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ORIGINAL ARTICLE

QTL mapping and comparative genome analysis of agronomic traits including grain yield in winter rye

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Abstract

Key message **Genetic diversity in elite rye germplasm as well as $F_{2:3}$ testcross design enables fast QTL mapping to approach genes controlling grain yield, grain weight, tiller number and heading date in rye hybrids**.

Abstract Winter rye (*Secale cereale* L.) is a multipurpose cereal crop closely related to wheat, which offers the opportunity for a sustainable production of food and feed and which continues to emerge as a renewable energy source for the production of bioethanol and biomethane. Rye contributes to increase agricultural crop species diversity particularly in Central and Eastern Europe. In contrast to other small grain cereals, knowledge on the genetic architecture of complex inherited, agronomic important traits is yet limited for the outbreeding rye. We have performed a QTL analysis based on a $F_{2:3}$ design and testcross performance of 258 experimental hybrids in multi-environmental field trials. A genetic linkage map covering 964.9 cM based on SSR, conserved-orthologous set (COS), and mixed-phase dominant DArT markers allowed to describe 22 QTL with significant effects for grain yield, heading date, tiller number, and thousand grain weight across seven environments. Using rye COS markers, orthologous segments for these traits have been identified in the rice genome, which carry cloned and functionally characterized rice genes. The initial genome scan described here together with the existing knowledge on candidate genes provides the basis for subsequent analyses of the genetic and molecular mechanisms underlying agronomic important traits in rye.

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Introduction

Rye (*Secale cereale* L.) belongs to the Triticeae tribe of the grasses and contributes to increase crop species diversity particularly in European agroecosystems. Winter rye offers modest requirements to the germination temperature, low demands on soil and climatic conditions and excellent winter hardiness. This small grain cereal is a multipurpose crop whose grain is traditionally used for bread making and as feed for livestock. In addition, rye continues to emerge as a renewable energy source for the production of bioethanol and biomethane.

Rye is outstanding among the small grain cereals with respect to its outbreeding nature. Cytoplasmic male sterility (CMS) as genetic fertilization control mechanism as well as efficient restorer genes successfully enable hybrid breeding (Geiger and Miedaner 2009). The exploitation of heterosis by a systematic evaluation of the genetic divergent gene pools ‘Petkus’ and ‘Carsten’ (Hepting 1978) resulted in a significant gain in grain yield (Laidig et al. 2017), which is economically the most important trait in rye breeding.

Agronomic important traits like grain yield or grain weight reveal a continuous phenotypic variation and are genetically controlled by a complex network of multiple and interacting loci. Most of these quantitative trait loci (QTL) are sensitive to environmental conditions and have only small effects on the expression of a trait (Mackay et al. 2009). Currently, the phenotypic evolution of rye varieties is achieved by conventional breeding approaches, which select genotypes indirectly through their phenotypic performance. Genetic improvement by artificial selection is known to cause a reduction in genetic diversity (Wright et al. 2005; Yamasaki et al. 2005; Kovach and McCouch 2008), which might be forced in the future by an implementation of the concept of genomic selection (Meuwissen et al. 2001) in hybrid rye breeding programs (Wang et al. 2014; Auinger et al. 2016). Thus, the maintenance of genetic diversity for the long-term success of the genetic improvement of rye is gaining increasing importance. The analysis of functionally characterized genes as components of quantitative genetic variation in agronomic important traits (Hou et al. 2014; Qin et al. 2014; Jiang et al. 2015; Yue et al. 2015) provides an unbiased and precise assessment of crop genetic diversity in space and time. The largest progress among the cultivated cereals in cloning of QTL for grain yield components and other agronomic important traits has been achieved in rice (Yonemaru et al. 2010; Yamamoto et al. 2012), which has a significant impact on the genetic improvement of this important staple food (Xing and Zhang 2010). In species like rye with just emerging genomic tools (Martis et al. 2013; Bauer et al. 2017), knowledge on candidate genes represents an attractive opportunity to elucidate the molecular genetic basis in particular for quantitative inherited traits. The mining of valuable alleles (Kumar et al. 2010), especially those occurring with low frequencies in self incompatible rye germplasm collections, facilitates a targeted identification of new haplotypes, which can, in a first step, be precisely introgressed in self-fertile elite germplasm based on allele-specific markers and subsequently systematically evaluated in terms of their agronomic value. However, knowledge on QTL in the 8 Gb genome of rye (Bartos et al. 2008) is still in its infancy. The initial QTL mapping in rye was based on line per se performance of agronomic traits and RFLP maps of F_2-derived mapping populations (Börner et al. 2000; Milczarski and Masojć 2003). QTL governing agronomic traits in genetic resources of rye have been approached by a marker-assisted establishment of introgression line libraries (Falke et al. 2008, 2009). Further QTL mapping experiments were directed towards the dominant dwarfing gene *Ddw1* (Börner et al. 1999), in vitro response (Bolibok et al. 2007), α-amylase activity and related traits (Masojć and Milczarski 2005, 2008; Myśków et al. 2011), as well as morphological traits (Myśków et al. 2014) of rye.

The phenotyping of the first comprehensive analysis identifying QTL of yield- and quality related traits was performed on progenies of two elite bi-parental mapping populations within the 'Petkus' gene pool (Miedaner et al. 2012). However, knowledge on QTL of agronomic important traits including grain yield and heading date in the 'Carsten' gene pool is still lacking. We report on a QTL analysis in elite germplasm of rye hybrids using a phenotyping strategy based on testcrosses with 258 $F_{2:3}$ lines in multi-environmental field trials. The objectives of our study were to (1) identify QTL for grain yield, thousand grain weight, spikes per square meter, and heading date in the 'Carsten' gene pool, and to compare them to companion studies in the 'Petkus' gene pool, (2) identify orthologous segments in the rice genome for QTL carrying regions in rye, and (3) identify potential candidate genes based on a comparative genomics approach between rye and rice.

Materials and methods

Plant material

A bi-parental cross between two self-fertile elite inbred lines (HYB201 and HYB202) from the 'Carsten' (pollinator) gene pool was established by HYBRO Saatzucht GmbH & Co. KG and advanced to $F_{2:3}$ lines without selection as recently described (Haffke et al. 2014). In total, 258 out of 272 $F_{2:3}$ lines as well as both parental genotypes were successfully crossed with a CMS single-cross tester of the 'Petkus' (seed parent) gene pool resulting in three-way interpool hybrids. These experimental hybrids together with the six released hybrid varieties Minello, Visello, Palazzo, Brasetto, SU Drive, and SU Stakkato as checks were tested in field trials.

Field experiments and traits

Field experiments were conducted in 2 years (2011 and 2012) in Bornhof, Mecklenburg-Western Pomerania (53°49′N,12°89′W), Groß Lüsewitz, Mecklenburg-Western Pomerania (54°07′N, 12°33′W), Hohenheim, Baden Württemberg (48°72′N, 9°20′W), and Wulfsode, Lower Saxony (53°06′N, 10°24′W). The experiment in Bornhof 2012 failed due to severe pre-flowering drought stress, resulting in a total number of seven environments obtained as location × year combination. Entries were grown on drilled plots of 5–6 m^2 size. The experimental design within each trial was a randomized incomplete block design (alpha design, 34 blocks × 8 entries) with two replications except of the parental genotypes, which were repeated four times each.

Plots were harvested with a conventional plot harvester at full ripening (EC 92) for grain yield (GYD). GYD is reported as dt ha^{-1} at 14% moisture. Additional traits recorded for all plots were heading date (HDT, 1 = very late, 9 = very early), thousand grain weight (TGW, g) and spikes per square meter (SSM), the latter at EC71 and using a counting frame. For statistical analyses, the means of both experiments were calculated as no significant ($P > 0.05$) difference among experiments was found.

