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ORIGINAL PAPER

# Effect of a rye dwarfing gene on plant height, heading stage, and Fusarium head blight in triticale (×*Triticosecale* Wittmack)

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**Abstract**

***Key message*** **The rye-derived dwarfing gene *Ddw1* on chromosome 5R acts in triticale in considerably reducing plant height, increasing FHB severity and delaying heading stage.**

*Abstract* Triticale, an amphiploid hybrid between durum wheat and rye, is an European cereal mainly grown in Germany, France, Poland, and Belarus for feeding purposes. Dwarfing genes might further improve the genetic potential of triticale concerning lodging resistance and yield. However, they might have pleiotropic effects on other, agronomically important traits including Fusarium head blight. Therefore, we analyzed a population of 199 doubled haploid (DH) lines of the cross HeTi117-06 × Pigmej for plant height, heading stage, and FHB severity across 2 locations and 2 years. The most prominent QTL was detected on chromosome 5R explaining 48, 77, and 71 % of genotypic variation for FHB severity, plant height, and heading stage, respectively. The frequency of recovery in cross validation was ≥90 % for all three traits. Because the markers that detect dwarfing gene *Ddw1* in rye are also in our population the most closely linked markers, we assume that this major QTL resembles *Ddw1*. For FHB severity two, for plant height three, and for heading stage five additional QTL were detected. Caused by the considerable genetic variation for heading stage and FHB severity within the progeny with the dwarfing allele, short-strawed, early heading and FHB-resistant lines can be developed when population size is large enough.

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## Introduction

Dwarfing or reduced height (*rht*) genes were widely used in international wheat breeding starting with the green revolution (Gale and Youssefian 1985). Alleles like *Rht-B1b* (syn *Rht1*) or *Rht-D1b* (syn *Rht2*) reduce not only plant height, but increase spike fertility and allow a higher input of nitrogen and, thus, a higher grain yield (Law et al. 1978). In addition, they are known to enhance FHB severity considerably (e.g., Miedaner and Voss 2008; Srinivasachary et al. 2008). In rye, several dwarfing genes are known (*rf.* Börner et al. 1998) of which the dominant gene *Ddw1* located on chromosome 5RL (Korzun et al. 1996) is probably the most frequently used. It was introduced in rye breeding by introgressing the dwarfing mutant 'EM-1' from the Vavilov gene bank collection in St. Petersburg/Russia that was firstly described by Kobyljanski (1972) and originally named *Hl* (*Humilus*). *Ddw1* has been introduced into triticale by Polish breeders (Banaszak 2010). In triticale breeding programs, reducing plant height might be advantageous to increase grain yield due to a better partitioning of assimilates for the benefit of the ear and reducing the risk of lodging (Foulkes et al. 2011). Indeed, a semi-dwarf cultivar ('Pigmej') was introduced into the EU list of recommended cultivars in 2008 (BSL 2013). Pigmej has a plant height of 3 on the 1–9 scale (1 = very short, 9 = very tall) and is among the shortest triticale cultivars available to date (BSL 2013).

Hexaploid triticale (×*Triticosecale* Wittmack), the intergeneric hybrid between tetraploid wheat (*Triticum durum*

L.) as female and rye (*Secale cereale* L.) as male parent, is widely used as feed grain growing on about 3.2 million hectares in Europe in 2012 (FAOSTAT 2013). Main producers are Poland, Belarus, Germany, and France covering 71 % of the worldwide acreage. Triticale is affected by several necrotrophic fungal pathogens such as Septoria nodorum glume blotch, Septoria tritici blotch, and Fusarium head blight (FHB) according to the humid climate in these regions (Arseniuk 1996). FHB caused by *Fusarium graminearum*, *F. culmorum* and other *Fusarium* species leads not only to reduced thousand-grain weight and grain yield (Arseniuk et al.1993), but also to contamination by mycotoxins, e.g., deoxynivalenol (DON), nivalenol (NIV), or zearalenone (ZEA) that are harmful to animals and humans (Becher et al. 2013). Triticale grain is mainly used as feed for pigs and chicken. Swine, however, are the most sensitive farm animals responding to DON with a decrease in feed intake and a compromised immune system (EFSA 2004). FHB contamination must be minimized already in the field. Classical agronomic procedures and fungicide applications do not suffice in FHB control (Becher et al. 2013), therefore breeding for resistance is still the best method to reduce losses. Improving high-yielding cultivars for FHB resistance is a challenge for triticale breeders.

