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

Maria Buerstmayr and Hermann Buerstmayr

University of Natural Resources and Life Sciences Vienna, Department of Agrobiotechnology, Institute of Biotechnology in Plant Production, Konrad Lorenz Str. 20, A-3430 Tulln, Austria.

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ABSTRACT

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The semidwarfing alleles reduced height (*Rht*)-*D1b* and *Rht*-*B1b* are widely deployed in wheat breeding. Both alleles have similar effects on plant height but differ in their effect on Fusarium head blight (FHB) severity. A double-haploid population and a backcross population, segregating for *Rht-B1a/Rht-B1b* and *Rht-D1a/Rht-D1b*, were evaluated for FHB severity, plant height, and anther retention in field trials in three consecutive years. The

semidwarfing alleles reduced plant height and increased the proportion of retained anthers. Reduced plant height and a high proportion of retained anthers were associated with increased FHB severity. The *Rht-D1b* allele had a significantly greater impact on anther retention and FHB severity than the *Rht-B1b* allele. *Fusarium graminearum* establishes infection sites predominantly inside the floral cavity and retained anthers potentially support colonization and initial hyphal growth, leading to a higher disease level in genotypes with a higher proportion of retained anthers. This is the first report demonstrating that differences in disease severity associated with *Rht-D1b* and *Rht-B1b* can be partly explained by their different effect on the extent of anther retention.

The utilization of dwarfing or reduced height (*Rht*) genes in cereal crops has been a major component of the Green Revolution (Hedden 2003). Semidwarf plants have short, strong stalks and are highly resistant to lodging. An altered harvest index toward a higher ratio of grain yield to the total plant biomass enabled the application of more intensive agronomic measures and resulted in substantial yield increases. *Rht-B1b* (formerly *Rht1*) and *Rht-D1b* (formerly *Rht2*) are the most common mutant dwarfing alleles among a series of homeologous *Rht-1* alleles, which are located on chromosomes 4BS and 4DS (Börner et al. 1996; Gale and Youssefian 1985). The Japanese genotype ‘Norin 10’ is the source of *Rht-B1b* and *Rht-D1b*, and Norman Borlaug successfully incorporated these Norin 10 semidwarf alleles into productive bread and durum wheat cultivars (Borlaug 1968). They are now present in >70% of current commercial wheat cultivars worldwide (Evans 1998). Plant height variation in European winter wheat cultivars is mainly controlled by *Rht-B1b* and *Rht-D1b* (Würschum et al. 2015). The *Rht-D1b* allele was found in more than 35% of the German cultivars and more than 75% of the cultivars in Denmark, the United Kingdom, and France, while *Rht-B1b* was identified in approximately 5% of the cultivars (Knopf et al. 2008; Würschum et al. 2015). More than 90% of modern U.S. wheat cultivars possess either *Rht-B1b* or *Rht-D1b*. Among U.S. soft winter wheat cultivars, *Rht-D1b* is more frequent whereas, among hard winter wheat cultivars, *Rht-B1b* is more frequent (Guedira et al. 2010). In a study comparing four series of near-isogenic lines, the semidwarf alleles have been associated with improved agronomic traits, including increased grain yield (Lanning et al. 2012).

Rht-B1 and *Rht-D1* are orthologs of the *Arabidopsis thaliana* *gibberellin-insensitive (gai)* gene (Peng et al. 1997). The mutant *gai* alleles *Rht-B1b* and *Rht-D1b* encode single-nucleotide polymorphism (SNP) mutations in the DELLA domain that create premature stop codons and are responsible for reduced sensitivity to the phytohormone gibberellin, leading to shorter plant height (Peng et al. 1999). A pleiotropic role of DELLA on abiotic and biotic stress response was found in *A. thaliana* (Achard et al. 2006, 2008; Navarro et al. 2008), barley, and wheat (Chapman et al. 2007; Moriconi et al. 2012; Saville et al. 2012). *Rht-B1b* and *Rht-D1b* have been repeatedly mentioned in the context of increased susceptibility to Fusarium head blight (FHB).

FHB, primarily caused by *Fusarium graminearum* and *F. culmorum*, affects small grains throughout the cereal-growing regions of the world (McMullen et al. 1997). FHB epidemics result in substantial yield and quality losses. The pathogen overwinters saprophytically on crop debris, which acts as a reservoir of inoculum (Sutton 1982). During anthesis, ascospores or conidia reach the flowering wheat heads through splash water or by wind dispersion (Bai and Shaner 1994; Sutton 1982). Plant height potentially influences the number of ascospores or conidia reaching the wheat heads and, therefore, the likelihood of successful entry into the florets. Differences in microclimate such as relative humidity, leaf wetness duration, and temperature at spike height also affect FHB development (Hilton et al. 1999; Yan et al. 2011). As such, plant height constitutes a passive resistance to both initial infection with *Fusarium* spp. and fungal development.

Several field experiments collected data on plant height and FHB severity and almost all of these studies detected a positive effect of increased plant height on FHB resistance (Buerstmayr et al. 2009; Hilton et al. 1999; Mesterhazy 1995). Colocalization of quantitative trait loci (QTL) for FHB severity with plant height QTL has been found in numerous mapping populations, though not all plant height QTL were simultaneously associated with FHB resistance (Buerstmayr et al. 2011, 2012a; Draeger et al. 2007; Gervais et al. 2003; Häberle et al. 2009; Lu et al. 2013; Paillard et al. 2004;

Corresponding author: M. Buerstmayr; E-mail address: maria.buerstmayr@boku.ac.at

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Schmolke et al. 2005; Srinivasachary et al. 2008, 2009; Steiner et al. 2004; Voss et al. 2008). Mao et al. (2010) projected published data for both traits onto a consensus map and found 5 of 27 consensus plant height QTL overlapping with FHB resistance QTL. The negative effect of the semidwarfing allele *Rht-D1b* on FHB resistance was first demonstrated by Draeger et al. (2007) and has been repeatedly confirmed in mapping populations segregating for the *Rht-D1a/Rht-D1b* locus (Holzapfel et al. 2008; Srinivasachary et al. 2008, 2009; Voss et al. 2008). The *Rht-D1* gene had by far the largest impact on FHB resistance even in populations segregating for the major FHB resistance QTL *Fhb1* and the 'Sumai-3'-derived resistance QTL on chromosome 5A (Lu et al. 2011). Studies with near-isogenic lines (NIL) showed that FHB severity was remarkably higher on *Rht-D1b* genotypes compared with the *Rht-B1b* genotypes (Miedaner and Voss 2008; Srinivasachary et al. 2009). Srinivasachary et al. (2009) screened the double-haploid (DH) population 'Soissons' × 'Orvantis' which segregated for *Rht-B1a/Rht-B1b* and *Rht-D1a/Rht-D1b*. They observed that the *Rht-D1b* allele strongly increased FHB severity, while the *Rht-B1b* allele had no effect on FHB severity. Association mapping in European soft winter wheat confirmed a main effect QTL at *Rht-D1*; the locus *Rht-B1* had no significant main effect but showed a significant epistatic interaction with marker *Xbarc147*, a marker locus which is closely linked to *Fhb1* (Miedaner et al. 2011).

