a resistance QTL, and NILs containing solely *Fhb1* performed equally or better than NILs with *Qfhs.ifa-5A* only or no QTL (Fig S1). AUDPC remained generally low and was only significantly different in *Rht-D1b* haplotypes between NILs with both resistance QTL and NILs with no resistance QTL.

Discussion

Effects of *Rht1* haplotypes on plant height, anther retention and FHB resistance.

The association between increased FHB susceptibility and shorter plant height has been observed many times, leaving little doubt about the causal connection between plant height and FHB resistance. However, plant height ‘per se’ may not be the only driving factor, since for many plant height QTL no effect on FHB resistance has been reported (Buerstmayr et al. 2020). FHB is a floral disease and flowering related traits such as the amount of retained or extruded anthers (Buerstmayr et al. 2020; Kubo et al. 2013; Steiner et al. 2019) and the duration and the width of the opening of the florets during anther extrusion have been associated with FHB resistance (Gilsinger et al. 2005). Several independent studies identified an overlap of the *Rht1* loci with QTL for anther extrusion and for FHB resistance (Buerstmayr and Buerstmayr 2016; He et al. 2016; Lu et al. 2013; Xu et al. 2020; Zhu et al. 2021). Reduced plant height in combination with increased AR is thus particularly disadvantageous for FHB resistance and may explain why the *Rht1* loci regularly coincided with QTL for FHB resistance, where the *Rht1* loci commonly showed the strongest effect among the identified FHB QTL (Buerstmayr et al. 2020). Our analysis revealed an increase in AR in semi-dwarfing lines of all NIL groups (Figs. 1, 2). Effect size of the semi-dwarfing alleles on AR, however, depended on the genetic background and was highest in C1, followed by E4 and CM, and was lowest in MI. We also observed a stronger increase in AR% in *Rht-D1b* compared to *Rht-B1b* lines within NIL group C1 (Figs. 1, 2). A genotype dependent effect of *Rht-B1* alleles on anther extrusion was also reported by Okada et al. (2019), but unlike our results, they reported that the *Rht-D1b* allele did not change anther extrusion.

The strongest increase in susceptibility was observed in the semi-dwarfing NILs of group C1 and E4, followed by MI. This rank order largely corresponds with the relative increase in AR% which was also

highest in C1, followed by E4 and MI. FHB susceptibility among CM NILs was largely unaffected by the introgression of dwarfing alleles despite significantly more retained anthers in the shorter NILs. The higher proportion of retained anthers in *Rht-D1b* compared to the *Rht-B1b* lines in group C1 is also reflected in a higher susceptibility of the *Rht-D1b* lines. Since C1 *Rht-D1b* and *Rht-B1b* NILs have approximately equal plant height, we assume that the difference in FHB susceptibility is directly related to the differences in AR%. This supports our earlier findings in a double haploid population, where the *Rht-D1b* allele had a significantly greater impact on AR and FHB severity than the *Rht-B1b* allele (Buerstmayr and Buerstmayr 2016).

We expect only a small (if any) effect of plant height on FHB resistance in our greenhouse experiments. Most external factors that may play a role in field tests and passively influence FHB resistance in a plant height dependent manner, for instance wind, morning or evening dew, or rain-splashed spores, can be excluded under controlled greenhouse conditions. The influence of soil humidity derived from greenhouse pots is limited compared to conditions in field tests. The observed changes in resistance through introgression of semi-dwarfing *Rht-B1b* or *Rht-D1b* alleles may thus, at least under greenhouse conditions, primarily reflect the changes in AR%. However, we cannot rule out additional pleiotropic effects. Saville et al. (2012) proposed that DELLA proteins have a pleiotropic effect on disease resistance by altering stress response and controlled cell death, with DELLA accumulating semi-dwarfing *Rht1* lines being more susceptible to initial infection (type 1 resistance) but more resistant to the later colonization phase (type 2 resistance).