FHB resistance was extensively studied in wheat (Snijders 1990; Bai and Shaner 1994; Becher et al. 2013) and to some extent in rye (Miedaner and Geiger 1996). In wheat, hundreds of QTL have been described from an array of mapping populations (Buerstmayr et al. 2009; Löffler et al. 2009; Liu et al. 2009). In triticale, also a large genetic variation among cultivars was reported (Oettler and Wahle 2001). Resistant genotypes generally displayed a lower DON content. The correlation of FHB severity and DON content is in triticale, however, much lower than in wheat (Miedaner et al. 2004). A generation-means analysis across 15 triticale crosses showed that FHB resistance is quantitatively inherited with a preponderance of additive variance (Oettler et al. 2004) as previously known from wheat (Snijders 1990; Bai and Shaner 1994). Agronomic traits such as plant height, flowering date, and heading date in wheat are known to affect FHB infection, usually taller and later ripening cultivars are less infected (Mesterhazy 1995; Miedaner 1997). Several studies reported a strong association between plant height and FHB in wheat caused by the presence of *Rht* genes (Voss et al. 2008; Buerstmayr et al. 2009; Mao et al. 2010), which enhance FHB susceptibility. In addition, quantitative trait loci (QTL) for plant height linked to FHB QTL are another cause for a negative correlation between FHB severity and plant height (Holzapfel et al. 2008; Löffler et al. 2009).

In view of the dramatic effects of dwarfing genes *Rht-D1* and, to a lesser extent *Rht-B1*, in increasing FHB in wheat it is of utmost interest for the triticale breeders to further evaluate the effect of alternative dwarfing genes in triticale. The present study aimed to (1) analyze the effect of the dwarfing gene in the triticale cultivar Pigmej on FHB resistance, plant height, and heading stage, (2) determine correlations among these traits, and (3) localize and map the dwarfing locus in a doubled haploid (DH) triticale population of 199 lines developed from a cross between the two winter triticale parents HeTi117-06 and Pigmej.

## Materials and methods

### Plant materials and field trial

A doubled haploid (DH) population of 200 winter triticale lines (188 entries in the 1st year, 200 in the 2nd year) derived from the cross between two parents HeTi117-06 and Pigmej was used in this experiment. Twelve DH lines were tested in only 1 year due to limited seed availability. As a result of low general fitness, one DH line had to be discarded. Microspore culture procedure was followed to produce doubled haploid (DH) plants (Würschum et al. 2012). The DH population has been kindly provided by Saatzucht Dr. Hege GbR, Waldenbuch, Germany.

DH lines and check genotypes including the DH parents were evaluated for heading stage, plant height and Fusarium head blight severity under field conditions in two ecologically different locations: Hohenheim (HOH, longitude 9°11′15.5″, latitude 48°42′52.9″, altitude 401 m) and Oberer Lindenhof (OLI, longitude 9°18′15.6″, latitude 48°28′27.6″, altitude 707 m) across 2 years (2011, 2012) in Germany. Location–year combinations are referred to as environments in this paper. Field experiments followed an alpha design with three replications per location. Parents were planted three times within each replication. Entries were planted in one-row plots of 1.2 m length and 0.38 m width between rows; seed density was 30 kernels per row. Agronomic measures followed standard procedures at the respective locations. To prevent lodging, growth regulators were applied at HOH 2011, 2012 and OLI 2012. In all cases, Moddus® (Syngenta, 250 g $l^{-1}$ Thiamethoxam, 0.4 l $ha^{-1}$) was sprayed in BBCH 29-34 (Anonymus 2001). At HOH 2012, additionally CCC (BASF, 720 g $l^{-1}$ chlormequat chloride, 1.0 l $ha^{-1}$) in BBCH29 and Moddus® (0.4 l $ha^{-1}$) in BBCH34 were applied.

### Fusarium inoculation and trait assessment

To evaluate resistance to FHB, artificial inoculation was performed in all environments. The highly aggressive single-spore isolate of *Fusarium culmorum* FC46 (=IPO 39-01, Snijders and Perkowski 1990) was applied at a concentration of $7x10^5$ spores/ml. The inoculum was produced on

wheat grain medium as described by Miedaner et al. (1996) in detail. Conidia suspension was sprayed with an agricultural field sprayer (Hege 75, Waldenbuch, Germany) using an air pressure of 3 bar. Because of differences in flowering time, all genotypes were inoculated starting with the beginning of the flowering stage of early genotypes and repeated two to three times within a period of 5 days to ensure inoculation at mid-anthesis for each entry. FHB severity was rated visually at a minimum of three times as the percentage of infected spikelets per plot (0–100 %). We began with the onset of symptom development about 15 days after inoculation and continued at 3–5 days intervals until the beginning of the yellow ripening stage. Heading stage was recorded at an optimal date in terms of the respective growth stage of each plot from EC50 (very beginning of heading: inflorescence starts opening the flag leaf but is still not visible) to EC59 (end of heading: inflorescence fully emerged) according to Anonymus (2001). Plant height was measured once per plot after flowering in all environments.