The association of increased susceptibility in the presence of retained anthers was reported early (Dickson et al. 1921) and has since been verified in multiple studies (Buerstmayr and Buerstmayr 2015; Graham and Browne 2009; He et al. 2014; Kubo et al. 2013b; Lu et al. 2013; Pugh et al. 1933; Skinner et al. 2010; Tu 1953). Histological studies documented that *Fusarium* infection predominantly starts inside the floral cavity, forming hyphal networks on the inner surface of lemma, glume, and palea (Pugh et al. 1933; Zange et al. 2005). Partially retained anthers and pollen were found to enhance fungal growth and initial infection before spreading to other parts of the spikelet (Kang and Buchenauer 2000; Miller et al. 2004; Pugh et al. 1933). Strange et al. (1974) reported that anther extracts such as choline and betaine stimulated fungal growth. However, subsequent studies found that neither substance had an effect on fungal growth (Engle et al. 2004). Only a few studies on FHB resistance include anther retention (AR) as a morphological trait (Buerstmayr and Buerstmayr 2015; Lu et al. 2013; Skinner et al. 2010). In our previous study (Buerstmayr and Buerstmayr, 2015), two of the three identified QTL for AR overlapped with QTL for FHB severity; Lu et al. (2013) reported five QTL for anther extrusion and all of them coincided with QTL for FHB traits. Results of these studies indicate that most of the variability in anther extrusion and plant height appeared to be controlled by independent factors. Interestingly, the locus of semidwarfing gene *Rht-B1* was associated with both plant height and AR.

It remains to be demonstrated whether the effect on AR applies for *Rht-B1b* and *Rht-D1b* as well. For this purpose, two populations were screened, each segregating for both alleles, allowing a side-by-side comparison in different genetic backgrounds. This approach should allow for providing novel insights in possible differences in AR and the relation with FHB resistance due to different alleles at *Rht-B1* and *Rht-D1*.

MATERIALS AND METHODS

Plant material and field trials. Two different populations were used for this study: (i) a DH population of 177 DH lines from the cross of the semidwarf winter wheat 'Hermann' (*Rht-B1b*, *Rht-D1a*) and 'Skalmeje' (*Rht-B1a*, *Rht-D1b*) (abbreviated to H×S) was provided by Mike Taylor, Limagrain Seeds, Peine Rosenthal, Germany; and (ii) a backcross population, consisting of 79 BC₂F_{2.4} lines, was developed by using the tall and highly FHB-resistant breeding line 20812 (*Rht-B1a*, *Rht-D1a*) as recurrent parent and the semidwarf winter wheat Hermann (*Rht-B1b*, *Rht-D1a*) or 'Toras'

(*Rht-B1a*, *Rht-D1b*) as donor parents (abbreviated to H/T×20812). The tall breeding line 20812 was selected for high FHB resistance from the biparental cross Sumai-3 × 'Capo' at the authors' institute; it carries the *Fusarium* resistance QTL *Fhb1* and *Qfhs.ifa-5A*. The semidwarf winter wheat Hermann (breeder: Limagrain Europe-Research, Germany) and Toras (breeder: Syngenta Seeds, Germany) are moderately resistant and Skalmeje (breeder: KWS-Lochow, Germany) is moderately susceptible to FHB. Toras is a moderately FHB-resistant German wheat cultivar (ranked in class 2 on a scale of 1 [resistant] to 9 [susceptible]), despite the presence of the *Rht-D1b* dwarfing allele. Through marker-assisted back-crossing using foreground selection, we generated BC₂F₁ plants heterozygous at either *Rht-B1* (donor Hermann) or *Rht-D1* (donor Toras). BC₂F₁ plants were selfed and BC₂F₂ sister plants with contrasting homozygous allele status at *Rht-B1* or *Rht-D1* were selected using allele-specific DNA markers, according to Ellis et al. (2002). The selected BC₂F₂ plants were selfed and bulk propagated to the BC₂F_{2.4} for multienvironment testing. Among the backcross lines, 40 and 39 derived from the donor parents Hermann and Toras, respectively.

All lines of both populations, including the parents and several check genotypes, were evaluated for flowering date, plant height, AR, and FHB severity in field trials at IFA-Tulln (latitude 48°20'0" N, longitude 16°3'0" E, altitude 177 m) in the years 2010, 2011, and 2012. Experiments were arranged in a randomized complete block design with two blocks. Entries comprised double rows of 1 m in length and 17-cm row spacing. Sowing time was late autumn (October to November) in all years. The two blocks in each experiment were sown 2 to 3 weeks apart depending on the specific weather conditions, which delayed anthesis by 1 to 3 days for the later-sown blocks. Seed treatment, sowing density, and crop management were essentially the same as described by Buerstmayr et al. (2002).

***Fusarium* inoculation and trait assessments.** To promote FHB infection and disease development, artificial inoculation was performed in all experiments. A macroconidia suspension of the *F. culmorum* single-spore isolate IFA-91015, prepared as described by Buerstmayr et al. (2000), was used for inoculation. Conidia stock solutions were diluted with distilled water to a 500× concentration, and aliquots were stored at -80°C until use. At each inoculation day, the required amount of inoculum aliquots was thawed in lukewarm water and diluted using tap water to the desired macroconidia concentration of 2.5 × 10⁴ ml⁻¹. Prepared dilutions were immediately used for inoculation. Plots were individually inoculated twice, first when 50% of the heads within a plot were flowering and the second time 2 days later. Using a battery-driven backpack sprayer, inoculum at 100 ml m⁻² was sprayed onto the heads. Inoculations were carried out in the evenings, usually from 4:00 to 6:00 P.M. An automated mist-irrigation system triggered by leaf wetness measurement maintained humidity and kept plants wet during the first 20 h after inoculation. The percentage of infected spikelets per plot was visually estimated 10, 14, 18, 22, and 26 days after first inoculation (dai). Date of anthesis was recorded for each plot and converted into number of days counting from 1 May. AR was assessed on 20 florets of five randomly chosen heads 5 days after anthesis on all entries in 2 years: 2011 and 2012. The basal florets of four spikelets in the central part of each head were manually opened and characterized as retained as soon as one or more anthers remained within the floret or were trapped between lemma and palea. Plant height was measured in centimeters in each entry of all experiments.

Molecular genetic analysis. Total genomic DNA was isolated from young leaves of 10 pooled plants of each line and parent according to the protocol of Saghai-Marooof et al. (1984). For genotyping of plants during the back-crossing procedure for population H/T×20812 and all DH lines in the H×S population, the allele-specific SNP markers for *Rht-B1* and *Rht-D1* according to Ellis et al. (2002) were used.

Statistical analysis: Field data. FHB disease severity is given as percentage of infected spikelets at 26 dai (FHB%-26) and as area under the disease progress curve (AUDPC). AUDPC was calculated as described by Buerstmayr et al. (2000) and used as an integrated measure of intensity and progress of FHB disease severity from 10 to 26 dai. AR was converted to percentage of florets having at least one anther retained (AR%). Statistical analysis was carried out with R version 3.1.0 (R Development Core Team 2011). The phenotypic data were analyzed using the lme4 package (Bates et al. 2015) applying following model: $t_{ijk} = \mu + g_i + y_j + (gy)_{ij} + b_k(y_i) + e_{ijk}$, where t_{ijk} is the phenotypic observation for the i th genotype in the k th block of the j th year; μ is an intercept term, g and y , denote the effects of genotype and year, $b(y)$ the block within year, and gy the interaction effects between genotype and year; and e_{ijk} is the residual term. Variance components were determined by the restricted maximum likelihood method, assuming a random effects model. Heritability coefficients were estimated from variance components with the equation $H^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_{G \times Y} / y + \sigma^2_{E/yr})$, where σ^2_G is the genotypic variance, $\sigma^2_{G \times Y}$ the genotype-year interaction variance, σ^2_E the residual variance, y the number of years, and r the number of replications (Nyquist 1991). Pearson correlation coefficients between recorded traits in individual years and across years were calculated.