Effects of *Rht1* alleles in combination with FHB resistance QTL *Fhb1* and *Qfhs.ifa-5A* on plant height, anther retention and FHB resistance

The allele status at the *Rht1* genes and at the *Qfhs.ifa-5A* QTL affected AR, whereby the *Qfhs.ifa-5A* resistance allele reduced AR to at least the same level as the semi-dwarfing alleles increased it (Figs. 3, S1, Table 1). The proportion of retained anthers had a large impact on FHB resistance, accordingly absence of the *Qfhs.ifa-5A* resistance as well as the presence of dwarfing alleles increased FHB susceptibility. Retained anthers as potential susceptibility factors were particularly relevant in C1 lines where AR% was generally higher than in CM lines, changes in FHB resistance due to changes in allele status at the *Rht1* or *Qfhs.ifa-5A* genes were higher in the C1 than in the CM NILs.

There is clear evidence of a negative effect of retained anthers on FHB resistance, whereby absence of anthers enhances resistance to initial infection but does not protect plants from fungal spreading within spikes (Steiner et al. 2019). FHB infection starts inside the floral cavities and anthers partially trapped between the palea and lemma are important entry points for the fungus into the florets (Kang and Buchenauer 2000). Once the spores have successfully entered the florets, the floral cavities provide conditions that are highly conducive for pathogen development. Spore germination and hyphal colonization were much more enhanced on anthers, pollen and stigma compared to the surrounding tissues of the lemma and palea and were the preferred tissue at the onset of FHB infection (Kang and Buchenauer 2000; Miller et al. 2004; Pugh 1933). Successful anther extrusion depends on a multitude of

genes that may regulate different aspects of the flowering process, for instance lodicule swelling, anther dehiscence, filament elongation, rigidity of the filaments, flower opening angle and flower opening duration, but it may also be influenced by spike architecture or the shape of the glumes. The complex nature of anther extrusion likely explains the observed background dependence of the *Rht1* genes and the *Qfhs.ifa-5A* genes on modulating AR.

Gene expression analysis revealed a stress response NAC SECONDARY WALL THICKENING PROMOTING FACTOR1 (*NST1*)-like protein as a potential candidate gene for *Qfhs.ifa-5AS* (Buerstmayr et al. 2021). Genetic experiments in the model plant *Arabidopsis thaliana* showed that *NST1* is key regulator of secondary cell wall biosynthesis in anther endothecium cells (Mitsuda et al. 2007; Mitsuda et al. 2005). *NST1* also induced ectopic secondary wall thickening in various tissues, including filaments of stamens (Mitsuda et al. 2005), however, it is unknown if this has an influence on anther extrusion.

Rht1 genes control cell elongation, one may thus expect shorter stamen filaments as demonstrated in *Arabidopsis* (Cheng et al. 2004) or changes in tissue structures due to more compact cell-types. However, in a detailed search for optimal pollinator traits no association between *Rht-D1* status and anther filament length was observed, but anther length was significantly affected (Okada et al. 2021).

Fhb1 had no influence on AR and had only a very small impact on FHB resistance in the susceptible C1 background, whereas *Fhb1* was at least as good as *Qfhs.ifa-5A* in improving in FHB incidence in the highly resistant CM background. CM NILs without any resistance QTL reached FHB incidence levels that were almost as high as those in the C1 NILs, however, the respective AUDPC scores did not raise to the same level. This suggests that the genetic background of CM confers, in addition to *Fhb1*, excellent type 2 resistance that is able to block the fungus from spreading within the spike even under high disease pressure.

Conclusion

The current study confirms earlier published results that semi-dwarfing alleles have a negative impact on FHB resistance. The data shed new light on the dependence of this association on the genetic background. Studying NILs with greatly different background resistance illustrates that a highly resistant line, such as CM-82036, retains a high resistance level even if semi-dwarf *Rht1* alleles are introduced. A highly susceptible line, such as Michael, is susceptible almost regardless of its *Rht1* status. Introducing semi-dwarfing alleles into a moderately resistant line, such as C1, simultaneously leads to a significant increase in anther retention and FHB susceptibility. The well-known QTL *Qfhs.ifa-5A* is capable of increasing FHB resistance and anther extrusion as efficiently as semi-dwarfing alleles (*Rht-D1b* or *Rht-B1b*) decrease these traits. Thus introducing *Qfhs.ifa-5A* into semi-dwarf breeding populations is highly recommended in order to lay a baseline for safeguarding FHB resistance in wheat. Adding *Fhb1* on top will frequently lead to an even better level of FHB resistance and is therefore recommended for wheat breeding where FHB resistance is considered a key trait.