## Phenotypic data analysis

The phenotypic data were analyzed based on the following statistical model:

$$y_{ijkno} = \mu + c_i + g_i + l_j + y_k + (\mathrm{cl})_{ij} + (\mathrm{cy})_{ik} + (\mathrm{gl})_{ij} + (\mathrm{gy})_{ik} + (\mathrm{ly})_{jk} + (\mathrm{cly})_{ijk} + (\mathrm{gly})_{ijk} + r_{njk} + b_{onjk} + e_{ijkno},$$

where $y_{ijkno}$ was the phenotypic observation for the $i$th genotype at the $j$th location in the $k$th year of the $n$th replicate in the $o$th incomplete block, $\mu$ was an intercept term, $c_i$ was a factor with a single level for each check and a single level for each genotype group (DH population), $g$, $l$, $y$ denote the effects of genotype, location, year, respectively, and cl, cy, gl, gy, ly, cly, gly the respective interaction effects. In addition, $r$ and $b$ as effects of replicate and incomplete block were included in the model, $e_{ijkno}$ was the residual. Dummy variables were used to separate checks and genotypes and estimate variances for each group following Piepho et al. (2006), but for the sake of simplicity we suppressed dummies in the model stated above. Variance components were determined by the restricted maximum likelihood (REML) method considering $c_i$, $l_j$, $y_k$, $(\mathrm{cl})_{ij}$, $(\mathrm{cy})_{ik}$, $(\mathrm{ly})_{jk}$, and $(\mathrm{cly})_{ijk}$ as fixed and all other effects as random. Error and block variances were assumed to be heterogeneous among locations. Significance of variance component estimates was tested by model comparison with likelihood ratio tests in which halved $P$ values were used as approximation (Stram and Lee 1994). Heritability ($h^2$) on an entry-mean basis was estimated from the variance components as the ratio of genotypic to phenotypic variance following the formula: $h^2 = \sigma^2_{\mathrm{G}} / (\sigma^2_{\mathrm{G}} + \sigma^2_{\mathrm{G\times L}}/\mathrm{L} + \sigma^2_{\mathrm{G\times Y}}/\mathrm{Y} + \sigma^2_{\mathrm{G\times L\times Y}}/\mathrm{LY} + \sigma^2_{\mathrm{e}}/\mathrm{LYR}$ (Fehr 1987), where $\sigma^2_{\mathrm{G}}$ denotes the genotypic variance, $\sigma^2_{\mathrm{G\times L}}$ the genotype × location interaction variance, $\sigma^2_{\mathrm{G\times Y}}$ the genotype × year interaction variance, $\sigma^2_{\mathrm{G\times L\times Y}}$ the genotype × location × year interaction variance, and $\sigma^2_{\mathrm{e}}$ the error variance, L, Y, and R are the numbers of locations, years, and replications, respectively. Best linear unbiased estimates (BLUEs) were estimated across environments assuming fixed effects for the genotype $g_i$. Simple correlation coefficients ($r$) were calculated among all traits based on BLUEs of the DH lines. Significance of $r$ was tested using tabulated values based on Fisher's (1921) $z$ transformation. All statistical analyses were performed using ASReml 3.0 (Gilmour et al. 2009) and R (R Development Core Team 2012).

## Genetic data analysis

The DH lines were genotyped with DArT markers and a more detailed description of the DNA extraction and marker analysis has been published elsewhere (Alheit et al. 2011). In addition, the DH population has been genotyped with conserved ortholog set (COS) markers linked to the *Ddw1* gene in rye (Hackauf and Goldfisch pers. commun.). Genetic linkage map for this DH population was generated using JoinMap 4.1 (van Ooijen 2006) assuming Haldane's mapping function (1919) as an essential prerequisite for composite interval mapping as outlined by Zeng (1993). Markers were assigned to linkage groups at logarithm of odds (LOD) ≥3.0. In total, 668 polymorphic markers were used for genetic map construction. The map order of the markers within a defined linkage group was determined by application of a regression mapping algorithm. The map comprised 20 linkage groups that could be assigned to 20 chromosomes with chromosome 2R missing. The map spans totally across 1.889 cM with 537 cM for the A genome, 812 cM for the B genome and 540 cM for the R genome.

Quantitative trait loci (QTL) analysis was based on composite interval mapping (CIM), implemented in software PlabMQTL (Utz 2012), with a multiple regression approach (Haley and Knott 1992), was used to detect QTL positions and effects. The appropriate number of cofactors and the final genetic model were chosen through stepwise regression on the basis of the smallest values of the modified Bayes Information Criterion (mBIC; Baierl et al. 2006). Critical LOD thresholds were determined for each trait empirically according to Churchill and Doerge (1994) using 10,000 permutation runs. Depending on the trait, we applied LOD thresholds, which corresponded to genome-wide error rates of $\alpha \leq 10$ %. The proportion of $\sigma^2_{\mathrm{G}}$ explained by the regression model was calculated as $p_{\mathrm{G}} = R^2_{\mathrm{adj}}/h^2$ where $R^2_{\mathrm{adj}}$ is the adjusted proportion of phenotypic variance explained by the model. A 1-LOD support interval was specified around each QTL. In addition,

to determine the bias of $R^2_{adj}$ explained by detected QTL, fivefold cross validation (CV) was performed as follows: The entire data set (DS) was split into five genotypic subsamples, means from four out of five subsamples used as estimation set (ES) for QTL detection, localization and estimation of genetic effects. The remaining data group considered as test set (TS) in which prediction derived from the ES is tested for their validity by correlation predicted and observed data. Out of this analysis, we give the frequency of recovery, i.e., the percentage of validation runs detecting the respective QTL. A two-dimensional genome scan was performed and additive × additive digenic epistatic effects were tested.