Effect of *Rht* genes. Differences in trait performance between the genotypic groups classified by their *Rht* allele combinations in homozygous conditions (*Rht-B1a/Rht-D1a*, *Rht-B1b/Rht-D1a*, *Rht-B1a/Rht-D1b*, and *Rht-B1b/Rht-D1b*) were compared using the Tukey's multiple range test at $P < 0.05$. Estimated effects of the *Rht* genes on the evaluated traits were calculated with R version 3.1.0 (R Development Core Team 2011) using the R/qtl package 1.28-19-12 (Broman et al. 2003). A multiple regression model was fitted including genotypic data of *Rht-B1* and *Rht-D1* in the model. The overall fit of the full model (including both *Rht* genes) against

the null model (no *Rht* gene) was tested by analysis of variance (ANOVA). The effect of the individual *Rht* genes were determined by comparing the full model and the model with the respective *Rht* gene omitted (drop one model). From the drop-one ANOVA table the log of the likelihood values and the associated explained phenotypic variances of the individual *Rht* genes were calculated. Additive allelic effects were calculated as the regression coefficient of the phenotypic values on the respective genotypic values.

RESULTS

Parents of the H×S population differed in their resistance level to FHB and in AR but were similar for the traits plant height and flowering date. Average performance of the parental lines and the populations' means and ranges and the variance components and heritability coefficients of the evaluated traits are summarized in Table 1. Hermann had approximately 35% lower FHB severity (37% infected spikelets at 26 dai, AUDPC 349 units) than Skalmjeje (55% infected spikelets at 26 dai, AUDPC 541 units) and kept markedly fewer anthers retained (26 AR% versus 62 AR%). The recurrent parent 20812 of the H/T×20812 population was highly resistant to FHB, with an AUDPC of only 29 units (3.1% infected spikelets at 26 dai), whereas the donor parents Hermann and Toras had approximately 10- and close to 20-fold, respectively, higher percent infected spikelets and AUDPC values. AR% was approximately the same for 20812 and Hermann (20 and 26 AR%, respectively) but was approximately twice as high for Toras and Skalmjeje (59 and 62 AR%, respectively). Parent 20812 was 6 cm taller and flowered 2 days earlier than each of the donor parents. The high resistance level of the recurrent parent 20812 is also reflected in the low FHB severity mean of the H/T×20812 population. The BC₂-derived population has regained an expected average 87.5% of the genome of the resistant recipient line 20812. Population

TABLE 1. Means of the parents, means and ranges for individual experiments and across years, variance component estimates of genotype ($\sigma^2_{\text{Genotype}}$), year (σ^2_{Year}), block within year ($\sigma^2_{\text{Block within Year}}$), genotype-year ($\sigma^2_{\text{Genotype} \times \text{Year}}$), and the residual effects (σ^2_{error}) as well as line-mean heritability coefficients (H^2) for percentage of Fusarium head blight (FHB)-infected spikelets on day 26 after inoculation (FHB%-26), area under the disease progress curve (AUDPC) plant height, anther retention (AR), and flowering date (days after 1 May) of population H×S and population H/T×20812

Populations ^x	FHB%-26	AUDPC	Plant height (cm) ^y	AR (%) ^z	Flowering date ^y
Parents					
20812	3.1	29	87	20	28
Toras	27.2	313	81	59	33
Hermann	36.9	349	81	26	33
Skalmjeje	55.4	541	82	62	34
H×S					
Mean (range)	48 (10–83)	469 (107–941)	80 (50–107)	46 (7–100)	33 (31–38)
2010 (range)	71 (17–98)	799 (212–1521)	82 (51–107)	...	39 (37–42)
2011 (range)	44 (6–95)	372 (45–1042)	80 (48–107)	47 (6–100)	30 (26–35)
2012 (range)	30 (2–77)	236 (15–802)	78 (50–105)	46 (7–93)	32 (28–36)
Variance ± SD					
$\sigma^2_{\text{Genotype} \times \text{Year}}$	111 ± 10.5	17076 ± 130.7	4.2 ± 2.0	8.5 ± 2.9	0.05 ± 0.2
$\sigma^2_{\text{Genotype}}$	314 ± 17.7	37589 ± 193.9	159.7 ± 12.3	366.3 ± 19.1	1.16 ± 1.1
$\sigma^2_{\text{Block within Year}}$	10 ± 3.2	1302 ± 36.1	0.8 ± 0.9	19.9 ± 4.5	0.74 ± 0.9
σ^2_{Year}	447 ± 21.6	85909 ± 293.1	4.4 ± 2.1	0 ± 0.0	23.54 ± 4.9
σ^2_{error}	105 ± 10.3	10249 ± 101.2	10.2 ± 3.2	214.1 ± 14.6	0.89 ± 0.9
H^2	0.86	0.86	0.98	0.86	0.87
H/T×20812					
Mean (range)	8.9 (1.4–21.2)	101 (10–254)	86.0 (74–103)	56 (15–91)	30 (27–36)
2010 (range)	12.5 (1.8–30.0)	188 (14–477)	88.5 (77–107)	...	36 (33–42)
2011 (range)	8.0 (0.1–20.0)	67 (17–196)	86.9 (72–102)	54 (11–94)	26 (23–32)
2012 (range)	6.2 (0.1–22.5)	47 (2–180)	81.2 (70–100)	62 (27–92)	29 (25–36)
Variance ± SD					
$\sigma^2_{\text{Genotype} \times \text{Year}}$	5.1 ± 2.3	2442 ± 49.4	3.7 ± 1.9	84.7 ± 9.2	0.1 ± 0.3
$\sigma^2_{\text{Genotype}}$	20.9 ± 4.6	2884 ± 53.7	47.0 ± 6.8	243.5 ± 15.6	2.9 ± 1.7
$\sigma^2_{\text{Block within Year}}$	1.7 ± 1.3	185 ± 13.6	5.4 ± 2.3	0.3 ± 0.5	1.1 ± 1.0
σ^2_{Year}	9.8 ± 3.1	5636 ± 75.1	11.7 ± 3.4	29.9 ± 5.5	27.1 ± 5.2
σ^2_{error}	8.7 ± 2.9	1382 ± 37.2	10.2 ± 3.2	191.2 ± 13.8	0.9 ± 0.9
H^2	0.87	0.73	0.94	0.73	0.94

^x H×S = 177 genotypes and H/T×20812 = 79 genotypes. Variances = variance ± standard deviations (SD) and heritability coefficients.

^y For 3 years, two blocks within year.

^z For 2 years, two blocks within year.

H/T×20812 had a mean height of 86 cm, kept (on average) 56% of the anthers retained, and had a mean FHB severity of AUDPC 101 units (8.9% infected spikelets at 26 dai). The H×S population had a mean plant height of 80 cm, kept (on average) 46% of the anthers retained, and had a mean FHB severity of AUDPC 469 units (48% infected spikelets at 26 dai).