Phenotypic data analyses

Statistical analyses were based on plot data of 258 testcross progenies. Checks were calculated separately. All statistical computations were performed with the PLABSTAT software package in a two-step procedure (Utz 2010). Analyses of variance were first performed for all traits in each environment separately. The adjusted entry means from each location were used in a second step to estimate variance components based on the following linear model:

$$y = G + E + G \times E,$$

where G and E denote genotype and environment, respectively. Both factors were treated as random effects. Broad-sense heritability (h^2) on an entry-mean basis was estimated from the variance components as the ratio of genotypic to phenotypic variance (Fehr 1987). Simple correlation coefficients (r) were calculated among all traits based on entry means. Significance of r was tested using tabulated values based on Fischer's z transformation (Fischer 1921).

Genetic linkage map construction

The F_2 population was advanced to F_3 lines by selfing each F_2 plant and reconstituted by combining leaf tissue from 15 plants each of 272 derived individual $F_{2:3}$ progenies for DNA extraction. Analysis of SSR and COS markers on genomic DNA was performed in the mapping population as well as in disomic wheat-rye addition lines as previously described (Hackauf et al. 2009, 2012). Detailed information on assay conditions for both marker systems as well as primer sequences of the used COS markers are given in the electronic supplementary material 1 (ESM1). DArT markers have been assessed in the F_2 population as well as in the recombinant inbred line population L2039-NxDH (Martis et al. 2013) at Diversity Arrays Ltd., Australia, as described by Bolibok-Bragoszewska et al. (2009). As the majority of genotyped markers in the F_2 population followed a dominant inheritance, the linkage map was established by initially splitting the dominant markers into two groups with one group representing the dominant alleles from the female parent and the second group representing the dominant alleles from the male parent (Knapp et al. 1995). The resulting coupling-phase F_2 maps were integrated using the codominant SSR and COS markers and the linkage mapping software JoinMap 4.0 (Van Ooijen 2006). The Kosambi function was used to convert recombination values to genetic distances (cM). The assignment of the seven linkage groups to the seven rye chromosomes and the determination of their orientation were performed using previously mapped anchor markers as well as mapping of COS markers to individual rye chromosome arms in disomic wheat-rye addition lines (ESM3).

QTL analyses

QTL analyses were based on the genetic linkage map and adjusted entry means using software PLABMQTL (Utz 2012). Markers with a distance below 1 cM were excluded automatically by the software. Based on testcross performance of $F_{2:3}$ testcross progenies, main effect QTL for each trait contributing to the additive genetic variation can be detected. A 1-LOD support interval was specified around each QTL. Critical LOD thresholds were analyzed empirically for each trait according to Churchill and Doerge (1994) using 2000 permutation runs. It turned out that critical LOD thresholds corresponding to genome-wide error rates of $\alpha \leq 10\%$ were similar for all agronomic traits. Therefore, the highest LOD threshold (SSM = 3.47) was used for all agronomic traits. The proportion of genetic variance explained by the regression model was calculated as $p_G = R^2_{adj}/h^2$, where R^2_{adj} is the adjusted proportion of phenotypic variance explained by the model. In addition, 1000 cross-validation runs were applied to determine the bias of R^2. For this, the data were resampled independently 200 times at fivefold cross validations. A fivefold cross validation (CV) was performed as follows: the entire data set (DS) was split into five genotypic subsamples and means from four out of five subsamples were used as estimation set (ES) for QTL detection, localization, and estimation of genetic effects. The remaining data group served as test set (TS). Out of this analysis, we give the frequency of recovery, i.e., the percentage of validation runs detecting the respective QTL, and the mean QTL effects in ES and TS for comparison. The identified QTL were designated according to the recommended rules for loci and alleles controlling quantitative characters in wheat and related species (McIntosh et al. 2013).

Comparative QTL mapping

Sequence information on mapped COS markers was used to identify their orthologs in rice as previously described (Hackauf et al. 2009, 2012). The position of individual gene models was obtained from release 6.1 of the rice pseudomolecules (Kawahara et al. 2013). The genomic coordinates

of rice QTL were obtained from the Gramene database (Monaco et al. 2014, ESM2). Synteny maps between rye and rice were built using MapChart (Voorrips 2002).

Table 1 Means of two parental genotypes (HYB201, HYB202) and analysis of variance of their $F_{2:3}$ progenies

Material	Parameter[a]	HDT (1–9)[c]	SSM[b]	TGW (g)	GYD (dt ha^{-1})
HYB201		5.93	586.84	35.89	99.66
HYB202		5.93	559.93	35.98	95.60
Parental mean		5.93	573.39	35.94	97.63
TC-Population	MEAN	5.83	540.46	35.61	100.28
	MIN	4.79	285.88	38.45	94.28
	MAX	6.79	893.29	31.85	104.24
	$LSD_{5\%}$	0.61	50.22	0.95	3.23
	σ_G	0.10**	172.97**	0.89**	1.48**
	$\sigma_{G \times E}$	0.17**	242.00**	0.27**	3.78**
	σ_e	0.34	1724.09	0.43	5.71
	h^2	0.67	0.35	0.88	0.52

Means, ranges, estimates of variance components (genotypic, σ_G; genotype × environment interactions, $\sigma_{G \times E}$; and pooled error σ_e), heritabilities h^2, least significant difference at $P < 0.05$ (LSD 5%) for grain yield (GYD) as well as three agronomical important traits of 258 testcross (TC) progenies evaluated across seven environments

** Significant at 0.01 level of probability

[a] *HDT* heading date, *SSM* spikes per square meter, *TGW* thousand grain weight, *GYD* grain yield

[b] Results based on six environments

[c] HDT: 1 = very late, 9 = very early

Results

Pronounced genetic variation of experimental hybrids in agronomic traits

Phenotypic data of both parental genotypes were similar and the segregating progenies represented the mean testcross performance of their parents in most of the assessed traits (Table 1). All traits showed significant ($P < 0.01$) genotypic and genotype x environment interaction variances. The estimates of broad-sense heritability ranged from $h^2 = 0.35$ for SSM to $h^2 = 0.88$ for TGW with $h^2 = 0.52$ for GYD and $h^2 = 0.67$ for HDT (Table 1). None of the traits deviates from a normal distribution (Fig. 1). Both parental genotypes performed without significant phenotypic differences in HDT and TGW, while HYB201 performed superior to HYB202 in GYD and SSM (Table 1).