## Results

### Phenotypic data analysis

One hundred ninety-nine DH lines were successfully inoculated resulting in a mean FHB severity ranging from 16 to 24 % in individual environments (Table 1). FHB severity was 4 % higher in 2012 at both locations caused by higher temperature and humidity during flowering time in that year. Progenies ranged from 4 to 48 % at individual environments. Plants were generally shorter at HOH than at OLI in both years. Ranges for plant height were wide with the shortest progeny having 53 cm (HOH 2011) and the tallest 142 cm (OLI 2011). Mean heading stages were rather similar with the largest possible range at HOH 2011.

Heading stage and FHB severity were approximately normally distributed, while plant height tended to a bimodal distribution across environments (Fig. 1).

Parents did not differentiate much in heading stage across four environments (Table 1). Pigmej was on average 5 cm shorter and 2 % more infected than HeTi117-06 (Table 2). According to the considerable phenotypic ranges, genotypic variance component was significant for all traits ($P < 0.001$, Table 2) and by far the largest source of variation although the interaction variances genotype-by-year, genotype-by-location, and genotype-by-location-year were also significant ($P < 0.05$) for all traits except for genotype-by-location variance for heading stage. The interactions showed the highest importance for FHB severity. Error variances at the individual environments were similar for FHB severity and plant height, but variable for heading stage (data not shown). Entry-mean heritability estimates were high exceeding 0.67 for all traits.

The three traits were significantly ($P < 0.01$) intercorrelated. Plant height showed high coefficients of phenotypic correlation with both, heading stage (r = 0.762) and FHB

**Table 1** Means and ranges for 199 DH lines for Fusarium head blight (FHB) severity, heading stage (EC), and plant height for four environments

| Environment | FHB severity (%) | Heading stage (EC) | Plant height (cm) |
|---|---|---|---|
| HOH 2011 | 16.2 (5.4–37.7) | 53.1 (50.2–58.7) | 88.9 (53.0–120.6) |
| HOH 2012 | 20.4 (4.3–41.3) | 56.9 (52.5–59.2) | 96.2 (65.7–127.3) |
| OLI 2011 | 20.1 (8.7–47.5) | 55.0 (52.4–58.7) | 105.6 (67.0–142.1) |
| OLI 2012 | 23.9 (10.7–39.5) | 54.7 (51.2–58.7) | 103.5 (76.4–134.7) |

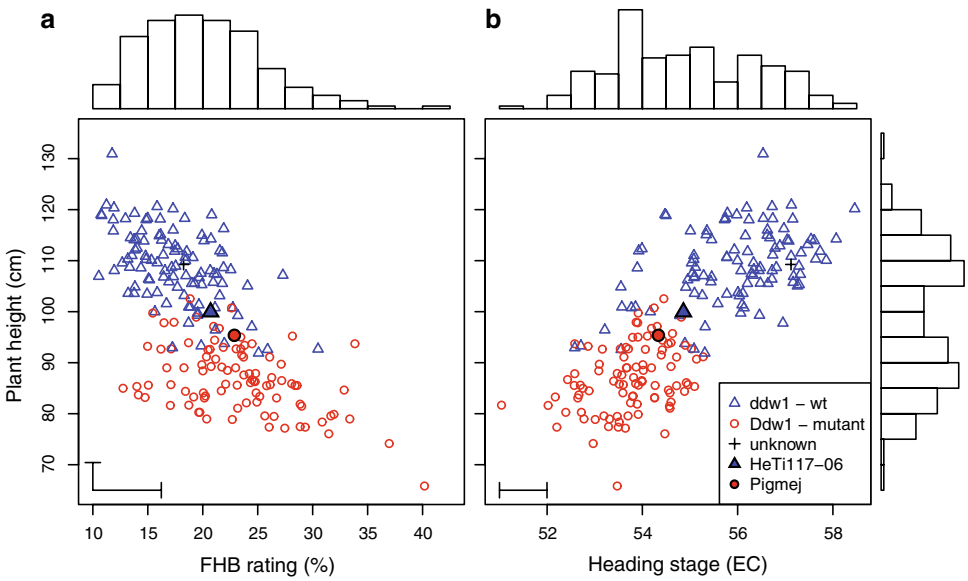


**Fig. 1** Associations and the corresponding marginal histograms **a** between Fusarium head blight (FHB) severity (%) and plant height (cm) and **b** between heading stage (EC) and plant height (cm) for 199 DH lines with the wild type (wt, $N = 103$ DH lines) and the mutant dwarf (mutant, $N = 95$ DH lines) allele at the *Ddw1* locus, respectively. One progeny could not be assigned to an allele caused by a recombination within the marker interval. Least significant differences ($P < 0.05$) are indicated by *bars*