Trait variation and trait correlations. All experiments showed continuous distributions for all evaluated traits. FHB severity, AR%, and flowering date were approximately normally distributed. Distribution of lines according to their plant height showed three peaks in population H×S and was bimodal in population H/T×20812 (Fig. 1; Supplementary Fig. S1). This corresponds to the frequency of the genotypes having combinations of either none, one, or both mutant forms of the *Rht-B1* and *Rht-D1* gene. Disease level differed among years and was highest in 2010 and lowest in 2012 for both populations. Correlation coefficients between years were highly significant for all traits and ranged from $r = 0.65$ to 0.73 for both AUDPC and FHB%-26 and from $r = 0.67$ to 0.74 for anthesis date, and were $r = 0.95$ for plant height and $r = 0.75$ for AR% in population H×S. Correlations coefficients between years for population H×S ranged from $r = 0.69$ to 0.75 for AUDPC and for FHB%-26, from $r = 0.84$ to 0.87 for plant height, and from $r = 0.81$ to 0.91 for anthesis date, and was $r = 0.61$ for AR in population H/T×20812. As expected, AUDPC and FHB%-26 were highly

correlated ($r = 0.96$ and 0.98 in population H×S and H/T×20812, respectively). Pearson correlation coefficients between traits for individual years and across years are provided as Supplementary Table S1. Correlations of FHB severity to AR% were highly significant and similar among years and populations ($r = 0.64$ to 0.70), whereas correlations of FHB severity to plant height were more variable and ranged from $r = -0.51$ to -0.74 in population H×S and from $r = -0.33$ to -0.69 in population H/T×20812. Taller plants were significantly less diseased and had fewer anthers retained. A high number of retained anthers increased FHB severity (Fig. 1). The association between FHB severity, plant height, and AR% is visualized in Supplementary Fig. S2. When looking at plants having approximately the same plant height, those with higher AR% were more diseased; and, the opposite, considering plants having approximately the same amount of AR%, the taller plants were less diseased. Correlation between FHB severity and flowering date varied and was significant in 2011 for population H×S and in 2010 and 2011 for population H/T×20812. Flowering date had no effect on plant height and AR%. Genotypes were a main source of variance for all traits (Table 1). Generally, $\sigma^2_{\text{Genotype} \times \text{Year}}$ were smaller than $\sigma^2_{\text{Genotype}}$. Variance component $\sigma^2_{\text{Genotype} \times \text{Year}}$ for FHB severity (AUDPC and FHB%-26) was strong in both populations and for AR% in population H/T×20812. Heritability coefficients were highest for plant height ($H^2 = 0.98$) in population H×S, and lowest

Fig. 1. Scatterplots with marginal histograms for overall means of Fusarium head blight (FHB) severity to plant height and anther retention and for anther retention to plant height of **A**, population H×S and **B**, population H/T×20814. Allele status at reduced height (*Rht*)-1 genes of individual genotypes is indicated by different symbols.

for FHB severity (AUPDC) and AR% ($H^2 = 0.73$) in population H/T×20812.

***Rht* genes and their association to FHB severity, AR, and plant height.** Lines of population H×S were subdivided according their *Rht* genotypes into four subgroups comprising double mutants (*Rht-B1b/Rht-D1b*), double wild types (*Rht-B1a/Rht-D1a*), and combinations thereof (*Rht-B1a/Rht-D1b* or *Rht-B1b/Rht-D1a*). For the H/T×20812 population, the grouping was similar but without the double mutant group. Overall means of individual groups and results of pairwise comparisons using Tukey's multiple range test are summarized in Table 2 and boxplots illustrate their distributions (Fig. 2; Supplementary Fig. S3). The ranking of the subgroups was constant in all experiments, with *Rht-B1a/Rht-D1a* < *Rht-B1b/Rht-D1a* < *Rht-B1a/Rht-D1b* < *Rht-B1b/Rht-D1b* for FHB disease symptom severity and AR%. Differences in the mean performance of the subgroups were significant in most though not all experiments. The double wild-type genotypes *Rht-B1a* and *Rht-D1a* had both the lowest disease severity and the lowest number of retained anthers, while double mutant genotypes *Rht-B1b* and *Rht-D1b* had the highest values. The mutant allele *Rht-D1b* had a significantly larger negative impact on disease level than the mutant allele *Rht-B1b* in all experiments of H/T×20812. Similar results were obtained in the H×S population, although differences were significant in 2011 and for means across experiments only. Amount of retained anthers was higher for the *Rht-D1b* genotypes compared with the *Rht-B1b* genotypes. These differences were significant in all experiments of population H×S and in the 2011 experiment of population H/T×20812. The subgroups having either the dwarfing genotype *Rht-B1b* or *Rht-D1b* had approximately the same plant height and were significantly shorter than the subgroup without dwarfing genes while, at the same time, they were significantly taller than the double dwarfs. The composition of the *Rht* genes had no influence on flowering date.

Multiple regression analysis of the genotypic value on the number of wild-type alleles showed equally strong effects of both

Rht genes on plant height (Table 3). Substituting either of the tall alleles by its dwarf counterpart reduced plant height, on average, by 9 cm in the H×S population and by 6 cm in the H/T×20812 population. Both genes together explained close to 90% of the phenotypic variance in the H×S population and more than 75% of the phenotypic variance in the H/T×20812 population. In population H×S, the *Rht-B1b* allele led to an increase of FHB severity by 75 AUDPC units (equivalent to 6.8% infected spikelets 26 dai) while the *Rht-D1b* allele increased FHB severity by 122 AUDPC units (10.5% infected spikelets). Their effects were considerably lower in the highly resistant genetic background of population H/T×20812, with an increase in FHB severity of 14 AUDPC units (1.6% infected spikelets) due to *Rht-B1b* and 58 AUDPC units (4.6% infected spikelets) due to *Rht-D1b*. The total phenotypic variance explained across experiments was 41% in population H×S and 58% in population H/T×20812. Multiple regression analysis of AR% on the number of *Rht-B1b* and *Rht-D1b* alleles calculated an increase of 6 and 12%, respectively, in population H×S and an increase of 10 and 14%, respectively, in population H/T×20812, with a total explained phenotypic variance close to 40 and 50% in population H×S and H/T×20814, respectively.

DISCUSSION

Several morphological traits have been found to be associated with FHB, among which plant height and anther extrusion are the most important ones (Buerstmayr et al. 2012b). Both populations used in the current study showed continuous variations of FHB severity and AR, whereas the variation of plant height had either two or three peaks, reflecting the composition of *Rht* alleles present in the individual genotypes (Fig. 1). The semidwarfing mutant alleles *Rht-B1b* and *Rht-D1b* were equally effective in reducing plant height and acted additively when combined (Fig. 2; Tables 2 and 3). *Rht-B1b* and *Rht-D1b* mutations contain nucleotide substitutions that create a premature stop codon (Peng et al. 1999). These mutant

TABLE 2. Means of reduced height (*Rht*) classes and Tukey's multiple range tests for Fusarium head blight (FHB) severity, anther retention (AR), plant height, and flowering date of individual years and across years^x