A genetic linkage map based on SSR, COS, and mixed-phase dominant DArT markers

We have established a genetic linkage map covering 964.9 cM of the rye genome (Table 2, ESM3). A set of 789 DArT markers, which were supplemented by 17 genomic and 40 EST-derived SSR as well as 66 COS markers, provided the framework to establish this map. The map length of the seven rye chromosomes varied between 105.5 cM of chromosome 3R and 165.9 cM of chromosome 5R. Integration of DArT markers in the recently published transcript map of the recombinant inbred line population L2039-NxDH allowed to identify 127 DArT markers (16.1%),

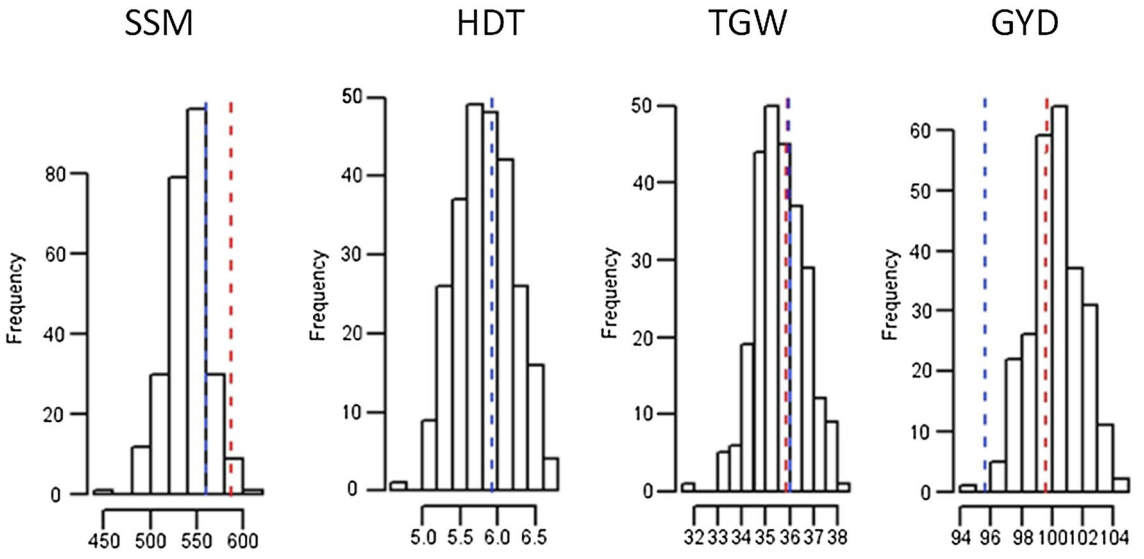

Fig. 1 Frequency distribution of the traits spikes per square meter (SSM), heading date (HDT), thousand grain weight (TGW), and grain yield (GYD) in the population HYB201 × HYB202. *Dashed lines* mark means of parents (*red* HYB201, *blue* HYB202) (color figure online)

Table 2 Survey of the genetic linkage map

Chr	Map length	DArT	COS	EST-SSR	SSR	Marker/chromosome
1R	158.8	101	11	5	4	121
2R	142.9	121	3	6	1	131
3R	105.5	70	3	5	1	79
4R	143.5	126	18	5	2	151
5R	165.9	94	17	13	2	126
6R	122.0	170	7	5	5	187
7R	126.4	107	7	1	2	117
Total	964.9	789	66	40	17	912

which could be mapped in both populations (ESM3). A comparison based on these common markers revealed almost perfect collinearity between both maps.

In total, 26 of the EST-derived SSR (65%) and 60 of the COS markers (91%) correspond to orthologous gene models in rice (ESM4). The collinearity between the rye genetic map of HYB201 × HYB202 and the rice genome (Fig. 2, ESM5-10) identifies 13 conserved blocks, which represent 28.4% of the rye map and 25.2% of the rice genome (Table 3). Each syntenic block has at least two markers in common between individual rye and rice segments. Synteny between this rye map and the rice genome is supported by 157 RFLP and EST-derived SSR markers, which have been mapped in independent populations and which have been integrated in our analysis based on their orthologous rice gene models (ESM4). The chromosomal localization of these 157 markers on individual rye chromosome arms approves the suggested orientation of the 7 rye chromosomes in the genetic map of HYB201 × HYB202 and allows a rough prediction of the centromere position for each chromosome (Fig. 2, ESM5-10). Complementary to the rice gene models with known map position in rye, we have included 156 cloned QTL/genes mapping to the 13 conserved blocks in rice (ESM4). The majority of these genes (62) control flowering time. In addition, we have integrated the identified rice gene models with orthologs of known map position in rye in the recently published virtual linear gene order model (genome zipper) comprising 22,426 rye genes (ESM4, Martis et al. 2013). As already described for the DArT markers, the comparison with the map of the genome zipper, which integrates data from three independent mapping populations, showed a good congruency in the order of the EST-derived SSR and COS markers mapped in the present study (ESM4). Likewise, the rye genome zipper supports the observed syntenic relationship between the genetic map of HYB201 × HYB202 and the rice genome. Furthermore, the rye genome zipper bridges the genetic maps of HYB201 × HYB202 and Lo115 × Lo90 based on 191 SNP markers, which have been mapped in Lo115 × Lo90 (Miedaner et al. 2012) as well as in the genome zipper (ESM4, sheets RyeZipper_1R to RyeZipper_7R). Thus, a comparative QTL mapping between HYB201 × HYB202 and Lo115 × Lo90 based on the collinearity between both maps and the genome zipper becomes feasible as well. This comparison, however, revealed that the orientation of chromosomes 2R, 3R, 4R, 5R, and 7R in Lo115 × Lo90 was published inverted compared to the integrated map of the genome zipper (ESM4).

Identification of QTL for four agronomic traits

Composite Interval Mapping with a multiple regression approach allowed to detect the positions of 22 QTL with an LOD threshold ≥3.47 (Table 4). Most QTL were found for TGW (10) and HDT (7). The explained genotypic variance of individual QTL ranged from 3 to 40%. The genotypic variance for HDT simultaneously explained by all seven detected QTL reached 85%. For the other traits, this estimate ranged from 44% for SSM to 70% for TGW. Our analysis revealed 11 major QTL explaining more than 10% of the genotypic variance. A major GYD QTL, *QGyd-2R* ($p_G = 40\%$), is located on the long arm of chromosome 2R (Fig. 2). Two major QTL for SSM, *QSsm-3R* ($p_G = 23.5\%$) and *QSsm-5R* ($p_G = 20.5\%$), are located on chromosome 3R and 5R, respectively (Table 4). Significant ($P < 0.01$) QTL × environment interaction variances could be observed for 10 QTL (45.5%, Table 4).

Comparative QTL mapping between rye and rice

The conserved synteny observed in the present study enables to superimpose QTL identified in rye and rice. For this purpose, we have used the rice genome as surrogate to integrate flanking markers of rye and rice QTL, which enabled to determine consensus among QTL for related traits (Fig. 2, ESM5-10).

Orthologous segments between 0.9 and 8.6 Mb on rice chromosomes R2, R3, R4, and R6 correspond to the six QTL *QHdt-2R.1*, *QHdt-2R.2*, *QHdt-4R.2*, *QHdt-5R*, *QHdt-6R,* and *QHdt-7R* controlling heading date in rye. The syntenic rice segments carry QTL *dth4.2, QHd4, hd6, QHd3c, QHd3b, Hd6, dth2.1,* and *QHd3b*, which have an