**Table 2** Means of the two parents (A, B) and means and ranges of 199 DH lines and estimates of variance components ($\sigma^2_G$, genotypic, $\sigma^2_{G\times L}$, genotype × location interaction, $\sigma^2_{G\times Y}$, genotype × year interaction, $\sigma^2_{G\times L\times Y}$, genotype × location × year interaction, $\sigma^2_e$, mean error variances across all environments) for Fusarium head blight (FHB) severity (%), plant height (cm), and heading stage (EC) across four environments

| Parameter | FHB severity (%) | Heading stage (EC) | Plant height (cm) |
|---|---|---|---|
| Means and ranges | | | |
| A (HeTi117-06) | 20.7 | 54.9 | 99.9 |
| B (Pigmej) | 22.9 | 54.3 | 95.4 |
| DH lines: mean | 20.3 | 54.9 | 98.4 |
| DH lines: range | 10.5–40.2 | 51.0–58.5 | 65.8–130.9 |
| Variances ($\sigma^2$) and heritability of DH lines: | | | |
| $\sigma^2_G$ | 18.44*** | 2.05*** | 163.43*** |
| $\sigma^2_{G\times L}$ | 2.30* | 0 | 2.56** |
| $\sigma^2_{G\times Y}$ | 9.82*** | 0.14*** | 2.35** |
| $\sigma^2_{G\times L\times Y}$ | 7.29*** | 0.12*** | 1.58* |
| $\sigma^2_e$ | 15.25 | 0.85 | 20.89 |
| Heritability | 0.67 | 0.92 | 0.97 |

*,**,*** significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

severity ($r = -0.673$, Fig. 1). The respective coefficient between heading stage and FHB severity was considerably lower ($r = -0.389$). Thus, short progenies tended to be later in heading, but more susceptible in FHB.

### QTL analysis

For FHB severity, plant height, and heading stage three, four, and six QTL, respectively, amounted the respective critical LOD value (Table 3). The most prominent QTL, however, was detected on chromosome 5R explaining 48, 77, and 71 % of genotypic variation for FHB severity, plant height, and heading stage, respectively (Table 3). The frequency of recovery for this QTL was ≥90 % in all three traits. The most closely linked markers were for all traits two of the COS markers *Xiac128*, *Xiac129* and *Xiac130* on the long arm of chromosome 5R (Fig. 2). A second major QTL for plant height was detected on the same chromosome, but about 50 cM distal. All QTL were additively inherited. A further QTL for heading stage on chromosome 6A explained 40 % of genotypic variance, the QTL on chromosome 2A and 5B were minor.

In addition, four epistatic interactions were detected for FHB severity, plant height, and heading stage, respectively (Table 3). They were of the additive x additive type of interaction and had similar effects than the non-5R QTL. Interestingly, the *Ddw1* locus on chromosome 5R is involved in all epistatic interactions.

The DH subpopulation with the mutant allele (dwarf) showed considerably different means for all three traits when compared with the DH subpopulation with the wild-type allele (tall, Table 4). Segregation in the unselected DH population equaled almost 1:1 ratio. A large difference between progenies with and without the dwarfing gene can be seen from the scatter plots (Fig. 1) where both subpopulations overlap only with a few progeny. However, progenies within each of both alleles showed still considerable variation for the three traits. One DH line could not be assigned to an allele caused by a recombination within the marker interval. All QTL and the epistatic interactions together explained about 60 % of FHB severity and >80 % of plant height and heading stage.

## Discussion

### Transgressive segregation for plant height, heading stage, and FHB resistance

Both parents of this study, HeTi117-06 and Pigmej, had similar trait means (Table 2). This is a common feature in practical plant breeding because breeders tend to cross "best × best". However, as a fundamental rule of quantitative genetics, this does not necessarily limit segregation variance (Falconer and Mackay 1996). For all three traits, we observed a highly significant genotypic variance (Table 3) and transgressive segregation (Fig. 1), i.e., progenies that were significantly differing from the better or worse parent. This transgressive segregation illustrates that the parents carry at least in part complementary alleles at different QTL (Tanksley 1993) that are newly combined in the progeny. The fact that the parents have similar trait means shows that they achieve this through different QTL which were identified in our QTL mapping experiment. Transgressive segregation is common in both natural and domesticated populations (Rieseberg et al. 2003) and one of the driving forces of new cultivars. High importance of transgressive segregation was also reported recently in winter rye for several agronomic traits (Miedaner et al. 2012).