Trait ^y <i>Rht</i> genotype	Population H×S					Population H/T×20812				
	N ^z	Across years	2010	2011	2012	N ^z	Across years	2010	2011	2012
FHB severity										
FHB%-26										
<i>B1a/D1a</i>	48	32.9 a	57.3 a	23.8 a	17.6 a	38	5.8 a	9.8 a	4.3 a	3.4 a
<i>B1b/D1a</i>	46	46.6 b	70.0 b	41.4 b	28.6 b	22	8.9 b	10.8 a	9.0 b	7.0 b
<i>B1a/D1b</i>	50	53.7 b	76.0 b	55.1 c	30.2 b	19	15.1 c	20.0 b	14.4 c	10.8 c
<i>B1b/D1b</i>	31	67.3 c	90.3 c	61.0 c	50.8 c	...	...	...	...	...
AUDPC										
<i>B1a/D1a</i>	48	312 a	610 a	199 a	127 a	38	65 a	139 a	35 a	22 a
<i>B1b/D1a</i>	46	422 b	730 ab	329 b	208 b	22	94 b	156 a	67 b	58 b
<i>B1a/D1b</i>	50	518 c	844 b	478 c	232 b	19	180 c	322 b	132 c	86 c
<i>B1b/D1b</i>	31	715 d	1,141 c	544 c	459 c	...	...	...	...	...
AR (%)										
<i>B1a/D1a</i>	48	29 a	...	30 a	30 a	38	45 a	...	39 a	51 a
<i>B1b/D1a</i>	46	41 b	...	42 b	41 b	22	66 b	...	60 b	72 b
<i>B1a/D1b</i>	50	54 c	...	55 c	54 c	19	74 b	...	76 c	72 b
<i>B1b/D1b</i>	31	66 d	...	66 d	66 d	...	...	...	...	...
Plant height (cm)										
<i>B1a/D1a</i>	48	94 a	96 a	95 a	90 a	38	92 a	94 a	94 a	87 a
<i>B1b/D1a</i>	46	81 b	82 b	82 b	79 b	22	80 b	84 b	82 b	77 b
<i>B1a/D1b</i>	50	79 b	82 b	79 c	77 b	19	79 b	83 b	78 c	76 b
<i>B1b/D1b</i>	31	58 c	59 c	57 d	58 c	...	...	...	...	...
Flowering date										
<i>B1a/D1a</i>	48	33 a	39 a	30 a	32 a	38	30 ab	36 ab	25 ab	28 ab
<i>B1b/D1a</i>	46	33 a	39 a	30 a	32 a	22	29 b	35 b	25 b	27 b
<i>B1a/D1b</i>	50	34 a	39 a	30 a	32 a	19	31 a	36 a	26 a	29 a
<i>B1b/D1b</i>	31	33 a	39 a	30 a	32 a	...	...	...	...	...

^x Classes are defined by combinations of *Rht* genotypes at loci *Rht-B1* and *Rht-D1*. Means of groups with different letters are significantly different at $P < 0.05$.

^y FHB%-26 = percentage of infected spikelets at 26 days after first inoculation, AUDPC = area under the disease progress curve, and Flowering date = days after 1 May.

^z Number of lines per genotypic group.

alleles result in reduced plant height, reduced response to gibberellin, and increased in planta gibberellin levels (Gale and Marshall 1973; Webb et al. 1998). Their genetic similarity presumably explains their similar effects on plant height (Gale and Youssefian 1985; Peng et al. 1999). The moderately susceptible population HxS as well as the highly *Fusarium* spp.-resistant population H/Tx20812 showed significant associations between disease level and plant height in each experiment. A negative correlation between plant height and increased FHB severity is a frequently observed phenomenon, either in segregating populations or in collections of diverse wheat genotypes, when evaluated under field conditions (Buerstmayr et al.

2009; Mesterhazy 1995). Yan et al. (2011) studied pairs of NIL for nine different *Rht* genes. When tested under natural plant height, all of the tall isolines showed higher resistance than their dwarf counterparts. The negative effect of most dwarf alleles disappeared when the dwarf isolines were physically raised so that their spikes were at the same height as those of their respective tall isolines. This strongly supports the theory that the observed association may be caused by direct and indirect effects of height differences per se, leading to a higher chance of disease escape due to distance of the heads from the soil surface in combination with a less favorable microclimate for disease development for taller genotypes.

Fig. 2. Boxplot of genotypes grouped by their reduced height (*Rht*) combination (*Rht-B1a/Rht-D1a*, *Rht-B1b/Rht-D1a*, *Rht-B1a/Rht-D1b*, and *Rht-B1b/Rht-D1b*) for Fusarium head blight (FHB) severity, anther retention, and plant height in **A**, population HxS and **B**, population H/Tx20814. Medians are indicated by solid bold lines. Means of groups with different letters are significantly different at $P < 0.05$ using Tukey's multiple range test.

TABLE 3. Individual and combined estimates for reduced height (*Rht*)-*B1* and *Rht*-*D1* determined by multiple regression analysis for Fusarium head blight (FHB) severity (area under the disease progress curve [AUDPC] and percentage of FHB-infected spikelets on day 26 after inoculation [FHB%-26]), plant height, and anther retention (AR) in populations HxS and H/Tx20812^z

Trait, experiment	Population HxS							Population H/Tx20812						
	<i>Rht-B1</i>			<i>Rht-D1</i>			Total	<i>Rht-B1</i>			<i>Rht-D1</i>			Total
	LOD	PV%	Add	LOD	PV%	Add	PV%	LOD	PV%	Add	LOD	PV%	Add	PV%
AUDPC														
Mean	7.3	12.4	74.7	17.0	33.1	122.5	41.4	1.8	4.6	14.1	14.8	57.5	57.5	58.0
2010	4.9	9.6	100.0	11.0	23.5	156.7	30.2	0.1	0.5	8.2	8.8	39.1	92.1	41.8
2011	2.5	4.7	51.3	13.3	28.8	127.1	31.3	5.6	10.5	16.3	22.1	72.4	48.6	72.5
2012	15.1	24.2	73.8	18.9	31.9	84.0	50.4	5.4	17.7	17.9	12.2	49.4	32.2	52.4
FHB%-26														
Mean	7.1	12.7	6.8	14.9	29.6	10.5	38.5	3.4	8.6	1.6	16.1	60.8	4.6	60.8
2010	4.3	9.0	6.8	8.5	18.8	9.8	25.2	0.1	0.5	0.5	8.6	38.4	5.1	41.2
2011	3.0	5.7	6.2	12.0	26.2	13.3	29.5	8.2	18.5	2.4	20.3	68.4	5.1	69.9
2012	12.0	21.4	7.7	14.1	25.8	8.4	42.2	3.7	12.7	1.8	18.9	46.2	3.7	47.6
Plant height (cm)														
Mean	64.2	45.8	-8.3	70.4	55.7	-9.0	89.5	20.4	53.0	-5.8	20.5	53.4	-6.1	76.8
2010	62.5	49.7	-9.1	63.2	50.7	-9.2	88.1	16.2	52.9	-5.8	12.9	37.8	-5.1	66.4
2011	74.6	43.6	-8.6	86.4	62.1	-10.3	92.7	23.8	46.3	-6.4	29.4	69.8	-8.3	84.7
2012	53.1	43.9	-7.2	59.0	53.5	-7.9	85.6	12.1	43.6	-5.2	10.3	34.9	-4.9	57.5
AR (%)														
Mean	5.4	8.8	6.1	18.9	37.2	12.5	42.4	6.8	24.6	10.4	10.1	41.1	14.3	49.1
2011	3.7	6.7	5.9	14.1	29.8	12.5	33.8	4.0	14.7	10.5	9.2	40.4	18.4	43.1
2012	5.2	8.8	6.3	17.8	35.5	12.6	38.9	6.4	28.1	10.6	5.6	23.9	10.2	38.4

^z LOD = log of the likelihood ratio, PV% = percentage of explained phenotypic variance, and Add = additive effects, positive values denote that the semidwarfing allele increases trait values relative to its respective wild-type allele.