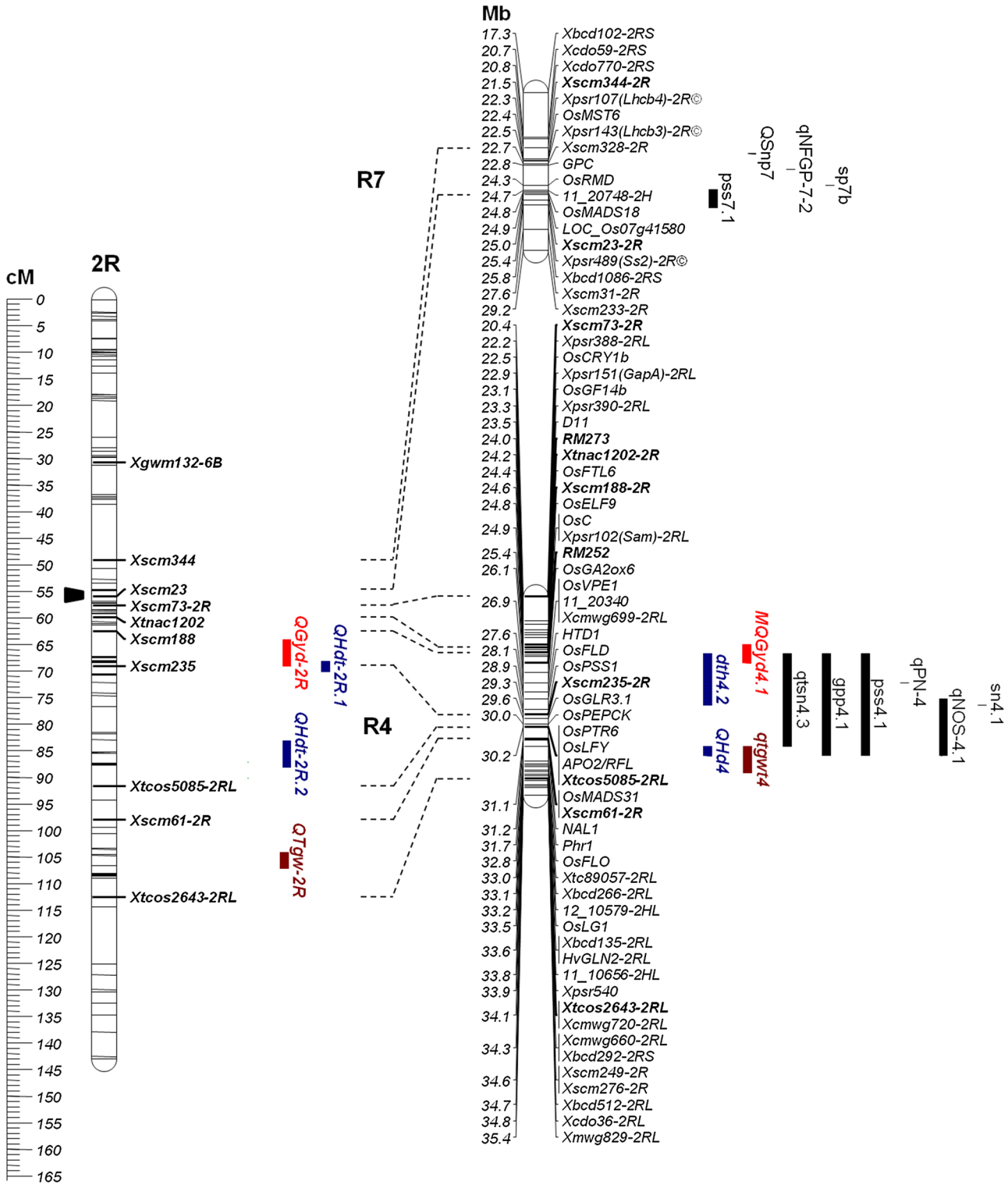

Fig. 2 Comparative QTL mapping between rye and rice. Gene-derived markers are given in *bold* and allow to integrate a 61.8 cM segment on the long arm of rye chromosome 2R in the physical map of rice chromosomes 4 (R4) and 7 (R7). The gene-derived markers and their orthologs in rice are connected by *dotted lines*. The positions of the markers in the rye map are given in cM and in the physical map of rice in Mb. The *vertical bars* and *QTL symbols* indicate the position of the following quantitative traits: QGyd-2R: grain yield, QHdt-2R.1, QHdt-2R.2: heading date, QTgw-2R: thousand grain weight, qtgwt4: 1000-seed weight, QHd4: heading date, MQGyd4.1: grain yield, sn4.1: spikelet number, qNOS-4-1: spikelet number, qPN-4:panicle number, qtsn4.3: spikelet number, gpp4.1: grain number, pss4.1: seed set percent, dth4.2: day to heading, pss7.1: seed set percent. A description of the genes indicated in the rice physical maps is given in ESM4

Table 3 Survey of conserved linkage blocks between rye and rice

Rye block	Start	End	Size (cM)	Map length covered	Rice block	Start	End	Size (Mbp)	Pseudomolecule covered
1R-1	32.8	67.4	34.6	0.218	R5-1	0.6	3.3	2.7	0.091
1R-2	98.2	131.0	32.8	0.207	R5-2	26.3	28.9	2.6	0.087
2R-1	49.0	55.9	6.9	0.048	R7	21.5	25.0	3.5	0.116
2R-2	57.6	112.4	54.8	0.383	R4	20.4	34.1	13.7	0.385
3R-1	46.6	53.4	6.8	0.065	R1	0.2	23.2	23.1	0.533
4R-1	127.3	138.9	11.6	0.081	R2-1	0.0	0.3	0.2	0.006
4R-2	53.4	81.8	28.4	0.198	R6-1	1.8	9.1	7.3	0.234
5R-1	61.9	69.7	7.8	0.047	R6-2	11.2	26.5	15.4	0.492
5R-2	93.0	112.5	19.5	0.118	R3-1	24.6	33.2	8.6	0.235
5R-3	118.6	139.3	20.7	0.125	R3-2	0.1	1.8	1.7	0.046
6R-1	8.4	35.6	27.2	0.223	R2-2	24.8	33.1	8.3	0.230
7R-1	12.2	23.1	10.9	0.086	R3-3	34.8	35.4	0.6	0.017
7R-2	45.0	57.4	12.4	0.098	R3-4	3.7	10.0	6.3	0.174
Total			274.4	0.284				93.9	0.252

impact on heading date in rice, as well (ESM2). Moreover, 18 cloned genes controlling heading date in rice are located on each of the syntenic fragments (ESM4).

For the four QTL *QTgw-2R*, *QTgw-4R.1*, *QTgw-4R.3,* and *QTgw-5R* controlling the yield component thousand grain weight in rye, we have identified syntenic segments between 0.8 and 5.2 Mb on rice chromosomes R3, R4, R6, and R11. Four of these syntenic rice segments harbour QTL *qtgwt4*, *tgwt11*, *gw6,* and *QKw3a* which govern TGW in rice, as well (ESM2). The rice genes, OsFLO, which is involved in the regulation of grain size and starch quality in rice (She et al. 2010) as well as HGW, which regulates grain weight and heading date in rice (Li et al. 2012b), are located in syntenic fragments of *QTgw-2R* and *QTgw-4R.3* (ESM4).

For the QTL *QSsm-5R* controlling the yield component spikes per square meter in rye, we have identified a syntenic segment on rice chromosome R9. This rice segment carries the QTL qTN-9-1 which governs tiller number in rice (ESM2, ESM8). The gene *OsEATB*, which is involved in the regulation of tiller number in rice (Qi et al. 2011), is located in this syntenic segment too (ESM4).We have identified two syntenic segments of 0.04 and 4.7 Mb on rice chromosomes R3 and R4 for the 2 QTL *QGyd-5R and QGyd-2R* controlling GYD in rye. These syntenic rice segments harbour GYD QTL *qgy3.1* and the meta QTL $MQTL_{4.1}$ for GYD (Swamy et al. 2011), the latter of which is indicated as *MQGyld4.1* in Fig. 2. The syntenic segment on rice chromosome R4 is delimited by *Xscm188* and *Xscm235* and carries additional QTL governing spikelet number (*qtsn4.3*, *qNOS-4.1*, and *sn4.1*), panicle number (*qPN-4*), grain number (*gpp4.1*), and seed set percent (*pss4.1*) (ESM2). Furthermore, this 4.7 Mb rice segment carries the gene *OsGA2ox6,* a Gibberellin (GA) 2-oxidase (ESM4).