### Dwarfing allele is associated with plant height and heading stage

In triticale population HeTi117-06 × Pigmej, we detected a dwarfing gene on the long arm of chromosome 5R with extremely high effects on plant height and heading stage and moderate effects on FHB severity. The effect of a dwarfing gene located on chromosome 5R on plant height and biomass production in the same population (called EAW78) was described recently on the basis of an integrated map of five populations (Alheit et al. 2014). However, in this companion

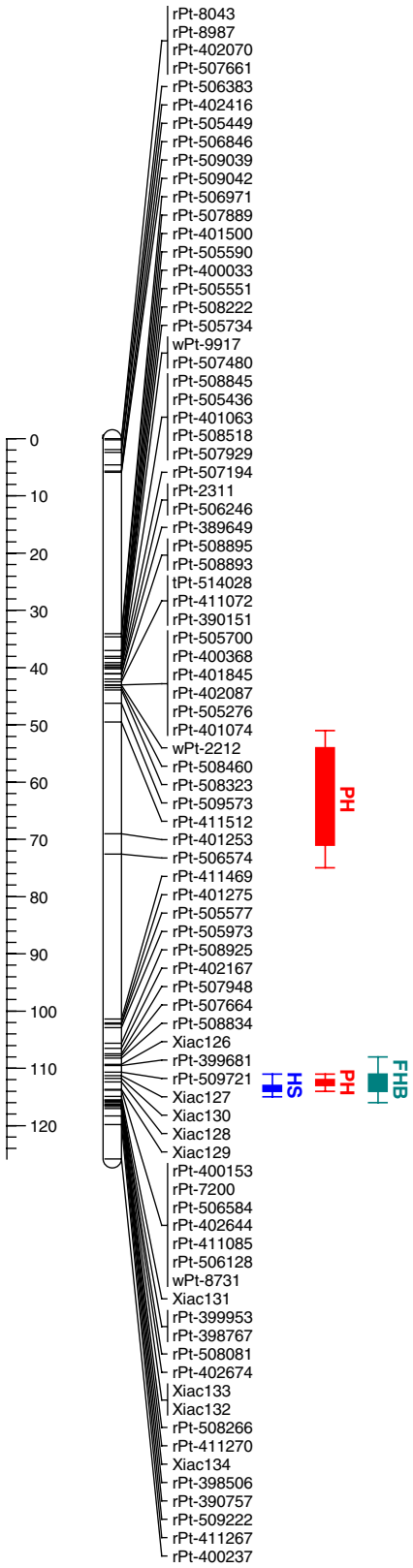


**Fig. 2** Map of chromosome 5R with the detected QTL for Fusarium head blight (FHB) resistance, plant height (PH), heading stage (HS)

study, chromosome 5R was given in opposite orientation of the chromosome as can be seen from the marker rPt-509721 (Fig. 2) that is common in both maps. In our study, the number of replications in the environments was higher and the map was calculated only for the population analyzed with a few additional markers on chromosome 5R closely linked to the rye dwarfing gene *Ddw1*. Therefore, we found a higher explained genetic variation for plant height by this gene than Alheit et al. (2014), although we applied growth regulators in our experiments. This is necessary and a common practice in high-yielding European triticale growing to avoid lodging. Lodging intensifies FHB severity by high humidity when the heads are near the ground and considerably reduces genotypic differentiation. Thus, without growth regulators, the experiment would not yield any meaningful results with regard to FHB resistance. But also for plant height, results would not have been useful when lodging occurs because the subpopulation with long-straw allele would suffer much more from lodging than that with the short-straw allele resulting in an inacceptable overestimation of the effects of *Ddw1*.

The dwarfing gene *Ddw1* has previously been mapped distally on the long arm of rye chromosome 5R (Korzun et al. 1996). A comparative genetic approach using model grass genomes as a blueprint (Hackauf et al. 2012) allowed to develop COS markers closely linked to *Ddw1* in rye (Hackauf and Goldfisch, pers. commun.). Several of these markers are linked to the major QTL for plant height in our triticale population as well (Fig. 2). This observation is a strong hint, that we indeed detected the rye gene *Ddw1* governing the major QTL for plant height in the triticale cultivar Pigmej, which has a rye inbred line in its pedigree (Henryk Wos, pers. commun.). Although the cultivar itself was only slightly shorter than the parent HeTi117-06 possessing the tall allele at this locus, some progenies were really short with plant height <80 cm (Fig. 1). Accordingly, progenies with the mutant allele were, on average, 22 cm shorter than those with the wild-type allele (Table 4). According to these results, the bimodal frequency distribution for plant height refers to a monogenic inheritance as well. Of course, several other QTL for plant height also play a role resulting basically in two overlapping normal distributions for each of the two allele classes. Indeed, we detected three other QTL in this population governing plant height (Table 3).