***Rht-B1b* and *Rht-D1b* and their different effect on FHB severity.** When considering the two semidwarfing genes independently, the effect of the *Rht-D1b* allele on FHB severity was always higher than that of the *Rht-B1b* allele, and a combination of both was most detrimental (Tables 2 and 3; Fig. 2). The effect of the *Rht-D1* locus was significant in 2 of 3 years in population H×S as well as in population H/T×20812 (Table 3). These results suggest that *Rht-1* genes affect FHB resistance even in highly resistant material. Comparing the two populations clearly shows that resistance level at *Rht-1* loci highly depends on genetic background. Looking at the performance of Skalmeye and Toras demonstrates that cultivars possessing the same *Rht* gene (here, the *Rht-D1b* allele) can markedly differ in their resistance level (Table 1). Association mapping for FHB resistance among European soft winter wheat revealed a significant main effect at *Rht-D1* and an epistatic effect for *Rht-B1* (Miedaner et al. 2011). Liu et al. (2013) reported negative pleiotropic effects of *Rht-B1b* and *Rht-D1b* on *Fusarium* resistance. Similarly, Miedaner and Voss (2008) screened NIL differing for several *Rht* genotypes and found that both *Rht-B1b* and *Rht-D1b* increased FHB severity but only *Rht-D1b* led to a significantly higher FHB severity. Congruently, in the mapping population Soissons × Orvantis segregating for both dwarfing genes, only the *Rht-D1* locus showed a strong association with FHB severity, though each gene had approximately the same plant height reducing effect (Srinivasachary et al. 2009). Because plant height of *Rht-B1b* and *Rht-D1b* genotypes are approximately the same, factors other than plant height per se must cause this gene-specific difference in FHB severity.

***Rht-B1b* and *Rht-D1b* and their effect on AR.** Screening the population for AR showed clear differences in the number of retained anthers according to their *Rht* genotype (Tables 2 and 3; Fig. 2). Analogous to FHB severity, *Rht-D1b* had a stronger impact on AR% than *Rht-B1b*, and double dwarfs had the highest number of retained anthers. The H×S population constantly showed significant differences among all four genotypic groups. Similarly, semidwarfing alleles increased the number of retained anthers in the H/T×20812 population, though the differences in AR between *Rht-D1b* and *Rht-B1b* genotypes were less pronounced and not always significant (Table 2; Fig. 2). AR is a complex quantitative trait (Buerstmayr and Buerstmayr 2015; Lu et al. 2013) and the current results suggest that AR, like many quantitative traits, is influenced by environment and genetic background. The mean AR % of population H/T×20812 was surprisingly high, considering that the parental lines 20812 and Hermann had a low AR% and only Toras showed a high number of retained anthers. Lines of the genotypic classes *Rht-B1b* and *Rht-D1a* had markedly more anthers retained than Hermann (donor of *Rht-B1b* allele for population H×S and H/T×20812). The genetic background of Hermann apparently promotes anther extrusion. Although plant height and flowering date were routinely included when screening for FHB severity under field conditions, few studies report results concerning AR or anther extrusion. All studies that investigated the association between the extent of anther extrusion and FHB resistance observed an increase of FHB severity in lines with increased proportion of retained anthers (Buerstmayr and Buerstmayr 2015; Dickson et al. 1921; Graham and Browne 2009; He et al. 2014; Kubo et al. 2013a, b; Lu et al. 2013; Skinner et al. 2010; Tu 1953). Colonization of spikes often starts on anthers retained within the floret. Possibly the tissue is more easily colonized and, as such, serves as a first reservoir for subsequent colonization of surrounding tissue (Kang and Buchenauer 2000; Miller et al. 2004; Pugh et al. 1933). Thus, the observed difference on FHB severity between *Rht-B1b* and *Rht-D1b* may be partially caused by their different proportion on florets with retained anthers. Among a number of publications examining FHB severity in populations segregating for *Rht-D1* or *Rht-B1*, only one included anther extrusion. Lu et al. (2013) investigated a recombinant inbred line population segregating at the *Rht-B1* locus. QTL analysis identified five QTL for anther extrusion and seven QTL for plant height. These included the *Rht-B1* locus, which had a

strong impact on plant height and anther extrusion and coincided with a major QTL for FHB severity after spray and grain spawn inoculation. All QTL for anther extrusion and five of the seven plant height QTL overlapped with QTL for FHB severity, whereby low anther extrusion and reduced height were associated with increased susceptibility. Results highlight both anther extrusion and plant height as relevant morphological factors, which need to be taken into account when breeding for FHB resistance. Apart from *Rht-B1*, most other QTL for anther extrusion and plant height mapped to different genomic regions, indicating that variability in anther extrusion and plant height may be controlled by *Rht* genes and by further height-independent genetic factors (Lu et al. 2013). Buerstmayr and Buerstmayr (2015) evaluated a mapping population descending from the cross Capo × 'Arina'; none of the parents possesses a major semidwarf allele. They found a highly significant correlation between AR% and plant height, although no coinciding QTL were identified, supporting the independent genetic control of AR% and plant height. Investigating population H×S and H/T×20812 for plant height and AR% simultaneously showed that lines of approximately the same plant height are more vulnerable when more anthers are retained. Likewise, lines having approximately the same AR% were more diseased for the shorter genotypes, indicating, that AR as well as plant height influence FHB severity. As a general feature, the *Rht-1* dwarfing genes reduce plant height by decreasing length of vegetative cells, while cell number is not affected (Miralles et al. 1998). Therefore, spikes will be more compact and glumes stiffer. A shorter spike phenotype may influence the microclimate surrounding spikes and contribute to increased FHB susceptibility. Cheng et al. (2004) reported an influence of the DELLA proteins on floral development. They found that gibberellin regulates cell elongation rather than cell division during *Arabidopsis* stamen filament elongation, leading to shorter filaments. Reduced length of filaments and stiffer glumes presumably facilitate AR. Thus, it might be a special property of the gibberellin-insensitive *Rht-1* semidwarfing alleles that they confer low anther extrusion and reduced plant height at the same time. Although *Rht-B1b* and *Rht-D1b* lead to approximately the same magnitude of plant height reduction, they differ markedly in their effect on AR% and FHB severity. One may hypothesize that the higher FHB disease-promoting effect of *Rht-D1b* compared with *Rht-B1b* might be due to its effect on increasing AR and, thus, indirectly promotes FHB infection conditions on the heads of *Rht-D1b*-carrying plants. Whether this effect is due to pleiotropic effects of the different *Rht* alleles themselves or to nearby genes that are in very high linkage disequilibrium with the *Rht* genes remains to be clarified. In light of these results, we suggest that the semidwarf allele *Rht-B1b* should be preferred in wheat breeding over *Rht-D1b*, whenever FHB resistance is an important trait. Still, when backcrossing semidwarf alleles into tall genotypes with a very high level of resistance such as breeding line 20812, semidwarf progeny with very good FHB resistance can be obtained when using *Rht-B1b* and lines with moderate to good FHB resistance when using *Rht-D1b*. Thus, if breeders want to use the *Rht-D1b* semidwarf allele in breeding, its negative effect should be counteracted by utilizing positive QTL alleles at other FHB resistance loci in order to obtain (moderately) resistant cultivars. In other words, it is feasible to select FHB-resistant semidwarf lines with either *Rht-B1b* or *Rht-D1b* but it is much easier with *Rht-B1b*.

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LITERATURE CITED

Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van der Straeten, D., Peng, J., and Harberd, N. P. 2006. Integration of plant responses to environmentally activated phytohormonal signals. *Science* 311:91-94.