Discussion

Rich genetic diversity for agronomic traits in elite rye germplasm

In this study, we have used an $F_{2:3}$ design (Austin and Lee 1996; Fisch et al. 1996) and analyzed 258 testcross progenies originating from two elite genotypes of a hybrid rye breeding program to identify QTL for four agronomic traits including grain yield in winter rye hybrids. Although, in this experimental design, only half of the total additive genetic variation can be exploited, this approach considers the limited predictive value of line per se performance for hybrid performance in yield-related traits in rye (Miedaner et al. 2014). A further serious challenge in using testcross performance for mapping in bi-parental populations is dominance. A strong dominant allele of a tester genotype may mask the effect of the allele contributed by the tested genotype. The limitations of strong elite testers have previously been discussed (Hallauer and Miranda 1988; Mihaljevic et al. 2005). However, despite these limitations, we observed a significant genetic variation ($P < 0.01$) for all analyzed traits. The observation of significant genetic variation between testcross progenies is particularly noteworthy, as in the $F_{2:3}$ lines, only half of the additive genetic variance was exploited compared to recombinant inbred line populations (Hallauer et al. 2010). Although the resolution of QTL mapping using recombinant inbred lines is higher due to an increased number of recombination events in successive selfing steps, the time consuming development of recombinant inbred lines per se can be biased by inbreeding depression in case of the allogamous rye.

Table 4 Quantitative trait loci of four agronomic traits including grain yield based on 10% LOD threshold at 3.47 (based on 2000 permutation test) and a 1000-fold cross validation

	QTL	Chr.	Pos	SIL	SIR	Left marker	Right marker	LOD	Effect[a]	p_G[b]	QTL × E[d]	Freq. CV[c]	EffectES[e]	EffectTS[f]
Heading date [1–9]	∑ 7									∑ 84.65				
	QHdt-2R.1	2R	69	68	70	XrPt-507619	XrPt-508957	6.304	−0.133	8.6	**	0.9000	−0.188	−0.205
	QHdt-2R.2	2R	86	83	88	XrPt-509592	XrPt-402599	5.313	−0.173	15.33	**	0.7470	−0.166	−0.143
	QHdt-4R.1	4R	6	0	14	XtPt-3302	XrPt-507297	5.882	0.109	9.5	**	0.7190	0.121	0.108
	QHdt-4R.2	4R	69	67	70	XrPt-509132	Xtc368556 g	6.868	0.103	8.76	**	0.3560	0.108	0.069
	QHdt-5R	5R	105	99	108	Xtcos1359	XrPt-400590	4.351	0.139	14.45	**	0.5570	0.145	0.121
	QHdt-6R	6R	9	7	10	Xtnac1727	XrPt-5403	4.477	0.105	7.57	**	0.3470	0.118	0.071
	QHdt-7R	7R	57	56	59	XrPt-402149	XrPt-399686	10.322	−0.165	20.44	**	0.8200	−0.152	−0.140
Spikes per m^2	∑ 2									∑ 43.98				
	QSsm-3R	3R	70	69	72	Xscm239-3R	XrPT-507655	3.470	−9.699	23.52		0.2550	−10.621	−8.185
	QSsm-5R	5R	49	48	52	XtPT-3980	Xtcos5220-5RL	3.807	9.267	20.46		0.3550	9.780	7.969
Grain yield (dt ha-1)	∑ 3									∑65.63				
	QGyd-2R	2R	66	64	69	Xscm188	XrPt-508470	18.8	−1.16	40.21	**	0.914	−1.127	−1.087
	QGyd-3R	3R	81	75	83	XrPt-401113	XrPt-398525	5.34	−0.55	12.66	–	0.254	−0.565	−0.429
	QGyd-5R	5R	126	124	128	Xtnac1388-5R	Xtcos3096-5R	4.926	0.608	12.76	*	0.599	0.634	0.438
1000-grain weight (g)	∑ 10									∑ 70.21				
	QTgw-1R.1	1R	69	67	70	XrPt-505603	XrPt-400866	18.485	0.545	14.68	–	0.664	0.536	0.516
	QTgw-1R.2	1R	133	130	138	Xscm171	XrPt-507839	4.406	0.205	2.77	–	0.174	0.268	0.116
	QTgw-2R	2R	106	104	107	XrPt-505455	XrPt-398612	4.399	−0.591	17.34	–	0.141	−0.522	−0.485
	QTgw-3R	3R	45	39	47	XrPt-506847	Xscm84-3R	4.816	0.277	4.24	–	0.15	0.298	0.137
	QTgw-4R.1	4R	31	25	36	XrPt-400085	Xscm352	5.236	−0.297	2.57	**	0.197	−0.413	−0.246
	QTgw-4R.2	4R	51	50	52	XrPt-400488	Xscm356	13.376	−0.536	7.24	–	0.616	−0.647	−0.634
	QTgw-4R.3	4R	69	68	70	XrPt-509132	Xtc368556 g	6.737	0.563	10.41	–	0.561	0.569	0.539
	QTgw-5R	5R	119	118	125	Xtnac1394	XrPt-506735	6.512	0.222	3.21	–	0.124	0.275	0.111
	QTgw-6R	6R	2	1	4	XrPt-508161	XrPt-399992	4.183	−0.234	3.4	–	0.096	−0.29	−0.132
	QTgw-7R	7R	5	4	7	XrPt-508123	XrPt-507064	4.311	0.242	4.35	–	0.413	0.262	0.166

QTL quantitative trait loci, *Chr* chromosome, *Pos* position in cM, *SIL* support interval left in cM, *SIR* support interval right in cM, *Left-M* left marker, *Right-M* right marker, *LOD* critical LOD value

[a] Additive effect, [b] part of the explained genetic variance, [c] Frequency in cross validation, [d] QTL by environment interaction tested for significance (sequentially rejective Bonferroni *F* test) at the 0.05 (*) and 0.01 (**) probability level, respectively, [e] mean QTL effect in the estimation sets of cross validation, [f] mean QTL effect in the test sets of cross validation

Thus, an $F_{2:3}$ design as described in the present study enables a fast and unbiased approach of QTL mapping in winter rye hybrids.

Notably, the parental mean was identical between both elite genotypes for HDT, which indicates that no major phenology-related gene, for example influencing photoperiod response, is segregating in the $F_{2:3}$ population. This genetic makeup of both parental elite genotypes minimized masking of QTL by confounding effects of loci governing plant development, a phenomenon that affected QTL analysis in wheat and barley (Fleury et al. 2010). Furthermore, the parental mean did not differ significantly ($P < 0.05$) from progeny mean in all traits, except for GYD, indicating a predominantly additive inheritance. Although we observed no significant phenotypic differences between the means of the two parents in the testcross performance for HDT and TGW, the analysis of their progeny indicated that both genotypes differ with respect to the genetic constitution at individual QTL for both traits. Our results are in line with research, e.g., in maize which revealed, that parental phenotypic difference in testcross means was only weakly or even unrelated to progeny genetic variances (Melchinger et al. 1998; Hung et al. 2012). Thus, our data represent a further example of the challenge to (1) predict genetic variances in segregating generations from phenotypic means of parental genotypes and (2) appropriately select parents based on their phenotypic performance in plant breeding programs. In contrast, genome-based approaches have a higher power to predict the breeding potential of base populations in rye (Wang et al. 2014; Auinger et al. 2016).