A large effect of the dwarfing gene on phenological traits, like heading stage, previously described by Börner et al. (2000) for flowering time in rye, was clearly detected in triticale for the first time. These large phenotypic effects are reflected by the results of our QTL analysis with high proportions of explained genotypic variance for plant height and heading stage (77 and 71 %, respectively), extremely high frequencies of recovery during cross-validation runs

**Table 3** QTL positions (pos), their confidence intervals (CI), epistatic effects, logarithm of odd (LOD), effect sizes, explained genotypic variances ($p_G$) and frequencies of occurrence in cross validation procedure after 1.000 runs (FreqCV) for Fusarium head blight (FHB) severity (%), heading stage (EC), and plant height (cm) of 199 DH lines across four environments

| Trait | Chromosome | Left marker | Right marker | Pos [CI] (cM) | LOD[a] | Additive effect[b] | $p_G$ (%) | Freq CV |
|---|---|---|---|---|---|---|---|---|
| FHB severity (%) | C_5A | wPt-9204 | wPt-6825 | 7 [2–11] | 3.34 | 1.141 | 10.17 | 0.211 |
| | C_5R | Xiac130 | Xiac128 | 112 [111–114] | 14.29 | 2.902 | 48.40 | 0.953 |
| | C_6R | rPt-401114 | tPt-513005 | 89 [88–90] | 4.06 | −1.113 | 9.58 | 0.246 |
| | C_5R:C_6R | | | | | −0.878 | 6.09 | |
| | $p_{G-total}$ | | | | | | 58.60 | |
| Plant height (cm) | C_6A | tPt-4209 | wPt-0902 | 51 [43–54] | 5.86 | 1.260 | 4.04 | 0.953 |
| | C_7B | tPt-513969 | wPt-1330 | 72 [62–88] | 3.61 | 1.648 | 6.80 | 0.299 |
| | C_5R1 | rPt-411512 | rPt-401253 | 63 [54–71] | 4.69 | 2.707 | 12.04 | 0.656 |
| | C_5R2 | Xiac130 | Xiac128 | 112 [111–114] | 52.11 | −11.029 | 76.70 | 0.959 |
| | C_6A:C_5R2 | | | | | −2.438 | 13.52 | |
| | $p_{G-total}$ | | | | | | 80.90 | |
| Heading stage (EC) | C_2A | wPt-3244 | wPt-2293 | 79 [76–80] | 4.88 | 0.217 | 8.61 | 0.480 |
| | C_6A | wPt-0902 | wPt-2077 | 53 [51–54] | 22.24 | 0.562 | 39.97 | 0.999 |
| | C_5B | wPt-8106 | tPt-3719 | 13 [12–14] | 6.10 | −0.214 | 8.23 | 0.375 |
| | C_4R | rPt-506948 | rPt-398820 | 30 [28–31] | 5.72 | 0.142 | 1.55 | 0.330 |
| | C_4R | rPt-508464 | rPt-509061 | 91 [86–95] | 7.49 | 0.247 | 5.00 | 0.763 |
| | C_5R | Xiac128 | Xiac129 | 113 [111–114] | 46.77 | −1.004 | 70.97 | 0.899 |
| | C_6A:C_6R | | | | | −0.280 | 13.12 | |
| | C_4R:C_5R | | | | | −0.213 | 5.19 | |
| | $p_{G-total}$ | | | | | | 84.50 | |

[a] Threshold for LOD value (after 10.000 permutations): 3.22 for FHB severity, 3.15 for plant height, and 3.15 for heading stage

[b] Negative effect indicates that Pigmej is contributing the allele for FHB susceptibility, shortness, and delayed heading, respectively

**Table 4** Number of progenies (N), means, and their difference between subpopulations (Δ) for Fusarium head blight (FHB) severity, heading stage (EC), and plant height for progeny with wild type and mutant allele, respectively, at the *Ddw1* locus on chromosome 5R across four environments; one progeny could not be assigned to an allele due to recombination within the marker interval

| Allele | N | FHB severity (%) | Δ | Plant height (cm) | Δ | Heading stage (EC) | Δ |
|---|---|---|---|---|---|---|---|
| *ddw1,* wild type | 103 | 17.5 | | 108.8 | | 55.9 | |
| *Ddw1*, mutant | 95 | 23.2 | 5.7 | 87.0 | −21.8 | 53.8 | 2.2 |

(>90 %) and only slightly reduced effects in the test set after cross-validation (data not shown). For all traits, epistatic interaction between the dwarfing gene and other loci has been detected. Such interactions are a main cause why the effects even of single genes are differing among genetic backgrounds/crossing partners.

## FHB resistance in triticale is primarily inherited by additive gene action

FHB resistance in triticale is quantitatively inherited with a predominantly additive gene action (Oettler et al. 2004; Miedaner et al. 2006) as previously reported from wheat (e.g., Snijders 1990; Bai and Shaner 1994). This is in agreement with our results as can be seen from the continuous variation of our biparental mapping population with a high heritability (Fig. 1). Moreover, mean of DH progeny did not deviate from parental mean illustrating that a larger number of loci is segregating and substantiating a preponderance of additive gene action (Falconer and Mackay 1996).