Declarations

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Conflict of interest/Competing Interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Authors contributions statement

MB conducted and supervised development of NILs, greenhouse and field experiments, MB analyzed the data and wrote the manuscript. HB initiated and guided the study and obtained funding. All authors read and approved the final manuscript.

Availability of data and material

The plant material and datasets employed in this study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Ethical standard

The authors declare that the experiments conducted for this publication comply with the current laws of Austria.

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References

1. Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J, Harberd NP (2006) Integration of plant responses to environmentally activated phytohormonal signals. *Science* 311:91-94. <https://doi.org/10.1126/science.11118642>

2. Acuña T, Rebetzke G, Condon T, Richards R (2001) The effect of *rht* genotype and temperature on coleoptile growth and dry matter partitioning in young wheat seedlings. *Funct Plant Biol* 28:417-423. <https://doi.org/10.1071/PP01010>
3. Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. 2015 67:48. <https://doi.org/10.18637/jss.v067.i01>
4. Boeven PHG, Longin CFH, Leiser WL, Kollers S, Ebmeyer E, Wurschum T (2016) Genetic architecture of male floral traits required for hybrid wheat breeding. *Theor Appl Genet* 129:2343-2357. <https://doi.org/10.1007/s00122-016-2771-6>
5. Borlaug NE (1968) Wheat breeding and its impact on world food supply. In: Finlay EW, Sheperd KW (eds) 3rd International Wheat Genet Symp. Australian Academy of Science, Canberra, Australia, pp 5-15
6. Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). *Theor Appl Genet* 104:84-91. <https://doi.org/10.1007/s001220200009>
7. Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. *Theor Appl Genet* 107:503-508. <https://doi.org/10.1007/s00122-003-1272-6>
8. Buerstmayr H, Steiner B, Lemmens M, Ruckenbauer P (2000) Resistance to fusarium head blight in winter wheat: Heritability and trait associations. *Crop Science* 40:1012-1018
9. Buerstmayr M, Buerstmayr H (2015) Comparative mapping of quantitative trait loci for Fusarium head blight resistance and anther retention in the winter wheat population Capo × Arina. *Theor Appl Genet* 128:1519-1530. <https://doi.org/10.1007/s00122-015-2527-8>
10. Buerstmayr M, Buerstmayr H (2016) The Semidwarfing Alleles *Rht-D1b* and *Rht-B1b* Show Marked Differences in Their Associations with Anther-Retention in Wheat Heads and with Fusarium Head Blight Susceptibility. *Phytopathology* 106:1544-1552. <https://doi.org/10.1094/phyto-05-16-0200-r>
11. Buerstmayr M, Steiner B, Buerstmayr H (2020) Breeding for Fusarium head blight resistance in wheat —Progress and challenges. *Plant Breeding* 139:429-454. <https://doi.org/10.1111/pbr.12797>
12. Buerstmayr M, Wagner C, Nosenko T, Omony J, Steiner B, Nussbaumer T, Mayer KFX, Buerstmayr H (2021) Fusarium head blight resistance in European winter wheat: insights from genome-wide transcriptome analysis. *BMC Genomics* 22:470. <https://doi.org/10.1186/s12864-021-07800-1>
13. Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, Luo D, Harberd NP, Peng J (2004) Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. *Development* 131:1055-1064. <https://doi.org/10.1242/dev.00992>
14. Ellis M, Spielmeier W, Gale K, Rebetzke G, Richards R (2002) "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theor Appl Genet* 105:1038-1042. <https://doi.org/10.1007/s00122-002-1048-4>