The significance of genotype-by-environment interaction observed for all traits illustrates that multi-environmental phenotyping of the investigated traits is imperative. The number of environments used in the present study fits the suggested prerequisite for a reliable estimation of h^2 (Schön et al. 2004). Entry-mean heritability was high for TGW ($h^2 = 0.88$) and medium for HDT ($h^2 = 0.67$), while GYD ($h^2 = 0.52$) revealed a moderate heritability estimate. Consequently, the chances of QTL detection for these traits with the given sample sizes were assumed to be high. The h^2 estimates for GYD of intrapool testcrosses of two bi-parental populations from the 'Petkus' genepool were higher ($h^2 = 0.70$, Miedaner et al. 2012) compared to the h^2 estimates for GYD in the present study. The moderate heritability for GYD estimated in the present study indicates a reasonable high impact of the different test environments on the performance of the experimental hybrids in our study. Indeed, the year 2011 was one of the five warmest years in Germany since 1881 with 9% less precipitation compared to the long-term average (Deutscher Wetterdienst 2011), while rainfall and temperature were almost close to the average in 2012 throughout Germany (Deutscher Wetterdienst 2012).

A novel genetic linkage map for the analysis of complex inherited traits in rye

The construction of an accurate genetic linkage map with 941 markers provides the pivotal basis for the mapping of QTL and subsequent applications including comparative mapping, positional cloning, as well as the transfer of these results in practical crop improvement programs. We have used dominant inherited DArT markers as an efficient, microarray-based DNA fingerprinting method, which delivered the framework to establish a genetic linkage map in rye. Up to now, DArT markers have successfully been applied in rye to establish genetic linkage maps based on populations of recombinant inbred lines (RIL, Milczarski et al. 2011; Miedaner et al. 2012). By splitting the data set into two complementary subsets, each containing shared codominant and dominant markers in the coupling-phase, and subsequent integrating both data sets into a single map based on the shared codominant markers, we were able to establish a genetic linkage map in a F_2 population, whose length (964.6 cM) compares well with data for previously published F_2, BC_1, and $F_{3:4}$ mapping populations in rye (Devos et al. 1993; Korzun et al. 2001; Ma et al. 2001; Hackauf et al. 2009; Miedaner et al. 2012). In a previous report, an almost 2.5-fold increase in length has been observed for a genetic linkage map based on DArT and SSR compared to a map based on SNP and SSR markers (Miedaner et al. 2012). The mapping strategy applied in the present study considered that in F_2 matings, recombination frequencies and locus orders of markers may be mis-estimated from dominant markers in mixed coupling phases (Knapp et al. 1995; Mester et al. 2003). We demonstrate that genetic map construction in rye can take advantage of an efficient genome-wide DArT fingerprinting without increasing the map length, if genotype data for a reasonable number of codominant markers are available as well. This requirement can be achieved for instance with SSR and COS markers. Likewise, SNP markers from the recently established high-density maps of rye (Martis et al. 2013; Bauer et al. 2017) as well as recently published sequence information on DArT markers (Gawroński et al. 2016) might be used and converted to allele-specific PCR assays for simple and accurate genotyping assays without sophisticated laboratory equipment.

The comprehensive sequence analysis by Gawroński and co-workers (2016) revealed inconsistencies concerning localization and order of DArT markers in a high-density consensus map (Milczarski et al. 2011) compared to the transcript map of rye (Martis et al. 2013). The observed collinearity between the rye map described in the present study and other genetic maps of rye as well as parts of the rice genome indicates a reliable marker order in the established genetic map. In total, 158 DArT markers

(20.1%) described here have been mapped in the population Lo115 × Lo117 (Miedaner et al. 2012), as well. Notably, collinearity between both maps could be observed for all chromosomes; however, the orientation of chromosomes 2R, 4R, and 5R in population Lo115 × Lo117 is inverted relative to the map established for the QTL analysis described in the present study (ESM3).

The genetic map described here is characterized by a comparable high marker density. It has recently been reported that high-density maps increase the precision of QTL localization as well as the precision of effect estimates for detected QTL, especially for small- and medium-sized QTL and the power to resolve closely linked QTL (Stange et al. 2013). A further attribute of the established map in terms of a subsequent dissection of individual QTL is the alignment with the recently published transcript map of rye (Martis et al. 2013), which enables a systematic enrichment of markers for individual QTL. Here, we report only on those QTL that surmount the threshold across all seven environments. Although this approach may have contributed to a relatively low number of QTL per trait, these environmentally stable QTL are most promising for breeding purposes.

Large-effect QTL for heading date in conserved segments of the rye genome

Flowering time is a key adaptive trait in wild and crop cereal species which is also known as heading date and warrant plants to switch from vegetative to reproductive growth at a most favourable time for pollination, seed development, and seed distribution. Breeding of varieties with reduced time to flowering has been reported as an effective strategy to increase yield in regions where drought commonly occurs during grain filling (Passioura 1996; Slafer and Whitechurch 2001). In our study, the parents of the $F_{2:3}$ population HYB201 and HYB202 are both elite winter rye genotypes adapted to the Central European climate. Although, thus, both parental genotypes reveal no phenotypic differences in heading date control, we were able to identify seven QTL explaining 85% of the genotypic variance for HDT in their testcross progenies. This observation indicates the impact of major genes in determining HDT of winter rye. Progress in dissecting the genetic and molecular basis of flowering time control in European elite winter wheat has recently been achieved based on genotypes of contrasting geographic origin, which revealed substantial differences in flowering time behaviour (Langer et al. 2014; Zanke et al. 2014). In both studies, the photoperiod regulator *Ppd-D1* was identified as the major factor controlling flowering time in the studied germplasm sets. Interestingly, Zanke and co-workers (loc. cit.) identified a marker locus on wheat chromosome 5BL with homology to the rice photoperiodism gene *Hd6*, which is associated with the determination of the heading date in wheat. Likewise, *QHdt-5R* maps to a genomic segment, which is syntenic to a sub-genomic region on rice chromosome R3 including the heading date QTL *Hd6*. This obvious correspondence between heading date QTL across taxa provides insights in the evolution of the underlying HDT phenotype. The observation suggests that corresponding genes might be involved in the evolution of the relevant phenotypes and identifies *QHdt-5R* as a rewarding target for a dissection and detailed characterization of a HDT QTL at the molecular level in rye. The further development of molecular tools for a precise genetic improvement of HDT will support the adaptation and performance of winter rye particularly in the light of predicted climate change.

QTL for thousand grain weight are located at different positions in both heterotic groups

In the present study, we have approached TGW as an important component of GYD. In total, 10 QTL were detected explaining 70% of the genotypic variance. Neither of the three major QTL *QTgw-1R.1* ($p_G = 14.68$), *QTgw-2R* ($p_G = 17.34$), and *QTgw-4R.3* ($p_G = 10.41$) identified in the present study reached a comparable high p_G value as the majority of major TGW QTL identified in testcrosses of the two bi-parental 'Petkus' populations (Miedaner et al. 2012).