This study is, to our best knowledge, the first QTL mapping of FHB resistance on a sub-genomic segment of the rye genome in triticale worldwide. The main effect of *Ddw1* on FHB severity was very large and only two additional QTL were found in this population. The second QTL on chromosome 6R [89 cM] did not match the critical LOD value after permutation test. Interestingly, this second locus was also involved in the epistatic interaction with *Ddw1* concerning FHB. Epistasis, i.e., the

interaction of non-linked loci, was previously reported for FHB resistance in wheat (Ma et al. 2006; Miedaner et al. 2011).

### Dwarf triticale lines have, on average, a higher FHB severity

The effect of the dwarfing gene on FHB severity, that was the main objective of our study, was large as judged from the percentage of genotypic variance explained by this gene (48 %), but considerably lower than for plant height and heading stage. In terms of severity, FHB susceptibility of the wild type was enhanced by 32 % when the dwarfing gene was introduced as calculated from Table 4. As a consequence, also DON content should be higher in progenies with *Ddw1*. Indeed, Pigmej is categorized as a cultivar with above-average DON content in the grain after FHB infection in Germany (DLG 2013). These findings are strong hints that *Ddw1* has pleiotropic effects not only on plant height but also on heading stage and FHB severity. An alternative explanation could be a linkage between *Ddw1* and several QTL for the other traits in a small segment of chromosome 5RL. However, considering the results with the wheat dwarfing allele *Rht-D1b*, where a perfect marker located directly in the gene leads to similar results (Voss et al. 2008), makes the first explanation more likely.

### Selection for short, FHB-resistant triticale is resource demanding, but feasible

In view of the positive effects of *Ddw1* on plant height, but the unwanted effects on heading stage and FHB severity, the challenge for the breeder is to use the dwarfing effect, but to avoid triticale lines that are later flowering and more susceptible to FHB. Despite the negative correlation between plant height and FHB severity, some short progenies are also superior in FHB severity (i.e., <20 % as an example) as illustrated by Fig. 1. This can be explained by the effects of other FHB resistance QTL alleles in the genomic background (i.e., on chromosomes other than 5RL) of some progenies that decrease the FHB severity despite the presence of *Ddw1*. From these QTL, only two have been detected in this study, probably because their effects are below the critical LOD value or they are not segregating in this population. Consequently, in practical breeding, short triticale lines with good FHB resistance can be achieved. The same is true for the association among heading stage and *Ddw1*. Here, indeed five other QTL besides *Ddw1* were detected that have an impact on the trait including one major QTL on chromosome 6A explaining 40 % of genotypic variance that was already described as an "earliness per se" locus (Griffiths et al. 2009).

Large population sizes are necessary to use the dwarfing allele of *Ddw1* in practical breeding. In our study of 199 progenies, only about 6 % of progeny with the dwarfing allele was superior in FHB resistance. If the heading stage is also considered as a selection trait, then the fraction would be reduced even more. Consequently, high efforts are necessary to select dwarf progeny without acquiring the negative aspects of this locus. Prerequisite is an efficient and reliable identification of plants carrying the dwarfing gene. The *COS* markers linked to the *Ddw1* gene basically comply with this requirement.

Whether *Ddw1* will be further used in practical breeding will be seen in the future but the example of *Rht-D1b* from wheat is encouraging. The reported side effects are not necessarily prohibitive for the use of *Ddw1* in triticale breeding as long as the breeder counteracts it with QTL positively affecting the respective other traits. In wheat, it has been shown that the negative effects of dwarfing allele *Rht-D1b* can be counterbalanced by a minimum of two major beneficial QTL for FHB resistance (Lu et al. 2011). In their study, the *Rht-D1* locus explained 38 % of the phenotypic variation for FHB severity, thus resembling the $R^2$ value of the *Ddw1* locus explained in our study (32 % as concluded from $p_G$ in Table 3).

In conclusion, a rye-derived dwarfing gene, *Ddw1*, makes elite triticale progenies considerably shorter but is associated with delayed heading and higher FHB susceptibility. In a companion study, *Ddw1* reduced grain yield on average for 0.43 Mg ha$^{-1}$ in this population harboring a QTL at this locus [112 cM] that explained 10.4 % of genotypic variance for grain yield (Maurer and Alheit, unpublished). The mapping results reported from this study are an encouraging first step for a further molecular dissection of this high-effect locus. With the advances in sequencing technologies, it might be possible to fine map and clone *Ddw1* in the future and to unravel its mode of action. At the moment, marker-assisted breeding of semi-dwarf, FHB-resistant and high-yielding triticale appears feasible in practical breeding.

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**Ethical standards** The experiments comply with the current laws of Germany in which they were performed.

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