15. Evans LT (1998) Feeding the ten billion: Plants and population growth. Cambridge: Cambridge University Press:133–150.
16. Flintham J, Börner A, Worland A, Gale M (1997) Optimizing wheat grain yield: Effects of *Rht* (gibberellin-insensitive) dwarfing genes. *The Journal of Agricultural Science* 128:11-25. <https://doi.org/10.1017/S0021859696003942>
17. Gilsinger J, Kong L, Shen X, Ohm H (2005) DNA markers associated with low *Fusarium* head blight incidence and narrow flower opening in wheat. *Theor Appl Genet* 110:1218-1225. <https://doi.org/10.1007/s00122-005-1953-4>
18. He X, Singh PK, Dreisigacker S, Singh S, Lillemo M, Duveiller E (2016) Dwarfing genes *Rht-B1b* and *Rht-D1b* are associated with both type I FHB susceptibility and low anther extrusion in two bread wheat populations. *PLoS One* 11:e0162499. <https://doi.org/10.1371/journal.pone.0162499>
19. Hedden P (2003) The genes of the Green Revolution. *Trends in Genetics* 19:5-9. [https://doi.org/10.1016/S0168-9525\(02\)00009-4](https://doi.org/10.1016/S0168-9525(02)00009-4)
20. Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom J* 50:346-363. <https://doi.org/10.1002/bimj.200810425>
21. Jenkinson P, Parry DW (1994) Splash dispersal of conidia of *Fusarium culmorum* and *Fusarium avenaceum*. *Mycological Research* 98:506-510. [https://doi.org/10.1016/S0953-7562\(09\)80468-1](https://doi.org/10.1016/S0953-7562(09)80468-1)
22. Jones S, Farooqi A, Foulkes J, Sparkes DL, Linforth R, Ray RV (2018) Canopy and Ear Traits Associated With Avoidance of *Fusarium* Head Blight in Wheat. *Frontiers in Plant Science* 9. <https://doi.org/10.3389/fpls.2018.01021>
23. Kang Z, Buchenauer H (2000) Cytology and ultrastructure of the infection of wheat spikes by *Fusarium culmorum*. *Mycological Research* 104:1083-1093. <https://doi.org/10.1017/S0953756200002495>
24. Kubo K, Fujita M, Kawada N, Nakajima T, Nakamura K, Maejima H, Ushiyama T, Hatta K, Matsunaka H (2013) Minor Differences in Anther Extrusion Affect Resistance to *Fusarium* Head Blight in Wheat. *J Phytopathol* 161:308-314. <https://doi.org/10.1111/jph.12060>
25. Kubo K, Kawada N, Fujita M, Hatta K, Oda S, Nakajima T (2010) Effect of cleistogamy on *Fusarium* head blight resistance in wheat. *Breeding Science* 60:405-411. <https://doi.org/10.1270/jsbbs.60.405>
26. Lu QX, Lillemo M, Skinnes H, He XY, Shi JR, Ji F, Dong YH, Bjornstad A (2013) Anther extrusion and plant height are associated with Type I resistance to *Fusarium* head blight in bread wheat line 'Shanghai-3/Catbird'. *Theor Appl Genet* 126:317-334. <https://doi.org/10.1007/s00122-012-1981-9>
27. McMullen M, Bergstrom G, De Wolf E, Dill-Macky R, Hershman D, Shaner G, Van Sanford D (2012) A unified effort to fight an enemy of wheat and barley: *Fusarium* Head Blight. *Plant Disease* 96:1712-1728. <https://doi.org/10.1094/pdis-03-12-0291-fe>
28. Miller SS, Chabot DMP, Ouellet T, Harris LJ, Fedak G (2004) Use of a *Fusarium graminearum* strain transformed with green fluorescent protein to study infection in wheat (*Triticum aestivum*). *Canadian Journal of Plant Pathology* 26:453-463. <https://doi.org/10.1080/07060660409507165>