- Achard, P., Renou, J. P., Berthome, R., Harberd, N. P., and Genschik, P. 2008. Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. *Curr. Biol.* 18:656-660.
- Bai, G., and Shaner, G. 1994. Scab of wheat: Prospects for control. *Plant Dis.* 78:760-765.
- Bates, D., Maechler, M., Bolker, B., and Walker, S. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67:1-48.
- Borlaug, N. E. 1968. Wheat breeding and its impact on world food supply. Pages 5-15 in: *Proc. 3rd Int. Wheat Genet. Symp.* E. W. Finlay and K. W. Sheperd, eds. Australian Academy of Science, Canberra, Australia.
- Börner, A., Plaschke, J., Korzun, V., and Worland, A. J. 1996. The relationships between the dwarfing genes of wheat and rye. *Euphytica* 89:69-75.
- Broman, K. W., Wu, H., Sen, S., and Churchill, G. A. 2003. R/qtl: QTL mapping in experimental crosses. *Bioinformatics* 19:889-890.
- Buerstmayr, H., Adam, G., and Lemmens, M. 2012b. Resistance to head blight caused by *Fusarium spp.* in wheat. Pages 236-276 in: *Disease Resistance in Wheat*. I. Sharma, ed. CABI, Wallingford, UK.
- Buerstmayr, H., Ban, T., and Anderson, J. 2009. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: A review. *Plant Breed.* 128:1-26.
- Buerstmayr, H., and Buerstmayr, M. 2015. Comparative mapping of quantitative trait loci for Fusarium head blight resistance and anther retention in the winter wheat population Capo $\times$ Arina. *Theor. Appl. Genet.* 128:1519-1530.
- Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, L., Steiner, B., Stierschneider, M., and Ruckebauer, 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.
- Buerstmayr, H., Steiner, B., Lemmens, M., and Ruckebauer, P. 2000. Resistance to fusarium head blight in winter wheat: Heritability and trait associations. *Crop Sci.* 40:1012-1018.
- Buerstmayr, M., Huber, K., Heckmann, J., Steiner, B., Nelson, J. C., and Buerstmayr, H. 2012a. Mapping of QTL for Fusarium head blight resistance and morphological and developmental traits in three backcross populations derived from *Triticum dicoccum $\times$ *Triticum durum*. *Theor. Appl. Genet.* 125:1751-1765.*
- Buerstmayr, M., Lemmens, M., Steiner, B., and Buerstmayr, H. 2011. Advanced backcross QTL mapping of resistance to Fusarium head blight and plant morphological traits in a *Triticum macha $\times$ *T. aestivum* population. *Theor. Appl. Genet.* 123:293-306.*
- Chapman, S. C., Mathews, K. L., Trethowan, R. M., and Singh, R. P. 2007. Relationships between height and yield in near-isogenic spring wheats that contrast for major reduced height genes. *Euphytica* 157:391-397.
- Cheng, H., Qin, L., Lee, S., Fu, X., Richards, D. E., Cao, D., Luo, D., Harberd, N. P., and Peng, J. 2004. Gibberellin regulates *Arabidopsis* floral development via suppression of DELLA protein function. *Development* 131:1055-1064.
- Dickson, J. G., Johann, H., and Wineland, G. 1921. Second progress report on the Fusarium blight (scab) of wheat. (Abstr.) *Phytopathology* 11:35.
- Draeger, R., Gosman, N., Steed, A., Chandler, E., Thomsett, M., Srinivasachary, Schondelmaier, J., Buerstmayr, H., Lemmens, M., Schmolke, M., Mesterhazy, A., and Nicholson, P. 2007. Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. *Theor. Appl. Genet.* 115:617-625.
- Ellis, M., Spielmeier, W., Gale, K., Rebetzke, G., and Richards, R. 2002. "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theor. Appl. Genet.* 105:1038-1042.
- Engle, J. S., Lipps, P. E., Graham, T. L., and Boehm, M. J. 2004. Effects of choline, betaine, and wheat floral extracts on growth of *Fusarium graminearum*. *Plant Dis.* 88:175-180.
- Evans, L. T. 1998. Feeding the Ten Billion: Plant and Population Growth. Cambridge University Press. Paperback, Cambridge, UK.
- Gale, M. D., and Marshall, G. A. 1973. Insensitivity to gibberellin in dwarf wheats. *Ann. Bot. (Lond.)* 37:729-735.
- Gale, M. D., and Youssefian, S. 1985. Dwarfing genes in wheat. Pages 1-35 in: *Progress in Plant Breeding*. G. E. Russell, ed. Butterworths, London.
- Gervais, L., Dedryver, F., Morlais, J. Y., Bodusseau, V., Negre, S., Bilous, M., Groos, C., and Trottet, M. 2003. Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. *Theor. Appl. Genet.* 106:961-970.
- Graham, S., and Browne, R. A. 2009. Anther extrusion and Fusarium head blight resistance in European wheat. *J. Phytopathol.* 157:580-582.
- Guedira, M., Brown-Guedira, B., Van Sanford, D., Sneller, C., Souza, E., and Marshall, D. 2010. Distribution of *Rht* genes in modern and historic winter wheat cultivars from the eastern and central USA. *Crop Sci.* 50:1811-1822.
- Häberle, J., Schweizer, G., Schondelmaier, J., Zimmermann, G., and Hartl, L. 2009. Mapping of QTL for resistance against Fusarium head blight in the winter wheat population Pelikan/Bussard/Ning8026. *Plant Breed.* 128:27-35.
- He, X. Y., Singh, P. K., Schlang, N., Duveiller, E., Dreisigacker, S., Payne, T., and He, Z. H. 2014. Characterization of Chinese wheat germplasm for resistance to Fusarium head blight at CIMMYT, Mexico. *Euphytica* 195:383-395.
- Heiden, P. 2003. The genes of the Green Revolution. *Trends Genet.* 19:5-9.
- Hilton, A. J., Jenkinson, P., Hollins, T. W., and Parry, D. W. 1999. Relationship between cultivar height and severity of Fusarium ear blight in wheat. *Plant Pathol.* 48:202-208.
- Holzappel, J., Voss, H., Miedaner, T., Korzun, V., Häberle, J., Schweizer, G., Mohler, V., Zimmermann, G., and Hartl, L. 2008. Inheritance of resistance to Fusarium head blight in three European winter wheat populations. *Theor. Appl. Genet.* 117:1119-1128.
- Kang, Z. S., and Buchenauer, H. 2000. Cytology and ultrastructure of the infection of wheat spikes by *Fusarium culmorum*. *Mycol. Res.* 104:1083-1093.
- Knopf, C., Becker, H., Ebmeyer, E., and Korzun, V. 2008. Occurrence of three dwarfing *Rht* genes in German winter wheat varieties. *Cereal Res. Commun.* 36:553-560.
- Kubo, K., Fujita, M., Kawada, N., Nakajima, T., Nakamura, K., Maejima, H., Ushiyama, T., Hatta, K., and Matsunaka, H. 2013a. Minor differences in anther extrusion affect resistance to Fusarium head blight in wheat. *J. Phytopathol.* 161:308-314.
- Kubo, K., Kawada, N., and Fujita, M. 2013b. Evaluation of Fusarium head blight resistance in wheat and the development of a new variety by integrating type i and ii resistance. *JARQ* 47:9-19.
- Lanning, S. P., Martin, J. M., Stougaard, R. N., Guillen-Portal, F. R., Blake, N. K., Sherman, J. D., Robbins, A. M., Kephart, K. D., Lamb, P., Carlson, G. R., Pumphrey, M., and Talbert, L. E. 2012. Evaluation of near-isogenic lines for three height-reducing genes in hard red spring wheat. *Crop Sci.* 52:1145-1152.
- Liu, S. Y., Griffey, C. A., Hall, M. D., McKendry, A. L., Chen, J. L., Brooks, W. S., Brown-Guedira, G., Van Sanford, D., and Schmale, D. G. 2013. Molecular characterization of field resistance to Fusarium head blight in two US soft red winter wheat cultivars. *Theor. Appl. Genet.* 126:2485-2498.
- Lu, Q. X., Lillemo, M., Skinner, H., He, X. Y., Shi, J. R., Ji, F., Dong, Y. H., and Bjørnstad, 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.
- Lu, Q. X., Szabo-Hever, A., Bjørnstad, A., Lillemo, M., Semagn, K., Mesterhazy, A., Ji, F., Shi, J. R., and Skinner, H. 2011. Two major resistance quantitative trait loci are required to counteract the increased susceptibility to Fusarium head blight of the *Rht-D1b* dwarfing gene in wheat. *Crop Sci.* 51:2430-2438.
- Mao, S., Wei, Y., Cao, W., Lan, X., Yu, M., Chen, Z., Chen, G., and Zheng, Y. 2010. Confirmation of the relationship between plant height and Fusarium head blight resistance in wheat (*Triticum aestivum* L.) by QTL meta-analysis. *Euphytica* 174:343-356.
- McMullen, M. P., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Dis.* 81:1340-1348.
- Mesterhazy, A. 1995. Types and components of resistance to Fusarium head blight of wheat. *Plant Breed.* 114:377-386.
- Miedaner, T., and Voss, H. 2008. Effect of dwarfing *Rht* genes on Fusarium head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. *Crop Sci.* 48:2115-2122.
- Miedaner, T., Würschum, T., Maurer, H., Korzun, V., Ebmeyer, E., and Reif, J. 2011. Association mapping for Fusarium head blight resistance in European soft winter wheat. *Mol. Breed.* 28:647-655.
- Miller, S. S., Chabot, D. M. P., Ouellet, T., Harris, L. J., and Fedak, G. 2004. Use of a *Fusarium graminearum* strain transformed with green fluorescent protein to study infection in wheat (*Triticum aestivum*). *Can. J. Plant Pathol. Rev. Can. Phytopathol.* 26:453-463.
- Miralles, D. J., Calderini, D. F., Pomar, K. P., and D'Ambrogio, A. 1998. Dwarfing genes and cell dimensions in different organs of wheat. *J. Exp. Bot.* 49:1119-1127.
- Moriconi, J. I., Buet, A., Simontacchi, M., and Santa-Maria, G. E. 2012. Near-isogenic wheat lines carrying altered function alleles of the *Rht-1* genes exhibit differential responses to potassium deprivation. *Plant Sci.* 185-186:199-207.
- Navarro, L., Bari, R., Achard, P., Lison, P., Nemri, A., Harberd, N. P., and Jones, J. D. G. 2008. DELLAs control plant immune responses by modulating the balance and salicylic acid signaling. *Curr. Biol.* 18:650-655.
- Nyquist, W. E. 1991. Estimation of heritability and prediction of selection response in plant populations. *Crit. Rev. Plant Sci.* 10:235-322.
- Paillard, S., Schnurbusch, T., Tiwari, R., Messmer, M., Winzeler, M., Keller, B., and Schacherer, G. 2004. QTL analysis of resistance to Fusarium head blight in Swiss winter wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 109:323-332.
- Peng, J. R., Carol, P., Richards, D. E., King, K. E., Cowling, R. J., Murphy, G. P., and Harberd, N. P. 1997. The *Arabidopsis* *GAI* gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes Dev.* 11:3194-3205.