Based on DArT markers, which have been mapped in the populations Lo115 × Lo117 ('Petkus' gene pool) as well as HYB201 × HYB202 ('Carsten' gene pool, ESM3), a direct comparison of QTL governing TGW in both bi-parental populations is possible. *QTgw-1R.1* maps to an interval which is defined by markers *XrPt-399643* mapping at position 147.6 cM and *XrPt-401076* mapping at position 175.6 cM on chromosome 1R in Lo115 × Lo117 and can, thus, be classified as not identical to the grain weight QTL #1, which has been mapped distally to the *XrPt-399643/XrPt-401076*-interval on chromosome 1R (Miedaner et al. 2012). This conclusion can be drawn for *QTgw-1R.2* as well, which maps distally to the shared marker *XrPt-507636*, a marker located at position 270.0 cM in Lo115 × Lo117 (Miedaner et al. 2012). No thousand grain weight QTL have been mapped on chromosomes 2R, 3R, and 4R in population Lo115 × Lo117, thus, turning a comparison for *QTgw-2R*, *QTgw-3R*, *QTgw-4R.1*, *QTgw-4R.2,* and *QTgw-4R.3* not feasible. *QTgw-5R* is located in an interval marked by *XrPt-507948* and *XrPt-410783*, which have been mapped at positions 41.9 cM and 72.5 cM in Lo115 × Lo117 (Miedaner et al. 2012). Based on the map position of these markers relative to the distally located genetic interval carrying the QTL for TGW on chromosome 5R in Lo115 × Lo117, it can largely be excluded that

QTgw-5R corresponds to the thousand grain weight QTL #2 in *Lo115xLo117*. Rather, *QTgw-5R* is located in the interval defined by *XrPt-400590* and *XrPt-507373*. These markers are located on the long arm of chromosome 5R in the RIL population L2039-NxDH. In this RIL population, the wheat microsatellite marker *Xgwm6-5R* maps to this particular interval as well (ESM3). Using its synonymous identifier *WMS6, Xgwm6-5R* has been reported as being linked with the major gene KW_5 governing TGW in rye (Wricke 2002). This observation renders it likely that *QTgw-5R* corresponds to KW_5. However, additional markers linked to *Xgwm6-5R* in population L2039-NxDH need to be mapped in population HYB201 × HYB202 to validate this hypothesis. The QTL *QTgw-6R* is located distally from *XrPt-411507* and appears to be different from QTL #3, which has been mapped proximal to *XrPt-411507* in population Lo115 × Lo117 (Miedaner et al. 2012). Finally, *QTgw-7R* maps to a segment on the short arm of chromosome 7R, which is located distally from *XrPt-508478* at position 17.2 cM in Lo115 × Lo117 (Miedaner et al. 2012), indicating that this QTL does not correspond to the thousand grain weight QTL #4 on the long arm of chromosome 7R in the 'Petkus' population. The observed contrasting results in both bi-parental populations with respect to the explained genetic variance and particularly the different map positions of individual QTL indicate that in hybrid rye breeding, the genetic improvement of TGW needs to be independently examined in both heterotic genepools. In conclusion, all QTL detected in this population for TGW are different from those reported earlier in other populations.

A conserved major QTL for grain yield on the long arm of chromosome 2R

The QTL analysis described here allowed to detect a major QTL *QGyd-2R*, which is characterized by a high frequency of cross validation. In the two recently analyzed mapping populations representing the 'Petkus' gene pool, 1 and 7 QTL governing GYD in rye have been reported (Miedaner et al. 2012). Likewise, based on a data set of almost 1000 testcross progenies evaluated in 19 environments, 8 QTL for GYD have been identified in maize at a significance threshold of LOD = 3.21 (Schön et al. 2004). Schön and co-workers (2004) demonstrated by cross validation of experimental data, that increasing sample sizes as well as number of environments increased the number of detected QTL. As a consequence of this so-called Beavis effect (Beavis 1998; Xu 2003), it appears to be evident that the experiment described here was underpowered for QTL analysis of GYD and predisposed us to find large-effect variants, while we could not detect QTL with smaller effects that essentially caused variation in this trait. Indeed, our observation raises the question, if the effect for *QGyd-2R* is just overestimated or if biological reasons could explain that QTL variants like *QGyd-2R* with such a substantial influence on a quantitative trait like GYD have arisen under the infinitesimal model, which predicted that heritable quantitative traits would be specified by an innumerable number of minute effects (Fisher 1918)?

The mapping of individual QTL explaining a large proportion of the inherited variability is a common finding from QTL mapping experiments (Farrall 2004; Schön et al. 2004; Phillips 2005). For example, in rice, a major QTL for GYD was identified on chromosome R1 (Vikram et al. 2011), while in wheat, major QTL for GYD have been identified on chromosomes 3A and 6A (Rustgi et al. 2013). The identification of *QGyd-2R* in rye hybrids is in line with these observations. Interestingly, in the population Lo115 × Lo117, the GYD QTL #3 was mapped to that genetic interval on chromosome 2RL, which harbours *QGyd-2R* as well and which is defined by the flanking marker loci *XrPt-401315* and *XrPt-399800*, respectively. The effect of GYD QTL #3 was less pronounced compared to *QGyd-2R*. This might be attributed to the fact that *QGyd-2R* in our study was identified in testcrosses of the mapping population with a single-cross elite tester from the opposite gene pool, which includes heterotic effects. The congruent map positions of *QGyd-2R* and GYD QTL #3 in both genetic divergent gene pools suggest that mutations at corresponding genetic loci on chromosome 2RL contribute to determine GYD in rye. Random mutations of large effect were proposed to have a high probability of fixation if they were favourable (Kimura 1983). This proposed modification of the infinitesimal model predicts that mutations of an intermediate size will drive adaptation and balances Fisher's expectation that most favourable mutations will have small effects with correspondingly small fixation probabilities (Farrall 2004). This process might be enforced by the artificial selection in plant breeding. Likewise, the observed large effect of *QGyd-2R* fits to another model which suggests that natural selection validates mutations with large effects at the beginning of an adaptation process with a maximum of adaptive space, while later on in the process when the organism has essentially reached its optimum state, the space is narrowed and successful mutations must have smaller effects (Orr 1998). Thus, according to Orr's model, *QGyd-2R* might belong to few QTL with (relatively) large effects, which determine the quantitative variation of GYD in rye together with a cumulative number of genes with smaller effects. A comparative genomics approach and the progress achieved in the isolation of rice genes controlling agronomic traits provide a first indication of a candidate gene with such a pronounced effect on grain yield.

Candidate genes exist across the identified QTL regions

In rye, comparative genomic approaches have proven to be successful for the development of molecular markers in different sub-genomic regions carrying major genes on the short arm of chromosome 1R (Mago et al. 2005), the long arm of chromosome 2R (Hackauf and Wehling 2005), the long arm of chromosome 4R (Hackauf et al. 2012), and the short arm of chromosome 7R (Miftahudin and Gustafson 2004; Collins et al. 2008). The observed collinearity between rye and rice in the present study matches the previously established virtual linear gene order model (Martis et al. 2013) almost perfectly and provides the opportunity to investigate overlaps among QTL in the genomes of rye and rice for individual traits.

The syntenic relationships between rye chromosome 3R and rice chromosome R8, 4R and R8, 5R and R6, 6R and R1, as well as 7R and R6 have not yet been described (Martis et al. 2013), and provide further insights in the evolution of the rye genome. The syntenic relationship between 6R and R1 is supported by the map positions of the genomic wheat SSR markers *GWM391*, *GWM247,* and *GWM340* on the long arm of rye chromosome 6R, which have been described in a previous study (Khlestkina et al. 2004) and could be confirmed here. These markers have been mapped on homeologous group 3 chromosomes in wheat (Röder et al. 1998), which have been shown to correspond to rice chromosome R1 (Munkvold et al. 2004).