29. Mitsuda N, Iwase A, Yamamoto H, Yoshida M, Seki M, Shinozaki K, Ohme-Takagi M (2007) NAC Transcription Factors, NST1 and NST3, Are Key Regulators of the Formation of Secondary Walls in Woody Tissues of Arabidopsis. *Plant Cell* 19:270-280. <https://doi.org/10.1105/tpc.106.047043>
30. Mitsuda N, Seki M, Shinozaki K, Ohme-Takagi M (2005) The NAC Transcription Factors NST1 and NST2 of Arabidopsis Regulate Secondary Wall Thickenings and Are Required for Anther Dehiscence. *Plant Cell* 17:2993-3006. <https://doi.org/10.1105/tpc.105.036004>
31. Muqaddasi QH, Pillen K, Plieske J, Ganai MW, Roder MS (2017) Genetic and physical mapping of anther extrusion in elite European winter wheat. *Plos One* 12:17. <https://doi.org/10.1371/journal.pone.0187744>
32. Okada T, Jayasinghe J, Eckermann P, Watson-Haigh NS, Warner P, Hendrikse Y, Baes M, Tucker EJ, Laga H, et al. (2019) Effects of *Rht-B1* and *Ppd-D1* loci on pollinator traits in wheat. *Theor Appl Genet* 132:1965-1979. <https://doi.org/10.1007/s00122-019-03329-w>
33. Okada T, Jayasinghe J, Eckermann P, Watson-Haigh NS, Warner P, Williams ME, Albertsen MC, Baumann U, Whitford R (2021) Genetic factors associated with favourable pollinator traits in the wheat cultivar Piko. *Funct Plant Biol* 48:434-447. <https://doi.org/10.1071/fp20181>
34. Parry DW, Jenkinson P, McLeod L (1995) Fusarium ear blight (scab) in small grain cereals—a review. *Plant Pathology* 44:207-238. <https://doi.org/10.1111/j.1365-3059.1995.tb02773.x>
35. Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP (1997) The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes Dev* 11:3194-3205. <https://doi.org/10.1101/gad.11.23.3194>
36. Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, et al. (1999) 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* 400:256-261. <https://doi.org/10.1038/22307>
37. Pestka J (2010) Toxicological mechanisms and potential health effects of deoxynivalenol and nivalenol. *World mycotoxin journal* 2010 v.3 no.4:pp. 323-347. <https://doi.org/10.3920/wmj2010.1247>
38. Pugh GW (1933) Factors Affecting Infection of Wheat Heads by *Gibberella saubinetii*. *J. Agric. Res.* 46:771-779.
39. R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>
40. Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganai MW (1998) A microsatellite map of wheat. *Genetics* 149:2007-2023. <https://doi.org/10.1093/genetics/149.4.2007>
41. Saville RJ, Gosman N, Burt CJ, Makepeace J, Steed A, Corbitt M, Chandler E, Brown JK, Boulton MI, et al. (2012) The 'Green Revolution' dwarfing genes play a role in disease resistance in *Triticum aestivum* and *Hordeum vulgare*. *J Exp Bot* 63:1271-1283. <https://doi.org/10.1093/jxb/err350>
42. Schweiger W, Steiner B, Ametz C, Siegwart G, Wiesenberger G, Berthiller F, Lemmens M, Jia HY, Adam G, et al. (2013) Transcriptomic characterization of two major Fusarium resistance quantitative trait