- Peng, J. R., Richards, D. E., Hartley, N. M., Murphy, G. P., Devos, K. M., Flintham, J. E., Beales, J., Fish, L. J., Worland, A. J., Pelica, F., Sudhakar, D., Christou, P., Snape, J. W., Gale, M. D., and Harberd, N. P. 1999. 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* 400:256-261.
- Pugh, G. W., Johann, H., and Dickson, J. G. 1933. Factors affecting infection of wheat heads by *Gibberella saubinetii*. *J. Agric. Res.* 46:771-779.
- R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
- Saghai-Marouf, M. A., Soliman, K., Jorgensen, R. A., and Allard, R. W. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci. USA* 81:8014-8018.
- Saville, R. J., Gosman, N., Burt, C. J., Makepeace, J., Steed, A., Corbitt, M., Chandler, E., Brown, J. K. M., Boulton, M. I., and Nicholson, P. 2012. The 'Green Revolution' dwarfing genes play a role in disease resistance in *Triticum aestivum* and *Hordeum vulgare*. *J. Exp. Bot.* 63:1271-1283.
- Schmolke, M., Zimmermann, G., Buerstmayr, H., Schweizer, G., Miedaner, T., Korzun, V., Ebmeyer, E., and Hartl, L. 2005. Molecular mapping of Fusarium head blight resistance in the winter wheat population Dream/Lynx. *Theor. Appl. Genet.* 111:747-756.
- Skinnes, H., Semagn, K., Tarkegne, Y., Maroy, A. G., and Bjornstad, A. 2010. The inheritance of anther extrusion in hexaploid wheat and its relationship to Fusarium head blight resistance and deoxynivalenol content. *Plant Breed.* 129:149-155.
- Srinivasachary, Gosman, N., Steed, A., Hollins, T., Bayles, R., Jennings, P., and Nicholson, P. 2009. Semi-dwarfing *Rht-B1* and *Rht-D1* loci of wheat differ significantly in their influence on resistance to Fusarium head blight. *Theor. Appl. Genet.* 118:695-702.
- Srinivasachary, Gosman, N., Steed, A., Simmonds, J., Leverington-Waite, M., Wang, Y., Snape, J., and Nicholson, P. 2008. Susceptibility to Fusarium head blight is associated with the *Rht-D1b* semi-dwarfing allele in wheat. *Theor. Appl. Genet.* 116:1145-1153.
- Steiner, B., Lemmens, M., Griesser, M., Scholz, U., Schondelmaier, J., and Buerstmayr, H. 2004. Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. *Theor. Appl. Genet.* 109:215-224.
- Strange, R. N., Majer, R. J., and Smith, H. 1974. Isolation and identification of choline and betaine as 2 major components in anthers and wheat-germ that stimulate *Fusarium graminearum* in vitro. *Physiol. Plant Pathol.* 4:277-290.
- Sutton, J. C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. *Can. J. Plant Pathol.* 4:195-209.
- Tu, D. S. 1953. Factors affecting the reaction of wheat blight infection caused by *Gibberella zeae*. Ph.D. thesis, Ohio State University, Columbus.
- Voss, H., Holzapfel, J., Hartl, L., Korzun, V., Rabenstein, F., Ebmeyer, E., Coester, H., Kempf, H., and Miedaner, T. 2008. Effect of the *Rht-D1* dwarfing locus on Fusarium head blight rating in three segregating populations of winter wheat. *Plant Breed.* 127:333-339.
- Webb, S. E., Appleford, N. E. J., Gaskin, P., and Lenton, J. R. 1998. Gibberellins in internodes and ears of wheat containing different dwarfing alleles. *Phytochemistry* 47:671-677.
- Würschum, T., Langer, S. M., and Longin, C. F. H. 2015. Genetic control of plant height in European winter wheat cultivars. *Theor. Appl. Genet.* 128:865-874.
- Yan, W., Li, H., Cai, S., Ma, H., Rebetzke, G., and Liu, C. 2011. Effects of plant height on type I and type II resistance to fusarium head blight in wheat. *Plant Pathol.* 60:506-512.
- Zange, B. J., Kang, Z., and Buchenauer, H. 2005. Effect of Folicur® on infection process of *Fusarium culmorum* in wheat spikes. *Z. Pflanzenkrankh. Pflanzenschutz J. Plant Dis Prot.* 112:52-64.