Flowering time is a trait that is largely conserved across taxa and the flowering time pathway in temperate grasses is well characterized at the molecular level (Higgins et al. 2010). Thus, the flowering time genes located in corresponding segments of the six QTL *QHdt-2R.1*, *QHdt-2R.2*, *QHdt-4R.2*, *QHdt-5R*, *QHdt-6R,* and *QHdt-7R* provide candidates to further dissect the molecular basis of flowering time control in rye. An interesting candidate gene appears to be the rice *HGW* gene, which is located 0.02 Mb distally to a 1.7 Mb segment on rice chromosome R6, that is defined by *Xtcos1747* and *Xtc368556g* and that is syntenic to a segment on rye chromosome 4R including the colocalized QTL *QTgw-4R.3* and *QHdt-4R.2*. HGW encodes an ubiquitin-associated (UBA) domain protein, which delays heading and reduces grain weight in rice (Li et al. 2012a). Likewise, the rice gene *OsFLO* is involved in the regulation of grain size and starch quality in rice (She et al. 2010) and provides a candidate gene for the QTL *QTgw-2R* on rye chromosome 2R.

A comprehensive analysis of the official German variety trials, where experimental hybrids are tested for their value for cultivation and use, highlighted that the tremendous genetic gain achieved in grain yield of hybrid rye varieties between 1989 and 2014 is mainly driven by their ability to increase the density of SSM (Laidig et al. 2017). We have identified two QTL, *QSsm-3R* and *QSsm-5R*, explaining 44% of the genotypic variance for SSM in our mapping population. This result suggests the impact of major genes in determining SSM of winter rye. The synteny to rice chromosomes R9 discovers the rice gene *OsEATB*, which encodes an ERF protein associated with tillering and panicle branching (Qi et al. 2011), as an initial target for studying the genetic makeup of *QSsm-5R* in rye at the sequence level (ESM4).

Interestingly, the meta QTL $MQTL_{4.1}$ for GYD in rice is located on the segment on rice chromosome R4 (Swamy et al. 2011) corresponding to the *QGyd-2R*-interval on rye chromosome 2RL. This observation identifies the *Xscm188/Xscm235* interval on rye chromosome 2RL and rice chromosome R4 particularly attractive for further research to improve our understanding concerning the genetic basis of GYD in cereals. Both flanking microsatellite markers *Xscm188* and *Xscm235* enable a marker-assisted introgression of *QGyd-2R* in different genetic backgrounds for the validation with the current and alternative CMS single-cross testers in rye. Furthermore, *QGyd-2R* represents an attractive target to counterbalance linkage drag effects on grain yield that have been observed for efficient restorer-of-fertility genes in rye hybrids (Miedaner et al. 2017).

The co-localization of the grain yield $MQTL_{4.1}$ with QTL governing spikelet number (*qtsn4.3*, *qNOS-4.1*, *sn4.1*), panicle number (*qPN-4*), grain number (*gpp4.1*), and seed set percent (*pss4.1*) in the syntenic *Xscm188/Xscm235*-segment on rice chromosome R4 reveals information on important yield components. In rye, a high amount of heterosis has been reported for seed set percent and grain number (Geiger and Wahle 1978). Thus, these traits provide a possible explanation of the large genetic effect of *QGyd-2R* on grain yield in the present study compared to the GYD QTL #3 identified in testcrosses within the 'Petkus' gene pool (Miedaner et al. 2012) and should be assessed in subsequent experiments on *QGyd-2R*. Noteworthy, a putative gibberellin (GA) 2-beta-dioxygenase, *OsGA2ox6*, is located in the syntenic *Xscm188/Xscm235*-interval on rice chromosome R4 as well. A Gain-of-function *GA2ox6*-mutant showed increased tillers and adventitious root growth in rice (Lo et al. 2008). Overexpression of *GA2ox6* with a mutation at A141E in rice has been reported to result in a significant increase of grain yield by, on average, 24% as compared to the non-transformed control and by, on average, 39% to transgenic rice plants with overexpression of the allele G343A in three tested environments (Lo et al. 2016). Although *GA2ox6* is not listed among the candidate genes residing at the $MQTL_{4.1}$ region (Swamy et al. 2011), this knowledge of gene function as well the strong correlation between tiller number and grain yield in hybrid rye (Laidig et al. 2017) qualifies *GA2ox6* as an interesting target to approach allelic

variants of both parental genotypes (HYB201, HYB202) and beyond to elucidate effects on grain yield in rye. The negative heterosis observed for tiller number of rye hybrids compared to the midparent values (Geiger and Wahle 1978) does not exclude *GA2ox6* as a candidate gene for *QGyd-2R*, as light incidence is higher in a plant population of inbred lines, which, in addition, are less vigorous compared to hybrid plants and, thus, have a lower demand for nutrients and water. Altogether, these attributes probably result in a lesser reduction of established tillers in inbred lines at stem elongation compared to hybrids (Geiger and Wahle 1978). However, we observed no co-localization between *QGyd-2R* and QTL for tiller number, a trait that we have recorded as SSM. This might be explained by the comparable low heritability estimate ($h^2 = 0.35$) of SSM in our study, as the power of QTL detection is strongly affected by the heritability of a trait (Viana et al. 2017). Likewise, linkage significantly reduced detection power in a simulation study (Li et al. 2012a) and might have compromised to detect a QTL for SSM linked to *QGyd-2R*. Thus, further research on *QGyd-2R* including high-resolution mapping is needed to precisely determine the number and linkage relationships of genes with an impact on GYD.

In contrast to the progress achieved in dissecting the molecular basis of complex agronomic traits particularly in rice (Zuo and Li 2014), large knowledge gaps still exists on the genetic and molecular control of biological processes related to grain yield and yield parameters in other small grain cereals (Valluru et al. 2014). Using the rice genome data as a blueprint, comparative genomic approaches enabled to identify genes associated with nitrogen use efficiency (Quraishi et al. 2011a), grain fibre content (Quraishi et al. 2011b), carotinoid content (Dibari et al. 2012), grain weight (Su et al. 2011; Zhang et al. 2012), spikelet number (Zhang et al. 2015), and grain size (Ma et al. 2016) in wheat. These examples demonstrate the potential of comparative genomics approaches to isolate conserved genes controlling QTL in large Triticeae genomes. Hence, the initial genome scan described in the present study can open new perspectives to understand the genetic basis of the analyzed agronomic important traits in rye and to strengthen our understanding of the genetic and molecular mechanisms underlying cereal yield traits.

Conclusions

The QTL analysis of agronomically important traits including grain yield and heading date in the present study revealed a high degree of polymorphism in elite rye genotypes. This rich genetic diversity of the outbreeding rye together with a phenotyping strategy based on a $F_{2:3}$ design and testcross performance in multi-environmental field trials argues for a stronger utilization of rye in QTL analysis for the identification of genes that genetically control agronomically important traits in small grain cereals.

The large-effect QTL, like those found here for GYD, HDT, SSM, and TGW, could be candidates for successful marker-assisted selection programs in rye practical breeding. In addition, the primary mapping population described here will be advanced to near isogenic lines (NILs) for further fine-mapping and cloning individual QTL. For this purpose, the whole-genome draft sequence of rye (Bauer et al. 2017) will make a crucial contribution to enhance our understanding of complex agronomic traits in this close relative of wheat.

Author contribution statement The work presented here was carried out in collaboration between all authors. BH, FJF, and TM defined the research theme and supervised the project. BH and FJF conceived the design of this study and coordinated the experiments. FJF developed the plant materials. SH contributed to phenotyping, analyzed and interpreted the phenotypic data statistically, and performed the QTL analysis. BK and SR contributed to the collection of phenotypic data. AK guided the DArT fingerprinting and validated marker scores. DM contributed to the collection of phenotypic data and integrated DArT markers in the L2039-NxDH map. BH guided the genotyping with sequence-specific markers, established the genetic linkage map, performed the comparative mapping, interpreted the results, and drafted the manuscript. TM discussed analyses, interpretation, and presentation. All authors have read and approved the final version of the manuscript.

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Compliance with ethical standards

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

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