- loci (QTLs), *Fhb1* and *Qfhs.ifa-5A*, identifies novel candidate genes. Molecular Plant Pathology 14:772-785. <https://doi.org/10.1111/mpp.12048>
43. Schweiger W, Steiner B, Vautrin S, Nussbaumer T, Siegwart G, Zamini M, Jungreithmeier F, Gratl V, Lemmens M, et al. (2016) Suppressed recombination and unique candidate genes in the divergent haplotype encoding *Fhb1*, a major Fusarium head blight resistance locus in wheat. Theor Appl Genet 129:1607-1623. <https://doi.org/10.1007/s00122-016-2727-x>
 44. Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105-1114. <https://doi.org/10.1007/s00122-004-1740-7>
 45. Steiner B, Buerstmayr M, Michel S, Schweiger W, Lemmens M, Buerstmayr H (2017) Breeding strategies and advances in line selection for Fusarium head blight resistance in wheat. Tropical Plant Pathology 42:165-174. <https://doi.org/10.1007/s40858-017-0127-7>
 46. Steiner B, Buerstmayr M, Wagner C, Danler A, Eshonkulov B, Ehn M, Buerstmayr H (2019) Fine-mapping of the Fusarium head blight resistance QTL *Qfhs.ifa-5A* identifies two resistance QTL associated with anther extrusion. Theor Appl Genet 132:2039-2053. <https://doi.org/10.1007/s00122-019-03336-x>
 47. Steiner B, Lemmens M, Griesser M, Scholz U, Schondelmaier J, Buerstmayr H (2004) Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theor Appl Genet 109:215-224. <https://doi.org/10.1007/s00122-004-1620-1>
 48. Su Z, Jin S, Zhang D, Bai G (2018) Development and validation of diagnostic markers for *Fhb1* region, a major QTL for Fusarium head blight resistance in wheat. Theor Appl Genet 131:2371-2380. <https://doi.org/10.1007/s00122-018-3159-6>
 49. Van De Velde K, Thomas SG, Heyse F, Kaspar R, Van Der Straeten D, Rohde A (2021) N-terminal truncated *RHT-1* proteins generated by translational reinitiation cause semi-dwarfing of wheat Green Revolution alleles. Molecular Plant 14:679-687. <https://doi.org/10.1016/j.molp.2021.01.002>
 50. Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Froberg RC (1999) RFLP Mapping of QTL for Fusarium Head Blight Resistance in Wheat. Crop Science 39:crosci1999.0011183X003900030032x. <https://doi.org/10.2135/crosci1999.0011183X003900030032x>
 51. Würschum T, Langer SM, Longin CFH (2015) Genetic control of plant height in European winter wheat cultivars. Theor Appl Genet 128:865-874. <https://doi.org/10.1007/s00122-015-2476-2>
 52. Xu K, He X, Dreisigacker S, He Z, Singh PK (2020) Anther extrusion and its association with Fusarium head blight in CIMMYT wheat Germplasm. Agronomy-Basel 10. <https://doi.org/10.3390/agronomy10010047>
 53. Zhu H, Gilchrist L, Hayes P, Kleinhofs A, Kudrna D, Liu Z, Prom L, Steffenson B, Toojinda T, et al. (1999) Does function follow form? Principal QTLs for Fusarium head blight (FHB) resistance are coincident with QTLs for inflorescence traits and plant height in a doubled-haploid population of barley. Theor Appl Genet 99:1221-1232. <https://doi.org/10.1007/s001220051328>

54. Zhu Z, Xu X, Fu L, Wang F, Dong Y, Fang Z, Wang W, Chen Y, Gao C, et al. (2021) Molecular Mapping of Quantitative Trait Loci for Fusarium Head Blight Resistance in a Doubled Haploid Population of Chinese Bread Wheat. Plant Dis:Pdis06201186re. <https://doi.org/10.1094/pdis-06-20-1186-re>

Figures

Figure 1

Boxplots of near isogenic lines (NILs) grouped by *Rht1* allele status for the recurrent NIL groups C1, CM, E4 and MI for plant height, anther retention, infection sites per head, and area under the disease progress curve (AUDPC). Medians are indicated by solid bold lines and outliers by open circles. Comparisons are based on best linear unbiased estimators (BLUEs) across greenhouse trials. Bonferroni correction was used to adjust for multiple pairwise contrasts. Different letters below boxes indicate significant differences among NIL groups ($p < 0.05$), and bars between boxes indicate significant differences among

Rht1 haplotypes within NIL groups. Signif. codes: '***' $p < 0.001$; '**' $0.001 < p < 0.01$; '*' $0.01 < p < 0.05$. Interaction plots depict BLUEs and standard errors of *Rht1* haplotypes by NIL group interaction

Figure 2

Boxplots of near isogenic lines (NILs) grouped by *Rht1* allele status for the recurrent NIL groups C1, CM, E4 and MI for plant height, anther retention, FHB incidence, and area under the disease progress curve (AUDPC). Medians are indicated by solid bold lines and outliers by open circles. Comparisons are based on best linear unbiased estimators (BLUEs) across field trials. Bonferroni correction was used to adjust for multiple pairwise contrasts. Different letters below BLUEs indicate significant differences among NIL groups ($p < 0.05$), and bars between boxes indicate significant differences among *Rht1* haplotypes within NIL groups. Signif. codes: '***' $p < 0.001$; '**' $0.001 < p < 0.01$; '*' $0.01 < p < 0.05$. Interaction plots depict BLUEs and standard errors of *Rht1* haplotypes by NIL group interaction

Figure 3

Interaction plots depict best linear unbiased estimators (BLUES) and standard errors for plant height, anther retention, FHB incidence and area under the disease progress curve (AUDPC) for combinations of *Rht1* haplotypes and QTL within NIL group C1 (a) and CM (b)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [EMS1.pdf](#)
- [EMS2.pdf](#)